Association Analysis Identifies New Loci for Resistance to Chinese Yr26-Virulent Races of the Stripe Rust Pathogen in a Diverse Panel of Wheat Germplasm

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Abstract

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most destructive fungal diseases of wheat worldwide. The expanding Yr26-virulent *Pst* race (V26) group overcomes almost all currently deployed resistance genes in China and has continued to accumulate new virulence. Investigating the genetic architecture of stripe rust resistance in common wheat is an important basis for a successful utilization of resistance in breeding programs. A panel of 410 exotic wheat germplasms was used for characterizing new stripe rust resistance loci. This panel was genotyped using high-density wheat 660K single-nucleotide polymorphism (SNP) array, and phenotypic evaluation of seedlings for stripe rust resistance was performed using multiple *Pst* races. Thirty-five loci conferring resistance were identified through genome-wide association mapping, and explained phenotypic variances ranged from 53 to 75%. Of these, 14 were colocated in the proximity of the known loci, including cataloged *Yr* genes *Yr9*, *Yr10*, *Yr26*,

Yr33, *Yr47*, *Yr56*, *Yr57*, *Yr64*, *Yr67*, *Yr72*, and *Yr81* and three temporarily designated as *YrCen*, *YrNP63*, and *YrRC* detected in our quantitative trait locus (QTL) mapping studies. Seven of them (*Yr9*, *Yr10*, *Yr24/26*, *Yr81*, *YrCEN*, *YrNP63*, and *YrRC*) were confirmed by molecular detection or genetic analysis. New loci that were identified to be different from reported *Yr* genes need further confirmation. Nine QTL with significantly large phenotypic effect on resistance to all tested races were considered as major loci for effective resistance. The identified loci enrich our stripe rust resistance gene pool, and the linked SNPs should be useful for marker-assisted selection in breeding programs.

Keywords: cereals and grains, cultivar/resistance, disease management, field crops, genome-wide association study, GWAS, linkage disequilibrium, *Puccinia striiformis*, seedling resistance, *Triticum aestivum*

With the human population expected to increase from 7 to approximately 10 billion over the next 30 years, there will be immense pressure to increase yields to ensure global food security. Triticeae crops provide >30% of total grain production, and increased yield is the only assurance of food security. Most of the global annual production of common wheat (*Triticum aestivum* L.) of >700 million metric tons is produced in Europe, East and South Asia, North America, and Oceania (FAO; www.fao.org/faostat). China is currently the largest producer with >130 million tons. It is predicted that climate change will continue to be a major challenge for maintaining production levels. It is predicted that biotic stresses, including fungal diseases, will threaten future wheat production. Among them, stripe rust or yellow rust caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*) is currently one of the most destructive diseases worldwide. Fungicides are effective for stripe rust control; however, such practice adds

to production costs, and there are growing concerns of pesticide effects on human safety, environment, and the likelihood that pathogens will become resistant or tolerant (Chen 2005; Chen and Kang 2017). Consequently, continuous efforts to maintain and increase genetic resistance in commercially relevant wheat cultivars are economically important for future disease control.

Based on historical epidemiological data of stripe rust, the wheat-growing regions in China can be divided into the western oversummering region, the overwintering region, and the eastern epidemic region (Li and Zeng 2002; McIntosh et al. 2018; Wan et al. 2004). Previous studies demonstrated that *Pst* urediniospores in wheat seedlings from the oversummering region transit to the overwintering region and then, spread eastward to eastern China at adult plant stage (Brown and Hovmöller 2002; Chen and Kang 2017). Longnan (Gansu province) is considered to be an oversummering region, whereas Sichuan Basin (Sichuan province), Longdong (Gansu province), and Guanzhong (Shaanxi province) are considered as overwintering regions and serve as “green bridges” for the pathogen to migrate from the western oversummering region to the eastern epidemic region. The top priority is to decrease the pathogen buildup in oversummering and overwintering regions by breeding wheat cultivars with seedling resistance (Kang et al. 2015).

In China, 34 Chinese *Pst* races (CYR1 to CYR34) have been well characterized and documented. The emergence of the races in the post-Yr26-virulent race group (hereafter referred to as post-V26), including CYR34, has changed the structure of Chinese *Pst* populations, and they are currently becoming the most prevalent races in the region (Bai et al. 2018; Han et al. 2015; Wu et al. 2016). Race CYR34 with a broad virulence spectrum is considered more aggressive than those previously characterized races, and it defeated several race-specific *Yr* resistance genes, which were effective in the past (Mu et al. 2019b; Wu et al. 2016). Despite the huge impact of this unique race group on wheat production in China, little is known on the genetic resistance for the control of post-V26 races, and better understanding of host resistance and deployment into elite cultivars will enhance resistance breeding. Unexploited wheat germplasm can be potentially valuable sources of genetic diversity that can enhance

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and enrich breeding germplasm with needed traits and sustainable improvement of modern cultivars (Hao et al. 2011a), and it has played an important role in stripe rust resistance breeding in China (Zhuang 2003).

The complexity of the hexaploid wheat genome initially limited molecular genetic studies at the whole-genome level, but next generation sequencing (NGS) technologies radically changed the landscape (Wang et al. 2015, 2018). NGS enabled high-throughput discovery of DNA variants and facilitated development of new generation markers based on single-nucleotide polymorphisms (SNPs) (Winfield et al. 2016). With whole-wheat genome sequencing and development of high-density SNP chips, massive amounts of genetic data are becoming more easily and more cheaply procurable. Several high-density wheat SNP arrays were constructed for genetic studies and breeding (Rasheed et al. 2017). These achievements led to genome-wide association studies (GWAS) in wheat, a powerful method for dissecting complex genetic variation and for estimating individual gene or quantitative trait locus (QTL) effects on phenotype. GWAS has enabled rapid identification of genetic components of stripe rust resistance in large germplasm collections (Bulli et al. 2016; Liu et al. 2017; Maccaferri et al. 2015; Yao et al. 2019).

The purpose of the present study was to search for new genes/QTL conferring resistance to V26 *Pst* races using a GWAS approach. A collection of 410 exotic wheat accessions was evaluated in seedling tests with multiple *Pst* races and genotyped with the wheat 660K SNP array. The specific objectives were to 1) dissect the genetic basis of stripe rust resistance in the panel, 2) validate the use of GWAS approach by detection of known *Yr* genes, and 3) identify individual chromosome regions and tag them with SNP markers that would potentially allow improvement of stripe rust resistance in molecular-assisted breeding.

Materials and Methods

Association mapping panel. The 410 wheat accessions were provided by the China Agriculture Research System (Fig. 1A and Supplementary Table S1), and they included landraces, registered cultivars, cultivated germplasm, breeding lines, and genetic stocks from public or private wheat breeding programs worldwide. Two wheat lines (Avocet S [AvS] and Mingxian 169 [MX169]) were used as susceptible controls. In addition, a set of *Yr* single-gene lines based on AvS and another set of Chinese differential cultivars and certain *Yr* gene donor lines were included in the study as reference stocks for individual stripe rust resistance gene.

