A High-Resolution Map of Wheat QYr.ucw-1BL, an Adult Plant Stripe Rust Resistance Locus in the Same Chromosomal Region as Yr29

Nicolas Cobo, Humphrey Wanjugi, Evans Lagudah, and Jorge Dubcovsky*

The appearance of highly virulent and more aggressive races of Puccinia striiformis f. sp. tritici (Pst) during the last two decades has led to stripe rust epidemics worldwide and to the rapid erosion of effective resistance genes. In this study, we mapped an adult-plant resistance locus from the Argentinean wheat [Triticum aestivum L.] cultivar Klein Chajá, which is effective against these new Pst races. By using wheat exome capture data and a large population of 2480 segregating plants (4960 gametes), we mapped QYr.ucw-1BL within a 0.24-cM region [332 kb in International Wheat Genome Sequencing Consortium (IWGSC) RefSeq version 1.0] on chromosome arm 1BL. This region overlaps with current maps of the adult-plant Pst resistance gene Yr29, which has remained effective for more than 60 yr. An allelism test failed to find recombination between QYr.ucw-1BL and Yr29 and yielded similar resistance phenotypes for the two loci. These results, together with similar haplotypes in the candidate region, suggested that QYr.ucw-1BL and Yr29 might represent the same gene. However, we cannot rule out the possibility of tightly linked but different genes because most of the 13 genes in the candidate region are annotated with functions associated with disease resistance. To evaluate their potential as QYr.ucw-1BL/Yr29 candidate genes, we characterized their polymorphisms between resistant and susceptible haplotypes. Finally, we used these polymorphisms to develop high-throughput markers to accelerate the deployment of these Pst resistance loci in wheat breeding programs.

**Wheat stripe rust** (also known as yellow rust) is caused by *Pst*. In the past, this pathogen was restricted to regions with relatively cool climates (Kolmer et al., 2015), where it caused significant reductions in crop yield (Smith et al., 1986) and quality (Dimmock and Gooding, 2002). However, with the appearance of more virulent and aggressive *Pst* races that are better adapted...
to higher temperatures (Milus et al., 2008; Markell and Milus, 2008; Hovmøller et al., 2016). Stripe rust epidemics have expanded to warmer areas (Hovmøller et al., 2010). Genotypic data from a worldwide collection of *Pst* races have suggested that the highly virulent races detected in the last two decades originated in the Middle East or East Africa and spread rapidly, probably aided by human activities (Ali et al., 2014). These new races have caused *Pst* epidemics affecting most major wheat-producing areas in Africa, Asia, Australia, Europe, North and South America, and the Middle East (Milus et al., 2009; Hovmøller et al., 2010, 2011; Dong et al., 2017; Cobo et al., 2018) and have resulted in the rapid erosion of effective resistance genes (Lowe et al., 2011).

The replacement of susceptible wheat cultivars with new ones that are resistant to the new *Pst* races is a good strategy to control the current epidemics (Hovmøller et al., 2010) and prevent their expansion (Pink, 2002; Cao et al., 2012). However, the genetic plasticity, rapid evolution, and potential for long-distance dispersal of the wheat rust populations represent a constant challenge to this strategy (Hovmøller et al., 2002, 2008; Milus et al., 2009; Chaves et al., 2013), and has promoted a growing interest in the identification of more durable sources of resistance (Lin and Chen, 2009).

Wheat stripe rust resistance genes can be classified into race-specific and non-race-specific categories (Lowe et al., 2011; Chen et al., 2013). Race-specific genes (also referred to as major genes) typically encode nucleotide-binding leucine-rich repeat proteins. These proteins can detect the presence of pathogen effectors or the modifications they produce in guarded host proteins. This recognition triggers either hypersensitive reactions (Periyannan et al., 2013; Saintenac et al., 2013; Mago et al., 2015; Steuernagel et al., 2016; Marchal et al., 2018) or the coordinated upregulation of pathogenesis-related genes that reduce pathogen growth (Zhang et al., 2017; Chen et al., 2018). Because deletions or amino acid changes in the effectors can help the pathogen to avoid detection by the nucleotide-binding leucine-rich repeat genes, race-specific genes often remain effective only for a few years after their deployment in commercial cultivars (Chen et al., 2017b; Salcedo et al., 2017; Cobo et al., 2018).

Adult-plant resistance (APR) genes often present incomplete resistance and have historically been more durable than race-specific genes (Boyd, 2006; Krattinger et al., 2009; Lowe et al., 2010). Among the three wheat stripe rust APR genes cloned so far, *Yr18* has the longest history of resistance. *Yr18* encodes a modified ATP-binding cassette transporter (Krattinger et al., 2009) that has remained effective for almost 100 yr (Ellis et al., 2014). *Yr46*, which encodes a hexose transporter, is prevalent in landraces from the Punjab region of India (Moore et al., 2015), suggesting that it may have been effective for a long time. Finally, *Yr36* encodes a kinase-steroidogenic acute regulatory protein-related lipid-transfer lipid-binding protein (Fu et al., 2009; Gou et al., 2015) and has remained effective against the new and more aggressive *Pst* races.

Two of the three cloned APR genes have been shown to confer partial resistance to multiple pathogens, including stripe rust (*Yr* genes), leaf rust (*Lr* genes), stem rust (*Sr* genes), and powdery mildew (*Pm* genes). The cloning of *Yr18* confirmed that it was the same gene as *Lr34*, *Sr57*, and *Pm38*, whereas the cloning of *Yr46* confirmed its identity with *Lr67*, *Sr55*, and *Pm46*. These results suggest that these APR genes may affect conserved processes that are required by multiple biotrophic fungal pathogens to infect wheat. The 1BL locus, *Yr29/Lr46/Sr58/Pm39*, is also associated with resistance to multiple pathogens (William et al., 2003; Lillemo et al., 2008; Singh et al., 2013) but the causal gene has not been identified so far.

The *Yr29/Lr46* locus is prevalent in CIMMYT wheats and may have originated from different geographical sources. The wheat cultivar Americano 25e, proposed to carry *Lr46*, was derived from landraces in Uruguay in the early twentieth century (Kohli, 1986) and was used as a parent in the wheat-breeding programs in Uruguay and Argentina in the 1920s (Kolmer, 2015). The tall Indian cultivars New Pusa 876 and Sujata, which are also proposed to carry the *Yr29/Lr46* locus, have remained resistant to stripe and leaf rust since their release in the 1960s and 1980s, respectively (Lan et al., 2015; Ponce-Molina et al., 2018). However, it is still not known if this multipathogen resistance locus is the result of the pleiotropic effects of a single gene or of multiple linked genes. In this study, our focus was on resistance to stripe rust, so we compared our results only with studies that mapped stripe rust resistance (*Yr29*) in this chromosome region.

