Combined linkage and association mapping reveals two major QTL for stripe rust adult plant resistance in Shaanmai 155 and their haplotype variation in common wheat germplasm

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Abstract

The development and deployment of diverse resistance sources in new wheat cultivars underpin the durable control of stripe rust. In the present study, two loci for adult plant resistance (APR), QYrSM155.1 and QYrSM155.2, were identified in the Chinese wheat breeding line Shaanmai 155. QYrSM155.1 was mapped to a 3.0-cM interval between the single-nucleotide polymorphism (SNP) markers AX-109583610 and AX-110907562 on chromosome arm 2BL. QYrSM155.2 was mapped to a 2.1-cM interval flanked by the SNP markers AX-110378556 and AX-86173526 on chromosome arm 7AS. A genome-wide association study was used to identify markers associated with APR in a panel of 411 spring wheat lines. Thirteen and 11 SNPs were significantly associated with QYrSM155.1 and QYrSM155.2, respectively, corresponding to physical intervals of 653.75–655.52 Mb on 2BL and 81.63–83.93 Mb on 7AS. To characterize the haplotype variation and the distribution of these QTL, haplotype analysis was performed based on these SNPs in an independent panel of 1101 worldwide wheat accessions. Three major haplotypes (2B_h1, 2B_h2, and 2B_h3) for QYrSM155.1 and four major haplotypes (7A_h1, 7A_h2, 7A_h3, and 7A_h4) for QYrSM155.2 were identified. Accessions individually harboring QYrSM155.1_h1 and QYrSM155.2_h1 haplotypes and their combination displayed resistance. Additional assays of 1306 current Chinese cultivars and breeding lines using markers flanking QYrSM155.1 and QYrSM155.2 indicated that the resistance haplotypes of the two QTL were present in respectively 1.45% and 14.16% of lines. Increasing resistance haplotype frequencies at these two loci using marker-assisted selection should benefit wheat production in China.

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1. Introduction

In 2017, the world production of common wheat (Triticum aestivum L.) was around 735 million tonnes (FAOSTAT data, http://www.fao.org/). According to FAO estimates, an increase of 70% in production will be required by 2050 to provide food security for the increasing global population. The primary goal of most wheat breeding programs is to increase grain yield. However, wheat production is impaired by multiple fungal diseases [1] and yield increases should be protected by disease control. Stripe or yellow rust (YR), caused by Puccinia striiformis Westend. f. sp. tritici Erikss. (Pst), is one of the most harmful diseases of wheat [2,3]. The main challenge for stripe-rust control is the constant emergence of new virulent races able to overcome the protection provided by overused resistance genes (or potentially overused fungicides), as illustrated by several recurrent “boom-and-bust” cycles in China [4–6]. Mutation and somatic hybridization in Pst populations are the most common causes of genetic variation. However, Pst sexual recombination has also attracted increasing concern [7]. Although stripe rust can be suppressed by timely fungicide application, it is often insufficient to prevent yield losses in...
severely infected fields [8]. The use of fungicides also increases production costs and leads to environmental and human health problems. For this reason, host-plant resistance should be preferred for disease control, and continuous efforts are required to increase the resistance level and durability in contemporary wheat cultivars. Because stripe rust resistance in the adult plant stage is often quantitatively inherited, quantitative trait locus (QTL) mapping is the method used to identify genetic bases of the resistance phenotype. QTL mapping also provides a basis for the map-based cloning of underlying resistance genes and for marker-assisted selection (MAS) during breeding [9]. QTL mapping is usually performed by genotyping a population of plants that are progeny of a biparental cross, a labor-intensive, time-consuming, and costly procedure. Pooling or bulked-segregant analysis (BSA) is an alternative method proposed by Giovannoni et al. [10] and Michelmore et al. [11]. BSA involves the genotyping of selected and pooled DNA samples from sets of plants with contrasting phenotypes to provide a simple and effective way to rapidly search for markers linked to a target trait.