Ten *Pst* races identified as CYR23, CYR29, CYR31, CYR32, CYR33, V26/CH42, CYR34/Gansu (CYR34), V26/Sichuan (V26/SC), V26/Shaanxi (V26/SX), and V26/Laboratory (V26/LAB) were used to test seedlings in the greenhouse. All races had been collected from the overwintering region in Gansu province or the overwintering regions of Sichuan Basin and Shaanxi province, and they are maintained by the Institute of Plant Pathology, Northwest A&F University. The virulence/avirulence patterns of the races were confirmed by testing on AvS *Yr* single-gene lines, Chinese differential cultivars, and certain *Yr* gene donor lines used to differentiate *Pst* races (Table 1).

Molecular profiling and SNP filtering. All of the 410 accessions were profiled using the wheat 660 K genotyping array by Capital Bio Technology Co. Ltd. (Beijing, China; <http://www.capitalbiotech.com>). The polyploid version of the Affymetrix Genotyping Console software (Affymetrix, Santa Clara, CA) was used to conduct SNP allele clustering and genotype calling on the raw SNP data. To ensure genotyping accuracy, SNP markers with minor allele frequencies (MAFs) <0.05, missing data frequencies >10%, and Hardy-Weinberg equilibria >0.01 were excluded from further analysis. Polymorphism information content (PIC) was calculated using self-programming in Perl. PIC estimates the detection power and informative SNP markers (Botstein et al. 1980). The formula is $PIC = 1 - \sum (P_i)^2$, where P_i is the proportion of the population carrying the i th allele. In addition, the extent of genetic differentiation among populations was determined by calculating expected heterozygosity (H_{exp}). H_{exp} was estimated at each marker locus, and it was

averaged across all markers for a global characterization of each chromosome and subgenome (Nei 1978). H_{exp} is analogous to allelic variation in a random mating population and describes the average distance between individuals within a population. Values close to zero indicate that the individuals within the population are similar.

Molecular detection of previously identified *Yr* genes or QTL was undertaken to genotype all accessions using tightly linked or cosegregated Kompetitive allele-specific PCR (KASP) or simple sequence repeats (SSR) markers. The genes/alien chromosome segments included *Yr5* (Marchal et al. 2018), the 1RS/1BL translocation (*Yr9*) (Zeng et al. 2014), *Yr10* (Liu et al. 2014; Yuan et al. 2018), *Yr15* (Klymiuk et al. 2018), *Lr37/Yr17/Sr38* (Liu et al. 2019), *Yr24/26* (Wu et al. 2018b), and *Yr81* (Gessese et al. 2019).

Population structure, kinship, and linkage disequilibrium analysis. The population structure of the panel was assessed by the model-based Bayesian clustering software STRUCTURE 2.3.3 (Pritchard et al. 2000). A burn-in period of 10,000 iterations and 20,000 Markov chain Monte Carlo iterations across five independent repeats was implemented to estimate K (number of subpopulations) ranging from 2 to 10. The most likely number of subpopulations was determined using the ΔK method based on the rate of change in Log likelihood $\ln P(D)$ between successive K values as previously described (Earl and VonHoldt 2012). The identity by state relative K matrix was calculated between pairs of accessions using PLINK (Purcell et al. 2007). Heat maps of the kinship were generated on the basis of the K matrix using the pheatmap R package v1.0.8.

Genome-wide linkage disequilibrium (LD) analyses for the whole genome and each subgenome (A, B, and D) were estimated as the squared allele frequency correlation (r^2) using PLINK software. Pairwise comparisons were measured among filtered SNP markers based on r^2 . The parameters for calculating r^2 were set to $-r2\text{-ld-window-kb } 30000\text{-ld-window } 1000\text{-ld-window-r2 } 0$. For each subgenome, we calculated r^2 between pairs of SNP loci within a distance of 30,000 kb. For LD decay analysis, 30,000-kb LD regions covering SNP pairs were divided into bins of 100 kb, and r^2 values were plotted against distance in millions of base pairs. A locally weighted regression (LOESS) curve was drawn to fit the data using second degree locally weighted scatterplot smoothing in the R program. The genetic distance corresponding to $LD \leq 0.1$ was considered as the critical distance spanning a QTL. All of the high confidence-associated loci that were included within half-decay distances on the same chromosome were defined as the same QTL.

Stripe rust assessment and analysis of phenotypic data. All accessions were evaluated at the seedling stage with the *Pst* races in 2016 and 2017. Eight to 10 seeds of each accession were planted in a single well of 5×10 -well trays (each well size: $5 \times 5 \times 10$ -cm depth). Seedlings at the two-leaf stage (14 days after planting) were inoculated with urediniospores mixed with talc at a ratio of approximately 1:20 and then, incubated in a dew chamber at 10°C for 24 h in darkness before being moved to a rust-free greenhouse at $17 \pm 2^\circ\text{C}$ with a 14-h light (22,000 lx) daily cycle. Stripe rust reactions of individual accessions were recorded based on a 0 to 9 infection-type (IT) scale (Line and Qayoum 1992) when AvS and MX169 showed full sporulation at 18 to 20 days postinoculation. Stripe rust reactions were assessed at least twice. Accessions with IT scores of 0 to 3 were retested with the same race to validate resistant responses. Statistical analyses were performed on the phenotypic data using the basic package in R (R Core Team 2014). Analysis of variance (ANOVA) was used to test for additive variance between races, genotypes, and race $\times$ genotype interactions.

Association analysis. GWAS was conducted using a univariate linear mixed model with GEMMA software (Zhou and Stephens 2012). The threshold for P value was calculated using a modified Bonferroni correction (Genetic type 1 Error Calculator, version 0.2) with a suggestive threshold of P value = $1/Ne$ (Ne = effective SNP number) (Li et al. 2012a, b). The suggestive P -value thresholds ranged from 1.29×10^{-4} to 8.6×10^{-4} for each chromosome (Table 2), and a mean value of 2.90×10^{-4} was adopted as the criterion for genome-wide significance. Significant markers from the GWAS were visualized using Manhattan plots, and P -value

distributions were visualized by quantile-quantile plots, both drawn by the qqman package in R 3.0.3 (<http://www.r-project.org/>). The phenotypic variance explained (R^2) by significant SNPs was evaluated by genome-wide complex trait analysis (GCTA) software (Yang et al. 2011).