*Yr29* was first described in ‘Pavon 76’ (William et al., 2003) and has provided partial APR to leaf and stripe rust for more than 60 yr (Kolmer, 2015). *Yr29* has been mapped in multiple studies with different genetic backgrounds in the distal region of the long arm of chromosome 1B (reviewed in Cobo et al., 2018). We recently mapped the adult-plant stripe rust resistance locus *QYr.ucw-1BL* on a 25.5-cM region that overlaps with *Yr29* (Cobo et al., 2018). This locus has remained effective to the highly virulent *Pst* races present in California during the last 6 yr, revealing potential for durable resistance against this pathogen. The objectives of this study were to generate a high-resolution map for *QYr.ucw-1BL*, to compare it with current *Yr29* maps, and to identify potential candidates for these resistance genes. In addition, we used exome-capture data to characterize the resistant and susceptible haplotypes and to develop high-throughput markers to accelerate the deployment of these genes in wheat breeding programs.

**MATERIAL AND METHODS**

**Population Development**

The segregating populations used in this study were derived from recombinant inbred lines (RILs) generated from the cross between the Argentinean common wheat cultivars Klein Chajá (KC) and Klein Proteo (KP). Four quantitative trait loci (QTL) for stripe rust resistance
were identified in this population, including \( QY_{uowo-1BL} \), \( QY_{uowo-2BS} \), and \( QY_{uowo-3D} \) (centromeric) from KC and \( QY_{uowo-4DL} \) from KP (Cobo et al., 2018). To facilitate the high-resolution mapping of \( QY_{uowo-1BL} \), we selected parental RILs that differed in \( QY_{uowo-1BL} \) (present in RIL55 and absent in RIL66) but were homozygous susceptible for \( QY_{uowo-2BS} \) and \( QY_{uowo-3D} \).

At the time of the initial cross, we were not aware of the existence of \( QY_{uowo-4DL} \), a minor resistance QTL present in RIL66 (Fig. 1). This QTL explained on average 10% of the variation in \( Pst \) resistance observed in the original RIL population (Cobo et al., 2018). To avoid any potential confounding effect of \( QY_{uowo-4DL} \) in the critical recombinant families segregating for in \( QY_{uowo-1BL} \), we prioritized families that were homozygous for \( QY_{uowo-4DL} \). For the few families segregating for both \( QY_{uowo-1BL} \) and \( QY_{uowo-4DL} \), we genotyped both loci and separated their effects via factorial ANOVAs.

The high-resolution map for \( QY_{uowo-1BL} \) was constructed in two phases, referred hereafter as Fine Mapping 1 (FM1) and Fine Mapping 2 (FM2) (Fig. 1). For the FM1 phase, 121 \( F_2 \) plants from the RIL55 \( \times \) RIL66 cross were screened for recombination events between the \( QY_{uowo-1BL} \) flanking markers \( IWA695 \) and \( IWA7892 \) (Fig. 1a). Progeny tests with 10 to 23 \( F_3 \) plants from the families with selected recombination events were evaluated for \( Pst \) resistance in the University of California – Davis (UCD) experimental field station in the 2014–2015 growing season (Fig. 1b). For the FM2 phase, we selected nonrecombinant \( F_3 \) plants that were homozygous for both flanking markers and screened \( F_4 \) plants for recombination events between these markers (Fig. 1c). We selected lines carrying recombination events in the candidate region and evaluated their \( F_{4.5} \) progeny in the UCD experimental field station in the 2015–2016 growing season (Fig. 1d).

An allelism test between \( QY_{uowo-1BL} \) and \( Yr29 \) was performed with an \( F_2 \) population derived from the cross between ‘Lalbahadur’ (Pavon-1B) and RIL55. Lalbahadur (Pavon-1B) is a Pavon 76 chromosome 1B substitution line (Herrera-Foessel et al., 2018). We used the simple sequence repeat marker \( wmc776 \) that was completely linked to \( Yr60 \) (Herrera-Foessel et al., 2015) to account for the additional variability introduced by this gene.

**Field Experiments**

Field experiments were initiated in mid-November at the University of California field station near Davis, California (38°31’N, 121°46’W). Fertilization consisted of 224 kg ha\(^{-1}\) applied as ammonium sulfate, half at pre-planting and the rest at the beginning of jointing. Trials were flood-irrigated as needed (two to five per season). For progeny tests, five seeds were sown in 1-m rows; for each family, four rows were used (0.4 m between rows). In addition, RIL55, RIL66, KC, KP, and the lines carrying \( Yr29 \) (i.e., Lalbahadur (Pavon-1B) and Avocet-Yr29) were included as checks within each trial as 1-m head rows (30 seeds). The highly susceptible common wheat line ‘DS6301’, used as spreader border, was inoculated with a mix of \( Pst \) spores collected at the UCD experimental field station during the previous season to ensure strong and even inoculum pressure.

We used two indices to estimate plant reactions to \( Pst \), infection type (IT) and severity. Infection type was estimated using a scale from 0 (resistant) to 9 (susceptible) described previously (Line and Qayoum, 1992). Severity was estimated as the proportion of the flag leaf affected by rust (Peterson et al., 1948). Experiments were scored between the heading (Z30) and grain filling (Z80) stages (Zadoks et al., 1974). The \( Pst \) races detected at the UCD field during the 2015 and 2016 seasons, together with their virulence profiles were described previously (Cobo et al., 2018).

**High-Density Map Marker Development and Genotyping**

For the \( QY_{uowo-1BL} \) flanking markers used in the first phase of the high-resolution mapping (\( IWA7892 \) and \( IWA695 \))(Fig. 2a), we developed a cleaved amplified polymorphic sequence (CAPS) assay and a Kompetitive allele specific polymerase chain reaction (PCR) assay (KASP). For the second phase of the high-density mapping, \( FM1 \) (Fig. 1a, 1b), we genotyped the parental lines with Illumina’s Infinium Wheat 90K single nucleotide
polymorphism (SNP) assay (Wang et al., 2014) and designed TaqMan assays (Applied Biosystems, CA, USA) for eight polymorphic SNPs located in the target region (Fig. 2b). Single nucleotide polymorphism and primer sequences are presented in Supplemental Table S1. Polymerase chain reactions were performed with the TaqMan GTXpress Master Mix (Life Technologies, Grand Island, NY) following the manufacturer’s manual and using a program with the following conditions: 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing, and extension at 60°C for 60 s.

