Adult Plant Leaf Rust Resistance Derived from Toropi Wheat is Conditioned by *Lr78* and Three Minor QTL

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ABSTRACT

Leaf rust caused by *Puccinia triticina* is an important disease of wheat in many regions worldwide. Durable or long-lasting leaf rust resistance has been difficult to achieve because populations of *P. triticina* are highly variable for virulence to race-specific resistance genes, and respond to selection by resistance genes in released wheat cultivars. The wheat cultivar Toropi, developed and grown in Brazil, was noted to have long-lasting leaf rust resistance that was effective only in adult plants. The objectives of this study were to determine the chromosome location of the leaf rust resistance genes derived from Toropi in two populations of recombinant inbred lines in

a partial Thatcher wheat background. In the first population, a single gene with major effects on chromosome 5DS that mapped 2.2 centimorgans distal to *IWA6289*, strongly reduced leaf rust severity in all 3 years of field plot tests. This gene for adult plant leaf rust resistance was designated as *Lr78*. In the second population, quantitative trait loci (QTL) with small effects on chromosomes 1BL, 3BS, and 4BS were found. These QTL expressed inconsistently over 4 years of field plot tests. The adult plant leaf rust resistance derived from Toropi involved a complex combination of QTL with large and small effects.

Leaf rust caused by *Puccinia triticina* Erikss. is a common disease of wheat worldwide (Saari and Prescott 1985; Samborski 1985). Losses due to leaf rust can be severe if the crop is infected early in development and the cultivars are susceptible (Marasas et al. 2004). Wheat breeding programs in many countries have included leaf rust resistance as an important trait for released cultivars. In South America (Germán et al. 2007) and North America (Kolmer and Hughes 2015), populations of *P. triticina* are highly variable, with many different virulence phenotypes or races detected annually. Wheat cultivars having race-specific leaf rust resistance (*Lr*) genes that are effective in seedlings have selected races of *P. triticina* with the corresponding virulence, rendering the resistance ineffective. Although more than 70 *Lr* genes have been given gene designations (McIntosh et al. 2017), most of these are seedling resistance genes and relatively few give resistance to the current *P. triticina* populations. *Lr* genes that express best in adult plants and provide an incomplete type of resistance to all races have proven to be the most durable (Lagudah 2011).

Leaf rust is the most prevalent and severe wheat disease in Uruguay, southern Brazil, and Argentina. Losses to leaf rust in this region can exceed 50% if susceptible cultivars are grown and fungicides are not applied (Germán et al. 2007). Wheat cultivars from South America have been a valuable source of long-lasting resistance to *P. triticina*. The cultivar Frontana from Brazil was the source of adult plant resistance genes *Lr13* and *Lr34* (Dyck et al. 1966; Kolmer et al. 2008) for many cultivars grown in South America and North America. Although *Lr13* is a race-specific adult plant resistance gene that no longer provides much effective resistance in many countries, *Lr34* has remained effective for more than 50 years and continues to be

an important part of leaf rust resistance in wheat cultivars in North America (Kolmer et al. 2008; Oelke and Kolmer 2005), South America (Germán and Kolmer 2012), and elsewhere. Other cultivars from Brazil (Kolmer and Liu 2001), Uruguay (Germán and Kolmer 2013), and Argentina (Dyck 1989) have *Lr34* and possibly other adult plant resistance genes that have not yet been characterized. Landrace-derived wheat cultivars from Uruguay were found to have the adult plant resistance gene *Lr46* (Kolmer 2015) and other undefined resistance genes (Kolmer et al. 2007).

The wheat cultivar Toropi (Frontana/Quaderma A//Petiblanco 8) from Brazil was released in 1965 (Kohli 1986), grown widely for 15 years, and has remained leaf rust resistant since its release. Toropi is resistant at the adult plant stage in field plots but is susceptible at the seedling stage. Previous genetic studies (Barcellos et al. 2000; Rosa et al. 2016) of Toropi have indicated that at least two recessive genes condition the adult plant leaf rust resistance. The objectives of this research were to characterize and map the leaf rust resistance derived from Toropi in a Thatcher wheat background to enable direct comparison of its resistance with other Thatcher lines that are near-isogenic for adult plant and seedling *Lr* genes (McIntosh et al. 1995). Two different recombinant inbred line (RIL) populations with leaf rust resistance derived from Toropi were developed in order to separate the segregating resistance genes.

