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RESEARCH

Mapping QTL for Resistance to New Virulent Races of Wheat Stripe Rust from Two Argentinean Wheat Cultivars

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ABSTRACT

During the last two decades, new virulent and aggressive races of *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*) have spread worldwide, causing devastating epidemics and prompting the search for new sources of resistance in wheat (*Triticum aestivum* L.). Between 2012 and 2017, we mapped four stripe rust resistance quantitative trait loci (QTL) effective against the *Pst* races present in California, USA, using recombinant inbred lines (RILs) developed from the cross between the Argentinean cultivars 'Klein Proteo' and 'Klein Chajá'. The RIL population showed transgressive segregation in all six growing seasons relative to the parental lines, which showed moderate levels of *Pst* resistance. Analyses by year detected QTL conferring adult plant resistance on chromosomes 1BL, 2BS, 3D centromeric (from Klein Chajá), and 4DL (from Klein Proteo). *QYr.ucw-1BL*, mapped in the *Yr29* resistance gene region, was significant in all seasons ($P < 0.01$) and explained on average 31.0 to 32.8% of the observed variation. *QYr.ucw-2BS* showed a stronger effect than *QYr.ucw-1BL* in 2013 but was ineffective in 2014 and 2016. This QTL also conferred seedling resistance, suggesting that it is an all-stage resistance gene. Centromeric *QYr.ucw-3D* and *QYr.ucw-4DL* showed smaller effects than the previous QTL and were significant only in some of the experiments. No significant interactions were detected among QTL, indicating the absence of digenic epistatic effects. The molecular markers identified in this study can be used to combine these genes and accelerate their deployment in wheat breeding programs.

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Abbreviations: APR, adult plant resistance; IT, infection type; IWGSC, International Wheat Genome Sequencing Consortium; KC, Klein Chajá; KP, Klein Proteo; LOCO-LMM, "leave-one-chromosome-out" linear mixed model; LOD, logarithm of odds; LS mean, least squares mean; NBS-LRR, nucleotide binding site–leucine-rich repeat; PVE, phenotypic variation explained; *Pst*, *Puccinia striiformis* f. sp. *tritici*; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SSR, simple sequence repeat.

Common wheat (*Triticum aestivum* L.) is the second most widely grown crop worldwide, with ~750 Tg produced every year (FAO, 2018). To maintain and increase global wheat production, it is necessary to minimize losses generated by different pathogens. Wheat stripe rust (also called yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*), is a devastating disease (Hovmøller et al., 2008; Chen et al., 2010) that causes significant reductions in both yield (Smith et al., 1986) and grain quality (Dimmock and Gooding, 2002; Lowe et al., 2011). During the last two decades, new *Pst* races with broader virulence profiles, increased aggressiveness, and tolerance to high temperatures have defeated many of the previously known stripe rust resistance genes (Milus et al., 2008; Markell and Milus, 2008; Hovmøller et al., 2016). In 2000 alone, 21 new, highly virulent races were identified in the United States, and >60 additional races have been found since then. In

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2003, these new *Pst* races caused grain yield losses of >25% in California, where these new races were initially detected (Jackson et al., 2003). Highly virulent races were reported later in Australia, Europe, and North Africa (Dong et al., 2017). Two of the *Pst* genotypes, identified in Europe and North Africa in 2015 to 2016, were detected in 2017 in Argentina, where they affected >3 million ha, causing the worst epidemics of stripe rust since the 1930s (Global Rust Reference Centre, 2018).

Although fungicides can be used to control this disease, they are expensive and pose health risks when not used properly. Breeding resistant cultivars is a more effective, economical, and environmentally friendly way to control stripe rust in wheat (Cao et al., 2012). However, the implementation of this strategy requires continuous efforts to identify and deploy new sources of resistance against the rapidly evolving *Pst* populations.

Wheat rust resistance genes are classified into all-stage and adult plant resistance (APR) genes. All-stage resistance genes (also called major or seedling resistance genes) are effective starting at early stages of plant development and typically encode nucleotide binding site–leucine-rich repeat (NBS-LRR) resistance proteins. These proteins recognize pathogen effectors (or the modified host proteins) and trigger 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* (*PR*) genes that reduce pathogen growth (Zhang et al., 2017; Chen et al., 2018). By contrast, the few wheat rust APR resistance genes cloned so far encode a more diverse set of proteins than the NBS-LRR, which include an ATP-binding cassette (ABC) transporter (Krattinger et al., 2009), a kinase-START lipid binding protein (Fu et al., 2009; Gou et al., 2015), and a hexose transporter (Moore et al., 2015).

Changes in pathogen effectors, including amino acid changes in the contact surface, loss-of-function mutations, or deletions, can help the pathogen avoid detection by the corresponding NBS-LRR genes (Chen et al., 2017; Salcedo et al., 2017). As a result, many all-stage resistance genes are defeated within a few years of their commercial deployment by the rapidly evolving rust populations. By contrast, resistance conferred by rust APR genes has been relatively durable (Krattinger et al., 2009). To improve the durability of deployed rust resistance genes, wheat breeders pyramid multiple all-stage resistance genes, multiple APR genes, or combinations of both (Singh et al., 2000; Lowe et al., 2011; Nelson et al., 2018). However, as some of the genes in these pyramids are defeated, it is important to discover new sources of resistance and develop linked molecular markers to incorporate them in new pyramids and accelerate their deployment.

The main objective of this study was to map quantitative trait loci (QTL) for field resistance to the new aggressive *Pst* races detected in California after the year 2000. Additional objectives included the study of the QTL epistatic interactions to select the best combinations, and their comparison with previously mapped *Pst* resistance genes to determine their novelty. To explore sources of resistance different from the ones frequently used in our breeding program, we selected a recombinant inbred line (RIL) population derived from the cross between the partially resistant Argentinean cultivars 'Klein Proteo' (KP) and 'Klein Chajá' (KC).

MATERIALS AND METHODS

Population Development

A segregating mapping population of 96 RILs was developed by crossing the Argentinean common spring wheat cultivars KP (KAVKAZ/K-4500-L.A.4//VEERY/3/KLEIN-COBRE/4/KL-H-1928-M-132) and KC (NANJING/3/BUCKBUCK//H-697/DEKALB-LAPACHO). Both lines showed intermediate levels of *Pst* resistance, but no named stripe rust resistance genes have been previously reported from these lines. By contrast, leaf rust (*Puccinia triticina* Erikss.) resistance genes have been identified both in KC (*Lr17*) and KP (*Lr3a* and *Lr10*) (Vanzetti et al., 2011). The RIL population was genotyped at F_6, and $F_{6:7}$ head rows were planted for seed increases. $F_{6:8}$ seeds were used for all the following field experiments.

Linkage Map Construction

Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980) and resuspended in 200 mL of Tris-HCl ethylenediaminetetraacetic acid (EDTA, pH 8.0). Genotyping was conducted at the USDA-ARS genotyping laboratory in Fargo, ND, using the Illumina Infinium Wheat single nucleotide polymorphism (SNP) 9K iSelect assay, developed by the International Wheat SNP Consortium (Cavanagh et al., 2013). Illumina SNP data were processed with GenomeStudio 2011.1 (Illumina, 2011). In addition, 108 polymorphic simple sequence repeats (SSRs, Grain Genes database, http://wheat.pw.usda.gov/GG3/) were mapped to facilitate the comparison with previously published maps. Co-segregating markers were combined into one representative marker for map construction and QTL analyses. A linkage map was built with MAPMAKER/EXP 3.0 (Lincoln et al., 1993) using the Kosambi mapping function (Kosambi, 1943). Markers were grouped into linkage groups using a minimum logarithm of odds (LOD) threshold of 3.0, and a three-point linkage analysis was used to determine the most likely order of markers. Linkage groups were assigned to chromosomes using a previous consensus map as a reference (Cavanagh et al., 2013).