For the second phase, FM2, (Fig. 1c,d), we designed seven KASP assays for SNPs identified by exome capture (Fig. 2c). The KASP assays were designed as recommended by the manufacturer (Semagn et al., 2014) and the primers for each assay are presented in Supplemental Table S1. Polymerase chain reactions were performed in a 5-μL reaction volume (2.5 μL 2× KASP Master Mix, 0.07 μL KASP primer assay mix and 2.5 μL genomic DNA at 2–20 ng μL⁻¹). We used a two-step touchdown program with the following conditions: 94°C for 15 min, followed by 10 cycles of touchdown of 94°C for 20 s, annealing from 61 to 55°C for 1 min (dropping 0.6°C per cycle), followed by 26 cycles of 94°C for 20 s, and annealing at 55°C for 1 min. Both the KASP and TaqMan results were analyzed with a FLUOstar Omega F plate reader (BMG LABTECH, Ortenberg, Germany) with the software package KlusterCaller (LGC Genomics).

Exome Capture
Genomic DNA was extracted from KC and KP and from the Yr29/Lr46 donor lines Lalbahadur (Pavon-1B) and Pavon 76 (Singh et al., 1998; William et al., 2003). Captures were performed with a wheat exome capture designed by NimblGene-Roche (140430_Wheat_TGAC_D14_REZ_HX1, Roche NimbleGen Inc., Madison, WI). Fragmentation, genomic library construction, and exome capture were conducted by following a previously published methodology (Krasileva et al., 2017; Mo et al., 2018). After we removed the low-quality reads and adaptor sequences, processed sequence reads were aligned with ‘Chinese Spring’ (CS) IWGSC RefSeq version 1.0 (International Wheat Genome Sequencing Consortium, 2018). On average, 84,400,549 reads were mapped per line, with an average read length of 150 bp. Coverage per mapped base pair (78-fold coverage) was calculated by multiplying the number of reads by their length and dividing the product by the captured coverage space reported for ‘Cadenza’ (162.4 Mb)(Krasileva et al., 2017). Variant calling was performed for each line individually, and SNPs with a sequencing depth lower than three reads or a mapping quality lower than 25 were removed from the analysis.
Table 1. Families† with critical recombination events in the first mapping phase (FM1). Family size, average infection type (IT), and ANOVA P-values are presented below the genotypes for each family.

<table>
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<th>FM1_46</th>
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<td>H</td>
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</table>

*All families were homozygous susceptible for QYr.ucw-4DL. Family FM1_61 showed recombination between IWB34935 and IWB72918, but was excluded from the table because it was affected by *Barley yellow dwarf virus* infection.*

† The closest flanking markers to QYr.ucw-1BL.

§ The genotypes for QYr.ucw-1BL are indicated in bold and were inferred from the statistical analysis for resistance.

¶ H, heterozygous; C, resistant (Klein Chajá); P, susceptible (Klein Proteo).

Statistical Analysis

For the progeny tests of the families carrying the critical recombination events, we performed one-way ANOVAs for infection type by using the allelic classes (homozygous KP, homozygous KC and heterozygous) of the markers segregating at the QYr.ucw-1BL target region. To test the overall effect of QYr.ucw-1BL, we performed an ANOVA with QTL genotype and families as factors. The overall effect of QYr.ucw-4DL was tested by performing one-way ANOVAs within each allele of QYr.ucw-1BL (QYr.ucw-4DL segregated between but not within families). Assumptions of the normality of residuals and homogeneity of variances were tested via the Shapiro-Wilk and Levene tests, respectively, and the data were transformed when necessary to meet these assumptions. Variance components were estimated via the restricted maximum likelihood model. All statistical analyses were conducted in SAS version 9.4 (SAS Institute Inc., 2013). The degree of dominance was calculated as [(heterozygote value – midpoint value between the two homozygotes) ÷ additive effect] (Falconer, 1960).

RESULTS

The High-Density Genetic Map of QYr.ucw-1BL

The mapping of QYr.ucw-1BL was conducted in two phases to limit the number of simultaneous progeny tests required for families carrying recombination events in the target region. In the first round (FM1), we identified 50 plants with recombination events between the flanking markers IWA695 and IWA7982 in a population of 121 F2 plants derived from the RIL55 × RIL66 cross (Fig. 1a and Fig. 2a). These 50 plants were genotyped with eight new TaqMan markers developed for SNPs identified in the region from the 90K SNP chip (Fig. 2b).

Progeny tests were performed for 50 F3 families derived from the F2 plants carrying recombination events in the target region. Ten to 23 individual F3 plants from each family were genotyped with the new markers and scored for IT and severity under strong *Pst* infection in the field. One-way ANOVAs for IT among allelic classes of heterozygous markers linked to QYr.ucw-1BL were performed to identify families that were homozygous or heterozygous for the resistance gene. Twenty-one families showed significant differences among allelic classes and were classified as heterozygous for QYr.ucw-1BL, whereas the other 23 showed nonsignificant differences and were classified as homozygous (six families were excluded due to severe *Barley yellow dwarf virus* infection). Genotyping results and statistical analyses are presented in Table 1 for the 10 families with the closest recombination events to QYr.ucw-1BL.

Families showing significant segregation were scored as heterozygous for QYr.ucw-1BL and the homozygous were classified as resistant or susceptible based on their relative scores and comparison with controls. This strategy showed that QYr.ucw-1BL was located one recombination event (family FM1_46) distal to IWB34935 and five recombination events proximal to IWB72918 (Table 1 and Fig. 2b). The total distance between IWB34935 and IWB72918 in Fig. 2b was further (2.9 cM) than expected from the six recombination events (2.5 cM) presented in Table 1 because there was an additional family with a recombination event in this region for which we have genotypic but not phenotypic data (lost because of *Barley yellow dwarf virus* infection).
The QYr.ucw-1BL flanking markers IWB34935 and IWB72918 identified in FM1 were used in the second phase of the high-resolution mapping to screen 2359 F₄ plants derived from selected heterozygous F₃ plants. We identified 124 F₄ plants with recombination events between these two flanking markers (Fig. 1). These lines were genotyped with seven new KASP markers targeting SNPs identified in the exon capture that were located in the new candidate region. We also added the marker csLV46G22, which has been used in several Yr29 mapping studies (Ren et al., 2017).

Progeny tests for the 124 F₄ families (14–22 plants per family) revealed 43 heterozygous families with significant differences in IT and severity among allelic classes of the markers segregating in the target region. No significant differences were detected within the 60 families that were classified as homozygous susceptible or resistant on the basis of their relative scores and by comparison with the controls. Twenty-one families were excluded because no phenotypic data were available. Twenty-one families were excluded because no phenotypic data were available. Twenty-one families were excluded because no phenotypic data were available. Twenty-one families were excluded because no phenotypic data were available. Twenty-one families were excluded because no phenotypic data were available.