Comparisons with previously published *Yr* genes and QTL. To determine the relationships between significant loci identified in the GWAS and previously reported *Yr* genes/QTL, we compared the physical locations of loci based on the Chinese Spring reference. For previously reported *Pst* resistance genes/QTL, the closest flanking markers were used to generate confidence intervals (Bulli et al. 2016; Chen and Kang 2017; Maccaferri et al. 2015). Whether loci identified in the GWAS were new depended on the haplotype block interval. Moreover, to validate the GWAS results, the markers

linked to previously identified *Yr* genes or QTL detected in this study were also incorporated into the GWAS analyses. Three recombinant inbred line (RIL) populations from our previous studies were used for genetic linkage analysis of *YrCEN* (Mu et al. 2019a, b), *YrNP63* (Wu et al. 2017a, b), and *YrRC* (Wu et al. 2018a, b).

Results

Genetic diversity, population structure, and LD analysis. After removing low-quality SNP markers (MAF < 0.05 and missing data > 0.1), a total of 383,621 SNPs were identified in the 410 accessions (Table 2). Available position assignments of 377,666 were distributed across all 21 chromosomes. The B genome showed the highest number of SNPs (167,579) followed by the A (152,380) and D

Fig. 1. Population structure of 410 wheat accessions. **A**, Geographic distribution of the global wheat germplasm collection. **B to D**, Subgroups inferred by Ward's clustering, kinship, and structure analysis. Vertical lines indicate genetic similarity thresholds used to classify accessions into two main groups (dashed line) and five subgroups (dotted lines). **C**, The 410 × 410 kinship matrix based on simple matching of genetic similarities (identity by state [IBS]). Separations among Ward-based groups are shown as the horizontal dashed line for main groups and dotted lines for subgroups. **D**, Matrices of membership coefficients of accessions corresponding to two to five hypothetical subpopulations derived from the STRUCTURE analysis. **E**, Genome-wide average linkage disequilibrium (LD) decay over physical distances. Plots of pairwise single-nucleotide polymorphism LD r^2 values as a function of intermarker map distance (millions of base pairs) based on IWGSC RefSeq v.1.0. Different colored curves represent the fits to LD decay of A, B, D, and the whole genome. The black bold line represents the population-specific critical r^2 value (0.1) beyond which LD is likely owing to linkage.

(57,707) genomes. The map lengths of subgenomes A, B, and D were 4,934.47, 5,179.38, and 3,950.83 million base pairs (Mb), respectively, corresponding to average marker densities of 30.88, 32.36, and 14.61 markers per 1 Mb, respectively. The highest marker density varied among chromosomes at a minimum of 11.13 markers per 1 Mb for chromosome 4D and a maximum of 49.35 markers per 1 Mb for 3B. The mean values of $H_{e_{exp}}$ and PIC for the three subgenomes ranged from 0.53 to 0.71 and from 0.22 to 0.28, respectively. Among chromosomes, 5B had the highest $H_{e_{exp}}$ (0.76) and PIC (0.30) values; in contrast, chromosome 4D exhibited the lowest values (0.47 and 0.20, respectively). Approximately 75% of markers had PIC values exceeding 0.20, demonstrating their informativeness.

Population structure was also determined using STRUCTURE V2.3.4 with all cleaned SNPs according to the ΔK method of Bayesian clustering. In this study, growth habit traits (vernalization requirement) and geographical origin (Africa, Americas, Asia, Europe, and

Oceania) were major factors determining diversity in this panel. The population was first structured into two main subpopulation groups based on vernalization requirement (winter versus spring) (Fig. 1B to D). Five subpopulations proved to be the best grouping (henceforth referred to as Sp1 to Sp5) (Fig. 1B to D and Supplementary Table S1). Sp1 containing 56 spring accessions was well represented by the AvS *Yr* single-gene lines and accessions from Australia. Sp2 consisting of 67 spring accessions mainly included landraces and cultivars from Asia (India, Pakistan, Turkey, and Iran) and Africa (Ethiopia and Egypt). Sp3 comprised 132 spring accessions mainly from Europe (Italy, France, and Germany), the Americas (Mexico, the United States, Chile, and Brazil), and North Africa (Morocco). Sp4 consisting of 46 winter accessions was mainly represented by accessions from Eastern Europe (Czech Republic, the former Yugoslavia, Poland, and the former East Germany) and Western Asia (Turkey). Sp5 including 109 winter accessions was mainly from

Table 1. Virulence (V) and avirulence (A) of *Puccinia striiformis* f. sp. *tritici* races used in this study and their virulence spectra on *Yr* single-gene lines based on Avocet S, Chinese differential cultivars, and other *Yr* gene donor lines at seedling stage

