Genome-Wide Association Mapping of Adult-Plant Resistance to Stripe Rust in Common Wheat (*Triticum aestivum*)

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Abstract

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a globally devastating disease of common wheat (*Triticum aestivum* L.), resulting in substantial economic losses. To identify effective resistance genes, a genome-wide association study was conducted on 120 common wheat lines from different wheat-growing regions of China using the wheat 90K iSelect SNP array. Seventeen loci were identified, explaining 9.5 to 21.8% of the phenotypic variation. Most of these genes were detected in the A (seven) and B (seven) genomes, with only three in the D genome. Among them, 11 loci were collocated with known resistance genes or quantitative trait loci reported previously, whereas the other six are likely

new resistance loci. Annotation of flanking sequences of significantly associated SNPs indicated the presence of three important candidate genes, including E3 ubiquitin-protein ligase, F-box repeat protein, and disease resistance RPP13-like protein. This study increased our knowledge in understanding the genetic architecture for stripe rust resistance and identified wheat varieties with multiple resistance alleles, which are useful for improvement of stripe rust resistance in breeding.

Keywords: 90K SNP array, GWAS, marker-trait association, *Puccinia striiformis* f. sp. *tritici*, wheat

Stripe rust (yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most destructive fungal diseases in temperate or middle altitude and maritime wheat-growing regions and can significantly reduce grain yield in wheat (*Triticum aestivum* L.) (Stubbs 1985; Wan et al. 2007). Although stripe rust can be controlled by fungicides, this adds a significant cost and causes environmental pollution. Use of resistant cultivars is an economical, effective, and environmentally friendly way to control the disease (Singh et al. 2000).

Stripe rust resistance can be categorized into all-stage (also known as seedling) resistance (race-specific or complete resistance) and adult-plant resistance (APR) (race-nonspecific or partial resistance) (Carter et al. 2009; Singh et al. 2000). Generally, all-stage resistance is conferred by major genes and shows immune or highly resistant responses to specific races throughout the entire growth cycle (Carter et al. 2009; Singh et al. 2000). However, such resistance is usually not durable and is readily overcome by new pathogen races (Carter et al. 2009). In contrast, APR is usually conferred by minor genes and is more likely effective against all *P. striiformis* f. sp. *tritici* races (Singh et al. 2000). Combinations of four to five APR genes can confer a high level of resistance that is more durable than race-specific genes (Chen 2005; Singh et al. 2005). For example, *Yr18* in combination with

two to four minor genes has provided durable resistance to stripe rust globally for nearly 100 years (Krattinger et al. 2009). To achieve long-term control of stripe rust, APR has been increasingly emphasized in wheat breeding for its race nonspecificity and durability (Fu et al. 2009; Li et al. 2014; Rosewarne et al. 2013; Singh et al. 2005).

Traditional linkage mapping is widely used to identify quantitative trait loci (QTLs) underlying complex traits. Rosewarne et al. (2013) summarized about 140 QTLs for stripe rust resistance distributed across 49 chromosomal regions. However, QTLs identified in biparental populations represent only a small fraction of the natural variation, and only two alleles at each locus can be captured in each population (Brescighello and Sorrells 2006; Liu et al. 2017; Zhu et al. 2008). Alternatively, genome-wide association study (GWAS) provides a powerful approach for QTL mapping and has been widely used to identify genomic regions for complex quantitative traits because it can capture a larger portion of the natural variation present in diverse panels (Brescighello and Sorrells 2006; Zhu et al. 2008). Compared with traditional linkage mapping, GWAS has advantages in reducing cost and time for developing biparental populations, permitting a more representative gene pool and a higher mapping resolution, and mapping many traits in one set of genotypes (Brescighello and Sorrells 2006; Flint-Garcia et al. 2003; Zhu et al. 2008). Furthermore, it is feasible to genotype large germplasm collections with high-density molecular markers using 35K (Allen et al. 2011), 90K (Wang et al. 2014; Wen et al. 2017), and 660K (Cui et al. 2017) single-nucleotide polymorphism (SNP) arrays. GWAS has been successfully used to identify QTLs for grain yield (Ain et al. 2015; Rasheed et al. 2014), disease resistance (Liu et al. 2017; Maccaferri et al. 2015), and end-use quality in wheat (Dong et al. 2016).