Field Experiments

The RIL population and the parental lines KC and KP were sown in mid-November at the University of California Field Station near Davis, CA (38°31′ N, 121°46′ W) in a Yolo loam soil (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents). Fertilization consisted of 224 kg N ha^{-1} applied as $(NH_4)_2SO_4$, half at preplanting and the rest at the beginning

of jointing. Trials were flood irrigated as needed (two to five irrigations). Because of limited seed supply, four RILs were excluded and only 92 RILs were used in these experiments. Each line was planted in 1-m head rows (30 seeds per row), with a spacing of 0.4 m between rows. The field experiment was repeated for six consecutive years (2012–2017), with two replications of the RIL population used in each year.

The highly susceptible common wheat line DS6301 was used as a spreader border to provide a strong and even inoculum pressure. Although natural and strong *Pst* infections occurred regularly in this region (Maccaferri et al., 2015), we inoculated the susceptible borders with a mix of *Pst* spores collected at the University of California–Davis experimental field station during the previous season to ensure a strong disease pressure. No fungicides were applied.

Disease Evaluation

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 leaf affected by rust (Peterson et al., 1948). The RIL population was scored twice each season (i.e., during heading [*Z50*] and grain-filling [*Z80*] stages; Zadoks et al., 1974) to minimize the effect of differences in phenology among lines. We used the observation showing the strongest and most even infection, which was, in most cases, the second observation. Each season, samples of infected leaves were sent to the USDA-ARS Wheat Health, Genetics and Quality Research Unit in Pullman, WA, to identify the *Pst* races present in the field, which are summarized with their virulence formulas in Supplemental Table S1.

QTL Analysis

A QTL analysis was conducted using R/qtl2 version 0.4-21 (Broman, 2018), with phenotypic data collected during six seasons (2012–2017) and the linkage map described above. Infection type and severity were analyzed independently for each year, using the mean of the two replications as the response variable. One RIL (K-96) was removed from the analyses because of its high number of missing marker data (32%). Interval mapping was conducted using a "leave-one-chromosome-out" linear mixed model (LOCO-LMM), with a 1-cM step. Linear mixed models account for potential polygenic effects by modeling the covariance between phenotypes and genotypes as a random effect (Gonzales et al., 2017). In addition, LOCO-LMM models reduce potential overestimations of Type I and Type II error rates, compared with linear mixed models with a single kinship matrix (Yang et al., 2014; Gonzales et al., 2017). Twenty-one kinship matrices were calculated, each one excluding a different chromosome, and each chromosome was evaluated using the kinship matrix constructed with the remaining chromosomes only. The LOD threshold for QTL significance ($P < 0.05$) for each trait × year combination was calculated by performing 1000 permutations.

Statistical Analysis

For each QTL, the marker associated with the highest LOD score across years was designated as the peak marker. These peak markers were used as classification variables in a factorial ANOVA for IT and severity conducted independently for each growing season using the PROC GLM statement in SAS version 9.4 (SAS Institute, 2013). The statistical model included the peak markers for each QTL and their first-order interactions. Normality of residuals was tested using the Shapiro–Wilk test implemented in PROC UNIVARIATE, and the phenotypic variation explained (PVE) was calculated using PROC VARCOMP for each QTL. To validate the selected peak markers, we also explored the closest flanking markers in factorial ANOVAs including all four QTL and environments as blocks. For each QTL, we conducted three separate ANOVAs, with the peak marker and the two closest flanking markers (maintaining the selected peak markers at the other three QTL). We then compared the *F* values of the selected peak markers and the flanking markers and confirmed that the selected peak marker was the most significant in the combined analyses. Correlation coefficients (*r*) between IT and severity from different years were calculated using the PROC GLM statement in SAS version 9.4.

For the PVE calculation, the nonsignificant interactions were excluded from the model. The effect of individual QTL–QTL combinations was estimated by calculating the least squares means (LS means) for IT and severity of RILs sharing the same alleles at the four QTL peak markers. The groups with different resistance allele combinations were compared with RILs with no resistance QTL using a Dunnett multiple comparison test. Error bars represent the SEMs.

Comparisons with Previously Mapped Resistance Genes and QTL

To compare the location of the QTL identified in this study with previously published *Pst* resistance genes and QTL, sequence-based markers flanking the QTL were aligned to the most recent *T. aestivum* reference sequence of Chinese Spring (IWGSC RefSeq v1.0) developed by the International Wheat Genome Sequencing Consortium (IWGSC, 2018). We then used the physical position of the markers on the reference sequence and the MapChart 2.2 (Voorrips, 2002) program to generate comparative maps.

RESULTS

Linkage Map

The linkage map generated for the RIL population has a total length of 2903 cM and includes 2806 polymorphic markers (2698 SNPs and 108 SSRs), resulting in an average of one marker per centimorgan. After merging co-segregating markers, 747 unique polymorphic loci were mapped. A smaller number of SNP markers were mapped on the D genome chromosomes (232 SNPs) relative to those in the A (1315 SNPs) or B (1151 SNPs) genome chromosomes, a result similar to previous maps constructed using the same SNP assay (Cavanagh et al., 2013; Dong et al., 2017). A spreadsheet with all mapped markers and mapped distances is included as Supplemental File S1.

Stripe Rust Infection

Both parental lines showed moderate levels of field resistance to the *Pst* races present in the six experiments

(Supplemental Table S1). On average, KP showed slightly higher IT and severity values (IT = 5.5, severity = 60%) than KC (IT = 4.7, severity = 49.2%). The RIL population showed transgressive segregation (Supplemental Fig. S1). Correlation coefficients (*r*) between IT and severity were high for all six seasons (*r* = 0.84–0.97, Table 1). Pairwise comparisons of the IT or severity values across years were slightly lower than the comparisons within years, but were all significant ($P < 0.001$, Table 1).

QTL and Statistical Analyses

Four QTL were significant for at least one season for both IT and severity ($P < 0.05$, LOD > 3.3). The alleles for *Pst* resistance of the QTL located on chromosome arms 1BL, 2BS, and 3D centromeric were derived from KC, whereas the allele for resistance on chromosome arm 4DL was derived from KP. Figure 1 represents a summary of the IT and severity effects of the individual QTL across the 6 yr of this study.

The factorial ANOVA model including the QTL peaks as classification variables and all pairwise interactions explained, on average, 61.6% of the variation in the population for IT and 64.0% for severity. No significant interactions were detected between any pair of QTL. However, some caution in the interpretation of this result is required because our RIL population was relatively small, and some small but real interactions may appear as nonsignificant. The significance and percentage of PVE for each QTL in each year is summarized in Table 2.

