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ORIGINAL ARTICLE

Rapid identification of an adult plant stripe rust resistance gene in hexaploid wheat by high-throughput SNP array genotyping of pooled extremes

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Abstract

Key message **High-throughput SNP array analysis of pooled extreme phenotypes in a segregating population by KASP marker genotyping permitted rapid, cost-effective location of a stripe rust resistance QTL in wheat.**

Abstract German wheat cultivar "Friedrichswerther" has exhibited high levels of adult plant resistance (APR) to stripe rust in field environments for many years. $F_{2:3}$ lines and F_6 recombinant inbred line (RILs) populations derived from a cross between Friedrichswerther and susceptible landrace Mingxian 169 were evaluated in the field in 2013, 2016 and 2017. Illumina 90K iSelect SNP arrays were used to genotype bulked extreme pools and parents; 286 of 1135 polymorphic SNPs were identified on chromosome 6B. Kompetitive Allele-Specific PCR (KASP) markers were used to verify the chromosome region associated with the resistance locus. A linkage map was constructed with 18 KASP-SNP markers, and a major effect QTL was identified within a 1.4 cM interval flanked by KASP markers *IWB71602* and *IWB55937* in the region 6BL3-0-0.36. The QTL, named *QYr.nwafu-6BL*, was stable across environments, and explained average 54.4 and 47.8% of the total phenotypic variation in $F_{2:3}$ lines and F_6 RILs, respectively. On the basis of marker genotypes, pedigree analysis and relative genetic distance *QYr.nwafu-6BL* is likely to be a new APR QTL. Combined high-throughput SNP array genotyping of pooled extremes and validation by KASP assays lowers sequencing costs compared to genome-wide association studies with SNP arrays, and more importantly, permits rapid isolation of major effect QTL in hexaploid wheat as well as improving accuracy of mapping in the QTL region. *QYr.nwafu-6BL* with flanking KASP markers developed and verified in a subset of 236 diverse lines can be used in marker-assisted selection to improve stripe rust resistance in breeding programs.

Introduction

Stripe rust or yellow rust (YR) caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) leads to reduced yield and grain quality in common wheat (*Triticum aestivum* L.) (Hovmøller et al. 2010; McIntosh et al. 1995). Although chemical control can be effective there are long-term environmental concerns regarding the use of fungicides (Chen 2014). The optimal strategy to control wheat rusts is resistant cultivars, a major focus of many breeding programs worldwide (Chen 2005; Wellings 2011).

Approximately 80 *Yr* genes have been permanently designated (McIntosh et al. 2016, 2017). However, most of these *Yr* genes are all-stage (ASR) or race-specific resistances that are currently ineffective against prevalent races in China and elsewhere (Han et al. 2015; Sharma-Poudyal et al. 2013). Only a few named ASR genes such as *Yr5*, *Yr15*, *Yr53*,

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Yr61, *Yr65* and *Yr69* are still widely effective and these can be used in breeding programs (Hou et al. 2016; Wu et al. 2017b; Xu et al. 2013; Zeng et al. 2015; Zhou et al. 2014). Increasing emphasis is also being placed on genes that confer partial resistance at post-seedling growth stages and are effective against a broad spectrum of *Pst* races (Brown 2015; Niks et al. 2015). Historically, some adult plant resistance (APR) or high-temperature adult plant resistance (HTAPR) genes have remained effective for long time periods suggesting that at least some sources of APR or HTAPR may be multi-genic and non-race specific (Chen 2013). Over the last decade, more than 200 quantitative trait loci (QTL) for stripe rust resistance have been identified and allocated to 49 chromosomal regions on 20 of the 21 wheat chromosomes (McIntosh et al. 2016, 2017; Rosewarne et al. 2013). Several APR/HTAPR genes or QTL, such as *Yr18*, *Yr30*, *Yr32*, *Yr36*, *Yr39*, *Yr46*, *Yr52*, *Yr54*, *Yr59* and *Yr62*, have contributed to major portions of phenotypic variation and have remained effective in China (Basnet et al. 2014b; Lu et al. 2014; Wu et al. 2016; Zeng et al. 2015; Zhou et al. 2015). Three of these APR/HTAP genes, *Yr18*, *Yr36* and *Yr46*, have been cloned and shown to have different mechanisms of action form the NBS-LRR proteins that characterize many race-specific disease and pest resistance genes (Fu et al. 2009; Krattinger et al. 2009; Moore et al. 2015). Although the levels of protection conferred by APR/HTAPR genes individually may not be sufficient for protection against loss, combining such genes usually improves the level and stability of protection. The availability of molecular markers greatly assists the combining process in breeding programs (Chen 2013; Ellis et al. 2014) although similar results can be achieved in well-planned disease nurseries due to the additive nature of such genes (Singh et al. 2011).

It is an unavoidable reality that the ~17 Gb genome size in hexaploid wheat with extensive stretches of repetitive DNA (> 80%) have resulted in lack of robust, tightly linked markers for many genes or QTLs for use in breeding programs (Wang et al. 2015). Huge advances in genome sequencing, including the recently released *T. aestivum* cv. Chinese Spring and *T. turgidum* ssp. *dicoccoides* cv. Zavitan genome sequences (IWGSC RefSeq v1.0, http://www.wheatgenome.org/; WEWSeq v1.0, Avni et al. 2017) have opened many opportunities for gene location and marker development. Single nucleotide polymorphisms (SNP) are now the preferred state-of-art marker type as they are highly abundant, lower cost, locus-specific and highly polymorphic compared to previous molecular marker systems (Varshney et al. 2014; Yang et al. 2015). Several wheat SNP arrays such as 9K (Cavanagh et al. 2013) and 90K (Wang et al. 2014) arrays based on BeadArray™ technology from Illumina (http://www.illumina.com) have been developed and are widely used in identifying markers closely linked to agronomic traits. These SNP platforms have accelerated genome-wide association studies (GWAS) and QTL mapping of resistance to rusts (Gao et al. 2016; Hou et al. 2015; Liu et al. 2015, 2017; Maccaferri et al. 2015). Sometimes it is a worthwhile option to genotype all lines, particularly for permanent populations such as recombinant inbred lines (RILs) or double haploid lines (DH) to map multiple traits or multiple QTL for a single trait. However, it is not cost-efficient or sensible to genotype all individuals in large temporary segregating populations with SNP arrays. Moreover, crop genetic improvement and breeding often demands only small to moderate numbers of SNPs linked to key agronomic trait loci because highly multiplexed platforms have relatively high per-sample costs. For these reasons, chip-based platforms may be less suitable for applications requiring low to medium marker densities for a large number of samples. In contrast, Kompetitive Allele Specific PCR (KASP™, http://www.Lgcgenomics.com) is a flexible and now widely used technology (Rasheed et al. 2016, 2017; Semagn et al. 2014).

Bulked segregant analysis (BSA) (Michelmore et al. 1991) involving selected and pooled DNA samples from contrasting sets of phenotypes provides a simple and rapid approach to search for markers linked to specific genomic regions conferring a trait of interest. Combining the BSA strategy with high-throughput next-generation sequencing technology is a common practice for gene identification and QTL mapping (Zou et al. 2016). Many studies have outlined methodologies and applications of high-throughput sequencing in BSA for qualitative and quantitative traits (Abe et al. 2012; Ramirez-Gonzalez et al. 2015a; Takagi et al. 2013; Wu et al. 2017a).

More than 1000 common wheat accessions were screened for resistance to stripe rust under controlled greenhouse conditions and in field nurseries at Yangling in Shaanxi province and at Tianshui in Gansu since 2008 and many lines with effective resistance to prevalent Chinese *Pst* races were identified (Han et al. 2010, 2012). Wheat cultivar Friedrichswerther, named after a breeding station in Germany, was accessioned in the Gatersleben GenBank in 1964 (http://genbank.vurv.cz/wheat/pedigree/pedigree.asp). Although susceptible in seedling tests this line displays high levels of APR to current Chinese *Pst* races and little is known about these genetic basis of resistance. The objectives of this study were to identify and map a genomic region containing an APR QTL using SNP arrays following BSA, and to develop and verify KASP markers for marker-assisted selection in breeding programs.

Materials and methods

Population development

Friedrichswerther (FRIED) was crossed with susceptible winter wheat landrace Mingxian 169, and 177 F_2 plants were

produced from a single F_1 plant. One hundred and fifty-eight $F_{2:3}$ lines were obtained from 177 F_2 plants in 2012 and 150 F_5-derived F_6 recombinant inbred lines (RILs) were obtained from the 158 $F_{2:3}$ lines in 2015 through single-seed descent. Mingxian 169 (MX169) and Xiaoyan 22 (XY22) were used as susceptible controls throughout this study. Two hundred and thirty-six wheat cultivars and landraces were used to evaluate polymorphisms of molecular markers flanking the resistance locus. They included 54 leading cultivars, 68 advanced lines from major winter wheat areas in China (Zeng et al. 2014), 28 landraces and 85 foreign germplasms with resistance to stripe rust (Han et al. 2012).

Greenhouse trials

Seedling and adult plant tests were conducted under controlled greenhouse conditions to characterize the adult plant resistance of FRIED. Seven *Pst* races (CYR29, CYR31, CYR32, CYR33, CYR34, Su11-7 and V26/CH42) were used. Their virulence/avirulence characteristics were previously reported by Wu et al. (2016). For seedling tests 10–15 plants of MX169 and FRIED were grown in 9 × 9 × 9 cm pots, and for adult-plant tests three plants were grown in larger 20 × 20 × 15 cm pots. Seedlings at the two-leaf stage (14 days after planting) and adult-plants at the booting stage were separately inoculated with urediniospores of each race mixed with talc (approximately 1:20). Inoculated plants were incubated at 10 °C in a dew chamber in darkness for 24 h, and then transferred to a greenhouse at 17 ± 2 °C with 14 h of light (22,000 lx) daily. 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. To confirm and clarify ITs of the entries, the tests were repeated three times.