Accession name	Postulated gene	CYR23	CYR29	CYR31	CYR32	CYR33	CYR34	V26/CH42	V26/Lab	V26/SC	V26/SX
Avocet S	Unknown	V	V	V	V	V	V	V	V	V	V
AvSYr1NIL	<i>Yr1</i>	V	V	V	V	V	V	A	V	V	V
AvSYr5NIL	<i>Yr5</i>	A	A	A	A	A	A	A	A	A	A
AvSYr6NIL	<i>Yr6</i>	A	A/V	V	V	V	V	V	V	V	V
AvSYr7NIL	<i>Yr7</i>	V	V	V	V	V	V	V	V	V	V
AvSYr8NIL	<i>Yr8</i>	V	V	V	V	A	V	V	V	A	V
AvSYr9NIL	<i>Yr9</i>	A	V	V	V	V	V	A	V	V	V
AvSYr10NIL	<i>Yr10</i>	A	A	A	A	A	V	V	V	V	V
AvSYr15NIL	<i>Yr15</i>	A	A	A	A	A	A	A	A	A	A
AvSYr17NIL	<i>Yr17</i>	A	V	V	V	V	V	V	V	V	V
AvSYr18NIL	<i>Yr18</i>	V	V	V	V	V	V	V	V	V	V
AvSYr24NIL	<i>Yr24</i>	A	A	A	A	A	V	V	V	V	V
AvSYr26NIL	Unknown	A	A	V	V	V	V	V	V	V	V
AvSYr27NIL	<i>Yr27</i>	A	A	A	V	A	V	A	V	V	V
AvSYr28NIL	<i>Yr28</i>	A	A	V	V	V	V	V	V	A	V
AvSYr29NIL	<i>Yr29</i>	V	V	V	V	V	V	V	V	V	V
AvSYr31NIL	<i>Yr31</i>	V	V	A	V	V	V	V	V	V	V
AvSYr32NIL	<i>Yr32</i>	A	A	A	A	A	V	A	V	A	A
AvSYr43RIL	<i>Yr43</i>	A	A	V	V	V	V	V	V	V	V
AvSYr44RIL	<i>Yr44</i>	V	V	V	V	V	V	V	V	V	V
AvSYrExp2RIL	<i>YrExp2</i>	V	V	V	V	V	V	V	V	V	V
AvSYrSPNIL	<i>YrSP</i>	A	A	V	V	V	V	A	V	V	V
AvSYrTr1NIL	<i>YrTr1</i>	A	A	A	A	A	A	A	V	A	A
Trigo Eureka (CD1) ^a	<i>Yr6</i>	V	V	V	V	V	V	A	V	V	A
Fulhard (CD2)	Unknown	V	V	V	V	V	V	V	V	V	V
Baochun-128 (CD3)	Unknown	V	V	V	V	V	V	V	V	V	V
Nanda-2419 (CD4)	Unknown	V	V	V	V	V	V	V	V	V	V
Virgilio (CD5)	<i>YrVir1</i> , <i>YrVir2</i>	A	V	V	V	V	V	V	V	V	V
Abbondanza (CD6)	Unknown	V	V	V	V	V	V	V	V	V	V
Early-Premium (CD7)	Unknown	V	V	V	V	V	V	V	V	V	V
Funo (CD8)	<i>YrA</i> , +	V	V	V	V	V	V	V	V	V	V
Danish-1 (CD9)	<i>Yr3</i>	V	V	V	V	V	V	A	V	V	A
Jubileina-II (CD10)	<i>YrJu1</i> , <i>YrJu2</i> , <i>YrJu3</i> , <i>YrJu4</i>	A	A	A	V	V	V	V	V	V	V
Fengchan-3 (CD11)	<i>Yr1</i>	V	V	V	V	V	V	V	V	V	V
Lovrin-13 (CD12)	<i>Yr9</i> , +	A	V	V	V	V	V	A	V	V	V
Kangying-655 (CD13)	<i>Yr1</i> , <i>YrKy1</i> , <i>YrKy2</i>	A	A	A	V	V	V	V	V	V	V
Suwon-11 (CD14)	<i>YrSu</i>	A	A	V	V	V	V	V	V	V	V
Zhong-4 (CD15)	Unknown	A	A	A	A	A	A	A	A	A	A
Lovrin-10 (CD16)	<i>Yr9</i>	A	V	V	V	V	V	A	V	V	V
Hybrid46 (CD17)	<i>Yr4b</i> , <i>YrH46</i>	A	A	V	V	A	V	A	A	V	A
<i>Triticum spelta</i> (CD18)	<i>Yr5</i>	A	A	A	A	A	A	A	A	A	A
Guinong-22 (CD19)	<i>Yr26</i>	A	A	A	A	A	V	V	V	V	V
Siete Cerros T66	<i>Yr2</i>	A	V	V	V	V	V	V	V	V	V
Hugenoot	<i>Yr25</i>	A	A	A	A	V	V	A	A	V	V
92R137	<i>Yr26</i>	A	A	A	A	A	V	V	V	V	V
Pindong 34	<i>Yr61</i>	A	A	A	A	A	A	A	A	A	A
CH786	<i>Yr69</i>	A	A	A	A	A	A	A	V	A	A
Tyee	<i>Yr76</i>	A	A	A	A	A	V	V	V	V	V

^a CD, Chinese differential host.

Western Europe (France, Italy, Spain, and the United Kingdom) and North America (the United States and Canada). The results from Ward's clustering, genotypic similarities, and subpopulation coefficients based on STRUCTURE are shown in Figure 1B and C. The STRUCTURE membership coefficients revealed a high degree of admixture in a large number of accessions, particularly among cultivars and modern breeding materials. This result was consistent with the widespread germplasm exchange that characterized the period from the Green Revolution to the early 2000s.

The extent of LD was estimated using 152,380, 167,579, and 57,707 SNP loci from the A, B, and D subgenomes, respectively. LD analysis was assessed based on the 717,701,068 pairwise comparisons from 377,666 SNPs, and pairwise LD was estimated using the squared allele frequency correlation (r^2). Plots of LD estimation (r^2) as a function of physical distance (millions of base pairs) indicated clear LD decay with increasing physical distance. A critical value for significance of r^2 was selected as 0.1, and the point in the curve that intercepted the critical r^2 was determined as the average LD decay. The average LD decay distance for the whole genome was approximately 4.0 Mb; the lowest LD decay distance was 2.2 Mb for the D genome and about 2.5 and 4.9 Mb for the A and B genomes, respectively (Fig. 1E). Significant associated loci within a 4.0-Mb genomic region in the same chromosome were regarded as the same QTL block.

Seedling response. In all seedling experiments, the panel of wheat accessions had significantly different IT scores (Supplementary Table S1). The susceptible checks AvS and MX169 consistently had susceptible reactions (IT 9). Thirty-one accessions displayed resistance to all tested races. Among them, 13 accessions showed stable, high-level resistance (IT 0 to 3) to all races (Supplementary Table S1). Based on ANOVA of single-race seedling tests, there were highly significant differences ($P < 0.0001$) among races, genotypes, and race $\times$ genotype interactions, indicating race-specific resistance. The distributions of IT exhibited bimodal distributions (Fig. 2) consisting of resistant (IT from 0 to 6) and susceptible (IT from 7 to 9) peaks. The proportion of susceptible accessions with pre-V26 races of CYR23, CYR29, CYR31, CYR32, and CYR33 ranged from 38 to 58%. With the V26 races (V26/CH42, V26/Lab, V26/SC, V26/SX,

and V26/TS), 59 to 71% of accessions were susceptible (IT 7 to 9), 9 to 21% were resistant (IT 0 to 3), and 19 to 25% were moderately resistant (IT 4 to 6) (Fig. 2).

Genome-wide association analyses. GWAS based on the univariate linear mixed model was performed on the IT values for each race. Significant SNP trait associations using the suggestive threshold P value $< 2.90 \times 10^{-4}$ ranged from 108 to 1,144 (Supplementary Table S1). There were 35 high-confidence QTL associated with seedling resistance to at least two races ($P < 0.001$). These were distributed on all chromosomes except 3D and 4D (Figs. 3 and 4 and Table 3). For convenience, tagged SNPs for each QTL were

Fig. 2. Box plot distributions of seedling infection type (IT) for 10 races of *Puccinia striiformis* f. sp. *tritici* (*Pst*). Solid horizontal lines show medians. The half violin plots show the seedling-stage resistance density distribution in the wheat panel tested with 10 *Pst* races. The raw data points overlying the box plots display the frequency of infection types of wheat accessions tested with different *Pst* races. The IT scores were also placed in three groups; R (0 to 3), MR (4 to 6), and S (7 to 9). The frequency values of different groups are shown in parentheses.

