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ORIGINAL PAPER

Identification and characterization of pleiotropic and co-located resistance loci to leaf rust and stripe rust in bread wheat cultivar Sujata

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Abstract

Key message ***Two new co-located resistance loci, QLr.cim-1AS/QYr.cim-1AS and QLr.cim-7BL/YrSuj, in combination with Lr46/Yr29 and Lr67/Yr46, and a new leaf rust resistance quantitative trait loci, conferred high resistance to rusts in adult plant stage.***

Absract The tall Indian bread wheat cultivar Sujata displays high and low infection types to leaf rust and stripe rust, respectively, at the seedling stage in greenhouse tests. It was also highly resistant to both rusts at adult plant stage in field trials in Mexico. The genetic basis of this resistance was investigated in a population of 148 F_5 recombinant inbred lines (RILs) derived from the cross Avocet × Sujata. The parents and RIL population were characterized in field trials for resistance to leaf rust during 2011 at El Batán, and 2012 and 2013 at Ciudad Obregón, Mexico, and for stripe rust during 2011 and 2012 at Toluca, Mexico; they were also characterized three times for stripe rust at seedling stage in the greenhouse. The RILs were genotyped with diversity arrays technology and simple sequence repeat markers. The final genetic map was constructed with 673 polymorphic markers. Inclusive composite interval mapping analysis detected two new significant co-located resistance loci, *QLr.cim-1AS/QYr.cim-1AS* and *QLr.cim-7BL/YrSuj*, on chromosomes 1AS and 7BL, respectively. The chromosomal position of *QLr.cim-7BL* overlapped with the seedling stripe rust resistance gene, temporarily designated as *YrSuj*. Two previously reported pleiotropic adult plant resistance genes, *Lr46/Yr29* and *Lr67/Yr46*, and a new leaf rust resistance quantitative trait loci derived from Avocet were also mapped in the population. The two new co-located resistance loci are expected to contribute to breeding durable rust resistance in wheat. Closely linked molecular markers can be used to transfer all four resistance loci simultaneously to modern wheat varieties.

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Introduction

Wheat leaf (or brown) rust (LR) and stripe (or yellow) rust (YR), caused by *Puccinia triticina* and *P. striiformis* f. sp. *tritici*, respectively, are important diseases causing significant losses to wheat annually. Worldwide LR occurs more often than YR (Khan et al. 2013) and causes yield losses of up to 40 % in susceptible cultivars, primarily by reducing kernel weight and number (Knott and Padidam 1988). Stripe rust is one of the most devastating diseases of wheat in many cool, temperate regions of the world (Wan et al. 2007). It affects up to 40 % of wheat production area in countries such as Mexico, India, Pakistan, Bangladesh,

and China (Dubin and Brennan 2009) and more than 20 epidemics have been reported worldwide (Wellings 2011). Growing resistant wheat cultivars is the most effective way to manage rust diseases. However, resistant cultivars often become susceptible due to the evolution and selection of new, virulent rust races and their rapid and widespread dissemination.

Two types of rust resistance genes are often defined in wheat. Race-specific resistance genes usually confer protection throughout the growth cycle and, therefore, resistance conferred by them is also called all-stage resistance (Chen 2013). These resistance genes cause various degrees of hypersensitive reactions in the host if the pathogen possesses corresponding avirulence genes (Flor 1942). In contrast, race non-specific minor genes that confer adult plant resistance (APR) are usually present together with other similar genes and, therefore, associated with quantitative inheritance (Das et al. 1992; Johnson and Law 1973). Most cultivars with multiple genes for APR are susceptible at the seedling stage but later display resistance to a number of races (Bjarko and Line 1988). To date, 74 LR and 67 YR genes have been catalogued (McIntosh et al. 2013). Of these, *Lr34/Yr18/Pm38/Sr57* (Singh et al. 2012), *Lr46/Yr29/Pm39/Sr58* (Singh et al. 2013), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2014) confer pleiotropic adult plant resistance (PAPR) to leaf rust, stripe rust, powdery mildew, and stem rust. Genes encoding PAPR *Lr34* (Krattinger et al. 2009) and adult plant resistance gene *Yr36* (Fu et al. 2009) differ from genes that code for race-specific resistance and are predicted to mediate different signaling pathways for APR expression.

Quantitative trait loci (QTL) mapping has been used to identify genomic regions in wheat that contribute to quantitative resistance to LR and YR and to quantify the respective contribution of each region (McIntosh et al. 2013; Rosewarne et al. 2013). More than 80 LR and 140 YR QTL have been reported in wheat (Li et al. 2014; Rosewarne et al. 2013). However, many of these QTL are identical or represent closely linked loci that are bred into diverse backgrounds by phenotypic selection of breeding materials under rust pressure. Potentially eight closely linked/co-located QTL on chromosomes 1BS, 2AL, 2BS (2), 2DL, 5BL, 6AL, and 7BL showed resistance to leaf rust, stripe rust, and powdery mildew simultaneously, according to a review of over 50 QTL mapping publications during the past 15 years (Li et al. 2014).

Sujata, a mutant selection for hybrid necrosis gene *Ne1* present in a popular tall Indian wheat variety C306, was released in 1983 for cultivation under rainfed conditions in Madhya Pradesh, the Central Zone of India, where C306 was already popular (Hasabnis et al. 2003). Sujata's popularity among Indian farmers despite its tall plant type derives from its good agronomic traits, rust resistance, and appealing chapatti quality (Hasabnis et al. 2003).

The objectives of this study were to (1) investigate the genetic basis of LR and YR resistance in an Avocet × Sujata F_5 RIL population, (2) identify PAPR and other resistance loci for both rusts at the adult plant stage using molecular markers, and (3) evaluate the single QTL effect on LR and YR at the adult plant stage.

Materials and methods

Plant materials

We developed and used 148 F_4-derived F_5 recombinant inbred lines (RILs) from the cross of susceptible parent Avocet-*YrA* with Sujata. Avocet-*YrA* (hereafter referred to Avocet) is a reselection from Australian cultivar Avocet and lacks the resistance gene *YrA*. Sujata (pedigree: Regent/Czechoslovakia-3//2*C-591/3/C-217/Niphad-4//C-281) possesses APR to predominant Mexican races of *Puccinia triticina* and displays an intermediate YR infection type in seedlings, whereas Avocet is susceptible to these races at all growth stages. The RIL population was developed using the single spike descent approach described by Basnet et al. (2013). After multiplication of the RILs and parents at El Batán, Mexico, the same seed plot was used in all field and greenhouse studies and for genotyping.