Disease assessments in the field

The 158 $F_{2:3}$ lines and parents were evaluated for APR to stripe rust in Shaanxi and Gansu provinces during 2012–2013; the 150 RIL population and parents were evaluated at three field sites: Yangling in Shaanxi province during 2015–2016 and 2016–2017, Jiangyou in Sichuan province during 2015–2016 and 2016–2017, and Tianshui in Gansu province during 2015–2016. An individual trial at each site in each year was considered a single environment. Tianshui and Jiangyou are hotspot regions for natural stripe rust development on a regular basis. The Yangling nursery was inoculated with urediniospores of prevalent race CYR32 suspended in liquid paraffin (1:300) sprayed onto MX169 at flag leaf emergence. All RIL trials were arranged in randomized complete blocks with three replicates, whereas the $F_{2:3}$ line trials had two replicates. An individual plot consisted of a single 1 m row with 30 cm between adjacent rows. Each plot was sown with approximately 30 seeds. The parents and susceptible variety XY22 were planted every 20 rows throughout the field. The susceptible control MX169 was planted in 5-row blocks after every 50 rows and around the field area. XY22 and MX169 served as inoculum spreaders to ensure uniform disease development throughout the field. Infection types (IT) and disease severities (DS) were recorded three times, when MX169 had 60–100% and XY22 had 30– > 90% severity during the period April 1–20 at Jiangyou, May 1–25 at Yangling, and May 20–June 10 at Tianshui. Disease severity was assessed visually using percentage diseased leaf area 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. Disease severities for each line were converted to area under the disease progress curve (AUDPC) values (Chen and Line 1995). Relative AUDPC (rAUDPC) values were calculated for each line and parent as a percentage of the mean AUDPC value of the susceptible parent MX169 (Lin and Chen 2007). Both the rAUDPC and IT data were used in QTL mapping.

Statistical analysis

Mean IT and rAUDPC of each $F_{2:3}$ line and F_6 RIL were used in analyses of variance (ANOVA). Analyses of variance and Pearson's correlation coefficients were performed with AOV functionality in the QTL IciMapping V 4.1 software package (http://www.isbreeding.net/). Broad-sense heritability (h_b^2) of stripe rust resistance was calculated as $h_b^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{ge}^2/e + \sigma_\varepsilon^2/re)$ (Allard 1960), where σ_g^2 is $(MS_f - MS_{fe})/re$, σ_{ge}^2 is $(MS_{fe} - MS_e)/r$ and σ_ε^2 is MS_e; σ_g^2 = genetic variance, σ_{ge}^2 = genotype × environment interaction variance, σ_ε^2 = 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.

DNA sample preparation and bulk segregant analysis

DNA samples were extracted from fresh leaves of parents, F_2 plants and the F_5 population used to produce the F_6 lines at the jointing stage in the field (following Song et al. 1994). BSA was performed to identify markers polymorphic between the resistant and susceptible parents, and between the resistant (R-bulk) and susceptible (S-bulk) DNA bulks. Based on the stripe rust response phenotypes of the $F_{2:3}$ lines and F_6 RILs in all environments, F_2 and F_6 DNA bulks of eight homozygous resistant (IT 1–2, DS ≤ 10) and eight

homozygous susceptible (IT 8–9, DS ≥ 90) were constituted as resistant and susceptible bulks, respectively. The F_2 bulks and parents were genotyped with the 90K SNP arrays from CapitalBio Corporation (Beijing; http://www.capitalbio.com). SNP genotype calling and clustering was processed with the polyploid version of GenomeStudio software (Illumina, http://www.illumina.com). SNP filtering criteria were followed: monomorphic SNP and those showing call rates less than 85%, ambiguity in calling, and minor allele frequencies below 5% were removed from the dataset. The polymorphic SNPs associated with resistance in BSA contained homozygous and heterozygous genotypes. Only homozygous genotype differences were localized to chromosomes based on the high-density 90K map (Wang et al. 2014) and were calculated as number of SNPs per centiMorgen.

KASP marker assays and genotyping

Following chromosome location based on polymorphic SNPs, those SNPs in the likely target region were selected for conversion to KASP markers following PolyMarker (Ramirez-Gonzalez et al. 2015b). The specific-chromosome KASP markers were used to screen the parents and bulks to confirm polymorphism before genotyping the entire population. KASP assays were performed in 384-well plate format following the protocol of LGC Genomics. Reaction mixtures consisted of final volumes of 5 µL containing 2.5 µL of genomic DNA (50–100 ng), 2.5 µL of 2 × KASP master mix (V4.0, LGC Genomics), 0.014 µL of primer mix (12 µM of each allele-specific primer and 30 µM of common primer). A Veriti 384 well Thermal Cycler (Applied Biosystems) was used with cycling conditions of denaturation at 95 °C for 15 min, nine cycles of 95 °C for 20 s, touchdown starting at 65 °C for 60 s (decreasing by 0.8 °C per cycle), and followed by 30–40 cycles of amplification (95 °C for 20 s; 57 °C for 60 s). End-point fluorescence data were visualized with a microplate reader (FLUOstar Omega, BMG LABTECH, Germany) and analyzed using Klustering Caller software (LGC, Middlesex, UK).

Genetic linkage map and QTL analysis

Chi-squared (χ^2) tests for goodness of fit were performed to determine the deviations of observed segregation ratios from theoretically expected ratios. Linkage between markers was established using JoinMap version 4.0 software and a logarithm of odds (LOD) score threshold of 3.0 (Van Ooijen 2006). Recombination fractions were converted to centiMorgans (cM) using the Kosambi function (Kosambi 1943). The linkage map was graphically visualized with Mapchart V2.3 (Voorrips 2002). Chromosome bin assignment of the resistance locus in a linkage group was based on previously published deletion maps (Sourdille et al. 2004) and 90K integrated wheat maps (Maccaferri et al. 2015).

Quantitative trait loci (QTL) mapping was conducted by inclusive composite interval mapping of additive functionality (ICIM-ADD) in the QTL IciMapping V 4.1 software package (Meng et al. 2015; Wang 2009). A walk speed of 1.0 cM with a stepwise regression probability of 0.001 was chosen for QTL detection. Combining the calculated value by 1000 permutations at a probability of 0.01 with the formula of Sun et al. (2013), the LOD score to determine significant QTL was 2.6–5.0 in all five environments, thus a LOD threshold of 5.0 was set to declare a significant QTL. In addition, single marker analysis (SMA) functionality was also used to detect LOD scores for each marker. The mean IT and rAUDPC score for each $F_{2:3}$ line and each F_6 RIL at each field site were used in the respective QTL analyses. The phenotypic variances explained (*PVE*) by individual QTL were also obtained using ICIM.

Genome reference and gene annotation

To obtain physical positions of polymorphic SNP markers the SNP probes were aligned with respect to the newly released Chinese Spring sequence through a BLAST search (Reference Sequence v1.0, the International Wheat Genome Consortium (IWGSC), http://www.wheatgenome.org/). The SNP probes were also blasted to the Zavitan genome sequences (the International Wild Emmer Wheat Genome Sequencing Consortium, WEWseq). More recently, IWGSC RefSeq v1.0 and WEWSeq v1.0 with annotation of genes became available on websites https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations and http://wewseq.wixsite.com/consortium, respectively. Annotated genes in the target region were extracted for analyzing the genes involved in plant disease resistance.

Results

Phenotypic evaluation

In greenhouse experiments with *Pst* races CYR29, CYR31, CYR32, CYR33, CYR34, Su11-7 and V26/CH42, FRIED was susceptible (IT 8–9) in seedling tests but highly resistant (IT 1–2) in adult-plant stage tests (Fig. S1). The susceptible parent MX169 was susceptible (IT 8–9) at both growth stages. In field tests, stripe rust developed to adequate levels for scoring high quality phenotypic data. In all experiments MX169 was susceptible (IT 9, DS ≥ 90) at the second or third scoring dates, and FRIED was resistant (IT 1–2, DS ≤ 10). The mean rAUDPC values of FRIED ranged from 4.4 to 10.6%, whereas MX169 had mean rAUDPC values of 90–100% in all trials (Fig. 1b, d, f; Table S1A,

B). Both IT and rAUDPC data for the $F_{2:3}$ lines and F_6 RIL population showed continuous distributions, indicating that APR resistance in FRIED was quantitatively inherited (Fig. 1). The broad-sense heritability values based on all data sets for rAUDPC and IT of $F_{2:3}$ lines were 0.90 and 0.88 (Table 1), with correlation coefficients of 0.89 and 0.93 ($P < 0.001$) (Table 2), respectively. Correlation coefficients, ranging from 0.80 to 0.92, for either rAUDPC or IT of the F_6 RILs among the five field environments were all significant ($P < 0.001$) (Table 2), and heritabilities were 0.90 and 0.92, respectively (Table 1). The ANOVA showed significant phenotypic variation in both rAUDPC and IT among lines, environments and line × environment interactions in the field experiments (Table 1); no significant variation was detected among replications within experiments; lines were the main significant sources of phenotypic variation based on the high heritability. These results suggested that the expression of APR was consistent across environments and QTL controlling APR had a very large effect on reducing stripe rust severity.

