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RESEARCH

Identification of Quantitative Resistance to *Puccinia striiformis* and *Puccina triticinia* in the Soft Red Winter Wheat Cultivar 'Jamestown'

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ABSTRACT

Disease resistance is critical in soft red winter wheat (*Triticum aestivum* L.) cultivars. Leaf rust caused by *Puccinia triticina* Eriks and stripe rust caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. are destructive pathogens of wheat. Phenotypic data were collected at diverse locations for resistance to leaf rust (North Carolina, Texas, and Virginia) and stripe rust (Arkansas, North Carolina, Georgia, Texas, and Virginia) in a Pioneer '25R47' /'Jamestown' (P47/JT) population composed of 186 $F_{5:9}$ recombinant inbred lines (RILs). The P47/JT RILs were genotyped with a public 90K iSelect single-nucleotide polymorphism array. Analysis of the P47/JT population identified two quantitative trait loci (QTL) for leaf rust resistance on chromosome 5B and two QTL for stripe rust resistance on chromosomes 3B and 6A. These QTL were associated with both infection type and disease severity. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of Jamestown on 5B was as high as 22.1%. Variation explained by the putative stripe rust resistance QTL of Jamestown on 3B and 6A was as high as 11.1 and 14.3%, respectively. Introgression and pyramiding of these QTL with other genes conferring resistance to leaf and stripe rusts via marker-assisted selection will facilitate development of soft red winter wheat cultivars having more durable resistance.

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Abbreviations: HTAP, high-temperature adult plant; LOD, logarithm of odds; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single-nucleotide polymorphism; SSR, simple sequence repeat.

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide (Bolton et al., 2008) and can be found on every continent with the exception of Antarctica (Huerta-Espino et al., 2011). In the southeastern US soft red winter wheat region, disease severity will typically peak during April in Georgia and at the end of May in Virginia (Kolmer and Hughes, 2013). Losses from leaf rust are typically less severe than those resulting from the other two common rust diseases, stem rust (*Puccinia graminis* Pers.:Pers.) and stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.), but leaf rust causes greater overall losses due to its wider distribution and occurrence (Huerta-Espino et al., 2011). Selection pressure forced on the pathogen population by the presence of only a few resistance genes deployed among the predominant wheat cultivars has resulted in extensive genetic diversity among *P. triticina* virulence

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phenotypes (Kolmer, 1992). Control of leaf rust through deployment of diverse and durable genetic resistance in cultivars, as opposed to reliance on fungicides, has been shown to be the most cost-effective method, with an estimated 27:1 benefit to cost ratio (Marasas et al., 2003). There are >74 leaf rust resistance genes that have been mapped to chromosome locations and given gene designations, as well as numerous temporarily designated leaf rust resistance genes (McIntosh et al., 2013). Due to the highly variable nature of *P. triticina*, durable leaf rust resistance in wheat cultivars has been difficult to achieve.

Stripe rust (*P. striiformis*) of wheat is considered to be one of the most widely destructive plant diseases in the world and one of the most important diseases of wheat since the 1960s (Line, 2002). Stripe rust reduces grain and forage yield and quality. Seed harvested from crops infected with stripe rust often exhibit low vigor and emergence (Chen, 2005). Stripe rust consumes water and nutrients from the host plant, weakening the plant (Chen, 2005). In 2000, 21 new races of stripe rust were identified in the United States, and among these new races, eight had combinations of virulence to resistance genes that were previously known to provide exceptional resistance in the United States (Chen et al., 2002). From 2000 to 2007, there were a total of 115 identified races of stripe rust (Chen et al., 2010), and by 2010, that number had grown to 146 (Wan and Chen, 2014). New races were identified that are able to germinate at warmer temperatures, allowing for disease development later in the season. This new stripe rust population has increased adaptation and fitness yet contains many virulence alleles that are not required to overcome resistance in soft red winter wheat cultivars east of the Rocky Mountains (Chen, 2005; Dracatos et al., 2016). There are >60 stripe rust resistance genes that have been mapped to chromosome locations and given gene designations, and there are also numerous temporarily designated stripe rust resistance genes (McIntosh et al., 2013). The common incorporation of race-specific resistance genes into commercial cultivars due to their simple inheritance makes use of such genes that are appealing in breeding programs. However, the most severe epidemics caused by stripe rust were the result of failure of race-specific resistance genes in widely grown cultivars. Stripe rust genes are classified into two categories: all-stage resistance (race-specific, hypersensitive resistance), or qualitative resistance, and high-temperature adult plant resistance (HTAP), or quantitative resistance (Chen, 2007). Expression of these genes results in various amounts of chlorosis and/or necrosis, depending on the level of resistance of the plant and environmental factors such as temperature. Due to the highly variable nature of *P. striiformis*, durable stripe rust resistance in wheat cultivars has been difficult to achieve.

Adult plant resistance, or quantitative resistance, is more durable and effective against multiple races of a pathogen. Introgression of multiple seedling resistance genes and adult plant resistance genes into elite cultivars will result in broader spectrum and more durable resistance (Griffey and Allan, 1988). Multiline cultivars and gene pyramiding have been successfully used to control stripe rust (Chen, 2007). Development of gene pyramids requires the identification of diverse genes and QTL for resistance, and their combined incorporation into a high-yielding cultivar (Singh et al., 1992).

