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ORIGINAL ARTICLE

Comparative genome-wide mapping versus extreme pool-genotyping and development of diagnostic SNP markers linked to QTL for adult plant resistance to stripe rust in common wheat

Jianhui Wu[1] · Shuo Huang[1,2] · Qingdong Zeng[1] · Shengjie Liu[1,2] · Qilin Wang[1] · Jingmei Mu[1,2] · Shizhou Yu[1,2] · Dejun Han[1,2] · Zhensheng Kang[1,3]

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Abstract

Key message **A major stripe rust resistance QTL on chromosome 4BL was localized to a 4.5-Mb interval using comparative QTL mapping methods and validated in 276 wheat genotypes by haplotype analysis.**

Abstract CYMMIT-derived wheat line P10103 was previously identified to have adult plant resistance (APR) to stripe rust in the greenhouse and field. The conventional approach for QTL mapping in common wheat is laborious. Here, we performed QTL detection of APR using a combination of genome-wide scanning and extreme pool-genotyping. SNP-based genetic maps were constructed using the Wheat55 K SNP array to genotype a recombinant inbred line (RIL) population derived from the cross Mingxian 169 × P10103. Five stable QTL were detected across multiple environments. After comparing SNP profiles from contrasting, extreme DNA pools of RILs six putative QTL were located to approximate chromosome positions. A major QTL on chromosome 4B was identified in $F_{2:4}$ contrasting pools from cross Zhengmai 9023 × P10103. A consensus QTL (LOD = 26–40, *PVE* = 42–55%), named *QYr.nwafu-4BL*, was defined and localized to a 4.5-Mb interval flanked by SNP markers *AX-110963704* and *AX-110519862* in chromosome arm 4BL. Based on stripe rust response, marker genotypes, pedigree analysis and mapping data, *QYr.nwafu-4BL* is likely to be a new APR QTL. The applicability of the SNP-based markers flanking *QYr.nwafu-4BL* was validated on a diversity panel of 276 wheat lines. The additional minor QTL on chromosomes 4A, 5A, 5B and 6A enhanced the level of resistance conferred by *QYr.nwafu-4BL*. Marker-assisted pyramiding of *QYr.nwafu-4BL* and other favorable minor QTL in new wheat cultivars should improve the level of APR to stripe rust.

Abbreviations

ANOVA	Analysis of variance
APR	Adult plant resistance
BSA	Bulked segregant analysis
CIMMYT	International Maize and Wheat Improvement Center
DArT	Diversity arrays technology
DS	Disease severity
EST	Expressed sequence tag
GWAS	Genome wide association studies
HTAP	High-temperature adult plant
ICIM	Inclusive composite interval mapping
IT	Infection type
IWGSC	International wheat genome consortium
KASP	Kompetitive allele-specific PCR
LOD	Logarithm of odds
MAS	Marker-assisted selection
Pst	*Puccinia striiformis* f. sp. *tritici*
PVE	Phenotypic variance explained
QTL	Quantitative trait loci

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Shuo Huang and Qingdong Zeng contributed equally to this work.

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✉ Dejun Han
handj@nwsuaf.edu.cn

✉ Zhensheng Kang
kangzs@nwsuaf.edu.cn

[1] State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

[2] College of Agronomy, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

[3] College of Plant Protection, Northwest A&F University, Yangling 712100, Shaanxi, People's Republic of China

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RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
SNP	Single nucleotide polymorphisms
SSR	Simple sequence repeats
STS	Sequence tag site

Introduction

It is predicted that the world population will approximate 10 billion by 2050 (United Nations 2015). As a staple food source, wheat provides about 20% of total grain production and significant growth in production must continue to meet global demand in the foreseeable future (www.fao.org/faostat). However, breeders are still faced with a huge challenge to advance or even maintain production, especially in light of predicted climate change accompanied by biotic stresses, such as the rusts (Hovmøller et al. 2010; McIntosh et al. 1995). Stripe or yellow rust (Yr) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is world threatening and can cause 5–25% yield losses in almost all wheat-growing regions (Chen 2005; Wellings 2011). Despite the effectiveness of chemical control, numerous factors should be taken into account such as costs of fungicides and their application, long-term environmental concerns and loss of efficacy (Chen 2014). Deployment of cultivars with inbuilt diverse types of resistance is the optimal strategy to control rusts, especially in developing countries (Chen 2005; Wellings 2011).

Currently, the number of permanently designated *Yr* genes exceeds 80 (Feng et al. 2017; McIntosh et al. 2016, 2017, pers. comm. 2018). Unfortunately, many of these genes are not effective, no longer effective, or not yet widely used in China and elsewhere (Sharma-Poudyal et al. 2013; Zeng et al. 2015). The reason for loss of effectiveness is that many of these genes confer all-stage, race-specific resistance that is vulnerable to being negated by new or rare pathogen races when deployed in cultivars, especially when singly present (Chen 2005; Li and Zeng 2002). By comparison, many sources of adult plant resistance (APR), sometimes referred as high-temperature adult plant (HTAP) resistance, have proved to be more durable, but usually exhibit quantitative, partial resistance that is expressed at later growth stages (Ellis et al. 2014). When acceptable (more resistant) levels of this type of resistance are subject to genetic analysis, it is common that more than one resistance gene is involved and that the genes are additive in effect. The component genes are usually considered to be non-race specific, but in any case resistance based on multiple genes should not fail as a result of a single mutational change in the pathogen, thus the greater durability of this type of resistance (Brown 2015; Chen 2013; Niks et al. 2015). Due to repeated failures of all-stage resistance sources partial adult plant resistance is continuing to be favored by breeders. More than 200 APR genes/QTL are dispersed across all 21 wheat chromosomes (Bulli et al. 2016; McIntosh et al. 2017; Rosewarne et al. 2013). Among stripe rust APR genes or QTL, *Yr18*, *Yr36* and *Yr46* have been cloned (Fu et al. 2009; Krattinger et al. 2009; Moore et al. 2015). Because "Rusts never sleeps" we continue to search for more genes conferring resistance and to integrate them into breeding programs for which both the resistance gene and markers are required for their selection.

Significant advances in genome sequencing and high-throughput SNP-based genotyping in wheat have radically changed the methods of wheat research (Uauy 2017). Over the past few years, the newest reference genome of hexaploid wheat (AABBDD) cv. Chinese Spring and wild emmer (AABB) cv. Zavitan have been released (Avni et al. 2017; International Wheat Genome Consortium (IWGSC), http://www.wheatgenome.org/). In addition, the progenitor species and other bread wheat and durum wheat cultivars have been sequenced (Chapman et al. 2015; Jia et al. 2013; Ling et al. 2013; Luo et al. 2017; Uauy 2017). All of the above of resources provide tools for high-throughput discovery of DNA variants and facilitate faster development of new generation markers such as single nucleotide polymorphism (SNP) (Allen et al. 2011; Kumar et al. 2012; Yang et al. 2015). Compared to earlier markers, SNP and their alleles and haplotypes have the advantage of high resolution of genetic diversity and gene function (Xu et al. 2017). Subsequently several high-density wheat SNP arrays and platforms such as Illumina Bead Array™, Affymetrix Gene Chip™ and Kompetitive Allele-Specific PCR (KASP) have been developed (Rasheed et al. 2017). The shift to SNP-based platforms has accelerated gene/QTL mapping, genome-wide association studies (GWAS) and marker-assisted selection in cereal rust research (Bulli et al. 2016; Hou et al. 2015; Maccaferri et al. 2015; Wu et al. 2017a, 2017b). The Axiom® Wheat55 K SNP array containing 53,063 SNPs was specifically selected from the Wheat660 K SNP array by analyzing polymorphism in a global set of wheat accessions (Jia and Zhao 2016). This new SNP array has lower cost, less "ascertainment bias" and more scorable markers across multiple genetic backgrounds (Jizeng Jia, pers. comm.).

Conventional gene/QTL mapping requires phenotyping and genotyping of every individual in a mapping population, a time-consuming, laborious and costly process (Xu et al. 2017). In contrast, bulked segregant analysis (BSA) based on pooling of small numbers of phenotypically similar individuals provides a simple and rapid approach to search for DNA variants linked to specific genomic regions conferring a trait of interest (Giovannoni et al. 1991; Michelmore et al. 1991). Combining high-throughput genotyping technology with a BSA strategy is a powerful tool for accelerating gene/QTL identification in many crops (Liu et al. 2012; Ramirez-Gonzalez et al. 2014; Schlötterer et al. 2014; Singh et al.