Mapping QTL for stripe rust resistance with $F_{2:3}$ lines and F_6 RIL data

A total of 1135 SNPs showed polymorphisms between the DNA bulks after genotyping by the 90K SNP array; 286 SNPs were located on chromosome 6B and the others were distributed across other chromosomes (Fig. 2a). Moreover, the proportion of SNPs overlapping between bulks and parents was highest for chromosome 6B (Fig. 2a). The numbers of markers on chromosomes 2B and 7B were also higher. These results indicated that SNPs in 6B were extremely likely to be associated with the major resistance locus and there may be minor QTL on 2B and 7B. Most of the linked

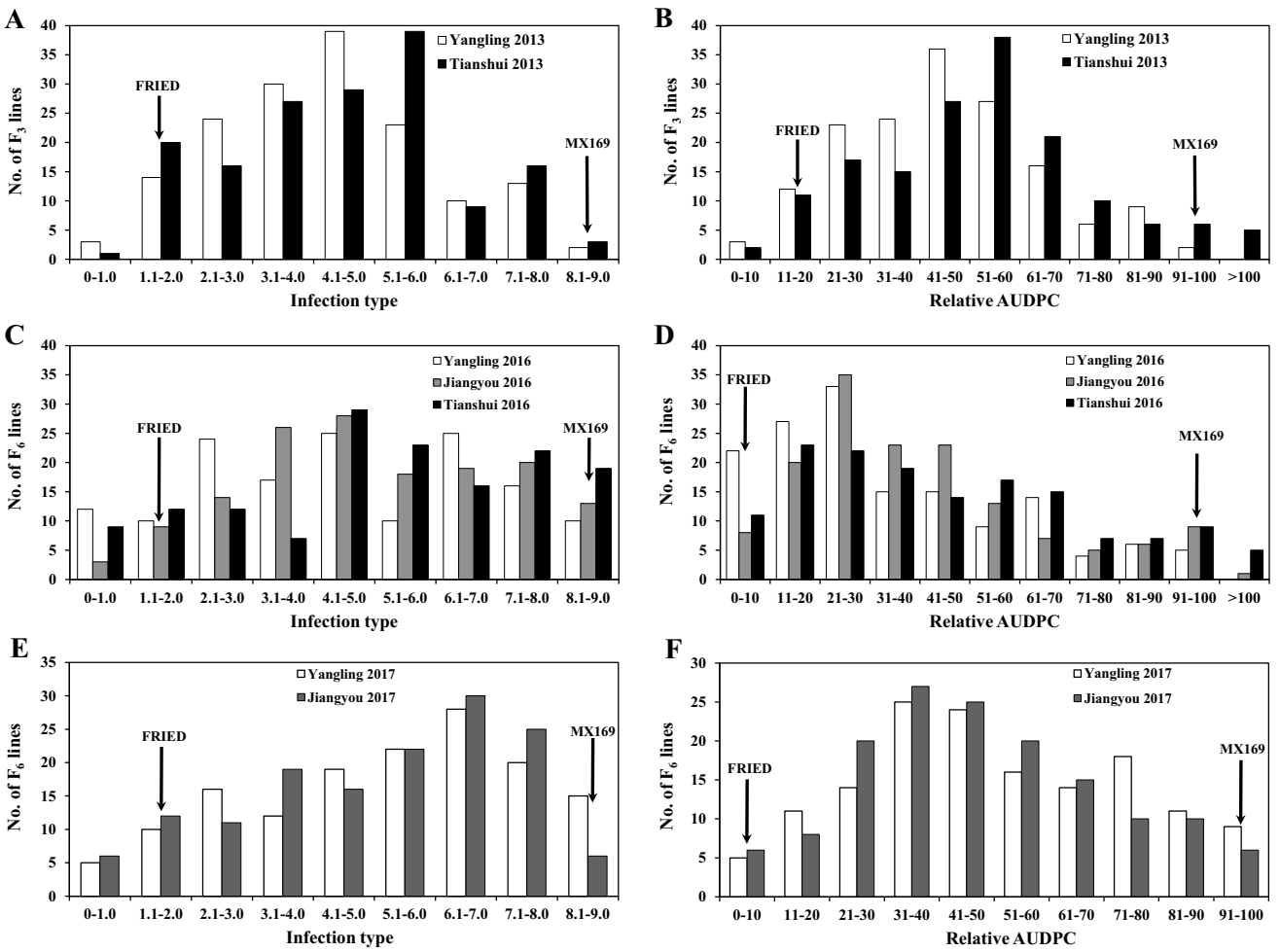

Fig. 1 Phenotypes of MX169, FRIED and their progenies across all environments. Frequency distribution of mean infection types (IT) and relative area under the disease progress curve (rAUDPC) for 158 $F_{2:3}$ lines at Yangling and Tianshui in 2013 (**a**, **b**). Frequency distribution of mean IT and rAUDPC for 150 F_6 RILs at Yangling, Jiangyou and Tianshui in 2016 and 2017 (**c**–**f**). Black arrows indicate values of the parental lines

Table 1 Analysis of variance and estimates of broad-sense heritability (h_b^2) of relative area under the disease progress curve (rAUDPC) and infection type (IT) scores of $F_{2:3}$ lines and an F_6 recombinant inbred lines (RIL) population derived from MX169 × FRIED

Source of variation	rAUDPC			IT		
	df	Mean square	*F* value	*df*	Mean square	*F* value
$F_{2:3}$ lines	157	1551.70	30.66*	157	10.79	18.30*
Replicates/environment	1	55.89	2.21	1	0.04	0.11
Environments	1	2583.20	51.04*	1	19.27	32.69*
Line × environment	156	164.70	3.25*	156	1.41	2.39*
Error	313	50.61		313	0.59	
h_b^2	0.90			0.88		
F_6 RILs	149	10,703.52	167.77*	149	59.11	91.38*
Replicates/environment	2	148.82	2.33	2	0.64	0.98
Environments	4	8125.72	127.36*	4	40.38	62.42*
Line × environment	594	288.37	4.52*	594	2.28	3.51*
Error	1483	63.80		1483	0.65	
h_b^2	0.90			0.92		

*Significant at $P = 0.001$

Table 2 Correlation coefficients (*r*) of mean relative area under the disease progress curve (rAUDPC) and infection type (IT) of the FRIED × MX169-derived $F_{2:3}$ lines across two environments and F_6 recombinant inbred lines tested in five environments

Environment (location, year)	Yangling 2013	Tianshui 2013	Yangling 2016	Tianshui 2016	Jiangyou 2016	Yangling 2017	Jiangyou 2017
Yangling 2013	1						
Tianshui 2013	0.89 (0.93)[a]	1					
Yangling 2016	–	–	1				
Tianshui 2016	–	–	0.85 (0.81)	1			
Jiangyou 2016	–	–	0.90 (0.81)	0.87 (0.82)	1		
Yangling 2017	–	–	0.90 (0.81)	0.87 (0.80)	0.89 (0.92)	1	
Jiangyou 2017	–	–	0.90 (0.87)	0.87 (0.81)	0.88 (0.89)	0.92 (0.86)	1

[a] *r* values based on IT data are given in parentheses. All *r* values were significant at $P = 0.001$

SNPs on 6B were within the interval 57.0–72.1 cM (total map length 127.5 cM) in the 90K SNP map (Wang et al. 2014) (Fig. 2b). Forty-nine chromosome-specific SNPs selected for conversion to KASP markers were screened in the parents and bulks to confirm their polymorphisms before genotyping the entire population; 31 of 49 markers failed to distinguish the parents and bulks. The sequences of the polymorphic KASP markers are listed in Supplementary Table S2.

A genetic map constructed using the 18 KASP markers and data from the 150 F_6 RILs spanned 33.1 cM (Table S3A). Using the genetic linkage map and mean rAUDPC and IT data from each environment a major effect QTL, *QYr.nwafu*-6BL, was mapped to chromosome 6B by ICIM. The peak of the QTL was located in a 1.4 cM interval spanned by KASP markers *IWB71602* (Tdurum_contig45370_324) and *IWB55937* (RAC875_c26487_118) (Fig. 2d). A second genetic map constructed for the $F_{2:3}$ population using the same markers was quite similar to that for the F_6 population (Fig. 3b; Table S3B). According to previously published deletion maps of Sourdille et al. (2004) and the integrated genetic map in Maccaferri et al. (2015) the QTL was located in bin 6BL3-0-0.36 (Fig. 2e). The resistance conferred by *QYr.nwafu-6BL* was stable across environments and explained an average 54.4 and 47.8% of the phenotypic variation in rAUDPC for the $F_{2:3}$ lines and F_6 RILs, respectively, and an average 34.5 and 35.3% of the phenotypic variation in IT for $F_{2:3}$ lines and F_6 RILs, respectively (Table 3). Genotypic data for F_2 and F_6 populations from the KASP assays are provided in Fig. S2A, B, C, D and E and in Table S4.

