Meta-QTLs for multiple disease resistance involving three rusts in common wheat (Triticum aestivum L.)

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Abstract

Rust diseases in wheat are a major threat to global food security, therefore, the development of multiple disease-resistant cultivars (resistant to all three rusts) is a major goal in all wheat breeding programs worldwide. In the present study, meta-QTLs and candidate genes for multiple disease resistance (MDR) involving all the three rusts were identified using 152 individual QTL mapping studies for resistance to leaf rust (LR), stem rust (SR), and yellow rust (YR). From these 152 studies, a total of 1,146 QTLs for resistance to the three rusts were retrieved, which included 368 QTLs for LR, 291 QTLs for SR, and 487 QTLs for YR. Of these 1,146 QTLs, only 718 QTLs could be projected onto the consensus map saturated with 234,619 markers. Meta-analysis of the projected QTLs resulted in the identification of 86 MQTLs, which included 71 MDR-MQTLs. Ten of these MDR-MQTLs were referred to as the 'Breeders' MQTLs'. Seventy-eight of the 86 MQTLs could also be anchored to the physical map of the wheat genome and 54 MQTLs were validated by marker-trait associations identified during earlier genome-wide association studies. Twenty MQTLs (including 17 MDR-MQTLs) identified in the present study were co-localized with 42 known R genes. *In silico* expression analysis allowed identification of several differentially expressed candidate genes encoding proteins carrying different domains including the following: NBS-LRR, WRKY domains, F-box domains, sugar transporters, transferases, etc. The introgression of these MDR loci into high-yielding cultivars should prove useful for developing high yielding cultivars with resistance to all three rusts.

Key Message

In wheat, multiple disease resistance meta-QTLs (MDR-MQTLs) and underlying candidate genes for the three rusts were identified which may prove useful for development of resistant cultivars.

Introduction

Wheat production suffers major losses due to three rusts including leaf rust (LR, caused by *Puccinia triticina* f. sp. *tritici*), stem rust (SR, caused by *P. graminis* f. sp. *tritici*), and yellow rust (YR, caused by *P. striiformis* f. sp *tritici*) (Joshi et al. 1985; Rana et al. 2021). According to available estimates, LR causes yield losses of up to 50% in favorable conditions (Knott 1989; Huerta-Espino et al. 2011), SR causes yield losses of up to 100% during epidemic outbreaks (Soko et al. 2018), and YR causes yield losses ranging from 10–70%. The extent of yield losses in these estimates, sometimes also depends upon the cultivar used, time of infection, rate of disease development, and duration of the disease (Chen, 2005; Afzal et al. 2007). Chemical control and development of resistant cultivars are the two major options for managing the yield losses as above. Since the pathogens develop resistance against fungicides rather quickly (Oliver, 2014), the development of resistant cultivars is the preferred approach to overcome the losses due to rust infections. This requires adequate knowledge of the genetics of resistance.

During the last several decades, many studies have been conducted to identify genes for resistance to individual diseases (Li et al. 2020). As a result, different R genes or gene systems have become available for resistance against different diseases. These R genes can be race-specific or race-non-specific, the latter providing broad-spectrum resistance (Kou and Wang, 2010; Kaur et al. 2021). Currently, a large number of R genes are known for each of the three rusts, which include ~80 genes for LR, >70 genes for SR and ~83 genes for YR (McIntosh et al. 2016; Pradhan et al. 2020). These genes have regularly been used for breeding for disease resistance, although only a fraction of these genes has been utilized in developing resistant cultivars

The development of resistant cultivars generally involves the transfer of one or more genes for a specific disease, although efforts have also been made to develop resistance for multiple diseases in a breeding programme. This has generally been achieved through the pyramiding of genes (Gupta et al. 2021; Sharma et al. 2021; Rana et al. 2021). The method of pyramiding genes through conventional breeding is, however, demanding and requires a long time to achieve the desirable phenotype. In order to overcome this limitation, the concept of multiple disease resistance (MDR) was put forward initially for legumes (Nene, 1988) followed by several other reports in different crops including wheat (Singh et al. 2012; Zwart et al. 2010; Jighly et al. 2016; Mago et al. 2011). MDR differs from broad-spectrum resistance and APR, both generally providing resistance against most prevalent races for a particular pathogen, and not the resistance against several pathogens (Wiesner-Hanks and Nelson, 2016).

MDR has been described as "the holy grail" for several crops including barley (Paterson, 2014), and wheat (Pooja et al. 2014). Inheritance of MDR has also been studied (Ali et al. 2013; Pooja et al. 2014; Schweizer and Stein 2011; Wiesner-Hanks and Nelson 2016); in these inheritance studies, the occurrence of MDR has been inferred from a high level of positive correlations observed among resistance to individual diseases within the same crop (Randhawa et al. 2018). This MDR has been attributed to either the tightly linked clusters of R genes or pleiotropy (see later for some details). Some of the examples of tightly linked genes in wheat for resistance against several diseases involving three rusts include the following: (i) *Lr34/ Yr18/ Sr57/ Pm38/Ltn1* (7DS; Krattinger et al. 2009), (ii) *Lr46/ Yr29/ Sr58/ Pm39/Ltn2* (1BL, Singh et al. 2013), (iii) *Sr2*/Lr27/ *Yr30/Pbc1* (3BS; Mago et al. 2011), (iv) *Lr67/Yr46/Sr55/Pm46/Ltn3* (4DL; Herrera-Foessel et al. 2014).

MDR has also been identified in naturally occurring wheat cultivars. For instance, long back in history, wheat cultivars Hope and H-44, were both shown to carry resistance to not only LR and SR, but also for loose and covered smuts (Ausemus et al. 1943). Multi-trait (MT) analysis for quantitative disease resistance (QDR) involving more than one correlated disease also allowed identification of QTLs conferring resistance to multiple diseases, suggesting that there may be complex loci, which control resistance against more than one disease (Singh et al. 2012). In some recent reports, mainly involving genome-wide association studies (GWAS), MDR has also been reported in 5-10% of the naturally occurring wheat genotypes, including some synthetic wheats (Friesen et al. 2008; Gurung et al. 2009; Miedaner et al. 2020; Kumar et al. 2020A; Saini et al. 2022a). The details of these reports are summarized in Table S1.

The source of MDR identified in wheat genotypes used for several GWA studies may not be known, but we assume that pyramiding of genes through conventional breeding or occurrence of tight linkage or pleiotropy should be responsible for MDR in these cultivars. In recent years, the pyramiding of genes

for resistance against more than one disease has also been facilitated through markers assisted selection (MAS). One good example is the pyramiding of genes for the thousand-grain weight (TGW) along with three rust resistance genes (*Yr15* from one source and *Lr57-Yr40* from another source) into two popular Indian cultivars, namely PBW343 and PBW550 (Kaur et al. 2020).

The idea of MDR also prompted studies involving the identification of meta-QTLs using known QTLs for each of several individual diseases. This resulted in the identification of MDR-MQTLs in barley (Schweizer and Stein, 2011), maize (Ali et al. 2013), rice (Kumar and Nadarajah 2020b), and wheat (Saini et al. 2021a). In the present study, meta-QTL analysis was conducted for the identification of MDR-MQTLs providing resistance to three rusts, namely LR, SR, and YR in wheat; for this purpose, QTLs reported for resistance against all the three rusts were utilized. MDR-MQTLs thus identified were also compared with MTAs reported earlier using GWAS and with the available results of transcriptomics undertaken to uncover potential genomic regions and key candidate genes (CGs) that influence MDR in wheat. We hope that the MDR MQTLs identified during the present study should prove useful for the transfer and clustering of MQTLs and CGs for achieving MDR in wheat breeding programs.

Materials And Methods

Collection of information on QTLs associated with LR, SR, and YR diseases

Using PubMed and Google Scholar, an extensive search was made for QTLs already reported to be associated with resistance to LR, SR, and YR (till August 2021). The information thus obtained was further supplemented by the wheat QTL database (WheatQTLdb; www.wheatqtldb.net; Singh et al. 2021). Following information about individual QTLs was collected and compiled from each such study: (i) type and size of the mapping population, (ii) flanking markers and their genetic positions on the map, (iii) peak position, (iv) phenotypic variation explained (PVE) or R^2 value, and (v) logarithm of the odds (LOD) score for each QTL. In cases, where no information was available on the peak position of QTL, the mid-position of the two flanking markers was taken as the peak. Also, when the confidence interval (CI) for the QTL was missing, the CI (95%) was estimated using the following population-specific equations developed through different simulations:

(i) F_2 and BC populations (Darvasi and Soller, 1997): CI (95%) =530/(R^2×N)..... (i)

(ii) For DH populations (Visscher and Goddard, 2004): CI (95%) =287/(R^2×N)..... (ii)

(iii) For RIL populations (Guo et al. 2006): CI (95%) =163/(R^2×N)..... (iii)

From each study, the following two types of input data text files were prepared using the instructions available in the BioMercator v3 manual (Sosnoswski et al. 2012): (i) genetic map file and (ii) QTL information file. The generated map file for each study included information on the type and size of the population, mapping function, map units, and positions of different markers on linkage groups. Studies that lacked essential data (e.g., PVE or R^2; marker positions) were excluded from the analysis.