Recently developed high-throughput genotyping technologies, based on microarrays and next-generation sequencing (NGS), are becoming widely adopted for wheat genome analyses [12]. The reference genome [13] of the wheat line Chinese Spring enables the efficient high-throughput discovery of DNA variants. Among them, single nucleotide polymorphisms (SNPs) are primary markers for genetic analyses based on chip hybrid. Current SNP assay platforms, including Illumina Bead Chip, Affymetrix Gene Chip, Kompetitive Allele-Specific PCR (KASP) [14], and allele-specific quantitative PCR (AQPCR) have been used in mapping and MAS [15]. Combined with these technologies, BSA can readily identify large numbers of markers linked to target genes or QTL that can be directly mapped in the reference genome [16]. Accordingly, this strategy is becoming increasingly useful for the rapid identification of genes or QTL [17–19].

In previous studies [20–22], we assembled more than 5000 wheat lines, including domestic and foreign cultivars and landraces from the China Agriculture Research System (CARS). During the evaluation of these lines for stripe rust response in multiple artificially or naturally inoculated field nurseries over several years, we identified different accessions with high resistance to prevalent Chinese wheat Pst races. Among them, the advanced breeding line Shannmai 155 (SM155), developed by the Northwest A&F University, displayed a moderate but consistent stripe rust resistance level following its release in 2009 [23]. Because little was known about the genetic basis of the potentially durable resistance in this line, the present study aimed to identify the genetic bases of SM155 adult plant resistance (APR), perform haplotype analyses of germplasm panels to predict the distribution of the identified QTL in other genotypes, and develop AQPCR markers linked to the identified QTL for MAS.

2. Materials and methods

2.1. Plant materials

The parental lines used were the susceptible line Avocet S (AvS) and the resistant line Shannmai 155 (SM155). AvS is an Australian spring wheat selection that is highly susceptible to most Pst races in China [22]. SM155 is an advanced disease-resistant breeding line developed in Shaanxi province. The AvS × SM155 population consisted of 140 F2:7 recombinant inbred lines (RILs). A panel of 411 breeding lines from the International Maize and Wheat Improvement Center (CIMMYT) and the International Center for Agricultural Research in the Dry Areas (ICARDA) (panel 1) was used in a genome-wide association study (GWAS) to confirm QTL mapping results [24]. An independent global collection of 1101 wheat accessions (panel 2), for which wheat 660 K SNP genotypic data were available [24], was used for haplotype analysis. A panel of 1306 current Chinese wheat cultivars and breeding lines (panel 3), from CARS was used to predict the prevalence and distribution of the resistance genes based on flanking SNP markers identified in SM155.

2.2. Field experiments and phenotypic evaluation

SM155 was susceptible in seedling tests in a previous study [23] but conferred APR to the most recent post-V26 Pst races. The 140 RILs were evaluated for APR in three field environments: Yangling in Shaanxi province during the 2017–2018 (18YL) and 2018–2019 (19YL) cropping seasons and Jiangyou in Sichuan province during 2017–2018 (18JY). Lines were planted in randomized complete blocks with two replicates; 15–20 seeds of each line were planted in 1-m rows with a 25-cm row spacing. Xiaoyan 22 (XY22) (susceptible control) was planted after every 20 test rows. Mingxian 169 (MX169) served as an inoculum spreader to ensure uniform disease development throughout the field. In mid-March of each year, trials at Yangling were inoculated with a urediniospore mixture of the predominant Pst strains, CYR32 and CYR34, suspended in a light oil, and sprayed onto MX169 and XY22. Previous studies [22] have identified the avirulence/virulence characteristics of these races.

Infection type (IT) and disease severity (DS) were used to evaluate adult-plant reactions. IT was recorded on a 0–9 scale with 0–6 considered resistant and 7–9 susceptible [25]. DS was based on the modified Cobb scale [26]. These values were first recorded when stripe rust DS on AvS and MX169 reached approximately 80% during April 5–25 at JY and May 3–17 at YL. Disease assessments were performed at least twice to ensure phenotypic data reliability.