The objectives of this study were to (i) evaluate the genetic diversity and population structure of the wheat panel, (ii) perform GWAS to identify SNP markers significantly associated with stripe rust resistance, and (iii) search for candidate genes for further study.

Materials and Methods

Plant materials. The association panel comprised 120 wheat accessions mainly from different regions of China, including 54 modern cultivars, 54 advanced lines, and 12 landraces (Supplementary Table S1).

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Seedling tests. Seedling tests for stripe rust response were conducted under controlled greenhouse conditions according to the method of Li and Zeng (2002). Seedlings of 120 wheat accessions were evaluated using prevalent *P. striiformis* f. sp. *tritici* races CYR32, CYR34, and a mixture of CYR32, CYR33, CYR34, Zhong 4-1, Shuiyuan 11-4, Shuiyuan 11-7, Hybrid 46-4, and Hybrid 46-8, respectively. Mingxian 169 was used as a susceptible control. Seeds of each accession were grown in pots of 10 cm diameter. After seeds were planted for about 10 days (the first leaf was fully expanded), seedlings were inoculated with fresh urediniospores of the above *P. striiformis* f. sp. *tritici* races mixed with talc at a 1:20 ratio. After inoculation, the seedlings were placed in a dew chamber at 9°C and 100% relative humidity for 24 h and then transferred to a greenhouse maintained in a 14-h light and 10-h dark cycle at 14 to 17°C. Infection types (ITs) were scored about 2 weeks after inoculation, when rust was fully developed on the control Mingxian 169, based on the 0 to 4 scale (Bariana and McIntosh 1993; Li et al. 2006). Plants with IT 0 to 2 were considered resistant and those with IT 3 to 4 susceptible (Bariana and McIntosh 1993; Li et al. 2006).

Field trials and phenotypic evaluation. All 120 accessions were evaluated for response to stripe rust at the Pixian Experimental Station (30°05'N, 102°54'E), Sichuan Academy of Agricultural Sciences, and at the Gangu Experimental Station (34°45'N, 105°20'E), Gansu Academy of Agricultural Sciences, during the 2014 to 2015 and 2015 to 2016 cropping seasons, and at Zhongliang Experimental Station (34°36'N, 105°39'E), Tianshui Institute of Agricultural Science in Gansu province, during the 2014 to 2015 cropping season. All three locations are hotspots for stripe rust in China, having favorable conditions for the disease. Field trials were conducted in randomized complete blocks with three replications. Each plot contained three 1.5-m rows spaced 20 cm apart. Every 10th row was planted with the highly susceptible control, Huixianhong. Inoculations in the field were performed by spraying a mixture of prevalent Chinese *P. striiformis* f. sp. *tritici* races, including CYR32, CYR33, and CYR34, at the jointing stage.

Maximum disease severities (MDSs) were scored 18 to 20 days postflowering when stripe rust severities on the control cultivar Huixianhong reached a maximum level. The MDS from each environment and the best linear unbiased predictor (BLUP) across the five environments for each accession were used for subsequent analysis.

Statistical analysis. Analysis of variance (ANOVA) was performed using SAS version 9.3 (SAS Institute, Cary, NC). Broad sense heritability (h_b^2) of MDS was calculated according to the method of Liu et al. (2017).