Table 2. Genetic diversity parameters and suggestive threshold of P values for 410 wheat accessions for subgenomes and individual chromosomes

Chromosome	No. of markers	Effective no.	Suggestive P value	Markers (%)	Length (Mb)	Marker density	He_{exp}^a	PIC ^b
1A	25,780	5,684	1.76E-04	6.83	594.02	43.40	0.70	0.28
2A	26,455	6,004	1.67E-04	7.00	780.76	33.88	0.67	0.27
3A	18,265	4,514	2.22E-04	4.84	750.73	24.33	0.67	0.27
4A	21,308	4,379	2.28E-04	5.64	744.54	28.62	0.67	0.27
5A	21,354	4,853	2.06E-04	5.65	709.76	30.09	0.75	0.30
6A	14,187	3,616	2.77E-04	3.76	617.97	22.96	0.72	0.29
7A	25,031	6,354	1.57E-04	6.63	736.69	33.98	0.68	0.27
1B	19,274	4,968	2.01E-04	5.10	689.38	27.96	0.70	0.28
2B	25,764	6,945	1.44E-04	6.82	801.25	32.15	0.71	0.29
3B	40,996	7,752	1.29E-04	10.86	830.70	49.35	0.59	0.24
4B	12,790	2,731	3.66E-04	3.39	673.47	18.99	0.73	0.29
5B	30,625	6,340	1.58E-04	8.11	713.02	42.95	0.76	0.30
6B	22,140	5,778	1.73E-04	5.86	720.95	30.71	0.73	0.29
7B	15,990	3,641	2.75E-04	4.23	750.61	21.30	0.72	0.29
1D	8,812	2,421	4.13E-04	2.33	495.44	17.79	0.58	0.24
2D	9,456	3,123	3.20E-04	2.50	651.81	14.51	0.59	0.24
3D	7,092	6,634	4.81E-04	1.88	615.48	11.52	0.53	0.22
4D	5,675	1,163	8.60E-04	1.50	509.85	11.13	0.47	0.20
5D	9,023	2,544	3.93E-04	2.39	566.04	15.94	0.52	0.22
6D	7,400	2,351	4.25E-04	1.96	473.56	15.63	0.54	0.22
7D	10,249	1,033	9.07E-04	2.71	638.65	16.05	0.51	0.21
A genome	152,380			40.35	4,934.47	30.88	0.69	0.28
B genome	167,579			44.37	5,179.38	32.36	0.71	0.28
D genome	57,707			15.28	3,950.83	14.61	0.53	0.22
Total	377,666		2.90E-04		14,064.68	26.85	0.64	0.26

^a He_{exp} , expected heterozygosity.

^b PIC, polymorphism information content.

selected based on the strongest association with stripe rust response considering the smallest marker-wise P value, the largest phenotypic variance explained (R^2) by the marker, and the largest number of races to which a significant association was detected by the marker. The 35 loci (SNP-tagged QTL) were present on the specific chromosome arms according to the 660K SNP genetic and physical maps (Table 3). The frequency of marker alleles associated with resistance ranged from 0.03 to 0.62, although marker alleles are not necessarily indicative of functional resistance alleles. Only 2 of the 35 most significant loci had relatively high allele frequencies of >50% across accessions. The phenotypic variation explained (PVE) by individual QTL ranged from 0.9 to 6.7%, and the total value of PVE contributed by all QTL was 53 to 75%. Fifteen loci conferred resistance to all V26 races; eight of these QTL conferred resistance to all races, and their cumulative PVE values were 17 to 35%. Seventeen of the 35 loci were potentially new based on unique chromosome locations when referenced to the consensus and physical maps.

Comparison and confirmation of *Yr* resistance genes or QTL. Diagnostic marker data for *Yr9*, *Yr10*, *Yr26*, and *Yr81* were incorporated into the current GWAS. Because of low allele frequencies, markers for *Yr5* (1%), *Yr15* (0.2%), and *Yr17* (2%) were excluded. As expected, at resistance allele frequencies of >5%, *Yr9*, *Yr10*, *Yr26*, and *Yr81* showed the most significant associations with specific races (Table 3). These results validated the effectiveness of the univariate linear mixed model for stripe rust resistance data of this collection. Three loci on 1DS (*SNP8*), 2BS (*SNP11*), and 4BL (*SNP18*) were mapped in previous studies. *QYr.nwafu-2DS.2* conferred resistance to specific V26 races, and it was consistent with *YrCEN* from German cultivar Centrum (Mu et al. 2019a). SNPs

AX-94487286 (*QYr.nwafu-2BS.2*) and *AX-89362089* (*QYr.nwafu-4BL*) were closely linked with *YrNP63* and *YrRC*, respectively. These genes were defined as adult plant resistance (APR) genes, but they had small effects on seedling response in our previous studies (Wu et al. 2017a, b; 2018a). Therefore, the loci on 2BS and 4BL identified by GWAS were likely to be *YrNP63* and *YrRC*, respectively.

Alignment of putative QTL to previously reported *Yr* genes and QTL. As a matter of convenience, these 35 loci identified by this GWAS were classified into seven homologous groups for specific description based on the reference genomes of Bulli et al. (2016), Chen and Kang (2017), and Maccaferri et al. (2015) that are shown in Figure 4.

Group 1. *QYr.nwafu-1AS* (*AX-109565026*, *SNP1*) was located at 28.72 Mb in chromosome 1AS and conferred resistance to four of the five V26 races. *QYr.Ma.wgp-1AS* with seedling resistance was mapped at 9.10 Mb on chromosome 1AS (Liu et al. 2018). Although *QYr.nwafu-1AS* is about 20 Mb away from *QYr.Ma.wgp-1AS*, the relationship between them needs to be further studied. *QYr.nwafu-1AL* (*AX-108783897*, *SNP2*) and *QYr.wsu-1A.2* (*IWA3215*) were both located within the confidence interval of *QYr.tam-1AL* (Basnet et al. 2014). However, they were probably different because *QYr.nwafu-1AL* (556.30 Mb) was about 37 Mb away from *QYr.wsu-1A.2* (593.30 Mb), and *QYr.tam-1AL* conferred APR. More than 50 SNPs covering almost the entire chromosome 1BS were detected, and further LD analysis combined with race specificity suggested that this large genomic region harbored four seedling resistance loci designated as *QYr.nwafu-1BS.1*, *QYr.nwafu-1BS.2*, *QYr.nwafu-1BS.3*, and *QYr.nwafu-1BL*. *QYr.nwafu-1BS.1* (*AX-109418656*, *SNP3*), *QYr.nwafu-1BS.2* (*AX-108898523*, *SNP4*), and *QYr.nwafu-1BL*

Fig. 3. Genome-wide association analysis results for seedling response to 10 races of *Puccinia striiformis* f. sp. *tritici*. Quantile-quantile and Manhattan plots of significantly associated single-nucleotide polymorphisms. The horizontal lines show the genome-wide significant threshold $-\log_{10}(P)$ value of 3.5. The A, B, and D genomes are in blue, green, orange, and purple, respectively.