2.3. Phenotypic statistical analyses

Mean IT and DS values were used for phenotypic and QTL analyses. Analysis of variance (ANOVA) was used to estimate genotype (G), environment (E), and G × E interaction effects. Pearson’s correlation coefficient (r) and ANOVA were calculated with the “AOV” tool in QTL icliMapping 4.2 software [27] and R 4.0.4 [28]. Broad-sense heritabilities (h2) of stripe rust resistance were estimated using the formula h2 = σ2g/(σ2g + σ2e/n + σ2r/mr) where σ2g is the genetic variance, σ2e is the genotype × environment interaction variance, σ2r is the error variance; σ2e = (MSe − MSbe)/n, σ2r = (MSr − MSbr)/r and σ2r = MSr, where MSe is the mean square of genotypes, MSbe is the mean square of genotype × environment interaction, MSr is the mean square of error, r is the number of replications, and n is the number of environments. Best linear unbiased predictions (BLUPs) were estimated using genotype and environment data for each line as random effects in the lme4 linear mixed model package in R. The BLUPs were used to evaluate general effects and to find likely QTL positions.

2.4. BSA and molecular marker development

Fresh leaves were collected from F2:6 RILs at the joint stage and genomic DNA was extracted using the CTAB protocol [29]. DNA from RILs with extreme phenotypes was pooled in equal amounts to establish resistant (IT 1–2, DS < 10) and susceptible (IT 8–9, DS > 90) DNA bulks. RILs’ and parents’ bulked DNA were sent to CapitalBio Corporation (Beijing, China; http://www.capitalbio.com) for genotyping with the Affymetrix Wheat 660K SNP
array (http://www.affymetrix.com/support/index.affx). SNP geno-
type calling and clustering were performed with Affymetrix Geno-
typing Console (GTC) software. A subset of polymorphic SNPs lying in
the target region between the two bulks and between the par-
ents was developed into AQP markers for RIL population genotyping.
Experimental procedures for AQP marker screening in population genotyping were previously described [15].

2.5. Genetic linkage map construction and QTL analyses

The SM155 genotype was defined as A and the AvS as B. Linkage
map construction and QTL analyses were based on the 25 AQP
markers identified from BSA. After these markers were genotyped in
the RIL population, JoinMap version 4.0 [30] was used to con-
struct a genetic linkage map using a logarithm of odds (LOD) score
of 3.0 as the threshold. Genetic distances were calculated in
centimorgans (cM) based on the Kosambi mapping function [31].
The mean IT and DS values were used to identify QTL using the
inclusive composite interval mapping with the additive tool
(ICIM-ADD) in IciMapping 4.1 [27]. The phenotypes averaged over
multiple environments were used to determine each line’s equilib-
rium value, and to perform QTL analysis for each environment. The
walking speed selected for QTL detection was 1.0 cM, with
P = 0.001 in stepwise regression. Likelihood of odds (LOD) signifi-
cance thresholds were calculated with 1000 permutations at
P < 0.01. The phenotypic variances explained by individual QTL
and the additive effects at LOD peaks were also calculated.

2.6. Association, haplotype, and molecular detection analysis

Association analysis was performed for panel 1 in a previous study [24]. After integration of QTL mapping and GWAS results, the QTL candidate intervals associated with APR were refined. For panel 2, the 660K SNP genotyping data were extracted to identify haplotypes within target QTL regions. Representative or informative SNPs, obtained after filtering, were used in Haplovie 4.1 (https://sourceforge.net/projects/haplovie/) for haplotype analy-

sis. Phylogenetic analysis was performed using MEGA 7.0.14 (https://www.megasoftware.net/) to cluster marker groups and to identify lines based on the genotypic similarity corresponding to phenotypes. Based on the genetic mapping results, specific SNP markers flanking target QTL were converted into AQP primers and were used to test parents and selected RILs, as described by Wu et al. [18]. These AQP markers were used for molecular detec-
tion in SM155 (control) and in panel 3 to estimate the frequencies of the identified gene(s) or QTL in Chinese wheat breeding germplasm.