Construction of the consensus map and QTL projection

The consensus genetic map was constructed using the R package "LPmerge" (Endelman and Plomion 2014) utilizing the following high-quality genetic maps: (i) the 'Wheat Consensus SSR 2004' map with 1235 markers (Somer et al. 2004), (ii) the 'Wheat Composite 2004' map with 4403 markers, available at the GrainGenes database (http://wheat.pw.usda.gov), (iii) 'Integrated 2013' durum wheat map with 3669 markers (Marone et al. 2013), and (iv) four SNP maps developed using following different SNP arrays: 'Illumina 9K iSelect Beadchip Array' (with 7,504 SNPs) (Cavanagh et al. 2013), the 'AxiomR, Wheat 660K SNP array' (with 119,566 SNPs) (Cui et al. 2017), 'Illumina iSelect 90K SNP Array' (46,977 SNPs) (Wang et al. 2014), and 'Wheat 55K SNP array' (with 56,505 SNPs) (Winfield et al. 2016). Additional markers flanking each QTL reported in individual studies for LR, SR, and YR resistance genes were also included on the consensus genetic map.

QTLs were projected onto the above consensus map using the projection tool (QTLProj) available in Biomercator v4.2 (Chardon et al. 2004); QTLProj uses a dynamic algorithm to determine the best context for projection. An optimal context consists of a pair of common markers that flank the QTL in the original map and for which the distance is consistent between the original map and the consensus map. The behavior of the algorithm to find such a configuration is controlled by the lower values of the ratio of the distances between flanking markers and of the p-value obtained by testing the homogeneity of these distances in the original map and the consensus maps (Veyrieras et al. 2007).

Meta-QTL analysis and identification of MQTL hotspots

Meta-QTL analysis was performed using the position of each input QTL and the variance of this position, assessed through CI values; the analysis was based on Biomercator v4.2 (Arcade et al. 2004; Sosnowski et al. 2012). Two different approaches were used, one involving cases, where the number of QTLs on an individual chromosome was ≤10, (Goffinet and Gerber 2000), and the other including cases, where this number was >10 (Veyrieras et al. 2007). The model with the lowest Akaike Information Criterion (AIC) value was chosen as the best fit in the first approach. In the second approach, the best model was chosen among the AIC, corrected AIC (AICc and AIC3), Bayesian Information Criterion (BIC), and Average Weight of Evidence (AWE) models. The MQTLs located in the overlapping or adjacent genomic regions were considered as MQTL hotspots.

MQTLs were named based on their genetic positions; for instance, MQTLs mapped on chromosome 1A were designated as MQTL1A.1, MQTL1A.2, and so on. The PVE value and LOD score of a MQTL were calculated as the mean of the PVE values and LOD scores of the initial QTLs, on which the MQTL is based. The nucleotide sequences of flanking markers of each MQTL were BLASTed against the wheat reference genome sequence to find out their physical

positions in the genome. Physical positions of GBS-SNPs were obtained using the JBrowse wheat genome browser (https://wheat-urgi.versailles.inra.fr/Tools/Jbrowse).

Comparison of MQTLs with GWAS-based MTAs

In order to validate the MQTLs identified in the present study, data from 23 independent GWA studies (including 4 GWA studies on LR, 6 on SR, 7 on YR, 6 studies including at least two different rusts) was collected. These GWA studies utilized populations of wheat including durum wheat, hexaploid wheat (e.g., spring, and winter wheat) and synthetic hexaploid wheats (SHW) with population sizes ranging from 100 (Leonova et al. 2020) to 23,346 (Juliana et al. 2020), phenotyped across the 17 different countries. The details of population size, associated diseases, genotyping platform/number of SNP markers used, and MTAs detected in these studies are available in Table 1. Physical positions of each significant and stable SNP associated with the trait were obtained from the respective studies or JBrowse-WHEAT URGI database (https://urgi.versailles.inra.fr/jbrowseiwgsc/) and CerealsDB (https://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/indexNEW.php). Subsequently, the physical positions of these MTAs were compared with the physical coordinates of the MQTLs; MQTL co-localizing each with at least one MTA was considered as GWAS-validated/verified MQTL.

Association of MQTLs with known R genes

The Association of MQTLs with known R genes for the three rusts was also examined. For this purpose, sequences of R genes or their associated markers were BLASTed against the wheat reference genome to obtain the physical positions of the genes. The physical positions of the genes were then compared with the physical coordinates of the MQTLs to ascertain the co-localization of MQTLs with known R genes.

Identification of candidate genes in MQTL regions and their GO analysis

The BioMart tool of EnsemblPlants database (https://plants.ensembl.org/biomart/martview) was used to identify candidate genes (CGs) within 2Mb regions flanking the individual MQTL regions. In some cases, peaks of individual MQTLs had to be worked out before the search for CGs. The method employed to estimate the physical positions of MQTL peaks has been reported earlier (Jan et al. 2021). The InterPro database (https://www.ebi.ac.uk/interpro/) was used to extract the physical coordinates, function descriptions, and gene ontology (GO) terms of the identified gene models.

In silico expression analysis of the candidate genes

In silico expression analysis of the above CGs was conducted using expVIP (http://www.wheat-expression.com/; Ramírez-González et al. 2018) for stripe rust and genevestigator database (https://genevestigator.com/; Hruz et al. 2008) for leaf and stem rusts. Unfortunately, expression data for CGs for all the three rusts was not available in the same expression database. In the genevestigator database, 11 studies were available out of which, only 4 studies carried the expression data of our putative candidate genes including two studies each for LR and SR (Cloutier et al. 2008; Rutter et al. 2017; Salcedo et al. 2017; Yadav et al. 2016).

The expression data used in the present study for YR resistance belonged to two different studies (Zhang et al. 2014; Dobon et al. 2016). The data on expression was available as $\log_2$ transformed tpm (transcripts per million) values. Only genes (CGs) showing FC $\geq$ 2 (upregulation, 2-fold or more) or FC $\leq$ -2 (downregulation, 2-fold or more) were accepted as showing differential expression in the form of fold changes estimated by comparing tpm values under stress vs. control. The representative heatmap of the DECGs was generated using an online tool ClustVis (https://biit.cs.ut.ee/clustvis/).

Homoeologous relationship

MQTLs identified during the present study were also examined for homoeologous relationships, using corresponding physical positions on the known homoeologous chromosomes. For this purpose, genes were identified from the complete MQTL region, and these genes were subjected to BLAST analysis against the wheat genome database available at EnsemblPlants to identify the corresponding homoeologs on different wheat chromosomes. Homoeologs were extracted with their physical positions from the database, which were then compared with the physical coordinates of the MQTLs identified on the concerned chromosomes. MQTLs located on homoeologous chromosomes having similar genes were accepted as homoeologous MQTLs.

Results

QTLs associated with LR, SR, and YR

Information of 1146 wheat QTLs (368 for LR, 291 for SR, and 487 for YR) from 152 interval mapping studies were collected (Table S2). These 152 studies included 31 studies for LR, 24 studies for SR, 66 studies for YR, and 31 studies each associated with at least two different rusts (Table S2, Fig. 1a). A total of 160 mapping populations were utilized in these 152 studies, which included 117 sets of RILs, 31 sets of DH lines, and 12 F3/backcross populations (some studies utilized more than one population). The population size in different studies ranged from 58 (Zhang et al. 2016) to 1020 (Agenbag et al. 2014), and the number of QTLs in individual studies ranged from one each in several individual studies (e.g., Kolmer et al. 2011; Wang et al. 2015; Zhang et al. 2016; Zwart et al. 2008) to a maximum of 59 QTLs in one study (Bajgain et al. 2016). The distribution of QTLs for three individual rusts and among 21 chromosomes along with the distribution of QTLs according to PVE and LOD score are presented in Fig. 1.