Genetic diversity, population structure, and linkage disequilibrium (LD). Total genomic DNA was extracted using a modified cetyltrimethylammonium bromide method (Saghai-Marouf et al. 1984). All 120 accessions were genotyped using the wheat 90K SNP chip (Wang et al. 2014) by Compass Biotechnology (Beijing, China; <http://www.kangpusen.com/>). To ensure call accuracy, SNP clustering was validated visually. Genetic diversity (D), polymorphism information content (PIC), and minor allele frequency (MAF) were computed by PowerMarker version 3.25 (Liu and Muse 2005). To avoid spurious marker-trait associations (MTAs), SNP markers with MAF < 0.05 and missing data > 20% were excluded from further analyses. The details of the consensus genetic map were obtained from Wang et al. (2014), whereas the physical positions of SNP markers from the wheat 90K array were obtained from the International Wheat Genome Sequencing Consortium website (IWGSC, <http://www.wheatgenome.org/>). Genetic diversity (D) was calculated following Botstein et al. (1980):

$$\hat{D}_l = 1 - \sum_{i=1}^k \hat{p}_{li}^2$$

and the PIC value was estimated using the following formula:

$$\widehat{PIC}_l = 1 - \sum_{u=1}^k \hat{p}_{lu}^2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^k 2\hat{p}_{lu}\hat{p}_{lv}$$

where P_{lu} and P_{lv} are the frequencies of the u^{th} and v^{th} alleles for marker l , respectively. To ensure the accuracy of MTAs, SNPs with

missing data > 20% and MAF < 5% were excluded in subsequent analysis.

Population structure was analyzed using 1,000 polymorphic SNP markers (one marker/17 Mb) from the 90K SNP array with Structure version 2.3.4 (Botstein et al. 1980) following the method of Liu et al. (2017). Principal component analysis (PCA) and neighbor-joining (NJ) trees were also used to validate population stratification with the software Tassel version 5.0 (Bradbury et al. 2007) and PowerMarker version 3.25 (Liu and Muse 2005), respectively. LD among markers were calculated by Tassel version 5.0 with 38,841 SNP markers following the method of Liu et al. (2017).

Genome-wide association analysis and in silico annotation of SNPs. To control background variation and eliminate spurious MTAs, GWAS for stripe rust response was analyzed using the mixed linear model (MLM) (Q+K) by Tassel version 5.0. Markers with an adjusted $-\log_{10}(P) \geq 3.0$ were regarded as significant for stripe rust response. Loci identified in at least three environments were included in the results. Manhattan plots and quantile-quantile (Q-Q) plots were drawn using the CMplot package in R language (<https://github.com/YinLiLin/R-CMplot>). Alleles with positive effects for lower MDS were referred to as “favorable alleles”, whereas those for higher MDS were “unfavorable alleles” at each locus. The frequencies of favorable and unfavorable alleles and their allelic effects were calculated based on the representative SNPs at the resistant loci.

The following steps were conducted to identify candidate genes for important loci. First, all genes located in a 100-Kb interval around the peak SNP (± 50 kb) of each important locus were found from IWGSC version 1.0 (<http://www.wheatgenome.org/>). Then, all available SNPs located inside of these genes were searched. These genes (except for expressed proteins, hypothetical proteins, transposon proteins, and retrotransposon proteins) with one or more SNPs in the coding regions that could lead to sense mutations were considered candidate genes for stripe rust resistance.

Results

Variations in stripe rust response in the greenhouse and field.

The 120 wheat accessions were evaluated in the seedling stage for stripe rust resistance in the greenhouse (Supplementary Table S1). Except for a few accessions (such as Aikang 58, Beijing 0045, and Jimai 22 resistant to CYR32), most of the accessions were susceptible to CYR32, CYR34, and the mixed races.

Accessions in this panel showed diverse responses to stripe rust, and a continuous variation of MDS was observed in the field across five environments (Supplementary Table S1). The BLUPs across environments ranged from 7.1 to 83.3% with an average of 40.5% (Supplementary Table S1; Fig. 1; Supplementary Fig. S2), presenting a wide range of reaction across the diverse panel. ANOVA revealed highly significant differences ($P < 0.001$) among the genotypes, environments, and genotype $\times$ environment interactions (Table 1). Broad-sense heritability (h_b^2) for MDS across all environments

Fig. 1. The maximum disease severity for stripe rust evaluated in 120 wheat accessions. Reported values are best linear unbiased predictors across five environments.

was estimated to be 0.64, indicating less environmental variance compared with genotypic variance.