MATERIALS AND METHODS

Seed of Toropi (PI 344200) were obtained from P. L. Dyck, Agriculture and AgriFood Canada, Cereal Research Centre, Winnipeg MB, Canada. Initial crossing between Toropi and the leaf rust susceptible spring wheat Thatcher (CI 1003) (Tc) was performed in the glasshouse. Plants were grown in 15-cm-diameter pots filled with a soil, peat, and sand mixture at 18 to 25°C with 8 h of fluorescent and incandescent lighting. F₁ plants were generated by using Toropi as the male parent. The F₁ seed was then backcrossed as the male parent to Thatcher. Eighty BC₁F₁ seeds were planted in a greenhouse in 15-cm pots and selfed to obtain BC₁F₂ families. Fifty seeds from each BC₁F₂ family were planted in 3-m rows spaced 30 cm apart in field

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plots near Winnipeg, MB, Canada. The plots were planted perpendicular to spreader rows of cultivars Thatcher and Little Club. The spreader rows were inoculated with a mixture of *P. triticina* races that were common in Manitoba in the mid-1990s (Kolmer and Liu 1997). The plots were inoculated when the spreader rows were at jointing stage. The BC₁F₂ families were evaluated for leaf rust when the flag leaves of Thatcher were at 70 to 80% severity based on the modified Cobb scale (Peterson et al. 1948). Leaf rust response in the adult plants was rated as resistant (R) = small uredinia surrounded by necrosis, moderately resistant (MR) = moderate size uredinia surrounded by necrosis, moderately susceptible (MS) = moderate size uredinia surrounded by chlorosis, and susceptible (S) = large uredinia without necrosis or chlorosis.

Seed of selected BC₁F₂ families that expressed resistance in field plots were planted in 15-cm pots and grown to the adult stage in a greenhouse at 18 to 25°C with 16 h of supplemental metal halide lighting. Plants were inoculated at the flag leaf stage with a *P. triticina* isolate of virulence phenotype BBBD (Long and Kolmer 1989), which is highly avirulent to most *Lr* genes in wheat. Seed was harvested from plants that had clearly discernable fleck infection types (IT) and small to moderate size uredinia surrounded by chlorosis. The selected BC₁F₃ seed were planted and grown to the adult stage and flag leaves were inoculated with isolate BBBD. The BC₁F₃ plants from family number 3 had low infection of 22+ (small to moderate size uredinia, with chlorosis) and plants from family number 4 had IT of ;1- (hypersensitive flecks, with small uredinia

surrounded by necrosis). Seed from BC₁F₃ plants 3A and 4A were advanced to the F₄ and F₅ generations by single-seed descent. The BC₁F₄ lines 3A1, 4A2, and Toropi had a high IT of 3+ when tested as seedlings for response to isolates of *P. triticina* races BBBD and THBJG. BC₁F₅ adult plants 3A12A and 4A21A had IT of ;2 (flecks with small uredinia and chlorosis) and were advanced to the F₆ generation by single-seed descent. The Tc*2/Toropi lines 3A12A and 4A21A were crossed with Thatcher, and Tc*3/Toropi 3A12A F₆ and Tc*3/Toropi 4A21A F₆ RIL were derived by single-seed descent. The additional cross to Thatcher allowed the adult plant leaf rust resistance derived from Toropi to be evaluated in a background that was close to uniformity for maturity and plant height, which is helpful in the evaluation of adult plant leaf rust resistance. Seed from each RIL set was increased once in the greenhouse.

One hundred RIL from the Tc*3/Toropi 3A12A population were grown and tested for leaf rust resistance in plots at St. Paul, MN in 2010, 2012, and 2013. This RIL set was also tested for resistance in plots at Crookston, MN in 2010. One hundred RIL from the Tc*3/Toropi 4A21A RIL set were grown and tested for leaf rust resistance in plots at St. Paul, MN in 2011, 2012, and 2013. In the field plot tests at St. Paul, 50 to 60 seeds of the parents and each RIL were planted in 2 m rows spaced 30 cm apart perpendicular to spreader rows of the wheat cultivars Thatcher, Morocco, Max, and Little Club, all of which are susceptible to leaf rust. Plots in Crookston in 2010 had a row of Max planted every 20 rows of RIL. The plots were inoculated with isolates of *P. triticina* races MLDSB, TDBGG, MFPSB,

Fig. 1. Distribution of leaf rust severity (%) in adult plants of **A**, Thatcher*3/Toropi 4A21A and **B**, Thatcher*3/Toropi 3A12A recombinant inbred line (RIL) populations in field plot tests.