Consensus map for QTL projection

The three maps (other than the SNP maps) shared 566 markers, whereas, 'Wheat Composite 2004' had 3531 unique markers, 'Wheat Consensus SSR 2004' had 81 unique markers, and 'Integrated 2013' durum wheat map had 2351 unique markers. The number of unique and shared (or common) markers among these maps has been shown via a Venn diagram (Fig. 2). Markers showing ordinal conflicts were removed while constructing the consensus map using LPmerge package of R programming. The consensus map developed for the present study included 234,619 loci, which included both marker loci (SNPs, DArT, SSR, AFLP, RAPD, STS, EST-SSR, SRAP, ISSR, KASP) and gene loci (*Vrn*, *Ppd*, *Rht*, *Glu*, etc.) (Table S3). The length of the consensus map was 7637.09 cM (Fig. S1), giving a density of 30.72 loci/cM (Table S4). The marker densities for individual chromosomes are presented in Fig. S1. The marker density for individual chromosomes ranged from 7.78 markers/cM for 4D to 71.62 markers/cM for 1A (Table S4). Sub-genome 'A' had a genetic length of 2565.38 cM with 91,856 loci (35.8 markers/cM), sub-genome 'B' had a length of 2938.05 cM with 106,811 loci (36.35 markers/cM), and sub-genome 'D' had a length of 2133.66 cM with 35,952 loci (16.85 markers/cM).

For QTL projection, only 718 QTLs, which had flanking markers available on the consensus map were used. These projected QTL included 175 QTLs for LR, 222 QTLs for SR, and 321 QTLs for YR. The distribution of these projected QTLs on three sub-genomes was 209 (65.11%) for A subgenome, 367 (59.09%) for B subgenome and 139 (68.14%) QTLs for D subgenome. Similarly, the number of projected QTLs for individual chromosomes ranged from 9 (4D, 6D) to 93 (2B) with an average of around 34 QTLs per chromosome. Mean CIs in original and projected QTLs also differed for LR, SR, and YR (Fig. 3). Most of the projected QTLs for these diseases showed higher CIs, with respective mean values of 11.84 cM and 14.64 cM for original and projected LR CIs, of 5.63 cM and 6.45 cM for original and projected SR CIs, and of 10.77 cM and 11.2 cM for original and projected YR CIs. For LR, 39.67% and 24.45% of the original and projected QTLs had CIs greater than 10 cM, respectively. For SR, 20.62% and 18.55% of the original and projected QTLs possessed CI values greater than 10 cM, respectively. Lastly, for YR, 32.85% and 21.97% of the original and projected QTLs had CIs greater than 10 cM, respectively.

MQTLs and their salient features

The number of MQTLs obtained was 86, which involved only 596 of the 718 QTLs that were projected (Fig. 4, Table 2, Table S5). The remaining 122 projected QTLs included (i) 110 QTLs, for which the predicted QTL peaks were not included within any MQTL, and (ii) 12 singletons, each with solitary QTL in a predicted MQTL. The 86 MQTLs were distributed on all wheat chromosomes except chromosome 6D (Fig. 4), involving a maximum of 9 MQTLs on 6A and a minimum of only two MQTLs each on 3D, 6B, 7A, and 7D (Fig. 5a). Moreover, the number of MQTLs did not depend on the number of QTLs on individual chromosomes utilized for MQTL analysis, for instance, chromosomes 3B, 6B, and 7D each had many more initial/original QTLs but relatively fewer MQTLs (Table S5).

Only 71 of the 86 MQTLs could be classified as MDR-MQTLs, which included 28 MQTLs for all the 3 rusts, and 43 for two rusts each (8 for LR and SR; 14 for LR and YR; 21 for SR and YR) (Fig. 5b). The remaining 15 MQTLs each provided resistance for only one rust (1 for LR; 6 for SR and 8 for YR) (Table 2, Table S5). The number of QTLs per MQTL ranged from ≤ 5 in 52 MQTLs to ≥ 20 QTLs in the three MQTLs (viz., MQTL2B.3, MQTL3B.1, and MQTL7D.1) (Fig. 5c). At least 34 MQTLs (39.53%) were derived after clustering QTLs from four or more different studies involving distinct parental lines; they should be more stable across environments.

The CI of the MQTLs ranged from 0.04 to 14.3 cM (mean 1.58 cM), while that of projected QTLs ranged from 0.008 to 83.5 cM (mean 10.57 cM); this amounts to a range of 21.6 fold reduction in MQTL on 6B and ~14-15 fold reduction in MQTLs on 3B, 7D, 2B, with a mean of 6.69 fold reduction in the CI of all MQTLs (Fig. 5d). These lengths of CI in cM (genetic distance) corresponds to a physical distance, which ranged from 19 Kb (MQTL6A.1) to 604.3 Mb (MQTL2D.1) with a mean of 51.3 Mb in 78 of the 86 MQTLs (barring 1A.1, 1A.2, 1B.7, 1D.4, 1D.5, 2A.1, 4B.7, 4B.8); CI in 47 of these MQTLs each covered a physical distance of <20 Mb. The LOD score of individual MQTLs ranged from 3.09 to 28.05 (mean 8.33), while the PVE ranged from 4.74 to 51.02% (mean 16.33%).

Ten MQTLs were selected to be the most important for breeders and were, therefore, named breeders' MQTLs. Following criteria were used for the identification of breeders MQTLs: (i) MQTLs involving QTLs for all the three rusts, (ii) CI <2 cM, (ii) average PVE >15%, (iii) average LOD >8, and (iv) involvement of at least 5 QTLs within the MQTL. These MQTLs included the following: MQTL1B.1, 2A.3, 2A.5, 2B.1, 2B.2, 2B.3, 3B.1, 4A.1, 6A.1, and 7D.2 (Table 3). Five MQTL hotspots were also identified, one each on chromosomes 1B, 2A, 2B, 3B and 4B. Each of the five hotspots consisted of 2 MQTLs as follows: (i) MQTL1B.2 and MQTL1B.3, containing 10 QTLs, each controlling all the three rusts (ii) MQTL2A.3 and MQTL2A.4, containing 23 QTLs, each controlling all the three rusts, (iii) MQTL2B.2 and MQTL2B.3, containing 49 QTLs, each controlling all the three rusts (iv) MQTL3B.2 and MQTL3B.3 comprising 10 QTLs, each controlling two rusts (v) MQTL4B.5 and MQTL4B.6 containing 4 QTLs, each controlling two rusts (Fig. 4; Table 3).

MQTLs overlapping GWAS-MTAs

In order to identify MQTLs with a higher level of confidence, the above 78 MQTLs (only 78 of 86 were physically anchored) were compared with 1,926 MTAs for the three rusts, reported in 23 GWAS; this resulted in the identification of 54 MQTLs overlapping 497 MTAs (Table S6); 22 MQTLs overlapped MTAs for all the three rusts while 17 MQTLs overlapped MTAs, each for at least two of the three rusts; 15 MQTLs overlapped MTAs controlling only one the three rusts. The number of MTAs for each MQTL also varied, ranging from 1 MTA (for several MQTLs) to 78 MTAs (for only one MQTL, namely MQTL6A.9) (Table S6).

MQTLs carrying R genes

R genes overlapping MQTLs were also identified; 42 R genes (9 Lr genes: 5 Sr genes and 28 Yr genes) were colocalized with 20 MQTLs (Table S5); MQTL2B.5 co-localized with the following 13 R genes: *Lr35*, *Sr36*, *YrSP*, *Yr5*, *Yr7*, *Yr43*, *Yr44*, *Yr53*, *Yr72*, *Lr13*, *Yr27*, *Sr9*, and *Sr28* genes; MQTL1B.2 colocalized with *Yr10* and *Yr64* and MQTL2A.2 colocalized with *Lr17* and *Lr37* (Table S5). Overall, 3, 1, and 11 MQTLs colocalized with the genes conferring resistance to LR, SR, and YR, respectively. The only MQTL with R genes for all the three rusts is MQTL2B.5.

Candidate genes (CGs) and their gene ontology (GO) terms

In total, 1707 putative CGs were identified for 77 MQTLs (no CG for MQTL4B.3) revealing several GO terms involved in various biological, molecular, and cellular processes. Some of the important GO terms include those involved in processes like the following: defense response, toxin activity, DNA binding, phosphorylation, protein ubiquitination, proteolysis, transmembrane transport, oxidation-reduction processes, catalytic activity, ATP binding, protein binding, heme binding, iron ion binding, metal ion binding, transmembrane transporter activity, oxidoreductase activity, etc. (Table S7).