Marker coverage and genetic diversity. After removing SNPs with MAF < 5% and missing data > 20%, 38,841 SNPs were used for subsequent analyses (Supplementary Table S2). The total length of the map was 3,674.16 cM, with a mean genetic distance of 0.095 cM/SNP. Among the SNPs, 14,918 (38.4%), 18,012 (46.3%), and 5,911 (15.3%) were from the A, B, and D genomes, respectively, with corresponding map lengths of 1,297.0, 1,197.9, and 1,325.6 cM, respectively. The B genome had the highest marker density (14.3 SNPs/cM), followed by the A (11.1 SNPs/cM) and D (4.5 SNPs/cM) genomes. The SNP markers showed different levels of genetic diversity, ranging from 0.090 to 0.500 with a mean of 0.351, and PIC ranged from 0.090 to 0.375 with a mean of 0.278. The B genome exhibited the highest genetic diversity and PIC values (0.365 and 0.285, respectively), followed by the A (0.359 and 0.285, respectively) and D (0.329 and 0.266, respectively) genomes (Supplementary Table S2).

Table 1. Analysis of variance of maximum disease severity for stripe rust in 120 wheat accessions

Source of variation	df	Mean square	F value ^a
Replicate (nested in environments)	9	4,106	8.5***
Environment	4	511,350	498.9***
Genotype	119	5,128	36.9***
Genotype × environment	476	782	4.3***
Error	1,650	153	

^a Three asterisks (***) indicates significant at $P < 0.0001$.

Population structure and linkage disequilibrium. A clear peak at $K = 2$ indicated that the optimal population structure for further analyses was to divide this panel into two subgroups, which was consistent with the results of PCA and NJ tree analysis (Fig. 2). Subpopulation 1 included 77 accessions, mainly from Gansu, Beijing, Henan, and Shandong, whereas subpopulation 2 comprised 43 accessions, all from Henan province.

In total, 38,841, 14,918, 18,012, and 5,911 SNPs were used to evaluate LD decay for the whole genome as well as the A, B, and D genomes, respectively. The mean genome-wide LD pattern graphically displayed by scatter plots of pairwise LD r^2 over genetic distance revealed that LD decayed rapidly with increasing genetic map distance (Supplementary Fig. S1). The LD decay distances were about 8, 9, 14, and 9 cM for the A, B, D, and whole genomes, respectively.

MTAs. One hundred eighty-two MTAs for stripe rust resistance at 17 loci (Table 2; Supplementary Table S3) were identified; these were distributed on chromosomes 1A, 1B, 2A, 2D, 3A (two), 3B, 3D, 4A, 4B (two), 5D, 6A, 6B (two), 7A, and 7B, respectively, explaining 9.5 to 21.8% of the phenotypic variations (Table 2; Supplementary Table S3; Fig. 3). Most of these loci were on the A (seven) and B (seven) genomes, followed by the D genome (three). All resistance loci were detected in three or more environments (Table 2; Fig. 3). The Q-Q plots representing expected and observed probabilities of associations of SNPs are presented in Supplementary Figure S3.

Relationship between stripe rust response and number of resistance alleles. To further understand the combined effects of alleles on stripe rust response, we examined the number of favorable and unfavorable alleles in each accession. Individual accessions contained eight to 16 favorable alleles (Fig. 4; Supplementary Table S1).

Fig. 2. Population structure analysis of 120 wheat accessions. **A**, Two subgroups inferred by structure analysis; **B**, neighbor-joining tree; and **C**, principal components (PC) analysis plots. **D**, Estimated ΔK of structure analysis.

Linear regression ($r^2 = 0.87$) showed a dependence of MDS on the numbers of favorable or unfavorable alleles (Fig. 4). Thus, accessions with more favorable alleles and fewer unfavorable alleles, such as Lovrin 13 (12 alleles), Zhou 8425B (13), Zhongmai 175 (13), Lanhangxuan 122 (15), Lantian 25 (15), and Libellula (16), were more resistant to stripe rust.