THBJG, TNRJ, and MCRKG in 2011; MHDSB, MFPSB, MLDSB, TBBGJ, TFBQJ, and TFBGQ in 2012; MHDSB, MFPSB, MLDSB, TBBGJ, TFBQJ, and TFBGQ in 2013; and MHDSB, MFPSB, MLDSB, TBBGJ, TFBQJ, TFBGQ, and TBBGS in 2014. These races were commonly found in the United States from 2009 to 2013, and are virulent to many seedling-effective *Lr* genes and race-specific adult plant resistance genes. Urediniospores were mixed with Soltrol 170 oil (Chevron-Phillips Petroleum, Bartlesville, OK) and atomized onto the spreader rows. The plots were rated when the susceptible parent Thatcher had leaf rust severity of 60 to 80% with a susceptible response. Three to five flag leaves collected from different parts of the row were assessed for severity and response. The leaf rust severities (percentage of leaf area covered) of the RIL in field tests were tested for correlation using Pearson's coefficient with PROC CORR in Statistical Analysis Software (SAS Institute, Cary, NC).

High-quality DNA from the parents and 92 RIL of Tc*3/Toropi 3A12A and 94 RIL of Tc*3/Toropi 4A21A was isolated using the cetyltrimethylammonium bromide buffer method recommended by Diversity Arrays Technology (DARt) (Triticarte Pty. Ltd., Canberra, Australia) (Akbari et al. 2006). The Tc*3/Toropi 3A12A RIL were genotyped with DARt methodology. Polymorphic markers were indicated as present (1) or absent (0). The Tc*3/Toropi 3A12A population was also genotyped with 33 simple-sequence repeat (SSR) markers (Supplementary Table S1). A 10- μ l polymerase chain reaction (PCR) mix was made of 20 to 40 ng of template DNA, 0.4 mM each reverse and M13-tailed forward primer, 0.4 mM fluorescence-labeled M13 primer, 0.08 mM each dNTP, 1.2 μ l of 10 $\times$ PCR buffer, 1 mM MgCl₂, and 0.6 U of *Taq* polymerase. PCR was performed using a touch-down program in a DNA Engine Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Four different plates were pooled into one plate using a Biomek NX^P liquid handling system (Beckman Coulter Inc., Brea, CA). The pooled PCR products labeled with one of the four dyes (FAM, VIC, NED, and PET) were analyzed in an ABI Prism 3730 DNA Sequencer (Applied Biosystems, Foster City, CA) and fragment length and polymorphism of amplicons were scored using GeneMarker (SoftGenetics LLC, State College, PA). The Tc*3/Toropi 3A12A RIL were also genotyped with the *csLV46* marker, which is linked to *Lr46* (E. Lagudah, CSIRO Canberra, personal communication).

The RIL of Tc*3/Toropi 4A21A were genotyped with the Infinium iSelect 9K wheat bead chip array described in Cavanagh et al. (2013). A single-nucleotide polymorphism (SNP) genotype calling for the RIL and parental lines was performed manually using GenomeStudio (v.2011.1; Illumina, San Diego, CA). Twenty RIL were also selectively genotyped with the Infinium iSelect 90K wheat bead chip

array described by Wang et al. (2014). Of these RIL, 10 were resistant in the field plot tests and the other 10 were susceptible. Based on the genotyping data from the 90K chip, 39 Kompetitive allele-specific PCR (KASP) primer sequences for selected 90K SNP markers on chromosomes 4A, 5D, 6A, 7A, and 2D that showed polymorphisms between two contrasting genotype groups were downloaded from Polymarker (polymarker.tgac.ac.uk). The KASP markers were assayed on the 20 selected RIL following the manufacturer's instructions on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). Eighty RIL of Tc*3/Toropi 4A21A were genotyped with 12 selected KASP markers. The Tc*3/Toropi 4A21A RIL were also genotyped with SSR markers on chromosome 5DS.

For both RIL populations, markers were assembled with quantitative trait loci (QTL) ICI Mapping (Meng et al. 2015) and redundant markers (100%) were removed using the BIN function. Linkage groups were assembled with Mapmaker v2.0 for Macintosh (Lander et al. 1987) using the Kosambi mapping function with a logarithm of odds (LOD) of 10 and $r = 0.3$. Linkage groups were assigned to wheat chromosomes based on previously published chromosomal assignments for each marker. Because the two resistant parents were derived from Thatcher, it would be expected that most markers (SNP, DARt, and SSR) would not be polymorphic between the parents in regions not associated with leaf rust resistance, resulting in an incomplete overall genetic map. Because the resistant parents were selected for leaf rust resistance compared with Thatcher, it would be expected to find polymorphic markers in the regions associated with the leaf rust resistance.

QGENE (Nelson 1997) was used to calculate the coefficient of determination (R^2), and LOD scores for each marker interval at a significance level of $\alpha = 0.05$ with 1,000 permutations of the dataset. Analysis of variance of markers and disease severity was conducted with PROC GLM in SAS.

