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Crop Science

ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Quantitative trait loci mapping reveals the complexity of adult plant resistance to leaf rust in spring wheat 'Copio'

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Abstract

The spring wheat (*Triticum aestivum* L.) line 'Copio' has exhibited high level of adult plant resistance (APR) to the leaf rust (*Puccinia triticina*) pathogen in Mexico during field evaluations. To elucidate the genetic basis of leaf rust resistance in Copio, 176 F_4-derived F_6-recombinant inbred lines (RILs) from a cross of wheat lines 'Apav' and Copio were phenotyped in the field for two seasons in the United States and Mexico. A total of 762 genotyping-by-sequencing (GBS) single nucleotide polymorphic (SNP) markers were used to develop linkage maps. Composite interval mapping identified seven quantitative trait loci (QTL), all contributed by Copio. Three QTL on chromosome arms 1BL (*QLr.umn-1B*), 2AS (*QLr.umn-2A*), and 3BS (*QLr.umn-3B*) were consistently expressed across all four environments. The QTL on 1BL represents *Lr46*, which is a pleiotropic APR gene, while the QTL on 2AS is colocated to the *Lr37* gene in the 2NS/2AS translocation fragment. The QTL on 3BS, mapped to the *Sr2/Yr30/Lr27* genomic region, is more likely to be a unique locus conferring APR to leaf rust races because all phenotyping environments had *Lr27* virulent pathotypes. Moreover, the functionality of *Lr27* is complimented by *Lr31* on chromosome 4BS, which is lacking in both parents. Marker haplotypes identified seven RILs carrying a combination of resistance alleles at all three loci. This combination reduced leaf rust coefficient of infection up to 52 and 36% in the Mexican and U.S. environments, respectively. This study reports the complex genetic mechanism of APR to leaf rust in Copio and its importance as a potential resistance source for gene pyramiding through recombination breeding.

1 | INTRODUCTION

Leaf rust is a common disease of hexaploid wheat (*Triticum aestivum* L.), tetraploid wheat (*Triticum turgidum* L.), and triticale (× *Triticosecale* Wittmack [*Secale* × *Triticum*])—a hybrid of wheat and rye (Roelfs et al., 1992). It is caused by the fungal pathogen *Puccinia triticina* (hereafter abbreviated as *Pt*), formerly known as *Puccinia recondita* f. sp.

Abbreviations: APR, adult plant resistance; CIMMYT, International Maize and Wheat Improvement Center; COI, coefficient of infection; GBS, genotyping-by-sequencing; IT, infection type; LOD, logarithm of odds; PCR, polymerase chain reaction; Pt, *Puccinia triticina*; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism.

tritici. Leaf rust is less destructive than stem rust (*Puccinia graminis* f. sp. *tritici*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) but is responsible for more significant losses due to its frequent occurrence and widespread distribution worldwide (Huerta-Espino et al., 2011; Kolmer, 2005; Roelfs et al., 1992). Leaf rust causes severe losses when susceptible varieties are infected at early growth stages (Marasas et al., 2004).

Development and deployment of resistant wheat cultivars is a sustainable and eco-friendly process to control leaf rust (Oelke & Kolmer, 2004; Pink, 2002; Singh & Rajaram, 1991; Singh et al., 2000). Genetic resistance to wheat rusts is mainly characterized as race-specific seedling resistance, which is also known as all stage resistance, race-specific adult plant resistance (APR), and race-nonspecific APR, which is also described as partial resistance or slow rusting (Chen, 2013; Das et al., 1992; Johnson & Law, 1973). So far, 15 leaf rust APR genes have been designated. Among them, seven genes (*Lr12, Lr13, Lr22a/b, Lr35, Lr37, Lr48*, and *Lr49*) are race-specific, while eight could be race-nonspecific (McIntosh et al., 1995; 2008, 2016). Among race-specific APR genes, *Lr12, Lr13, Lr22b, Lr35*, and *Lr37* are qualitative and provide hypersensitive reactions but are functional only at the adult plant stage (McIntosh et al., 1995; Singh & Bowden, 2011). Race-specific resistance that is controlled by seedling genes is generally less durable, and the pathogen can more easily overcome it by evolving new races through mutation and selection (Jones & Dangl, 2006; Lowe et al., 2011). Many all-stage resistance genes became ineffective after a few years of deployment. For example, two genes, *Lr10* and *Lr16*, from the Canadian wheat cultivar Selkirk became ineffective within 2–8 yr of deployment (McCallum et al., 2016). Similarly, gene *Lr9* was deployed in the eastern United States during the 1970s, and within a few years, it was overcome by virulent *Pt* races (Kolmer et al., 2009). Moreover, in the United States, race-specific genes *Lr24*, *Lr26*, *Lr41*, and *Lr50* were also overcome by the pathogen (Kolmer et al., 2009). More recently, *Lr21*, which was common in many spring wheat cultivars grown in Minnesota, was defeated in 2010 by a new virulent race (Kolmer & Anderson, 2011).

Race-nonspecific resistance genes are generally long-lasting but do not provide high levels of resistance when used alone; however, they do provide adequate resistance when used in combination with other race-specific or race-nonspecific genes (Singh et al., 2000). The race-nonspecific APR gene *Lr34* has been deployed in many wheat cultivars worldwide and has proven to be a durable gene for leaf rust resistance (Krattinger et al., 2009; Lagudah et al., 2009; Singh et al., 2000). Combining *Lr34* gene with other APR genes, namely *Lr46*, *Lr67*, and *Lr68*, has significantly reduced damage from leaf rust (Silva et al., 2015). Currently, eight leaf rust resistance genes, *Lr34* (Dyck, 1977; Dyck, 1987; Lagudah et al., 2009), *Lr46* (Singh et al., 1998), *Lr67* (Dyck & Samborski, 1979), *Lr68* (Herrera-Foessel et al., 2012), *Lr74* (Chhetri et al., 2016), *Lr75* (Singla et al., 2017), *Lr77* (Kolmer et al., 2018a), and *Lr78* (Kolmer et al., 2018b), are considered as race-nonspecific APR genes. The determination of their genetic nature through cloning is important to predict their durability. Among these race-nonspecific APR genes, three have been demonstrated to be pleiotropic: *Lr34/Yr18/Pm38/Sr57* (Singh et al., 2012), *Lr46/Yr29/Pm39/Sr58* (Singh et al., 2013), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al., 2014). They confer partial resistance to all three rust pathogens plus powdery mildew caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici*. (Lillemo et al., 2008; William et al., 2003).