Stripe rust evaluations in the greenhouse

Seedling evaluations of Avocet, Sujata, and the RIL population were conducted in the greenhouse using predominant Mexican *P. striiformis* isolate Mex96.11 in 2011, 2013, and 2014. Twenty-seven differential lines with known YR resistance genes (mostly in the Avocet-background) were also included in the seedling experiment. Seedlings were inoculated at the two-leaf stage by spraying urediniospores suspended in light-weight mineral oil Soltrol 170 (Chempoint.com) with an atomizer. Inoculated plants were placed in a dew chamber at 7 °C for 48 h and then transferred to the greenhouse. In the 2013 experiment, a data logger (LogTag analyzer Ver. 1.9) was installed in the greenhouse and programmed to measure air temperature every 15 min. The minimum, maximum, and mean post-inoculation greenhouse temperatures were 6.3, 33.8, and 17.8 °C, respectively. Infection type (IT) data were recorded 2 weeks post-inoculation based on a modified 0–9 scale (McNeal et al. 1971), where 0 = no visible infection; 1 = necrotic/chlorotic flecks without sporulation; 2 = necrotic/chlorotic blotches/stripes without sporulation; 3 = necrotic/chlorotic blotches/stripes with trace sporulation; 4 = necrotic/chlorotic blotches/stripes with light sporulation; 5 = necrotic/chlorotic blotches/stripes with intermediate sporulation; 6 = chlorotic stripes with moderate sporulation; 7 = stripes

without chlorosis/necrosis and with moderate sporulation; 8 = stripes without chlorosis/necrosis and with sufficient sporulation; and 9 = stripes without chlorosis/necrosis and abundant sporulation. The three datasets were used to determine whether the RILs were resistant, susceptible or segregating.

Field experiments

The RILs and parents were evaluated for APR to LR at two Mexican field sites: El Batán in 2011 (LR2011 experiment) and Ciudad Obregón in the 2011–2012 and 2012–2013 growing seasons (LR2012 and LR2013 experiments, respectively). Similarly, two YR experiments were conducted at CIMMYT's research station in Toluca, Mexico, during the 2011 and 2012 growing seasons (hereinafter referred to YR2011 and YR2012 experiments). Field plots consisted of 0.7-m paired rows with approximately 60 plants of each line. A mixture of Morocco and the Avocet near-isoline for *Yr24/26* was used as the LR spreader, whereas a mixture of six susceptible wheat lines derived from an Avocet/Attila cross, Morocco, and Avocet near-isoline for gene *Yr31* was used as the YR spreader in field trials. The spreaders were planted around the experimental area and as hill plots in the middle of a 0.3-m pathway on one side of each experimental plot. The LR epidemic was initiated by spraying the LR spreader with an equal mixture of *P. triticina* races MBJ/SP and MCJ/SP suspended in Soltrol 170. The avirulence/virulence formulas of MBJ/SP and MCJ/SP were described in Herrera-Foessel et al. (2012). Similarly, *P. striiformis* races Mex96.11 and Mex08.13 were sprayed onto YR spreaders within and around the experimental areas. Isolate Mex08.13 belongs to the lineage of the aggressive *P. striiformis* f. sp. *tritici* race first detected in California, USA (Chen 2005) and possesses virulence to genes *Yr*(*2*), *6*, *7*, (*8*), *9*, *31*, & *A*, whereas Mex96.11 is virulent to *Yr2*, *6*, *7*, *9*, *27* & *A*. Disease severity (DS) on parents and RILs was recorded thrice in each experiment according to the modified Cobb's Scale (Peterson et al. 1948) and host response to YR infection was determined according to Roelfs et al. (1992), where R is necrotic/chlorotic stripes without sporulation; MR is necrotic/chlorotic stripes with some sporulation; M (or MRMS) is necrotic/chlorotic stripes with intermediate sporulation; MS is stripes without chlorosis/necrosis but with moderate sporulation; and S is stripes without chlorosis/necrosis and with abundant sporulation. As all the RILs displayed a susceptible infection response to leaf rust at the adult plant stage, only DS was recorded. In case of repeated DS data, the first note was recorded when the susceptible parent Avocet displayed approximately 80 % severity and repeated about a week later when it reached 90–100 %. For multiple disease readings, the area under the disease progress curve (AUDPC) was calculated using the method suggested by Bjarko and Line (1988).

Genetic and statistical analyses

The F_5 RILs and their parents were evaluated using an augmented design in the LR2012, LR2013, and YR2012 experiments, where 30 RIL entries were replicated twice and randomly distributed across the population. The LR2011 and YR2011 field experiments were conducted with a single replication. Based on disease severity and infection response, RILs were classified into three phenotypic categories following Singh and Rajaram (1992): homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS), and lines whose responses were different from those of the two parents (OTHER).

The number of LR and YR APR genes in the RIL population was estimated using Mendelian segregation analysis (Knott and Padidam 1988; Singh and Rajaram 1992), where the observed frequencies for each category (HPTR:HPTS:OTHER) were tested against the expected frequencies for different numbers of additive genes using Chi squared (χ^2) analysis. Moreover, the minimum number of APR genes was also estimated using the quantitative approach described by Wright (1968) as $n = (\mathrm{GR})^2/4.57(\sigma_g^2)$, where GR (genotypic range) = phenotype range $\times$ h^2 (narrow-sense heritability), σ_g^2 = genetic variance of the F_5 RILs in the present population, $h^2 = \sigma_g^2/\sigma_g^2 + \sigma_e^2$. An analysis of variance (ANOVA) and a correlation analysis were carried out using SAS 9.2 (SAS Institute, Cary, NC) with the final disease severity (FDS) in each environment. For the analysis of the YR seedling resistance gene, RILs with infection types 3–4 to 5–6 were considered resistant and those with types 6–7 to 9 were considered susceptible. Segregating RILs consisted of seedlings with both resistant and susceptible reactions.

Molecular markers, linkage map construction, and QTL analysis

The DNA of parents and RILs was extracted from approximately 20 plants per line using the CTAB method (CIMMYT 2005). Both parents and 132 selected RILs were analyzed with 1,450 diversity arrays technology (DArT) markers and 520 simple sequence repeat (SSR) primers for a polymorphism survey. Finally, 673 polymorphic markers were used to construct the linkage maps with Joinmap 4.1 (Van Ooijen 2006), including the post-flowering leaf tip necrosis (LTN) morphological marker. The LTN was scored in the RILs as absent (0) or present (1) in the LR2013 experiment at Ciudad Obregón. Linkage maps were graphically visualized with MapChart (Voorrips 2002). QTL mapping of FDS and AUDPC from each

experiment and mean of final disease severity (LRM and YRM) across experiments for both LR and YR were carried out using inclusive composite interval mapping (ICIM) (https://www.integratedbreeding.net/supplementary-toolbox/genetic-mapping-and-qtl/icimapping). The logarithm of odds (LOD) threshold to declare a QTL significant at $P = 0.05$ for each trait was determined based on 1,000 permutation tests and an LOD of 2.5 was indicated in the QTL figures. Stepwise regression was used to detect the percentages of phenotypic variance explained (PVE) by individual QTL and additive effects at the LOD peaks. Marker orders and chromosomal assignments of linkage groups were based on a wheat consensus genetic map and the physical bin location of DArT markers (Francki et al. 2009; Huang et al. 2012; Somers et al. 2004; Wilkinson et al. 2012). QTL designations were assigned following recommended practices (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro).