Comparisons with reported resistance genes/QTLs in chromosome 6B

SSR markers linked with previously reported genes/QTLs in chromosome 6B were evaluated on MX169, FRIED and wheat lines with the corresponding QTL to determine if

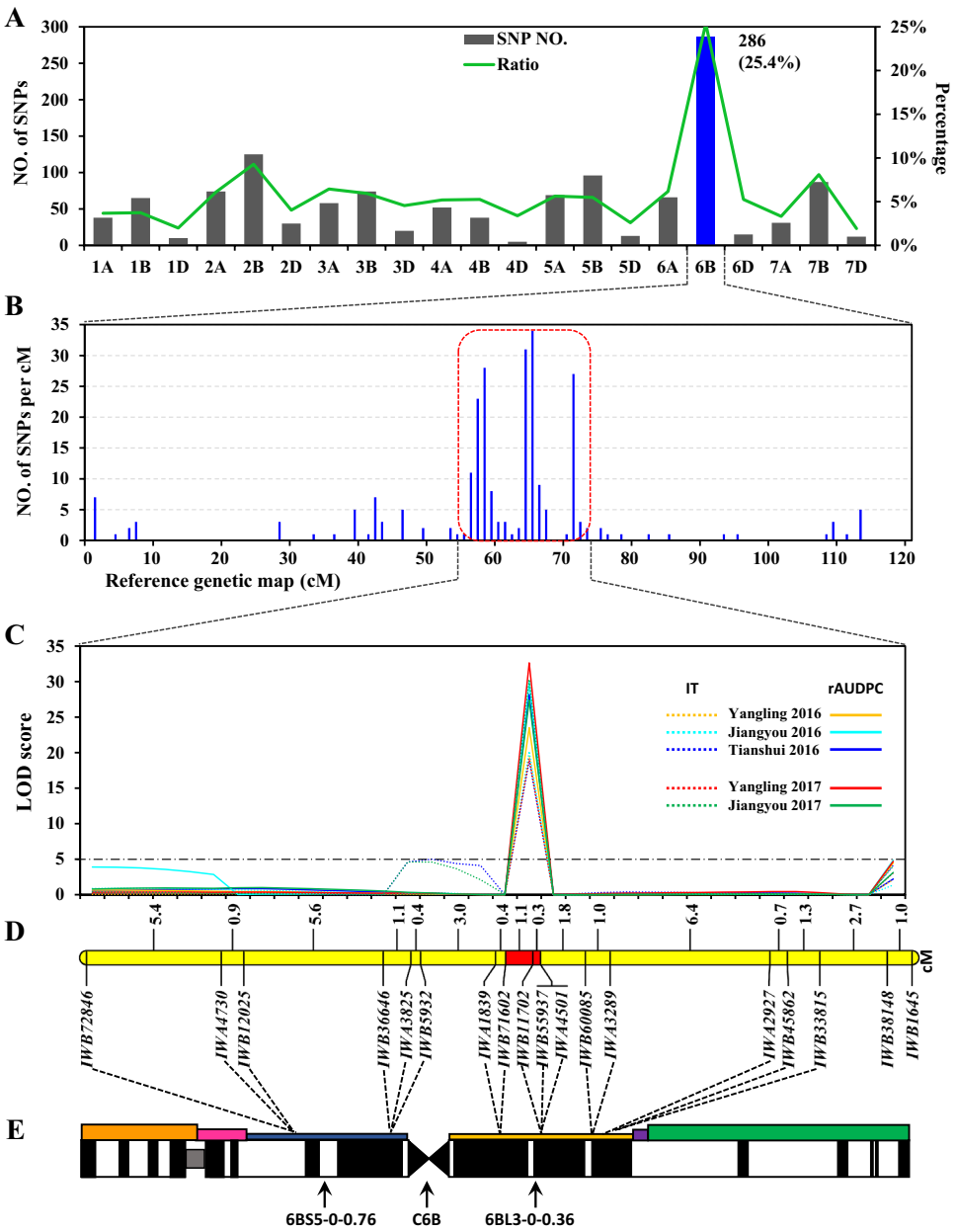

Fig. 2 Overview of analyses. **a** Distribution of polymorphic SNPs in each chromosome identified by the 90K SNP array and corresponding percentages. **b** Positions of SNPs in chromosome 6B based on the 90K SNP map (Wang et al. 2014). **c** Selected SNPs in red dotted boxes were subjected to KASP assays. **d** Genetic linkage map of stripe rust resistance locus *QYr.nwafu-6BL* on wheat chromosome arm 6BL based on the F_6 RIL data. **e** Deletion bin map of *QYr.nwafu-6BL* (color figure online)

QYr.nwafu-6BL was distinct (Table 4). All SSR markers except for *Xgwm136* and *Xbarc136* had alleles that were different from those in FRIED. Additionally, KASP markers linked to *QYr.nwafu-6BL* were assayed on wheat lines Druchamp, LM168a, RSL65 (*Yr36*), Stephens, Pavon 76, Pingyuan 50 and VPM1 with previously described genes/QTL on chromosome 6B. These lines matched one of the six KASP markers in FRIED, but the KASP alleles at *IWB71602*, *IWB55937*, *IWA4501* and *IWB60085* in FRIED were unique (Table 5, Fig. S2G, H, I). Hence, there was no clear evidence of resistance gene/QTL commonality in the molecular detections.

FRIED and its F_6 lines with/without the 6BL QTL marker alleles were compared with wheat lines Druchamp, LM168a, RSL65 (*Yr36*), Stephens, Pavon 76, Pingyuan 50 and VPM1 using *Pst* race CYR34 in the greenhouse. As shown in Fig. 4, Fig. S1 and Table 5, FRIED was susceptible to race CYR34 in the seedling stage, whereas FRIED and its F_6 lines with the 6BL QTL marker alleles conferred high resistance (DS = 5–20%) at the adult plant stage; lines with reported 6B genes/QTL displayed moderate resistance to moderate susceptibility (DS = 40–80%); F_6 lines without the 6BL QTL marker alleles and susceptible checks were highly susceptible (DS = 80–100%).

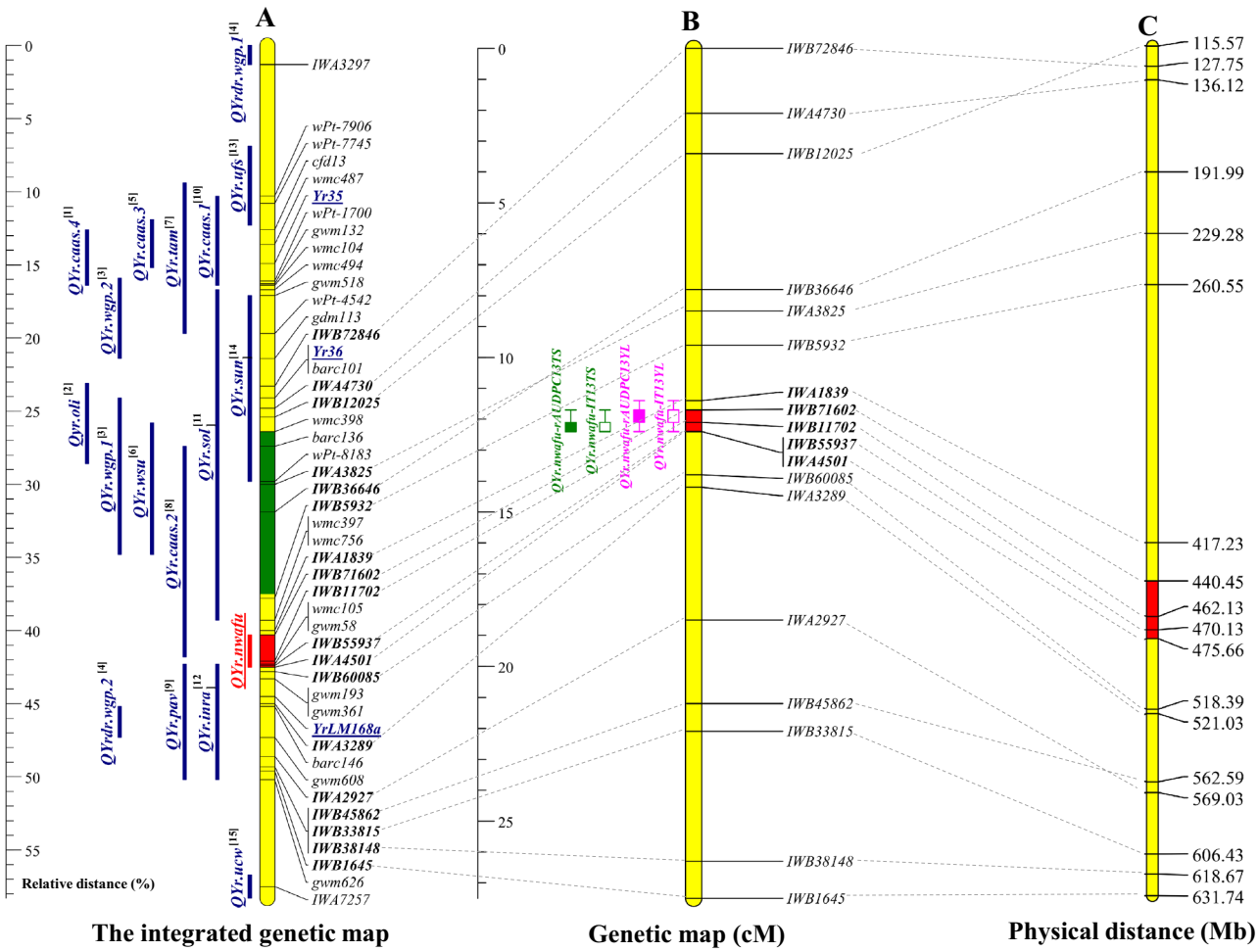

Fig. 3 The location of *QYr.nwafu-6BL* on wheat chromosome 6B. **a** Identified QTL (red bar with underlined font and red region on chromosome 6B) in this study and previously mapped *Pst* resistance genes and QTLs (blue bars) were positioned based on integrated genetic maps in Maccaferri et al. (2015). Chromosome length was standardized to relative length. Centromere region is colored green. Confidence intervals of QTLs are indicated with blue lines. References [1]Ren et al. (2012b), [2]Suenaga et al. (2003), [3]Santra et al. (2008), Dong et al. (2017), [4]Hou et al. (2015), [5]Bai et al. (2012), [6]Bulli et al. (2016), [7]Basnet et al. (2014a), [8]Lan et al. (2010), [9]William et al. (2006), [10]Ren et al. (2012a), [11]Christiansen et al. (2006), [12]Dedryver et al. (2009), [13]Prins et al. (2011), [14]Bariana et al. (2010); [15]Maccaferri et al. (2015). **b** Genetic linkage map of wheat chromosome 6B. The QTL region was identified by QTL mapping using phenotypic and marker data from $F_{2:3}$ lines. **c** The physical map of wheat chromosome 6B according to the Chinese Spring IWGSC RefSeq v1.0 sequence (color figure online)