The evolution of new *Pt* races and the rapid ineffectiveness of race-specific genes have diverted the attention of rust pathologists and the wheat-breeding community to identify and utilize race-nonspecific APR genes for sustainable resistance. Advancements in DNA technologies and reduced genotyping costs have revolutionized quantitative trait loci (QTL) mapping and resulted in greatly improved marker coverage. Next-generation sequencing and release of the wheat reference genome (IWGSC, 2018) have facilitated more precise QTL mapping and QTL-position comparisons with other genotypes. This study utilized a bi-parental recombinant inbred line (RIL) mapping population developed by the Global Wheat Program at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico. The objective of our study was to identify QTL associated with leaf rust APR in the United States and Mexico using genotyping-by-sequencing (GBS) – a next-generation sequencing approach.

Core Ideas

- Seven quantitative trait loci (QTL) for leaf rust resistance were mapped in spring wheat 'Copio'.
- A QTL on 2AS was colocalized to *Lr37* in the 2NS/2AS translocation in Copio.
- A QTL on 3BS consistently mapped to the *Sr2/Yr30/Lr27* region can be a new leaf rust adult plant resistance locus.
- Allelic combination of *Lr46* and the QTL on 2AS and 3BS reduced leaf rust coefficient of infection up to 52% in Mexico.

2 | MATERIALS AND METHODS

2.1 | Plant material and seedling evaluations

This study used 176 F_4-derived F_6 RILs from a cross of spring wheat lines 'Apav' and 'Copio'. Both parental lines

were tested against Mexican *Pt* races MBJ/SP and BBG/BP at the seedling stage. The avirulence and virulence formula of race MBJ/SP is *Lr2a, 2b, 2c, 3ka, 9, 16, 18, 19, 21, 24, 25, (26), 28, 29, 30, 32, 33, 36/1, 3, 3bg, 10, 11, 13, 15, 17, 20, 23, 27 + 31, 37*. Race MBJ/SP has partial virulence for *Lr26* (Herrera-Foessel et al., 2012) and is shown inside parentheses. The other race BBG/BP is a variant of BBG/BN that is virulent to race-specific gene *Lr12* and qualitative complimentary genes *Lr27 + Lr31* (Huerta-Espino et al., 2009). The avirulence and virulence formula of race BBG/BP is *Lr1, 2a, 2b, 2c, 3, 3ka, 3bg, 9, 13, 14a, 15, 16, 17, 18, 19, 21, 22a, 24, 25, 26, 28, 29, 30, 32, 35, 37/Lr10, 11, 12, 14b, 20, 23, 27 + 31, 33, 72* (Huerta-Espino et al., 2011).

The RIL population and both parental lines were also tested against two U.S. *Pt* races BBBDS (virulent to *Lr14a*) and MCDSB (virulent to *Lr1, 3, 10, 17, 14a, 26* and *B*). Race BBBDS is widely avirulent while MCDSB is virulent to many leaf rust genes. In seedling assays, lines carrying known genes mostly in the Thatcher background were also included (Suppl. Table 1). Apav and Copio along with differential lines were planted using 5–7 seeds per line. For inoculation, urediniospores stored at -80 ˚C were heat-shocked in a 45 ˚C water bath for 15 min and then rehydrated overnight at 80% relative humidity inside a humidity chamber. Plants were inoculated at the two-leaf stage with urediniospores suspended in light mineral oil (approximately 12–15 mg of urediniospores per 0.8 ml of Soltrol 170 oil) using an atomizer. Inoculum concentration was approximately 0.15 mg on each plant. Inoculated plants were placed on a bench for about 60 min to allow the oil to evaporate because it may cause phytotoxicity. After oil evaporation, plants were placed in a dew chamber overnight (15 ˚C) to provide enough moisture for urediniospores germination and then transferred to a greenhouse with controlled temperature of 12—18 ˚C. At 12 to 14 d after inoculation, infection types (ITs) were assessed on plants using the 0–4 scale described by Long and Kolmer (1989). According to this scale, infection type "0" = no visible disease symptoms, ";" = only flecks and no uredinia, "1" = small sized uredinia which are encircled by necrosis, "2" = small to medium sized uredinia surrounded by chlorosis or necrosis, "3" = medium-sized uredinia with no chlorosis or necrosis, and "4" = large-sized uredinia without chlorosis or necrosis. Infection types 0–2 and their variations (0;, ;1, ;2, and 12) were considered resistant host and avirulent pathogen, while ITs 3 and 4 were considered susceptible host and virulent pathogen. The 0–4 seedling score on the RIL mapping population was converted into a linearized 0–9 scale using the modified Perl script as described by Gao et al. (2016). The Perl script is mainly based on the seedling data conversion scale proposed by Zhang et al (2014) with some modifications. For simple scores like 0, 2+, and 3, it only used Zhang's 0–9 scale, but if the scored values were more complex (e.g., 13+), then the first value was weighted double, and the final score was obtained based on the arithmetic means. Based on the linear scale, lines with a 1–6 score were considered resistant, while 7–9 were susceptible. Chi-squared (χ^2) tests were performed to check the assumptions of expected fitting-ratios for seedling gene segregation in the RIL population.

2.2 | Field evaluations for leaf rust

The RIL mapping population and parents were evaluated for leaf rust (*Pt*) in four environments at the adult plant stage. The phenotyping environments included Mexico 2016 and 2017 (hereafter referred to as Mex16 and Mex17) and Saint Paul 2016 and 2017 (hereafter referred to as Stp16 and Stp17).

For adult plant resistance evaluations, parents and the mapping population were planted at the CIMMYT Experimental Station at Ciudad Obregon in the Yaqui Valley during the 2015–2016 and 2016–2017 growing seasons (Mex16 and Mex17). Experimental lines of 0.7 m long were sown in paired rows with 0.3 m between rows. An 'Avocet' near-isogenic line (*Yr24*/*Yr26*) was used as a spreader. The experimental lines were surrounded by the spreader lines, and along one side of the trial, spreader hill plots were sown in the alley. To initiate rust infection and develop epidemic conditions, spreader rows were inoculated using an equal proportion of MBJ/SP and MCJ/SP *Pt* races. The only difference between these races is that MBJ/SP has partial virulence, while MCJ/SP has complete virulence to *Lr26* (Lan et al., 2014). The urediniospores of both races were suspended in Soltrol 170 (1 g L^{-1}) and sprayed on spreader rows. One liter of mixture was applied on approximately 122 m of spreader rows (8 mg m^{-1}).

In St. Paul, the mapping population and parental lines were included in a nursery inoculated with both leaf rust and stem rust during the 2016 and 2017 growing seasons. Single rows of 2 m with 0.2 m of distance between rows were planted in an augmented design with 2-m alleys. Wheat lines 'Morocco' and 'LMPG-6' were planted surrounding the experimental lines with a continuous row on the alternate alleys as spreader rows. A mixture of six *Pt* races (MHDSB, MFPSB, MLDSD, TFBGQ, TBBGS, and MJBJG) was suspended in Soltrol 170 oil (Chevron-Phillips Petroleum) and inoculated on the spreader rows following the same procedures described above. These races are virulent to the race-specific resistance genes *Lr12, Lr13*, and *Lr37* in U.S. wheat cultivars (Kolmer et al., 2018a). *Lr12* and *Lr37* are known to be effective only at the adult plant stage.