All the markers presented in the high-resolution map (Table 2, Fig. 2c) were polymorphic not only between KC and KP, but also between the isogenic lines ‘Avocet’ and ‘Avocet’ and between the single chromosome substitution lines Lalbahadur (Pavon-1B) and Lalbahadur, which differ in Yr29. Therefore, combinations of these markers may be useful to predict the presence of Yr29 and/or QYr.ucw-1BL in breeding populations.

**Genes Annotated in the QYr.ucw-1BL Candidate Region**

The 115-kb candidate region between ucw.k34 and ucw.k18 includes only one complete gene (TraesCS-1B01G454100)(Table 3), which is annotated as a putative RECEPTOR-LIKE PROTEIN KINASE (RLK). This candidate region also includes the last exon and 3' untranslated region of the gene.

### Table 2. Families† with critical recombination in the second mapping phase (FM2). Family size, average infection type (IT) associated with each allele, and ANOVA P-values are presented for each family below the genotypes.

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</tr>
<tr>
<td>IWB72918</td>
<td>2.56</td>
<td>C</td>
<td>P</td>
<td>C</td>
<td>C</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family size</td>
<td>17</td>
<td>20</td>
<td>19</td>
<td>16</td>
<td>20</td>
<td>17</td>
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<td></td>
</tr>
<tr>
<td>Average IT</td>
<td>3.2</td>
<td>6.6</td>
<td>3.4</td>
<td>5.2</td>
<td>5.0</td>
<td>4.3</td>
<td>4.6</td>
<td>4.1</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.1414</td>
<td>0.2598</td>
<td>0.6871</td>
<td>0.0131</td>
<td>3.5E-5</td>
<td>0.0022</td>
<td>0.0003</td>
<td>0.4229</td>
<td>0.0532</td>
<td></td>
</tr>
</tbody>
</table>

† Two families (FM2_104 and FM2_119) with recombination events between ucw.k31 and ucw.k34 and one (FM2_27) with a recombination event between ucw.k23 and csLV46G22 are not included in the table because no phenotypic data were available.
‡ Markers flanking the larger and more conservative candidate gene region.
§ QYr.ucw-1BL flanking markers.
†† The genotype of QYr.ucw-1BL was presented in the last row (+, homozygous resistant; -, homozygous susceptible).
# The genotype of QYr.ucw-1BL# is presented in the last row (+, homozygous resistant; -, homozygous susceptible).
H, heterozygous;
P, susceptible (Klein Proteo).
Table 3. Exome capture single nucleotide polymorphisms (SNPs) in the candidate region and their predicted effect using sorting intolerant from tolerant (SIFT) scores (Kumar et al., 2009).

<table>
<thead>
<tr>
<th>Gene (strand)</th>
<th>Gene predicted function</th>
<th>Position (bp)</th>
<th>Allele variant</th>
<th>Variant effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TraesCS1B01G†</td>
<td>Sorting nexin</td>
<td>669,901,546</td>
<td>T C C C C C</td>
<td>KP KC Lalb Pavon CS SIFT¶</td>
</tr>
<tr>
<td>453700</td>
<td>Glucan endo-1,3-beta-glucosidase</td>
<td>669,924,312</td>
<td>G A A A A A</td>
<td>HS40R 1.00</td>
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<tr>
<td>454000</td>
<td>RLK, putative, expressed</td>
<td>670,025,488</td>
<td>A A C A A A</td>
<td>K461T 0.11</td>
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<td>454100</td>
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<td>670,034,402</td>
<td>A G G G G G</td>
<td>R43Q 0.11</td>
</tr>
<tr>
<td>454200</td>
<td>Glucan endo-1,3-beta-glucosidase</td>
<td>670,142,853</td>
<td>G A A A A A</td>
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<td>454400</td>
<td>RLK, putative, expressed</td>
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<td>G G G G T G</td>
<td>L464I 0.00</td>
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<tr>
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<td>670,167,425</td>
<td>T C C C C C</td>
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<td>WRKY transcription factor</td>
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<tr>
<td>455000</td>
<td>WRKY transcription factor</td>
<td>670,202,728</td>
<td>G A A A A A</td>
<td>NT56S 1.00</td>
</tr>
</tbody>
</table>

† TraesCS1B01G453800 and TraesCS1B01G454300 were excluded because they are annotated as transposable elements and have no exome capture data. TraesCS1B01G454700 and TraesCS1B01G454900 were also excluded because they are likely to be nonexpressed pseudogenes.

‡ RLK, , receptor-like kinase; WRKY, transcription factor with the WRKY domain.

§ KP, Klein Proteo; KC, Klein Chajá; Lalb, Lalbahadur (Pavon-1BL); Pavon, Pavon 76; CS, Chinese Spring.

¶ Bold indicates SIFT < 0.05 (a high probability of affecting protein structure and function) or premature stop codons.

# Names in italics indicate the marker used to map the polymorphism.

†† 7-bp insertion: CTCGGGA.
untranslated region of *TraesCS1B01G454000* (also annotated as an RLK) and the promoter and first 397 bp of *TraesCS1B01G454200* (annotated as a GLUCAN ENDO-1,3-BETA-GLUCOSIDASE).

The larger 332-kb candidate interval between *ucw.k31* and *cisLV44G22* includes 10 annotated genes, in addition to the three described above. These 10 genes include four additional RLKs, two additional GLUCAN ENDO-1,3-BETA-GLUCOSIDASE, one SORTING NEXIN, one SLEEPER, one SUGAR TRANSPORTER, and a member of the family transcription of factors carrying the WRKY domain (Table 3). These genes would be valid candidates for *QYr.ucw-1BL* only if the phenotype of the lines carrying the three critical recombination events that delimit the reduced candidate region turns out to be incorrect (e.g., through errors or an unexpected combination of epistatic effects confounding the interpretation of the resistance phenotype).

### Polymorphisms between KC and KP in the Candidate Genes

To explore the potential of the genes identified in the candidate region to be the cause of the observed differences in *Pst* resistance, we examined their polymorphisms between resistant (KC) and susceptible (KP) parental alleles by using exome capture data (Table 3). Within the 332-kb candidate region, KP exome capture sequences showed an average of 159.8 ± 3.2 SNPs (99.13% DNA identity, Supplemental Fig. S1) compared with the other four genotypes included in Table 3. By contrast, the number of SNPs detected among KC, CS, Pavon 76, and Lalbahadur (Pavon-1B) within the same region was more than 13-fold lower (11.8 ± 1.7 SNPs, 99.94% DNA identity; Supplemental Fig. S1). Chinese Spring was included in Table 3 as a reference, even though we currently do not know which allele of *QYr.ucw-1BL* is present in this cultivar.