RESULTS

Tc*3/Toropi 4A21A. The Tc*2/Toropi 4A21A parent had 10% leaf rust severity with an MR response in all three tests (Fig. 1A). The susceptible parent Thatcher had 60 to 70% leaf rust severity in three tests. The St. Paul 2011 test had fewer RIL with 70% severity compared with the other two tests. The St. Paul 2012 and 2013 tests had the highest correlation of 0.81 for leaf rust severity between the RIL, and the St. Paul 2011 and 2012 tests had the lowest correlation at 0.70 (Table 1).

In total, 587 markers were polymorphic (571 SNP, four SSR, and 12 KASP) in this population. After removing completely (100%) linked markers, 181 markers remained, which were used to construct 33 linkage groups that covered 701 centimorgans (cM). Single-marker regression and composite interval mapping of the genotyped RIL identified a region on chromosome 5DS, designated as *QLr.cdl-5DS*, that was associated with lower leaf rust severity. Three markers—the SSR *cfa2104* at 0.0 cM, the SNP *IWA6289* at 10.2 cM, and the SSR *cfd189* at 23.4 cM—were mapped to chromosome 5DS, with the LOD peak at 8.0 cM, closest to *IWA6289* (Fig. 2). The LOD scores for *QLr.cdl-5DS* were >9.0 in all three tests, and R^2 values

TABLE 1. Pearson's correlation coefficient of leaf rust severity ratings between different years of field plot tests of the Tc*3/Toropi 4A21A and Tc*3/Toropi 3A12A recombinant inbred line populations^a

Cross, population	Correlation coefficient				
	St. Paul 2010	Crookston 2010	St. Paul 2011	St. Paul 2012	St. Paul 2013
Tc*3/Toropi 4A21A					
St. Paul 2011	...	...	...	0.70*	0.74*
St. Paul 2012	...	...	...	...	0.81*
Tc*3/Toropi 3A12A					
St. Paul 2010	...	0.56*	...	0.68*	0.26*
Crookston 2010	...	...	...	0.72*	0.32*
St. Paul 2012	...	...	...	...	0.53*

^a An asterisk (*) indicates significant at $P < 0.01$.

Fig. 2. Composite interval mapping (IM) of leaf rust resistance in the Thatcher*3/Toropi 4A21A recombinant inbred line population. LOD = logarithm of odds, LS = leaf rust severity, and cM = centimorgans.

were >0.35 in all tests (Table 2). In the analysis of variance (ANOVA) of leaf rust severity, there was no significant genotype–environment interaction ($P = 0.18$), which allowed the overall mean severity and R^2 to be used. The 41 RIL homozygous for the *IWA6289* (G) allele inherited from Tc*3/Toropi 4A21A had an average leaf rust severity of 35.8%, whereas the 44 lines homozygous for the (A) allele from Thatcher had an average severity of 62.8% (Table 3). This indicated that a single adult plant resistance gene with major effect was segregating. Averaged over the three tests, the RIL homozygous for the *IWA6289* (G) allele from Tc*3/Toropi 4A21A had leaf rust severities of 10 to 60%, and RIL homozygous for the (A) allele from Thatcher had severities of 40 to 75% (Fig. 3). Of the 13 RIL with the *IWA6289* (G) allele that had average severity of 45 to 55%, 8 RIL had MRMS or MS response in all 3 years and 5 RIL had S response in only 1 year and MRMS or MS response in the other 2 years. One RIL with the *IWA6289* (G) allele had an average severity of 60%, and had an S response in 2 years and MS response in the third year. This RIL may be a recombinant between the resistance gene and *IWA6289*. Of the five RIL that had the *IWA6289* (A) allele and average leaf rust severity of 40 to 50%, three RIL had an S response in 1 year and MS responses in the other 2 years. The other two RIL had MRMS or MS responses in all 3 years. Some of the RIL with the *IWA6289* (A) allele that had lower severity and MR to MS response may also be recombinants. *IWA6289* had an overall R^2 value of 0.50 in the three tests. The KASP marker developed for *IWA6289* had allele calls for the 80 tested RIL that were identical to those obtained from the 9K SNP genotyping array for the entire population. In the physical map of Chinese Spring wheat, *IWA6289* is located on scaffold 16669 on chromosome 5D (Supplementary Table S2).