2.3 | Leaf rust phenotyping and data analysis

Nurseries were evaluated in all four environments for leaf rust severity on a scale of 0–100% using the modified Cobb

Scale (Peterson et al., 1948). In all environments, at least two readings were taken after anthesis (10.51 Feekes growth stage; Feekes, W., 1941) when disease severity was about 80% on the spreader rows. Infection response was also recorded on the RILs and both parents at the same time and classified into four categories: R = resistant (necrosis surrounded by small uredinia); MR = moderately resistant (necrosis surrounded by moderate-sized uredinia); MS = moderately susceptible (chlorosis surrounded by moderate- to large-sized uredinia); and S = susceptible (large-sized uredinia without necrosis or chlorosis). For all leaf rust trials, disease severity was recorded on a whole-plot basis, considering the percent of flag leaf area covered with disease. Terminal rust score was considered as the final disease severity. Field scores based on final disease severity were converted into coefficient of infection (COI) values using a customized Perl script described by Gao et al. (2016). This Perl script defined "severity" as percentage of diseased leaf area, and "response" (field IT) as 0–1 numeric scale, where 0 is resistant (R) and 1 is susceptible (S). Field data (disease severity and response) were automatically converted into three categories; severity, linearized infection response, and COI. Linearized infection response is infection response converted into a 0–1 scale, and COI is the product of severity and linearized infection response. Coefficient of infection values were used for all the phenotyping and QTL mapping analysis. Histograms and Pearson coefficient correlations were developed among all the phenotyping environments using the COI values in the R program (R. Core Team, 2018) and IBM SPSS 1.0.0.1174 (IBM Corp.).

Analysis of variance (ANOVA) of the RIL population was conducted in individual and combined environments for the leaf rust COI using the AOV function in the software IciMapping Version 4.1 (Meng et al., 2015). A linear model was fitted by considering the overall mean as fixed effects and all other factors as random effects. The observed trait response Y_{ij} of the genotype (G) *i* in the environment (E) *j* was modeled using the following equation:

$$Y_{ij} = \mu + G_i + E_j + (G \times E)_{ij} + e_{ij}$$

Where μ is the overall mean, and *e* is the random error.

Broad-sense heritability was estimated using the following formula:

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

Where $\sigma_G{}^2$ is the genotypic variance, and $\sigma_P{}^2$ is the phenotypic variance. The phenotypic variance was calculated using the formula below:

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

The $\sigma_{G\times E}{}^2$ is the variance of genotype-by-environment ($G \times E$) interaction, $\sigma_e{}^2$ is the error variance, and *n* is the number of environments.

2.4 | Genotyping and QTL mapping

Parents were genotyped with known markers of seedling and APR leaf rust resistance genes, *Sr2/Yr30/Lr27* (*csSr2*), *Lr34/Yr18/Sr57* (*csLV34*), *Lr46/Yr29/Sr58* (*csLV46G22*), *Lr68* (*csGS*), and *Lr67/Yr46/Sr55* (*Lr67SNP*) . For each marker, 10 μl polymerase chain reaction (PCR) reaction mix was prepared that contained 120 ng genomic DNA, 0.3 U Taq DNA polymerase, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 0.25 μM each of forward and reverse primers. The denaturation was performed at 94 ˚C for 5 min, followed by 35–40 cycles of amplification (94 ˚C for 45 s or 55 to 65 ˚C for 60 s, depending on primer pair, and 72 ˚C for 60 s) and finally the extension step at 72 ˚C for 7 min. Polymerase chain reaction products were visualized following the MASWheat protocols (https://maswheat.ucdavis.edu/protocols/leaf_rust_protocols). We were unable to test other known APR genes that have been recently designated, namely *Lr74* (Chhetri et al., 2016), *Lr75* (Singla et al., 2017), *Lr77* (Kolmer et al., 2018a), and *Lr78* (Kolmer et al., 2018b) due to the lack of diagnostic markers. Both parents were also tested for the 2NS/2AS translocation using 10 GBS markers associated with this translocation segment (Juliana et al., 2019) and gene-linked Kompetitive Allele Specific PCR marker (Helguera et al., 2003). This study utilized a genotyping data set from a previous study involving the same mapping population (Rauf et al., 2022). Genotyping data were generated by sequencing two 96-plex GBS libraries on Illumina Hi-Seq 2500 from which more than 220 million reads were obtained from each genomic library. The sequencing reads from both libraries were aligned to the 'Chinese Spring' wheat reference genome assembly v1.0 (IWGSC, 2018) in Burrows-Wheeler Aligner using the ALN function (Li & Durbin, 2009). For SNP identification, samtools was used to process the aligned sequences (Li, 2011). The single nucleotide polymorphism (SNP) filtration process was carried out at 30% minor allele frequency, and SNP calls were accepted using a minimum criterion of ≥3 alignments read-depth and ≥25 for the read-mapping quality. After the filtration process, 2,575 polymorphic markers with less than 20% missing data were used to develop linkage maps in QTL IciMapping Version 4.1 (Meng et al., 2015). Markers were assigned to 21 linkage groups, each representing a single chromosome. For marker ordering, a 'nnTwoOpt' algorithm was used, and 33 redundant markers were removed. Marker rippling was performed at a window size of eight, using the sum of adjacent recombination fractions criteria, and the Kosambi

TABLE 1 Seedling infection types of recombinant inbred line (RIL) parents Apav and Copio to *Puccinia triticina* (*Pt*) races

	Mexican *Pt* races		US *Pt* races	
Parent lines	**MBJ/SP**	**BBG/BP**	**BBBDB**	**MCDSB**
Apav	4	3+	;	3+
Copio	3	3	;12	3

mapping function was used to convert the recombination frequency into genetic distances (Kosambi, 1943) between markers.

For QTL mapping, redundant markers that had higher proportions of missing data were removed, and 762 SNP markers representing unique loci were retained. The composite interval mapping function in Windows QTL Cartographer 2.5 (Wang et al., 2012) was used for leaf rust QTL mapping. The composite interval mapping function used backward and forward regression at a walk speed of 1cM across the linkage groups. Quantitative trait locus/loci were declared significant at a threshold of 2.5 logarithm of odds (LOD) value. The detected QTL were named following the McIntosh et al. (2013) gene nomenclature and visualized on wheat chromosomes through MapChart 2.3 (Voorrpis, 2002).