3. Results

3.1. Phenotypic evaluation

As expected, the AvS line was susceptible (IT 8–9, DS ≥ 80) and SM155 was resistant (IT 2–3, DS ≤ 30) in all field tests (Fig. 1a, b; Table S1). Both IT and DS data for the RIL population showed con-

tinuous distributions in each environment (Fig. 1a, b; Table S1). The IT and DS distributions were close to their mean values in all environments, ranging from 5.6 to 6.1 and from 49.4% to 56.0%, respectively (Fig. 1c, d; Table S1). These values indicated that the SM155 resistance was conferred by APR and its stripe-rust responses were quantitative. Pearson’s correlation coefficients for IT and DS between pairs of environments were all significant and ranged from 0.74 to 0.76 and 0.86 to 0.89 (P < 0.001) (Fig. 1e, f), respectively. These values indicated that stripe-rust responses were consistent across environments and suggested that the same

resistance genes conferred resistance in all three environments. The broad-sense heritability values for IT and DS were 0.64 and 0.80, respectively (Table 1). ANOVA showed significant phenotypic variation in both IT and DS among lines, environments, and line × environment interactions (Table 1). No significant differ-

ences were detected among replicates within environments. Together, these results showed that APR expression was consistent but was influenced by the environment.

3.2. BSA and QTL analysis

Genotyping the DNA bulks with the wheat 660 K SNP array revealed 10,030 SNPs polymorphic between the R and S bulks. Of these, 2737 and 1677 were located on chromosomes 2B and 7A, respectively. The remaining 5616 SNPs were unevenly distributed on other chromosomes (Figs. 2a, 3a). The SNPs on 2B and 7A most strongly associated with resistance were in the 550–770 Mb and 40–130 Mb physical intervals, respectively (Figs. 2b, 3b). These results implied that resistance genes were present on chromo-
somes 2B and 7A. Respectively 25 and 19 SNPs in the potential tar-
target regions on chromosomes 2B and 7A were selected for conversion to AQP markers. They were screened on parents and bulks to confirm polymorphism before being genotyped in the entire population. Fourteen of the 25 markers on chromosome 2B and 5 of the 19 on 7A could not distinguish the parents and bulks. The remaining 25 (11 on 2B and 14 on 7A) were used to genotype the RILs. The polymorphic AQP marker sequences are listed in Table S2.

The chromosome 2B genetic map was constructed using 11 AQP markers spanning 28.9 cM (Fig. 2c; Table S1). A locus detected using IciMapping 4.1, temporarily named QYrSM155.1, was located in a 3.0 cM interval between markers AX-105983610 and AX-110907562 on chromosome arm 2BL (Fig. 2c) with a corresponding physical interval of 651.96–657.26 Mb (Fig. 2g). The IT and DS scores from all three environments, QYrSM155.1 explained 38.7%–44.0% of phenotypic variation for IT and 45.9%–49.9% for DS across environments (Table 2). Similarly, the chromosome 7A genetic map was constructed using 14 AQP markers spanning 69.1 cM (Fig. 3c; Table S1). A QTL detected on the chromosome 7A short arm, temporarily named QYrSM155.2, was identified in a 2.1 cM interval flanked by markers AX-110378556 and AX-86713526 (Fig. 3c), corresponding to the physical interval 80.54–84.75 Mb (Fig. 3g). QYrSM155.2 explained 25.7%–29.2% of phenotypic variation of for IT and 28.5%–34.9% for DS (Table 2).

The resistance alleles of both QTL were derived from the resistant parent SM155. The RILs were classified into four genotypic groups to estimate individual QTL or combined-QTL effects: no QTL had a mean IT of 7.8 and DS of 80.2% across environments, similar to the susceptible parent. Lines with only QYrSM155.2 had a mean IT of 5.7 and DS of 53.3%, lines with only QYrSM155.1 had a mean IT of 5.2 and DS of 47.7%, and lines with resistance alleles at both loci had a mean IT of 3.6 and DS of 24.2%, similar to the resistant parent (Fig. 1c; Table S1). These results indicated that the combination of the resistance alleles of both QTL conferred higher resistance than the resistance allele of either QTL alone, reaching resistance levels similar to those of the resistant parent.