Results

Seedling and adult plant response to leaf rust and stripe rust

Based on a 0–9 scale, the seedling infection types of Avocet and Sujata against *P. striiformis* isolate Mex96.11 were 7–8 and 2–3, respectively. The distribution of resistant, susceptible, and segregating RILs in the population conformed to the expected frequency for a single stripe rust resistance gene, as confimed using χ^2 analysis (Table 1). This resistance gene was temporarily designated as *YrSuj*. The seedling infection type displayed by the two parents against *P. triticina* races MBJ/SP was 3+ based on a 0–4 scale.

In the field experiments, the final disease severity (FDS) and reaction to LR were 90 S for Avocet and 5–15 MSS for Sujata at the adult plant stage during the three crop seasons. Mean LR severity on RILs ranged between 33.9 and 48.2 % during the 3 years of evaluation (Table 2). The frequency distribution of RILs for LR severity showed continuous and approximately normal distributions over the three environments (Fig. 1a), indicating the polygenic inheritance of APR to LR. Genetic analyses by Mendelian segregation analysis (Table 1) and Wright's method (Table 2) indicated the presence of 3–4 APR genes that confer resistance to LR in the Avocet × Sujata population.

The FDS and infection response to YR for Avocet and Sujata were 80 S and 1 R, respectively. Mean YR severity on RILs ranged between 12.1 and 14.9 % during 2 years (Table 1). The frequency distributions of RILs for YR severity were continuous with a pronounced skewness towards resistance (Fig. 1b), indicating the possible presence of a large-effect YR locus in the Avocet × Sujata population. Approximately three to four YR genes were estimated based on two methods of genetic analysis (Tables 1, 2).

Pearson correlation coefficients (r) for disease severities of RILs were high among three LR ($r = 0.78$–0.82) and two YR environments ($r = 0.86$) (Table 3). Slightly lower but significant correlations were also observed between LR and YR severities ($r = 0.47$–0.56, $P < 0.0001$) in all environments.

Table 1 Estimated number of resistance genes that confer seedling resistance to stripe rust and adult plant resistance to leaf rust and stripe rust in 148 Avocet × Sujata F_5 recombinant inbred lines (RILs) based on Mendelian segregation analysis

Response category of RILs	No. of RILs (adult plant)[a]					No. of RILs (seedling)[b]
	LR2011	LR2012	LR2013	YR2011	YR2012	Mex96.11
HPTS[c]	7	14	7	7	3	57
HPTR[d]	7	8	15	15	18	68
OTHER[e]	131	118	123	123	124	20
Missing	3	8	3	3	3	3
Total	148	148	148	148	148	148
No. of gene(s)	4	3	3	3	3	1
P value[f]	0.58	0.44	0.23	0.29	0.01	0.56

[a] Disease severity and host response to infection determined for leaf rust at El Batán 2011 (LR2011); Ciudad Obregón during the 2011–2012 (LR2012) and 2012–2013 (LR2013) seasons, and for stripe rust at Toluca during the 2011 (YR2011) and 2012 (YR2012) seasons to determine the adult plant response category of RILs

[b] Seedling tests with *P. striiformis* race Mex96.11 conducted thrice in the greenhouse to determine the seedling response category of RILs

[c] Homozygous parental type susceptible

[d] Homozygous parental type resistant

[e] Lines with responses different from the two parents

[f] *P* value is for the χ^2 test. The expected ratio of RILs grouped under HPTS, HPTR, and OTHER are 0.438:0.438:0.124, 0.084:0.084:0.832, and 0.037:0.037:0.926 for segregation of 1, 3, and 4 independently inherited genes, respectively, in the F_4-derived F_5 generation

Table 2 Summary of final disease severity in the Avocet × Sujata RIL population phenotyped for leaf rust during 2011 at El Batán (LR2011), and at Ciudad Obregón in 2011–2012 (LR2012) and 2012–2013 (LR2013), and for stripe rust in 2011 (YR2011) and 2012 (YR2012) at Toluca; also shown is estimated minimum number of segregating resistance genes using the Wright method

Parent/parameter	LR2011	LR2012	LR2013	YR2011	YR2012
Avocet	90 S	90 S	90 S	80 S	80 S
Sujata	15 MSS	5 MSS	10 MSS	1 R	1 R
Population mean	48.2	33.9	34.5	12.1	14.9
Low range	1	1	1	1	1
High range	90	90	90	80	90
σ_g^2	–	656.5	313.3	–	290.0
σ_e^2	–	35.1	36.5	–	42.9
h^2	–	0.95	0.90	–	0.87
No. of genes	–	2.4	4.4	–	4.5

'–' values not estimated for field experiments LR2011 and YR2011 because replicates were not used. ANOVA is necessary for estimating gene number when using Wright's method

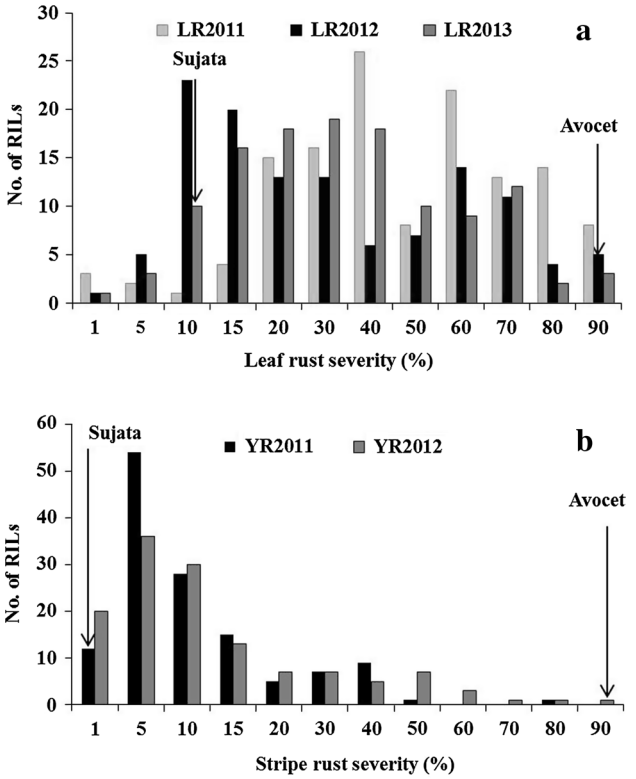

Fig. 1 Frequency distributions of Avocet × Sujata recombinant inbred lines (RILs) for final leaf rust severity (**a**) in field trials at El Batán in 2011 (LR2011), Ciudad Obregón during 2011–2012 (LR2012) and 2012–2013 (LR2013); for final stripe rust severity (**b**) at Toluca during 2011 (YR2011) and 2012 (YR2012). Mean values for the parents, Avocet and Sujata, are indicated by *arrows*

Table 3 Phenotypic correlations among final leaf rust severities in three environments (El Batán LR2011, Ciudad Obregón LR2012 and LR2013) and final stripe rust severities in two environments (Toluca YR2011 and YR2012) in the Avocet × Sujata RIL population

Environment	LR2011	LR2012	LR2013	YR2011
LR2012	0.79**			
LR2013	0.78**	0.82**		
YR2011	0.48**	0.47**	0.49**	
YR2012	0.52**	0.53**	0.56**	0.86**

**$P < 0.0001$

Linkage map construction and mapping the YR seedling resistance gene *YrSuj*

A total of 673 DArT and SSR polymorphic markers were used to construct the genetic linkage map; they spanned 1,403, 1,515, and 852 cM in the A, B, and D genomes, respectively. In all, 22 linkage groups were defined with representatives from each chromosome. Of these linkage groups, chromosomes 3B and 5A had 40 and 38 cM gaps, respectively. Only genetic maps of relevance (i.e., those relating to the location of seedling resistance gene and QTL) are reported here.