3 | RESULTS

3.1 | Seedling and adult plant phenotyping

Both parents of the RIL population, Apav and Copio, had high infection response against the Mexican *Pt* races at the seedling stage (Table 1). Infection type was recorded as 4 and 3 for MBJ/SP and 3+ and 3 for BBG/BP on Apav and Copio, respectively. Both Apav (;) and Copio (;12) showed resistant reactions to the U.S. race BBBDB but high reactions (3+ for Apav and 3 for Copio) to the other U.S. race MCDSB. Seedling assays were performed on the RIL population using both U.S. *Pt* races. For race BBBDB, 129 lines were resistant, 32 were susceptible, and 15 lines were heterogeneous. Chi-squared tests fit a two-gene model ($\chi^2 = 2.65$; p-value = .11) for seedling resistance to race BBBDB in the RIL population. To check gene segregation assumptions, heterogeneous lines were dropped from the analysis. All RILs were highly susceptible (IT = 3 and 4) when tested against the *Pt* race MCDSB, hence no seedling gene was segregated for resistance to this race. Seedling evaluations of differential lines showed that the race BBBDB was virulent to *Lr14a*, *Lr14b*, and *Lr20*, while race MCDSB was virulent to *Lr1*, *Lr3*, *Lr26*, *Lr17*, *LrB*, *Lr10*, *Lr14a*, *Lr3bg*, *Lr14b*, *Lr20*, and *Lr23* genes (Suppl. Table 1).

Disease development was excellent in all four field environments. Apav had high disease in both Mexican (COI = 90–100%) and U.S. (COI = 70–90%) environments. Copio was highly resistant (COI = 0–10%) across all four environments. The mean COI of the RIL population was 33.6% (Mex16) and 41.5% (Mex17) in Mexico and 73.1% (Stp16) and 33.0% (Stp17) in the U.S. environments. High disease pressure was observed in the 2016 St. Paul nursery, and disease frequency distribution was more skewed towards susceptibility (Figure 1). Histograms reflected the continuous disease distribution spectrum across all environments, which also represented all severity classes in the RIL mapping population (Figure 1).

Analysis of variance for individual and combined environments revealed significant ($P < .01$) differences among genotypes for the leaf rust COI. Furthermore, the genotype-by-environment interaction for leaf rust COI was also significant (Table 2). The estimates of the broad-sense heritability for the leaf rust COI were relatively high in Mexico at 0.91 to 0.94, as compared with St. Paul environments at 0.68 to 0.85 during the 2016 and 2017 seasons, respectively (Table 2).

Pearson coefficient of correlations (r) among leaf rust COIs was high and significant ($r = .40$ to $.80$) in all phenotyping environments at 1% significance level (Table 3). The highest correlation was observed between Mex16 and Mex17 environments ($r = .80$), followed by Mex17 and Stp17 ($r = .57$).

3.2 | Genotyping and QTL mapping

Marker assays revealed that Apav did not carry any known APR genes (*Lr34*, *Lr46*, *Lr67*, and *Lr68*), while Copio likely contained pleiotropic APR gene *Lr46*. Moreover, Copio was positive for the 2NS/2AS alien translocation fragment that harbors race-specific APR gene *Lr37* (Table 4). Seven QTL were identified that conferred resistance to leaf rust and were all derived from Copio. The QTL harbored by chromosomes 1BL (*QLr.umn-1B*), 2AS (*QLr.umn-2A*) and 3BS (*QLr.umn-3B*) were consistently detected across all four environments. In this study, the QTL *QLr.umn-1B* represents the pleiotropic APR gene *Lr46/Yr29/Sr58* and will be abbreviated as *Lr46* hereafter. The *QLr.umn-3B* locus explained the maximum phenotypic variation (up to 18%) followed by the *QLr.umn-2A* (up to 17%). *Lr46* was significant in one environment, but LOD peaks below the significance threshold (<2.5) were observed in the other three environments. Chromosomes 2B, 3A, 3D, and 5D each had one QTL detected in one environment (Table 5). Quantitative trait locus/loci on each chromosome along with the environments in which they were detected are presented in Figure 2.

Based on marker haplotype, RILs that carried resistant alleles on *Lr46*, *QLr.umn-2A*, and *QLr.umn-3B*, and all possible

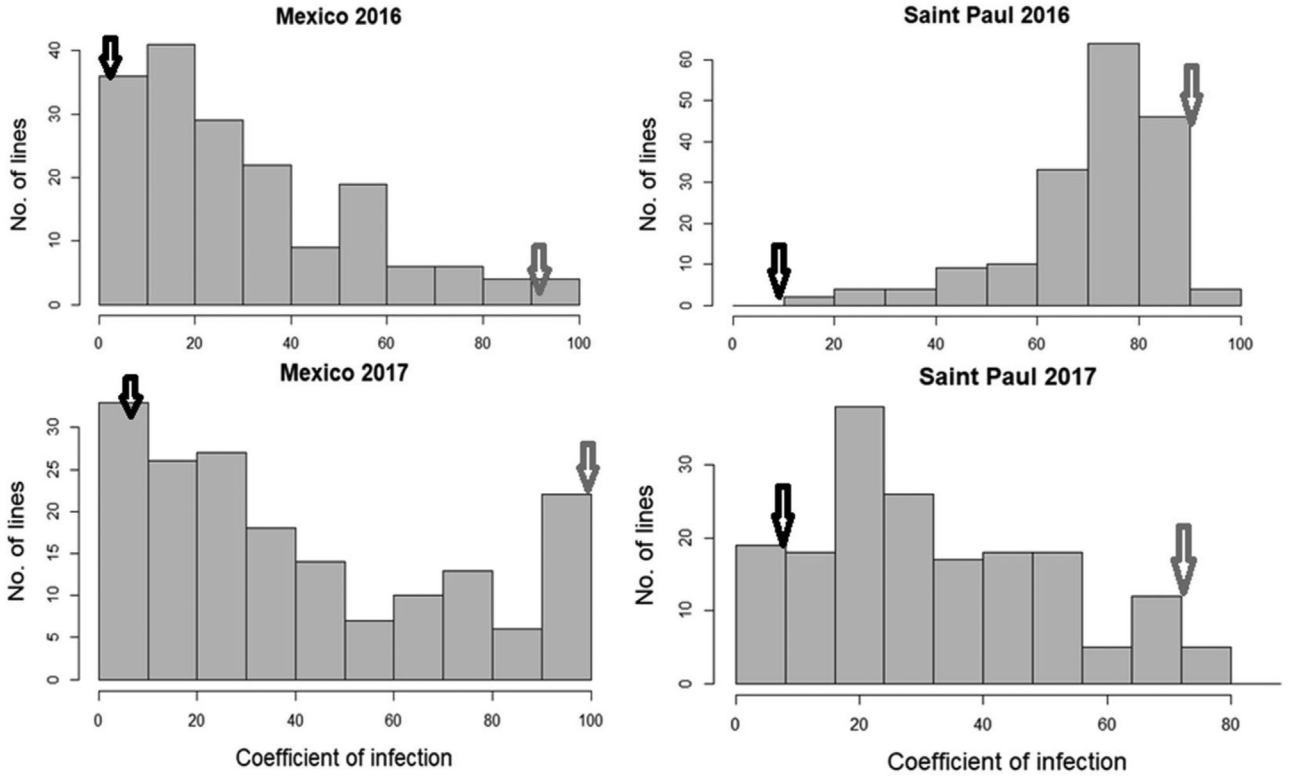

FIGURE 1 Frequency distribution of leaf rust coefficient of infection (COI) in the Apav × Copio recombinant inbred line (RIL) mapping population in four field environments. Black (Copio) and gray (Apav) arrows shown on the bars represent the COI values of the two parents in each environment