KASP marker validation for marker-assisted selection

A subset of 236 diverse wheat genotypes (Table S5) was used to assess the robustness of KASP markers linked to *QYr.nwafu-6BL* for marker-assisted selection. Markers *IWA1839* and *IWB11702* failed to distinguish resistant and susceptible lines and their use resulted in a high level of misclassification (Fig. S2F). Markers *IWB71602*, *IWB55937* and *IWA4501* amplified 5, 2 and 13 false positives among susceptible cultivars, respectively (Fig. S2G, H). In addition, the allele at *IWB60085* associated with resistance was present in 7 susceptible genotypes and absent in 229 genotypes (Fig. S2I). However, no wheat line had both flanking SNP markers, indicating that the combination of the two closest markers (*IWB71602* and *IWA4501/IWA55937*) can be used for marker-assisted selection of *QYr.nwafu-6BL.* Furthermore, this combination of KASP also differentiated *QYr. nwafu-6BL* in FRIED from other genes on 6B (Table 5).

Physical map and candidate genes analysis

The QTL underlying resistance to stripe rust was initially mapped between KASP markers *IWB71602* and *IWB55937* representing a genetic distance of 1.4 cM corresponding to a physical interval of 35.2 Mb in the Chinese Spring RefSeq v1.0 sequence (Fig. 3c). According to information on gene annotation (IWGSC RefSeq v1.0), this 35.2 Mb interval harbours 462 gene models (Table S6A). Further

Table 3 Summary of stripe rust resistance QTL detected by ICIM in the MX169 × FRIED $F_{2:3}$ population across two environments and F_6 population across five environments

Environment	Position[a]	Marker interval	IT			rAUDPC		
			LOD	Add	PVE	LOD	Add	PVE
Yangling2013	12	*IWB71602–IWB55937*	15.5	−1.6	37.8	17.1	−0.19	49.6
Tianshui2013	12	*IWB71602–IWB55937*	17.1	−1.5	41.1	29.5	−0.18	59.1
Average					34.5			54.4
Yangling2016	18	*IWB71602–IWB55937*	19.5	−1.6	32.3	23.5	−0.22	38.8
Tianshui2016	18	*IWB71602–IWB55937*	18.8	−2.9	42.1	28.2	−0.26	45.6
Jiangyou2016	18	*IWB71602–IWB55937*	20.2	−1.5	31.4	29.9	−0.24	48.1
Yangling2017	18	*IWB71602–IWB55937*	19.1	−1.5	33.5	30.6	−0.27	48.2
Jiangyou2017	18	*IWB71602–IWB55937*	30.2	−2.4	37.2	27.4	−0.24	58.3
Average					35.3			47.8

LOD logarithm of odds score, *Add* additive effect of the resistance allele, *PVE* percentage of phenotypic variance explained by individual QTL

[a]Peak position in centiMorgans from the first linked marker of the relevant linkage group

Table 4 SSR markers associated with known 6B stripe rust QTL assayed on FRIED, MX169 and carrier lines with corresponding QTL to determine commonality of positive (+) alleles

Marker	MX169	FRIED	Stephens	Pavon 76	Pingyuan 50	Renan	Solist	LM168a
Xbarc136	−	+	+	ND	ND	ND	ND	ND
Xgdm113	−	−	+	ND	ND	ND	ND	ND
Xbarc101	−	−	+	ND	ND	ND	ND	ND
Xgwm132	−	−	+	ND	ND	ND	ND	ND
Xgwm58	−	−	ND	+	ND	ND	ND	ND
Xgwm626	−	−	ND	+	ND	ND	ND	ND
Xgwm136	−	+	ND	ND	+	ND	ND	ND
Xgwm361	−	−	ND	ND	+	ND	ND	ND
Xgwm193	−	−	ND	ND	ND	+	ND	ND
Xgwm518	−	−	ND	ND	ND	ND	+	ND
Xwmc494	−	−	ND	ND	ND	ND	+	ND
Xwmc397	−	−	ND	ND	ND	ND	+	ND
Xwmc105	−	−	ND	ND	ND	ND	+	ND
Xbarc146	−	−	ND	ND	ND	ND	ND	+
Xwmc756	−	−	ND	ND	ND	ND	ND	+

ND no detection

analysis of the 5 environmental phenotypic values for F_6 RILs along with the genetic map indicated that most of LOD peaks were located in the *IWB11702–IWB55937* overlapping confidence interval (Table S3C) spanning about 8.0 Mb (6B: 462,132,021–6B: 470,132,934, Table S6A). Hence, the predicted genes related to disease resistance in this target region can be considered candidate genes. Some of the genes within this region, share hallmarks of typical R-genes, i.e., leucine-rich repeats (LRR) (*TraesCS6B01G494600LC.1*) and nucleotide binding site-leucine-rich repeats (NBS-LRR) (*TraesCS6B01G498300LC.1*); other genes were similar to receptor-kinase (*TraesCS6B01G259100.1*), receptor-like protein kinase (*TraesCS6B01G498000LC.1*) and protein kinase (*TraesCS6B01G257800.1*); some genes encoded transporters, such as a sugar transporter (*TraesCS6B01G257400.1*) and an ATP-binding cassette (ABC) transporter (*TraesCS6B01G493700LC.1*) (Table 6).

The flanking sequences of KASP-SNPs were also blasted to the Zavitan WEWSeq v1.0 sequence and a physical interval of 35.3 Mb containing 165 gene models was obtained (Table S6B). After analysis of the 9.9 Mb interval between *IWB11702* and *IWB55937* (6B: 444,647,048–6B: 454,544,231, Table S6B), most of genes were undescribed and only one gene encoding a receptor-like protein kinase (*TRIDC6BG042370*) can be considered a candidate gene.

Discussion

Widespread development and deployment of resistant cultivars is the best option for durable control of stripe rust

Table 5 Phenotype and alleles of KASP markers flanking *QYr.nwafu-6BL* in FRIED, MX169, susceptible checks and 9 wheat accession

Wheat line	KASP markers						Severity (%) and reaction at adult-plant stage							
	IWA1839	*IWB71602*	*IWB11702*	*IWB55937*	*IWA4501*	*IWB60085*	Yangling				Tianshui			
							2014	2015	2016	2017	2014	2015	2016	2017
FRIED	CC[a]	AA	GG	AA	GG	TT	5R[b]	1R	5R	5R[c]	5R	5R	5R	5R
MX169	TT	GG	AA	GG	AA	CC	100S	100S	100S	100S	100S	100S	100S	100S
MFRIL-15[d]	CC	AA	GG	AA	GG	TT	–[e]	–	10R	10R	–	–	10R	–
MFRIL-45	CC	AA	GG	AA	GG	TT	–	–	20R	10MR	–	–	5R	–
MFRIL-58	CC	AA	GG	AA	GG	TT	–	–	20MR	20R	–	–	10R	–
MFRIL-11	TT	GG	AA	GG	AA	CC	–	–	90S	90S	–	–	80S	–
MFRIL-102	TT	GG	AA	GG	AA	CC	–	–	100S	100S	–	–	90S	–
MFRIL-111	TT	GG	AA	GG	AA	CC	–	–	90S	85S	–	–	90S	–
Avocet S (CK)	TT	GG	AA	GG	AA	CC	100S	100S	100S	100S	100S	100S	100S	100S
XY22 (CK)	TT	GG	AA	GG	AA	CC	100S	95S	100S	100S	100S	100S	100S	100S
Druchamp	TT	GG	GG	GG	AA	CC	20S	10MS	20MS	40MS	10R	15MS	20MR	10R
LM168a	TT	GG	AA	GG	AA	CC	–	–	–	80MS	–	–	–	–
Pavon 76	TT	GG	GG	GG	AA	CC	50M	40MR	40MR	60MS	50S	70MS	60MS	50S
Pingyuan 50	CC	GG	AA	GG	AA	CC	30MR	20MR	30MR	50MS	40S	50MS	40MS	50S
Renan	TT	GG	GG	GG	AA	CC	–	–	–	50S	–	–	–	–
RSL65 (*Yr36*)	TT	GG	AA	GG	AA	CC	80R	60R	60MR	40M	40S	60MS	70S	40MR
Solist	CC	GG	AA	GG	AA	CC	–	–	–	60MS	–	–	–	–
Stephens	CC	GG	AA	GG	AA	CC	40S	50MS	40S	50M	60R	60MS	50MS	40MR
VPM1	TT	GG	AA	GG	AA	CC	25MS	20MS	30S	80MS	20MR	10R	20MR	60MS

[a]TT means the SNP locus

[b]A response to rust was a combination of disease severity recorded as a single value for each accession at each site from 0 to 100% of the foliage infected using Peterson et al. scale (1948), and *R* resistant, *MR* moderately resistant, *M* moderately resistant to moderately susceptible, *MS* moderately susceptible, *S* susceptible

