Identifying Loci Conferring Resistance to Leaf and Stripe Rusts in a Spring Wheat Population (*Triticum aestivum*) via Genome-Wide Association Mapping

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ABSTRACT

A previous genome-wide association study (GWAS) for leaf rust (caused by *Puccinia triticina*) resistance identified 46 resistance quantitative trait loci (QTL) in an elite spring wheat leaf rust resistance diversity panel. With the aim of characterizing the pleiotropic resistance sources to both leaf rust and stripe rust (caused by *P. striiformis* f. sp. *tritici*), stripe rust responses were tested in five U.S. environments at the adult-plant stage and to five U.S. races at the seedling stage. The data revealed balanced phenotypic distributions in this population except for the seedling response to *P. striiformis* f. sp. *tritici* race PSTv-37. GWAS for stripe rust resistance discovered a total of 21 QTL significantly associated with all-stage or field resistance on chromosomes 1B, 1D, 2B,

3B, 4A, 5A, 5B, 5D, 6A, 6B, 7A, and 7B. Previously documented pleiotropic resistance genes *Yr18/Lr34* and *Yr46/Lr67* and tightly linked genes *Yr17-Lr37* and *Yr30-Sr2-Lr27* were also detected in this population. In addition, stripe rust resistance QTL *Yrswp-2B.1*, *Yrswp-3B*, and *Yrswp-7B* collocated with leaf rust resistance loci *2B_3*, *3B_12*, and *7B_4*, respectively. Haplotype analysis uncovered that *Yrswp-3B* and *3B_12* were either tightly linked genes or the same gene for resistance to both stripe and leaf rusts. Single nucleotide polymorphism markers *IWB35950*, *IWB74350*, and *IWB72134* for the 3B QTL conferring resistance to both rusts should be useful in incorporating the resistance allele(s) in new cultivars.

Wheat (*Triticum* spp.) is one of the three major staple cereal crops in the world, together with rice and maize, and ranks first as a protein source. Wheat is the most important food crop in North Africa and West and Central Asia, where it supplies half of the total calories consumed in these areas (International Maize and Wheat Improvement Center 2015). Although global wheat production has reached >700 million tons at an annual increase rate of 1.6% (Food and Agriculture Organization of the United Nations 2019), it is insufficient to meet the accelerating global demand (Singh et al. 2016).

One main constraint to improving global wheat production is diseases, including leaf, stripe, and stem rusts (Aktar-Uz-Zaman et al. 2017; Singh et al. 2016). Leaf rust, incited by fungal pathogen *Puccinia triticina*, is more common than either stripe or stem rust. Yield losses caused by leaf rust occur in almost all wheat production

areas of the world, varying from trace amounts to >50% of the production. Severe leaf rust epidemics in the United States incurred yield loss of greater than 3 million tons between 2000 and 2004, with a total economic loss estimated at > US\$350 million (Huerta-Espino et al. 2011). Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is more frequently detected in the temperate regions of cooler and wetter environments (Liu et al. 2017b). Compared with leaf rust, stripe rust is adapted to a relatively narrower range of environmental conditions, but the damage attributable to stripe rust tends to be more severe. According to Beddow et al. (2015), an average of 5.47 million tons of global wheat, equivalent to US\$979 million, are lost annually as a result of stripe rust. Since 2000, with the emergence of new aggressive races, stripe rust has spread into the wheat-growing regions of warmer and dryer environments (Chen et al. 2010), which enhances the chances of multiple rusts simultaneously infecting wheat crops. Consequently, deployment of resistance to multiple diseases is desirable to mitigate losses of wheat production caused by both leaf rust and stripe rust.

Pyramiding of two or more resistance genes against rusts in individual cultivars is a common strategic goal for modern wheat breeding programs worldwide. Combinations of a couple of all-stage resistance (ASR) and adult-plant resistance (APR) genes can offer near immunity and long-lasting resistance (Ellis et al. 2014; Singh et al. 2001; Tiwari et al. 2014). However, the gene-pyramiding process is time-consuming, and all genes in the pyramid should only be deployed together for maximum efficacy and longevity. Another type of desirable resistance that has been

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exploited in wheat breeding programs is the pleiotropic or tightly linked resistance loci against more than one disease. *Yr18/Lr34*, *Yr29/Lr46*, and *Yr46/Lr67* are pleiotropic effect genes conferring nonrace-specific APR to both stripe and leaf rusts during the late growth stages (Herrera-Foessel et al. 2014; Krattinger et al. 2009; Williams et al. 2003). Instead of providing complete immunity to certain rust races, these pleiotropic genes retard the infection process or reduce symptom development across all races of the pathogens. Cloning of *Yr18/Lr34* uncovered a transporter-mediated mechanism, which allowed a better understanding of its nonrace-specific nature (Krattinger et al. 2009). Independent but tightly linked ASR genes also confer resistance to leaf and stripe rusts. The majority of these multigenic resistance sources were derived from translocations or introgressed segments of wheat relatives. Examples are *Yr9-Lr26*, *Yr17-Lr37* (Helguera et al. 2003), *Yr30-Lr27* (Mago et al. 2011; Singh et al. 2000), *Yr35-Lr53* (Dadhodaie et al. 2011), *Yr37-Lr54* (Marais et al. 2005), *Yr38-Lr56* (Marais et al. 2006), *Yr40-Lr57* (Kuruparthi et al. 2007), *Yr42-Lr62* (Marais et al. 2009), *Yr47-Lr52* (Qureshi et al. 2017), and *Yr70-Lr76* (Bansal et al. 2016). Closely linked molecular markers have been identified for these translocated segments, enabling expedient multipathogen resistance breeding with effectively diagnostic DNA markers.