Soft red winter wheat cultivars Jamestown and Pioneer '25R47' were crossed and used to develop recombinant inbred lines (RILs) to map QTL associated with resistance to leaf and stripe rust. Jamestown has been documented to have leaf rust resistance and displayed HTAP resistance to stripe rust in regional nurseries across the United States. The soft red winter wheat cultivar Jamestown (PI 653731) is productive in the southern Corn Belt, the Deep South, and throughout the mid-Atlantic region. This can be attributed to its resistance to leaf rust, stripe rust, and Hessian fly (*Mayetiola destructor*) (Griffey et al., 2010) The objectives of this study were to characterize QTL conferring resistance to leaf rust and stripe rust, and to identify or develop diagnostic DNA markers that can be used in marker-assisted breeding to pyramid these genes with other complementary genes to provide effective and durable resistance.

MATERIALS AND METHODS

Plant Materials

One hundred and eighty-six RILs were derived from the cross of Pioneer '25R47' (PI 631473) (Lively et al., 2004) by Jamestown (PI 653731) (Griffey et al., 2010). The cultivars are adapted to the eastern US soft red winter wheat growing region. The cultivar Jamestown was derived from the cross 'Roane'/Pioneer '2691' developed by Virginia Tech. Pioneer Hi-Bred International developed cultivar 25R47 from the cross WBE-2190-B-1 ('Frankenmuth' /Pioneer '2555' sib//Pioneer '2551' sib)/WBA-416-H-2 ('Houser' /MO-9545//W-4034-D/'Augusta')//Pioneer '2552'.

Field Assessment

The RIL population was evaluated in replicated field tests composed of 1.2-m rows as the experimental unit arranged in a randomized complete block design. Disease-resistant and susceptible checks, along with both parents of the population, were spaced throughout the design. Disease spreader strips of the leaf rust-susceptible cultivar 'Massey' (PI 17953) (Starling et al., 1984) and stripe rust-susceptible line VA10W-21 (Z00-5018/VA01W-158) bordered the RIL population at each location.

Leaf rust assessments of the RIL population were conducted at Warsaw, VA (one replication in 2013–2014 and two replications in 2014–2015), where susceptible borders were inoculated with leaf rust race TNRJ using Soltrol light mineral oil in addition to natural infection. The RIL population was also evaluated

at Castroville, TX (one replication), and Plymouth, NC (two replications), under natural infection in 2014 and 2015.

Stripe rust assessments of the RIL population was conducted at Blacksburg, VA (one replication in 2013–2014 and two replications in 2014–2015), where susceptible borders were infected by transplanting inoculated seedlings of VA10W-21 infected with the race PST-100 in addition to any naturally occurring infection. Race PST-100 was classified and designated under the old US race nomenclature system and differential host set and is similar to predominant races *PSTv-32* and *PSTv-52*, which were identified using the newer set of 18 stripe rust single-gene line differentials. The RIL population was also evaluated at Castroville, TX (one replication), and Laurel Springs, NC (two replications), under natural infection in both years. In addition, the population was evaluated 1 yr each in Fayetteville, AR (two replications), in 2014 and in Griffin, GA (two replications), in 2015, both under natural infection.

Disease ratings were assessed when the susceptible checks displayed no less than 30% severity. The population was assessed for infection type using a 0-to-9 scale (Line and Qayoum, 1992; Singh et al., 1992) and disease severity from 0 to 100% based on the modified Cobb scale (Peterson et al., 1948) for both *P. triticina* and *P. striiformis*. Each rating was recorded one to three times at each location per growing season, dependent on optimal infection levels of *P. triticina* and *P. striiformis*.

DNA Extraction

Tissue of each RIL was collected when seedlings reached the three-leaf stage and placed into 2-mL test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low-temperature (−80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder for 15 s or until finely ground. The DNA extraction was then implemented using a modified cetrimonium bromide (CTAB) method (Saghai-Maroof et al., 1984).

Microsatellite Assay

Over 400 simple sequence repeat (SSR) markers were analyzed using bulk segregant analysis of 142 samples with 71 of each extreme phenotype. The SSR primer pairs were synthesized by Applied Biosystems (Carlsbad, CA) and Integrated DNA Technologies (Coralville, IA). Primers were directly labeled with a fluorescent dye or indirectly labelled with an M13 fluorescent tail (5′-ACGACGTTGTAAAACGAC-3′ or 5′-CACGACGTTGTAAAACGAC-3′). Simple sequence repeats were run using similar procedures to Christopher et al. (2013). The polymerase chain reaction (PCR) products of four separate M13-PCRs were combined for analysis in an Applied Biosystems 3130xl Genetic Analyzer. The PCR products were transferred to a 96-well PCR plate with each well containing 9.9 μL of Hi-Di formamide and 0.1 μL of size standard. Samples were denatured at 95°C for 5 min. The PCR products were visualized on an Applied Biosystems 3130xl Genetic Analyzer, and the generated data were analyzed using the genotyping software Genemarker version 1.70 (SoftGenetics, 2007).

Single-Nucleotide Polymorphism Array

A 90K iSelect single-nucleotide polymorphism (SNP) genotyping assay was performed on the RIL population at the USDA-ARS Small Grains Genotyping Laboratory in Fargo, ND. Genome Studio 2.0 software (Illumina, 2016) was used to analyze the SNPs according to genotype. Procedures for calling SNP(s) were similar to the procedures used in Cavanagh et al. (2013) and Wang et al. (2014). In summary, there were multiple parental clusters called, similar to Liu et al. (2016), which were then converted to the A, B, H format (Supplemental Table S1). Manual scoring was assessed for each SNP cluster that could not be categorized by the default algorithm. Each SNP cluster was manually authenticated by visually assessing each cluster. KASPar oligos were ordered from Integrated DNA Technologies, with primers carrying standard FAM or VIC compatible tails (FAM tail: 5′-GAAGGTGACCAAGTTCATGCT-3′, VIC tail: 5′-GAAGGTCGGAGTCAACGGATT3′) and the target SNP in the 3′ end. Primer mix was set up as recommended by LGC Genomics (46 μL distilled H_2O, 30 μL common primer [100 μM], and 12 μL of each tailed primer [100 μM]). The KASPar primers were used for functional validation of results found in the QTL analysis.