[c]The resistance evaluation was tested with *Pst* race CYR34 in greenhouse

[d]ID of F_6 lines with/without the 6BL QTL marker alleles derived from MX169 × FRIED

[e]No data

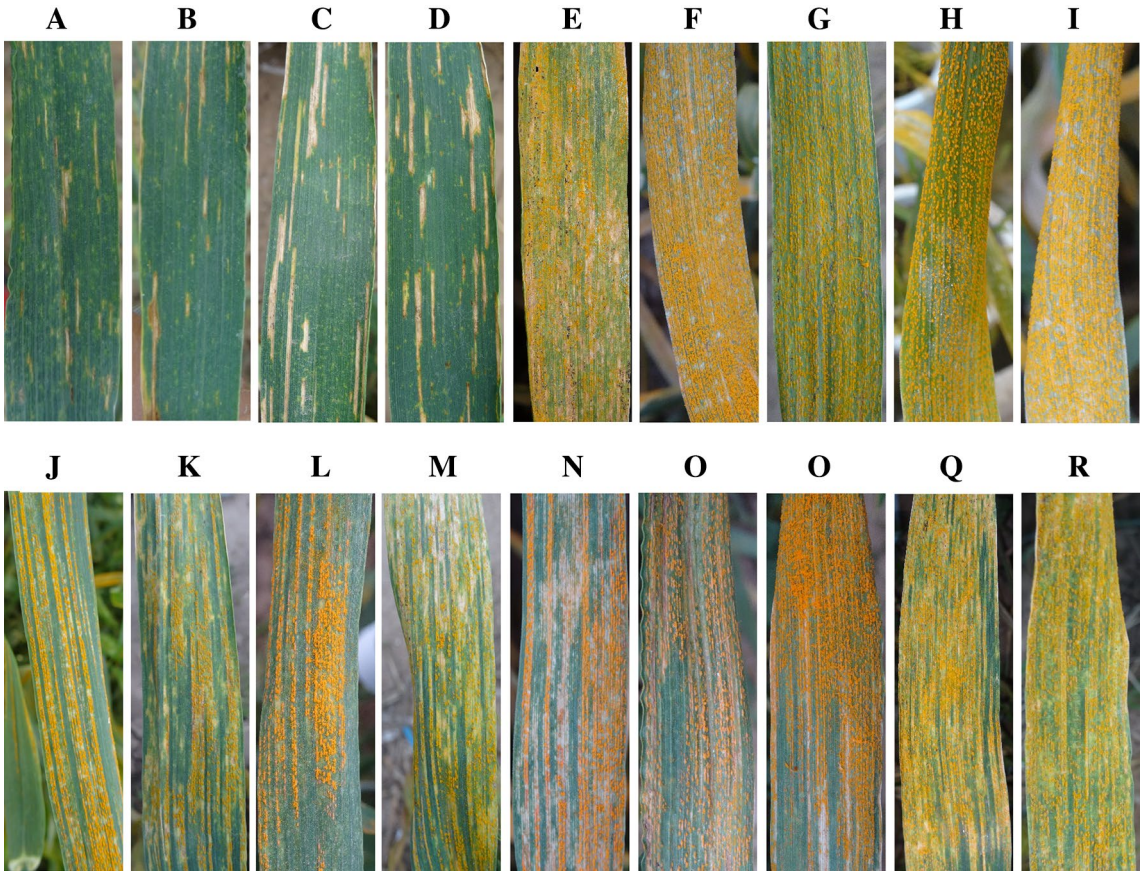

Fig. 4 Adult plants responses produced by FRIED, its F_6 lines with/without the 6BL QTL marker alleles, susceptible checks and other lines with reported genes/QTL on chromosome 6B when infected with *Pst* CYR34 race in greenhouse. **a** FRIED; **b** MFRIL-15; **c** MFRIL-45; **d** MFRIL-58; **e** MFRIL-11; **f** MFRIL-101; **g** MFRIL-111; **h** Avocet S; **i** MX169; **j** Druchamp; **k** RSL65; **l** Pingyuan 50; **m** Stephens; **n** Pavon 76; **o** Solist; **p** Renan; **q** VPM1; **r** LM168a

in wheat (McIntosh et al. 1995; Li and Zeng 2002). This emphasizes the importance of identifying new resistance genes, especially those effective against a broad spectrum of pathogen races. The wheat cultivar FRIED is susceptible at the seedling growth stage, but is highly resistant at the adult plant stage and hence possess adult-plant resistance (Han et al. 2012; Fig. S1).

In the present study, we combined BSA with the 90K SNP array to mine SNPs associated with resistance. A candidate genomic region was rapidly located on chromosome 6B due to the availability of consensus maps (Maccaferri et al. 2015; Wang et al. 2014). In the next step, all SNPs in the region were used to design KASP markers in the website of PolyMarker. Only chromosome-specific SNPs in the SNP-concentrated region were used initially in KASP assays to verify polymorphisms in the bulks and parents. Finally, polymorphic KASP markers were employed to generate a genetic map and the position of the target QTL was identified. Thus, combining high-throughput SNP array sequencing of pooled extremes for QTL identification in hexaploid wheat is feasible and efficient, and in the present study, was successful when using ether F_3 lines or F_6 RILs. Furthermore, validation of SNP genotyping with KASP assays lowered sequencing costs relative to genome-wide association studies, and more importantly, permitted rapid isolation of major effect QTL with improved accuracy of mapping.

Four *Yr* genes have been reported on chromosome 6B, namely *Yr4*, *Yr35*, *Yr36* and *YrLM168a*. Fan 6 and its derivatives allegedly containing *Yr4* are ineffective against current Chinese *Pst* races (Chen et al. 2009). *Yr35* derived from *T. dicoccoides* provides all-stage resistance (Marais et al. 2005), and *Yr36* also from *T. dicoccoides* confers HTAP resistance that is completely linked to SSR marker *Xbarc101* on chromosome arm 6BS (Uauy et al. 2005). These genes should be different from *QYr.nwafu-6BL*. *YrLM168a*, conferring APR, was located on chromosome arm 6BL of CIMMYT-derived cultivar Milan and flanked by SSR markers *Xwmc756* and *Xbarc146* (Feng et al. 2015). Milan originated from French wheat VPM1 which was selected from the cross "*Aegilops ventricosa*/*T. persicum*//3*T/*aestivum* cv. Marne" (Doussinault and Dosba 1981). FRIED is an old German cultivar dating from 1964 or earlier (Zeven and

Table 6 List of candidate genes in the *IWB71602–IWA4501* and gene annotations based on Chinese Spring IWGSC RefSeq v1.0

Gene ID	Hit-start[a]	Hit-end	Human-readable-description
TraesCS6B01G248300.1	445,008,132	445,012,017	Bidirectional sugar transporter SWEET
TraesCS6B01G478800LC.1	445,044,549	445,047,362	Serine/threonine-protein kinase AFC1
TraesCS6B01G479400LC.1	445,492,161	445,492,550	Pleiotropic ABC efflux transporter of multiple drugs
TraesCS6B01G248800.1	446,302,843	446,304,084	Cysteine-rich RLK (Receptor-like protein kinase) 32
TraesCS6B01G480700LC.1	447,740,079	447,740,393	UTP-glucose-1-phosphate uridylyltransferase
TraesCS6B01G250000.1	449,447,043	449,447,705	Temperature-induced lipocalin
TraesCS6B01G250800.1	450,083,928	450,084,128	Protein kinase superfamily protein
TraesCS6B01G251200.1	450,589,590	450,604,475	Leucine-rich repeat (LRR) family protein
TraesCS6B01G485100LC.1	450,798,608	450,809,526	Leucine-rich repeat (LRR) family protein
TraesCS6B01G485200LC.1	450,805,407	450,808,335	Disease resistance protein (NBS-LRR class) family
TraesCS6B01G251900.1	451,928,029	451,933,514	UDP-sugar transporter-like protein
TraesCS6B01G252200.1	452,188,836	452,190,856	Transporter-related family protein
TraesCS6B01G486800LC.1	453,260,809	453,261,513	LRR receptor-like protein kinase family
TraesCS6B01G487000LC.1	453,265,682	453,266,258	Protein kinase, putative
TraesCS6B01G252800.1	453,277,206	453,280,530	Receptor-kinase, putative
TraesCS6B01G252900.1	453,930,774	453,932,102	Leucine-rich repeat protein kinase protein
TraesCS6B01G253500.1	455,413,653	455,416,101	GDSL esterase/lipase
TraesCS6B01G256300.1	460,274,455	460,276,943	Receptor-like protein kinase
TraesCS6B01G493700LC.1[b]	461,963,573	461,963,761	ABC-2 type transporter family protein
TraesCS6B01G257400.1	462,024,836	462,031,751	Hexosyltransferase
TraesCS6B01G257800.1	462,148,520	462,149,515	Kinase family protein
TraesCS6B01G494600LC.1	462,625,667	462,626,050	FBD/LRR domains containing protein
TraesCS6B01G498000LC.1	465,849,854	465,850,114	Interleukin-1 receptor-associated kinase-like 2
TraesCS6B01G498300LC.1	465,885,913	465,886,407	MLO-like protein 2
TraesCS6B01G259100.1	467,457,750	467,460,908	Receptor kinase
TraesCS6B01G262100.1	472,234,061	472,236,324	UDP-glycosyltransferase
TraesCS6B01G262900.1	473,086,672	473,088,971	F-box/LRR protein

[a]Physical position of the gene on chromosome 6B

[b]Genes with bold font located in the *IWB11702–IWB55937* overlapping confidence interval

Zeven-Hissink 1976) and thus pre-dates VPM1-derived materials. Moreover, FRIED did not have the alleles of *Xwmc756* and *Xbarc146* that flanked *YrLM168a*. Thus, the resistance gene in FIRED appears to be different from those listed above.