The seedling responses of parents and RILs to *P. striiformis* isolate Mex96.11 were relatively consistent across the three different seedling experiments and monogenic inheritance was confirmed for YR seedling resistance. RILs classified into discrete R and S classes based on low (IT 34–56) or high infection types (IT 67–9), respectively, were incorporated in linkage mapping. *YrSuj* was mapped to the long arm of chromosome 7B, flanked by SSR markers *Xcfa2040* and *Xwmc526* (Fig. 2e).

QTL mapping for PAPR and co-located resistance loci

Two PAPR and two co-located resistance loci for LR and YR were detected in the Avocet × Sujata RIL population as determined using ICIM with 1,000 permutations based the LOD threshold. The first QTL, *QLr.cim-1AS/QYr.cim-1AS*, flanked by markers *wPt-9752* and *Xgdm33* on chromosome 1AS, was detected in all YR experiments as well as in LR2011 and LRM. It explained 3.2–8.2 and 10.5–13.8 % of phenotypic variation for LR and YR, respectively (Table 4; Fig. 2a). The second QTL was the known PAPR gene *Lr46/Yr29* based on the closely linked marker *csLV46G22*. It explained 7.4–16.5 and 7.7–13.5 % of variation for LR and YR severities, respectively, and was flanked by markers *wPt-8168* and *Xwmc216* (Table 4; Fig. 2b). The most consistent locus with the largest effect, *Lr67/Yr46*, was flanked by the morphological marker *LTN* and *Xgwm192*. It explained 33.6–57.9 and 8.1–13.9 % of total phenotypic variation for LR and YR severities, respectively (Table 4;

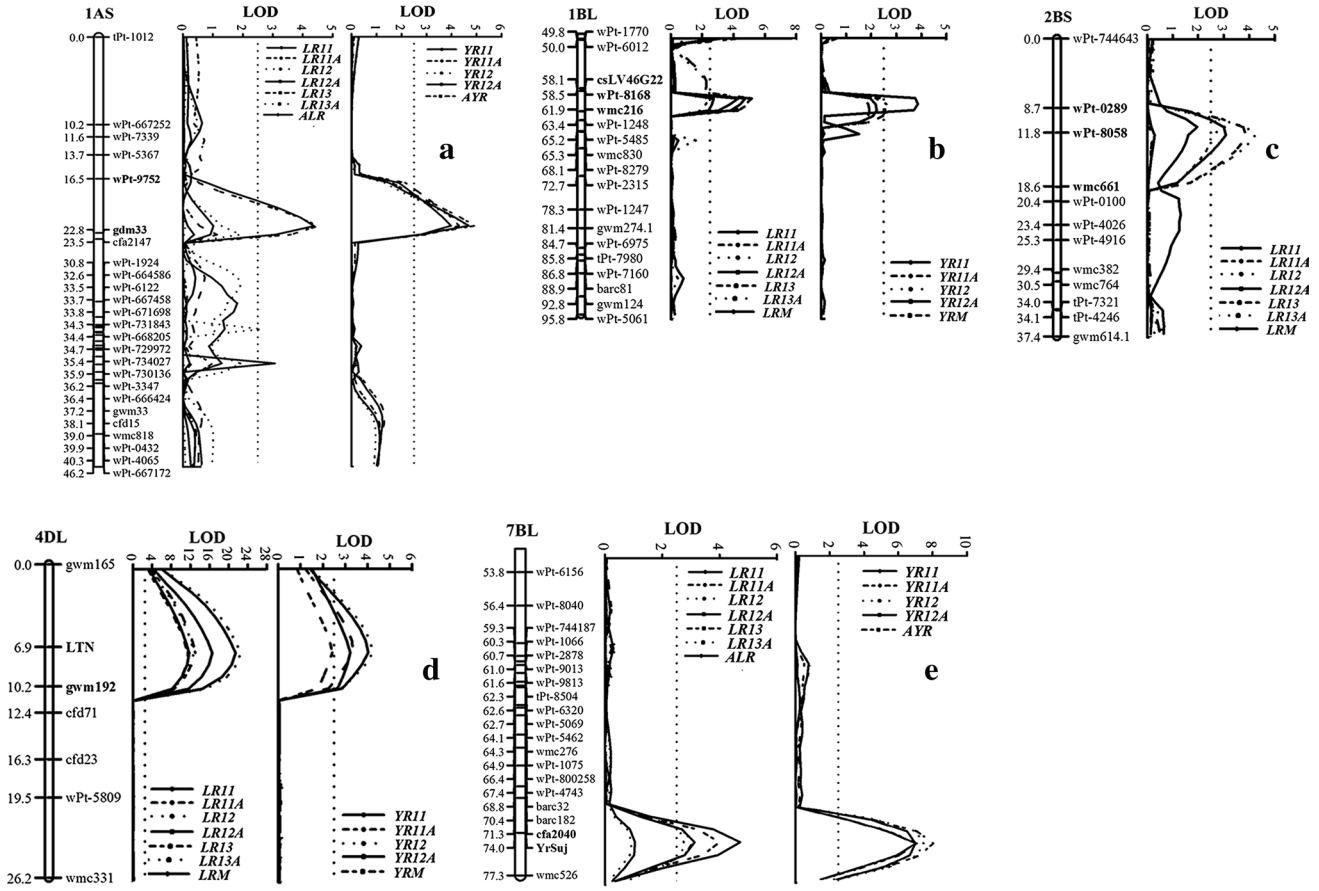

Fig. 2 Likelihood plots of quantitative trait loci (QTL) for adult plant resistance (APR) to leaf rust on chromosomes 1AS (**a**), 1BL (**b**), 2BS (**c**), 4DL (**d**), and 7BL (**e**), and for stripe rust resistance on chromosomes 1AS (**a**), 1BL (**b**), 4DL (**d**), and 7BL (**e**), respectively, identified by IciMapping 3.3 in the Avocet × Sujata RIL population. Significant LOD thresholds were detected based on 1,000 permutations. Positions (in cM) of the molecular markers along chromosomes are shown on the *vertical axes*; cumulated genetic distances of linkage group are also shown. LR11, LR12, and LR13: leaf rust phenotypic data at El Batán in 2011 and Ciudad Obregón during 2011–2012 and 2012–2013, respectively; YR11 and YR12: stripe rust phenotypic data at Toluca in 2011 and 2012, respectively; LR11A, LR12A, LR13A, YR11A, and YR12A: area under the disease progress curve (AUDPC), LRM, and YRM: mean of final disease severity over test environments. QTL flanking markers are shown in *bold*

Fig. 2d). The fourth co-located resistance QTL, *QLr.cim-7BL/YrSuj*, was centrally located over the mapped location of the YR seedling resistance gene *YrSuj*. This locus explained 9.2–15.2 and 21.7–24.5 % of the LR and YR variation, respectively, in adult plants. It was detected across all YR experiments in Toluca, whereas for LR *QLr.cim-7BL* was detected in LR2011 and LR2013 experiments as well as LRM across three experiments (Table 4; Fig. 2e). All these QTL were derived from the resistant parent Sujata.