Group 2. *QYr.nwafu-2AS* (AX-108928357, *SNP9*) conferred resistance to all tested races. *QYr.nwafu-2AS*, *Yr17*, *Yr56*, *Yr69*, and *YrR61* almost cluster to the terminal region of chromosome 2AS. *Yr17* is no longer effective against Chinese *Pst* races (Wu et al. 2016; Zeng et al. 2014); *Yr69*, putatively derived from *Thinopyrum ponticum*, conferred resistance to most races but not to V26-Lab (Table 1). *YrR61* identified in the soft winter wheat cultivar Pioneer 26R61 displayed APR (Hao et al. 2011b). *Yr56* with all-stage resistance (ASR; also known as seedling resistance) was characterized in durum accession number AUS 91575 (Bansal et al. 2011). Further allelism analysis needs to be performed for confirmation of their relationship. *QYr.nwafu-2BS.1* (AX-95244139, *SNP10*) should be different from *Yr27*, *Yr31*, and *Yr41* because it also exhibited consistent resistance to all tested races compared with *Yr27*, *Yr31*, and *Yr41*, which are ineffective in China (Wu et al. 2017a, b). *QYr.nwafu-2BS.2* (AX-94487286, *SNP11*) turned out to be related to *YrNP63* based on our previous study. Although *QYr.nwafu-2BL* (AX-94915683, *SNP12*) was approximately 10 Mb from ASR gene *Yr72* in AUS27506 (Robert McIntosh, personal communication), the confidence intervals overlapped based on the integrated genetic map (Fa Cui, personal communication). Therefore, *QYr.nwafu-2BL* might be *Yr72*. Because of lack of information of marker positions, the relationship between *QYr.nwafu-2DS* (AX-110483654, *SNP13*)

Group 5. *QYr.nwafu-5AS* (AX-89369801, *SNP19*) was located at 26.45 Mb in chromosome 5AS and thus, seems to be a new resistance gene. *QYr.nwafu-5AL* (AX-108821981, *SNP20*), located at 538.85 Mb in chromosome 5AL, conferred resistance to all 10 races with PVE of 3.6 to 4.8%. *QYr.nwafu-5AS* was likely to be different from *Yr34/48* located at 702.50 Mb (Qureshi et al. 2018). *QYr.nwafu-5BS* (AX-95658136, *SNP21*) was mapped in the same region as *Yr47* in Iranian common wheat landraces AUS28183 and AUS28187 (Bansal et al. 2011). *OYr.nwafu-5BL* (AX-111247705, *SNP22*) was

among a large number of QTL covering almost the entire chromosome 5BL. *QYr.nwafu-5DS* (AX-89648518, *SNP23*) spanned the confidence intervals of ASR genes *Yr40* and *Yr70* derived from *Aegilops geniculata* and *Aegilops umbellulata*, respectively.

Group 6. *QYr.nwafu-6AS* (AX-109992036, *SNP24*) spanned the confidence interval of *Yr81* in the Chinese landrace Aus27430 (Gessese et al. 2019). *QYr.nwafu-6AS* with a large PVE of 3.9 to 7.1% showed resistance to all tested Chinese races, and *Yr81* exhibited a median level of resistance (IT 4 to 6) to currently prevalent Australian *Pst* races. Moreover, the KASP marker *KASP_3077* associated with *Yr81* was assayed on the diversity panel, and it

showed a significant association with stripe rust resistance. Most important of all, the *Yr81* carrier was from Chinese landrace, and therefore, we can perform molecular detection of *Yr81* in Chinese landraces. The Chinese landraces with adapted agronomic backgrounds can mitigate linkage drag effects, which will rapidly facilitate the process of gene introgression and pyramiding into existing elite cultivars. *QYr.nwafu-6AL* (AX-108849013, *SNP25*) was >100 Mb from the nearest gene *YrLM168*, which was identified in a Chinese breeding line (Feng et al. 2014). *QYr.nwafu-6BS* (AX-94832659, *SNP26*) was located in a resistance gene-rich region, and tests of allelism will be required to resolve their relationships. *QYr.nwafu-6BL*

Table 3. Significant quantitative trait locus (QTL) for seedling resistance to races of *Puccinia striiformis* f. sp. *tritici*

QTL name	Chr. ^a	Tag marker ^b	Genetic position (cM) ^b	Physical position (Mb)	R allele and its ratio ^c	−log ₁₀ (P)	R ² (%)	Race	Postulated or linked genes
<i>QYr.nwafu-1AS</i>	1AS	AX-109565026 (<i>SNP1</i>)	34.28	28.72	<u>C</u> /T (0.26)	3.98–6.96	1.5–3.0	CYR23, CYR29, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>YrMa?</i> (<i>QYrMa.wgp-1AS</i>)
<i>QYr.nwafu-1AL</i>	1AL	AX-108783897 (<i>SNP2</i>)	87.74	556.30	<u>A</u> /G (0.08)	4.20–5.01	1.6–2.9	CYR29, CYR32, CYR33, CYR34	None
<i>QYr.nwafu-1BS.1</i>	1BS	AX-109418656 (<i>SNP3</i>)	21.61	17.54	<u>A</u> /C (0.11)	3.96–4.18	3.1–4.4	CYR23, CYR29, CYR31, CYR32, CYR33	<i>Yr10</i>
<i>QYr.nwafu-1BS.2</i>	1RS	AX-108898523 (<i>SNP4</i>)	90.21	39.83	<u>A</u> /G (0.32)	3.79–3.98	4.8–6.7	CYR23, V26/CH42	<i>Yr9</i>
<i>QYr.nwafu-1BS.3</i>	1BS	AX-110711112 (<i>SNP5</i>)	44.43	203.10	<u>T</u> /C (0.07)	3.36–5.78	2.6–4.9	CYR23, CYR29, CYR31, CYR32, CYR33, V26/Lab, V26/SX	<i>Yr64?</i>
<i>QYr.nwafu-1BL</i>	1BL	AX-109873988 (<i>SNP6</i>)	45.57	326.00	<u>A</u> /G (0.18)	3.95–6.99	4.4–5.2	CYR23, CYR29, CYR31, CYR32, CYR33	<i>Yr24/26</i>
<i>QYr.nwafu-1DS.1</i>	1DS	AX-94849392 (<i>SNP7</i>)	1.24	2.49	<u>G</u> /A (0.15)	4.11–7.23	2.8–6.1	CYR23, CYR29, CYR31, CYR32, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr25?</i>
<i>QYr.nwafu-1DS.2</i>	1DS	AX-108917513 (<i>SNP8</i>)	73.38	134.45	<u>A</u> /G (0.09)	3.89–4.90	1.3–3.6	V26/CH42, V26/Lab, V26/SX, V26/SC	<i>YrCEN</i>
<i>QYr.nwafu-2AS</i>	2AS	AX-108928357 (<i>SNP9</i>)	1.13	1.50	<u>C</u> /T (0.07)	4.00–7.95	1.8–3.3	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr56?</i>
<i>QYr.nwafu-2BS.1</i>	2BS	AX-95244139 (<i>SNP10</i>)	8.55	34.06	<u>T</u> /C (0.11)	4.47–5.29	2.3–4.2	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	None
<i>QYr.nwafu-2BS.2</i>	2BS	AX-94487286 (<i>SNP11</i>)	82.36	153.86	<u>T</u> /A (0.13)	4.10–7.58	0.9–3.0	CYR34, V26/CH42, V26/SX, V26/SC	<i>YrNP63</i>
<i>QYr.nwafu-2BL</i>	2BL	AX-94915683 (<i>SNP12</i>)	203.45	796.80	<u>C</u> /G (0.63)	3.95–5.45	1.7–2.6	CYR23, CYR31, CYR32, V26/SX, V26/SC	<i>Yr72</i>
<i>QYr.nwafu-2DS</i>	2DS	AX-110483654 (<i>SNP13</i>)	1.42	5.92	<u>T</u> /C (0.06)	5.69–7.91	2.8–4.7	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	
<i>QYr.nwafu-3AL</i>	3AL	AX-109327388 (<i>SNP14</i>)	135.63	721.32	<u>C</u> /G (0.07)	3.89–4.67	1.3–3.1	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/SX, V26/SC	Novel
<i>QYr.nwafu-3BS</i>	3BS	AX-110739849 (<i>SNP15</i>)	4.32	8.77	<u>G</u> /A (0.06)	4.37–9.09	1.0–1.8	CYR32, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr57</i>
<i>QYr.nwafu-3BL</i>	3BL	AX-109859163 (<i>SNP16</i>)	185.22	791.26	<u>T</u> /C (0.09)	4.01–5.52	1.2–1.7	CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/SX, V26/SC	<i>Yr80 + Yr82</i>
<i>QYr.nwafu-4AL</i>	4AL	AX-109357331 (<i>SNP17</i>)	111.17	686.38	<u>T</u> /G (0.20)	3.89–6.11	1.0–2.1	CYR33, CYR34, V26/CH42, V26/SX	<i>Yr51 + Yr60</i>
<i>QYr.nwafu-4BL</i>	4BL	AX-89362089 (<i>SNP18</i>)	91.75	640.10	<u>A</u> /C (0.17)	3.98–7.60	1.4–3.4	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel + <i>YrRC</i>