TABLE 2. Composite interval mapping of adult plant leaf rust resistance in the Tc*3/Toropi 4A21A and Tc*3/Toropi 3A12A recombinant inbred line populations^a

Cross, QTL	Peak position (cM)	Marker (cM)	Test	R^2 value	LOD
Tc*3/Toropi 4A21A					
<i>QLr.cdl-5DS</i>	8.0	<i>IWA6289</i> (10.2)	St. Paul 2011	0.37	9.30
			St. Paul 2012	0.53	15.41
			St. Paul 2013	0.44	12.26
Tc*3/Toropi 3A12A					
<i>QLr.cdl-1BL</i>	38.0	<i>csLV46</i> (39.6)	St Paul 2010	0.11	2.31
			Crookston 2010	0.10	2.07
			St. Paul 2012	0.06	1.25
			St. Paul 2013	0.00	0.00
<i>QLr.cdl-3BS.2</i>	56.0	<i>barc147</i> (54.3)	St Paul 2010	0.01	0.28
			Crookston 2010	0.14	3.01
			St. Paul 2012	0.14	2.95
			St. Paul 2013	0.02	0.35
<i>QLr.cdl-4BS</i>	16.0	<i>wPt-5497</i> (15.6)	St Paul 2010	0.04	0.82
			Crookston 2010	0.02	0.34
			St. Paul 2012	0.10	2.03
			St. Paul 2013	0.16	3.37

^a Abbreviations: QTL = quantitative trait loci, cM = centimorgans, and LOD = logarithm of odds.

Tc*3/Toropi 3A12A. The Tc*2/Toropi 3A12A parent had leaf rust severity of 10 to 30% with an MR to MS response in the 4 years of field plot tests (Fig. 1B). The susceptible parent Thatcher varied between 70 and 80% severity in the four tests. The Tc*3/Toropi 3A12A RIL varied from 10 to 80% severity in the field plot tests. In the St. Paul 2010 test, more RIL had a severity of 30 to 60% compared with the other 3 years, where the RIL were more evenly distributed for severity. The Crookston 2010 test had the largest number of RIL with a severity of 70%, whereas the St. Paul 2012 test had the largest number of RIL with 80% severity. The RIL showed a low to moderate correlation for severity between the four field plot tests (Table 1). The Crookston 2010 and St. Paul 2012 tests had the highest correlation of 0.72, whereas the St. Paul 2010 and St. Paul 2013 tests had the lowest correlation of 0.26.

The 92 RIL and parents were genotyped for a total of 370 polymorphic markers (336 DArT, 33 SSR, and *csLV46*). After removing completely linked (100%) markers, the 185 remaining markers were used to construct 28 linkage groups that covered 1,095 cM. Single-factor regression and composite interval mapping of the genotyped RIL identified regions on chromosomes 1BL, 3BS, and 4BS that were associated with lower leaf rust severity. In an ANOVA of leaf rust severity, there was no significant interaction between genotype and the four tests ($P = 0.106$), which allowed overall severity means and R^2 values for the individual QTL to be combined.

The region on chromosome 1BL associated with leaf rust severity was designated as *QLr.cdl-1BL*. Marker *csLV46* was closest to the LOD peak (Fig. 4A; Table 2). In the single-factor regression, *csLV46* significantly ($P < 0.001$) reduced leaf rust severity in all four tests, with an R^2 of 0.03. The RIL with the *csLV46* 560-bp allele inherited from Tc*3/Toropi3A12A had an average severity of 48.8%, whereas lines with the allele inherited from Thatcher had an average severity of 55.0%. However, *csLV46* had LOD scores <3.0 in all four tests. The highest LOD scores were 2.31 and 2.07 in St. Paul 2010 and Crookston 2010, respectively. The LOD scores for the St. Paul 2012 and 2013 tests were <1.5. St. Paul 2010 and Crookston 2010 also had the highest R^2 values, and St. Paul 2012 and St. Paul 2013 had the lowest values.

TABLE 3. Mean leaf rust severity (percent area covered) in multiple tests of combinations of adult plant resistance quantitative trait loci (QTL) in the Tc*3/Toropi 4A21A and Tc*3/Toropi 3A12A recombinant inbred line populations

Cross, genotype ^a	Number of observations	Mean leaf rust severity (%)	Group ^b
Tc*3/Toropi 4A21A			
<i>QLr.cdl-5DS-S</i>	132	62.8	A
<i>QLr.cdl-5DS-R</i>	123	35.8	B
Tc*3/Toropi 3A12A			
<i>QLr.cdl-1BL-S QLr.cdl-3BS-S QLr.cdl-4BS-S</i>	60	62.7	A
<i>QLr.cdl-1BL-S QLr.cdl-3BS-S QLr.cdl-4BS-R</i>	54	54.0	B
<i>QLr.cdl-1BL-R QLr.cdl-3BS-S QLr.cdl-4BS-S</i>	76	53.2	B
<i>QLr.cdl-1BL-S QLr.cdl-3BS-R QLr.cdl-4BS-S</i>	44	52.5	B
<i>QLr.cdl-1BL-R QLr.cdl-3BS-R QLr.cdl-4BS-S</i>	32	47.2	BC
<i>QLr.cdl-1BL-R QLr.cdl-3BS-S QLr.cdl-4BS-R</i>	48	46.8	BC
<i>QLr.cdl-1BL-S QLr.cdl-3BS-R QLr.cdl-4BS-R</i>	24	41.0	C
<i>QLr.cdl-1BL-R QLr.cdl-3BS-R QLr.cdl-4BS-R</i>	20	40.0	C