With the objective of identifying pleiotropic or tightly linked resistance genes to both leaf and stripe rusts, a genome-wide association study (GWAS) for stripe rust resistance was performed on a spring wheat population that has already been characterized for leaf rust resistance (Gao et al. 2016).

MATERIALS AND METHODS

Plant materials. A collection of 294 spring wheat breeding accessions provided by the U.S. Department of Agriculture Agricultural Research Service Cereal Disease Laboratory was used in this study (Supplementary Data File S1). This collection was sampled from a larger GWAS panel of 381 genotypes (Gao et al. 2016) based on the seed accessibility. Fifty-eight accessions were leaf rust resistance gene near-isogenic lines (NILs) in the Thatcher background. The other accessions were cultivars or advanced breeding lines derived from the public or private wheat breeding programs mainly in North and South America.

Stripe rust phenotyping. Race-specific resistance to stripe rust at the seedling stage was conducted under the controlled conditions in a greenhouse. The 294 accessions were tested with *P. striiformis* f. sp. *tritici* races PSTv-4, PSTv-14, PSTv-18, PSTv-37, and PSTv-40. For each race test, Avocet S (AvS) was used as a susceptible check, and 18 *Yr* single-gene lines that are used to differentiate *P. striiformis* f. sp. *tritici* races (Wan and Chen 2014) were included to confirm the race identity. Three seeds of each accession were planted in one well of 96-well trays, and each race test was repeated once. The inoculated seedlings were kept in a dew chamber at 10°C and 100% humidity without light for 24 h, and then they were moved to a rust-free growth chamber with condition settings of a diurnal temperature cycle gradually changing from 4°C at 2:00 AM to 20°C at 2:00 PM with a 16-h photoperiod. The stripe rust reaction of individual accessions was recorded using the infection type (IT), on a scale of 0 to 9 (Line and Qayoum 1992), when AvS had full sporulation, about 18 to 20 days after inoculation.

Stripe rust phenotyping of the 294 accessions was conducted in fields near Pullman, Central Ferry, and Mount Vernon, Washington, in 2014 to 2016, following methods as previously described (Liu et al. 2017a, b, c). Seeds of each accession (5 g) were sown into 0.5-m length at 0.3-m spacing in the early spring. AvS was planted every 20 rows. Either AvS (for the Mount Vernon location) or another susceptible spring wheat Lemhi (for the Spillman and Central Ferry locations) was planted surrounding the field as the inoculum spreaders to ensure adequate and uniform infection. The collection

was evaluated in five environments: the Spillman Agronomy Farm (46° 43' 47.1972'' N 117° 10' 54.2568'' W) near Pullman, Washington in 2014 (SPM14), 2015 (SPM15), and 2016 (SPM16); the Central Ferry Farm (46° 38' 9.816'' N 117° 47' 24.0792'' W) about 60 miles southwest of Pullman in 2015 (CLF15); and the Mount Vernon Northwest Washington Research and Extension Center (48° 25' 16.3776'' N 122° 20' 2.5692'' W) about 340 miles northwest of Pullman in 2015 (MTV15). All field trials used a nonreplicated completely random design. Plants in the trials were naturally infected by *P. striiformis* f. sp. *tritici* (Liu et al. 2017a). Stripe rust reactions were recorded as IT and disease severity (SEV), estimated as the percentage (0 to 100%) of the infected area of flag leaves (Peterson et al. 1948) from the boot to soft dough stages (GS 45 to 85) (Zadoks et al. 1974) depending on location. Heading date and plant height of each accession were scored in SPM15 and SPM16.

Statistical analyses of phenotypic data. Pairwise Pearson correlation coefficients among five environments were computed using the R function `cor.test`. Broad-sense heritability (H^2) was calculated with the following formula:

$$H^2 = \sigma_G^2 / \sigma_P^2$$

where σ_G^2 is the genotypic variance and σ_P^2 is the phenotypic variance. The formula for phenotypic variance is as follows:

$$\sigma_P^2 = [\sigma_G^2 + (\sigma_{E \times G}^2 + \sigma_e^2) / n]$$

where $\sigma_{E \times G}^2$ is the variance of environment by genotype interaction, σ_e^2 is the residual variance, and n is the environment number (Piepho and Möhring 2007). These variance components were dissected by analysis of variance generated from the PROC MIXED COVTEST procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC). Genotype, environment, and genotype-by-environment interaction were random variables in the analysis of variance (ANOVA) random-effects model. The PROC MIXED procedure was also used for estimating the best linear unbiased estimator (BLUE) of IT and SEV across five environments, with genotypes as the fixed effect and the other variables as random effects (Wolfinger et al. 1997).

Genotypic profile. The entire panel was genotyped using the Illumina iSelect 90K single nucleotide polymorphism (SNP) array that was previously described in Gao et al. (2016). The SNP marker profile was downloaded from the Triticeae Toolbox (T3) database (<https://triticeaetoolbox.org/wheat/>). Heterozygous alleles were converted to missing data. The SNPs with a missing marker frequency > 0.1 and a minor allele frequency < 0.1 were removed. After filtering, a total of 15,594 high-quality polymorphic SNPs were retained for further analyses. Additionally, diagnostic markers for pleiotropic resistance genes *Yr18/Lr34* and *Yr46/Lr67* and tightly linked genes *Yr17-Lr37* and *Yr30-Sr2-Lr27* were used to genotype the entire collection, and the marker data were included for association analysis. Information on these diagnostic markers is provided in Supplementary Table S1.