3.3. Association analysis and potential candidate genes

The panel 1 BLUP values for IT and DS were used for association tests based on univariate linear mixed model analysis. Using a threshold of P < 2.99 × 10−6, QTL were detected on several chromo-
somes, including 2B and 7A (Figs. 2d, 3d) [24]. Thirteen and 11 associated SNPs were detected in the QTL-mapping target regions

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on chromosomes 2B and 7A, respectively (Figs. 2e, 3e). All 13 markers on chromosome 2B were located in the interval 653.75b–655.52 Mb (Fig. 2f, g), and the 11 markers on 7A were located in the interval 81.63–83.93 Mb (Fig. 3f, g). Annotated genes in target regions were extracted from IWGSC RefSeq v1.0 [13] and three resistance genes were predicted in the QYrSM155.1 candidate region: TraesCS2B01G459500, TraesCS2B01G459600, and TraesCS2B01G459700 (Fig. 2g; Table S3). A colinearity analysis of these genes, using 10 + wheat genomes (http://wheat.cau.edu.cn/TGT/) data, indicated high genomic syntenies (Fig. S2). Similarly, seven resistance gene candidates (TraesCS7A01G127100, TraesCS7A01G127200, TraesCS7A01G127300, TraesCS7A01G127400, TraesCS7A01G127500, TraesCS7A01G127600, and TraesCS7A01G127900) were predicted in the QYrSM155.2 region (Fig. 3g; Table S3) with high colinearities (Fig. S2). All predicted genes were of high confidence and most were annotated as protein kinases (Table S3).

3.4. Haplotype variation and distribution

A haplotype survey of all 24 significant SNP markers identified in the panel 2 GWAS was conducted. Genotype data for 114 and 266 SNPs in the QYrSM155.1 and QYrSM155.2 intervals from the 1512 accessions in panels 1 and 2 were used for phylogenetic

<table>
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<th>Source of variation</th>
<th>IT</th>
<th>DF</th>
<th>Mean square</th>
<th>F-value</th>
<th>DS</th>
<th>DF</th>
<th>Mean square</th>
<th>F-value</th>
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*, significant at $P < 0.05$; **, significant at $P < 0.01$. 

Fig. 1. Disease distributions and correlations among environments. (a, b) Mean infection type (IT) and disease severity (DS) frequency distributions for 140 RILs grown at Yangling and Jiangyou in 2018 and Yangling in 2019. The values for the parents AvS and SM155 are indicated by arrows. (c, d) Violin plots of the IT and DS probability density distributions for RILs in all environments. (e, f) Correlation coefficients ($r$) for mean IT and DS of the RIL population across environments. All $r$ values are significant at $P < 0.001$. 

Table 1

Analysis of variance and estimates of broad-sense heritability ($h_2^b$) of infection type (IT) and disease severity (DS) for the RIL population derived from AvS × SM155.
analyses. Three main haplotype blocks were detected in the QYrSM155.1 region: 2B_h1, 2B_h2, and 2B_h3 (Fig. 4a; Table S4). These haplotypes were present in respectively 602 (39.8%), 396 (26.2%) and 514 (34.0%) accessions (Fig. 4a; Table S4). Haplotype 2B_h1 was common among CIMMYT and ICARDA-derived lines and was also present in some Chinese landraces and older cultivars. In contrast, 2B_h2 or 2B_h3 were present in most Chinese modern cultivars (Table S4). Four main haplotype blocks were detected in the QYrSM155.2 confidence interval: 7A_h1, 7A_h2, 7A_h3, and 7A_h4 (Fig. 4b; Table S4). These haplotypes were present in respectively 282 (18.7%), 318 (21.0%), 402 (26.6%), and 510 (33.7%) lines (Fig. 4b; Table S4). They were also widely present in Chinese cultivars, landraces and breeding lines, and exotic germplasm. Haplotype 7A_h1 was very common in Chinese lines prefixed “Xiaoyan” and their derivatives.