2016; Takagi et al. 2013; Zou et al. 2016). P10103 with the pedigree Ronbin.2-Fnd × CMH74A.630 is an advanced wheat line from CIMMYT (CIMMYT 1983). We have tested P10103 in greenhouse and field nurseries and consistently found that it was highly resistant to stripe rust at the adult plant stage, despite having intermediate reactions to some older races in seedling tests (Han et al. 2012). However, little is known about the genetic basis of resistance. The goals of this study were to: (1) detect stripe rust resistance loci using SNP-based genome-wide scanning across multiple environments, (2) dissect QTL using extreme pooling with high-throughput genotyping, and validate the major stable QTL in different genetic backgrounds, and (3) test the applicability of linked KASP markers for marker-based selection.

Materials and methods

Plant materials

The parental lines for this study were the susceptible lines Mingxian 169 (MX169) and Zhengmai 9023 (ZM9023) and resistant line P10103. Mingxian 169 is an old Chinese winter wheat landrace that is susceptible to all Chinese *Pst* races. Zhengmai 9023 is a leading commercial cultivar, but is moderately to highly susceptible to current *Pst* races (Xue et al. 2014). One mapping population of 168 $F_{6:7}$ recombinant inbred lines (RILs) was developed from the cross MX169 × P10103 (M/P); the other comprised 211 $F_{2:4}$ lines from cross ZM9023 × P10103 (Z/P) and was used to validate the stability of target QTL. A panel of 276 diverse local and foreign wheat cultivars and landraces was used to validate SNP markers flanking the identified QTL. All of the accessions were obtained from the China Agriculture Research System (CARS). Avocet S (AvS) and Xiaoyan 22 (XY22) were used as susceptible controls.

Greenhouse trials

Seedling and adult plant tests were conducted under controlled greenhouse conditions to characterize the stripe rust APR in P10103 and to compare an APR gene on chromosome 4B, with others reported in lines CH223 (*Yr50*), PI 192252 (*Yr62*) and Zhoumai 22 (*YrZH22*). In addition to six *Pst* races (CYR29, CYR31, CYR32, CYR33, Su11-7 and V26/CH42) used in previous studies (Han et al. 2012; Zeng et al. 2014; Zhou et al. 2015), we also used CYR34 (V26/Gui22-9). The avirulence/virulence characteristics of the races were reported by Wu et al. (2016). For seedling tests 10–15 plants of MX169, ZM9023, P10103 and its derivatives and other *Yr* gene donors were grown in 9 × 9 × 9 cm pots, and for adult plant tests, three plants were grown in larger 20 × 20 × 15 cm pots. Details of inoculation were described previously (Wu et al. 2018). Infection types (IT) were recorded 18–21 days after inoculation using a 0–9 scale (Line and Qayoum, 1992). Plants with ITs 0–6 were considered resistant, and plants with ITs 7–9 were considered susceptible. In order to confirm and clarify ITs of the entries, all tests were repeated three times.

Field assessment of stripe rust

Stripe rust assessments in the field were conducted at Yangling, Shaanxi province (34°17′ N, 108°04′ E, altitude 519 m), and Jiangyou in Sichuan province (31°53′N, 104°47′E, altitude 571 m) during 2015–2017. All trials were arranged in randomized complete blocks with two replicates. Each line with approximately 30 seeds was planted in a 100-cm row with 30 cm between rows. The parents and susceptible check XY22 were planted after every 20 rows throughout the field. Spreader rows containing mixtures of *Pst*-susceptible genotypes MX169 and AvS were planted around the plot area to produce an epidemic environment. Jiangyou is part of a natural over-wintering region for stripe rust and nurseries regularly become infected without artificial inoculation. Trials at Yangling were inoculated with a mixture of prevalent *Pst* races (CYR32 + CYR34) suspended in liquid paraffin (1:300) sprayed onto MX169 and AvS at flag leaf emergence. Test rows were visually rated for infection type (IT) and stripe rust severity (DS) 18–20 days post-flowering when severity levels on the susceptible checks reached maximum levels of 90–100% (15–20 May at Yangling and 10–15 April at Jiangyou). IT data were recorded for each line based on the 0–9 scale as described by Line and Qayoum (1992), with 1 being very resistant and 9 very susceptible. Disease severity scores were based on the modified Cobb scale (Peterson et al. 1948). IT and DS of homozygous lines were recorded as single values; and for segregating lines IT and DS were recorded as two or more values, but later averaged for each line.

Analysis of phenotype data

Mean IT and DS of each $F_{2:3}$ line and F_7 RIL were used to examine the variance within individual environments. Analyses of variance (ANOVA) and Pearson's correlation coefficients were performed using the "AOV" tool in QTL IciMapping V 4.1 software (Meng et al. 2015; Wang 2009; http://www.isbreeding.net/). Broad-sense heritability (*h2 b*) of stripe rust resistance was estimated as $h2\ b = \sigma 2\ g / (\sigma 2\ g + \sigma 2\ ge/\mathrm{e} + \sigma 2\ \varepsilon/re)$, where $\sigma 2\ g$ is $(MS_f - MS_{fe})/\mathrm{re}$, $\sigma 2\ ge$ is $(MS_{fe} - MS_e)/r$ and $\sigma 2\ \varepsilon$ is MS_e; $\sigma 2\ g$ = genetic variance, $\sigma 2\ ge$ = genotype × environment interaction variance, $\sigma 2\ \varepsilon$ = error variance, MS_f = mean square of genotypes, MS_{fe} = mean square of genotype × environment interaction,

MS_e = mean square of error, r = number of replications and e = number of environments.

Molecular genotyping and bulked segregant analysis

Fresh leaves of F_2 plants, and F_6-derived progeny and their corresponding parental lines were collected at the jointing stage in the field for genomic DNA extraction using the method described in Song et al. (1994). Each RIL ($N = 125$ from the 168 genotypes) and parent was genotyped by the 55 K iSelect SNP array. To compare genome-wide QTL scanning with mapping by extreme pool-genotyping and saturate the density of target QTL regions, we subsequently combined BSA with the Wheat660 K SNP array. Based on the stripe rust reactions of the $F_{2:3}$ lines and F_7 RILs in all environments, F_2 and F_7 DNA bulks of 10 homozygous resistant (IT 1–2, DS ≤ 10) and 10 homozygous susceptible (IT 8–9, DS ≥ 90) lines were pooled as resistant and susceptible bulks, respectively. Then, F_2 and F_7 bulks and parents were genotyped with the 660 K SNP array. Genotyping of the SNP arrays was performed by the CapitalBio Corporation, Beijing (http://www.capitalbio.com). SNP genotype calling and allele clustering were processed with the polyploid version in Affymetrix Genotyping Console™ (GTC) software. SNP filtering criteria were as follows: monomorphic and poor quality SNP loci with more than 10% missing values, ambiguous SNP calling, and minor allele frequencies below 5% were excluded from further analysis. Polymorphic SNPs associated with resistance in BSA contained homozygous and heterozygous genotypes. Homozygous genotype differences were localized to chromosomes based on the high-density 660 K genetic map (Jizeng, Jia pers. comm.). The closest SNP markers to the QTL peak from the 55 and 660 K wheat SNP arrays were converted to KASP genotyping format (see Table S1 for primer sequences). The $F_{2:3}$ populations were genotyped using cost-efficient KASP assays (LGC, Middlesex, UK). The procedure of SNP conversion to KASP markers and selective KASP assays was described in Ramirez-Gonzalez et al. (2015) and Wu et al. (2017a).

Linkage maps and QTL analysis

Genotypic markers were tested using Chi-squared (χ^2) tests to exclude markers with distorted segregation before constructing genetic linkage maps ($P > 0.001$). The numbers of redundant markers showing identical segregation were firstly reduced using the "BIN" tool in IciMapping V4.1 (Meng et al. 2015; Wang 2009), and one marker was chosen to represent each bin on the basis of the least amount of missing data or random choice of one when frequencies of missing data were equal. The filtered markers were used to construct linkage maps with the "MAP" tool in IciMapping V4.1. To finalize the linkage map, marker ordering and ripping were performed using the RECORD and COUNT algorithms, respectively, with the logarithm of odds (LOD) score thresholds set at 5–10. Genetic maps were drawn using Mapchart v2.3 software (Voorrips 2002). The positions of linkage groups were determined based on 660 K integrated maps (Jizeng, Jia pers. comm.).

Inclusive composite interval mapping with the additive tool (ICIM-ADD) in IciMapping V 4.1 was carried out to detect QTL for IT and DS for both populations. QTL analyses were conducted for both populations at each location using arithmetic means across environments to obtain balanced values for each line. The parameter settings used for one dimensional ICIM were followed: likelihood-odds (LOD) significance threshold calculated by 1000 permutations at a p value ≤ 0.01, and a walk speed genome scanning step of 1.0 cM. The phenotypic variances explained (*PVE*) by individual QTL and additive effect at the LOD peaks were also obtained.