To visualize the individual and combined effect of the four QTL on IT and severity, we used the alleles at the QTL peak markers to group RILs with the same allele combinations and obtain their mean and SEs across years (Fig. 2). All QTL combinations, with the exception of the RILs with the 2BS + 4DL combination, were significantly different ($P < 0.05$) from the RILs with no resistance alleles. On average, RILs carrying the single QTL for 1BL and 2BS were less susceptible than the RILs carrying the single 3D and 4DL QTL. The 2BS QTL showed the largest variability, which is consistent with the contrasting results observed in different years. The RILs with two alleles for resistance were, on average, better than the ones with a single one, and those with the 1BL + 2BS and 1BL + 4DL combination showed the best resistance within this group (Fig. 2). Among the RILs with three alleles for resistance, the 1BL + 2BS + 3D and 1BL + 4DL + 3D combinations showed the lowest IT values (severity values were more homogeneous, Fig. 2). The RILs with the four resistance alleles showed the best *Pst* resistance. Taken together, these results indicated that the effects of these four QTL were mainly additive.

1BL QTL (*QYr.ucw-1BL*)

The *QYr.ucw-1BL* allele for *Pst* resistance originated in KC. The peak of *QYr.ucw-1BL* was mapped in the distal region of chromosome arm 1BL associated with markers *IWA8581* and *csLV46G22*. The latter marker has been mapped close to stripe rust resistance gene *Yr29* in several studies (see Discussion). A 1-LOD score confidence interval defined a 25.5-cM interval delimited by markers *IWA3998* and *IWA198* (Fig. 1).

Among the QTL discovered in this study, *QYr.ucw-1BL* was the only one that was significant ($P < 0.01$) across all years for IT and severity. This QTL explained, on average, 32.8 and 30.9% of the observed variation on IT and severity, respectively (Table 2). When compared with RILs carrying susceptible alleles for all four QTL, the RILs including only *QYr.ucw-1BL* (Fig. 2) showed a reduction in LS means of 21.7% for IT ($P < 0.0001$) and 27.6% for severity ($P < 0.0001$).

2BS QTL (*QYr.ucw-2BS*)

The peak of QTL *QYr.ucw-2BS* was associated with SNP marker *IWA2885* (and linked marker *IWA3622*) and flanked by markers *IWA8420* and *wmc477* that delimited a 12.8-cM 1-LOD interval. When compared with RILs carrying susceptible alleles for all four QTL, the RILs including only *QYr.ucw-2BS* (Fig. 2) showed a reduction

Table 1. Correlation coefficients (*r*) between infection type (IT) and severity (S) for year–trait combinations.

	2012		2013		2014		2015		2016		2017	
Year–trait	IT	S	IT	S	IT	S	IT	S	IT	S	IT	S
IT2012	1.00											
S2012	*0.96*†	1.00										
IT2013	0.71	0.68	1.00									
S2013	0.68	0.65	*0.90*	1.00								
IT2014	0.65	0.64	0.52	0.46	1.00							
S2014	0.72	0.70	0.59	0.55	*0.90*	1.00						
IT2015	0.73	0.70	0.62	0.59	0.79	0.78	1.00					
S2015	0.74	0.72	0.68	0.70	0.61	0.65	*0.84*	1.00				
IT2016	0.61	0.59	0.44	0.38	0.76	0.75	0.77	0.64	1.00			
S2016	0.56	0.54	0.44	0.38	0.74	0.73	0.75	0.62	*0.94*	1.00		
IT2017	0.65	0.63	0.59	0.63	0.53	0.56	0.59	0.55	0.57	0.54	1.00	
S2017	0.63	0.61	0.59	0.63	0.48	0.54	0.57	0.54	0.52	0.50	*0.97*	1.00

† Correlation between IT and S in the same year are presented in italics. All correlations were significant ($P < 0.001$, $n = 96$).

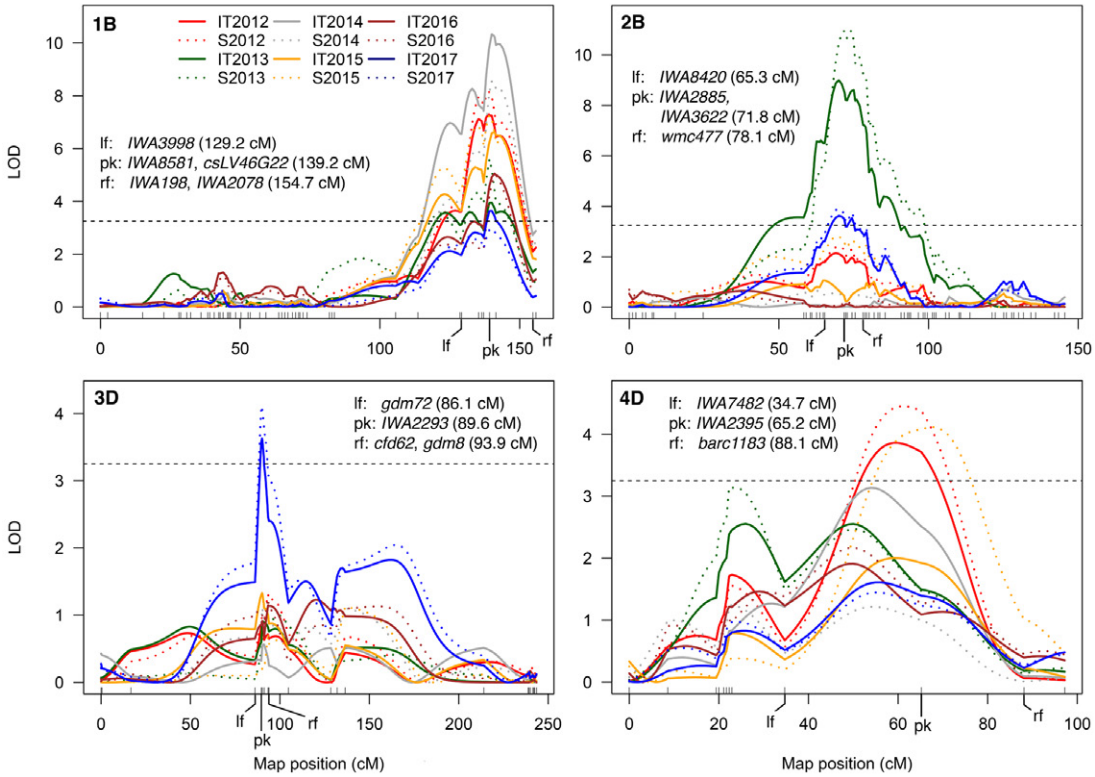

Fig. 1. Logarithm of the odds (LOD) plots for the significant quantitative trait locus (QTL) regions located on chromosomes (chr.) 1B, 2B, 3D, and 4D. The LOD score used as a threshold for significance is represented by a horizontal dashed line. In all plots, chromosomes are oriented with the telomere of the short arm to the left and tick marks along the abscissa indicate the positions of mapped markers. Solid lines represent results for infection type (IT) and dashed lines for disease severity (S) (see color key in figure). pk, QTL peak marker; lf, left flanking marker; rf, right flanking marker. Only a subset of the co-segregating markers is shown. All markers in the QTL region are included in Fig. 3 for chr. 1B, Fig. 4 for chr. 2B, Fig. 5 for chr. 3D, and Fig. 6 for chr. 4D. The complete genetic map is available in Supplemental File S1.

in LS means of 19.4% for IT (P = 0.0021) and 30.4% for severity (P = 0.014). The *QYr.ucw-2BS* allele for *Pst* resistance originated in KC.