QTL for APR to leaf rust in Avocet

A minor leaf rust resistance QTL derived from Avocet, *QLr.cim-2BS*, was detected in LR2012, LR2013, and LRM data. It was flanked by molecular markers *wPt-8058* and *Xwmc661* (Table 4; Fig. 2c) and explained 3.8–8.6 % of the total phenotypic variance.

Effects on LR and YR of the two new co-located resistance loci

Based on the closest linked markers, LR and YR severity distributions were observed among the RILs in the presence or absence of two co-located resistance loci, *QLr.cim-1AS/QYr.cim-1AS* and *QLr.cim-7BL/YrSuj*. The mean LR and YR severities of RILs with the *QLr.cim-1AS/QYr.cim-1AS* ranged from 1 to 70 % (with the exception of entry 197 with 90 % LR DS) and 1–30 %, respectively, whereas in the RILs without these loci the ranges were 5–90 % (Fig. 3a) and 1–90 % (Fig. 3b), respectively. A significant mean YR severity reduction of 10.3–13.5 % was observed in RILs with *QYr.cim-1AS*, compared to RILs without it (Table 5) across 2 years, whereas the effect of this genomic region on LR was significant only in LR2011 and LRM data with a 10.2–12.3 % DS reduction, respectively (Table 5). *YrSuj*

Table 4 Position and effects of quantitative trait loci (QTL) for adult plant resistance (APR) to leaf rust (LR) and stripe rust (YR), area under the disease progress curve (AUDPC, viz. LR2011A), and mean final disease severity over all environments (LRM and YRM) using inclusive composite interval mapping (ICIM) by IciMapping 3.3 in the Avocet × Sujata RIL population

QTL[a]	Environment	Position[b]	LeftMarker	RightMarker	LOD[c]	PVE (%)[d]	Add[e]
QLr.cim-1AS	LR2011	22	*wPt-9752*	*Xgdm33*	4.4	7.9	5.1
	LR2011A	22	*wPt-9752*	*Xgdm33*	4.5	8.2	23.5
	LRM	38	*Xgwm33.2*	*Xcfd15*	3.1	3.2	3.9
QYr.cim-1AS	YR2011	22	*wPt-9752*	*Xgdm33*	4.0	10.5	4.5
	YR2011A	22	*wPt-9752*	*Xgdm33*	4.3	11.2	54.5
	YR2012	22	*wPt-9752*	*Xgdm33*	4.7	13.1	6.3
	YR2012A	22	*wPt-9752*	*Xgdm33*	4.7	13.2	80.4
	YRM	22	*wPt-9752*	*Xgdm33*	4.9	13.8	5.3
Lr46	LR2011	59	*wPt-8168*	*Xwmc216*	4.3	12.7	8.4
	LR2011A	59	*wPt-8168*	*Xwmc216*	5.2	14.8	31.1
	LR2012	59	*wPt-8168*	*Xwmc216*	2.5	7.4	5.5
	LR2012A	59	*wPt-8168*	*Xwmc216*	2.7	8.6	39.5
	LR2013	59	*wPt-8168*	*Xwmc216*	5.2	16.5	7.5
	LR2013A	59	*wPt-8168*	*Xwmc216*	4.8	14.9	101.1
	LRM	59	*wPt-8168*	*Xwmc216*	4.7	14.1	8.2
Yr29	YR2011	60	*wPt-8168*	*Xwmc216*	3.9	13.5	1.8
	YR2012	61	*wPt-8168*	*Xwmc216*	2.7	9.0	5.1
	YR2012A	61	*wPt-8168*	*Xwmc216*	2.2	7.7	60.2
	YRM	61	*wPt-8168*	*Xwmc216*	2.6	8.7	4.2
Lr67	LR2011	7	*LTN*	*Xgwm192*	11.7	34.1	10.3
	LR2011A	7	*LTN*	*Xgwm192*	11.5	33.6	46.8
	LR2012	7	*LTN*	*Xgwm192*	22.5	57.9	19.2
	LR2012A	7	*LTN*	*Xgwm192*	21.4	56.2	100.9
	LR2013	7	*LTN*	*Xgwm192*	13.0	36.7	12.8
	LR2013A	7	*LTN*	*Xgwm192*	12.7	36.1	157.3
	LRM	7	*LTN*	*Xgwm192*	16.6	44.4	14.6
Yr46	YR2011	7	*LTN*	*Xgwm192*	3.2	10.7	1.6
	YR2011A	7	*LTN*	*Xgwm192*	2.4	8.1	45.8
	YR2012	7	*LTN*	*Xgwm192*	4.2	13.9	6.4
	YR2012A	7	*LTN*	*Xgwm192*	1.0	13.2	79.1
	YRM	7	*LTN*	*Xgwm192*	3.4	11.1	4.7
QLr.cim-7BL	LR2011	74	*Xcfa2040*	*Xwmc526*	4.7	15.2	6.9
	LR2011A	74	*Xcfa2040*	*Xwmc526*	4.0	13.1	29.3
	LR2013	74	*Xcfa2040*	*Xwmc526*	3.1	10.2	6.8
	LR2013A	74	*Xcfa2040*	*Xwmc526*	2.7	9.2	79.6
	LRM	74	*Xcfa2040*	*Xwmc526*	3.1	10.4	7.1
YrSuj	YR2011	74	*Xcfa2040*	*Xwmc526*	7.0	21.8	6.5
	YR2011A	74	*Xcfa2040*	*Xwmc526*	7.1	21.9	75.5
	YR2012	74	*Xcfa2040*	*Xwmc526*	7.5	23.0	8.2
	YR2012A	74	*Xcfa2040*	*Xwmc526*	7.0	21.7	101.9
	YRM	74	*Xcfa2040*	*Xwmc526*	8.0	24.5	7.0
QLr.cim-2BS	LR2012	11	*wPt-0289*	*wPt-8058*	3.1	4.9	−4.8
	LR2013	12	*wPt-8058*	*Xwmc661*	3.9	7.4	−5.4
	LR2013A	12	*wPt-8058*	*Xwmc661*	4.2	8.6	−82.6
	LRM	12	*wPt-8058*	*Xwmc661*	3.1	3.8	−4.6

[a] QTL that extend across single one-log support confidence intervals were assigned the same symbol