Linkage Map Construction and QTL Analysis

Linkage maps were constructed using JoinMap 4.0 (Van Ooijen, 2006), the Kosambi mapping function (Kosambi, 1943) was used to estimate map distance, and linkage groups were constructed based on a minimum logarithm of odds (LOD) threshold value of 3.0. Windows Cartographer (WinQTLCart version 2.5) (Wang et al., 2007), R/QTL (Broman et al., 2003), and IciMapping 4.1.0 (Wang et al., 2012) were used to identify QTL via interval mapping and composite interval mapping. The critical LOD value of 3.0 to declare a QTL significant (p = 0.05) was based on 1000 permutations (Doerge and Churchill, 1996) for all traits and linkage groups. MapChart 2.2 (Voorrips, 2002) was used to draw linkage maps.

RESULTS

Linkage Maps

Markers were placed on all 21 chromosomes, but there was low coverage on the D genome due to a majority of the markers being in complete correlation. The SSRs found to be polymorphic among the bulk segregant analysis were genotyped for the entire population. A total of 1760 unique SNP (1685) and SSR markers (75) were polymorphic between Jamestown and Pioneer '25R47'. Among these markers, 1682 were mapped in the final linkage analysis use to segregation distortion or poor quality of some SNPs and SSRs.

QTL Analysis

Four QTL were identified in Jamestown including two associated with leaf rust resistance on chromosome 5B and two for stripe rust resistance residing on chromosomes 3B and 6A. Markers linked to each QTL with the highest LOD scores in each year for each trait are presented in

Tables 1 and 2. The QTL on chromosome 5B conferring leaf rust resistance has the largest effect and was identified in each year for both infection type and disease severity. The smaller-effect QTL on 3B and 6A for stripe rust infection type were identified in each year.

Leaf Rust Resistance QTL on Chromosome 5B

The LOD peak of the QTL located on chromosome 5B, designated QLr.vt-5B.1 (Fig. 1), was located at SNP markers IWB7835 and IWB24418 between positions 22 and 25 cM, respectively. The second QTL, designated QLr.vt-5B.2 on 5B, was located between positions 38 and 39 cM at SNP markers IWB32871 and IWB26068 (Fig. 1). The QTL QLr.vt-5B.1 and QLr.vt-5B.2 are located at separate physical positions on the current survey sequence (Supplemental Table S2) (IWGSC RefSeq v1.0) and multiple other genetic maps (International Wheat Genome Sequencing Consortium, 2014; Chapman et al., 2015). Phenotypic variation in infection type explained by QLr.vt-5B.1 was highest (22.1%) in the 2015 Virginia test, 3.7% in the 2014 Virginia test, and 1.7% in the 2014