QYr.ucw-2BS was significant (P < 0.01) for both IT and severity only in 2012, 2013, and 2017. In 2013, this QTL explained 43.4% of the observed variation in IT and 46.4% of the variation in severity (Fig. 1, Table 2). The effects were smaller in 2017 (PVE, IT = 17.9%, severity = 18.8%) and 2012 (PVE, IT = 8.2%, severity = 8.6%) and only significant for severity in 2015 (PVE, severity = 13.6%, Table 2). The nonsignificant effects in 2014 and 2016 suggested that this gene could be an all-stage, race-specific gene. This hypothesis was supported by seedling resistance tests performed at Washington State University,

Table 2. ANOVA results from a factorial model by year, with each quantitative trait locus (QTL) for infection type (IT) and severity (S) represented by its peak marker.

		2012		2013		2014		2015		2016		2017	
QTL (peak marker[s])†	Trait	IT	S	IT	S	IT	S	IT	S	IT	S	IT	S
QYr.ucw-1BL (IWA8581)	*P* value‡	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	PVE (%)§	37.1	36.9	18.4	22.5	54.6	47.0	34.3	35.8	32.7	28.3	19.6	15.1
QYr.ucw-2BS (IWA2885, IWA3622)	*P* value	0.045	<0.01	<0.01	<0.01	0.336	0.900	0.460	<0.01	0.446	0.316	<0.01	<0.01
	PVE (%)	8.2	8.6	43.4	46.4	0	0	2.5	13.6	0	0	17.9	18.8
QYr.ucw-3D (IWA2293)	*P* value	<0.01	<0.01	0.084	0.072	0.077	<0.01	<0.01	0.107	<0.01	<0.01	<0.01	<0.01
	PVE (%)	2.4	4.1	1.5	0.4	0	2.7	5.0	0	4.3	5.9	21.6	25.4
QYr.ucw-4DL (IWA2395)	*P* value	<0.01	<0.01	0.015	0.046	0.028	0.140	0.126	< 0.01	0.448	0.233	0.163	0.136
	PVE (%)	19.3	22.0	6.5	3.7	13.4	7.3	9.9	15.3	5.3	6.0	4.0	3.7
Total model PVE (%)¶		67.0	71.5	69.8	73.0	68.0	57.0	51.8	64.7	42.3	40.3	63.1	63.0

† Peak markers used in the analysis are shown in parenthesis under each QTL name.

‡ P values of the QTL with significant effects are presented in italics.

§ PVE, phenotypic variation explained for individual QTL in each year.

¶ Total model PVE represents the phenotypic variation explained by a model including the peaks of all four QTL (nonsignificant interactions were excluded from the model).

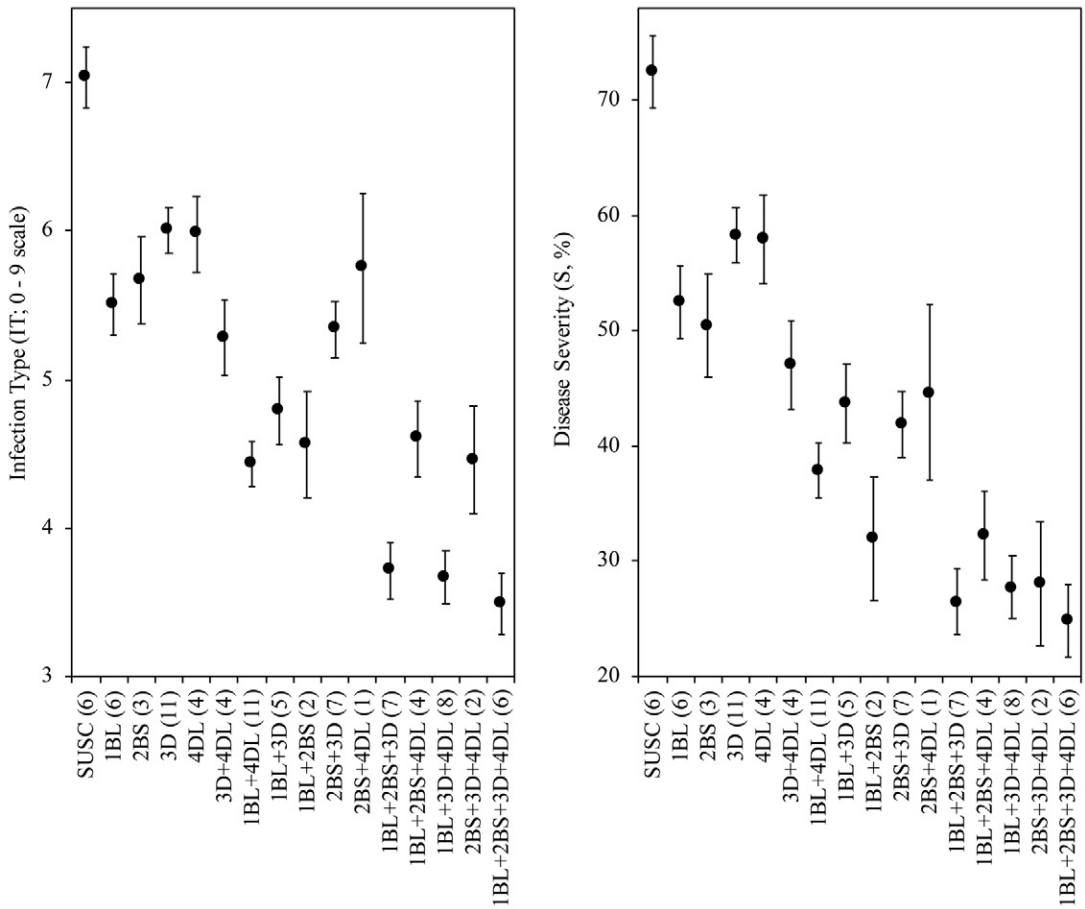

Fig. 2. Effect of individual quantitative trait locus (QTL) and QTL combinations on infection type (IT) and severity (S). SUSC represents recombinant inbred lines (RILs) that carry the susceptible allele for all four QTL. For the rest, the resistance alleles present in the group are indicated by the name of the chromosome where the QTL is located (e.g., RILs with the resistant allele of *QYr.ucw-1BL* and *QYr.ucw-2BS* are designated as 1BL + 2BS). The number of RILs grouped in each class is indicated in parentheses after each class name. The IT and S scores are means over genotypes and years. Error bars represent ± 1 SEM.

which showed that an RIL carrying only the 2BS QTL was resistant to *Pst* races PSTv-4 (IT = 4), PSTv-17 (IT = 4), PSTv-3 (IT = 2), PSTv-43 (IT = 2), and PSTv-45 (IT = 2, 4). In the same experiment, the control line Avocet S was completely susceptible to these races (IT = 9). When this RIL was tested at the adult plant stage under high-temperature conditions, it showed susceptibility to PSTv-37 (but not to PSTv-14, PSTv-40, and PSTv-51), providing additional evidence that this QTL represents a race-specific resistance gene.

3D QTL (*QYr.ucw-3D*)

QYr.ucw-3D was identified in the centromeric region of chromosome 3D and was associated with peak marker *IWA2293*. The 1-LOD confidence interval defined a 7.8-cM interval flanked by SSR markers *gdm72* and *cfd62/gdm8*. This QTL exceeded the 3.3-LOD threshold only in 2017 (Fig. 1) but was significant in the factorial ANOVA for both IT and severity in the field experiments performed in 2012, 2016, and 2017, for severity only in 2014, and for IT only in 2015 (Table 2). *QYr.ucw-3D* explained, on average, 5.8% of the phenotypic variation for IT and 6.4% for severity. When compared with RILs carrying susceptible alleles for all four QTL, the RILs including only *QYr.ucw-3D* (Fig. 2) showed a reduction in LS means of 14.6% for IT (P = 0.0011) and 19.6% for severity (P = 0.0015). The allele for resistance was conferred by KC.