Population structure and kinship. The population structure of the spring wheat panel was dissected by both Bayesian model-based clustering and principal component analysis (PCA). Bayesian model-based clustering was estimated using STRUCTURE version 2.3.4 (Falush et al. 2003; Pritchard et al. 2000) and followed parameter settings as previously described (Liu et al. 2017a, b, c). PCA was estimated using JMP Genomics version 6.0. The number of subpopulations that best represented this collection was determined by the ad hoc statistic Δk plot of Bayesian model-based clustering (Evanno et al. 2005) combined with the scree plot of PCA. To further explore the genetic relatedness of pairwise accessions in the collection, a kinship matrix (K) was generated in JMP Genomics version 6.0 using the identical-by-state (IBS) algorithm.

Association analysis. The R package Genome Association and Prediction Integrated Tool (GAPIT) (Lipka et al. 2012) was used to conduct the marker-trait association (MTA) analysis. A couple of models were performed on this collection, including the general linear model (GLM) (Riethmüller et al. 2002), the GLM with the membership coefficients of three subpopulations generated by Bayesian model-based clustering (Q), the mixed linear model (MLM) with K as a random variable, and the MLM with Q and K as variables. The optimal model that best fits the seedling and adult-stage phenotypes was MLM with K (hereafter, MLM+ K) according to the Bayesian information criterion values (Supplementary Table S2). Pearson correlation coefficients were calculated between field resistance (BLUE_IT, BLUE_SEV), heading date, and plant height. Plant height was significantly ($P < 0.05$) correlated with both BLUE_IT and BLUE_SEV, but heading date was not. In this case, plant height was added into the MLM+ K model as a covariate when analyzing adult-plant phenotype data. GWAS tests were performed separately for IT and SEV in each environment as well as the BLUEs generated from data across five environments. We used the marker-wise P value of 0.001 as the threshold to declare significant MTAs and the false discovery rate-corrected P value of 0.1 to select the highly significant MTAs. The physical positions of significant SNPs were obtained from the International Wheat Genome Sequencing Consortium (IWGSC) website (IWGSC RefSeq version 1.0, <http://www.wheatgenome.org>), and the genetic positions of significant SNPs were obtained from the 90K SNP consensus map (Wang et al. 2014).

The QTL interval boundaries were determined using the pairwise linkage disequilibrium (LD) correlation coefficients of significant markers with $r^2 \geq 0.5$ coupled with the genetic distances ≤ 10 cM. The pairwise LD r^2 was calculated using Haploview version 4.2 (Barrett et al. 2005). We chose the tagging marker for each QTL with the criteria of the smallest marker-wise P value, the largest phenotypic variation (PVE), and the largest number of races/environments in which significant associations were detected (Gao et al. 2016; Liu et al. 2017d; Naruoka et al. 2015). The PVE for each QTL was determined by the PVE of the tagging SNP.

To identify the pleiotropic/tightly linked QTL for stripe and leaf rust resistance, we compared the physical positions of stripe rust resistance QTL identified in the current study to the leaf rust resistance QTL identified in the same panel (Gao et al. 2016) based on IWGSC RefSeq version 1.0 (Appels et al. 2018). The R package Rldeogram (Hao et al. 2018) was used to visualize and map the identified stripe rust and leaf rust resistance QTL onto wheat chromosomes. The length of each chromosome and its centromere position were obtained from IWGSC RefSeq version 1.0 (Appels et al. 2018).

An integrated genetic map consisting of 235 previously documented *Yr* genes/QTL as well as different genetic marker

types, including SNPs, simple sequence repeats, diversity array technology, and expressed sequence tag markers, generated by Maccaferri et al. (2015), was used to compare the colocalizations between the QTL identified in the current study and the previously reported *Yr* genes or QTL.

RESULTS

Phenotypic variation in stripe rust resistance. In the tests with individual *P. striiformis* f. sp. *tritici* races at the seedling stage, PSTv-37 was more virulent to the current panel than any of the other four races, with 92, 6, and 2% of accessions showing susceptibility (IT from 7 to 9), moderate susceptibility (IT from 4 to 6), and resistance (IT from 0 to 3), respectively. To avoid false-positive error, the phenotype to PSTv-37 was not used for GWAS analysis because there were not enough resistant accessions in the population (Fig. 1). The IT distributions against *P. striiformis* f. sp. *tritici* races PSTv-4, PSTv-14, and PSTv-40 were skewed to susceptibility, with susceptible accession frequencies of 65, 76, and 79%, respectively. However, the IT distribution against PSTv-18, the least virulent race identified thus far in North America, was skewed to resistance with a resistant accession frequency of 74%.

At the adult-plant stage in the field tests, IT and SEV distributions were nearly normal or skewed toward low IT and SEV in most of the environments, except MTV15, where the population had a skewed distribution toward high IT and SEV, with the mean IT and SEV equal to 6.0 and 60.3%, respectively; in contrast, the mean IT and SEV within the other four environments ranged from 3.7 to 5.1 and 14.8 to 32.0%, respectively (Fig. 2). Pearson correlation coefficients across five environments ranged from 0.49 to 0.74 for IT and from 0.36 to 0.66 for SEV. Environment MTV15 displayed lower correlations with the other environments for both IT and SEV, with a range from 0.49 to 0.74 for IT and from 0.36 to 0.66 for SEV. As expected, correlations between IT and SEV within the same environment were greatest, ranging from 0.61 to 0.91 (Fig. 3). Across the five environments, the broad-sense heritability was 0.89 for IT and 0.77 for SEV.

Population structure and relationship with stripe rust resistance phenotype. Both model-based and PCA-based structure analyses found three major subpopulations in this collection, which is the same as population structure analysis in Gao et al. (2016). Accessions in subpopulation 1 were Thatcher NILs or had Thatcher in their pedigree. The first subpopulation, hence, was called the “Thatcher group,” hereafter. The other two subpopulations consisted of cultivars or breeding lines mainly collected throughout North and South America. Further details of the population structure are described in Gao et al. (2016).