Discussion

Identification and deployment of new sources of host resistance are essential for developing resistant cultivars for effective control of stripe rust. In this study, we characterized 120 wheat accessions from different ecological regions of China for reaction to stripe rust across multiple field environments to identify SNPs associated with stripe rust reaction and selected highly resistant cultivars to facilitate resistance breeding by marker-assisted selection (MAS).

Knowledge about genetic diversity, population structure, and LD decay is essential for GWAS to identify SNPs significantly associated with quantitative traits (Breseghello and Sorrells 2006; Chen et al. 2012; Lopes et al. 2015; Rasheed et al. 2014). The mean genetic diversity and PIC for these accessions were 0.351 and 0.278, respectively, indicating that this panel had high genetic diversity and was suitable for GWAS. Both the genetic diversity and PIC values were higher for the B genome than for the A and D genomes, in accordance with previous reports (Chen et al. 2012; Gao et al. 2017; Hao et al. 2017).

Structure analysis indicated that this wheat panel could be divided into two subgroups (Fig. 2A), in agreement with PCA (Fig. 2B) and NJ-tree analysis (Fig. 2C). The characterization of the subgroups was largely consistent with geographic origins and pedigrees, with few exceptions. For example, CA0958, CA1119, CA1113, CA0548, CA1090, CA9722, CA1055, and Zhongmai 175 were clustered together with their parent line Jing 411 in subgroup 1. Zhongmai 875 and Zhongmai 895 were derived from Zhoumai 16, and they were clustered in subgroup 2. Many studies have shown that the lack of appropriate correction for population structure can lead to spurious MTAs (Botstein et al. 1980; Breseghello and Sorrells 2006; Chao et al. 2007; Pritchard et al. 2000). Furthermore, PCA showed that the first three principal components explained 30.3% of the total variation. Thus, MLM with both the Q (PCA) and kinship among individuals was used

for GWAS to eliminate spurious MTAs resulting from population structure.

The distance of LD decay determines the marker density needed to perform GWAS and also affects the precision of GWAS (Breseghello and Sorrells 2006; Cormier et al. 2014; Khatkar et al. 2008). The LD decay is influenced by various factors such as population structure, recombination rate, and allele frequency (Chao et al. 2010; Hao et al. 2011; Sukumaran et al. 2015; Yu and

Fig. 3. Circle Manhattan plots for stripe rust resistance in 120 wheat accessions by the mixed linear model in Tassel version 5.0. Environments plotted from inside to outside are Gangu 2015, Gangu 2016, Pixian 2015, Pixian 2016, Zhongliang 2015, and best linear unbiased predictors for maximum disease severity across five environments, respectively. The $-\log_{10}(P)$ values from a genome-wide scan are plotted against positions on each of the 21 chromosomes.

Table 2. Marker-trait association for adult-plant resistance to stripe rust in 120 wheat accessions