Fig. 2. Overview of QYrSM155.1 analyses on chromosome 2B. (a) Polymorphic SNP distribution on each chromosome identified by the 660K SNP array. (b) The distribution of polymorphic SNPs is based on their physical locations on chromosome 2B. (c) Genetic linkage map of QYrSM155.1 on wheat chromosome 2B based on genotype data from RILs. The red bar indicates the candidate interval for QYrSM155.1. (d) Stripe-rust response GWAS for panel 1 consisting of 411 spring wheat lines. The horizontal line shows the genome-wide significance threshold – \(\log_{10}(P)\) value of 3.4 (same for e, f). (e, f) Local Manhattan plot of SNPs associated with stripe-rust resistance surrounding the chromosome 2B peak. The region enclosed in gray dotted lines represents the potential candidate region. (g) Predicted genes in the QYrSM155.1 candidate region.

Table 2

<table>
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<th>QTL</th>
<th>Environment</th>
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<th>Marker interval</th>
<th>LOD</th>
<th>PVE (%)</th>
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</table>

Chr., chromosome; LOD, logarithm of odds score; PVE, percentage of phenotypic variance explained by individual QTL; Add, additive effect of resistance allele.

a  YL, Yangling; JY, Jiangyou; 2018 and 2019 represent the field experiments for the RIL population during the 2017–2018 and 2018–2019 cropping seasons, respectively.

b  The physical positions of two flanking markers.
environments by 6.8%–13.6% and 17.9%–29.1% in panels 1 and 2, respectively (Fig. 4c; Table S4). Significant differences among DS for chromosome 7A haplotypes were detected, with 7A\_h1 being more resistant in three environments (DS-19YL, DS-19TS, and DS-20YL) than the other haplotypes (Fig. 4d; Table S4), and considered a favorable haplotype. For convenience, we defined 2B\_h1 as QYrSM155.1\_h1, and the others as collectively qyrsm155.1\_h2. Likewise, we defined 7A\_h1 as QYrSM155.2\_h1, and the others as...

Fig. 3. Overview of QYrSM155.2 analyses on chromosome 7A. (a) Polymorphic SNP distribution on each chromosome identified by the 660 K SNP array. (b) The distribution of polymorphic SNPs is based on their physical location on chromosome 7A. (c) Genetic linkage map of QYrSM155.2 on wheat chromosome 7A based on genotype data from RILs. The red bar indicates the candidate interval for QYrSM155.2. (d) Stripe rust responses GWAS in panel 1. The horizontal line shows the genome-wide significance threshold – log10(P) value of 3.4 (same for e, f). (e, f) Local Manhattan plot of SNPs associated with stripe rust resistance surrounding the chromosome 7A peak. The region enclosed in gray dotted lines represents the potential candidate region. (g) Predicted genes in the QYrSM155.2 candidate region.

Fig. 4. Haplotype analysis of the 1512 accessions from panels 1 and 2 using SNPs identified in the QYrSM155.1 and QYrSM155.2 regions, respectively. (a, b) Phylogenetic trees for haplotypes in QYrSM155.1 and QYrSM155.2 regions. (c, d) Stripe-rust responses of haplotype groups in QYrSM155.1 and QYrSM155.2 candidate regions across multiple environments. Asterisks indicate significant differences among groups or lines at P < 0.05 (Student’s t-test). Detailed results based on 660K wheat SNP markers are provided in Table S4.
4. Discussion