### Polymorphisms within the Reduced Candidate Gene Region

Within this region, we identified 27 SNPs, six of which were predicted to generate nonsynonymous amino acid changes in *TraesCS1B01G454100*. Four of these amino acids differences exhibited “sorting intolerant from tolerant” (SIFT) scores (Kumar et al., 2009) lower than 0.05, which predict disruptive effects in the structure and/or function of the encoded protein (Table 3).

We also detected a polymorphic miniature inverted-repeat transposable element in the promoter of *TraesCS1B01G454100*. By using primers specific for this insertion (Supplemental Table S2), we detected its presence in CS, KC, Pavon 76, and Lalbahadur (Pavon-1B) but not in KP (Supplemental Fig. S2). The miniature inverted-repeat transposable element insertion cosegregated with five amino acid changes that differentiate KP from the other four cultivars in Table 3. Only one amino acid change (S90P) was shared by CS and KP (Table 3).

The 3’ region of *TraesCS1B01G454000* included in the reduced candidate region showed two SNPs in the last intron and two in the 3’ untranslated region but none in the coding region. The rest of the gene located outside the reduced candidate region showed two nonsynonymous SNPs but the predicted amino acid changes showed high SIFT scores predictive of nondisruptive changes (Table 3). The proximal 5’ region of the *TraesCS1B01G454200* region included in the reduced candidate region showed three synonymous SNPs and three within an intron. Outside the reduced candidate region, we detected multiple nonsynonymous SNPs (all with high SIFT scores). However, these SNPs are unlikely to be relevant for the differences in *Pst* resistance because the coding sequence of this gene is interrupted in CS and in the resistant lines KC, Pavon 76, and Lalbahadur (Pavon-1B) by a 5538 insertion flanked by a TACTGAC host direct duplication (Supplemental Fig. S3). This insertion showed similarities to a terminal inverted repeats DNA transposon and encoded a protein 73% similar to a RICE SLEEPER2-like protein. This insertion, which is annotated in IWGSC RefSeq version 1.0 as a transposable element, is absent in KP. In the absence of the insertion, *TraesCS1B01G454200* encodes a complete GLUCAN ENDO-1,3-BETA-GLUCOSIDASE protein.
KC, Lalbahadur (Pavon-1B), and Pavon 76, \textit{TraesCS1B01G454400} encodes a complete RLK protein. However, in KP, a 7-bp frame-shift insertion generates a premature stop codon and a truncated protein. Two additional amino acid changes differentiate KP from the other lines in Table 3. This RLK also shows amino acid changes that are unique to KC, Lalbahadur (Pavon-1B), and Pavon 76 (Table 3). These unique mutations detected in the exome capture will require further validation by Sanger sequencing.

\textit{TraesCS1B01G454500} encodes a complete GLUCAN ENDO-1,3-BETA-GLUCOSIDASE protein. The susceptible cultivar KP has a 1-bp deletion that generates a premature stop and six amino acid changes that differentiate this cultivar from the other lines in Table 3.

\textit{TraesCS1B01G454600} encodes an RLK protein closely related to \textit{TraesCS1B01G454100} (Supplemental Fig. S4). However, a 41-bp frame-shift deletion in the sixth exon results in a truncated kinase domain, which probably impairs the function of this protein. This frame-shift deletion was detected in all the accessions listed in Table 3, suggesting that \textit{TraesCS1B01G454600} is a pseudogene.

\textit{TraesCS1B01G454700}, \textit{TraesCS1B01G454800}, and \textit{TraesCS1B01G45900} are described together because they were involved in a series of complex structural changes, which are described in Supplemental Fig. S5. In CS (IWGSC RefSeq version 1.0), a region of roughly 5.6 kb including the last two exons of \textit{TraesCS1B01G454800} (a putative SUGAR TRANSPORTER) and \textit{TraesCS1B01G454900} (a putative RLK) is duplicated and inserted close to the 3’ end of the coding region of \textit{TraesCS1B01G454900}. In IWGSC RefSeq version 1.0, this duplicated RLK is annotated as \textit{TraesCS1B01G454700}. By using specific primers for these different structural changes (Supplemental Table S2), we confirmed that the resistant lines KC, Pavon 76, and Lalbahadur (Pavon-1B) are similar to CS but KP does not have this duplication (Supplemental Fig. S5).

\textit{TraesCS1B01G454700} has a 5.2-kb transposon insertion flanked by host direct duplications (TCCGGGAC) in CS, KA, Pavon 76, and Lalbahadur (Pavon-1B) but not in KP (Supplemental Fig. S5). Since the transposon insertion eliminates a critical splicing site, the resulting protein is likely to be nonfunctional. In IWGSC RefSeq version 1.0, \textit{TraesCS1B01G454700} is annotated with an alternative start codon located after the transposon insertion. However, removal of the insertion restores the frame with an earlier start codon that predicts amino acids similar to the start of other RLKs (Supplemental Fig. S5).

\textit{TraesCS1B01G454800} encodes a putative SUGAR TRANSPORTER and shows only a single amino acid difference between KP and the other four lines (Table 3). This amino acid change has a low but nonsignificant SIFT score (0.1) (Table 3).

\textit{TraesCS1B01G454900}: The start of this gene is annotated in IWGSC. RefSeq version 1.0 at 670,181,369 (antisense strand). However, an alternative earlier start at position 670,184,517 results in 252 additional amino acids that are 99% identical to those in \textit{TraesCS1B01G454700} (without the retrotransposon). This additional protein sequence includes a premature stop codon that was detected in CS, KC, Pavon 76, and Lalbahadur (Pavon-1B). We were not able to amplify the region including the premature stop codon in KP by PCR, suggesting that \textit{TraesCS1B01G454900} is a pseudogene in all the genotypes analyzed in this study.

\textit{TraesCS1B01G455000} encodes a putative WRKY transcription factor. Two SNPs were predicted to generate polymorphic amino acid changes, one between KP and the other four lines (N156S) with a conservative SIFT score (1.00) (Table 3) and the other one differentiating KP and CS from the three resistant lines (P158L). This mutation has a relatively low SIFT score (0.15) (Table 3) and a very negative Blocks Substitution Matrix 62 score (-3), which predicts potential detrimental effects in this protein function.