(Continued on next page)

^a Chr., chromosome.

^b Position of each single-nucleotide polymorphism (SNP) based on the 660K wheat consensus map (Cui et al. 2017).

^c Resistance allele for each QTL is underlined with its ratio in this population.

(AX-94925274, SNP27) and *QYr.nwafu-6DL* (AX-109450046, SNP28) were at the ends of 6BL and 6DL, respectively, where no stripe rust resistance genes were reported previously.

Group 7. *QYr.nwafu-7AS* (AX-109436402, SNP29) conferred race-specific resistance to several *Pst* races, whereas *Yr61* conferred ASR to all tested *Pst* races (Table 1), and *Yrxy1* was characterized as high-temperature adult plant (HTAP) resistance (Zhou et al. 2011, 2014a, b). Taking these factors and physical distance into account, it seems that *QYr.nwafu-7AS* is a new resistance gene. *QYr.nwafu-7AL* (AX-108869002, SNP30) should be different from *Yr75* based

on position. Because of its broad spectrum effectiveness, *QYr.nwafu-7AL* is worthy of further attention. *QYr.nwafu-7BS* (AX-111450131, SNP31) was >100 Mb from *Yr6* and *Yr63*, and therefore, it should be unique. *QYr.nwafu-7BC* (AX-94443704, SNP32) was located in a resistance gene-rich segment. *QYr.nwafu-7BL* (AX-109913626, SNP33) overlapped the positions of *Yr2*, *Yr67*, and *YrZH84* and HTAP/APR genes *Yr52*, *Yr59*, and *YrKB*, implying that it was likely one allele of them (Huang et al. 2019; Li et al. 2006; Ren et al. 2012; Xu et al. 2014; Zhou et al. 2014b). Although *QYr.nwafu-7DS* (AX-109802588, SNP34) was very close to multiresistance

Table 3. (Continued from previous page)

QTL name	Chr. ^a	Tag marker ^b	Genetic position (cM) ^b	Physical position (Mb)	R allele and its ratio ^c	−log ₁₀ (P)	R ² (%)	Race	Postulated or linked genes
<i>QYr.nwafu-5AS</i>	5AS	AX-89369801 (SNP19)	62.92	26.45	C/T (0.31)	3.88–4.17	1.0–1.4	CYR23, CYR29, CYR31, CYR32, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel
<i>QYr.nwafu-5AL</i>	5AL	AX-108821981 (SNP20)	14.77	538.85	A/G (0.59)	3.86–4.19	3.6–4.8	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel + <i>Yr34/48</i>
<i>QYr.nwafu-5BS</i>	5BS	AX-95658136 (SNP21)	35.36	40.79	C/G (0.16)	3.91–6.94	1.0–1.2	CYR29, CYR31, CYR32, CYR33, CYR34, V26/SX, V26/SC	<i>Yr47</i>
<i>QYr.nwafu-5BL</i>	5BL	AX-111247705 (SNP22)	62.72	522.83	G/T (0.05)	4.77–6.70	4.8–6.2	CYR34, V26/SX, V26/SC	Novel
<i>QYr.nwafu-5DS</i>	5DS	AX-89648518 (SNP23)	13.64	33.70	T/C (0.10)	3.86–7.80	3.6–5.3	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr70</i>
<i>QYr.nwafu-6AS</i>	6AS	AX-109992036 (SNP24)	47.93	89.65	T/C (0.05)	3.92–6.40	3.9–7.1	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr81</i>
<i>QYr.nwafu-6AL</i>	6AL	AX-108849013 (SNP25)	131.39	562.27	C/T (0.16)	5.05–8.22	2.8–3.3	CYR34, V26/SX, V26/SC	Novel
<i>QYr.nwafu-6BS</i>	6BS	AX-94832659 (SNP26)	109.97	146.84	G/A (0.07)	3.75–4.75	1.4–3.0	CYR23, CYR29, CYR31, CYR32, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel
<i>QYr.nwafu-6BL</i>	6BL	AX-94925274 (SNP27)	204.29	711.76	C/T (0.19)	4.28–5.44	2.2–4.6	CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/SX, V26/SC	Novel
<i>QYr.nwafu-6DL</i>	6DL	AX-109450046 (SNP28)	113.12	457.24	T/C (0.03)	3.99–7.18	2.8–4.6	CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel
<i>QYr.nwafu-7AS</i>	7AS	AX-109436402 (SNP29)	62.81	133.03	C/A (0.07)	3.85–4.32	1.0–1.6	CYR23, CYR31, CYR32, CYR34, V26/Lab, V26/SX, V26/SC	Novel
<i>QYr.nwafu-7AL</i>	7AL	AX-108869002 (SNP30)	259.74	569.40	T/G (0.22)	3.85–7.05	1.3–3.2	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel + <i>Yr75</i>
<i>QYr.nwafu-7BS</i>	7BS	AX-111450131 (SNP31)	72.27	134.02	A/G (0.21)	4.04–4.54	1.1–2.1	CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	Novel
<i>QYr.nwafu-7BC</i>	7BC	AX-94443704 (SNP32)	93.57	329.36	C/T (0.11)	4.14–6.33	1.0–3.6	CYR23, CYR29, CYR31, CYR32, CYR33, V26/CH42, V26/SX	Novel
<i>QYr.nwafu-7BL</i>	7BL	AX-109913626 (SNP33)	231.95	708.96	C/T (0.22)	3.84–5.62	1.1–2.4	CYR23, CYR29, CYR31, CYR32, CYR33, CYR34, V26/CH42, V26/SX, V26/SC	<i>YrZh84</i>
<i>QYr.nwafu-7DS</i>	7DS	AX-109802588 (SNP34)	38.917	45.92	G/A (0.07)	3.35–6.61	1.0–2.8	CYR32, CYR34, V26/CH42, V26/SX, V26/SC	Novel + <i>Yr18</i>
<i>QYr.nwafu-7DL</i>	7DL	AX-111225948 (SNP35)	148.35	619.89	A/T (0.04)	3.74–4.15	1.0–1.7	CYR23, CYR29, CYR31, CYR33, CYR34, V26/CH42, V26/Lab, V26/SX, V26/SC	<i>Yr33?</i>