SNP probes were positioned to the newest reference hexaploid genome sequence (IWGSC RefSeq v.1.0) through a BLAST search to obtain the genomic locations of all polymorphic markers. The BLAST hits criteria included an e value threshold of 10^{-5} and minimum similarity cutoff higher than 95% between query and database sequences.

Identification of robust markers for marker-assisted selection

The diverse wheat panel was genotyped with the Wheat660 K SNP array to validate the robustness of SNP-based markers for marker-assisted selection. SNP markers surrounding target QTL were then used to construct phylogenetic neighbor-joining trees. Phylogenetic analysis was performed using MEGA v7.0.14 software to cluster marker groups. All data were visualized using the visualize model online operation provided by the ITOL website (http://itol.embl.de/).

Results

Phenotypic values and statistical analysis

In all field experiments, lines in both populations along with parental lines showed significant genetic variation for APR. The response of P10103 was consistently low (IT 1–2, DS < 10%), and the susceptible parents MX169 and ZM9023 were high (IT 9, DS > 95%; IT 9, DS > 90%) (Table 1 and Fig. S1). Infection types and disease severities for both populations were normally distributed in all environments indicating that the responses were quantitative. Broad-sense

Table 1 Summary of adult plant stripe rust responses for Mingxian 169 (MX169) × P10103 recombinant inbred line (RIL) population (M/P) and Zhengmai 9023 (ZM9023) × P10103 $F_{2:4}$ population (Z/P) during the 2015–2016, 2016–2017 cropping seasons in Yangling and Jiangyou

Environment	Population size	MX169	ZM9023	P10103	Mean	Range	*σ2 g*	SD	*h2 b*
Infection type (IT)									
[a]YL2015-16 M/P	168	9	–	0	5.2	1–9	3.7	1.9	0.89
JY2015-16 M/P	168	9	–	1	5.7	1–9	3.4	1.9	0.92
YL2016-17 M/P	168	9	–	1	5.4	1–9	4.3	2.1	0.91
JY2016-17 M/P	168	9	–	1	5.6	1–9	3.6	2.0	0.90
Average	168	9	–	0.8	5.5	1–9	3.7	2.0	0.90
YL2015-16 Z/P	211	–	9	1	4.7	1–9	5.8	2.1	0.91
JY2015-16 Z/P	211	–	9	0	4.6	0.5–9	4.2	2.1	0.88
YL2016-17 Z/P	211	–	9	1	4.6	1–9	3.3	2.0	0.86
JY2016-17 Z/P	211	–	9	1	5.0	1–9	3.6	1.9	0.90
Average	211	–	9	0.8	4.7	1–9	3.7	2.0	0.88
Disease severity (DS)									
[a]YL2015-16 M/P	168	100	–	5	47.6	4–100	427.8	19.1	0.89
JY2015-16 M/P	168	100	–	7.5	53.4	7.5–100	439.7	20.8	0.89
YL2016-17 M/P	168	100	–	10	51.7	5–100	532.5	22.8	0.92
JY2016-17 M/P	168	100	–	5	54.9	7.5–95	454.3	22.0	0.90
Average	168	100	–	7.5	51.9	6–97.5	446.1	21.2	0.90
YL2015-16 Z/P	211	–	90	5	45.4	3–97.5	681.6	23.0	0.90
JY2015-16 Z/P	211	–	95	5	44.4	0.5–100	512.5	23.6	0.84
YL2016-17 Z/P	211	–	85	5	46.9	3.5–100	427.6	22.4	0.84
JY2016-17 Z/P	211	–	95	10	50.4	1–100	479.2	22.6	0.88
Average	211	–	90	5	46.8	2–100	471.2	22.9	0.88

[a]*YL* Yangling, *JY* Jiangyou

heritabilities based on all data sets for both populations were generally high (Table 1). In the MX169/P10103 population heritabilities ranged from 0.89 to 0.92 with correlation coefficients from 0.78 to 0.90 ($P < 0.001$) (Tables 1 and 2); when all data were combined the heritability was 0.90. In the ZM9023/P10103 population, heritabilities ranged from 0.84 to 0.91 across environments and corresponding correlation coefficients ranged from 0.86 to 0.92; the heritability for combined environments was 0.88 (Tables 1 and 2). These results indicated that the APR was consistent across environments and that QTL controlling APR had large effects in reducing stripe rust severity.

Genome-wide SNP scanning analysis and linkage mapping

One hundred and twenty-five RILs were genotyped with the 55 K SNP array; 16,235 of 53,063 SNP loci showed

Table 2 Correlation coefficients (*r*) for mean disease severity (DS) and infection type (IT) of the MX169 × P10103 (M/P) RIL population and ZM9023 × P10103 (Z/P) $F_{2:4}$ population across four environments

Environment[a] (location, year)	*r* values based on MDS (IT)[b]							
	YL2016 M/P	JY2016 M/P	YL2017 M/P	JY2017 M/P	YL2016 Z/P	JY2016 Z/P	YL2017 Z/P	JY2017 Z/P
YL2016 M/P	1							
JY2016 M/P	0.78 (0.86)	1						
YL2017 M/P	0.82 (0.90)	0.78 (0.82)	1					
JY2017 M/P	0.85 (0.89)	0.83 (0.90)	0.82 (0.83)	1				
YL2016 Z/P	–	–	–	–	1			
JY2016 Z/P	–	–	–	–	0.90 (0.89)	1		
YL2017 Z/P	–	–	–	–	0.91 (0.90)	0.86 (0.87)	1	
JY2017 Z/P	–	–	–	–	0.90 (0.89)	0.92 (0.91)	0.87 (0.88)	1

[a]*YL* Yangling, *JY* Jiangyou

[b]*r* values calculated for IT are shown in parentheses. All *r* values are significant at $P = 0.001$

polymorphism between the parents. Among polymorphic SNPs, 1257 were removed due to more than 10% missing data or severe segregation distortion ($P < 0.001$) (Table S2). The remaining 14,978 SNPs fell into 2965 bins as 12,013 SNPs were redundant. After deletion of 10 unlinked SNP markers that fell outside the LOD 5.0–10.0 set by the permutation test, 2955 markers were used for construction of linkage groups. Finally, the 2955 markers were distributed in 36 linkage groups spanning a total length 6049.2 cM. The linkage groups were integrated into chromosome maps based on the 660 K integrated map. The A, B and D genomes included 1287 (48.2%), 1267 (44.7%) and 401 (7.1%) markers covering lengths of 2,456.3, 2,194.0 and 1,393.5 cM with average marker/bin intervals of 1.9, 1.7 and 3.5 cM, respectively. Chromosomes 1B, 1D 3B, 4D, 6B and 7D each had single linkage group; chromosomes 1A, 2A, 2B, 2D, 3A, 4A, 5A, 5B, 5D, 6D and 7B each had two linkage groups; 3D and 7A each had three linkage groups; 4BS/6AS and 4BL/6AL were translocation lines (Table 3). Here, we report only genetic maps of chromosomes with QTL.

QTL analysis of RIL population

Seven QTL contributing to stripe rust resistance in the M/P RIL population were identified in chromosome arms 2AL, 4AL, 4BL, 5AL, 5BS, 5BL and 6AL using the ICIM method; all were from P10103. QTL detected using both IT and DS data in four environments, and in the combined analysis, were regarded as stable. The five stable QTL were designated *QYr.nwafu-4AL*, *QYr.nwafu-4BL*, *QYr.nwafu-5AL*, *QYr.nwafu-5BL* and *QYr.nwafu-6AL*. The QTL with largest effect, *QYr.nwafu-4BL*, located in a 2.5-cM interval spanned by SNP markers *AX-110963704* and *AX-110564812*, explained 30.5–42.0% of the phenotypic variation across environments. *QYr.nwafu-4AL*, linked to markers *AX-111100419* and *AX-89434097*, explained 10.2–17.4% of the phenotypic variance. *QYr.nwafu-5AL*, with flanking

Table 3 Distribution and density of single nucleotide polymorphism (SNP) markers on wheat chromosomes in the RIL population derived from cross MX169 × P10103