^a S = susceptible and R = resistant allele at QTL.

^b Genotypes within each cross with a different letter differ significantly for leaf rust severity based on honest significance difference in PROC GLM in SAS.

The region on chromosome 3BS associated with leaf rust severity was designated as *QLr.cdl-3BS.2*. The SSR marker *barc147* was the closest marker to the LOD peak (Fig. 4B; Table 2). In the single-factor regression, *barc147* significantly reduced leaf rust severity over the four tests, with an R^2 of 0.04 ($P < 0.001$). The RIL with the *barc147* allele from Tc*3/Toropi 3A12A had an average severity of 47.1%, whereas lines with the allele inherited from Thatcher had an average severity of 54.6%. The LOD scores for the 3BS QTL were 3.01 and 2.95 in Crookston 2010 and St. Paul 2012, respectively. The LOD scores for the St. Paul 2010 and 2013 tests were <0.5 . Crookston 2010 and St. Paul 2012 had R^2 values of 0.14, whereas the other two tests had much lower values.

The region on chromosome 4BS associated with leaf rust severity was designated as *QLr.cdl-4BS*. The DArT marker *wPt-5497* was closest to the LOD peak (Fig. 4C; Table 2). In the single-factor regression, *wPt-5497* reduced leaf rust severity across the four tests, with an R^2 of 0.06 ($P < 0.001$). The RIL having the *wPt-5497* allele inherited from Tc*3/Toropi 3A12A had an average leaf rust severity of 46.1%, whereas lines with the Thatcher allele had an average severity of 54.8%. The 4BS QTL had a LOD score of 3.37 in the St. Paul 2013 test, 2.03 in the St. Paul 2012 test, and very low LOD scores in the other two tests. Similarly, R^2 values were also highest for the St. Paul 2013 and 2012 tests and very low for the other two tests.

The 92 RIL genotypes were placed into eight groups based on the presence of resistant and susceptible alleles at *QLr.cdl-1BL*, *QLr.cdl-3BS.2*, and *QLr.cdl-4BS* (Table 3). Lines with missing data at these loci or lines that were heterozygous were excluded. RIL with susceptible alleles at all three QTL had significantly higher leaf rust severity scores compared with any line that had one or more resistant alleles. RIL with all three resistant alleles and lines with resistant alleles at *QLr.cdl-3BS.2* and *QLr.cdl-4BS* had the lowest leaf rust severity. All RIL with two resistant alleles did not differ significantly for severity compared with lines with all three resistant alleles. RIL having a single resistant allele did not differ significantly for severity. In all individual tests, there was no significant interaction ($P > 0.05$) between any of the three QTL, indicating that the resistant alleles acted additively to reduce leaf rust severity.

DISCUSSION

Adult plant leaf rust resistance derived from Toropi was found in this study to be additive, with a single adult plant gene on 5DS and minor QTL on 1BL, 3BS, and 4BS. The adult plant gene on 5DS expressed consistently in all three field plot tests conducted at St. Paul, where the Tc*3/Toropi 4A21A parent showed resistance that was more effective than Thatcher lines with the adult plant

resistance genes *Lr34*, *Lr46*, and *Lr67*. Most RIL with the 5DS gene had an MR to MS response, with uredinia that were surrounded by necrosis and chlorosis that were easily distinguished from susceptible RIL. There was some overlap for leaf rust severity between RIL with the resistant allele for *IWA6289* and the susceptible allele. Almost all RIL that had a higher severity level still had MRMS to MS responses in the three tests. The higher severity levels in these RIL may have resulted from higher inoculum loads in certain areas of the plots. The single RIL with the resistant allele for *IWA6289* that had high severity and an MS and S response in the different years of testing may be a recombinant between the marker and the 5DS gene. The RIL that had the susceptible allele but had lower leaf rust severity also may be recombinants. The distance between *IWA6289* and the LOD peak was 2.2 cM in this population, which would allow for some of the RIL to be recombinants. Thatcher single-gene lines with other adult plant resistance genes that condition partial resistance such as *Lr46* (Kolmer et al. 2012) and *Lr34* can often appear to be susceptible, depending on the inoculum load and level of rust infection.