Differentially expressed CGs (DECGs) in MQTL regions

In-silico expression analysis was carried out for CGs of only 27 MQTLs (of 77 MQTLs that were used for identification of CGs), each with QTLs for all the three rusts (541 CGs were available). Only 81 CGs belonging to 22 MQTLs (from 27 MQTLs) were differentially expressed. These 81 DECGs were also analyzed for GO enrichment. GO terms were not available for 14 of these genes. The remaining 67 genes revealed important GO terms (Fig. 6a). These DECGs encoded proteins belonging to the following categories: R-domain containing proteins, transcription factors (Zn finger binding proteins, SANT/Myb domains, NAC domain, bHlH TFs), transporters (ABC transporter, proton/oligo-peptide transporter, MFS transporter domain), protein kinases, proteins involved in calcium signaling, peptidases, proteins involved in oxidoreductive processes (RuBisCO_sc, G6P_DH, FAD/NAD(P)-binding domain), etc. (Table S8 and S9). Interestingly, 8 out of the 81 DECGs showed differential expression for all the three rusts. A representative heat map of these DECGs is presented in Fig. 6b.

Homoeology among MQTLs

In the present study, homoeologous genes were identified for the 78 MQTLs located on 20 wheat chromosomes. The number of genes that were conserved among different MQTLs at corresponding position, ranged from few to hundreds. The information regarding the number of total genes available from the MQTL regions and those conserved between the MQTLs located on different chromosomes is reported in Table S10. In addition to the detection of homoeology among the MQTLs located on chromosomes of a certain homoeologous group, partial homoeology was also observed among the MQTLs located on chromosomes belonging to different homoeologous groups (Fig. 7).

Discussion

In the past, disease resistance in crops was largely treated as a qualitative trait, where an individual R gene controls resistance against a particular race of a pathogen following a gene-for-gene relationship (Flor 1942, 1971). These R genes have also been pyramided involving several genes either for the same disease or for multiple diseases, thus providing MDR. More recently, with the availability of DNA-based molecular markers and statistical tools, disease resistance could be treated as a quantitative trait and QTL analysis (involving both interval mapping and GWAS) could be used for the identification of markers associated with QTLs for major individual diseases. In some cases, markers have also been developed for MDR involving more than one diseases. A beginning has also been made in using associated markers for MAS for resistance against diseases in several crops including wheat (Kaur et al. 2020; Gupta et al. 2021; Sharma et al. 2021). Examples are also available for achieving MDR against more than one disease, through genes/QTLs or using complex/pleiotropic loci (Table S11).

We believe that if QTLs, each carrying resistance for more than one disease are available, then one may do away with the difficult procedure of pyramiding genes and achieve MDR through the transfer of individual QTLs or meta-QTLs (MQTLs) for MDR using only one set of associated markers for MAS. The present study is one such effort, where MQTLs were identified for MDR involving resistance to all the three rusts (LR, SR, and YR). This study is one of the few studies, where MQTLs for MDR have been identified, thus providing the same set of markers for MDR. In the published literature, the following are the only three studies, where MQTLs for MDR were identified: (i) in barley, for powdery mildew, net blotch, spot blotch, leaf blotch, brown rust, etc. (Schweizer and Stein, 2011); (ii) in rice, for the blast, sheath blight and bacterial leaf blight (Kumar et al. 2020b), and (iii) in maize, for northern leaf blight, gray leaf spot, and southern leaf blight (Ali et al. 2013). In wheat, the only other study for identification of MQTL for MDR was our own study involving the following five diseases: septoria tritici blotch, septoria nodorum blotch, fusarium head blight, karnal bunt, and loose smut (Saini et al. 2021a).

In an old review on MDR in legumes, MDR was defined simply as “host-plant resistance to two or more diseases” (Nene, 1988). In a relatively recent review, four different mechanisms for MDR were also described for achieving MDR (Wiesner-Hanks and Nelson, 2016; Fig. 8). Among these four methods, pyramiding or stacking of genes is the simplest method and has been successfully utilized in the past through conventional breeding. However, if we have complex loci or QTLs imparting resistance to multiple diseases, it will certainly help the breeders. Such naturally occurring complex loci for multiple diseases will be a boon for the plant breeders. Following are the four examples of such complex loci each involving two or more rusts in wheat including: (i) *Lr34/ Yr18/ Sr57/ Pm38Ltn1* (7DS; Krattinger et al. 2009), (ii) *Lr46/ Yr29/ Sr58/ Pm39Ltn2* (1BL; Huerta-Espino et al. 2020), (iii) *Sr2*/Lr27/ *Yr30/Pbc1* (3BS; Mago et al. 2011), (iv) *Lr67/Yr46/Sr55/Pm46/Ltn3* (4DL; Herrera-Foessel et al. 2014). These loci have often been described as pleiotropic, although evidence for pleiotropy against close linkage is not unequivocal.

Among the 1146 QRLs (quantitative resistance loci; often described as QTLs) reported in 150 interval mapping studies that were used in the present study, most QRLs were each focused on single rust and some studies reported QRLs, each conferring resistance to two or all the three rusts suggesting the occurrence of MDR loci in some current wheat cultivars (Bemister et al. 2019; Prins et al. 2011; Singh et al. 2013; for details, see Table S2). Availability of individual MQTLs each for resistance to two or all the three rusts, as observed in the present study and reported in three other crops (listed above), is yet another evidence in support of the MDR hypothesis (Wiesner-Hanks and Nelson, 2016).

The number of QTLs used for projection in the present study (718/1146 = 62.65%) is higher than those in one of the two earlier studies on MQTL analysis for individual rusts (LR and YR) in wheat, where in one study, only 44.03% QTLs were projected (Soriano and Royo, 2015). In the other study, 60.62% QTLs were

projected, which is not very different from the present study (Jan et al. 2021). A higher proportion of projected QTL may be attributed to the availability of more detailed information about many more QTLs and to the use of ultra-high density consensus map during the present study. The 86 MQTLs detected during the current study were obtained from 596 QTLs, which indicated a roughly seven-fold (596/86) reduction in redundancy for the genomic regions conferring resistance to LR, SR, and YR in wheat. This interesting observation is in sharp contrast to several earlier meta-QTL studies, where only 3 to 5-fold reductions in redundancy of genomic regions were reported; these earlier studies include meta-QTL analysis for LR (Soriano ad Royo, 2015), SR (Jan et al. 2021), fusarium head blight (Venske et al. 2019) and tan spot (Liu et al. 2020) diseases in wheat.

In the present study, >32% (28/86) of MQTLs were involved in providing resistance to all the three rusts studied. This frequency is much higher than 5-10% MTAs for MDR reported in naturally occurring cultivars (Table S1). The MQTLs for MDR identified in the present study and those reported in naturally occurring cultivars can be used to provide resistance against any of the two or all the three rusts in wheat. An earlier study also discovered the phenomenon of co-localization of QTLs for MDR in wheat, where 13 QTLs spread across nine wheat chromosomes were significantly associated with resistance to four different diseases, namely, LR, YR, tan spot, and Karnal bunt (Singh et al. 2012). In another study, Zwart et al. (2010) discovered a QTL representing a cluster of tightly linked loci on chromosome 3D for resistance to several foliar diseases (STB, tan spot, LR, SR, and YR). Some of these QTLs for MDR reported in naturally occurring wheat germplasm may correspond to the MQTLs for MDR identified in the present study. Such naturally occurring MDR QTLs include the following: *QSr.sun-3D*, *QStb.wai-3D*, *QRlnn.lrc-3D*, *QYls.lrc-3D*, *QTs.cimmyt-3AS*, *QYr.cimmyt-2AS* (Zwart et al. 2010; Singh et al. 2012). It is thus apparent that only a small fraction of MDR MQTLs identified during the present study are currently known to occur in nature, thus underlining the importance of the present study.