TABLE 2 Analysis of variance in four environments for leaf rust coefficient of infection and estimation of heritability on plot mean basis

Environments[a]	Source	MS[a]	*F*-value	σ_G^2	σ_e^2	σ_E^2	$\sigma_{G \times E}^2$	*H*
Stp16	Genotypes	519.8	3.9**	193.6	132.7			0.68
Stp17	Genotypes	744.5	36.3**	362.0	20.5			0.85
Mex16	Genotypes	1,221.6	127.0**	606.0	9.6			0.91
Mex17	Genotypes	1,947.4	134.2**	966.5	14.5			0.94
Combined ANOVA	Genotypes	2,882.0	65.0**	354.7	44.3			0.85
	Environments	11,8967.2	2,684.1**			337.8		
	G × E	517.1	11.7**				236.4	

Note. σ_G^2, genotypic variance; σ_e^2, error variance; σ_E^2, environment variance; $\sigma_{G \times E}^2$, genotype-by-environment interaction variance; *H*, broad-sense heritability.
[a]Stp, St. Paul; Mex, Mexico; 16 and 17 are the years 2016 and 2017 during which nurseries were phenotyped for leaf rust in the field.
[b]MS = Mean squares.
**Significant at $P < .01$ level.

combinations of these genes/QTL, were identified (Figure 3 and Suppl. Table 2). Generally, the RILs carrying more resistant alleles exhibited better resistance. The RILs that carried resistant alleles on all three loci had 36 and 52% less disease compared with the lines with none of these genes/QTL in the U.S. and Mexican environments respectively. This gene/QTL and the different combinations were more effective in the Mexican environments than the U.S. environments.

4 | DISCUSSION

Both parents of the RIL mapping population were susceptible at the seedling stage (IT ≥ 3) against the Mexican *Pt* races

TABLE 3 Pearson correlation (r) among coefficient of infection values on Apav × Copio recombinant inbred lines in four environments for leaf rust

Environment	Mex16	Mex17	Stp16
Mex17	.80**		
Stp16	.47**	.40**	
Stp17	.56**	.57**	.46**

Note. Mex16 and Mex17, Mexico 2016 and 2017, respectively; Stp16 and Stp17, Saint Paul 2016 and 2017, respectively.
**Correlation is significant at $p < .01$.

MBJ/SP and BBG/BP, which are virulent to race-specific genes *Lr1, 3, 3bg, 10, 11, 13, 15, 17, 20, 23, (26), 27 + 31, 37*, and *Lr10, 11, 12, 14b, 20, 23, 27 + 31, 33, 72*, respectively (Table 1). It was hypothesized that these genes were not associated with field resistance in adult-plant evaluations in Mexican environments. In addition to race MBJ/SP, we also used race MCJ/SP for field inoculations in Mexico, and the difference between these races is that the former race has partial virulence on *Lr26* while the latter race has complete *Lr26* virulence (Herrera-Foessel et al., 2012). *Lr26* was a spontaneous introgression from rye (*Secale cereale* L.) into wheat along with *Sr31/Yr9/Pm8* and is commonly known as the Kavkaz translocation of T1BL:1RS (Zeller, 1973). Based on the seedling phenotyping data against the MBJ/SP race, we eliminated the possibility of *Lr26* being present in either parent. Both parents were resistant (IT < 3) against the most avirulent U.S. *Pt* race BBBDB, and the Chi-squared (χ^2) tests of RIL data showed that two seedling resistance genes were segregating in our population for this race. The other U.S. *Pt* race MCDSB, which is widely virulent to many leaf rust genes, had high IT > 3 on both parents and the RIL population.

Copio had very low disease (i.e., COI) in all field environments, and this strong APR is likely due to multiple QTL with additive effects. A continuous disease distribution spectrum was observed in the RIL population, representing all phenotypic classes across all environments, indicating quantitative resistance (Figure 1). Disease severities of the RIL population were significantly correlated across all four environments. Stp16 was relatively less correlated with Mex17, which can be expected due to different environmental conditions and *Pt* races. The G × E effect can result in lower correlations, arising from environmental conditions, along with inoculum pressure and different rust races across environments. High heritability was observed across all four environments.

The QTL on chromosomes 2B (*QLr.umn-2B*), 3A (*QLr.umn-3A*), 3D (*QLr.umn-3D*), and 5D (*QLr.umn-5D*) were inconsistent and only detected in one environment. These QTL explained 6−13% of the phenotypic variance. Several studies involving biparental mapping populations and genome-wide association mapping have identified small- to medium-effect inconsistent QTL in CIMMYT wheat germplasm corresponding to the same chromosomes (Basnet et al., 2014; Gao et al., 2016; Lan et al., 2014; Yuan et al., 2020). It is more likely that these four QTL represent the previously reported leaf rust resistance loci in CIMMYT lines. A genome-wide association study was conducted by Gao et al. (2016) to identify leaf rust seedling and APR genes/QTL using a collection of 338 wheat lines from public and private sectors in Americas. The genome-wide association study panel had approximately 140 lines from CIMMYT. The study utilized wheat 90K SNP assay and reported 46 QTL involved in seedling and field resistance to leaf rust at variable levels. Association mapping identified genomic regions for leaf rust resistance on chromosomes 2B, 3A, and 5D. In another study, a QTL was reported on chromosome arm 3DS in the CIMMYT line 'Arableu#1', which explained up to 4% phenotypic variance (Yuan et al., 2020). Arableu#1 has one parent line common with Copio in their pedigrees. Although these QTL had minor effects, they can still contribute to reduce disease severity when combined with large-effect QTL and genes.

Medium- to large-effect QTL derived from Copio were consistently detected on chromosomes 1B (*QLr.umn-1B*), 2A (*QLr.umn-2A*), and 3B (*QLr.umn-3B*) and contributed substantial phenotypic variance for leaf rust. *QLr.umn-1B* was mapped on the long arm at 80.2 cM, and its physical position was 670.2 Mb on chromosome 1B. This QTL explained

TABLE 4 Molecular marker assays for known leaf rust adult plant resistance and seedling genes

Gene	Chromosome	Marker/assay	Apav	Copio	References
Lr46	1BL	*csLV46G22*	–	+	E. S. Lagudah, unpublished, 2016
Lr37/Sr38/Yr17	2AS	*KASP*	–	+	Helguera et al., 2003
Lr37/Sr38/Yr17	2AS	*GBS*	–	+	Juliana et al., 2019
Sr2/Yr30/Lr27	3BS	*csSr2*	–	+	Mago et al., 2011
Lr67	4DL	*Lr67SNP*	–	–	Hiebert et al., 2010
Lr68	7BL	*csGS*	–	–	Herrera-Foessel et al., 2012
Lr34	7DS	*csLV34*	–	–	Lagudah et al., 2006

Note. + and – represent that the gene was present or absent, respectively.