4DL QTL (*QYr.ucw-4DL*)

QYr.ucw-4DL was the only QTL discovered for which the allele for *Pst* resistance originated in KP. The peak of this QTL was linked to marker *IWA2395*, and the flanking markers defining the 53.4 cM 1-LOD confidence interval were *IWA7482* and *barc1183* (Fig. 1). This QTL exceeded the LOD threshold in 2012 for both IT and severity and in 2015 for severity (Fig. 1). In the factorial ANOVA, *QYr.ucw-4DL* showed significant effects for both IT and severity in 2012 and 2013, for IT only in 2014, and for severity only in 2015. This QTL explained, on average, 9.7% of the observed variation in IT and severity (Table 2). When compared with RILs carrying susceptible alleles for all four QTL, the RILs including only *QYr.ucw-4DL* (Fig. 2) showed a reduction in LS means of 15% for IT (P = 0.017) and 20.1% for severity (P = 0.0465).

DISCUSSION

The genotyping of a collection of 409 *Pst* races suggested that the more aggressive and high-temperature adapted *Pst* races detected in the last two decades originated in the Middle East or East Africa and spread rapidly through human activities (Ali et al., 2014). The appearance of these new races caused severe epidemics in North America, South America, Australia, Europe, and Africa (Dong et al., 2017; Global Rust Reference Centre, 2018) and a rapid erosion of effective resistance genes (Lowe et al., 2011).

As part of the global efforts to find new sources of resistance against these more aggressive *Pst* races, we evaluated a population from the cross of two Argentinean commercial cultivars that showed moderate levels of APR to *Pst* under field conditions in California. We found that these two parental lines carried different *Pst* resistance genes, which explained the strong transgressive segregation observed in the derived RIL population (Supplemental Fig. S1). The correlations observed across years for IT or severity values were smaller than the correlations observed between IT and severity scores within years (Table 2). This can be explained by the different *Pst* races detected during these 6 yr in the fields where the population was grown, which were likely the cause of the different effects of *QYr.ucw-2BS* across the years (Table 2). In addition, the severe weather variation observed in California during the years of this study likely affected the effectiveness of some of the QTL. The 2012 to 2014 period was one of the hottest and driest on record for California (Mann and Gleick, 2015) and was followed by above average rainfall in 2015 and 2016. Although these changing weather conditions made QTL detection more challenging, they provided a strong test for the consistency of the reported QTL.

Comparison with Previously Mapped Resistance Genes and QTL

The large yield losses caused by the new *Pst* races provided a strong incentive for the search for novel *Pst* resistance genes. This, together with the more powerful marker platforms developed for wheat, resulted in a significant increase in the mapped *Pst* resistance genes and QTL (Maccaferri et al., 2015; Hou et al., 2015; Calvo-Salazar et al., 2015; Ren et al., 2017; Dong et al., 2017; Ponce-Molina et al., 2018). The proliferation of mapping studies, together with the different sets of molecular markers used in these studies, have complicated the comparison among QTL mapped on the same chromosome arms.

Fortunately, the recent release of the wheat reference sequence (IWGSC RefSeq v1.0) provides a common reference to anchor the sequence-based markers used in the different studies. In the following discussion, and in Fig. 3 to 6, we compared the position of the QTL detected in this study (white rectangles) with the position of previously mapped *Pst* resistance genes (shaded rectangles) and QTL (black rectangles).

QYr.ucw-1BL

QYr.ucw-1BL was the most consistent QTL found in this study, and it was significantly associated with resistance to *Pst* in every season. The distal region of chromosome 1BL, where *QYr.ucw-1BL* was mapped, has been associated before with resistance to multiple pathogens, including stripe rust (*Yr29*), leaf rust (*Lr46*), stem rust (*Puccinia graminis* subsp. *graminis* Pers.:Pers., *Sr58*), and powdery mildew [*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal, *Pm39*] (William et al., 2003; Lillemo et al., 2008; Singh et al., 2013). It has been suggested that these different genes may represent pleiotropic effects of a single gene conferring resistance to a broad range of fungal pathogens. However, with the current resolution of the different mapping studies, it is not possible to rule out the alternative hypothesis of closely linked genes conferring resistance to the different pathogens. For this reason, we will focus only on those studies that observed significant effects for *Pst* resistance in this region in the discussion below.

Yr29 is an APR gene that was first identified in the cultivar 'Pavon 76' (William et al., 2003) and has since been mapped in multiple studies using different genetic backgrounds (William et al., 2006; Melichar et al., 2008; Bariana et al., 2010; Zwart et al., 2010; Bansal et al., 2014; Kolmer et al., 2015). According to the Catalogue of Gene Symbols for Wheat (McIntosh et al., 2013), *Yr29* is located in the distal region of chromosome arm 1BL between SSR markers *wmc44* and *gwm140* (Fig. 3). These two markers define a region of 22.7 Mb (between 662.2 and 684.9 Mb in IWGSC RefSeq v1.0) that overlaps very well with the 1-LOD score confidence interval for *QYr.ucw-1BL* identified in this study. Moreover, the peak marker of our QTL *IWA8581* co-segregated with *csLV46G22*, which has been reported to be in close linkage with *Yr29* in several 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). Taken together, these results suggested that *QYr.ucw-1BL* might correspond to *Yr29*.

QYr.ucw-2BS

The large effect of *QYr.ucw-2BS* on APR during some years and its complete lack of effect in other years (Table 2) suggested that *QYr.ucw-2BS* could be a major all-stage resistance QTL. This hypothesis was supported by the results from an RIL carrying only *QYr.ucw-2BS* that showed resistance to five *Pst* races at the seedling stage and susceptibility to one *Pst* race at the adult stage.

Several named *Pst* resistance genes, including *Yr27*, *Yr31*, *Yr41*, *YrC51-YrP81*, *YrF*, *YrH9014*, and *YrKK*,