[b] Peak position in centi-Morgans from the first linked marker of the relevant linkage group

[c] Logarithm of odds (LOD) score based on 1,000 permutations

[d] PVE is the proportion of phenotypic variance explained by the QTL

[e] Additive effect of phenotypic variance for each QTL

on chromosome 7BL conferred low seedling reaction to YR and also showed a significant effect on YR at the adult plant stage. The mean LR and YR severity on RILs ranged from 1 to 80 % (Fig. 3c) and 1–40 % (Fig. 3d), respectively, when *QLr.cim-7BL* and *YrSuj* were present, compared to 5–90 % when these loci were absent. For YR, a mean

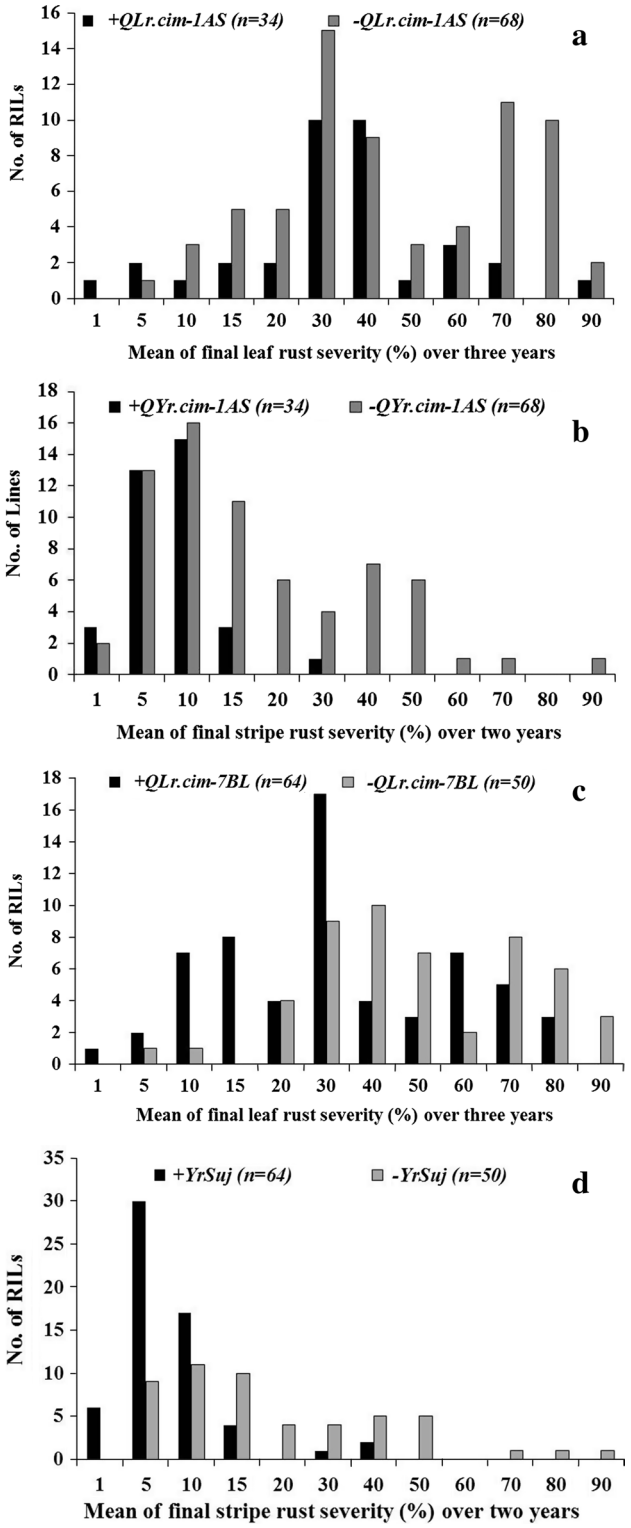

Fig. 3 Comparison of Avocet × Sujata recombinant inbred lines (RILs) for mean of final leaf rust severity and mean of final stripe rust severity in the presence or absence of two co-located QTL in field trials. **a** Effect of *QLr.cim-1AS* on leaf rust—present (+*QLr.cim-1AS*) and absent (−*QLr.cim-1AS*), **b** effect of *QYr.cim-1AS* on stripe rust—present (+*QYr.cim-1AS*) and absent (−*QYr.cim-1AS*), **c** effect of *QL.cim-7BL* on leaf rust—present (+*QL.cim-7BL*) and absent (−*QL.cim-7BL*), **d** effect of *YrSuj* on stripe rust—present (+*YrSuj*) and absent (−*YrSuj*). The number of RILs in each category is shown in *parentheses*

DS reduction of 11.2–14.0 % was observed for RILs with *YrSuj* compared to RILs without this gene across two readings and YRM (Table 5). This genomic region also showed a significant effect on LR, with a mean DS reduction of 13.7–16.8 % (Table 5), except in the LR2012 experiment.

Discussion

Qualitative and quantitative genetic analyses indicated that Sujata's resistance to LR and YR was controlled by about four genes. Consistent with the estimated gene number, two pleiotropic and two co-located resistance QTL were detected with ICIM analysis based on 1,000 permutations. In addition, one small-effect Avocet-derived QTL for LR was detected in the RILs. Among these QTL, two were known PAPR genes *Lr46/Yr29* and *Lr67/Yr46*, whereas the other two loci, *QLr.cim-1AS/QYr.cim-1AS* and *QLr.cim-7BL/YrSuj*, are potentially novel. It has been observed that the qualitative and quantitative approaches for estimating gene numbers are based on several theoretical assumptions of polygenetic quantitative inheritance, where each gene is considered to contribute equally to the phenotype. However, variable effects of different QTL on phenotypes are a common phenomenon in wheat genotypes. Thus, the estimated gene number usually represents the minimum number of polygenic loci segregating in a population, as is also evident in this study. Similar results were also reported in numerous other QTL mapping studies (Basnet et al. 2014; Rosewarne et al. 2013; Yang et al. 2013). Therefore, the estimated numbers of genes in Avocet × Sujata RILs based on the χ^2 and Wright methods were similar to the number of QTL detected, despite a slight discrepancy.