The Thatcher group was more susceptible to stripe rust than the other two groups in all field environments. The BLUE_IT and SEV values for the Thatcher group were significantly larger ($P < 0.05$) than the others (Table 1). The BLUE_IT and BLUE_SEV values for the second group were slightly smaller than those for the third group, but no significant difference was observed between these two groups. In the single-race seedling tests, the Thatcher group was significantly more resistant against PSTv-4 than the other two groups, whereas the second subgroup was significantly more resistant against PSTv-14 and PSTv-37 than the others. For PSTv-18, all three subgroups showed substantial resistance, but the Thatcher and the second subgroups were significantly more resistant than the third subgroup. There was no significant difference between the three subgroups for PSTv-40 responses (Table 1).

Dissection of genetic architecture of stripe rust resistance. The previous GWAS study for leaf rust resistance revealed pleiotropic rust resistance gene *Yr18/Lr34* on chromosome 7D associated with both ASR and APR to leaf rust in the collection (Gao et al. 2016). As an internal control, the diagnostic marker data for *Yr18/Lr34* were incorporated into the current GWAS for stripe rust

Fig. 1. The distributions of infection type at the seedling stage against five races of *Puccinia striiformis* f. sp. *tritici*.

resistance. Unsurprisingly, at a resistance allele frequency of 35%, *Yr18/Lr34* showed the most significant associations with IT and SEV in all environments except SPM14 at the adult-plant stage compared with the other MTAs, and it accounted for 3.7 to 12.3% of the PVE (Table 2). This result validates the effectiveness of the MLM+K model for stripe rust resistance data of this collection. *Yr17-Lr37* and *Yr46/Lr67* were also detected in the population, with resistance allele frequencies of 2.0, 1.0, and 6.8%, respectively. Because of the low allele frequency, markers of *Yr17-Lr37*, *Yr46/Lr67*, and *Yr30-Sr2-Lr27* were excluded when conducting the GWAS.

An additional 21 QTL that showed significant or highly significant associations with stripe rust resistance were detected on chromosomes 1B, 1D, 2B, 3B, 4A, 5A, 5B, 5D, 6A, 6B, 7A, and 7B (Table 2; Supplementary Data File S2). Among these QTL, 17 were associated with IT against the tested *P. striiformis* f. sp. *tritici* races at the seedling stage, and therefore were most likely conferring ASR. The PVE explained by the 17 QTL ranged from 2.1 to 14.1%. Four ASR QTL (*Yrswp-2B.1*, *Yrswp-2B.2*, *Yrswp-2B.3*, and *Yrswp-2B.4*) were present in tandem at a large interval of ~140 Mb on the long arm of chromosome 2B. These four QTL had highly significant associations with resistance to PSTv-4, and *Yrswp-2B.2* was also significantly associated with PSTv-18. On chromosome 3BS, *Yrswp-3B*, tagged by SNP *IWB35950*, was associated with not only resistance to PSTv-40 at the seedling stage but also to resistance as measured by IT in environment SPM16 and BLUE values across all environments at the adult-plant stage. *Yrswp-3B* accounted for 3.9% of PVE for ASR and for 2.8 to 4.6% for field resistance. Four QTL (*QYrswp-6B.1*, *QYrswp-6B.2*, *QYrswp-6B.3*, and *QYrswp-7A*) were exclusively associated with field resistance in at least two environments at the adult-plant stage, which potentially represents APR QTL. The PVE explained by individual QTL ranged from 3.2 to 15.5%, and the cumulative PVE values of these four QTL coupled with *Yr18/Lr34* were 43.9% for BLUE_IT and 34.4% for BLUE_SEV.

Pleiotropic or tightly linked QTL for stripe and leaf rust resistance. With the aim of assessing the possible pleiotropic or tightly linked QTL for both stripe and leaf rust resistance besides *Yr18/Lr34*, *Yr17-Lr37*, *Yr46/Lr67*, and *Yr30-Lr27*, the remaining stripe rust resistance QTL identified in the current study were compared with the leaf rust resistance QTL identified in the same panel (Gao et al. 2016). According to the wheat 90K SNP consensus map (Wang et al. 2014) and IWGSC RefSeq version 1.0 (Appels et al. 2018), three stripe rust resistance QTL (*Yrswp-2B*, *Yrswp-3B*, and *Yrswp-7B*) located at the same regions of leaf rust resistance QTL *2B_3*, *3B_t2*, and *7B_4* (Gao et al. 2016), respectively (Fig. 4; Table 2). In a comparison of significant SNPs of stripe rust resistance QTL with leaf rust resistance QTL, only *Yrswp-2B.1* shared common SNPs (*IWB72407* and *IWB64322*) with *2B_3*. *IWB72407* and *IWB64322* were in complete LD ($r^2 = 1.0$) with each other. Nevertheless, haplotype “CG” for *Yrswp-2B.1/2B_3* was the resistance-associated haplotype for stripe rust, whereas it was the susceptibility-associated haplotype for leaf rust. In other words, these two resistance genes were linked in repulsion.

Haplotype analysis was conducted to further determine the relationships between *Yrswp-3B* and *3B_t2* and *Yrswp-7B* and *7B_4*. *Yrswp-7B* was not linked with *7B_4* because *IWB60899* for *Yrswp-7B* and *IWB64015* for *7B_4* had a LD r^2 equal to 0.005. However, *Yrswp-3B* was tightly linked with *3B_t2* because *IWB35950* and *IWB72134* for *Yrswp-3B* and *IWB74350* for *3B_t2* were in high LD ($r^2 = 0.64$ to 0.78). Furthermore, the most frequent haplotype, “ACT,” of *3B_t2* and *Yrswp-3B*, which was present in 68% of the accessions, conferred resistance to both stripe and leaf rusts. This haplotype accounted for 2.8 to 4.6% of PVE for stripe rust resistance and 4% of PVE for leaf rust resistance. In summary, *Yrswp-3B/3B_t2* is a potential QTL for resistance to both leaf and stripe rusts.