\textbf{Relative Effect of QYr.ucw-1BL and QYr.ucw-4DL}

The large number of plants segregating for \textit{QYr.ucw-1BL} in the FM2 phase (479 plants from 25 F4:5 families) allowed us to estimate the dominance effect of this QTL and its contribution to \textit{Pst} resistance relative to \textit{QYr.ucw-4DL}. A negative degree of dominance of -35.7% was observed for IT values, indicating that the resistant allele of \textit{QYr.ucw-1BL} is partially dominant (Fig. 3). The proportion of IT phenotypic variance explained by \textit{QYr.ucw-1BL} (62.9%) in the factorial ANOVA was more than 20-fold larger than the proportion explained.
by \(Q_{Yr}.ucw-4DL\) (3.1%), confirming a relatively minor effect of this QTL in our segregating populations. The proportion of the variance explained by the interaction was only 1.6%, suggesting that the effects of these two genes are mainly additive (Fig. 3). However, because of the large number of plants included in the analysis, this interaction was significant \((P = 0.003)\). The analysis of the simple effects of \(Q_{Yr}.ucw-4DL\) within the different classes of \(Q_{Yr}.ucw-1BL\) showed that \(Q_{Yr}.ucw-4DL\) has larger effects when \(Q_{Yr}.ucw-1BL\) was homozygous for the susceptible allele \((P < 0.001)\), intermediate effects when it was heterozygous \((P = 0.004)\), and nonsignificant effects when the plants were homozygous for the resistant \(Q_{Yr}.ucw-1BL\) allele \((P = 0.057)\) (Fig. 3). On average, plants homozygous for the \(Q_{Yr}.ucw-4DL\) allele showed a 6.4% reduction in IT relative to plants homozygous for the susceptible allele (Fig. 3). All segregating families analyzed in this study were homozygous for the \(Q_{Yr}.ucw-4DL\) allele; therefore, it was not possible to estimate the degree of dominance for this allele.

**Allelism Test**

To test if \(Q_{Yr}.ucw-1BL\) and \(Yr29\) were the same gene, we screened 733 \(F_2\) plants from the cross between RIL55 (KC allele) and Lalbahadur (Pavon-1B). This population was also segregating for \(Yr60\), so we used the linked marker \(wmc776\) (Herrera-Foessel et al., 2015) to account for its effect. By using this marker, we selected 148 \(F_2\) plants that were homozygous for the susceptible allele of \(Yr60\) and used them for the \(Q_{Yr}.ucw-1BL\)-\(Yr29\) allelism test.

The parental lines used in this allelism test showed few polymorphisms (Table 3), which limited the number of markers that could be developed for the region including both resistance genes. On the proximal side, we used the marker IWB57627 located 2.6 cM proximal to \(Q_{Yr}.ucw-1BL\) (Fig. 2b) and, on the distal side, the marker IWB72918 located 1.45 cM distal to \(Q_{Yr}.ucw-1BL\) and 1.35 cM distal to \(csLV46G22\) (Fig. 2c), a marker tightly linked to \(Yr29\) (Rosewarne et al., 2012).

We first selected the 39 \(F_2\) plants with recombination events between the flanking markers, because only these plants can exhibit recombination between \(Yr29\) and \(Q_{Yr}.ucw-1BL\). Among these plants, we identified four \(F_2\) plants that were highly susceptible (IT 8.5 or higher) and were candidates for recombination events between the two partial resistance genes. We focused on the fully susceptible plants because they were easier to differentiate than the plants with slightly different levels of resistance. The progeny tests from the four highly susceptible plants did not validate the hypothesis of recombination between the two resistance genes. For the four families, we did not find significant differences in IT values between the progeny homozygous for the recombinant chromosome (which were expected to have the susceptible alleles in phase) and those homozygous for the nonrecombinant chromosomes. Although these results failed to demonstrate recombination between \(Q_{Yr}.ucw-1BL\) and \(Yr29\), they do not rule out the possibility of two very closely linked genes.

We also used the 552 plants that showed no recombination between the flanking markers IWB57627 and IWB72918 to estimate the relative effects of \(Q_{Yr}.ucw-1BL\) and \(Yr29\) on \(Pst\) resistance in the field. To account for the effect of \(Yr60\), we performed a factorial ANOVA including \(wmc776\) and IWB57627/IWB72918 as factors (and alleles as classes within each factor). In this analysis, \(Yr60\) showed a significant effect on adult-plant IT \((P < 0.0001)\) but no significant effects were detected for the markers for the \(Q_{Yr}.ucw-1BL/Yr29\) region \((P = 0.40)\) or for the interaction between the two genes \((P = 0.59)\). These results indicate that \(Q_{Yr}.ucw-1BL\) and \(Yr29\) confer similar levels of \(Pst\) resistance when challenged with the \(Pst\) races present in our field experiment (Fig. 4).

In summary, the close proximity of \(Q_{Yr}.ucw-1BL\) and \(Yr29\), their similar effect on \(Pst\) resistance, and the limited number of polymorphisms in the candidate region (Table 3) support the hypothesis that \(Q_{Yr}.ucw-1BL\) and \(Yr29\) may represent the same gene.

**DISCUSSION**

In this study, we mapped a locus for adult-plant stripe rust resistance within a 332-kb region of chromosome arm 1BL that is rich in genes with functions related to disease resistance. This \(Pst\) resistance locus, temporarily designated as \(Q_{Yr}.ucw-1BL\), overlaps with previous QTL for APR to stripe rust (\(Yr29\)), leaf rust (\(Lr46\)), stem rust (\(Sr58\)) and powdery mildew (\(Pm39\)) (Singh et al., 1998, 2013; William et al., 2003; Lillemo et al., 2008). The resistance genes present in this chromosome region are widely distributed in the CIMMYT wheat germplasm (Singh et al., 2011) and have remained effective for more than 60 yr (Singh et al., 1998; Kolmer, 2015). This extended durability has motivated the interest in cloning the causal resistance genes.

The previous cloning of the wheat multipathogen APR genes \(Lr34/Yr18/Sr57/Pm38\) and \(Lr67/Yr46/Sr55/\)
Pm46 demonstrated that a single gene can provide resistance to multiple pathogens (Krattinger et al., 2009; Moore et al., 2015) and this could also be the case for QYr.ucw-1BL/Yr29/Lr46/Sr58/Pm39. However, we cannot rule out the alternative hypothesis of a cluster of linked resistance genes, each effective to different pathogens (Lagudah, 2011). The concentration of genes associated with disease resistance in this chromosome region and the diverse geographical sources of the Yr29/Lr46 resistance alleles provide indirect support for this last hypothesis. At least two independent origins have been reported for Yr29/Lr46/Sr58/Pm39 and its partial inclusion in the 332-kb candidate gene region for QYr.ucw-1BL/Yr29 (Molina et al., 2018). In the next section, we compare our high-resolution mapping of QYr.ucw-1BL from KC with the available mapping data for Yr29 and discuss how these results support these two hypotheses.