Marker ^a	Chr ^b	Position ^c (cM)	Environment ^d	SNP ^e	P value	R ² (%) ^f	Reference
IWB72825	1A	72.6	E1, E2, E4, E6	<u>A/C</u>	3.2E-05	11.3	Bariana et al. (2006)
IWA7141	1BL	115.9	E3, E4, E5	<u>G/A</u>	1.0E-04	9.8	Lin and Chen (2007)
IWB32326	2AS	95.8	E1, E3, E4, E5, E6	<u>A/G</u>	1.8E-06	20.6	
IWB10277	2DL	82.8	E2, E3, E4, E6	<u>A/C</u>	1.6E-05	14.5	Agenbag et al. (2012)
IWB10785	3AS	15.1	E3, E4, E5, E6	<u>A/C</u>	3.8E-04	10.3	Lillemo et al. (2008)
IWB64845	3AL	117.7	E1, E2, E4, E6	<u>A/G</u>	3.1E-05	14.6	
IWB8630	3B	67.7	E1, E3, E4, E6	<u>A/G</u>	1.7E-04	13.6	Hao et al. (2011)
IWB16589	3D	143.0	E1, E2, E4, E6	<u>A/G</u>	4.7E-05	9.5	
IWA3027	4AS	48.5	E1, E2, E3, E4, E5, E6	<u>A/G</u>	2.3E-06	18.2	
IWB65475	4BS	38.9	E3, E4, E5, E6	<u>G/A</u>	3.6E-05	15.1	
IWB71836	4BS	60.4	E1, E2, E4, E5, E6	<u>A/G</u>	1.6E-07	21.8	Zwart et al. (2010)
IWB13808	5DS	67.5	E1, E2, E4, E5, E6	<u>A/G</u>	1.5E-07	19.1	
IWB53520	6A	74.2	E1, E3, E4, E5, E6	<u>A/G</u>	1.0E-04	11.9	Lillemo et al. (2008)
IWA2975	6B	59.9	E1, E2, E4, E5, E6	<u>G/A</u>	2.3E-05	12.6	Bariana et al. (2006)
IWB74862	6BL	111.9	E1, E2, E4	<u>G/A</u>	4.2E-05	15.3	Rosewarne et al. (2012)
IWB19819	7AS	135.8	E1, E2, E3	<u>G/A</u>	9.4E-05	12.9	Ren et al. (2012)
IWB74019	7BL	145.3	E2, E3, E5, E6	<u>A/G</u>	3.5E-05	11.2	Rosewarne et al. (2012)

^a Markers significantly associated with stripe rust resistance (P value $< 10^{-3}$).

^b Chr = chromosome.

^c The genetic positions of SNP markers based on the wheat 90K consensus map (Wang et al. 2014).

^d E1 = Gangu 2015; E2 = Gangu 2016; E3 = Pixian 2015; E4 = Pixian 2016; E5 = Zhongliang 2015; and E6 = BLUPs across all five environments.

^e Favorable allele (single-nucleotide polymorphism, SNP) is underlined.

^f Percentage of phenotypic variance explained by the marker-trait association.

Buckler 2006). In this panel, the LD decay was about 9 cM for the whole genome; however, it extended to 14 cM for the B genome (Supplementary Fig. S1). This suggested that fewer markers are needed for GWAS in the B genome than in the A and D genomes (Chao et al. 2010; Edae et al. 2014; Sukumaran et al. 2015). The marker densities for the A, B, and D genomes were 11.1, 14.3, and 4.5 SNPs/cM, indicating a high mapping resolution and strong QTL detection power for stripe rust resistance loci in the diverse panel.

During the past two decades, more than 150 QTLs/genes for stripe rust resistance in wheat were identified using linkage or association mapping (Rosewarne et al. 2013). Comparison of the QTLs detected in the present study with those reported previously based on the physical map of IWGSC version 1.0 and integrated map of Maccaferri et al. (2015) identified 11 common loci (McIntosh et al. 2017; Rosewarne et al. 2013).

Bariana et al. (2006) found two QTLs for stripe rust resistance on chromosomes 1A (*QRYr1A.1* and *Xgwm164*) and 6B (*QRYr6B.2* and *wPt-8183*) in a doubled haploid population (Kukri × Janz); these overlapped with *IWB72825-1A* and *IWA2975-6B* in the present study. Rosewarne et al. (2012) reported two stripe rust resistance loci on chromosomes 6BL (*QRYr6B.3* and *wPt-6329*) and 7BL (*QRYr7B.3* and *Xgwm577*) in an Avocet/Pastor recombinant inbred line population, corresponding to the loci *IWB74862-6B* and *IWB74019-7B* or *IWB41905-7B* in our study. Lillemo et al. (2008) identified two QTLs on chromosomes 3AS (*QRYr3A.1*, *Xstm844tcac*, and *Xbarc310*) and 6A (*QRYr6A.2* and *Xbarc3*) in