Distributions of favorable alleles of identified QTL among three subpopulations. The stripe rust resistance-associated allele frequencies of identified QTL in the entire

population and three subpopulations are summarized in Table 3. The resistance allele frequency for *Yr18/Lr34* was 0.35 in the entire population, and a higher frequency was found in subgroup 3 (0.43) than the other two subgroups (0.14 and 0.39). The favorable allele frequency for *Yrswp-3B/3B_t2* was 0.76 in the entire population. The Thatcher group (0.88) had a higher favorable allele frequency than either subgroup 2 (0.71) or subgroup 3 (0.73). In contrast, the resistance alleles for the three field resistance QTL on chromosome 6B (*QYrswp-6B.1*, *QYrswp-6B.2*, and *QYrswp-6B.3*) were nearly absent in the Thatcher group but had higher favorable allele frequencies in the other two groups, except that the resistance allele for *QYrswp-6B.3* was also very low in subgroup 3. Nine ASR QTL (*Yrswp-1D.1*, *Yrswp-2B.2*, *Yrswp-2B.3*, *Yrswp-2B.4*, *Yrswp-3B*, *Yrswp-4A*, *Yrswp-5A*, *Yrswp-5B.2*, and *Yrswp-6A*) had the highest resistance allele frequencies in the Thatcher group, whereas seven QTL (*Yrswp-1B*, *Yrswp-1D.2*, *Yrswp-5B.1*, *Yrswp-5D.1*, *Yrswp-5D.2*, *Yrswp-7A*, and *Yrswp-7B*) were almost absent.

DISCUSSION

In this study using GWAS, we identified stripe rust resistance loci in a spring wheat collection whose genetic architecture of leaf rust resistance has already been assessed (Gao et al. 2016). A total of 333 SNP markers within 46 unique QTL on 18 chromosomes were reported by Gao et al. (2016) to have significant associations with leaf rust resistance. In our stripe rust resistance study, 21 QTL (165 SNPs) on 12 chromosomes as well as *Yr18/Lr34* were detected.

Fig. 2. The distributions of stripe rust resistance at the adult-plant stage: **A**, infection type, and **B**, disease severity. Each environment was referred to as SPM14, SPM15, and SPM16 for trials at Spillman Agronomy Farm in 2014, 2015, and 2016, respectively; CLF15 for the trial at Central Ferry in 2015; and MTV15 for the trial at Mt. Vernon in 2015.

Among them, three stripe rust resistance QTL on chromosomes 2B, 3B, and 7B collocated with leaf rust resistance QTL, but only *Yrswp-3B* and *3B_t2* were potential multiple resistance QTL in this population.

On the long arm of chromosome 2B, *IWB72407* and *IWB64322* are commonly present in the stripe rust resistance QTL *Yrswp-2B.1* and the leaf rust resistance QTL *2B_3*. However, *Yrswp-2B.1/2B_3* is unable to simultaneously improve resistance to both rusts because

Fig. 3. Pearson's correlation coefficients of infection type (IT) and disease severity (SEV) among five field environments. Each environment is referred to as SPM14, SPM15, and SPM16 for trials at Spillman Agronomy Farm in 2014, 2015, and 2016, respectively; CLF15 for the trial at Central Ferry in 2015; and MTV15 for the trial at Mt. Vernon in 2015. All correlations were significant at $P < 0.05$.

TABLE 1. Means of infection type (IT) and disease severity (SEV) against stripe rust at seedling and adult-plant stages in the entire population and three subpopulations^z

Plant stage	Trait/race	Entire population	Subpopulation 1 (n = 65)	Subpopulation 2 (n = 132)	Subpopulation 3 (n = 97)
Adult-plant stage	BLUE_IT	4.6	5.6 ^a	4.3 ^b	4.5 ^b
	BLUE_SEV	30.6	35.3 ^a	28.6 ^b	30.2 ^b
Seedling stage	IT (PSTv-4)	6.5	3.7 ^a	7.1 ^b	7.4 ^b
	IT (PSTv-14)	7.1	7.6 ^a	6.6 ^b	7.5 ^a
	IT (PSTv-18)	2.8	2.1 ^a	2.6 ^a	3.6 ^b
	IT (PSTv-37)	7.6	8.0 ^a	7.4 ^b	7.7 ^a
	IT (PSTv-40)	7.1	7.1	7.0	7.1

^z Different superscript letters for the subpopulation means showed a significant difference ($P < 0.05$) using one-way analysis of variance. BLUE = best linear unbiased estimator.

resistance-associated alleles for *IWB72407* and *IWB64322* are actually susceptible alleles for leaf rust (Gao et al. 2016). Therefore, this QTL should be avoided by wheat breeding programs whose target areas have occurrence of stripe and leaf rusts, but the negative association is worthy of further study.