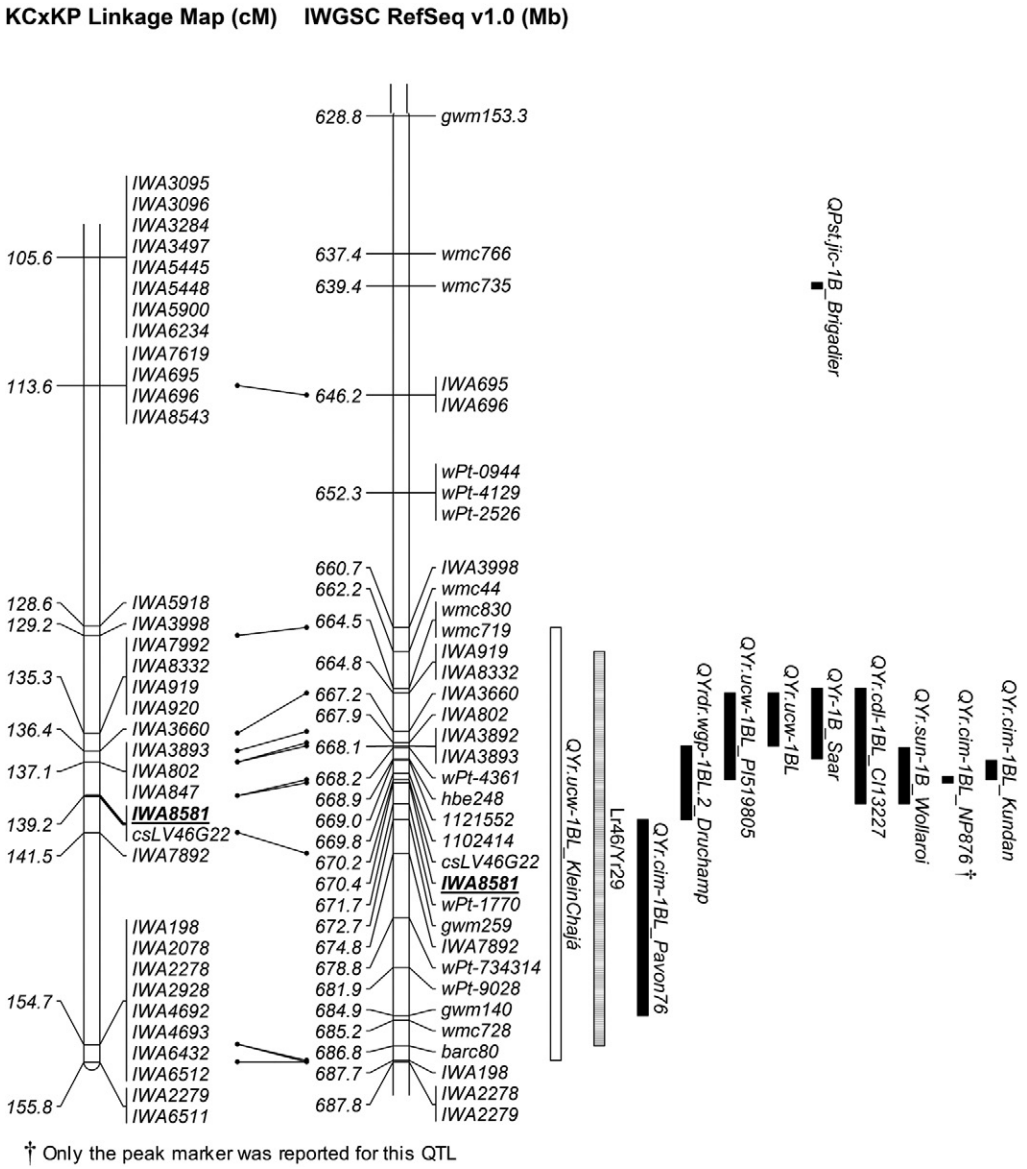

Fig. 3. *QYr.ucw-1BL* (white, 1-logarithm of the odds [LOD] confidence interval) compared with previously identified genes (gray) and quantitative trait loci (QTL) (black). Distances in the genetic map (left) are in centimorgans and in the physical reference in megabases (right). The genotype associated with the resistance allele is included after each QTL name. The name of *QYr.ucw-1BL* peak marker (*IWA8581*) is bolded and underlined. Lines connecting both maps indicate the position of the common markers on the map (not the positions of the marker names or distances). IWGSC, International Wheat Genome Sequencing Consortium; KC, Klein Chajá; KP, Klein Proteo.

have been mapped on the short arm of chromosome 2B (Rosewarne et al., 2013; Lan et al., 2014; Maccaferri et al., 2015; Wu et al., 2017). Most of these genes confer all-stage, race-specific resistance and have been defeated by Chinese *Pst* races (Wu et al., 2017). However, *YrKK* (derived from cultivar 'Kenya Kudu') conferred near immunity to adult plants in field trials in Toluca, Mexico, in 2010 and 2011 and showed limited effect on seedling resistance to three Mexican *Pst* isolates (Li et al., 2013). By contrast, *QYr.ucw-2BS* showed stronger seedling resistance to several *Pst* isolates and a weaker field resistance in adult plants (Fig. 2) than *YrKK*, suggesting that they are likely different genes. However, since different *Pst* races were used in the two studies, this hypothesis will require further validation.

Two independent results suggest that *QYr.ucw-2BS* is different from *Yr27*. First, RILs carrying only *QYr.ucw-2BS* were resistant to PSTv-4, PSTv-3, PSTv-17, PSTv-43, and PSTv-45, whereas the *Yr27* single-gene line (AvSYr27NIL) was susceptible (IT = 7–9) to the same races (Wan and Chen, 2014; Wan et al., 2016). In addition, in 2013 and 2017, when races virulent against *Yr27* were detected in the field at relatively high frequency (70 and 54%, respectively, Supplemental Table S1), *QYr.ucw-2BS* still conferred high levels of *Pst* resistance (>40% PVE in 2013 and 18–19% in 2017). Allelism tests will be required to determine if *QYr.ucw-2BS* is different from the overlapping *YrF* (from Francolin#1; Lan et al., 2014) and *YrH9014* (from *Psathyrotachys huashanica* translocation line H9014-14-4-6-1; Ma et al., 2013) (Fig. 4).