The High-Density Genetic Map of QYr.ucw-1BL and a Comparison with Yr29

The distal flanking marker of the 332-kb QYr.ucw-1BL candidate region, csLV46G22 (Fig. 2d), has been mapped closely linked to Yr29 in multiple studies (Kolmer et al., 2012; Rosewarne et al., 2012; Lan et al., 2014; Calvo-Salazar et al., 2015; Ren et al., 2017; Dong et al., 2017; Ponce-Molina et al., 2018). Pavon 76 and KC carry the same allele of csLV46G22 and show very similar exome capture haplotypes in this region (99.92% identical; Table 3 and Supplemental Fig. S1). However, this is not sufficient to demonstrate the presence of Yr29 in KC because it has been shown before that csLV46G22 is not a diagnostic marker for Yr29 (Ren et al., 2017). This is also supported by our mapping results, which show that QYr.ucw-1BL is four recombination events (0.1 cM) proximal to csLV46G22.

The most recent maps for Yr29/Lr46 from Pavon 76 place this locus between TraesCS1B01G453700 and csLV46G22 (Lagudah, unpublished data, 2018), a region that is very similar to the 332-kb candidate gene region for QYr.ucw-1BL identified in this study (Table 3). The Yr29 and QYr.ucw-1BL candidate gene regions differ only by the exclusion of TraesCS1B01G453700 from the Yr29 region and its partial inclusion in the 332-kb QYr.ucw-1BL region. The other complete genes listed in Table 3 can be considered as candidates for both QYr.ucw-1BL and Yr29/Lr46.

The tight linkage between QYr.ucw-1BL and Yr29 inferred from the comparative maps was also supported by the allelism test. In a population of 148 F1 plants segregating for both genes, none of the recombination events in the candidate region cosegregated with fully susceptible plants, which would have indicated recombination in phase of the two susceptible alleles. The other 552 lines analyzed in the allelism test, which showed no recombination events between the markers flanking the target region, provided valuable information about the relative effect of QYr.ucw-1BL and Yr29 in the same field experiment. We did not detect any significant difference in IT or severity between plants homozygous for QYr.ucw-1BL or Yr29 (Fig. 4), suggesting a similar resistance response. Taken together, the similar Pst resistance reactions and the colocation within a small chromosome region suggest (but do not demonstrate) that QYr.ucw-1BL and Yr29 might be the same gene.

Annotated Genes in the QYr.ucw-1BL Candidate Regions

The 12 high-confidence genes (excluding the TraesCS1B01G453800 repetitive element) located within the overlapping QYr.ucw-1BL/Yr29 candidate region in IWGSC RefSeq version 1.0 have annotated functions that are frequent among disease resistance genes. Six of these genes are RLKs, which encode proteins that are important for the recognition of extracellular signals and the initiation of intracellular signaling cascades in reaction to those stimuli (Chen et al., 2017a). The RLKs in this region (according to the modified annotations proposed for TraesCS1B01G454700 and TraesCS1B01G454900) encode proteins with two extracellular “Domains of Unknown Function 26” domains that are characteristic of the subgroup of CYSTEINE-RICH RECEPTOR KINASES (Chen, 2001). It has been proposed that members of this sub-group may be involved in redox signaling (Chen et al., 2004; Wrzaczek et al., 2010, 2013; Bourdais et al., 2015), plant–microbe interactions (Chen et al., 2004), and induction of cell death (Chen et al., 2003; Acharya et al., 2007; Yadeta et al., 2017). Among the CYSTEINE-RICH RECEPTOR KINASES in the QYr.ucw-1BL candidate region, TraesCS1B01G454100 is of particular interest as a candidate for QYr.ucw-1BL/Yr29 because of its location within the narrower candidate gene region delimited by ucw.k34 and ucw.k18 (Fig. 2c,d).

Three genes in the candidate gene region were annotated as GLUCAN ENDO-1,3-BETA-GLUCOSIDASES. These genes are widely distributed in seed-plant species and are involved in plant defenses against pathogen attack. These genes are frequently induced on detection of a pathogen infection and have been implicated in the hydrolysis of β-1,3-glucans present in the cell walls of pathogenic fungi (Beffa et al., 1993). In wheat, rapid accumulation of GLUCAN ENDO-1,3-BETA-GLUCOSIDASE transcripts was observed after Pst (Huang et al., 2013) or Puccinia graminis inoculation (Zhang et al., 2017; Chen et al., 2018).

This region also includes a single copy of a SLEEPER gene, a sugar transporter, and a member of the WRKY family of transcription factors. The protein encoded by TraesCS1B01G45300 (annotated as a transposable element) is significantly similar to RICESLEEPER2-like (XP_015645366). SLEEPER genes originate from active transposases in a process often referred as transposable element exaptation (Hoen and Bureau, 2015) or gene domestication (Knip et al., 2012). Previous reports have shown that these transposase-like genes can regulate gene expression and contribute to regulatory innovation, and that in some cases, can be essential for plant development (Knip et al., 2012; Hoen and Bureau, 2015).
TracesCS1B01G454800 encodes a putative sugar transporter. Polymorphisms in this class of genes can affect resistance to both leaf and stripe rust, as demonstrated by the cloning of the wheat rust resistance gene Lr67/Yr46 (Moore et al., 2015). Finally, TracesCS1B01G455000 encodes a WRKY transcription factor. Members of the WRKY family have been implicated in resistance against different pathogens, including Fusarium graminearum in wheat (TawWRKY45) (Bahrini et al., 2011), powdery mildew (MLA) in barley (Hordeum vulgare L.) (Chang et al., 2013), and rice blast (Pbl) (Inoue et al., 2013).

Polymorphisms between Resistant and Susceptible Genotypes in the Candidate Genes

The interpretation of the polymorphisms detected in the QYr.ucw-1BL/Yr29 candidate region depends on several assumptions, about which we currently have limited information. Therefore, if any of these assumptions are shown to be incorrect, the interpretations of the polymorphisms in this region should be re-examined. In the discussion presented below, we are making four assumptions: (i) QYr.ucw-1BL and Yr29/Lr46 are the same gene. (ii) Chinese Spring carries the susceptible allele for QYr.ucw-1BL/Yr29. (iii) The functional QYr.ucw-1BL/Yr29 allele confers resistance to Pst. (iv) Expression of the QYr.ucw-1BL/Yr29 resistance is not strongly affected by other loci segregating in the KC × KP mapping population.

Assumption 1 is based on the similar Pst resistance reactions of QYr.ucw-1BL and Yr29 and their close map location. Assumption 2 is based on a genetic study that failed to detect Lr46 in CS (Dyck, 1991). This negative result should be taken with caution because we observed that when QYr.ucw-1BL is present alone in the very susceptible ‘Avocet S’ background, it confers a limited level of resistance that delays the progression of the disease for only a few days. Under high Pst infection pressure, these plants are finally overwhelmed by the pathogen and appear to be susceptible. Assumption 3 is based on the partial dominance of the QYr.ucw-1BL resistance allele; and Assumption 4 on the consistent mapping results and the absence of unexpected close recombination events flanking QYr.ucw-1BL (Table 1 and Table 2).