Chromosome	Linkage groups	No. of markers	% markers	Bins	Length (cM)	Average marker/bin interval	[b]Marker density
1A	[a]G1 + G2	694	4.68	159	274.99	1.73	2.52
1B	G3	696	4.70	161	243.99	1.52	2.85
1D	G4	47	0.32	31	121.56	3.92	0.39
2A	G5 + G6	737	4.97	173	338.04	1.95	2.18
2B	G7 + G8	1151	7.77	266	408.8	1.54	2.82
2D	G9 + G10	136	0.92	64	245.62	3.84	0.55
3A	G11 + G12	1272	8.59	236	382.35	1.62	3.33
3B	G13	1187	8.01	225	413.56	1.84	2.87
3D	G14 + G15 + G16	176	1.19	43	178.13	4.14	0.99
4A	G17 + G18	1628	10.99	154	312.54	2.03	5.21
4BS/6AS	G19 442 + 124	566	3.82	79	209.04	2.65	2.71
4D	G20	81	0.55	45	169.03	3.76	0.48
5A	G21 + G22	1097	7.40	244	508.49	2.08	2.16
5B	G23 + G24	992	6.70	216	364.59	1.69	2.72
5D	G25 + G26	209	1.41	71	268.49	3.78	0.78
6AL/4BL	G27 586 + 215	801	5.41	132	287.59	2.18	2.79
6B	G28	1139	7.69	141	254.59	1.81	4.47
6D	G29 + G30	172	1.16	68	184.42	2.71	0.93
7A	G31 + G32 + G33	1006	6.79	192	372.56	1.94	2.70
7B	G34 + G35	801	5.41	176	284.62	1.62	2.81
7D	G36	227	1.53	79	226.22	2.86	1.00
A genome	14	7144	48.22	1287	2456.33	1.91	2.91
B genome	11	6623	44.70	1267	2194.03	1.73	3.02
D genome	12	1048	7.07	401	1393.47	3.47	0.75
Total	36	14815		2955	6049.22	2.05	2.45

[a]Chromosomes with two or more linkage groups

[b]Marker density (loci/cM) was calculated by dividing the number of unique loci by the genetic length

markers at 8.1 and 1.6 cM explained 15.3 and 12.0% of the phenotypic variation in IT and DS, respectively. *QYr.nwafu-5BL* was significant at a threshold LOD of 3.0 and explained 7.4–12.3% of the phenotypic variation across environments. The closest markers were *AX-110493865*, *AX-111488956* and *AX-110384205*, within a 5.2-cM interval. *QYr.nwafu-6AL*, explaining 4.2–13.1% of the phenotypic variance, was in interval *AX-110516770–AX-109575335* spanning 8.1 cM. The other two QTL were identified in only one location (Table 4, Fig. 1).

To investigate the QTL effects, the 125 lines were divided into genotypic groups based on the presence of closest markers to the five QTL (Table S3). As *QYr.nwafu-4BL* was a major QTL; the combinations of *QYr.nwafu-4BL* and the other QTL were separated and the rest of minor QTL on chromosome 4AL, 5AL, 5BL and 6AL were then grouped.

Table 4 Summary of stripe rust resistance QTL detected in the MX169 × P10103 RIL population and ZM9023 × P10103 $F_{2:3}$ population across four environments

QTL	Environment[a]	Marker interval	Infection type (IT)			Disease severity (DS)		
			LOD[b]	PVE[c]	Add[d]	LOD	PVE	Add
QYr.nwafu-2AL	YL2016	*AX-110126474—AX-109627180*	3.9	5.8	−0.4	3.5	5.9	−4.1
	YL2017	*AX-110126474—AX-109627180*	6.1	7.3	−0.5	4.4	6.2	−5.0
QYr.nwafu-4AL	YL2016	*AX-111100419—AX-89434097*	11.2	17.7	−0.7	6.7	11.8	−5.7
	JY2016	*AX-111100419—AX-89434097*	11.4	15.6	−0.7	8.2	11.3	−6.4
	YL2017	*AX-111100419—AX-89434097*	12.8	17.4	−0.7	9.7	14.7	−7.6
	JY2017	*AX-111100419—AX-89434097*	8.3	10.6	−0.6	7.3	10.2	−6.3
	Mean	*AX-111100419—AX-89434097*	10.9	15.3	−0.7	8.0	12.0	−6.5
QYr.nwafu-4BL	YL2016	*AX-110963704—AX-110564812*	19.8	38.0	−1.1	20.3	42.0	−11.7
	JY2016	*AX-110963704—AX-110564812*	20.3	35.9	−1.1	20.9	34.0	−12.3
	YL2017	*AX-110963704—AX-110564812*	20.9	33.2	−1.0	17.2	30.5	−11.2
	JY2017	*AX-110963704—AX-110564812*	17.0	38.8	−1.3	14.6	35.1	−13.5
	Mean	*AX-110963704—AX-110564812*	19.5	36.5	−1.1	18.2	35.4	−12.2
QYr.nwafu-5AL	YL2016	*AX-111654317—AX-109832565*	4.3	6.5	−0.4	3.9	7.7	−5.3
	JY2016	*AX-111654317—AX-109832565*	4.4	5.6	−0.4	4.5	6.3	−4.8
	YL2017	*AX-111654317—AX-109832565*	3.4	5.1	−0.3	5.6	7.9	−5.8
	JY2017	*AX-111654317—AX-109832565*	5.7	8.6	−0.5	6.5	9.2	−6.0
	Mean	*AX-111654317—AX-109832565*	4.4	7.8	−0.4	5.0	8.6	−6.2
QYr.nwafu-5BS	JY2016	*AX-109839576—AX-89370492*	3.1	4.2	−0.4	4.9	7.4	−5.2
	JY2017	*AX-89370492—AX-109402563*	5.7	7.2	−0.5	5.1	6.9	−5.2
QYr.nwafu-5BL	YL2016	*AX-110493865—AX-111488956*	7.3	10.9	−0.6	5.8	10.0	−5.3
	JY2016	*AX-111488956—AX-110384205*	9.0	12.1	−0.6	7.9	11.0	−6.4
	YL2017	*AX-110493865—AX-111488956*	7.2	8.7	−0.5	8.4	12.3	−7.1
	JY2017	*AX-111488956—AX-110384205*	6.8	8.7	−0.5	5.4	7.4	−5.4
	Mean	*AX-110493865—AX-110384205*	7.6	10.1	−0.6	6.9	10.2	−6.1
QYr.nwafu-6AL	YL2016	*AX-108785002—AX-109575335*	4.4	6.0	−0.4	4.2	7.0	−4.5
	JY2016	*AX-110516770—AX-108786424*	4.9	6.0	−0.4	3.5	4.2	−3.9
	YL2017	*AX-108785002—AX-109575335*	4.8	7.2	−0.5	5.2	8.2	−5.8
	JY2017	*AX-110516770—AX-108786424*	9.5	13.1	−0.7	5.0	6.2	−4.9
	Mean	*AX-108785002—AX-109575335*	5.8	8.3	−0.6	4.2	5.9	−4.6
QYr.nwafu-4BL	YL2016	*AX-110963704—AX-110519862*	35.3	50.2	−1.4	32.8	49.5	−17.8
	JY2016	*AX-110963704—AX-110519862*	30.5	45.6	−1.3	28.6	44.2	−24.5
	YL2017	*AX-110963704—AX-110519862*	34.0	47.3	−1.4	31.2	46.1	−16.3
	JY2017	*AX-110963704—AX-110519862*	39.7	55.0	−2.1	26.6	42.4	−24.6
	Mean	*AX-110963704—AX-110519862*	38.1	53.7	−1.6	35.4	50.9	−20.8