4.1. Emerging Pst races threaten wheat production

New physiological Pst races can evolve from asexual and sexual recombination events and may become predominant and cause large-scale epidemics, resulting from their selection in resistant cultivars combined with higher fitness and/or chance [6,32]. The magnitude of epidemics depends upon favorable climatic factors combined with the area sown to susceptible genotypes. The purpose of disease resistance breeding is to increase the planting area and overall degree of resistance deployed cultivars. Even moderate to low resistance levels can reduce disease buildups, leading to lower terminal disease levels and reduced crop losses [33]. The most recent epidemics in China have been caused by one or more pathotypes with virulence to cultivars carrying Yr26. Also, these pathotypes can be aggressive and carry virulence factors that might overcome unknown resistance genes deployed in other cultivars. They are accordingly considered a major current threat in China [6,34,35]. There is an urgent need to develop and deploy genetically diverse stripe rust-resistant cultivars, not only in regions where cultivars carrying Yr26 are currently grown but also in regions growing predominantly susceptible genotypes [20]. Cultivars, or groups of cultivars, with highly effective all-stage resistance (ASR), are prone to resistance loss, requiring alternative means of gene deployment. One resistance deployment strategy is to use APR genes combinations that are less effective individually but, when combined, present additive effects [33]. Since its release in 2009, the advanced breeding line SM155 has maintained moderate to high APR levels in field environments and was considered worthy of detailed genetic analyses. Although SM155 carries Yr9 (1BL.1RS translocation) for ASR [23], that gene has not been widely effective in China since the 1980s [36]. In the present study, we detected two QTL, QYrSM155.1 and QYrSM155.2, on SM155 chromosomes 2B and 7A, respectively. These QTL were stably expressed in all field environments and had significant effects reducing stripe rust in disease nursery levels, especially when combined (QYrSM155.1 + QYrSM155.2).

4.2. Comparing QTL and tracing their origins

4.2.1. QYrSM155.1

Previous studies identified several ASR genes on chromosome arm 2BL, including Yr3, Yr5, Yr7, Yr43, and Yr53 [32]. QYrSM155.1 confers APR and was expected to be different from previously identified 2BL seedling resistance genes. In our previous study [37], a major QTL, QYrqin.nwafu-2BL.1, was identified in the cultivar Qinnong 142. This QTL was flanked by SNP markers AX-94507002 and AX-94562871, corresponding to the physical interval 650–673 Mb, overlapping the QYrSM155.1 candidate region. Phylogenetic analyses revealed that QYrqin.nwafu-2BL.1 was in the same branch as QYrSM155.1. Both Qinnong 142 and Shaanmai 155 are from Shaanxi province and likely share a distant common parent [37]. In our previous integrated genetic map, several QTL identified in other studies also mapped to arm 2BL, including QYraq.caas-2BL in Aquileja, QYr.inra-2BL in Camp Remy, QYr.caas-2BL.2 in Naxos, QYrdr.wgp-2BL in Druchamp, QYr.caas-2BL.2 in Lumai 21, QYr.nafu-2BL in P9897, QYr.nwafu-2BL.1 (YrZ501) in Z501, and QYrnsb.
nwofu-2BL.3 (YrSnb.1) in Sunbird “S” [24,32]. These results indicate that at least some of these QTL might be the same, but further studies are required for confirmation. The QYrSM155.1 haplotype distributions revealed that the favorable haplotype 2B_h1 is widely dispersed in foreign wheat lines, especially those from CIMMYT and ICARDA, and is present in many Chinese landraces and their derivatives. Based on kinship and pedigree analyses, 2B_h1 is likely derived from ICARDA or CIMMYT-derived lines or from Chinese landraces. However, 2B_h1 is present at very low frequency (1.4%) in current Chinese wheat breeding lines. There is thus an opportunity to increase the frequency of this haplotype in Chinese cultivars.

4.2.2. QYrSM155.2

Only two major genes were previously identified on chromosome arm 7AS: Yr61 and Yrxy1. However, other QTL were reported from QTL mapping and GWAS [32]. In Pindong 34, Yr61 confers ASR whereas, in Xiaoyan 54, Yrxy1 is an all-stage, temperature-sensitive resistance gene. [38,39]. QYrSM155.2 conferred APR in our field environments and may be different from Yr61 and Yrxy1. However, haplotype and phylogenetic analyses revealed that Xiaoyan 54 was in the same haplotype branch as QYrSM155.2. Pedigree analysis indicated that SM155 is a Xiaoyan 54 descendendent through the common parent Xiaoyan 6, suggesting that QYrSM155.2 and Yrxy1 might be identical. Based on the IWGSC reference genome, the QTL QYr.sun-7AS in CPI133872 spanned 68–111 Mb [40], the QTL QYr.caas-2A in Jingshuang 16 spanned 70–116 Mb [41] and IWA2710. APR was located at 68 Mb [42]. All these QTL were within the region harboring QYrSM155.2, pointing again to commonality. Haplotype distribution analysis suggested that the favorable haplotype 7A_h1 is widely dispersed in both international and Chinese wheat germplasm, in particular in wheat lines from Shaanxi province carrying the “Xiaoyan” prefix. Xiaoyan 6 is a Chinese wheat breeding backbone parent and most Shaanxi province wheat lines are derivatives of it. In previous studies [43,44], Xiaoyan 6 conferred high-temperature resistance to stripe rust at the seedling stage. Xiaoyan 6 allegedly carries introgressions from tall wheatgrass (Thinopyrum ponticum), but further studies are required to determine whether QYrSM155.2/Yrxy1 is derived from tall wheatgrass.