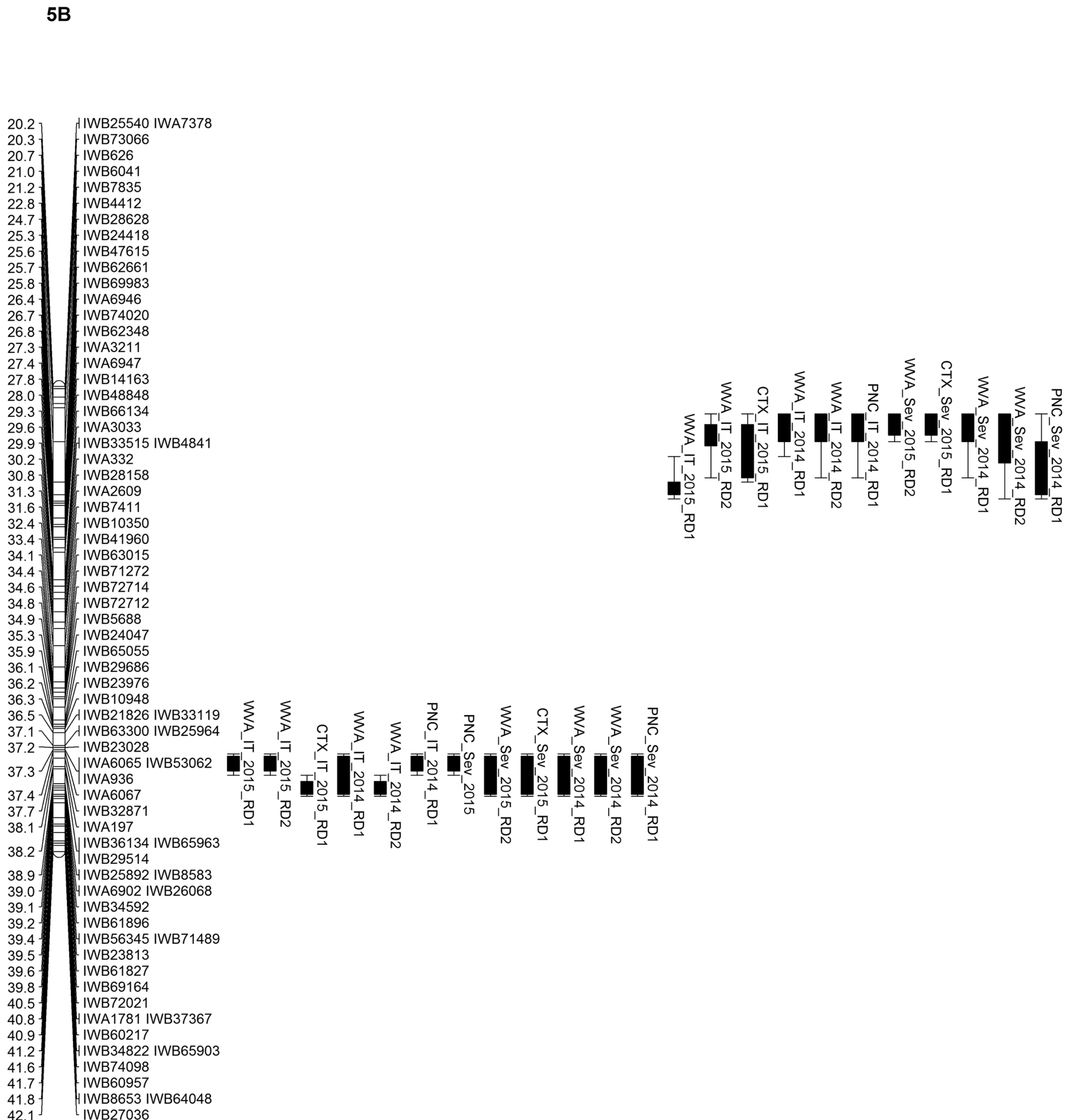

Fig. 1. Partial linkage map of chromosome 5B indicating location of traits associated with QLr.vt-5B.1 (above) and QLr.vt5B.2 (below).

North Carolina test (Table 1). The LOD scores were 4.6, 7.9, and 4.2, respectively. Phenotypic variation in leaf rust severity explained by QLr.vt-5B.1 also was highest (16.3%) in the 2015 Virginia test, 4.2% in the 2014 Virginia test, 10.7% in the 2015 Texas test, and 1.6% in the 2014 North Carolina test. The LOD scores were 9.1, 3.7, 4.2, and 3.1, respectively. Phenotypic variation in infection type explained by QLr.vt-5B.2 was highest for Virginia tests in 2015 (5.5%) and 2014 (4.0%), 3.3% in the 2015 Texas test, and 2.2% in the 2014 North Carolina test (Table 1). The LOD scores were 9.0, 9.5, 8.0 and 4.2, respectively. Phenotypic variation in disease severity explained by QLr.vt-5B.2 was highest (8.1%) in the 2015 North Carolina test, 5.1 and 3.5% in the 2015 and 2014 Virginia tests, and 2.4% in the 2015 Texas test. The LOD scores were 4.2, 6.8, 5.9, and 3.9, respectively. The variation of the phenotypic variation of QTL QLr.vt-5B.1 and QLr.vt-5B.2 can be explained by the difference in the infection type and severity from multiple environments, and subsequently the environment × genotype interactions (Fig. 2).

Stripe Rust Resistance QTL on Chromosome 3B

The LOD peak of the QTL located on chromosome 3B, designated QYr.vt-3B, was located at SNP markers IWB60584 and IWB23272 between positions 61 and 65 (Fig. 3). This QTL explained 8.9% of the phenotypic variation for infection type in the 2014 Virginia test, 6.9% in the Texas test, and 8.7 (2014) and 7.4% (2015) in the North Carolina tests (Table 2). The LOD scores were 7.1, 3.3, 7.0, and 4.4, respectively. For disease severity, QYr.vt-3B explained 11.1% of the variation in the 2015 North Carolina test and 8% in the 2014 Virginia test with LOD scores of 10.2 and 4.4, respectively. The slight variation of the of the phenotypic variation of QTL QYr.vt-3B can be described by the variance in the infection type and severity from Laurel Springs was more severe than in Blacksburg in both 2014 and 2015 (Fig. 4).

Table 1. Quantitative trait loci (QTL) associated with leaf rust infection type and severity in Pioneer '25R47'/Jamestown recombinant inbred lines (RILs) evaluated in 2014 and 2015 seasons.

Trait name†	Chr‡	Position	Confidence interval	Left marker	Right marker	LOD§	PVE¶	Add#
							%	
WVA_IT_2015_RD1	5B	25	23.5–25.5	IWB28628	IWB24418	3.4	5.0	−0.8
WVA_IT_2015_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	4.6	22.1	−0.6
CTX_IT_2015_RD1	5B	23	21.5–24.5	IWB4412	IWB28628	6.0	2.6	−0.6
WVA_IT_2014_RD1	5B	22	21.5–23.5	IWB7835	IWB4412	7.9	3.7	−1.1
WVA_IT_2014_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	8.2	3.3	−1.2
PNC_IT_2014_RD1	5B	22	21.5–24.5	IWB7835	IWB4412	4.2	1.7	−0.5
WVA_Sev_2015_RD2	5B	22	21.5–22.5	IWB7835	IWB4412	9.1	16.3	−3.6
CTX_Sev_2015_RD1	5B	22	21.5–22.5	IWB7835	IWB4412	4.2	10.7	−7.2
WVA_Sev_2014_RD1	5B	22	21.5–23.5	IWB7835	IWB4412	3.7	4.2	−2.2
WVA_Sev_2014_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	4.6	3.0	−3.3
PNC_Sev_2014_RD1	5B	24	21.5–25.5	IWB4412	IWB28628	3.1	1.6	−1.7
WVA_IT_2015_RD1	5B	38	37.5–38.5	IWB32871	IWA197	4.2	5.5	−0.8
WVA_IT_2015_RD2	5B	38	37.5–38.5	IWB32871	IWA197	9.0	5.5	−1.0
CTX_IT_2015_RD1	5B	39	38.5–39.5	IWA6902	IWB26068	8.0	3.3	−0.8
WVA_IT_2014_RD1	5B	38	37.5–39.5	IWB32871	IWA197	9.5	4.0	−1.2
WVA_IT_2014_RD2	5B	39	38.5–39.5	IWA6902	IWB26068	8.9	3.3	−1.3
PNC_IT_2014_RD1	5B	38	37.5–38.5	IWB32871	IWA197	5.9	2.2	−0.5
PNC_Sev_2015	5B	38	37.5–38.5	IWB32871	IWA197	4.2	8.1	−6.0
WVA_Sev_2015_RD2	5B	38	37.5–39.5	IWB32871	IWA197	6.8	5.1	−3.3
CTX_Sev_2015_RD1	5B	38	37.5–39.5	IWB32871	IWA197	3.9	2.4	−7.1
WVA_Sev_2014_RD1	5B	39	37.5–39.5	IWA6902	IWB26068	3.5	3.6	−2.3
WVA_Sev_2014_RD2	5B	39	37.5–39.5	IWA6902	IWB26068	5.9	3.5	−3.9
PNC_Sev_2014_RD1	5B	38	37.5–38.5	IWB32871	IWA197	3.6	1.6	−1.8