Known *Lr* genes on 5DS include *Lr57* derived from *Aegilops geniculata* (Kuraparthi et al. 2009), *Lr70* derived from common wheat (Hiebert et al. 2014), and *Lr76* derived from *A. umbellulata* (Bansal et al. 2017). However, because these three resistance genes are expressed in seedlings, the adult plant resistance gene on 5DS is most likely a new gene for leaf rust resistance. *Lr70* mapped to the distal region of 5DS, 5.6 cM distal to *barc130* and 7.4 cM distal to *wmc233* (Hiebert et al. 2014). Both *barc130* and *wmc233* are located on scaffold 68913 on chromosome 5D. The 5DS gene identified in this study was flanked by *cfa2104* and *cfcl189*, both of which are proximal to *barc130* and *wmc233* in the wheat SSR consensus map (Somers et al. 2004). *IWA6289* was also placed proximal to *Lr70* based on scaffold location of the associated SSR and SNP markers in the physical map of chromosome 5DS. Furthermore, *Lr57* and *Lr76* are derived from lower-ploidy relatives of wheat. The release of Toropi in 1965 predates the transfer of these genes to wheat. On this basis, the 5DS gene is a new *Lr* gene, and was designated as *Lr78*.

The SNP marker *IWA6289* was previously strongly associated with adult plant leaf rust resistance in an association mapping study (Turner et al. 2016). In that study 1,032 spring wheat accessions from worldwide sources, including 155 cultivars from South America, and Toropi were genotyped with the 9K iSelect Illumina chip. The accessions were tested for leaf rust resistance in multiple field plot tests and in seedling tests to specific *P. triticina* races. *IWA6289* was associated with resistance in field plot tests in St. Paul in 2011, 2012, and 2014 and in Crookston in 2012. The SNP *IWA6289* was also associated with seedling resistance to races BBBDB and BBBBD, both of which are highly avirulent to most seedling *Lr* genes. Although

Fig. 3. Frequency distribution of Thatcher*3/Toropi 4A21A recombinant inbred line (RIL) for leaf rust severity based on segregation at *IWA6289*. RIL are segregating for the (G) allele from Thatcher*2/Toropi 4A21A, and the (A) allele from Thatcher.

Toropi was susceptible to both races in seedling tests, the adult plant resistance associated with *IWA6289* may enhance the expression of seedling resistance genes when combined in a single genotype, as is the case with *Lr34* (Germán and Kolmer 1992). The longevity of the Toropi resistance in South America and the consistent expression of *Lr78* in the 3 years of testing with a mixture of *P. tritici* races suggest that this gene may condition race-nonspecific resistance. The lack of other SNP and SSR markers in the 5DS region is likely due to the high degree of relatedness between the parents and the low genetic

polymorphism of hexaploid wheat in the D genome. Turner et al. (2016) also noted a low number of SNP markers in the D genome.

The minor QTL on 1BL, 3BS, and 4BS expressed inconsistently over the 4 years of testing. The QTL on 1BL is most likely the adult plant resistance gene *Lr46*. This gene does not always condition a clearly distinguishable resistance response when segregating with other resistance genes, and may be influenced by temperature and moisture conditions in field tests. In Mexico, RIL with *Lr46* derived from the cultivar Chapio had much higher LOD scores for the 1BL

Fig. 4. Composite interval mapping (IM) of leaf rust resistance in the Thatcher*3/Toropi 3A12A recombinant inbred line population: **A**, chromosome 1BL; **B**, chromosome 3BS; and **C**, chromosome 4BS. LOD = logarithm of odds, LS = leaf rust severity, and cM = centimorgans.

QTL compared with the same RIL population in China (Rosewarne et al. 2015). Lagudah (2011) indicated that *Lr46* is not as effective in higher-temperature environments compared with cooler environments. The presence of other *Lr* genes may also influence the relative expression of *Lr46*. In RIL populations of Tc*3/CI13227 (Kolmer et al. 2012) and Tc*3/Americano 25e (Kolmer 2015) evaluated in Crookston and St. Paul in 2010 on the same date as the Tc*3/Toropi 3A12A RIL, the 1BL QTL region of *Lr46* had high LOD scores of 8.6 to 15.2 and 9.8 to 16.8, respectively. In the Tc*3/CI13227 RIL, *Lr46* was the only detected resistance QTL and, in the Tc*3/Americano 25e RIL, a minor QTL on 5BL enhanced the resistance of *Lr46*. The source of *Lr46* may also affect expression of this gene, because different alleles may vary for effectiveness of resistance, as in the case of *Lr34* (Cao et al. 2010). Although LOD scores for the 1BL QTL in the Tc*3/Toropi 3A12A RIL were <3.0 in all tests, RIL with the resistant allele at the 1BL QTL had significantly lower leaf rust severity compared with RIL that had susceptible alleles at all three QTL.