Moderate correlation coefficients ($r = 0.47$–0.56) were observed between the two diseases across experiments. This is likely due to the unequal effect of the pleiotropic and co-located QTL that confer LR and YR resistance and to the different magnitudes of additive effects among them, as evidenced by the near-normal distribution for LR and the skewed distribution of RILs towards resistance for YR severity. The possible presence of another undetected small-effect LR or YR QTL in the RIL population cannot be ruled out. Similar to our results, a moderate correlation between LR and YR ($r = 0.58$) was reported by Suenaga et al. (2003) in a double haploid Fukuho-komugi × Oligoculum population, in which three PAPR loci, *Lr34/Yr18*, *Lr46/Yr29,* and *Yr30/Sr2*, were mapped. A relatively higher correlation ($r = 0.68$–0.85) between LR and YR severities was found in the Avocet × Pavon 76 F_6 RILs (William et al. 2006), where three co-located resistance loci (on 1BL,

Table 5 *t* Tests for the comparison of final leaf rust (LR) and stripe rust (YR) severities in 132 Avocet × Sujata RILs when resistance loci *QLr.cim-1AS/QYr.cim-1AS* and *QLr.cim-7BL/YrSuj* were either absent or present

LR resistance locus	RIL (No.)	LR2011	LR2012	LR2013	LRM	YR resistance locus	YR2011	YR2012	YRM
−*QLr.cim-1AS*	68	53.5 A	37.9 A	38.5 A	42.7 A	−*QYr.cim-1AS*	16.6 A	21.1 A	19.0 A
+*QLr.cim-1AS*	34	41.2 B	28.0 A	31.5 A	32.5 B	+*QYr.cim-1AS*	6.3 B	7.6 B	7.1 B
−*QLr.cim-7BL*	50	57.0 A	39.6 A	42.8 A	46.5 A	−*YrSuj*	18.0 A	21.6 A	20.0 A
+*QLr.cim-7BL*	64	40.2 B	31.3 A	29.1 B	31.8 B	+*YrSuj*	6.8 B	7.6 B	7.3 B

Different letters within each column following the mean indicate significant differences based on the *t* test ($P < 0.01$)

4BL and 6AL) for both rusts were detected. In addition, Basnet et al. (2014) identified three LR and YR co-located resistance loci on chromosomes 1BL, 3BS, and 3D in 182 Avocet × Quaiu#3 RILs, with a statistically significant but relatively lower correlation ($r = 0.21$–0.32) between two rust diseases. Moreover, only one PAPR locus, *Lr46/Yr29*, was detected in 141 F5 Avocet × Francolin#1 RILs with a correlation coefficient of 0.39–0.76 between LR and YR across test environments (Lan et al. 2014).

Co-located resistance QTL on 1AS

Co-located resistance QTL *QLr.cim-1AS/QYr.cim-1AS* was detected near the distal end of chromosome 1AS in the present study. Several YR resistance QTL have been reported on chromosome 1A in lines such as Janz (Bariana et al. 2010), Renan (Dedryver et al. 2009), Pastor (Rosewarne et al. 2012), and Naxos (Ren et al. 2012a). However, all of these QTL were detected on the long arm of chromosome 1A (Rosewarne et al. 2013). No YR resistance genes or QTL have been mapped around the *QYr.cim-1AS* region. Leaf rust seedling resistance gene *Lr10* (Feuillet et al. 2003) was mapped on 1AS and located approximately 16 cM away from *QLr.cim-1A* based on a wheat consensus map (Somers et al. 2004). Gene *Lr10* originated from both hexaploid and tetraploid wheat and was ineffective against Mexican LR race MBJ/SP, which was used in the present study for field inoculation. This suggests that *QLr.cim-1AS* is not the same as *Lr10*; instead, it represents a new genomic region which confers resistance against both LR and YR at the adult plant stages.

Lr46/Yr29 on 1BL

Lr46/Yr29, located on wheat chromosome 1BL is one of the most utilized wheat PAPR loci, it is known to confer broad-spectrum resistance to four bio-trophic diseases: leaf rust (Singh et al. 1998), stripe rust (Singh et al. 1998), stem rust (Singh et al. 2013), and powdery mildew (Lillemo et al. 2008). *Lr46/Yr29* is also associated with premature senescence of the leaf tips, commonly referred to leaf tip necrosis (Rosewarne et al. 2006). *Lr46/Yr29* has been identified in many wheat lines and populations (Basnet et al. 2013; Lillemo et al. 2008; Rosewarne et al. 2012; Suenaga et al. 2003) and widely used in CIMMYT germplasm for partial resistance against LR and YR (Singh et al. 1998). However, the effectiveness of this PAPR locus in conferring partial resistance to rust diseases depends upon the environment and genetic background. It has been reported that *Lr46/Yr29* explained 16–55 and 11–43 % of total phenotypic variation for LR and YR, respectively, in parental populations under different experimental conditions (Basnet et al. 2013; Lan et al. 2014; Rosewarne et al. 2012).

Lr67/Yr46 on 4DL

Lr67/Yr46 was originally transferred from PI250413, a Pakistani wheat accession, to Thatcher-derived line RL6077 by Dyck (Dyck and Samborski 1979). Although this study reports for the first time the presence of *Lr67/Yr46* in an Indian cultivar, CIMMYT's wheat breeding history reveals that this gene has played a vital role in durable resistance to LR and YR for more than 60 years. Recent findings have proven that *Lr67/Yr46* is pleiotropic to stem rust APR gene *Sr55*, partial resistance gene to powdery mildew *Pm46* and *Ltn3*, and a new leaf tip necrosis gene (Herrera-Foessel et al. 2014). *Lr67/Yr46* explained 33.6–57.9 and 8.1–13.9 % of the variation for LR and YR, respectively, in our study. Several YR resistance QTL have also been mapped on 4D, for example, in Bainong 64 (Ren et al. 2012b), Pastor (Rosewarne et al. 2012), W-219 (Singh et al. 2000), and Oligoculum (Suenaga et al. 2003). Two QTL for LR resistance were detected in ND495 (Chu et al. 2009) and Forno (Messmer et al. 2000). The relationship between these QTL and *Lr67/Yr46* can only be confirmed through using a diagnostic gene sequence-based molecular marker currently being developed.

Co-located resistance genes on 7BL

The co-located resistance loci *QLr.cim-7BL* and *YrSuj* were mapped on the long arm of chromosome 7B, exactly at the same location as the YR seedling resistance locus *YrSuj*. The flanking markers *Xcfa2040* and *Xwmc526* were located

approximately 2.7 and 3.3 cM, respectively, from *QLr.cim-7BL/YrSuj* (Table 4; Fig. 2e). Four designated YR resistance genes *Yr39* (Lin and Chen 2007), *Yr52* (Ren et al. 2012c), *Yr59* (Chen, personal communication), and *Yr67* (Bansal, personal communication), as well as three LR resistance genes *Lr14a*, *Lr14b* (McIntosh et al. 1995), and *Lr68* (Herrera-Foessel et al. 2012) are located on chromosome 7BL.