† First two letters indicate states (WVA = Warsaw, VA; CTX = Castorville, TX; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).

‡ Chromosome.

§ Logarithm of odds value.

¶ Plant variation.

Level of additivity.

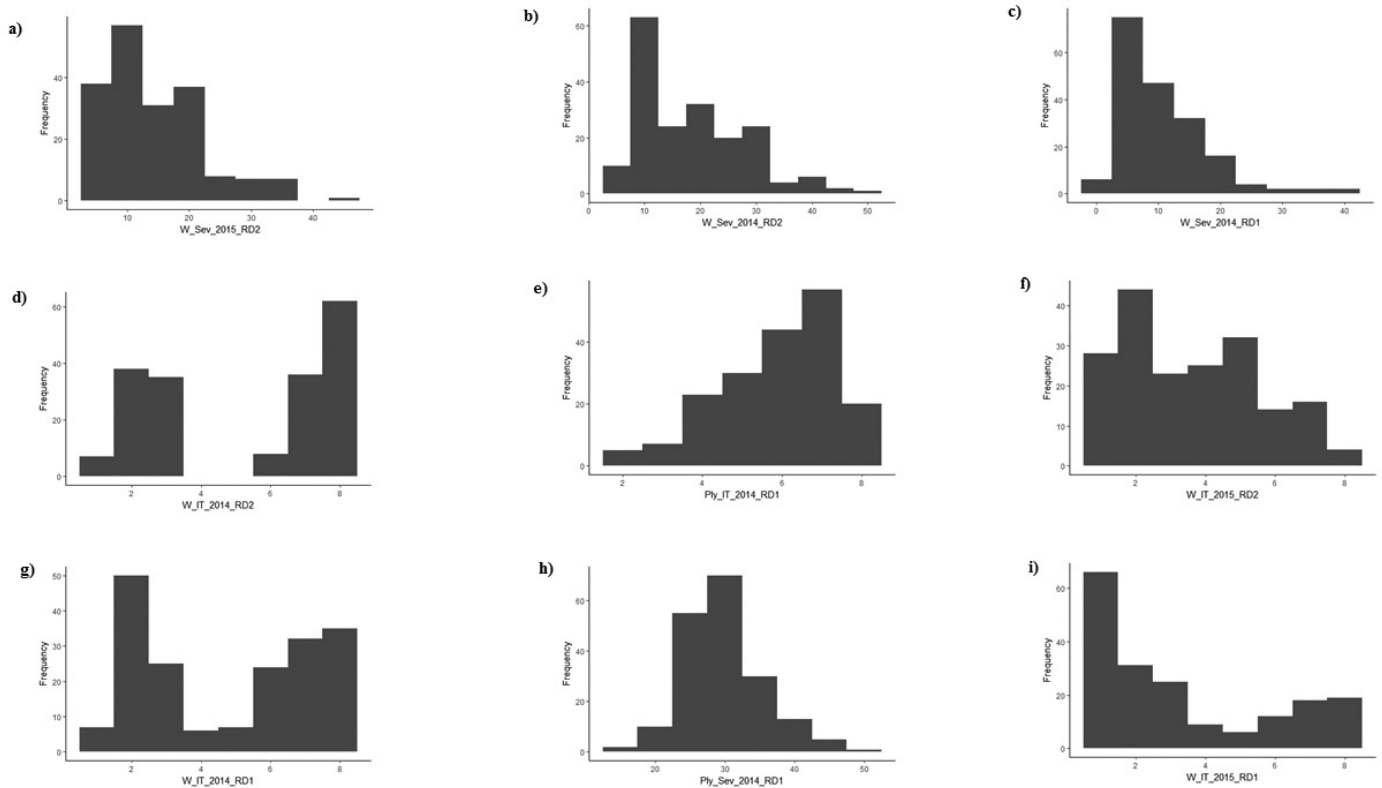

Fig. 2. Histograms of infection type and severity for significant locations associated with chromosome 5B.