Fifteen stripe rust APR QTLs have been reported on chromosome 6B (Fig. 3a). The fact that most of these QTLs had minor effect and explained phenotypic variations of less than 10% (Rosewarne et al. 2013) is seemingly sufficient evidence alone to suggest that they are different from *QYr.nwafu-6BL*. Only *QYrste.wgp-6BS.1* (*Yr78*) and *QYrste.wgp-6BS.2* in Stephens (Dong et al. 2017; Santra et al. 2008) and *QYr.pav-6BL* in Pavon 76 (William et al. 2006) explained phenotypic variation of more than 10%. Based on integrated genetic map (Maccaferri et al. 2015), most of these QTLs nearly covered the 6BS region and *QYr.nwafu-6BL* spanned the interval from 80.0 to 82.9 cM (Fig. 3a). *QYr.sol-6BS*, *QYrpin.caas-6BS.2*, *QYr.pav-6BL*, *QYr.inra-6BL*, and *QYrdr.wgp-6BL.2* overlapped the *QYr.nwafu-6BL* region, whereas other QTL were more than 10 cM away from *QYr.nwafu-6BL*. However, haplotype analysis of markers flanking these QTLs showed that FRIED shared no marker alleles with these cultivars and the KASP alleles encompassing *QYr.nwafu-6BL* were unique to FRIED. In our previous study, Pavon76, Pingyuan 50, RSL65, Stephens, VPM1, displayed moderate susceptibility or slow rusting with disease severities of 30–70% in the field (Table 5). In greenhouse tests with *Pst* race CYR34, the response of FRIED and the F_6 lines with the 6BL QTL marker alleles were more resistant than lines with other reported 6B genes/QTLs. Moreover, pedigree analyses revealed no relationship between FRIED and the other resistance sources (https://npgsweb.ars-grin.gov/gringlobal/search.aspx?). Thus, taking all factors into account it appears that *QYr.nwafu-6BL* is a new resistance gene and only further comparative laboratory and field tests will provide more clarity.

In wheat, ten race-specific resistance genes have been cloned including *Sr22*, *Sr33*, *Sr35*, *Sr45*, *Sr50* (resistance

to stem rust), *Lr1*, *Lr10*, *Lr21*, *Lr22* (resistance to leaf rust), and *Yr10* (resistance to stripe rust) and all code for nucleotide-binding and leucine-rich repeat (NLR) receptor proteins (Ellis et al. 2014; Krattinger and Keller 2016; Periyannan et al. 2017; Thind et al. 2017). However, the barley stem rust resistance gene *Rpg1* encodes a receptor-like serine/threonine kinase with tandem kinase domains (Brueggeman et al. 2002). In contrast to most NLR-encoding *R* genes, recent cloning of several adult plant stripe rust resistance genes has indicated that the underlying genetic bases are more variable (Brown 2015). For instance, *Yr36* encodes a chloroplast-localized protein containing kinase and START lipid-binding domains (Fu et al. 2009); the *Lr34/Yr18* and *Lr67/Yr46* genes encode an ATP-binding cassette (ABC) transporter and a hexose transporter, respectively (Krattinger et al. 2009; Moore et al. 2015). Based on the functions of the identified proteins, vesicle trafficking and protein/metabolite transportation are probably common physiological processes involved in adult plant resistance (Niks et al. 2015). In this study, by analyzing gene annotations in the target region based on Chinese Spring IWGSC RefSeqv1.0, wheat genes *TraesCS6B01G257800.1, TraesCS6B01G494600LC.1, TraesCS6B01G498000LC.1, TraesCS6B01G498300LC.1* and *TraesCS6B01G259100.1* were predicted to confer potential race-specific resistance to pathogens with features of classical R-genes or protein kinases. *TraesCS6B01G493700LC.1* and *TraesCS6B01G257400.1* encode an ABC-2 type transporter family protein and hexosyltransferase, respectively. Their functions in energy metabolism and transport are very similar to that of *Lr34/Yr18* and *Lr67/Yr46*, respectively. This information is valuable for future high-resolution mapping and map-based cloning of *QYr.nwafu-6BL*. Further genetic studies and more detailed analyses are needed to confirm the roles of candidate genes in stripe rust response.

Marker-assisted selection is an effective way to help breeders incorporate and pyramid resistance genes into breeding material thereby reducing disease severity (Chen 2013). However, the markers must be reliable, specific, easily used, and cost-effective. Ideal markers are based on actual resistance genes, but only a few stripe rust APR genes have been cloned. In the MX169 × FRIED $F_{2:3}$ and F_6 RIL populations we located a major effect QTL with flanking markers *IWB71602* and *IWB55937/IWA4501* that effectively distinguished presence/absence of *QYr.nwafu-6BL* when used in combination. These closely linked markers should enable effective selection in breeding programs.

Author contribution statement JHW conducted the experiments, analyzed the data, and wrote the manuscript. QLW and DJH identified the resistant parental line, made the cross and participated in field experiments. SJL, SH and JMM participated in field experiments and contributed to the genotyping experiment. QLW, SZY and QDZ assisted in analyzing the data. DJH and ZSK conceived and directed the project and revised the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

Ethical standards 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.

References

Abe A, Kosugi S, Yoshida K, Natsume S, Takagi H et al (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat Biotechnol 30:174–178

Allard RW (1960) Principles of plant breeding. Wiley, New York

Avni R, Nave M, Barad O, Baruch K, Twardziok SO et al (2017) Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357:93–97

Bai B, Ren Y, Xia XC, Du JY, Zhou G et al (2012) Mapping of quantitative trait loci for adult plant resistance to stripe rust in German wheat cultivar Ibis. J Integr Agric 11:528–536

Bariana HS, Bansal UK, Schmidt A, Lehmensiek A, Kaur J et al (2010) Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. Euphytica 176:251–260

Basnet BR, Ibrahim AMH, Chen XM, Singh RP, Mason ER et al (2014a) Molecular mapping of stripe rust resistance in hard red winter wheat TAM 111 adapted to the U.S. high plains. Crop Sci 54:1361–1373

Basnet BR, Singh RP, Ibrahim AMH, Herrera-Foessel SA, Huerta-Espino J et al (2014b) Characterization of *Yr54* and other genes associated with adult plant resistance to yellow rust and leaf rust in common wheat Quaiu 3. Mol Breed 33:385–399

Brown JK (2015) Durable resistance of crops to disease: a Darwinian perspective. Annu Rev Phytopathol 53:513–539

Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F et al (2002) The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. Proc Natl Acad Sci USA 99:9328–9333

Bulli P, Zhang JL, Chao SM, Chen XM, Pumphrey M (2016) Genetic architecture of resistance to stripe rust in a global winter wheat germplasm collection. G3 6:2237–2253

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S et al (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057–8062

Chen XM (2005) Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Can J Plant Pathol 27:314–337

Chen XM (2013) High-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sci 04:608–627

Chen XM (2014) Integration of cultivar resistance and fungicide application for control of wheat stripe rust. Can J Plant Pathol 36:311–326

Chen XM, Line RF (1995) Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant (HTAP) resistance and interaction of HTAP and race-specific seedling resistance to *Puccinia striiformis*. Phytopathology 85:573–578

Chen WQ, Wu LR, Liu TG, Xu SC, Jin SL et al (2009) Race dynamics, diversity, and virulence evolution in *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust in China from 2003 to 2007. Plant Dis 93:1093–1101

Christiansen MJ, Feenstra B, Skovgaard IM, Andersen SB (2006) Genetic analysis of resistance to yellow rust in hexaploid wheat using a mixture model for multiple crosses. Theor Appl Genet 112:581–591

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M et al (2009) Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. Phytopathology 99:968–973

Dong ZZ, Hegarty JM, Zhang JL, Zhang WJ, Chao SM et al (2017) Validation and characterization of a QTL for adult plant resistance to stripe rust on wheat chromosome arm 6BS (*Yr78*). Theor Appl Genet. doi:10.1007/s00122-017-2946-9

Doussinault G, Dosba F (1981) Analyse monosomique de la résistance a la rouille jaune du géniteur blé tendre VPM 1. Comptes rendus des seances de l'Academie d'agriculture de France, pp 133–138

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:1–13

Feng J, Chen G, Wei Y, Liu Y, Jiang Q et al (2015) Identification and mapping stripe rust resistance gene *YrLM168a* using extreme individuals and recessive phenotype class in a complicated genetic background. Mol Genet Genom 290:2271–2278

Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L et al (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science 323:1357–1360

Gao L, Turner MK, Chao S, Kolmer J, Anderson JA (2016) Genome wide association study of seedling and adult plant leaf rust resistance in elite spring wheat breeding lines. PLoS One 11:e148671

Han DJ, Wang QL, Zhang L, Wei GR, Zeng QD et al (2010) Evaluation of resistance of current wheat cultivars to stripe rust in Northwest China, North China and the Middle and Lower Reaches of Changjiang River epidemic area. Sci Agric Sin 43:2889–2896

Han DJ, Zhang PY, Wang QL, Zeng QD, Wu JH et al (2012) Identification and evaluation of resistance to stripe rust in 1980 wheat landraces and abroad germplasm. Sci Agric Sin 45:5013–5023

Han DJ, Wang QL, Chen XM, Zeng QD, Wu JH et al (2015) Emerging *Yr26*-virulent races of *Puccinia striiformis* f. sp. *tritici* are threatening wheat production in the Sichuan Basin, China. Plant Dis 99:754–760