Yr39 originated from US soft white spring wheat Alpowa (pedigree: Fielder/Potam70/2/Walladay/3/Walladay/Potam70) and is closely linked to the resistance gene analog polymorphism markers *Xwgp36* and *Xwgp45* (Lin and Chen 2007). It explained 59–64 % of the total phenotypic variation for YR at the heading-flowering stages in the Avocet × Alpowa population. *Yr39* was ineffective (IT = 8) against three US *P. striiformis* races at the seedling stage but conferred low to moderate resistance (IT 2–5 on a 0–9 scale) to these pathotypes at the adult plant stage (Lin and Chen 2007). On the other hand, *YrSuj* confers low resistance to Mexican YR isolates Mex96.11 at both seedling and adult plant stages, and is located approximately 65 cM away from *Yr39* based on wheat consensus maps (Somers et al. 2004). *Yr52* was detected in an Indian spring wheat accession PI 183527, which was introduced into the US in 1949. It was flanked by markers *Xbarc182* and *Xwgp5258* at a genetic distance of 36.5 ± 6.75 cM from *Yr39* (Ren et al. 2012c). PI 183527 was susceptible (IT = 8) to three US YR races at the seedling stage, but was resistant at the adult plant stage under a high-temperature profile and conferred infection type 12, i.e., short necrotic stripes without uredinia on flag leaves (Ren et al. 2012c). Based on common markers in different maps (Ren et al. 2012c; Somers et al. 2004), *Yr39* can be placed at about 30 cM from *YrSuj*. Similarly, the recently designated high-temperature APR gene *Yr59* was also mapped on chromosome 7BL in PI 660061, an Iraqi landrace introduced into the US in 1948 (Chen, personal communication). *Yr59*, flanked by markers *Xwgp5175* and *Xbarc32*, is located approximately 5–7 cM from *YrSuj* based on consensus maps (Somers et al. 2004) and the Avocet × Sujata genetic map. The approximate genetic distances between *Yr39* and *Yr52* and between *Yr52* and *Yr59* are 31.2 and 5.4 cM, consecutively (Zhou, personal communication). Based on map positions and different YR resistance responses, we believe that *YrSuj* is different from previously reported genes *Yr39*, *Yr52,* and *Yr59* on 7BL. However, *Yr67*, a recently designated stripe rust seedling resistance gene in the Indian tall variety C591 (and most likely the same as *YrC591* based on the origin of the resistance and the chromosome position; Xu et al. 2014), was closely linked to SSR marker *Xcfa2040*; i.e., within 8 cM (Bansal, personal communication). This gene may be the same as *YrSuj* because both Sujata and C591 displayed a similar infection type at the seedling stage against race Mex96.11 in greenhouse tests (data not presented). We also detected the effect of *YrSuj* on APR to leaf rust, which could be due to either its' pleiotropic effect on LR or due to another tightly linked gene.

Leaf rust APR gene *Lr68* was mapped on 7BL in CIMMYT wheat Parula between markers *Xgwm146* and *Psy1-1* (Herrera-Foessel et al. 2012) and was very tightly linked to race-specific resistance gene *Lr14b*. Sujata was susceptible to *P. triticina* race TCT/QB at the seedling stage, which is avirulent to *Lr14b* (data not shown). Thus, *QLr.cim-7BL* should be different from *Lr68* and *Lr14b*. In addition, LR QTL in Attila (Rosewarne et al. 2008), Pastor (Rosewarne et al. 2012), Forno (Schnurbusch et al. 2004), and Opata (Faris et al. 1999), as well as YR QTL in Attila (Rosewarne et al. 2008), Pastor (Rosewarne et al. 2012), and SHA3/CBRD (Ren et al. 2012a), were also mapped on chromosome 7BL. However, the LR and YR resistance QTL in Pastor were located at the distal end of chromosome 7BL, whereas the genetic distance between LR and YR QTL in Attila was about 10 cM (Rosewarne et al. 2012). Thus, *QLr.cim-7BL* may be different from these loci. Chromosome 7BL harbors various resistance genes and QTL. Therefore, further studies will be required to pinpoint more concise locations and determine the relationships among these genes/QTL.

LR QTL on 2BS from Avocet

A QTL for slow rusting APR to LR in Avocet, *QLr.cim-2BS*, was located in the interval between *wPt-0289* and *Xwmc661* in the distal region of chromosome 2BS. No other LR resistance QTL has been reported in this region, though seedling resistance gene *Lr16* has been located at 5 cM from *QLr.cim-2BS*, based on wheat consensus maps (Somers et al. 2004). Sujata's infection type against Mexican LR race MBJ/SP was 3+, compared with 3C for the *Lr16* differential line. This indicated that *QLr.cim-2BS* could be a new LR APR locus in Avocet. In addition, Rosewarne et al. (2008) found a YR resistance QTL in this region in the susceptible line Avocet; however, we did not detect any effect of *QLr.cim-2BS* on YR in the present study. Another YR resistance QTL in Avocet has been mapped on chromosome 6AL in several studies (Lan et al. 2014; Lillemo et al. 2008; Rosewarne et al. 2012; William et al. 2006), indicating that there are at least one minor LR and two YR resistance QTL in the widely used susceptible line Avocet.

Effects of the new co-located resistance loci

The effects of the presence or absence of each QTL/genes were estimated based on the assumption that the remaining QTL/genes were randomly distributed among RILs (Fig. 3).

QTL *QLr.cim-1AS* and *QLr.cim-7BL* displayed a clear distribution in RILs with a maximum final LR severity of 70 and 80 %, respectively, whereas final maximum LR severity was 90 % when the two APR loci were absent. However, one RIL with *QLr.cim-1AS* also displayed 90 % LR severity. This could be due to the misclassification of this line into the +QTL group as we used the flanking markers in the absence of cosegregating or tightly linked markers. YR FDS distribution of *QYr.cim-1AS* and *YrSuj* also indicated that RILs with these resistance loci were clearly skewed towards resistance with a maximum YR severity of 30 and 40 %, respectively, while some RILs that did not have these loci had maximum YR severities of 90 % (Fig. 3). The distribution, PVEs and *t* test indicated that co-located resistance loci *QLr.cim-1AS/QYr.cim-1AS* and *QLr.cim-7BL/YrSuj* had greater effects on YR than on LR, which in the latter case is due to the relatively greater effect of seedling resistance gene *YrSuj* in adult plants.

Conclusions

Based on conventional genetics and molecular mapping, the high level of LR resistance in Sujata is governed by two pleiotropic and two co-located resistance loci, whereas resistance to YR is due to the combination of seedling resistance gene *YrSuj* and three APR QTL. PAPR genes *Lr46/Yr29* and *Lr67/Yr46* identified in Sujata confer pleiotropic effects on resistance to LR and YR. Avocet contributed *QLr.cim-2BS*, a small-effect LR QTL. The co-located resistance QTL *QLr.cim-7BL* overlapped with the seedling resistance gene *YrSuj* that conferred low levels of resistance to YR in both seedlings and adult plants. The potentially new co-located resistance loci *QLr.cim-1AS/QYr.cim-1AS* in Sujata conferred moderate levels of APR to YR but had a smaller effect on LR. Sujata can be used effectively in wheat breeding programs to transfer multiple pleiotropic and co-located resistance genes from a single source using closely linked molecular markers.

Author contribution statement CX Lan phenotyped and genotyped the population with SSR markers, did QTL analysis, and wrote the manuscript. YL Zhang genotyped the population with SSR markers. SA Herrera-Foessel, BR Basnet, and RP Singh phenotyped the population for leaf and stripe rust in Mexico and reviewed the manuscript. J Huerta-Espino constructed the population for genetic analysis. ES Lagudah provided the molecular markers for genotyping the population and reviewed the manuscript.

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Conflict of interest No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

Ethical standards I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and is not under consideration for publication elsewhere. All the authors listed have approved the manuscript.

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