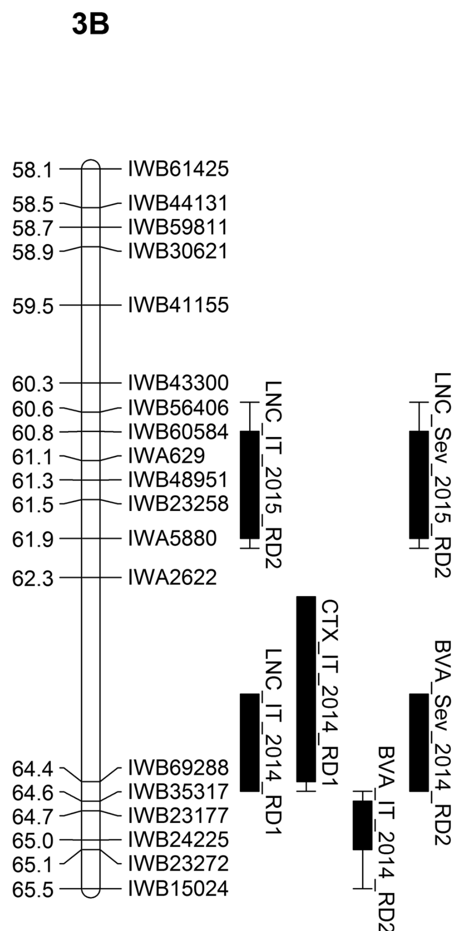

Fig. 3. Partial linkage map of chromosome 3B indicating location of traits associated with QYr.vt-3B.

Stripe Rust Resistance QTL on Chromosome 6A

The LOD peak of the QTL located on the long arm of chromosome 6A between positions 83 and 87.5, designated QYr.vt-6A (Fig. 5), was located at SNP markers IWB5971and IWB63000. The phenotypic variation in infection type explained by this QTL was highest for the Virginia test in 2015 (14.3%) and 2014 (12.2%), and 6.3% for the 2014 North Carolina test (Table 2). The LOD scores were 13.1, 3.7, and 4.0, respectively (Table 2), For severity, QYr.vt-6A accounted for 7.7 and 1.9% of the variation in the Virginia and North Carolina tests, respectively, with an LOD score of 3.9 for both tests. The variation of the of the phenotypic variation of QTL QYr. vt- 6A can be explained by the difference in the infection type and severity from Laurel Springs in 2014 was less severe than in Blacksburg in 2014 and 2015 (Fig. 6).

Phenotypic Effects of QTL Marker Loci on Leaf and Stripe Rust Resistance

To predict the effect of individual and pyramided QTL on leaf rust and stripe rust resistance, individuals (RILs) of the mapping population containing different combinations of the QTL were delineated into separate groups (Tables 3 and 4). The means of each group of RILs associated with disease infection type and severity were compared using a Students *t* test. Lines containing both QLr.vt-5B.1 and QLr.vt-5B.2 were similar to lines having only QLr.vt-5B.2 but had significantly lower

Table 2. Quantitative trait loci (QTL) associated with stripe rust infection type and severity in Pioneer '25R47'/Jamestown recombinant inbred lines (RILs) evaluated in 2014 and 2015 seasons.

Trait name†	Chr‡	Position	Confidence interval	Left marker	Right marker	LOD§	PVE¶	Add#
							%	
LS_IT_2015_RD2	3B	61	60.5–62.0	IWB60584	IWA629	4.4	7.4	−0.5
LS_IT_2014_RD1	3B	64	63.5–64.5	IWA2622	IWB69288	7.0	8.7	−0.6
C_IT_2014_RD1	3B	64	62.5–64.5	IWA2622	IWB69288	3.3	6.9	−0.3
BB_IT_2014_RD2	3B	65	64.5–65.5	IWB24225	IWB23272	7.1	8.9	−0.7
LS_Sev_2015_RD2	3B	61	60.5–62.0	IWB60584	IWA629	10.2	11.1	−4.6
BB_Sev_2014_RD2	3B	64	63.5–64.5	IWA2622	IWB69288	4.4	8.0	−3.1
BB_IT_2015_RD2	6A	83	82.5–83.5	IWB63000	IWB2065	13.1	14.3	−1.0
LS_IT_2014_RD1	6A	87	86.5–87.5	IWB35245	IWB5971	4.4	2.2	−0.6
LS_IT_2014_RD2	6A	84	82.5–83.5	IWB63000	IWB2065	4.0	6.3	−0.5
BB_IT_2014_RD2	6A	85	86.5–87.5	IWB35245	IWB5971	3.1	3.4	−0.6
BB_IT_2014_RD3	6A	84	84.0–85.5	IWA3487	IWB70137	3.7	12.2	−0.5
LS_Sev_2014_RD1	6A	87	86.5–87.5	IWB35245	IWB5971	3.2	1.2	−2.6
LS_Sev_2014_RD2	6A	84	84.0–85.5	IWA3487	IWB70137	3.9	1.9	−4.3
BB_Sev_2014_RD2	6A	84	84.0–85.5	IWA3487	IWB70137	3.9	7.7	−3.0

† First two letters indicate states (WVA = Warsaw, VA; CTX = Castorville, TX; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).

‡ Chromosome.

§ Logarithm of odds value.

¶ Plant variation.

Level of additivity.