[a] *YL* Yangling, JY Jiangyou

[b] *LOD* logarithm of odds score

[c] *PVE* percentage of the phenotypic variance explained by individual QTL

[d] *Add* additive effect of resistance allele

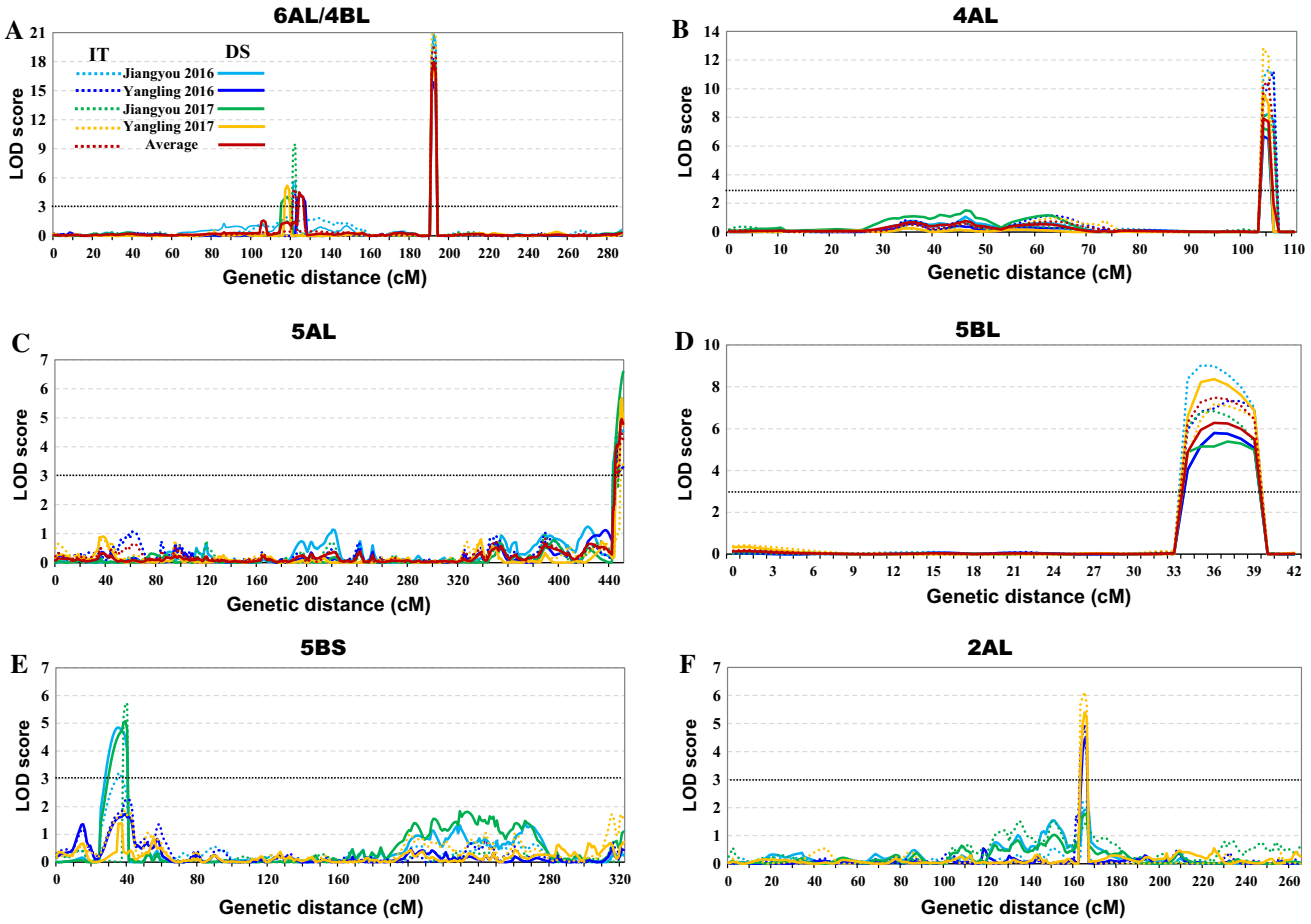


Fig. 1 Graphical display of QTL for stripe rust resistance in MX169×P10103 RIL population detected in four environments. Six linkage groups were identified with SNP markers. QTL were identified by inclusive composite interval mapping (ICIM), and the dashed black horizontal line indicates the threshold LOD score of 3 to declare significance. Environments where the QTL were detected are shown in different colors

Finally, there were 10 groups based on the number of QTL. The mean IT and DS values of the 10 groups are shown in Fig. 2. Lines with the highest numbers of QTL had the lowest IT and DS. The IT and DS values in lines with five QTL ranged from 1.6 to 2.1 and 11.9 to 18.8% across four environments, respectively, similar to the resistant parent (Fig. 2a, b; Table S3); corresponding to the total phenotypic variance explained in the combined analysis ranging from 72.1 to 78%. RILs with no QTL were highly susceptible (IT = 8–9, DS > 90%), similar to the susceptible parent. Grouping was performed using the "BIP" tool in IciMapping V 4.1 software.

Combining BSA with the 660 K SNP array

To compare genome-wide QTL scanning with mapping by extreme pool-genotyping and to saturate marker density in the major QTL region, we combined BSA with 660 K SNP arrays from the two populations. Approximately 2500 SNPs were polymorphic between the RIL DNA bulks; 331, 760, 294, 301 and 335 of these were located on chromosomes 4A, 4B, 5A, 5B and 6A, respectively, whereas the others were distributed across other chromosomes (Fig. S2A). The proportion of SNPs common between bulks and parents on chromosome 4B was the highest; the numbers of markers on chromosomes 4A, 5A, 5B and 6A were also relatively high (Fig. S2A). We calculated the number of SNPs based on their physical positions by window sizes of 1 Mb and upwards. Intervals of 541–650 Mb on 4BL, 450–719 Mb on 4AL, 668–689 Mb on 5AL, 461–480 Mb on 5AL and 522–559 Mb on 6AL clustered the highest numbers of SNPs (Fig. 3b, Table S4A). These genomic regions were consistent with the results of genome-wide QTL mapping. On the other hand, approximately 1300 SNPs showed polymorphisms in the F_2 DNA bulks, of which 969 SNPs were located on chromosome 4B (Fig. S2B) and most of the linked SNPs were within an interval 505–665 Mb (Fig. 3d, Table S4B). The other minor QTL detected in the RIL population were not

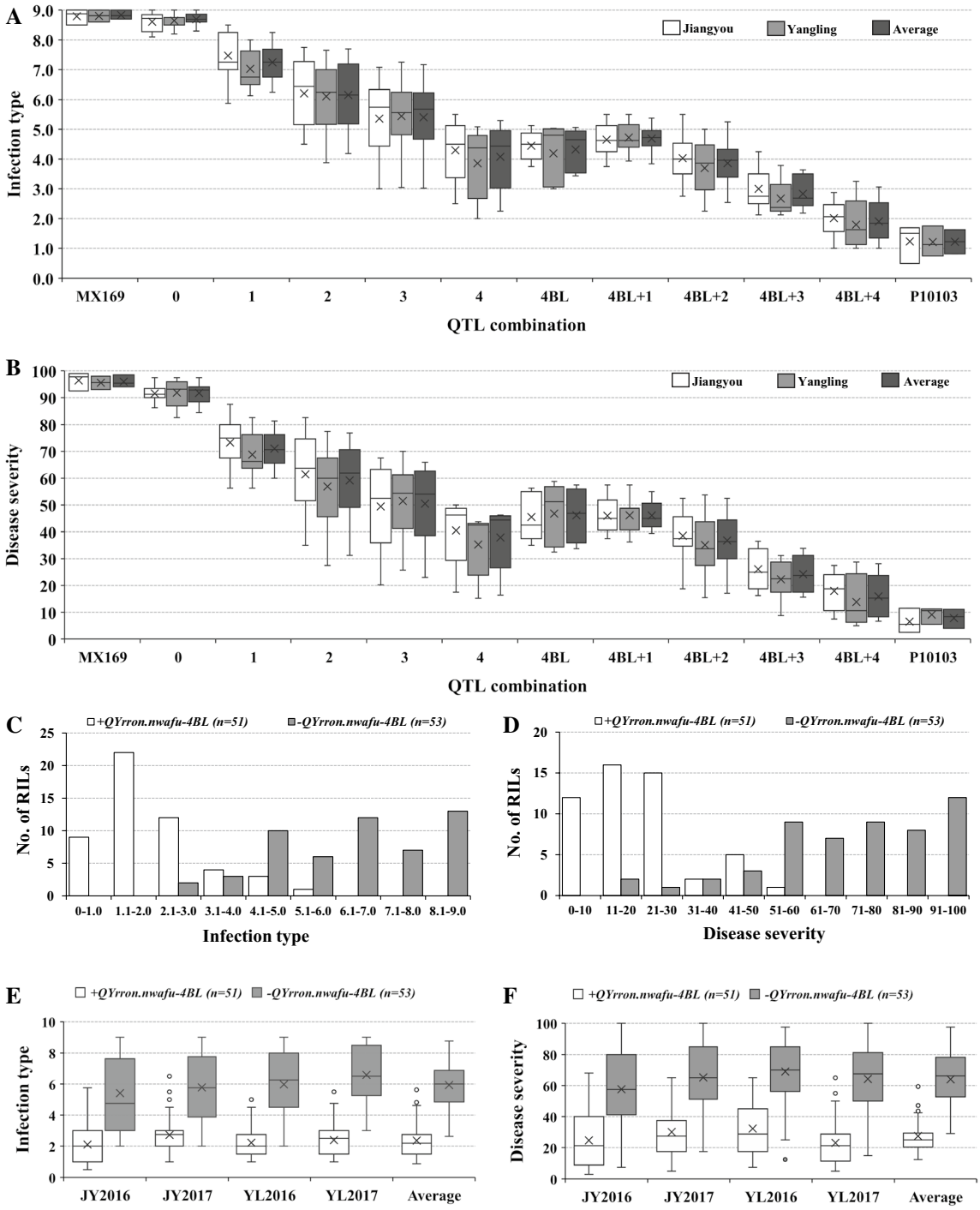


Fig. 2 Effects of *QYr.nwafu-4BL* and QTL combinations on stripe rust scores illustrated by mean infection type and disease severity scores of RILs from the MX169×P10103 population at Yangling, Jiangyou and combined environments (**a**, **b**); and from ZM9023×P10103 (**c**, **d**, **e**, **f**). As a major QTL, the combinations of *QYr.nwafu-4BL* and the other QTL were separated, and the minor QTL on chromosomes 4AL, 5AL, 5BL and 6AL were grouped. The numbers represent the combination of minor QTL (0: none; 1: 4AL, 5AL, 5BL and 6AL; 2: 4AL+5AL, 4AL+5BL, 4AL+6AL, 5AL+5BL, 5AL+6AL and 5BL+6AL; 3: 4AL+5AL+5BL, 4AL+5AL+6AL, 5AL+5BL+6AL; 4: 4AL+5AL+5BL+6AL). The box plots (quartiles are boxes, medians are continuous lines, means are crosses, whiskers extend to the farthest points that are not outliers, and outliers are black dots) for infection type and disease severity associated with the identified QTL and their combination