If both KP and CS carry the susceptible QYr.ucw-1BL/Yr29 alleles, then only two of the nonsynonymous SNPs reported in Table 3 can be considered as potential causal SNPs: S90P in TracesCS1B01G454100 and P158L in TracesCS1B01G455000. Among them, S90P in TracesCS1B01G454100 is of particular interest because this gene is included in the reduced 115-kb candidate gene region. This amino acid change is predicted to have a strong effect on protein structure and function (SIFT 0.01) (Table 3). If we assume that CS carries the susceptible QYr.ucw-1BL/Yr29 allele, then the mutation inverted-repeat transposable element insertion in the TracesCS1B01G454100 promoter and the five SNPs that differentiate KP from the other four cultivars including CS (Table 3) cannot be the causal polymorphisms.

The serine allele from the S90P polymorphisms was detected in KP and CS and in 14 accessions (24%) for which exome capture data are available in the Wheat T3 database (https://triticeaeatoolbox.org/wheat/, accessed 29 Oct. 2018). The proline allele associated with Pst resistance was detected in a larger number of accessions, including KC, Pavon 76, Lababadur (Pavon-1B), and 45 accessions (76%) with exome capture data in the Wheat T3 database. The proline allele is also present in the currently sequenced wheat genotypes Cadenza, ‘Kronos’, ‘Paragon’, ‘Robigus’, ‘Claire’, ‘ArinaLrFor’, ‘Jagger’, ‘Julius’, and ‘Landmark’. This result indicates that either QYr.ucw-1BL/Yr29 resistance is widely distributed, or that S90P is not the causal polymorphism.

The other SNP present in both CS and KP is P158L in TracesCS1B01G455000 (Table 3). This amino acid change is only six residues from the conserved WRKY site and has a very low “Blocks Substitution Matrix 62” (BLOSUM) score (-3), which is indicative of a disruptive change in protein structure and function. However, the associated SIFT score is not that low (0.15) because the change is located within a small indel that is variable across the grass genomes. The leucine alleles associated with resistance is present in 46 of the 59 lines (78%) that have exome capture data available in the Wheat T3 database.

The serine allele in S90P and the proline allele in P158L, which were associated with susceptibility in KP, were detected also in the cultivars Avocet S, ‘Rusty’, ‘Langdon’, and ‘Zavitan’, all of which showed highly susceptible reactions to Pst in the UCD experimental fields. The tetraploid cultivar Kronos showed the allele associated with susceptibility for TracesCS1B01G455000 (P158) but the allele associated with resistance for TracesCS1B01G454100 (S90P). Therefore, the determination of the QYr.ucw-1BL/Yr29 allele present in Kronos may provide valuable information regarding these two putative causal polymorphisms. Between these two polymorphisms, we favor the S90P as a candidate polymorphism because it is completely linked with QYr.ucw-1BL. The P158L polymorphism is separated from QYr.ucw-1BL by two recombination events in our high-density map (Table 2; families FM1_50 and FM2_120). However, if unusual epistatic interactions or undetected field conditions confound the interpretation of the resistance phenotype in these two families, the P158L polymorphisms should still be considered as potential candidates for QYr.ucw-1BL.

Although no other amino acid changes were consistent between KP and CS in the 332-kb candidate region, we cannot rule out changes in the regulatory regions of these genes. Expression studies of the different genotypes with and without pathogen inoculations will be required to determine if there are differences in expression associated with the differences in resistance. If we assume that QYr.ucw-1BL actively confers resistance to Pst (Assumption 3 above), all the genes with nonfunctional alleles in the resistant cultivars can be discarded as candidate genes. This criteria would eliminate TracesCS1B01G454200.
amplification of marker TraesCS1B01G454600 (a 41-bp frame-shift indel), TraesCS1B01G454700 (a transposon insertion), and TraesCS1B01G454900 (a premature stop codon) from the list of candidate genes.

Finally, we cannot rule out the possibility that the causal gene is absent in the CS reference genome. If this were the case, we would have missed the candidate genes in all our analyses. Sequencing the genome (or the QYr.ucw-1BL/Yr29 candidate region) from a wheat cultivar that carries the QYr.ucw-1BL/Yr29 resistance allele would be required to test this possibility.

CONCLUSIONS AND PRACTICAL APPLICATIONS

On the basis of the comparative maps, the similar resistance phenotype, and the similar haplotypes, we hypothesize that QYr.ucw-1BL and Yr29 might represent the same gene. Although cloning these genes would be necessary to provide a conclusive test for this hypothesis, the determination of the ability of QYr.ucw-1BL to confer APR to leaf rust, stem rust, and powdery mildew could add valuable information to test this hypothesis. These experiments will require the introgression of QYr.ucw-1BL into genetic backgrounds lacking additional resistance genes for these diseases or the development of segregating populations from crosses between KC and cultivars that are susceptible to these diseases.

The exome capture data were very useful for identifying polymorphisms and developing molecular markers for the high-density maps. These data also provided a detailed description of the haplotypes present in the QYr.ucw-1BL/Yr29 candidate region, which helped prioritize genes for further validation. The haplotype analysis also yielded SNPs that differentiate resistant and susceptible alleles for both QYr.ucw-1BL and Yr29, which represent a valuable tool for characterizing the presence of QYr.ucw-1BL/Yr29 in germplasm collections and accelerating their deployment in wheat breeding programs.

Supplemental Materials

Supplemental Table S1. Markers used in the high-density map of QYr.ucw-1BL.

Supplemental Table S2. Markers used to characterize the 332-kb candidate region.

Supplemental Fig. S1. DNA identity among lines in the 332-kb conservative candidate region.

Supplemental Fig. S2. Polymerase chain reaction amplification of marker ucw.crk-4100 for the miniature inverted-repeat transposable element insertion in the promoter of TraesCS1B01G454100.

Supplemental Fig. S3. TraesCS1B01G454200 and TraesCS1B01G454300 region.

Supplemental Fig. S4. Phylogenetic relationship among putative RECEPTOR LIKE KINASE proteins in the candidate gene region.

Supplemental Fig. S5. Characterization of the region including TraesCS1B01G454700, TraesCS1B01G454800, and TraesCS1B01G454900.

Author Contributions

NC performed most of the experimental work for this project and wrote the first version of the manuscript. HW designed the TaqMan markers and contributed to the genotyping of the segregating populations. EL provided marker csLV46G22, Yr29 mapping information, and information about the distribution of this gene. All authors reviewed the manuscript. JD designed and directed the project and wrote the final version of the manuscript.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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