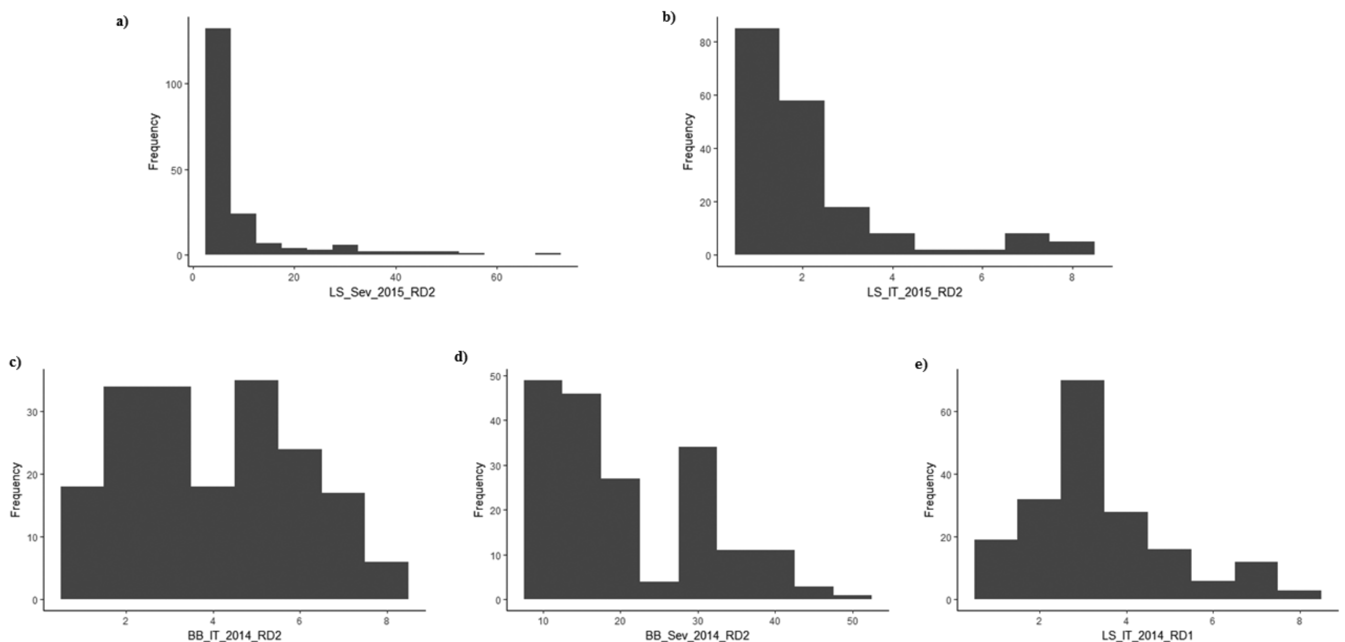

Fig. 4. Histograms of infection type and severity for significant locations associated with chromosome 3B.

infection type and severity than the lines containing only QLr.vt-5B.1. Lines containing both QLr.vt-5B.1 and QLr.vt-5B.2 were similar to lines having only QLr.vt-5B.2 but had significantly lower infection type and severity than the lines containing only QLr.vt-5B.1. This indicates that there may be some interaction between QLr.vt-5B.1 and QLr.vt-5B.2 (Table 3). Lines possessing a combination of QYr.vt-3B and QYr.vt-6A, QYr.vt-3B alone, or QYr.vt-6A alone were not statistically different, which indicates that these two QTL may not have major additive or epristatic effects (Table 4).

DISCUSSION

Leaf Rust Resistance QTL on Chromosome 5B

Two known leaf rust genes, *Lr18* and *Lr52*, have previously been reported to reside on chromosome 5B (McIntosh, 1983; Hiebert et al., 2005). It is unlikely that the source

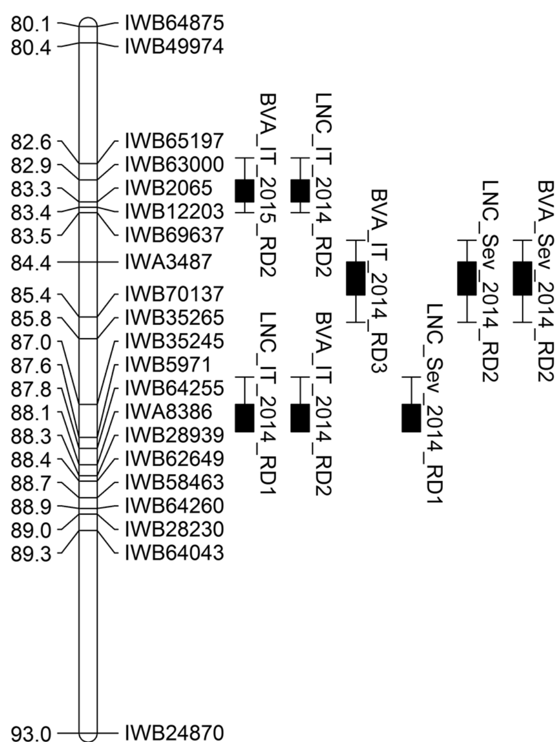

Fig. 5. Partial linkage map of chromosome 6A indicating location of traits associated with QYr.vt-6A.

Table 3. Mean leaf rust infection type and severities of recombinant inbred lines (RILs) and parent Jamestown with combinations of quantitative trait loci (QTL).

QTL combination†	Infection type	Severity	Individuals‡
Jamestown	3.05a	17.3a	–
QLr.vt-5B.1, QLr.vt-5B.2	3.57a	18a	48
QLr.vt-5B.2	3.65a	18.5ab	10
QLr.vt-5B.1	5.33b	25.6b	36
None	6.7c	32.2c	92

† Mean phenotype of QTL combination of RILs. Those with the same letter are not significantly different ($p < 0.05$). All markers within the respective QTL were used.

‡ Denotes number of RILs in each QTL combination.

Table 4. Mean stripe rust infection type and severities of recombinant inbred lines (RILs) and parent Jamestown with combinations of quantitative trait loci (QTL).

QTL combination†	Infection type	Severity	Individuals‡
Jamestown	3.00a	16.2a	–
QYr.vt-3B, QYr.vt-6A	3.03a	16.4a	24
QYr.vt-3B	3.11a	16.8a	57
QYr.vt-6A	3.19a	17.4a	48
None	5.23b	29.4b	57

† Mean phenotype of QTL combination of RILs. Those with the same letter are not significantly different ($p < 0.05$). All markers within the respective QTL were used.