TABLE 5 Quantitative trait loci (QTL) associated with leaf rust resistance in Apav × Copio recombinant inbred line (RIL) mapping population across four environments

Chr.[a]	Environments[b]	QTL	Peak marker	Position	LOD	R^2[c]	Additive
				cM		%	
1B	Mex16	*QLr.umn-1B*	chr1B_670207768	80.4	5.4	8	7.1
	Mex17¶	*QLr.umn-1B*	chr1B_670207768	80.4	1.3	2	4.8
	Stp16¶	*QLr.umn-1B*	chr1B_670207768	80.4	2.3	4	3.3
	Stp17¶	*QLr.umn-1B*	chr1B_670207768	80.4	0.5	1	1.9
2A	Mex16	*QLr.umn-2A*	chr2A_19914469	1.0	11.3	17	12.0
	Mex17	*QLr.umn-2A*	chr2A_19914469	1.0	7.4	13	13.9
	Stp16¶	*QLr.umn-2A*	chr2A_21005775	4.8	1.9	3	3.5
	Stp17	*QLr.umn-2A*	chr2A_21005775	4.8	5.7	11	7.6
2B	Mex16	*QLr.umn-2B*	chr2B_769717318	82.6	4.4	8	7.6
3A	Mex17	*QLr.umn-3A*	chr3A_688594297	95.4	3.1	7	8.9
3B	Mex16	*QLr.umn-3B*	chr3B_6071517	25.6	7.0	10	8.2
	Mex17	*QLr.umn-3B*	chr3B_6071517	25.6	9.3	17	13.6
	Stp16	*QLr.umn-3B*	chr3B_6396363	20.8	7.2	15	6.4
	Stp17	*QLr.umn-3B*	chr3B_4469125	25.2	7.9	18	8.5
3D	Stp16	*QLr.umn-3D*	chr3D_305491061	69.4	3.2	6	3.9
5D	Mex16	*QLr.umn-5D*	chr5D_21558380	15.9	4.6	13	9.7

¶*Note*. Environments in which QTL peaks were observed but logarithm of odds (LOD) values were nonsignificant (LOD < 2.5).

[a]Chromosomes (Chr.) on which QTL was detected.

[b]Leaf rust phenotyping environments (Mex16 and Mex17, Mexico 2016 and 2017, respectively; Stp16 and Stp17, Saint Paul 2016 and 2017, respectively).

[c]Leaf rust phenotypic variation explained by the QTL and presented as percentage.

a maximum phenotypic variation of 8.0% for leaf rust in the Mex16 environment (Figure 2). The *QLr.umn-1B* region is known to carry race-nonspecific APR gene *Lr46* (Singh et al., 2013) that is pleiotropic with *Sr58*, *Yr29*, and *Pm39*, conferring resistance to multiple pathogens. Molecular marker assay for *Lr46* (*csLV46G22*) was positive in Copio (Table 4). Yuan et al. (2020) mapped *Lr46* on 1BL spanning the 669.2–673.7 Mb interval. Several other studies have also identified *Lr46* in the same genomic region involving different populations, and comparative mapping found 672.6 to 673.8 Mb as the common sequence region (Yuan et al., 2020). Therefore, it is likely that *QLr.umn-1B* represents *Lr46* in Copio. Although composite interval mapping detected *Lr46* in only one environment, LOD peaks were observed below the significance threshold (LOD ≥ 2.5) in other environments (Suppl. Figure 1). It is established from previous studies that when *Lr46* is present with other resistance genes, it usually does not provide a clear resistance response. The expression of *Lr46* might be influenced by the environmental conditions (e.g., temperature, moisture), genetic background, and the presence of other *Lr* genes (Kolmer et al., 2018, b; Rosewarne et al., 2015; Kolmer et al., 2012; Lagudah, 2011).

The QTL *QLr.umn-2A* was mapped on chromosome 2AS and consistently detected in all environments (Suppl. Figure 2). This QTL explained up to 17% of the phenotypic variance. The short arm of chromosome 2A in Copio carries the 2NS/2AS chromosomal translocation from *Triticum ventricosum* (Tausch) (Maia, 1967). This translocation segment harbors three rust resistance genes: *Lr37*, *Yr17*, and *Sr38* (Helguera et al., 2003). The recent characterization by de novo assembly delineated the 2NS segment to be approximately 33 Mb in wheat cultivars 'Jagger' and 'CDC Stanley' (Gao et al., 2021). Helguera et al. (2003) characterized the translocation segment on chromosome 2AS using the restriction fragment length polymorphism markers. Furthermore, a cleaved amplified polymorphic sequence marker (*URIC/LN2*) was developed to amplify N and A alleles of the *Xcmwg682* locus. The physical position of the cleaved amplified polymorphic sequence marker *URIC/LN2* linked to *Lr37/Sr38/Yr17* (Helguera et al., 2003) was 3.9 Mb in the IWGSC RefSeq v1.0. In the present study, *QLr.umn-2A* was in the 19.9 to 21 Mb interval. The CIMMYT has also identified about 60 markers that tag the 2NS/2AS translocation.

Lr37 is a race-specific APR gene (McIntosh et al., 1995), and leaf rust pathogen races were virulent to *Lr37* in both the Mexican and the U.S. phenotyping environments. Seedling leaf rust resistance gene *Lr17* is also located on the short arm of chromosome 2A, but we eliminate this possibility because