MDR at the level of individual QTLs/genes or MQTLs (identified in the present study) may represent either the tightly linked loci or individual loci, each pleiotropic in nature. In case of closely linked multiple QTLs, these QTLs may be available either in the coupling phase or in the repulsion phase, resulting in positive and negative correlations between resistance to more than one disease. For instance, the wheat *Sr2* locus, which confers resistance to LR, SR, and powdery mildew (Mago et al. 2011), was tightly linked in the repulsion phase to the *Fhb1* locus, which confers resistance to fusarium head blight (Flemmig, 2012). Similarly, QRLs for STB and yellow leaf spot inherited from one parent were linked in repulsion to the *Lr24/Sr24* locus conferring resistance to leaf rust and stem rust inherited from the other parent (Zwart et al. 2010). The QTLs linked in the coupling phase can be readily introgressed together to provide resistance to two or more diseases, but introgression of the QTLs linked in the repulsion phase may be a trade-off, because the transfer of resistance to one disease is associated with susceptibility to another rust. As a result, it is obvious that in order to make effective use of a resistance source, a thorough understanding of the complexity of its inheritance is required.

The co-localization of ~69% (54/78) of the physically anchored MQTLs with known GWAS-MTAs also deserves attention. Such a comparison of MQTLs with GWAS-MTAs in earlier studies reported the following proportion of QTLs that could be verified by GWAS-MTAs: (i) 38.7% (Aduragbemi and Soriano, 2021); (ii) 51.6% (Pal et al. 2021); (iii) 61.4% (Yang et al. 2021); (iv) 63.3% (Saini et al. 2021a), (v) 78.6% (Saini et al. 2021b), (vi) 54.61% (Saini et al. 2022b). Our results seem to agree with the values of validation of MQTLs by GWAS-MTAs in the above earlier studies. These co-localized MQTLs provide a basis for accurate mining of high confidence CGs associated with MDR in wheat.

Some MDR MQTLs detected during the current study also overlap the known rust resistance R genes (Table S5) which included 7 cloned genes (*YrSP*, *Yr5*, *Yr7*, *Lr67/Yr46*, *Sr21*, *Sr33*, and *Yr36*; Zhang et al. 2020). These genomic regions are believed to be involved in controlling both qualitative and quantitative resistance, making them more important targets for introgression into susceptible wheat lines in order to improve resistance to the three rusts. Following are some examples: (i) MQTL2A.6 overlaps with two resistance genes *Sr21* and *Yr32.* The gene *Sr21* encodes a coiled-coil nucleotide-binding leucine-rich repeat (NLR) protein and confers resistance against races belonging to Ug99 group at high temperature (Chen et al. 2018), while, the other gene *Yr32* was characterized to confer resistance against YR, effective both at the seedling and adult growth stage (Eriksen et al. 2004). (ii) MQTL2B.5 is associated with two Lr genes (*Lr13* and *Lr35*), three Sr genes (*Sr9*, *Sr28* and *Sr36*), and eight Yr genes (*Yr5, Yr7, Yr27*, *Yr43*, *Yr44*, *Yr53*, *Yr72* and *YrSP*), making this region a hotspot that can be used for introgression of MDR in wheat. Amongst the 13 co-localized genes with MQTLs, three genes, namely, *Yr5*, *Yr7*, and *YrSP* have recently been cloned (Marchal et al. 2018); *Yr5*, which remains effective to a broad range of Pst isolates worldwide, is closely related yet distinct from *Yr7*, whereas *YrSP* is a truncated version of *Yr5* with 99.8% sequence identity (Marchal et al. 2018). These three Yr genes are members of a complex resistance gene cluster on chromosome 2B that encodes an NLR protein with a non-canonical N-terminal zinc-finger BED domain that differs from that found in non-NLR wheat proteins (Marchal et al. 2018). Diagnostic markers have also been developed for the above three Yr genes; similar markers can also be developed for other non-allelic Lr and Sr genes which may accelerate the haplotype analysis and expedite stacking of different genes through MAS. (iii) MQTL2D.1 is co-localized with a seedling LR resistance gene (*Lr15*) (Dholakia et al. 2013), two adult plant YR resistance genes (*Yr16*, *Yr54*) and an all-stage YR resistance gene (*Yr55*) (Rani et al. 2019). (iv) MQTL3A.1 overlap with *Lr63*, *Yr76*; *Lr63* conditions low to intermediate infection types to most *P. triticina* isolates, however in combinations with other effective seedling or AP resistance genes, it can be used to develop wheat cultivars with highly effective LR resistance. (v) MQTL4D.3 is associated with one cloned gene *Lr67* (synonymous to *Yr46*, *Sr55*, *Pm46*, and *Ltn3*, and observed to show co-segregation for AP for YR, SR and powdery mildew and leaf tip necrosis, respectively) (Herrera-Foessel et al. 2014; Moore et al. 2015). This gene encodes a predicted hexose transporter (LR67res), which differs from the susceptible form of the same protein (LR67sus) by two amino acids conserved across the orthologous hexose transporters. The protein LR67sus and related proteins encoded by homeoalleles function as high-affinity glucose transporters. Through heterodimerization with these functional transporters, LR67res shows a dominant-negative effect on glucose uptake. Changes in hexose transport in infected leaves could explain the plant's ability to suppress the growth of multiple biotrophic pathogen species (Moore et al. 2015).

The MDR MQTLs, which co-localize with known Lr, Sr, and Yr genes may also be important targets for introgression into susceptible wheat lines for enhancing the resistance against all the three rusts. Although there are hardly any examples of transfer of MDR QTL for all three rusts, examples for transfer of multiple QRLs for the same disease are available. For instance, Hu et al. (2020) transferred two QRLs (*Qyr.nafu-2BL* and *QYr.nafu-3BS*) for resistance to

SR from wheat line P9897 into three Chinese elite wheat cultivars, Chuanmai 42, Xiangmai 25, and Zhengmai 9023. They concluded that a combination of major known gene and QRLs may widen the resistance spectrum and enhance the resistance.

There are also examples, where CGs underlying MQTLs were identified for several traits including drought tolerance (Kumar et al. 2020c), tan spot resistance (Liu et al. 2020), and fusarium head blight resistance (Venske et al. 2019) in wheat. However, we used a strategy earlier used by Jan et al. (2021) in which the physical positions of MQTL peaks were determined, and then, the 1 Mb intervals on either side of the MQTL peaks was considered for the identification of CGs available in MQTL regions.

During the present study, a set of 81 differentially expressed CGs (DECGs) were also identified (Table S9 and S10). These DECGs encoded proteins carrying different domains, for instance: kinase domain, NBS-LRR domain, serine/threonine-protein kinase, UDP-glycosyltransferase, WRKY domains, F-box domain, glycosyl hydrolase, ABC transporter-like domain, WD40-repeat-containing domain, and MFS transporter. Following are some examples of CGs, which are known to have a role in disease resistance: (i) Serine-threonine protein kinase (*STPK-V*) gene, which is a member of *Pm21* family for resistance against powdery mildew (Cao et al. 2011; Xing et al. 2018). (ii) Genes with NBS-LRR domain, which resemble cloned Yr genes like *Yr10, Yr5*, etc., (Liu et al. 2014; Marchal et al. 2018); (iii) genes like *Sr21* for resistance to stem rust in wheat (Chen et al. 2018) and *Rpg5* in barley (Brueggeman et al. 2008), which encode proteins with nucleotide-binding-site, leucine-rich, and protein kinase domains. (iv) The above transporters may also possibly encode Yr genes like *Yr46* which was shown to encode for hexose transporter (Moore et al. 2015). (v) UDP-glucosyltransferases were earlier reported to show differential expression due to SR infection in wheat genotypes indicating their role in *Yr39* mediated SR resistance (Coram et al. 2008). (vi) WRKY domain containing genes were recently found to encode for proteins of cloned *YrU* gene (Wang et al. 2020). Similarly, (vii) F-box domain containing gene was identified as a CGs underlying the *YrR39* locus in wheat and it was shown to upregulate due to SR infection (Yin et al. 2018).

Bread wheat is a hexaploid species with A, B and D ancestral sub-genomes, carrying sets of triplicate genes on homoeologous chromosomes in most of the cases (Shitsukawa et al. 2007; Leach et al. 2014). It has been found that 64% of the wheat genes are present in all three genomes while only 45% are expressed from all the three homoeoloci (Leach et al 2014). Comparison of candidate genes underlying the MQTLs provided evidence for evolutionary conservation of MQTLs across the three sub-genomes. Identifying homoeologs is important for engineering the genetic basis for traits of interest in polyploidy crop such as wheat.

Conclusions

In the present study, we integrated the results of QTL mapping studies on LR, SR, and YR resistance leading to the identification of 86 MQTLs. More than half of these MQTLs were validated using GWAS results. Some of these MQTLs were found to be co-located with as many as 42 known major resistance genes. Further, 28 MQTLs provided resistance to all the three rusts, while each of other 43 MQTLs provided resistance to any of the two rusts. Putative CGs were identified and 81 CGs showed differential expression, encoding important proteins. Eight DECGs were such, which showed differential expression for all the three rusts. Ten promising MDR-MQTLs were recommended for use in marker-assisted breeding for the development of resistance to three rust diseases in wheat cultivars. This study can also help better define the various mechanisms associated with MDR in wheat.