‡ Denotes number of RILs in each QTL combination.

Fig. 6. Histograms of infection type and severity for significant locations associated with chromosome 6A.

of resistance in Jamestown is *Lr52*, as this gene provided high resistance in seedling tests conducted by Hiebert et al. (2005), whereas seedlings of Jamestown and RILs in the Pioneer '25R47'/Jamestown population expressed only moderate resistance (N.R. Carpenter, C. Griffey, S. Malla, Shiaoman Chao, G. Brown-Guedira, unpublished data, 2017). The QTL on 5B most likely are flanking gene *Lr18* and conceivably working epistatically or additively with *Lr18* (N.R. Carpenter, C. Griffey, S. Malla, Shiaoman Chao, G.Brown-Guedira, unpublished data, 2017). Two other regions on 5B have been reported to be a cluster of QTL associated with leaf rust resistance. The QTL located on 5B by Prins et al. (2011) (barc4) and Zhou et al. (2014) (barc128) were on the short arm of the chromosome, which is distant from QLr.vt-5B.1 and QLr.vt-5B.2 based on consensus maps (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). A QTL detected in cultivar Capo (wPt-7006) was located close to the same position as QLr.vt-5B.2 (Buerstmayr et al., 2014) indicating these may be the same QTL (Supplemental Table S3). QLr.vt-5B.2 on chromosome 5B contains SNP markers that are higher throughput and less costly to evaluate than the diversity arrays technology (DArT) marker located on Capo. The QTL QLr.cdl-5BL (Xfcp) and QLr.fcu-5BL (wPt-0837) are located on the more distal end of chromosome 5B (Chu et al., 2009; Kolmer, 2015) and 5 to 13 cM away from QLr.vt-5B.2 (Maccaferri et al., 2015). This indicates that QLr.vt-5B.2 is likely not in the same region as the QTL reported by Kolmer (2015) and Chu et al. (2009) (Supplemental Table S3). Further future research, requiring a larger fine mapping population would be necessary to definitively prove QLr.vt-5B.1 and QLr.vt-5B.2 are separate QTL and differ previously identified QTL.

Stripe Rust Resistance QTL on Chromosome 3B

Although numerous QTL for stripe rust have been reported on chromosme 3B, they are located a significant distance from QYr.vt-3B (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). These include QYr-3B.1-Pavon76, QYr.cim-BS_Chapio_Yr30, QYr.tam-3B_Quaiu, QYr.cim-3BS.2_Franklin, QYr-3B_Oligoculm, QYr-3B_Alturas, QYr.inra-3BS_Renan, QYr.ucw-3BS_UC1110, QYr.ucw-3BS.2, and QYr.uga_AGS2000. All of these QTL were mapped to the proximal end of the short arm of chromosome 3B, which is a significant distance from QYr.vt-3B (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). The QTL QYrpi.vt-3BL_VA00W-38 (wPt-0267) was originally mapped to the long arm of chromosome 3B; however, its location based on newer consensus maps may actually reside on the short arm of chromosome 3B like the QTL described above (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). The QTL QYR.sun-3B_Wollaroi (wPt-9577), QYrid.vi.ui-3B_Rio Blanco (gwm299), and QYrex.wgp-3BL_Express (gwm299) map to the long arm of chromosome 3B. However, these QTL are significantly closer to the distal end of the long arm of chromosome 3B than QYr.vt-3B (Supplemental Table S3).

Stripe Rust Resistance QTL on Chromosome 6A

QYr.vt-6A is located on the long arm of chromosome 6A. The QTL QYr.uga-6AS-26R61 (wPt-671561), QYr.wgp-6AS_Express (gwm334), and QYr.cim-6A_Avocet (wPt-2573) are located on the short arm (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). QYr.cim-6AL_Francolin (wPt-733679) is located closer to the proximal end of chromosome 6AL, whereas QYr.vt-6A is located closer to the distal end of 6AL. The QTL QYr.orr-6AL_Stephens (wPt-1642), QYr-6A_Saar (wPt-7063), and QYr.ufs-6A_Kariega (wPt-7181) are located at the same position on the consensus map as QYr.vt-6A (Supplemental Table S3). Although this indicates that QYr.vt-6A likely is not novel, it does confirm that Jamestown has at least one QTL in common with the HTAP-resistant cultivar Stephens.

Breeding Applications

Two QTL were detected on chromosome 5B associated with leaf rust resistance (Table 1) and two other QTL associated with stripe rust resistance were located on 3B and 6A (Table 2). Once markers closely linked to these QTL are validated, they can be used in marker-assisted selection (MAS) to incorporate and pyramid these QTL with other effective resistance genes. Stripe rust resistance conferred by QYr.vt-3B, QYr.vt-6A, or the combination of QYr.vt-3B and QYr.vt-6A was not statistically different; therefore, these QTL may not have major additive or epistatic effects with one another. Therefore, MAS for QYr.vt-3B and/or QYr.vt-6A may be equally effective in reducing stripe rust susceptibility. Lines containing both QLr.vt-5B.1 and QLr.vt-5B.2 had significantly lower leaf rust infection type and severity than lines containing only QLr.vt-5B.1. In addition, lines having QLr.vt-5B.2 also had significantly lower infection type than lines having QLr.vt-5B.1. Therefore, it likely will be beneficial to implement MAS for both QLr.vt-5B.1 and QLr.vt-5B.2 to reduce leaf rust susceptibility. Future work will include the development of kompetitive allele-specific markers to validate the QTL in cultivars with Jamestown in the genetic background for use and validation in the USDA Small Grains Genotyping Labs.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

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