4.3. Linkage mapping coupled with association analysis permits refinement of candidate regions

With high-throughput sequencing development and availability of common wheat reference genomes, more efficient mapping techniques (such as combined QTL mapping and GWAS) have been used to describe the genetic basis of complex traits and to identify potentially associated genes [45]. However, despite the recent reduction in large-scale sequencing costs, whole-genome sequencing of wheat remains non-cost-effective owing to the large size of its allohexaploid genome [13]. High-density SNP arrays are an alternative that substantially reduces sequencing costs. The updated wheat 660K-v2 SNP array, which includes 660,009 SNPs distributed across all chromosomes and covering most gene regions [46], has been widely used in genetic studies such as for fine mapping and gene cloning [24,47,48]. A rapid GWAS for genetic description based on the estimated functional importance of each nucleotide polymorphism, initially described for rice [49], was recently applied to identify genes for stripe-rust resistance in wheat [24]. In the present study, we applied the same method to detect 13 and 11 significant SNPs for candidate regions containing QYrSM155.1 and QYrSM155.2, respectively (Fig. 2g, 3g; Table S3). Sixty-three annotated genes were identified in these regions (Table S3).

Relatively few genes for stripe rust resistance have been cloned, including Yr5, Yr7, Yr15, Yr18, YrAS2388, Yr36, Yr46, YrSP, and YrU1 [50]. Among them, Yr5, Yr7, Yr15, YrAS2388, YrSP, and YrU1 encode nucleotide-binding sites and leucine-rich repeat proteins (NBS-LRR), and some harbor zinc-finger BED and WRKY domains [50]. Yr15 encodes a protein comprised of two tandem kinase (or pseudokinase) domains, belonging to serine/threonine protein kinases, that were previously [51] shown to be involved in plant immunity. The remaining Yr18, Yr36, and Yr46 are considered durable resistance sources and encode respectively a putative ATP-binding cassette (ABC) transporter, a protein with a kinase domain and a lipid-binding START domain, and a hexose transporter. Although the resistance conferred by each of these genes seems to represent a different molecular mechanism, the three are considered to involve passive loss of susceptibility by host reprogramming and are involved in energy metabolism and senescence [52].

Based on gene annotations and information about these cloned genes, TraesCS2B01G459500, TraesCS2B01G459600, and TraesCS2B01G459700 encode pathogenesis-related proteins and are considered resistance allele candidates for QYrSM155.1. Annotated genes within the QYrSM155.2 region (TraesCS7A01G127200, TraesCS7A01G127300, and TraesCS7A01G127600, TraesCS7A01G129300, TraesCS7A01G130100 and TraesCS7A01G130400) encode serine/threonine-protein kinases, Caffeoyl-CoA O-methyltransferase, and receptor-like kinases, and each member has been previously [53] shown to be involved in plant immunity. Linkage mapping coupled with association analysis can improve the detection efficiency of gene regions involved in natural variation in target traits. Sequenced genomes of common wheat and its near relatives allowed the prediction of candidate genes in QTL regions. Analytical tools such as sequence analysis, virus-induced gene silencing, CRISPR-Cas, and transformation can be used for further validation tests of individual candidate resistance genes. Closely linked markers in confidence intervals of QYrSM155.1 and QYrSM155.2 can be used or marker-assisted breeding.

CRediT authorship contribution statement


Declaration of competing interest

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

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Appendix A. Supplementary data

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References


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