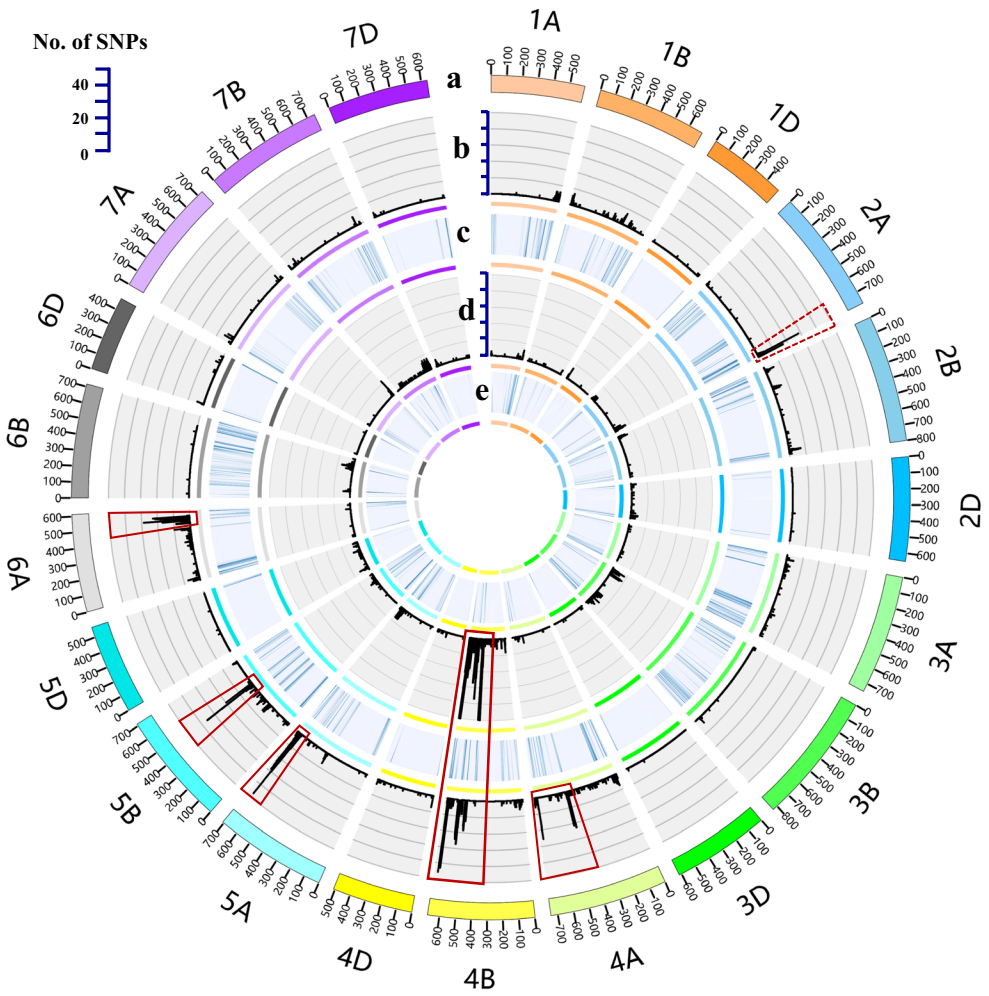


Fig. 3 Circos diagram showing physical distributions of single nucleotide polymorphisms (SNP) from extreme pool-genotyping. **a** Outer track is the physical size of each chromosome. Wheat chromosomes are represented in different colors. Heatmaps represent the numbers of SNPs in 30 Mb windows showing variation between Mingxian 169 and P10103 (**c**), and Zhengmai 9023 and P10103 (**e**). (**b:** RILs and **d:** $F_{2:4}$) Numbers of SNPs were calculated based on their physical positions by windows size of 1 Mb. The red boxes show the consistent QTL corresponding to those found by genome-wide QTL scanning. The most significant and stable QTL identified was on chromosome 4B (boxed segment)

identified in the F_2 bulks. The overlapping region on 4BL was most likely linked with the major resistance locus.

Validation of the major QTL using $F_{2:4}$ lines

Representing different genetic backgrounds, 211 $F_{2:4}$ Z/P lines were used to validate the major QTL on 4BL. Thirty-six chromosome-specific SNPs in the overlapping region were selected for conversion to KASP markers and then screened on the parents and bulks to confirm polymorphisms before being genotyped on the entire population; 19 failed to distinguish the contrasting parents and bulks. Sequences of the KASP markers are listed in Table S1. A genetic map was constructed using the 17 KASP markers genotyped on the 211 F_2 individuals spanned 35.2 cM. Using ICIM with mean IT and DS data, *QYr.nwafu-4BL* was identified and located in a 4.8-cM interval spanned by SNP markers *AX-110963704* and *AX-110519862* (Fig. 4b). It conferred a large effect and explained 42.4–55.0% of the phenotypic variation in all environments (Table 4). To assess resistance effect conferred by *QYr.nwafu-4BL*, the $F_{2:4}$ lines were divided into two groups: lines with and without *QYr.nwafu-4BL* based on the presence/absence of the most closely linked flanking markers. Mean infection type and disease severity for lines carrying *QYr.nwafu-4BL* ranged from 0.9 to 5.6 and 2 to 56.9%, respectively, whereas for those without the QTL ranged from 2.6 to 9.0 and 14.1 to 97.5%, respectively (Fig. 2c–e; Table S5A). Thus, *QYr.nwafu-4BL* reduced stripe rust severity by 32.9 to 41.1% (Fig. 3f, Table S5B).

Identification of the target genomic region

As the $F_{2:4}$ and RIL populations shared many common KASP-SNP markers and marker order (Fig. 4a, b), they were merged into a single consensus genetic map (Fig. 4c). For simplification, redundant SNPs in the non-targeted region were removed. Further analysis of the phenotypic values for both populations in eight environments along with the genetic maps indicated that the overlapping confidence interval was *AX-110963704–AX-110519862*, corresponding to a physical interval of 4.5 Mb (Fig. 4d, Table A in S6). According to the position of EST-derived probes in the 4B deletion bin map, *QYr.nwafu-4BL* was located in 4BL5-0.86-1.00 (https://wheat.pw.usda.gov/cgibin/westsql/map_locus.cgi; Fig. 4e, Table B in S6).

Comparisons with other genes or QTL located on chromosome 4BL

QYr.nwafu-4BL and other reported stripe rust resistance genes or QTL on chromosome 4BL were compared in two integrated genetic maps. The first map consisted of SSR markers and the Wheat90 K SNP array (Maccaferri et al. 2015); the second map contained known PCR markers (SSR, EST, STS, RAPD and RFLP), DArT, SNPs from Wheat90, Wheat660 and Wheat820 SNP arrays (Cui et al. 2017; Fa, Cui, pers. comm.). All genes/QTL were placed in the integrated genetic map based on the locations of flanking markers. Most of them were concentrated on the interval 55.0–73.5 cM (total map length 149 cM), whereas *QYr.nwafu-4BL* spanned the interval 88.3–92.1 cM (Fig. 4f).