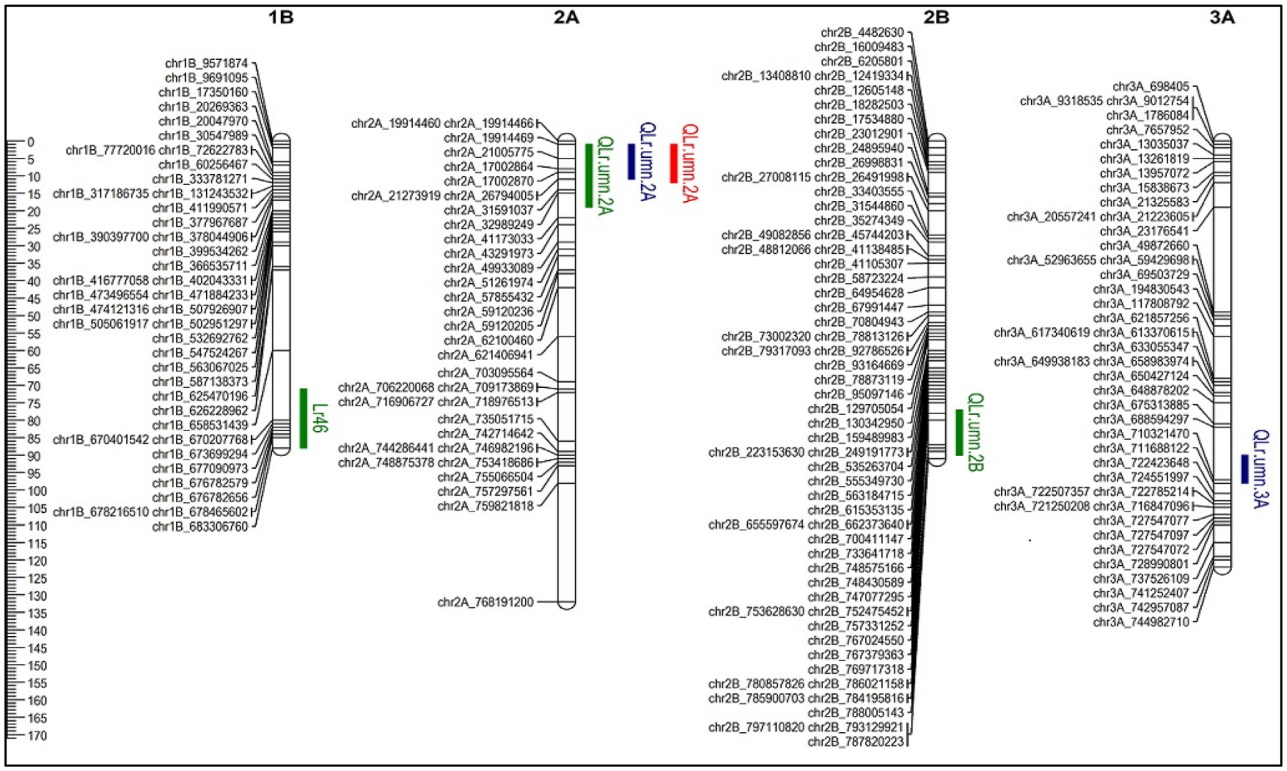

FIGURE 2 Chromosomes on which leaf rust quantitative trait loci (QTL) were detected across four environments. Genotyping-by-sequencing (GBS) single nucleotide polymorphism (SNP) markers are on the left side of each chromosome, and the detected QTL are on the right side shown in colored bars perpendicular to the chromosomes. Black, red, dark green, and blue colors represent that QTL were detected in St. Paul 2016, St. Paul 2017, Mexico 2016, and Mexico 2017, respectively. Quantitative traits loci stacking on each other at chromosomes 2A (*QLr.umn-2A*) and 3B (*QLr.umn-3B*) were detected in different environments and represent the same gene/QTL

the *Pt* races (MBJ/SP, MCJ/SP, MCDSB, MHDSB, MFPSD, and MLSDS) used for seedling assay and field evaluation are virulent to *Lr17*. Furthermore, the 2NS/2AS translocation and *Lr17* are in repulsion linkage (Bariana & McIntosh, 1994), which eliminates the possibility of Copio containing *Lr17*. Other studies have also reported leaf rust QTL co-located with *Lr37* (Wang et al., 2015; Azzimonti et al., 2014). The CIMMYT-Mexico also has enough data (CIMMYT, unpublished data, 2018) to support that the *Lr37/Yr17/Sr38* region harbors a slow rusting gene other than *Lr37* (R. P. Singh, personal communication, 2 Apr. 2019). Based on the physical position of *QLr.umn-2A* and virulence of the field inoculated races to *Lr37*, it is most likely that *QLr.umn-2A* is a colocalized QTL providing quantitative effects to leaf rust resistance.

A major QTL *QLr.umn-3B* was consistently detected across all the four testing environments (Mex16, Mex17, Stp16, and Stp17) on chromosome arm 3BS (Suppl. Figure 3). This QTL explained 10–17% of the phenotypic variance in Mexico and 15–18% in St. Paul for leaf rust severities. The *QLr.umn-3B* genomic region is known to affect the development of many fungal diseases with *Lr27* (Nelson et al., 1997), *Sr2* (Kota et al., 2006), *Yr30* (Singh et al., 2001), *Fhb1* (Liu et al., 2008), and *Pm* (Mago et al., 2011) genes. The race-specific seedling resistance gene *Lr27* is closely linked or allelic to the APR gene *Sr2* (Mago et al., 2011). Seedling gene *Lr27* on 3BS requires a complementary gene *Lr31* on chromosome 4BS for its function (Singh & McIntosh, 1984). In our study, genome-wide markers did not detect *Lr31* in either parental line. The *Pt* pathotypes (MBJ/SP, MCJ/SP, and BBG/BP) used for the seedling assays and field inoculation in Mexico were virulent to *Lr27* + *Lr31* (Herrera-Foessel et al., 2012; Huerta-Espino et al., 2011). Both parents of the RIL population had IT > 3 when challenged against these races at the seedling stage (Table 1). Current *Pt* races used for field tests in the United States are highly virulent to *Lr27* + *Lr31* (Kolmer et al., 2018b). The wheat cultivar 'Gatcher,' which carries *Lr10* and *Lr27* + *Lr31*, genes had high disease severity in St. Paul field nurseries in 2016 (Kolmer et al., 2018c). The absence of *Lr31* that compliments *Lr27* functionality and the presence of virulent pathotypes in all field environments suggests that leaf rust resistance in Copio was not conditioned by *Lr27*, although Copio was positive for the *Sr2* marker assay (Table 4).