Leaf rust resistance has also been previously found on chromosomes 3BS and 4BS. The complementary genes *Lr27* and *Lr31* are present on 3BS and 4BS, respectively; and, when present together, condition seedling resistance that is race specific (Singh and McIntosh 1984). *Lr27* is very tightly linked or allelic to *Sr2* (Mago et al. 2011). The cultivar Gatcher has *Lr27+Lr31* and is highly susceptible to leaf rust in field plot tests of current races of *P. triticina* in the United States (J. Kolmer, unpublished data). An allelic test in a cross between Gatcher and the Tc*2/Toropi 3A12a parent would be needed to determine whether the 3BS and 4BS QTL derived from Toropi are *Lr27+Lr31*. The cultivar Chapio also had an adult plant resistance QTL that mapped to 4BS (Rosewarne et al. 2015). The adult plant gene *Lr12* is also present on 4BS. In the field plot tests at St. Paul and Crookston, the Thatcher line with *Lr12* was highly susceptible, with a severity score of 60 to 80%. Because the 4BS QTL had LOD scores near 3.0 in the Crookston 2010 and St. Paul 2012 tests, it is not likely that this QTL is *Lr12*.

Other leaf rust resistance QTL have also been mapped to 3BS. Diéguez et al. (2014) identified an adult plant resistance gene with a hypersensitive response on 3BS, designated as *LrSV2*, which was very near or allelic to *Sr2*. Rosewarne et al. (2015) mapped an adult plant leaf rust resistance QTL on 3BS derived from Chapio, which was close to *Sr2*. Lan et al. (2014) mapped a QTL derived from the cultivar Francolin#1 to 3BS. Buerstmayr et al. (2014) mapped a QTL that was derived from the Austrian cultivar Capo in two different crosses on 3BS. *Lr74* is an adult plant resistance gene that was mapped to 3BS close to the *Sr2* locus and *LrSV2* in the cultivars BT-Schomburgk (Chhetri 2015) and Spark (Getie 2015). The relationship between the various adult plant leaf rust resistance QTL on 3BS that are close to *Sr2* will need to be investigated by either allelic tests or by fine-mapping in order to determine whether they are the same or unique genes.

The inheritance of leaf rust resistance in Toropi was examined previously. A selection designated as Toropi-6 was initially characterized as having two recessive genes for adult plant leaf rust resistance (Barcellos et al. 2000). Rosa et al. (2016) later determined that the same selection of Toropi had two recessive adult plant resistance genes, in addition to an adult plant race-specific resistance gene that was effective in New Zealand but not in Canada or Brazil. A QTL derived from Toropi for adult plant leaf rust and stripe rust resistance was mapped to chromosome 5AL (Rosa 2013), and a minor QTL for leaf rust resistance was mapped to 4BS. Using monosomic analysis, Da-Silva et al. (2012) located recessive adult plant *Lr* genes in Toropi on chromosomes 1A and 4D. The results from the previous studies disagree with our results in the two RIL populations derived from Toropi. One explanation is that Toropi is heterogeneous and that different selections of the cultivar were used in crosses with susceptible parents. Another explanation is that the entire leaf rust resistant genotype of Toropi may not have been evaluated in this study, because only two backcross lines with adult plant resistance were used as parents. The KASP marker for *IWA6289*, which is tightly linked to the 5DS resistance gene, can be used to assay different selections of Toropi.

The Tc*2/Toropi 4A21A line and Toropi can be used as sources to diversify adult plant leaf rust resistance in breeding projects. The resistance conditioned by *Lr78* was as effective as the resistance of the QTL on 1BL, 3BS, and 5BS combined. Genotypes with combinations of adult plant genes such as *Lr34* and *Lr68* with *Lr78* should be highly resistant. The KASP marker for *IWA6289* will facilitate selection of *Lr78*. However, the minor QTL detected on 1BL, 3BS, and 4BS in Tc*2/Toropi 3A12A would be difficult to retain in a breeding program because they condition relatively little resistance when present singly, and would be easily masked in the presence of more effective resistance genes. Robust diagnostic markers for these QTL would facilitate their retention in a breeding program. The longevity of the Toropi resistance characterized in this study was due to the combination of a single gene with a large effect combined with three QTL that had much smaller effects.

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