Declarations

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Author contributions

PKG, HSB, PKS, and SK conceived and planned this study. NP, IJ, AK, and KK collected the literature and tabulated the QTL data for meta-QTL analysis. NP, IJ, DKS prepared the input files and performed QTL projection and meta-QTL analysis. NP, IJ and DKS interpreted the results and wrote the first draft of the manuscript. PKG, HSB, PKS, and SK edited and finalized the manuscript with the help of NP, IJ and DKS.

Conflicts of interest

The authors declare no conflicts of interest.

Availability of data and material Additional data relevant to this paper is available in the form of supplementary material.

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Tables

Table 1: Summary of genome-wide association studies in wheat on LR, SR, and YR used in the present study

Type of wheat (Panel size)	Genotyping platform/Number	MTA's	Country	References
Leaf rust, stem rust and yellow rust				
Common wheat cultivars: from Kazakhstan and Europe (215)	20K (11510 SNPs)	44	Kazakhstan and Russia	Genievskaya et al. (2020)
Spring wheat (483)	35K (14650 SNPs)	438	India	Kumar et al. (2020a)
Spring wheat (148)	9K (5688 SNPs)	79	Australia	Kankwatsa et al. (2017)
Leaf rust and yellow rust				
Wheat accessions (268)	90K (12931 SNPs)	560	China	Zhang et al. (2021)
Winter durum wheat(328)	DArT (12,550)	7	Germany, Austria, and Hungary	Miedaner et al. (2019)
Yellow rust and stem rust				
European winter wheat diversity panel (158)	DArTs, SNP (21,543)	61	Germany	Miedaner et al. (2020)
Leaf rust				
Durum, tetraploid, and hexaploid winter wheat accessions (385)	90K (9570 SNPs)	133	Canada	Fatima et al. (2020)
Spring wheat (100)	15K (NA)	16	Russia	Leonova et al. (2020)
Spring wheat and soft red winter wheat accessions (331)	9K (5025 SNPs)	50	Georgia	Sapkota et al. (2019)
Leaf rust association mapping panel (381)	90K (18925 SNPs)	82	Minnesota (USA)	Gao et al. (2016)
Stem rust				
ICARDA spring wheat (245)	15K (9523 SNP)	44	Ethiopia	Shewabez et al. (2021)
Spring wheat panel (250)	GBS (9042 GBS)	66	Ethiopia and Germany	Edae et al. (2020)
Spring durum wheats (283)	GBS (26439 SNPs)	126	Ethiopia, Kenya	Megerssa et al. (2020)
Iranian Bread wheats (282)	GBS (8,959)	8	Iran	Saremirad et al. (2020)
Durum wheats (183)	SSR, DArT, STS (953)	8	Ethiopia	Letta et al. (2013)
Winter wheats from 1st and 3rd WWSRRNs (232)	DArT (4721 DArT)	7	Kenya	Yu et al. (2012)
Yellow rust				
Durum wheat landraces and cultivars (300)	35K (7093 SNPs)	35	Ethiopia	Alemu et al. (2021)
Spring wheat genotypes (465)	GBS (765 SNPs)	25	Pakistan, USA	Habib et al. (2020)
Chinese wheat cultivars (240)	90K (NA)	21	Wuhan (China)	Jia et al. (2020)
Advanced wheat breeding lines (CIMMYT) (23,346)	GBS (78662 SNPs)	31	India, Kenya and Mexico	Juliana et al. (2020)
CIMMYT pre-breeding wheat lines (419)	DArTseq (22415)	14	Mexico	Ledesma-Ramírez et al. (2019)
Spring wheat accessions (1000)	9K (4585 SNPs)	40	Washington state (USA)	Maccaferri et al. (2015)
Synthetic wheat accessions and bread wheat cultivars (192)	9K (2590 SNPs)	31	Ethiopia	Zegeye et al. (2014)

Table 2: MQTLs associated with multiple disease resistance identified in the present study

Chr.	MQTL/s (CI, in cM)	Flanking markers	QTLs involved (avg. LOD score; avg. PVE)
(a) Leaf rust, stem rust and yellow rust			
1A	MQTL1A.1 (59.93-61.88)	IWB7590/AX-109017398	4 (4.67; 10.72)
1B	MQTL1B.1 (1.72-2.59)	AX-94847267/ RAC875_c61512_173	6 (8.00;15.95)
1D	MQTL1D.2 (22.00-22.93); MQTL1D.6 (72.15-72.21)	AX-110480216/AX-111085909; IWB42815/IWB22866	3 (3.77; 22.72); 4 (5.12; 8.79)
2A	MQTL2A.3 (84.99-85.72); MQTL2A.4 (88.71-89.52); MQTL2A.5 (96.43-97.32)	wsnp_Ex_rep_c93362_82371891/Kukri_c84087_154; Excalibur_c92241_336/BobWhite_c2002_100; wsnp_Ex_c5412_9564478/GENE-1031_48	20 (11.36; 17.95); 3 (5.92; 14.69); 5 (11.93; 27.02)
2B	MQTL2B.1 (37.76-38.97); MQTL2B.2 (77.74-78.71); MQTL2B.3 (88.64-88.68)	IWB30384/ IWB44405.1; IWB10512/ IWB12081; IWB9434/Sr40;	14 (9.75; 24.30); 14 (15.50; 26.34); 35 (11.54; 21.39)
2D	MQTL2D.1 (9.13-11.03); MQTL2D.2 (32.54-34.78)	AX-109493327/AX-109949983; AX-109001452/Kukri_s110874_162	10 (5.91; 13.90); 13 (6.78; 20.02)
3A	MQTL3A.1 (21.06-21.76)	wPt-2757/Xgwm369	5(4.64; 8.21)
3B	MQTL3B.1 (12.86-13.06)	AX-109995200/AX-109881148	39 (9.56; 18.71)
3D	MQTL3D.1 (5.21-7.93)	AX-94395108/Kukri_c5252_107	7 (5.08; 10.16)
4A	MQTL4A.1 (18.02-19.62); MQTL4A.2 (45.06-45.80)	Xgwm165/wsnp_Ex_c2352_4405961; IWB12285/s21m40A	5 (14.74; 26.88); 7 (5.01; 11.53)
5B	MQTL5B.1 (64.30-66.33); MQTL5B.5 (157.42-162.48)	Xwmc734/ AX-110689592; IWB70171/ IWB8285	12 (5.27; 9.50); 3 (6.48; 14.87)
5D	MQTL5D.1 (18.20-20.68); MQTL5D.2 (34.73-36.80)	IWB4656/AX-111140170; IWB17760/ IWB17934	4 (7.42; 13.33); 4 (5.63; 9.22)
6A	MQTL6A.1 (7.63-7.93); MQTL6A.7 (88.37-88.94)	IWB5891/IWB10541; Xgwm334/wPt-8331	13 (12.84; 19.71); 6 (4.84; 13.70)
6B	MQTL6B.1 (74.04-74.93)	Xgwm644/ wsnp_Ku_c43368_50890819	15 (5.19; 13.78)
7A	MQTL7A.1 (87.18-89.89)	AX-95190652/AX-109338226	4 (18.00; 37.32)
7B	MQTL7B.2 (53.57-54.31)	IWB69807/IWB72961	5 (15.15; 11.70)
7D	MQTL7D.1 (49.34-51.88); MQTL7D.2 (122.25-122.53)	AX-111551132/AX-111453717; IWB15616/IWB16221	25 (16.00; 29.56); 7 (14.79; 24.42)
(b) Leaf rust and yellow rust			
1B	MQTL1B.3 (45.58-46.21); MQTL1B.4 (80.57-83.64); MQTL1B.5 (119.74-121.47); MQTL1B.6 (165.11-169.98)	IWB12256/AX-94409524; IWB12157/AX-95259256; IWB12619/IWA5779; IWB8812/IWB74944	3 (5.03; 14.77); 4 (5.41; 10.31); 13 (12.92; 28.12); 5 (5.35; 11.72)
2D	MQTL2D.3 (47.86-48.34)	AX-95022809/AX-108901497	4 (4.84; 8.20)
3A	MQTL3A.3 (89.72-90.21)	AX-89329833/AX-110020043	3 (6.45; 6.10)
3D	MQTL3D.2 (49.98-50.21)	AX-95205079/IWB42682	6 (6.38; 13.11)