Evaluation of markers linked to *QYr.nwafu-4BL* in various wheat genotypes

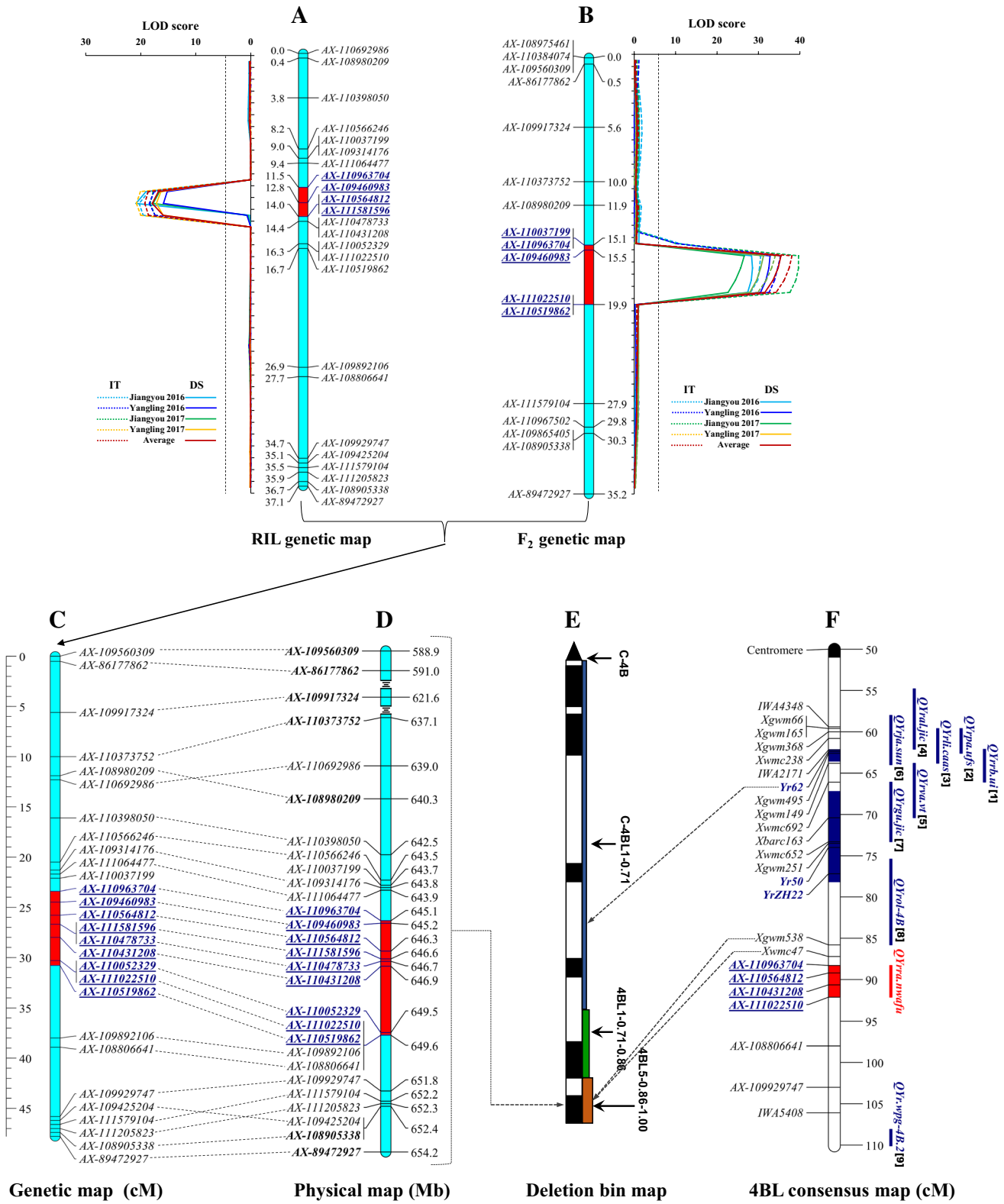
In order to evaluate the robustness of markers linked to *QYr.nwafu-4BL* and make comparisons with previous work 39 SNP markers covering a 17.1 Mb genomic region encompassing the *QYr.nwafu-4BL* locus were extracted to generate SNP genotypic data from 276 diverse wheat genotypes (Table S7). As shown in Fig. 5 and Table S7, the branch for the target region in line P10103 considerably differentiated from other branches for corresponding regions in lines without the target QTL or other genes on 4BL. The accessions within this branch displayed APR in field tests suggesting that they possibly contained the same locus. Pedigree analysis demonstrated that most of the accessions identical to P10103 were CIMMYT derivatives or associated with the CIMMYT breeding program (CIMMYT, 1983; http://wgb.cimmyt.org/gringlobal/search.aspx). All SNP alleles were located in the 637.1–654.2-Mb interval of the IWGSC chromosome 4B; their combinations differentiated the target group from other groups in the panel such as *AX-109460983* (G/A) and *AX-110519862* (G/A) or *AX-108980209* (C/T) and *AX-108905338* (A/G) (Table S7) indicating that these combinations of SNP markers could be used to detect *QYr.nwafu-4BL*.

Discussion

New *Pst* pathogenic groups such as race CYR34 (*Yr26*-virulent) that appeared from year 2008 are reported to be more aggressive and to have broader virulence profiles (Bai et al. 2017; Han et al. 2015; Liu et al. 2010). They are virulent to most previously named stripe rust resistance genes such as *Yr10* and *Yr26* and reduce the number of resistance sources available to wheat breeders. Expanding the number of resistance genes is ongoing work. In a previous study, wheat line P10103 was reported to confer a high level of APR in the field despite intermediate seedling responses to certain older races in greenhouse tests (Han et al. 2012). Here, resistance to stripe rust in two P10103 hybrid populations was characterized quantitatively and a major QTL and four consistently effective minor QTL were identified by ICIM analysis using genome-wide scanning of the M/P RIL population across multiple environments. Additionally, the potential QTL were identified by extreme pool-genotyping and the major stable QTL was also validated in a Z/P $F_{2:4}$ population.

Genome-wide scanning versus extreme pool-genotyping

The conventional approach to narrow QTL regions in common wheat is laborious and requires the development of DNA markers and the generation of a large number of advanced-generation progenies. The availability of next-generation sequencing (NGS) technologies and optimized reference genome sequences now available for wheat have improved the efficiency of genotyping and permit new strategies in QTL mapping for rapid marker discovery and map construction. We performed rapid QTL detection of stripe rust resistance using two SNP-based strategies. Genome-wide scanning QTL requires linkage maps with abundant markers, whereas QTL mapping by extreme pool-genotyping does not require assays for screening polymorphic markers or construction of linkage groups. However, the latter provides only an approximate position of a QTL, and additional QTL analysis is still needed for detailed mapping. SNP-based genetic maps were constructed using the Wheat55 K SNP array on the M/P RIL population and five stable QTL were detected across multiple environments. The potential QTL estimated by extreme pool-genotyping in RIL were basically the same, whereas only the major QTL on chromosome 4BL was apparent using $F_{2:3}$ extreme pools.



The different results from the RIL and $F_{2:4}$ pools were probably due to greater precision in genotyping of F_6 lines, QTL with minor effect and/or different genetic backgrounds.

Major QTL on chromosome 4BL

QYr.nwafu-4BL in P10103 with large effect was detected on chromosome arm 4BL. So far, there are only three *Yr*

◂**Fig. 4** The location of *QYr.nwafu-4BL* on wheat chromosome 4B. Genetic linkage map of *QYr.nwafu-4BL* on wheat chromosome arm 4BL produced from results of by using RILs (**a**) and $F_{2:4}$ lines (**b**). Markers surrounding the QTL were indicated with blue bold font and underline. **c** The consensus map of chromosome 4B was constructed by linkage maps from M/P and Z/P. **d** The physical map of wheat chromosome 4B according to the Chinese Spring IWGSC RefSeq v1.0 reference genome sequence was constructed using polymorphic markers from selected SNPs analyzed in KASP assays. **e** Deletion bin map of wheat chromosome long arm 4B. **f** Identified QTL (red bar with underlined font and red region on chromosome 4B) in this study and previously mapped *Pst* resistance genes and QTL (blue bars) were positioned based on integrated genetic maps (Maccaferri et al. 2015; Dr. Fa Cui, pers. comm.). Centromere region is colored black. Confidence intervals of QTL are indicated with blue lines. References [1] Chen et al. 2012; [2] Agenbag et al. 2012; [3] Lu et al. 2009; [4] Jagger et al. 2011; [5] Christopher et al. 2013; [6] Zwart et al. 2010; [7] Melichar et al. 2008; [8] Suenaga et al. 2003; [9] Naruoka et al. 2015