cultivar ‘Saar’, which collocated with *IWB10785-3A* and *IWB53520-6A*. In addition, five loci on chromosomes 1BL (*IWA7141*), 2DL (*IWB10277*), 3B (*IWB8630*), 4BS (*IWB71836*), and 7AS (*IWB19819*) coincided with *QYrex.wgp-1BL* (Lin and Chen 2007), *QRYr2D.2* (*Xgwm102*) (Agenbag et al. 2012), *QRYr3B.1* (*wPt-2557* and *Xbarc133*) (Hao et al. 2011), *QRYr4B.1* (*wPt-8543* and *Xwmc238*) (Zwart et al. 2010), and *QRYr7A.3* (*Xbarc127*) (Ren et al. 2012), respectively. The remaining six loci located on chromosomes 2A (*IWB32326*), 3A (*IWB64845*), 3D (*IWB16589*), 4A (*IWA3027*), 4B (*IWB65475*), and 5D (*IWB13808*) are likely new, which may contribute to better understanding stripe rust resistance and provide more opportunities for MAS breeding. The results confirmed that GWAS is a powerful and reliable tool for identification of complex quantitative genes. The alignment of several stripe rust resistance loci with previous studies also serves as a validation of the accuracy and powerfulness of the GWAS experiment.

Three candidate genes were identified by looking for the 100-Kb intervals around the significantly associated loci (Table 3). Bioinformatics analysis indicated that the SNP marker *IWB20306* on chromosome 1AL corresponds to E3 ubiquitin-protein ligase, which is required for host disease resistance in plants mediated by nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Chen et al. 2017; McHale et al. 2006; You et al. 2016). *IWB19819* on chromosome 7AS is associated with an F-box repeat protein in response to various pathogens through targeting substrates in the degradation machinery (Kim and Delaney 2002; Yin et al. 2017). The gene corresponding to SNP marker *IWB10785* on 3AS encoded a disease resistance RPP13-like protein that was proposed to play an essential role in regulation of responses to a variety of external stimuli, including biotic and abiotic stresses (Hameed et al. 2015; Ramachandran et al. 2017). Although bioinformatics analysis of trait-associated SNPs has proven to be an effective tool to find candidate genes (Ain et al. 2015; Zhai et al. 2016), more detailed experimental analyses are required to confirm the roles of these candidate genes in stripe rust reaction.

We demonstrated the potential of pyramiding favorable alleles to enhance stripe rust resistance. QTLs with consistent effects across multiple environments can be combined with other effective stripe rust genes for more durable resistance (Singh et al. 2000; Veldboom and Lee 1996). Accessions carrying more favorable alleles identified in the present study, such as Zhou 8425B (13 favorable alleles), Lovrin 13 (12), Zhongmai 175 (13), Lanhangxuan 122 (15), Lantian 25 (15), and Libellula (16) can be used as parental lines in breeding programs. All 17 loci identified in this study were detected in three or more environments and are recommended for resistance breeding. Furthermore, Singh et al. (2000) has reported that use of such QTLs as fixed effects in genomic selection models increases the prediction accuracy of genomic selection and could further accelerate the introgression of favorable alleles into breeding material. To efficiently perform MAS, the linked SNPs can be converted into kompetitive allele-specific PCR (Rasheed et al. 2017; Semagn et al. 2014) or semithermal asymmetric reverse PCR (Long et al. 2017) markers. In conclusion, this study has identified new APR genes to stripe rust and provided markers to facilitate wheat breeding for stripe rust resistance.

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Fig. 4. Linear regression between the number of unfavorable alleles (A) and favorable alleles (B) and the best linear unbiased predictors (BLUPs) of maximum disease severities.

Table 3. Candidate genes for single-nucleotide polymorphisms significantly associated with stripe rust resistance

Chromosome	Marker	Candidate gene	Sequence similarity (%)	Sequence coverage (%)	Quality parameters
1AL	<i>IWB20306</i>	E3 ubiquitin-protein ligase KEG	97	98	2 E ⁻¹⁴
3AS	<i>IWB10785</i>	Disease resistance RPP13-like protein	79	98	5 E ⁻⁰⁹
7AS	<i>IWB19819</i>	F-box/LRR-repeat MAX2 homolog	100	98	3 E ⁻¹²

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