4B	MQTL4B.1 (30.55-32.29); MQTL4B.2 (38.96-39.60)	IWB71989/IWA2125; AX-109923236/AX-108738231	8 (8.23; 18.65); 7 (5.61; 17.13)
5B	MQTL5B.3 (101.08-103.06); MQTL 5B.4 (129.34-130.98); MQTL 5B.6 (197.53-198.09)	AX-94942472/ AX-94503507; AX-94487193/ AX-95138970; GENE-3383_710/ Ex_c29928_1020	12 (6.60; 9.54); 3 (5.70; 8.38); 4 (5.54; 17.10)
6B	MQTL6B.2 (287.60-287.64)	Xwmc388.3/AX-94463796	15 (4.61; 10.42)
7B	MQTL7B.3 (70.00-71.53)	IWB3369/IWB10779	5 (3.36; 8.22)
(c) Leaf rust and stem rust			
1D	MQTL1D.1 (7.16-8.09); MQTL1D.3 (25.80-25.84); MQTL1D.5 (46.10-47.46)	Xmwg68/AX-110910133; AX-111475929/Xbarc152; XGli1/ IWA3124	2 (14.12; 23.13); 2 (5.40; 15.70); 4 (7.63; 20.04)
3A	MQTL3A.2 (68.62-69.56)	AX-109441469/AX-95153336	5 (7.51; 10.50)
4B	MQTL4B.5 (67.24-67.72)	IWB3256/wsnp_Ex_rep_c68248_67035459	2 (4.80; 12.10)
5A	MQTL5A.1 (69.56-71.38)	IWB20566/AX-94493739	6 (3.55; 6.32)
5D	MQTL5D.3 (93.03-99.12)	AX-108767468/AX-110967183	4 (9.77; 24.91)
7A	MQTL7A.2 (109.84-111.93)	AX-108833832/AX-108745312	5 (12.72; 15.22)
(d) Yellow rust and stem rust			
1A	MQTL1A.2 (78.79-79.24); MQTL 1A.4 (151.43-151.91)	wsnp_Ku_c5756_10191339/AX-109017398; Excalibur_rep_c110054_341/1132858	9 (6.05; 13.51); 3 (3.09; 8.84)
1B	MQTL1B.2 (31.19-32.10)	IWB11262/AX-94425009	7 (12.78; 19.99)
2A	MQTL2A.2 (58.97-59.63); MQTL2A.6 (114.15-114.78)	BS00068050_51/Kukri_c36139_292; AX-111707919/RAC875_c21013_1187	5 (5.77; 18.32); 11 (9.75; 15.54)
2B	MQTL2B.4 (103.74-104.30); MQTL2B.5 (156.51-156.65)	IWB9006/ IWB12154; 2B_310443339/Xwmc317a	16 (8.60; 18.69); 5 (9.44; 29.14)
3B	MQTL3B.2 (31.59-32.53); MQTL3B.3 (41.10-42.01)	AX-111494658/AX-111637887; AX-111684042/AX-111757878	7 (6.60; 14.21); 3 (5.17; 10.98)
4A	MQTL4A.3 (145.29-146.34)	IWB56172/Xbarc78	7 (18.62; 14.22)
4B	MQTL4B.4 (54.62-56.53); MQTL4B.8 (110.18-112.18)	AX-111719842/IWB6643; AX-109366086/RAC875_c10772_61	2 (9.76; 27.84); 5 (4.42; 5.74)
4D	MQTL4D.1 (0.03-0.05)	AX-111688098/AX-110768844	3 (5.85; 13.16)
5B	MQTL5B.2 (72.67-74.58)	AX-95205468/ IWA8391	6 (4.68; 8.38)
6A	MQTL6A.3 (11.14-11.76); MQTL6A.4 (23.33-24.19); MQTL6A.5 (36.545-37.09); MQTL6A.6 (47.76-48.67); MQTL6A.8 (116.48-118.32)	IWB11274/AX-110585473; 6A_16883183/Xwmc201; Xgwm427/Xbarc3; 6A_83918914/IWB61322; Xcfe80/IWB22389	4 (9.47; 31.99); 4 (28.05; 27.15); 5 (4.54; 6.78); 3 (6.03; 11.00); 3 (7.93; 14.82)
7B	MQTl7B.1 (44.85-44.22); MQTL7B.4 (138.35-152.65)	IWA1181/IWA7083; 1112830/Marker66313	18 (4.63; 10.84); 2 (5.60; 11.35)

Table 3 Features of selected Breeder's MQTLs and MQTL hotspots

Breeder's MQTLs	CI	No. of QTLs involved	LOD	PVE	Associated markers
MQTL1B.1	0.87	YR (2), SR (3), LR (1)	8	15.95	AX-94847267/RAC875_c61512_173
MQTL2A.3	0.73	YR (13), SR (1), LR (6)	11.36	17.95	wsnp_Ex_rep_c93362_82371891/Kukri_c84087_154
MQTL2A.5	0.89	YR (3), SR (1), LR (1)	11.93	27.02	wsnp_Ex_c5412_9564478/ GENE-1031_48
MQTL2B.1	1.21	YR (8), SR (4), LR (2)	9.75	24.3	IWB30384/ IWB44405.1
MQTL2B.2	0.97	YR (1), SR (11), LR (2)	15.5	26.34	IWB10512/ IWB12081
MQTL2B.3	0.04	YR (16), SR (11), LR (8)	11.54	21.39	IWB9434/IWB9825
MQTL3B.1	0.2	YR (12), SR (9), LR (18)	9.56	18.71	AX-109995200/ AX-109881148
MQTL4A.1	1.6	YR (1), SR (3), LR (1)	14.74	26.88	Xgwm165/ wsnp_Ex_c2352_4405961
MQTL6A.1	0.3	YR (7), SR (5), LR (1)	12.84	19.71	IWB5891/ IWB10541
MQTL7D.2	0.28	YR (3), SR (3), LR (1)	14.79	24.42	IWB15616/ IWB16221

MQTL Hotspots	CI	No. of QTLs involved	Associated markers	Co-localized genes
1 (1B.2 and 1B.3)	15.02	YR (6), SR (2), LR (2)	AX-94639223/AX-94409524	*Yr10,Yr64,Yr65*
2 (2A.3 and 2A.4)	4.53	YR (14), SR (2), LR (7)	Tdurum_contig42153_5854/BS00093990_51	-
3 (2B.2 and 2B.3)	10.94	YR (17), SR (22), LR (10)	IWB10512/IWB12081	-
4 (3B.2 and 3B.3)	10.43	YR (5), SR (5)	AX-111494658/AX-111757878	-
5 (4B.5 and 4B.6)	3.69	SR (3), LR (1)	IWB9483/BS00034148_51	*Yr68*

Figures

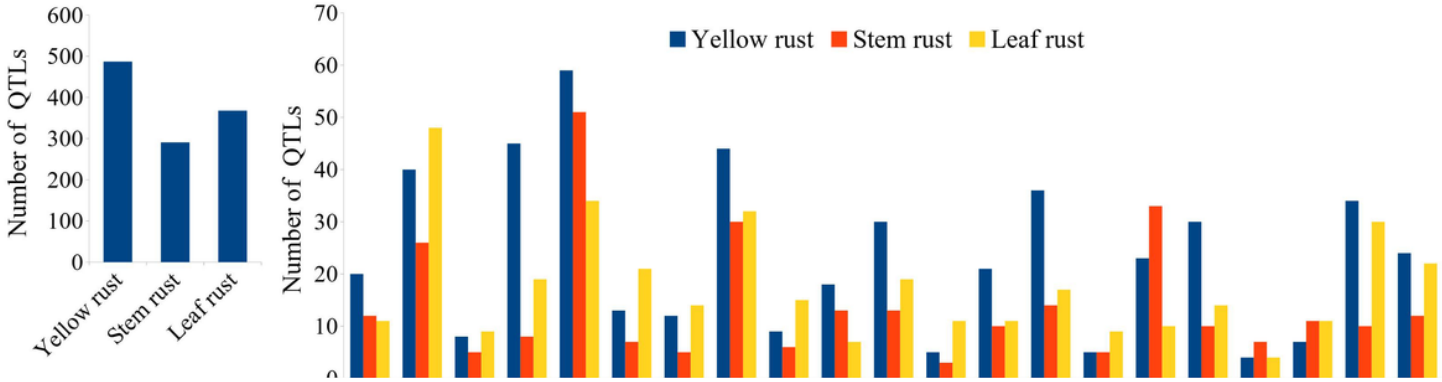

Figure 1

Distribution of QTLs (a) among three rusts, (b) on 21 individual wheat chromosomes, (c) with their PVE %, and (d) LOD scores.