The most promising multiple resistance QTL identified in this study, *Yrswp-3B/3B_t2*, was located in the telomere region of chromosome 3BS. *Yrswp-3B* was associated with resistance to *P. striiformis* f. sp. *tritici* race PSTv-40 as well as IT in environment SPM16 and BLUE. The leaf rust resistance QTL *3B_t2* was associated with response to a mixture of *P. triticea* races and multiple field environments (Gao et al. 2016). These associations suggest that the QTL complex *Yrswp-3B/3B_t2* appears to be effective against more *P. triticea* races than *P. striiformis* f. sp. *tritici* races. Previously documented tightly linked gene *Yr30-Sr2-Lr27* was also reported in chromosome 3BS close to the telomere region. However, the average LD r^2 between *Yrswp-3B/3B_t2* and *Yr30-Sr2-Lr27* in our population was 0.025. By exploring the resistance allele distributions, we found that the resistance

haplotypes for *Yrswp-3B/3B_t2* were widely present in all three subpopulations, with a resistance allele frequency of 88, 71, and 73%, respectively. However, the resistance allele for *Yr30-Sr2-Lr27* was exclusively present in subpopulation 2, with an allele frequency of 15%. These findings suggest that *Yrswp-3B/3B_t2* and *Yr30-Sr2-Lr27* were not linked with each other. However, biparental mapping was needed to further confirm whether *Yrswp-3B/3B_t2* and *Yr30-Sr2-Lr27* were the same pleiotropic gene. In a comparison of the genetic positions with documented rust resistance genes, *Yrswp-3B/3B_t2* did not overlap with any other previously published stripe rust and leaf rust resistance genes. Therefore, *Yrswp-3B/3B_t2* is probably a novel QTL. Because the resistance haplotype for *Yrswp-3B/3B_t2* was widely present in the current panel, this chromosomal segment is most likely under selection pressure throughout North and South American wheat breeding programs.

Besides *Yrswp-3B/3B_t2* we also detected the previously documented pleiotropic rust genes *Yr18/Lr34* and *Yr46/Lr67* and tightly linked genes *Yr17-Lr37* and *Yr30-Sr2-Lr27* in this population. Thatcher NILs RL6058, RL6081, and RL6077 in

TABLE 2. Significant quantitative trait loci (QTL) that associated with resistance to *Puccinia striiformis* f. sp. *tritici*

QTL	Type ^c	Chromosome	90K cons2014 position (cM) ^e	RefSeq version 1.0 position (bp) ^f	Tag-SNP ^g	Allele ^h	PVE (%) ^w	MTA ^s	Lr ^y	Yr ^z	References
<i>Yrswp-1B</i>	ASR	1B	107.4–109.7	632753937–636738140	IWB47288	T/G	4.5	PSTv-40	–	<i>YrExp1</i>	Lin and Chen (2007)
<i>Yrswp-1D.1</i>	ASR	1D	67.7	250140206	IWB6869	T/G	2.8	PSTv-14	–	–	–
<i>Yrswp-1D.2</i>	ASR	1D	115.6	435151463–435933420	IWB9896	A/G	3.6	PSTv-14	–	–	–
<i>Yrswp-2B.1</i>	ASR	2B	107.01–108.4	612686386–637574450	IWB72408	A/G	2.1–6.2	PSTv-4	<i>2B_3</i>	–	–
<i>Yrswp-2B.2</i>	ASR	2B	109.14–126.53	657844062–670837337	IWB12654	T/C	2.2–5.3	PSTv-4 , PSTv-18	–	<i>Yr5, Yr7, Yr44, Yr53, YrSP</i>	Feng et al. (2015), McGrann et al. (2014), Xu et al. (2013)
<i>Yrswp-2B.3</i>	ASR	2B	113.36–116.83	694051080–703976259	IWB19972	A/G	2.1–4.3	PSTv-4	–	<i>Yr5, Yr7, Yr44, Yr53, YrSP</i>	Feng et al. (2015), McGrann et al. (2014), Xu et al. (2013)
<i>Yrswp-2B.4</i>	ASR	2B	114.82–130.62	709423590–752485089	IWB34664	T/C	2.1–4.2	PSTv-4	–	<i>Yr5, Yr7, Yr44, Yr53, YrSP</i>	Feng et al. (2015), McGrann et al. (2014), Xu et al. (2013)
<i>Yrswp-3B</i>	ASR	3B	51.1	3240217–3228129	IWB35950	T/C	2.8–4.6	PSTv-40, SPM16_IT, BLUE_IT	<i>3B_t2</i>	–	–
<i>Yrswp-4A</i>	ASR	4A	108.7	663281171–663282832	IWB55277	A/G	2.3	PSTv-4	–	–	–
<i>Yrswp-5A</i>	ASR	5A	92.9–93.2	594143506–594576820	IWB26265	T/C	3.9	PSTv-40	–	–	–
<i>Yrswp-5B.1</i>	ASR	5B	68.6–78.0	520886418–539293256	IWA5478	A/G	14.1	PSTv-14	–	<i>QYrcwpg-5B_coda, QYr-5B_Oligoculm</i>	Case et al. (2014), Suenaga et al. (2003)
<i>Yrswp-5B.2</i>	ASR	5B	90.3	546827149–546849099	IWB3660	T/C	2.9	PSTv-14	–	–	–
<i>Yrswp-5D.1</i>	ASR	5D	67.5	132880061–154539793	IWB52508	T/C	2.8	PSTv-4	–	<i>Yrcaas-5DS_jingshuang 16</i>	Ren et al. (2015)
<i>Yrswp-5D.2</i>	ASR	5D	67.5	221012593	IWB34503	T/G	2.1	PSTv-4	–	<i>Yrcaas-5DS_jingshuang 16</i>	Ren et al. (2015)
<i>Yrswp-6A</i>	ASR	6A	15.7	3852742	IWA272	T/C	3.5	PSTv-4	–	<i>QYruga-6A_26R61</i>	Hao et al. (2011)
<i>QYrswp-6B.1</i>	APR	6B	33.8	30147276	IWA4010	T/C	3.2–9.7	CLF15_IT, SPM14_IT, SPM15_IT, SPM16_IT, BLUE_IT	–	<i>QYrufs-6B_Kariega, QYrTAM 111, QYrcaas-6BS.2_Naxos</i>	Prins et al. (2011), Ren et al. (2012)
<i>QYrswp-6B.2</i>	APR	6B	48.5–57.1	112885924–123747825	IWB20486	T/C	3.2–15.5	CLF15_IT, CLF15_SEV, MTV15_IT, SPM14_IT , SPM15_IT, SPM16_IT, BLUE_IT, BLUE_SEV, SPM15_SEV	–	<i>QYrswp-6BS.2_Stephens, QYrsum-6B_Janz</i>	Bansal et al. (2011), Santra et al. (2008)
<i>QYrswp-6B.3</i>	APR	6B	57.4	135112726	IWB33273	A/G	3.7–4.3	MTV15_SEV, SPM15_SEV, BLUE_SEV	–	–	–
<i>QYrswp-7A</i>	APR	7A	136.2	636889961	IWA6004	A/C	3.8–3.9	SPM15_IT, SPM16_IT	–	<i>IWA4996, Yrxy1</i>	Zegeye et al. (2014), Zhou et al. (2011)
<i>Yrswp-7A</i>	ASR	7A	228.4–232.11	731271409–732587216	IWB71711	T/C	4.1	PSTv-40	–	<i>IWA7592</i>	Bull et al. (2016)
<i>Yrswp-7B</i>	ASR	7B	159.0–171.1	731269292–731987449	IWB60899	A/G	3.9	PSTv-40	<i>7B_4</i>	<i>Yr59, Yr52</i>	McIntosh et al. (2014)
<i>Yr18/Lr34</i>	APR	7D	35	47411056–47425484	<i>Yr18/Lr34</i>	T/A	3.7–12.3	CLF15_IT , CLF15_SEV, MTV15_IT, MTV15_SEV , SPM15_SEV, SPM16_IT, SPM16_SEV , BLUE_IT , BLUE_SEV	<i>Lr34</i>	<i>Yr18/Lr34</i>	Suenaga et al. (2003)