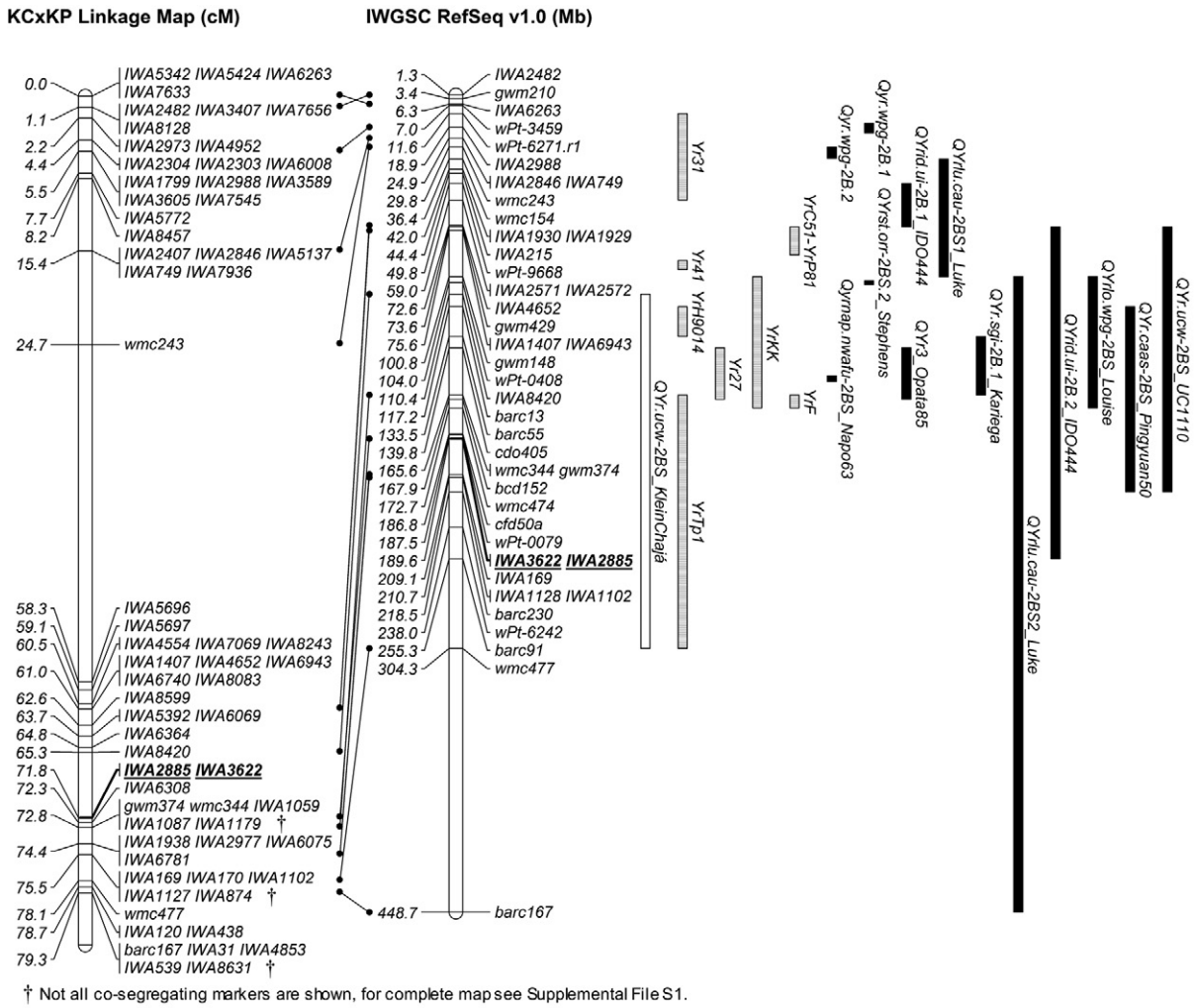

Fig. 4. *QYr.ucw-2BS* (white, 1-logarithm of the odds [LOD] confidence interval) compared with previously identified genes (gray) and quantitative trait loci (QTL) (black). Distances in the genetic map (left) are in centimorgans and in the physical reference in megabases (right). The name of genotype associated with the resistance allele is included after each QTL name. The name of *QYr.ucw-2BS* peak markers (*IWA2885* and *IWA3622*) are in bold and underlined. Lines connecting both maps indicate the position of the common markers on the map (not the positions of the marker names or distances). IWGSC, International Wheat Genome Sequencing Consortium; KC, Klein Chajá; KP, Klein Proteo.

In addition to the named *Yr* genes, multiple QTL for *Pst* resistance have been mapped on chromosome 2BS (Fig. 4). These include *QYr.sgi-2B.1* from 'Kariega' (Agenbag et al., 2014), *QYr3* from 'Opata85' (Boukhatem et al., 2002), *QYr.caas-2BS* from Pingyuan 50 (Lan et al., 2010), *QYrlu.cau-2BS2* from 'Luke' (Guo et al., 2008), *QYrlo.wpg-2BS* from 'Louise' (Carter et al., 2009), *QYr.ucw-2BS* from UC 1110 (Lowe et al., 2011), and *Qyrmap.nwafu-2BS* from 'Napo 63' (Wu et al., 2017). Napo 63 is related to Kenya Kudu through the common parent 'Florence' and shares the same haplotype at four Kompetitive allele-specific polymerase chain reaction (KASP) markers in the *YrKK* region. The same haplotype was also detected in Kariega, Opata85, Luke, and Louise (Wu et al., 2017), suggesting that these five lines might carry the *YrKK* resistance gene. Since we have established above that *YrKK* is likely different from *QYr.ucw-2BS*, these additional five QTL are also likely different. This hypothesis is further supported by the fact that the 2BS QTL in Louise confers APR resistance to PSTv-37 and *QYr.ucw-2BS* does not. Seedling tests have not been reported for the UC 1110 and Pingyuan 50 QTL that overlap with *QYr.ucw-2BS* (Fig. 4), and therefore allelism tests will be required to differentiate these three QTL.

QYr.ucw-3D

This QTL, identified in the centromeric region of chromosome 3D, was significant in five out of the six seasons for either IT or severity but explained only a small proportion of the phenotypic variation in most of the years (overall average = 5.8% in IT and 6.4% in severity, Table 2). No named *Pst* resistance genes have previously been reported in this chromosome region, but some *Pst* resistance QTL have been (Fig. 5). *QYr.inra-3DS* from the French cultivar 'Recital' was first described as a small-effect, slow-rusting resistance gene in this region (Dedryver et al., 2009). Later, *QYr.tam-3D* donated by Quaiu 3 (Basnet et al.,

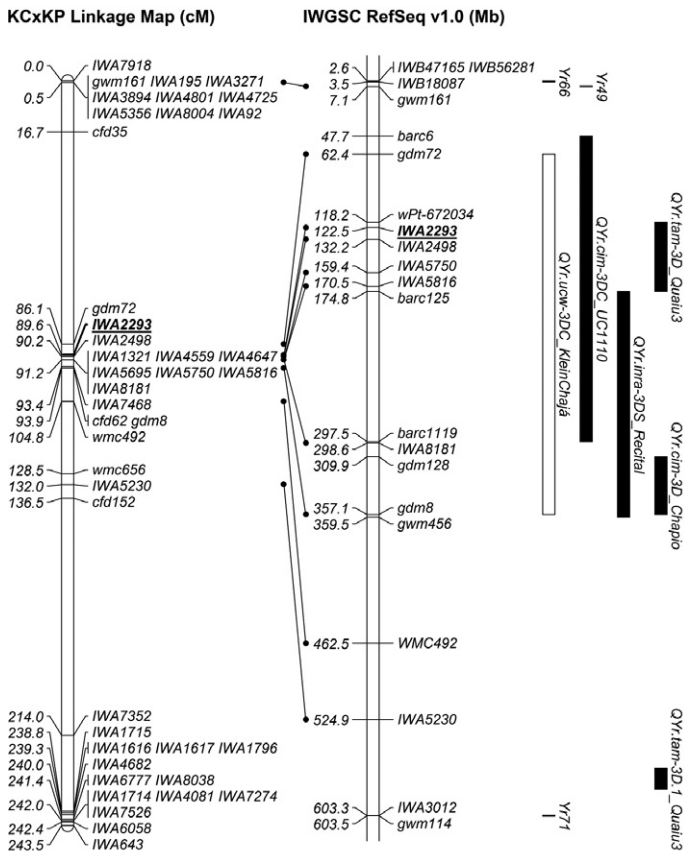

Fig. 5. *QYr.ucw-3D centromeric* (white, 1-logarithm of the odds [LOD] confidence interval) compared with previously identified genes (gray) and quantitative trait loci (QTL) (black). Distances in the genetic map (left) are in centimorgans and in the physical reference in megabases (right). The name of genotype associated with the resistance allele is included after each QTL name. The name of *QYr.ucw-3D* peak marker (*IWA2293*) is in bold and underlined. Lines connecting both maps indicate the position of the common markers on the map (not the positions of the marker names or distances). IWGSC, International Wheat Genome Sequencing Consortium; KC, Klein Chajá; KP, Klein Proteo.