Hou L, Chen X, Wang M, See DR, Chao S et al (2015) Mapping a large number of QTL for durable resistance to stripe rust in winter wheat Druchamp using SSR and SNP markers. PLoS One 10:e0126794

Hou L, Jia J, Zhang X, Li X, Yang Z et al (2016) Molecular mapping of the stripe rust resistance gene *Yr69* on wheat chromosome 2AS. Plant Dis 100:1717–1724

Hovmøller MS, Walter S, Justesen AF (2010) Escalating threat of wheat rusts. Science 329:369

Kosambi DD (1943) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Krattinger SG, Keller B (2016) Molecular genetics and evolution of disease resistance in cereals. New Phytol 212:320–332

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J et al (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Lan C, Liang S, Zhou X, Zhou G, Lu Q et al (2010) Identification of genomic regions controlling adult-plant stripe rust resistance in Chinese landrace Pingyuan 50 through bulked segregant analysis. Phytopathology 100:313–318

Li ZQ, Zeng SM (2002) Wheat rust in China. China Agriculture Press, Beijing

Lin F, Chen XM (2007) Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor Appl Genet 114:1277–1287

Line RF, Qayoum A (1992) Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America 1968–1987. US Department of Agriculture Technical Bulletin No. 1788, p 74

Liu JD, He ZH, Wu L, Bai B, Wen WE et al (2015) Genome-wide linkage mapping of QTL for adult-plant resistance to stripe rust in a Chinese wheat population Linmai 2 × Zhong 892. PLoS One 10:e145462

Liu W, Maccaferri M, Bulli P, Rynearson S, Tuberosa R et al (2017) Genome-wide association mapping for seedling and field resistance to *Puccinia striiformis* f. sp. *tritici* in elite durum wheat. Theor Appl Genet 130:649–667

Lu Y, Wang M, Chen X, See D, Chao S et al (2014) Mapping of *Yr62* and a small-effect QTL for high-temperature adult-plant resistance to stripe rust in spring wheat PI 192252. Theor Appl Genet 127:1449–1459

Maccaferri M, Zhang J, Bulli P, Abate Z, Chao S et al (2015) A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). G3 5:449–465

Marais GF, Pretorius ZA, Wellings CR, McCallum B, Marais AS (2005) Leaf rust and stripe rust resistance genes transferred to common wheat from *Triticum dicoccoides*. Euphytica 143:115–123

McIntosh RA, Wellings CW, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, East Melbourne, pp 20–26

McIntosh RA, Dubcovsky J, Rogers J, Morris C, Appels R et al (2016) Catalogue of gene symbols for wheat: 2015–2016 Supplement. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2015.pdf

McIntosh RA, Dubcovsky J, Rogers J, Morris C, Appels R et al. (2017) Catalogue of gene symbols for wheat: 2017 Supplement. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J 3:269–283

Michelmore RW, Paran I, Kesseli R (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Moore JW, Herrera-Foessel S, Lan CX, Schnippenkoetter W, Ayliffe M et al (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. Nat Genet 47:1494–1498

Niks RE, Qi X, Marcel TC (2015) Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. Annu Rev Phytopathol 53:445–470

Periyannan S, Milne RJ, Figueroa M, Lagudah ES, Dodds PN (2017) An overview of genetic rust resistance: from broad to specific mechanisms. PLoS Pathog 13:e1006380

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res Sect C26:496–500

Prins R, Pretorius ZA, Bender CM, Lehmensiek A (2011) QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega × Avocet S doubled haploid wheat population. Mol Breed 27:259–270

Ramirez-Gonzalez RH, Segovia V, Bird N, Fenwick P, Holdgate S et al (2015a) RNA-Seq bulked segregant analysis enables the identification of high-resolution genetic markers for breeding in hexaploid wheat. Plant Biotechnol J 13:613–624

Ramirez-Gonzalez RH, Uauy C, Caccamo M (2015b) PolyMarker: a fast polyploid primer design pipeline. Bioinformatics 31:2038–2039

Rasheed A, Wen W, Gao F, Zhai S, Jin H et al (2016) Development and validation of KASP assays for genes underpinning key economic traits in bread wheat. Theor Appl Genet 129:1843–1860

Rasheed A, Hao Y, Xia X, Khan A, Xu Y et al (2017) Crop breeding chips and genotyping platforms: progress, challenges, and perspectives. Mol Plant 10:1047–1064

Ren Y, He Z, Li J, Lillemo M, Wu L et al (2012a) QTL mapping of adult-plant resistance to stripe rust in a population derived from common wheat cultivars Naxos and Shanghai 3/Catbird. Theor Appl Genet 125:1211–1221

Ren Y, Li Z, He Z, Wu L, Bai B, Lan C, Wang C, Zhou G, Zhu H, Xia X (2012b) QTL mapping of adult-plant resistances to stripe rust and leaf rust in Chinese wheat cultivar Bainong 64. Theor Appl Genet 125:1253–1262

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX et al (2013) Quantitative trait loci of stripe rust resistance in wheat. Theor Appl Genet 126:2427–2449

Santra DK, Chen XM, Santra M, Campbell KG, Kidwell KK (2008) Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'. Theor Appl Genet 117:793–802

Semagn K, Babu R, Hearne S, Olsen M (2014) Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Mol Breed 33:1–14

Sharma-Poudyal D, Chen XM, Wan AM, Zhan GM, Kang ZS et al (2013) Virulence characterization of international collections of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*. Plant Dis 97:379–386

Singh RP, Huerta-Espino J, Bhavani S, Herrera-Foessel SA, Singh D et al (2011) Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175–186

Song WN, Ko L, Henry RJ (1994) Polymorphisms in the α-amy1 gene of wild and cultivated barley revealed by the polymerase chain reaction. Theor Appl Genet 89:509–513

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G et al (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genom 4:12–25

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890

Sun ZQ, Li HH, Zhang LY, Wang JK (2013) Properties of the test statistic under null hypothesis and the calculation of LOD threshold in quantitative trait loci (QTL) mapping. Acta Agron Sin 39:1–11

Takagi H, Abe A, Yoshida K, Kosugi S, Natsume S et al (2013) QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J 74:174–183

Thind AK, Wicker T, Šimková H, Fossati D, Moullet O et al (2017) Rapid cloning of genes in hexaploid wheat using cultivar-specific long-range chromosome assembly. Nat Biotechnol. doi:10.1038/nbt.3877

Uauy C, Brevis JC, Chen XM, Khan I, Jackson L et al (2005) High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. Theor Appl Genet 112:97–105

Van Ooijen JW (2006) JoinMap4, software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Varshney RK, Terauchi R, McCouch SR (2014) Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biol 12(6):e1001883

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang JK (2009) Inclusive composite interval mapping of quantitative trait genes. Acta Agron Sin 35:239–245

Wang S, Wong D, Forrest K, Allen A, Chao S et al (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol J 12:787–796

Wang M, Wang S, Xia G (2015) From genome to gene: a new epoch for wheat research? Trends Plant Sci 20:380–387

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129–141

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49:977–990

Wu JH, Wang QL, Chen XM, Wang MJ, Mu JM et al (2016) Stripe rust resistance in wheat breeding lines developed for central Shaanxi, an overwintering region for *Puccinia striiformis* f. sp. *tritici* in China. Can J Plant Pathol 38:317–324

Wu JH, Wang QL, Liu SJ, Huang S, Mu JM et al (2017a) Saturation mapping of a major effect QTL for stripe rust resistance on wheat chromosome 2B in cultivar Napo 63 using SNP genotyping arrays. Front Plant Sci 8:653

Wu JH, Wang QL, Xu LS, Chen XM, Li B et al (2017b) Combining SNP genotyping array with bulked segregant analysis to map a gene controlling adult-plant resistance to stripe rust in wheat line 03031-1-5 H62. Phytopathology. doi:10.1094/PHYTO-04-17-0153-R

Xu LS, Wang MN, Cheng P, Kang ZS, Hulbert SH et al (2013) Molecular mapping of *Yr53*, a new gene for stripe rust resistance in durum wheat accession PI 480148 and its transfer to common wheat. Theor Appl Genet 126:523–533

Yang H, Li C, Lam HM, Clements J, Yan G et al (2015) Sequencing consolidates molecular markers with plant breeding practice. Theor Appl Genet 128:779–795

Zeng QD, Han DJ, Wang QL, Yuan FP, Wu JH et al (2014) Stripe rust resistance and genes in Chinese wheat cultivars and breeding lines. Euphytica 196:271–284

Zeng QD, Shen C, Yuan FP, Wang QL, Wu JH et al (2015) The resistance evaluation of the *Yr* genes to the main prevalent pathotypes of *Puccinia striiformis* f. sp. *tritici* in China. Acta Phytopathol Sin 45:641–650

Zeven AC, Zeven-Hissink NC (1976) Genealogies of 14000 wheat varieties. The Netherlands Cereal Centre, Wageningen

Zhou XL, Han DJ, Chen XM, Gou HL, Guo SJ et al (2014) Characterization and molecular mapping of stripe rust resistance gene *Yr61* in winter wheat cultivar Pindong 34. Theor Appl Genet 127:2349–2358

Zhou XL, Zhan GM, Huang LL, Han DJ, Kang ZS (2015) Evaluation of resistance to stripe rust in eighty abroad spring wheat germplasms. Sci Agric Sin 8:1518–1526

Zou C, Wang P, Xu Y (2016) Bulked sample analysis in genetics, genomics and crop improvement. Plant Biotechnol J 14:1941–1955