^c QTL types include all-stage resistance (ASR) and adult-plant resistance (APR).

^e Position of each single nucleotide polymorphism (SNP) based on the 90K wheat consensus map (Wang et al. 2014).

^f Position of each SNP based on International Wheat Genome Sequencing Consortium IWGSC RefSeq version 1.0 (<http://www.wheatgenome.org>).

^g Tagging SNPs for each QTL were selected based on the strongest association with stripe rust response considering the smallest marker-wise *P* value, the largest phenotypic variance (PVE) explained by the marker, and the largest number of races/environments in which significant associations were detected with the marker.

^h The resistance allele for each QTL is bolded.

^w PVE explained by the tagging SNP for each QTL.

^s Marker-trait associations (MTAs) highlighted in bold had significance levels of false discovery rate-adjusted $P < 0.1$.

^y Leaf rust resistance (*Lr*) QTL identified in Gao et al. (2016) that collocated with stripe rust resistance QTL identified in this study.

^z Stripe/yellow rust resistance (*Yr*) QTL that were previously reported to overlap with QTL identified in this study.

subpopulation 1 were identified to carry resistance alleles for *Yr18/Lr34*, *Yr17-Lr37*, and *Yr46/Lr67*, respectively. This finding confirmed the accuracy of our genotyping results because these lines are the NILs for these genes in the Thatcher background (Draz et al. 2015). In addition, four cultivars (INIA_MIRLO, INIA_

CARANCHO, INIA_CARPINTERO, and INIA_CHIMANGO) from the Uruguayan program at La Estanzuela and advanced breeding line MN05209 bred by the University of Minnesota in subpopulation 2 also contained *Yr17-Lr37*; in addition, CHE-NAB70 from the International Maize and Wheat Improvement

Fig. 4. Chromosome positions of stripe rust and leaf rust resistance QTL. Stripe rust resistance QTL identified in the current study are presented on the left side of the chromosomes. Leaf rust resistance QTL identified previously are on the right side of the chromosomes. The numbers in the bracket were the start and end positions of QTL based on International Wheat Genome Sequencing Consortium IWGSC RefSeq version 1.0.

TABLE 3. The distribution of resistance allele frequencies for identified stripe rust resistance loci in three subpopulations

Locus	Type ^x	Allele ^y	Population	Resistance allele frequency ^z		
				Subpopulation 1, Thatcher group (n = 65)	Subpopulation 2 (n = 132)	Subpopulation 3 (n = 97)
<i>Yr18/Lr34</i>	APR	T/A	0.35	0.14	0.39	0.43
<i>QYrswp-6B.1</i>	APR	T/C	0.62	0.08	0.82	0.70
<i>QYrswp-6B.2</i>	APR	T/C	0.33	0.02	0.52	0.30
<i>QYrswp-6B.3</i>	APR	A/G	0.13	0	0.22	0.08
<i>QYrswp-7A</i>	APR	A/C	0.72	0.89	0.69	0.64
<i>Yrswp-1B</i>	ASR	T/G	0.32	0	0.25	0.63
<i>Yrswp-1D.1</i>	ASR	T/G	0.38	1.00	0.15	0.27
<i>Yrswp-1D.2</i>	ASR	A/G	0.14	0.05	0.26	0.03
<i>Yrswp-2B.1</i>	ASR	A/G	0.70	0.12	0.83	0.90
<i>Yrswp-2B.2</i>	ASR	T/C	0.63	0.86	0.19	0.26
<i>Yrswp-2B.3</i>	ASR	A/G	0.43	0.85	0.30	0.34
<i>Yrswp-2B.4</i>	ASR	T/C	0.40	0.92	0.34	0.144
<i>Yrswp-3B</i>	ASR	T/C	0.76	0.88	0.71	0.73
<i>Yrswp-4A</i>	ASR	A/G	0.73	0.98	0.54	0.84
<i>Yrswp-5A</i>	ASR	T/C	0.82	1.00	0.61	0.98
<i>Yrswp-5B.1</i>	ASR	A/G	0.16	0.05	0.32	0.03
<i>Yrswp-5B.2</i>	ASR	T/C	0.62	0.95	0.60	0.42
<i>Yrswp-5D.1</i>	ASR	T/C	0.10	0.03	0.18	0.04
<i>Yrswp-5D.2</i>	ASR	T/G	0.11	0.03	0.18	0.07
<i>Yrswp-6A</i>	ASR	T/C	0.35	0.94	0.14	0.25
<i>Yrswp-7A</i>	ASR	T/C	0.34	0.05	0.52	0.30
<i>Yrswp-7B</i>	ASR	A/G	0.34	0.05	0.50	0.31

^x QTL types include adult-plant resistance (APR) and all-stage resistance (ASR).
^y The resistance allele for each QTL is bolded.
^z Numbers in parentheses are the total numbers of accessions in each subpopulation.