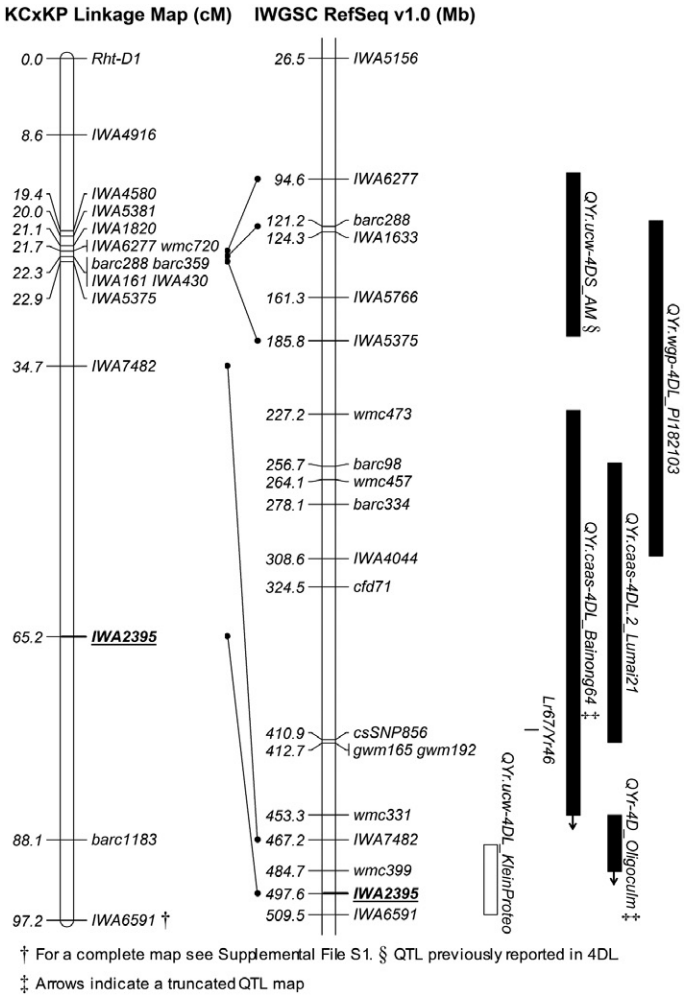

Fig. 6. *QYr.ucw-4DL* (white, 1- logarithm of the odds [LOD] confidence interval) compared with previously identified genes (gray) and quantitative trait loci (QTL) (black). Distances in the genetic map (left) are in centimorgans and in the physical reference in megabases (right). The name of genotype associated with the resistance allele is included after each QTL name. The name of *QYr.ucw-3D* peak marker (*IWA2395*) is in bold and underlined. Lines connecting both maps indicate the position of the common markers on the map (not the positions of the marker names or distances). IWGSC, International Wheat Genome Sequencing Consortium; KC, Klein Chajá; KP, Klein Proteo.

2014), *QYr.cim-3D* from 'Chapio' (Yang et al., 2013), and *QYr.cim-3DC* from UC 1110 (Lan et al., 2017) were identified in the same region. Since the 3D QTL reported in this study showed similar small effects relative to *Pst* resistance, allelism tests will be required to determine if they correspond to the same or different linked genes. Because of the reduced recombination in this centromeric region, it would be important to complement these allelism tests with haplotype studies and tests with multiple *Pst* races to determine if they exhibit the same resistance profile.

QYr.ucw-4DL

QYr.ucw-4DL, located in the distal part of the 4DL arm, was the only *Pst* resistance allele contributed by KP. In most years, this QTL explained a relatively small proportion of the observed phenotypic variation in IT and severity (overall average = 9.7%, Table 2) and was significant only in some of the years. A gene conferring APR to leaf rust and stripe rust, designated as *Lr67/Yr46*, was also mapped to the distal region of chromosome arm 4DL (Hiebert et al., 2010). The cloning of *Lr67/Yr46* revealed that resistance was associated with two nonsynonymous SNPs in an H+/monosaccharide transporter that moves hexoses across the plasma membrane (Moore et al., 2015). We sequenced this gene in KP and KC and confirmed that both have the susceptible allele, indicating that *QYr.ucw-4DL* is not *Lr67/Yr46*.

Four additional QTL have been reported on the long arm of chromosome 4D (Fig. 6). *QYr.wgp-4D*, identified in PI 182103, was mapped on the centromeric region of chromosome 4D, and its long arm border (*IWA4044*; Feng et al., 2018) was mapped >150 Mb proximal to *QYr.ucw-4DL*, indicating that they do not correspond to

the same resistance gene. *QYr.caas-4DL*, identified in the cultivar 'Bainong 64' (Ren et al., 2012), was associated with leaf rust and powdery mildew resistance, suggesting that it may be *Lr67/Yr46* (Ren et al., 2015). By contrast, *QYr.caas-4DL.2*, identified in 'Lumai 21', is likely not *Lr67/Yr46*, as its tightly linked marker *csSNP856* was not polymorphic in this population (Forrest et al., 2014; Ren et al., 2015). This QTL is also unlikely to be the same as *QYr.ucw-4DL* because their peaks are located ~190 Mb apart. *QYr.ucw-4DS* (originally reported as *QYr.ucw-4DL*) was identified in an association mapping study, with *IWA5375* as the peak marker (Maccaferri et al., 2015). Using the available IWGSC RefSeq v1.0 sequence, we determined that *IWA5375* and the linked markers *IWA6277* and *IWA5766* are actually in the short arm. The incorrect position of this QTL in Maccaferri et al. (2015) was the result of spurious linkage ($r^2 < 0.4$) between the peak SNP and the *Lr67/Yr46*-associated marker csSNP856, which are 225 Mb apart. *QYr-4D*, identified in Oligoculm, was detected only in one of the 3 yr tested and the map was distally truncated (Suenaga et al., 2003), precluding a precise mapping. This QTL overlaps with *QYr.ucw-4DL* and may represent the same gene. However, Oligoculm is a selection from an Israeli landrace that is not present in the pedigree of KP. Allelism tests will be required to determine if they correspond to the same or different genes.

Although the use of the IWGSC RefSeq v1.0 reference as a common reference to compare different QTL studies represents an advance relative to the comparison among genetic maps with different subsets of genetic markers, this comparison shares some of the same limitations. The comparison is only as good as the quality and resolution of the individual QTL maps. Only precise and robust QTL studies would yield informative comparisons.

Breeding Applications

The four *Pst* resistance QTL identified in this study showed additive effects (Fig. 2) and, when combined, provided effective resistance to stripe rust. The RILs containing alleles for resistance at all four QTL were, on average, the most resistant lines in the population (Fig. 2). The QTL mapped in this study can be combined with other APR and/or all-stage resistance genes to increase the diversity of deployed genes and, likely, extend the durability of the pyramided genes (Lowe et al., 2010; Nelson et al., 2018).

As more *Pst* resistance genes and QTL are mapped in wheat, overlapping QTL become more frequent (Fig. 3–6; Maccaferri et al., 2015). To use overlapping QTL in a breeding program, it is important to establish if these QTL are allelic or the effect of linked genes. In the latter scenario, linked genes can be recombined to place the resistance alleles in phase facilitating their deployment as single linkage blocks. Comparisons among QTL from different studies were complicated in the past because of the limited number of shared markers. However, the recent completion of the first complete wheat genome references (IWGSC RefSeq v1.0) provides a common set of coordinates that can be used as a common reference for sequence-based markers. Here, we established the physical coordinates of our four QTL and projected all previously published *Pst* resistance genes and QTL in the same genome reference. These analyses facilitated the comparisons among QTL identified in different studies and identified the QTL that require further allelism tests or haplotype analyses to determine their common or separate origin. As more *Pst* resistance genes and QTL are cloned, the precise relationship between linked QTL will be unequivocally established. A good example is the recent cloning of *Yr5* on chromosome arm 2BL, which established that *Yr5* and *YrSp* are allelic, and that *Yr7* is a related but different gene (Marchal et al., 2018).

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Acknowledgments

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Author contributions

N. Cobo led the experimental work and wrote the first version of the manuscript. L. Pflüger developed the recombinant inbred mapping population. X. Chen conducted seedling and APR tests for different *Pst* races. All authors reviewed the manuscript. J. Dubcovsky designed and directed the project and wrote the final version of the manuscript.

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