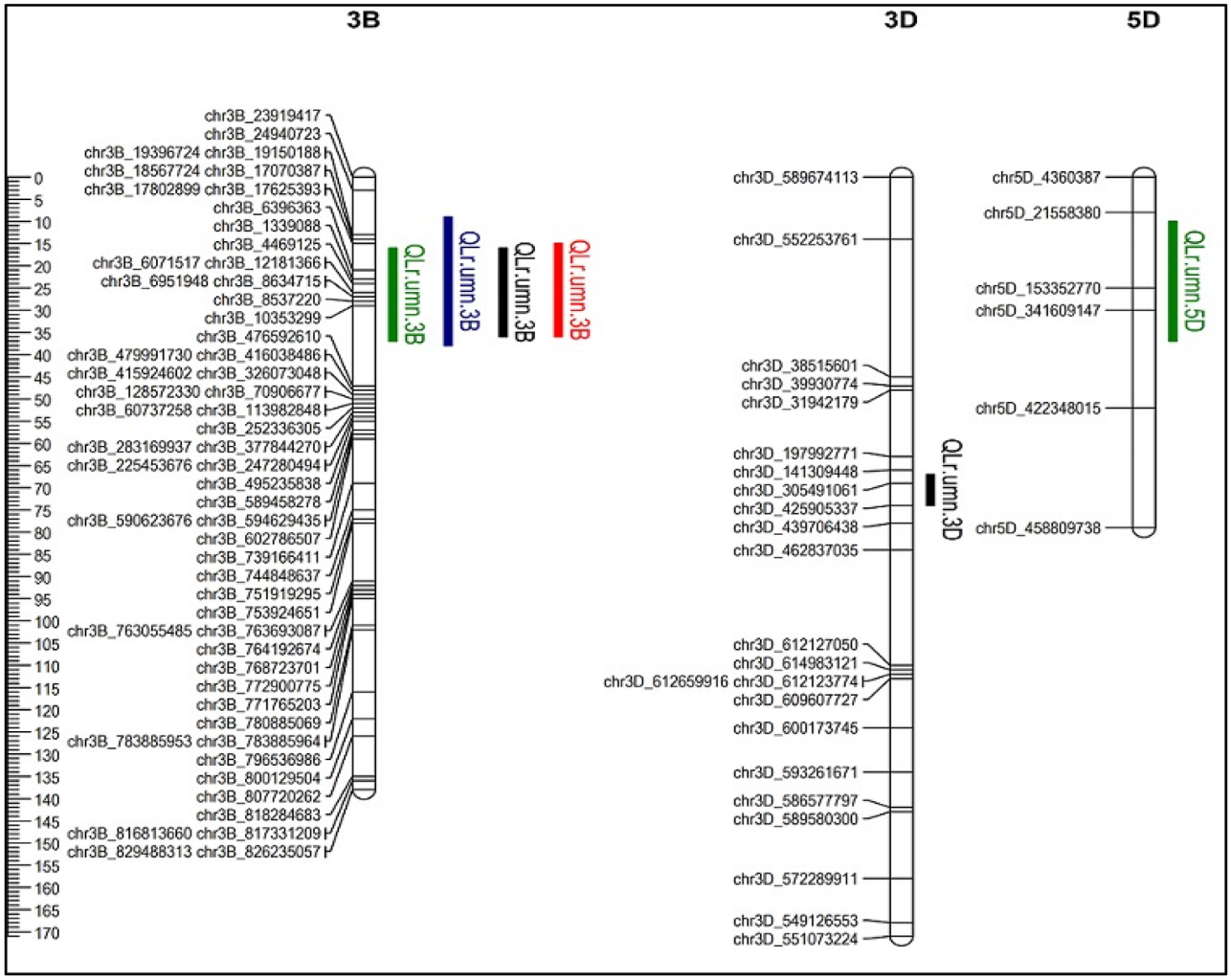

FIGURE 2 Continued

Some studies have reported that few wheat lines show *Lr27* specificity and do not confer resistance to stem rust (Singh & McIntosh, 1984). However, no wheat genotypes have been reported that carry *Sr2* gene but lack *Lr27* (Mago et al., 2011). Buerstmayr et al. (2014) reported a QTL (*QLr.ifa-3BS*) on 3BS in Austrian wheat cultivar 'Capo' that does not carry either *Lr27* or *Sr2*. This QTL confers resistance to both leaf and stripe rust. Our finding needs further investigation to reveal the relationship of *Sr2* and leaf rust QTL *QLr.umn-3B*. One possibility is that the *Sr2* allele confers resistance to leaf rust without *Lr27* functionality or that *QLr.umn-3B* is a unique APR QTL providing resistance against *Pt* races in Mexican and U.S. environments. Several QTL have been mapped on chromosome 3BS close to *Sr2*, which provided resistance to the leaf rust pathogen (Rosewarne et al., 2015; Lan et al., 2014; Dieguez et al., 2014; Buerstmayr et al., 2014). The *QLr.umn-3B* and other reported *Sr2* co-localized QTL need to be finely mapped to reveal if they are the same or different genes.

Based on marker haplotypes, seven RILs were identified that carried allelic combinations at three loci, namely *QLr.umn-1B* (represented as *Lr46*), *QLr.umn*-2A, and *QLr.umn-3BS*. This allelic combination had the lowest disease (i.e., COI) in all field environments, and pyramiding these loci was most effective for the Mexican environments.

Overall, this study revealed that seven genomic regions in Copio are associated with leaf rust resistance in four environments. Three QTL on chromosomes 1B, 2A, and 3B were consistently expressed and contributed substantial resistance to leaf rust across all phenotyping environments. The RILs that carried favorable alleles for all three QTL had up to 52 and 36% less disease in the Mexican and U.S. environments, respectively. This allelic combination needs to be further validated in cultivated wheat cultivars across diverse environments. Copio harbors several quantitative genomic regions that contribute minor to major effects for leaf rust APR that can be used as a resistance donor to develop resistant cultivars.

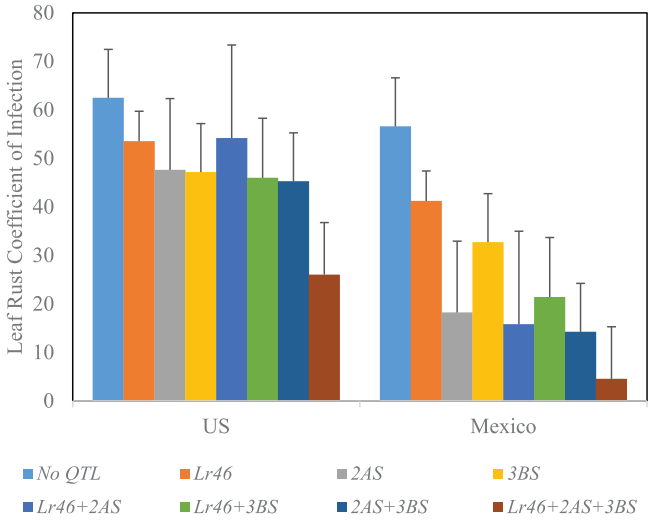

FIGURE 3 Leaf rust coefficient of infection (COI) of recombinant inbred lines in Mexico and US phenotyping environments by allelic combinations at three loci: *QLr.umn-1B* (represented as *Lr46*), *QLr.umn*-2A (represented as 2AS), and *QLr.umn-3BS* (represented as 3BS). Coefficient of infection (COI) in both environments is the mean of two phenotyping seasons. No quantitative trait loci (QTL) bar represents the COI when *Lr46*, *QLr.umn*-2A, and *QLr.umn-3BS* were absent. Whiskers on each bar represent the standard error

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AUTHOR CONTRIBUTIONS

Yahya Rauf: Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing – original draft. Caixia Lan: Data curation; Methodology; Resources. Mandeep Randhawa: Data curation, Methodology, Resources. Ravi P. Singh: Conceptualization; Resources; Validation; Writing – review & editing. Julio Huerta-Espino: Conceptualization; Resources; Writing – review & editing. James A. Anderson: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Validation; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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