Center (CIMMYT) and GIZA155 from Egypt in subpopulation 2 contained *Yr46/Lr67*. Twenty accessions from Argentina, Australia, Colombia, India, Kenya, CIMMYT, the United States, and Uruguay carried the resistance allele of *Yr30-Sr2-Lr27*. These cultivars can serve as direct donors of multiple rust resistance sources in their local wheat breeding programs. It was not unexpected that 35% of accessions ($n = 103$) had *Yr18/Lr34* in the entire population, because *Yr18/Lr34* has been widely detected in wheat accessions from CIMMYT and many other North American wheat breeding programs (Kolmer et al. 2008). Similar to the GWAS results reported in Gao et al. (2016), we also found that *Yr18/Lr34* had highly significant associations to stripe rust resistance in multiple environments at the adult-plant stage in this population, which validated the effectiveness of using the MLM+K model to perform GWAS analysis in our study.

Gao et al. (2016) observed a higher susceptibility against leaf rust in the Thatcher group than the other two groups in field tests, and several leaf rust resistance alleles were almost fixed in the Thatcher group. Similar results were also detected in the present study for stripe rust resistance. We detected that the stripe rust field responses of BLUE_IT and BLUE_SEV of the Thatcher group were significantly higher than the other two subpopulations, and the *QYrswp-7A* resistance allele for APR was almost fixed in the Thatcher group. Another interesting finding was that the mean seedling IT value tested with PSTv-4 was significantly lower in the Thatcher group than in the other groups, which was probably attributable to the fixation of PSTv-4-associated resistance alleles for *Yrswp-2B.2*, *Yrswp-2B.3*, *Yrswp-2B.4*, *Yrswp-4A*, and *Yrswp-6A*. Because 89% of accessions in the Thatcher group were Thatcher-derived NILs that had high genetic similarities with pairwise IBS coefficients ranging from 0.86 to 0.99, these resistance allele fixations may arise from the common Thatcher background (strong population structure). To test this hypothesis, we removed the 58 Thatcher NILs from this association panel and performed GWAS using the remaining 236 accessions. All significant loci except *Yrswp-1D.1* were reidentified, which verified that these loci were indeed significantly associated with the stripe rust resistance.

Based on the wheat consensus map (Wang et al. 2014) and hexaploid wheat integrated maps (Bulli et al. 2016; Maccaferri et al. 2015), we aligned the stripe rust resistance QTL identified in this study with previously published stripe rust resistance genes/QTL. Fourteen QTL overlapped or partially overlapped with previously reported stripe rust genes/QTL. On chromosome 2B proximal to *Yrswp-2B.1*, three ASR QTL (*Yrswp-2B.2*, *Yrswp-2B.3*, and *Yrswp-2B.4*) that loosely linked with one another (LD r^2 ranging from 0.1 to 0.4) were identified to cluster in chromosome 2BL. The three QTL overlapped with previously documented *Yr* genes *Yr5*, *Yr7*, *YrSP*, *Yr44*, and *Yr53* and were significantly associated with ASR to PSTv-14. None of the three QTL can be either *Yr5* or *Yr53* because both genes are effective against all U.S. *P. striiformis* f. sp. *tritici* races tested (Wan and Chen 2014; Xu et al. 2013). In addition, none of the three QTL can be either *Yr7* or *Yr44* because they are effective against PSTv-14, whereas both *Yr7* and *Yr44* are ineffective against this race (Wan and Chen 2014). *YrSP* could be one of the three chromosome 2B QTL identified in the present study, because it is effective against PSTv-14 and many other races (Feng et al. 2015; Wan and Chen 2014). Further studies are needed to dissect the chromosome 2B region harboring multiple genes for stripe rust resistance.

Yrswp-5D.1 and *Yrswp-5D.2* were considered as independent ASR QTL because they were loosely linked (LD $r^2 = 0.1$) and located 66 Mb apart on chromosome 5DS. An APR QTL, *Yrcaas-5DS_jingshuang 16*, from Chinese bread wheat cultivar Jingshuang 16 was mapped on the same chromosomal region (Ren et al. 2015). However, *Yrswp-5D.1* and *Yrswp-5D.2* should be different from the APR QTL because they confer ASR resistance only to PSTv-4.

Six of 21 QTL are most likely to be new QTL because they were located away from the previously reported genes/QTL. The six QTL are *Yrswp-1D.1*, *Yrswp-1D.2*, *Yrswp-4A*, *Yrswp-5A*, and *Yrswp-*

5B.2 for ASR and *QYrswp-6B.3* for APR to stripe rust. These novel QTL would enrich the wheat gene pool of stripe rust resistance genes and can be rapidly deployed for breeding because they were characterized from cultivars or advanced breeding lines. These donors possessed more improved agronomic traits and less linkage drag than landraces. Except for *Yrswp-1D.1* and *QYrswp-6B.3*, more than one significantly associated SNP was detected for these novel QTL. These molecular markers could help the introgression of stripe rust resistance QTL into wheat cultivars.

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