locus *Yr18*, it should be different because of its seedling resistance. *QYr.nwafu-7DL* (AX-111225948, *SNP35*) was mapped to a similar position as the previously reported *Yr33*.

In summary, several loci were confirmed as reported genes, such as *QYr.nwafu-1BS.1* (*Yr10*), *QYr.nwafu-1BS.2* (*Yr9*), *QYr.nwafu-1BL* (*Yr24/26*), *QYr.nwafu-2BS.2* (*YrNP63*), *QYr.nwafu-2DS.2* (*YrCen*), *QYr.nwafu-4BL* (*YrRC*), and *QYr.nwafu-6AS* (*Yr81*). The remaining QTL seemed to be new, but confirmation will require further studies.

Discussion

To substantially reduce pathogen spread from the oversummer survival region to eastern regions, it is important to characterize and deploy diverse resistance genes in wheat cultivars in southern Gansu, Sichuan Basin, and central Shaanxi (Han and Kang 2018). China is a relatively independent endemic region for stripe rust, and the *Pst* population is different from those in other countries (Stubbs 1985). Thus, we can introduce a large number of exotic wheat germplasms for improvement of stripe rust resistance. Exotic wheat germplasms are precious resources containing genetically abundant diversities, which could help enrich crop breeding pools and sustain improvement of modern cultivars (Hao et al. 2011a, b). Historically in China, exotic wheat germplasms have played an important role in stripe rust resistance breeding (Zhuang 2003). For example, during the 1940s and 1960s, American cultivars Quality, Early Premium, Ceres, and Triumph and Italian cultivars Nanda 2419 (selected from Mentana), Ardito, Abbondanza, Funo, and St1472/506 were widely popularized and became leading varieties, and/or they were also the backbone parents for breeding programs in China's major wheat-growing areas. In the 1970s, wheat cultivars with the 1BL/1RS translocation, such as Neuzucht and Lovrin accessions from Germany and Romania, and semidwarf wheat cultivars from the International Wheat and Maize Improvement Center were introduced to provide resistance, fertility, and semidwarfing genes. Moreover, cultivars Denmark 1, Jubileina I and II (Bulgaria), VPM series (France), Virgilio (Italy), Suwon 11 (Korea), India 798, and so forth were introduced as sources of resistance to stripe rust, and subsequently, cultivars with stripe rust resistance were developed and widely grown. However, the modern breeding system has led to a dramatic loss of genetic diversity in common wheat (Reif et al. 2005). In modern breeding systems, particularly in developing countries, yield is the most important goal, and most elite wheat cultivars are selected based on yield-related indices measured under conditions of high nutrition and optimum field management (Guzmán et al. 2017). Genetic resources from the primary gene pool of wheat are considered a promising approach to identify new and durable resistance sources that can be utilized in improvement of modern high-yielding varieties (Mujeeb-Kazi et al. 2013). In the present study, exotic lines identified as resistant included landraces, cultivars, and breeding lines originating from a range of countries, including the United Kingdom, Germany, France, Italy, Serbia, Ethiopia, Tunisia, the United States, South Africa, and Australia. These resources harboring abundant resistances genes would help enhance genetic diversity in modern wheat breeding programs. Therefore, 16 wheat lines (1748:Ae, Ethiopia-W15, Grifone-235, Kuro, Mishragani, Candeal [S], Penawa, Sample-Gray-1, Trigo-Blanco, Krac66, Vilmorin-27, Tupatecor, CMH83.2715, Fs056, H-159-1, and Leafen) showed resistance to all tested races, indicating the presence of large-effect resistance genes or their combinations.

In China, lack of seedling resistance is considered to be the bottleneck for resistance breeding in upper reaches of stripe rust-endemic areas. Major advantages of performing GWAS using exotic germplasm are exploration of the genetic composition of the widely used breeding materials, validation of effective loci, and discovery of novel loci that are significantly associated with the traits of interest. In the present study, a number of potentially novel or effective genes (including named *Yr* genes) conferring seedling resistance to V26 races were identified in the exotic wheat diverse panel; for example, 33 effective resistance loci, including postulated named genes *Yr25*, *Yr33*, *Yr47*, *Yr51*, *Yr56*, *Yr57*, *Yr70*, *Yr72*, and *Yr81*, were identified

as resistant to post-V26. It is promising that the resistance genes conferring high levels of seedling resistance identified in our study could solve the urgent need of resistance alleles. However, simultaneous consideration of all alleles in a breeding program is often challenging. Moreover, linkage drags always limit the improvement of resistance owing to undesirable traits when using germplasm with poor adaptation and need to be overcome. First, except for disease resistance, many agronomic traits and yield-related traits, including plant height, heading date, anthesis, spikelet number, kernels per spike, kernel weight, and so forth, were investigated in the field to confirm their adaptability (data not shown). In fact, these wheat lines with high resistance to stripe rust in seedling still displayed good resistance in the field (unpublished data). Second, a small subset of resistance alleles has been targeted by crossing with certain susceptible commercial varieties in breeding programs, thus supporting generation of high-value lines that carry favorable alleles of interest and selection of advanced breeding lines (Mu et al. 2019a, b; Wu et al. 2018a, b; Zeng et al. 2019). Third, most of the identified loci were surrounded by multiple clusters of SNPs in this study, and some tightly linked SNPs have been designed as KASP markers, which would contribute for the marker-assisted selection of the corresponding loci in future breeding programs.

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