genes reported on chromosome 4B, namely *Yr50*, *Yr62* and *YrZH22* (Liu et al. 2013; Lu et al. 2014; Wang et al. 2017). *Yr50*, allegedly derived from *Thinopyrum intermedium* in Chinese winter wheat line CH223, confers all-stage resistance (Liu et al. 2013). *Yr62*, identified in Portuguese spring wheat germplasm PI 192252, confers high-temperature adult plant resistance (Lu et al. 2014). *YrZH22* was mapped in Chinese winter wheat cultivar Zhoumai 22 (pedigree: Zhoumai 11/Wenmai 6//Zhoumai 13) which has strong seedling and adult plant resistance to *Pst* races CYR32, CYR33and CYR34 (Wang et al. 2017). In our previous study, *Yr50* and *YrZH22* conferred all-stage resistance to all tested races (Zeng et al. 2014; Han et al. unpublished data); and *Yr62* conferred a high level of resistance at the adult plant stage (Zhou et al. 2015). When tested with *Pst* race CYR34 in the greenhouse P10103, its RILs with only the 4BL QTLand PI 192252 (*Yr62*) showed resistance at the adult plant stage; Zhoumai 22 (*YrZH22*) was consistently resistant; and CH223 (*Yr50*) was susceptible. *Yr50* was mapped in the SSR marker interval *Xbarc1096–Xwmc47* spanning 15.2 cM. *YrZH22* shared SSR marker *Xwmc47* with *Yr50* indicating they may be located in close proximity (Wang et al. 2017). *Yr62* was flanked by markers *Xgwm495* and *IWA2171*, in a 7-cM interval including additional markers *IWA99*, *IWA1923*, *Xgwm251* and *Xgwm192*. Lu et al. (2014) reported that *Yr62* was 27.1 ± 8.6 cM from *Yr50* and demonstrated that *Yr62* was located in bin 4BL1-0-0.71, whereas *Yr50* was likely located in bin 4BL5-0.86-1.00. *QYr.nwafu-4BL* is also located in 4BL5-0.86-1.00 based on the physical positions of ESTs in 4BL chromosomal bins. The closest markers linked to *Yr50*, *Yr62* and *YrZH22* were neither polymorphic in our mapping population nor shared common alleles of SNP markers flanking *QYr.nwafu-4BL* (Fig. 5). *QYr.nwafu-4BL* was at least 10 cM from *YrZH22* and *Yr50* in the integrated genetic map. Moreover, pedigree analyses revealed no relationship between P10103 and the other resistance sources.

Several stripe rust resistance QTL have been reported on chromosome arm 4BL (Bulli et al. 2016; Rosewarne et al. 2013). Most of them were located between SSR markers *Xgwm165* and *Xgwm251* (Fig. 4f) (Bulli et al. 2016; Maccaferri et al. 2015). Based on the integrated genetic map, *QYr.nwafu-4BL* is more than 20 cM distal to this region. Although the location of *QYroli-4B* is near to *QYr.nwafu-4BL*, this QTL was not stable in field tests of Israeli line Oligoculm during 1999–2001 and explained only 1.8–12.3% of the phenotypic variance (Suenaga et al. 2003). Another QTL, *QYr.wpg-4B.2* (*IWA5408*), detected with seeding IT data in US Pacific Northwest winter wheat accessions was located more than 15 cM from *QYr.nwafu-4BL*. Moreover, none of the flanking markers reported for these QTL showed polymorphism in our population. Thus, taking all factors into account it appears that *QYr.nwafu-4BL* is new and only further comparative genotyping and phenotyping in field tests will provide more clarity.

Other QTL in P10103

Other medium to minor effect QTL were identified in P10103. *QYr.nwafu-4AL* with flanking markers *AX-111100419* and *AX-89434097* was detected near the distal end of chromosome 4AL. *Yr60* is also in this region closely linked to SSR markers *Xwmc776*, *Xwmc313* and *Xwmc219* (Herrera-Foessel et al. 2015). Common wheat line Almop with *Yr60* (pedigree: Avocet*3//Lalbmonol*4/Pavon) displayed moderate resistance to stripe rust in field tests in Mexico. Based on the integrated map, *AX-89434097* and *Xwmc313* are in the same region. In addition, both P10103 and Almop are from CIMMYT implying that *QYr.nwafu-4AL* could be *Yr60*. *QYr.nwafu-5AL* was in the 9.7-cM interval *AX-111654317–AX-109832565*. QTL *QYrpas.cim-5AL* identified in Pastor was in the overlapping region of the integrated map. Pastor is a CIMMYT breeding line containing several minor stripe rust and leaf rust APR QTL (Rosewarne et al. 2012). Therefore, *QYr.nwafu-5AL* and *QYrpas.cim-5AL* may be the same. Several QTL were previously reported on chromosome 5BL (Bulli et al. 2016); *QYr.nwafu-5BL* was mapped close to the terminal of 5BL. All QTL were at least 15 cM far from *QYr.nwafu-5BL* according to the integrated map (Maccaferri et al. 2015; Fa Cui, pers. comm.). *QYr.nwafu-5BL* should be a different QTL for resistance to stripe rust. *QYr.nwafu-6AL* was in the SNP marker interval between *AX-110516770* and *AX-109575335* harboring *QYr.caas-6AL* (Maccaferri et al. 2015). However, *QYr.caas-6AL* is unstable (Liu et al. 2015). Although we identified several QTL in the present study, further studies are required for concise location and confirmation of relationships with previously reported genes/QTL.

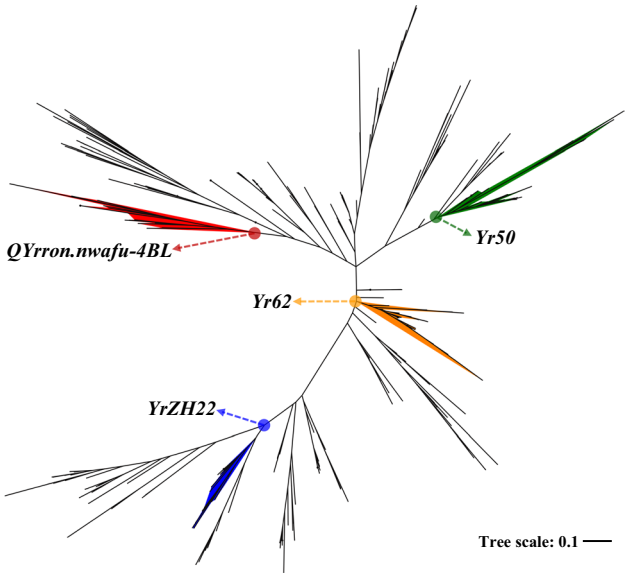


Fig. 5 Haplotype variation of the *QYr.nwafu-4BL* region among wheat genotypes revealed by SNPs distributed in the target region. Phylogenetic tree constructed with SNPs embracing *QYr.nwafu-4BL* region. Details of results based on Wheat660 K SNP markers are in Supplementary Table S7

Effectiveness of QTL and QTL stacking for MAS in wheat breeding

CIMMYT has made significant progress in disease resistance breeding (Guzmán et al. 2017). The Global Wheat Breeding Program was initiated in year 1944, and many wheat lines were introduced to China from Mexico during the following decades. *QYr.nwafu-4BL* from a CIMMYT-derived wheat line was stably expressed in two segregating populations (M/P and Z/P) and consistently accounted for 30–50% of the phenotypic variation in stripe rust response. It can be used in breeding for stripe rust resistance, especially in combination with other QTL in order to obtain a higher level of resistance and increased durability. Marker-assisted QTL stacking provides a targeted approach to detect and track gene/QTL in modern breeding programs. Increased selection efficiency due to accurate MAS can accelerate total genetic gain per year (Xu et al. 2017). The efficacy of a marker is dependent on its closeness to the gene/QTL underlying the target trait and the stability of expression of the gene in different genetic backgrounds. *QYr.nwafu-4BL* was surrounded by several SNP markers spanning an interval of less than 5 cM and was detected simultaneously in different populations. These closely linked markers should be useful for MAS. Further characterization of haplotypes associated with *QYr.nwafu-4BL* by SNP-based haplotype analysis will permit development of robust, breeder-friendly KASP markers.

Author contribution statement JH Wu conducted the experiments, analyzed the data, and wrote the manuscript. S Huang, QD Zeng and SZ Yu assisted in analyzing the data and preparing figures for the manuscript. QL Wang and DJ Han identified the resistant parental line, made the cross and participated in field experiments. SJ Liu, S Huang and JM Mu participated in field experiments and contributed to genotyping. DJ Han and ZS Kang conceived and directed the project and revised the manuscript.

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Compliance with ethical standards

Conflicts of interest The authors declare no competing interests.

Ethical standard I declare on behalf of my co-authors that the work described is original, previously unpublished research, and not under consideration for publication elsewhere. The experiments in this study comply with the current laws of China.

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