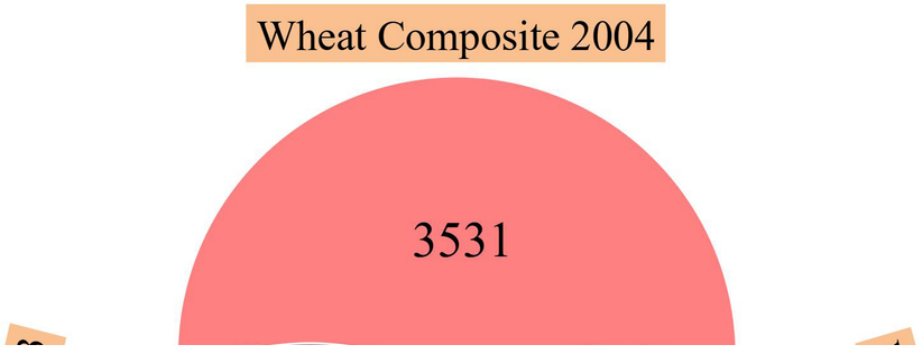

Figure 2

Venn diagram showing the number of unique and shared (or common) markers among the three maps (other than the SNP maps) used to construct the consensus map.

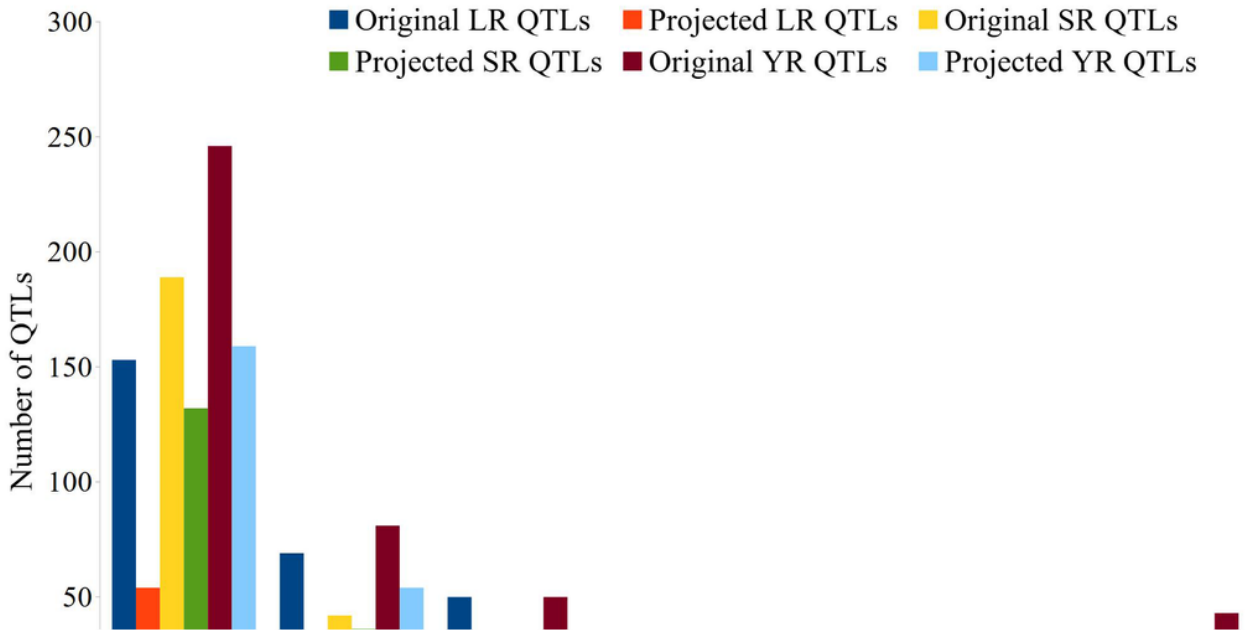

Figure 3

Comparison of confidence intervals (CIs) for original and projected QTLs associated with leaf, stem, and yellow rust.

Figure 4

Distribution of MQTLs on 20 different chromosomes of wheat. Vertical bars of different colours on the right of each chromosome indicate the position of MQTLs conferring resistance to different sets of rusts. Only the flanking markers most closely associated with MQTLs have been shown for better visualization. Known resistance genes co-localizing with the MQTLs are also shown.

Figure 5

Basic information of MQTLs obtained in meta-QTL analysis. (a) number of MQTLs on different wheat chromosomes, (b) MQTLs associated with different rust diseases, (c) number of MQTLs harboring different number of initial QTLs, (d) A comparison of mean CI for QTLs and MQTLs.

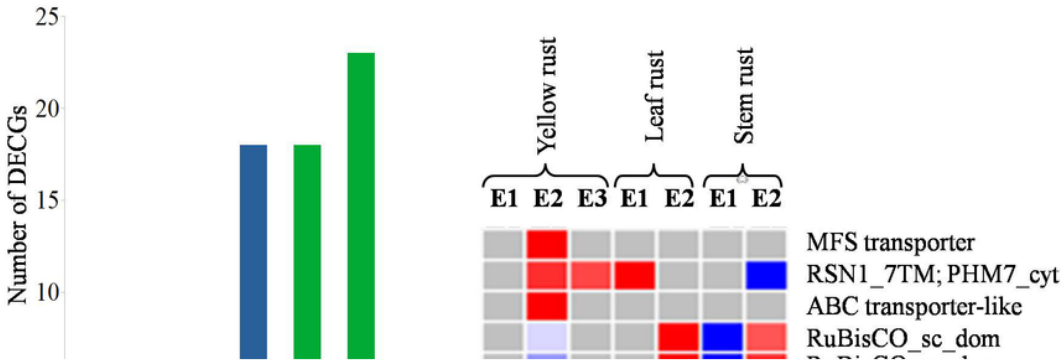

Figure 6

(a) Gene ontology (GO) terms for DECGs in the MQTL regions; (b) Representative heatmap of DECGs for all the three rusts.

Figure 7

Circos showing the homoeologous relationship of candidate genes underlying MQTLs.

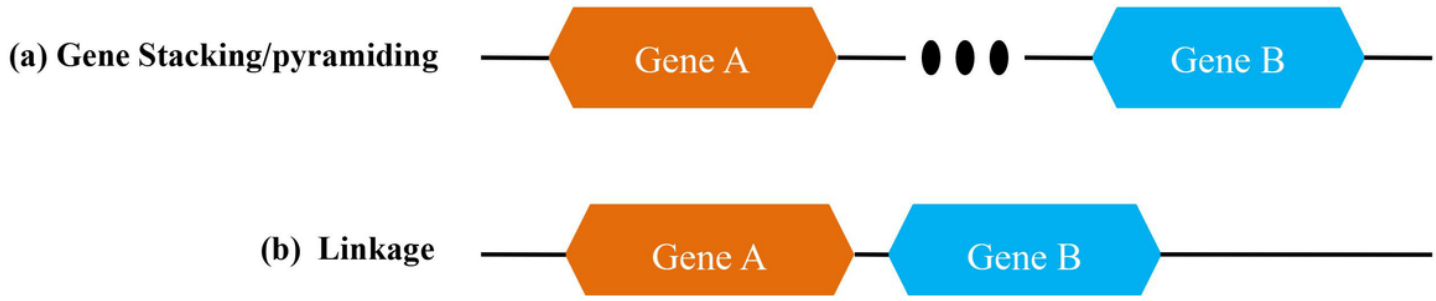

Figure 8

Methods for multiple disease resistance (MDR). (a) Loci conditioning resistance to single diseases (either R genes or QTLs) stacked/pyramided within a single genotype. (b) Loci conditioning resistance to individual diseases may be in tight linked. A single locus may have pleiotropic effects for resistance to multiple diseases, either with equal effects on more than one disease as shown in (c) or have effects on different diseases, which differ in size as shown in (d). The examples given here involve resistance. Only two diseases are shown here, but often resistance for more than two diseases is possible but sometimes resistance to one disease may be associated with susceptibility to another (a trade-off).

Supplementary Files

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

- FigS1.jpg
- SupplementaryTablesS1S11Revised.xlsx