Identification and Mapping of Adult Plant Resistance Loci to Leaf Rust and Stripe Rust in Common Wheat Cultivar Kundan

Y. Ren, Mianyang Institute of Agricultural Science/Mianyang Branch of National Wheat Improvement Center, Mianyang 621023, Sichuan, China; **R. P. Singh, B. R. Basnet,** and **C. X. Lan,** International Maize and Wheat Improvement Center (CIMMYT), 06600 México D.F., Mexico; **J. Huerta-Espino,** Campo Experimental Valle de México INIFAP, 56230 Chapingo, Edo. de México, Mexico; **E. S. Lagudah,** CSIRO Agriculture, Canberra, ACT 2601, Australia; and **L. J. Ponce-Molina,** National Institute of Agricultural and Livestock Researchs (INIAP-Ecuador), Santa Catalina Experimental Station, Quito, Ecuador

Abstract

Leaf rust (LR) and stripe rust (YR) are important diseases of wheat worldwide. We used 148 recombinant inbred lines (RIL) from the cross of Avocet × Kundan for determining and mapping the genetic basis of adult plant resistance (APR) loci. The population was phenotyped LR and YR for three seasons in field trials conducted in Mexico and genotyped with the diversity arrays technology sequencing (DArT-Seq) and simple sequence repeat markers. The final genetic map was constructed using 2,937 polymorphic markers with an average distance of 1.29 centimorgans between markers. Inclusive composite interval mapping identified two co-located APR quantitative trait loci (QTL) for LR and YR, two LR QTL, and three YR QTL. The co-located resistance QTL on chromosome 1BL corresponded to the pleiotropic APR gene *Lr46/Yr29*. *QLr.cim-2BL*, *QYr.cim-2AL*, and *QYr.cim-5AS* could be identified as new resistance loci in this population. *Lr46/Yr29* contributed 49.5 to 65.1 and 49.2 to 66.1% of LR and YR variations, respectively. The additive interaction between detected QTL showed that LR severities for RIL combining four QTL ranged between 5.3 and 25.8%, whereas the lowest YR severities were for RIL carrying QTL on chromosomes 1BL + 2AL + 6AL. The high-density DArT-Seq markers across chromosomes can be used in fine mapping of the targeted loci and development SNP markers.

Leaf (brown) rust (LR) and stripe (yellow) rust (YR), caused by *Puccinia triticina* and *P. striiformis* f. sp. *tritici*, respectively, are fungal diseases of common wheat (*Triticum aestivum* L.) that significantly reduce wheat production worldwide. Epidemics of wheat rusts can occur on a continental scale due to the widespread dispersal of urediniospores, which rapidly destroy leaf tissue and significantly reduce grain yield and quality (Khan et al. 2013; Roelfs et al. 1992; Wellings 2011). LR causes wheat yield losses ranging from 10 to 50% (Huerta-Espino et al. 2011), whereas losses from YR can range from 18 to 90% (Chen 2014), depending on the susceptibility of the cultivar, timing of the initial infection, rate of disease development, and duration of disease. Although wheat rusts can be managed through fungicide use, its effectiveness is limited by financial constraints, chemical availability, and inclement weather conditions affecting timely application. Therefore, growing resistant cultivars is the most economical and environment-friendly approach to managing these diseases.

Race-specific and race-nonspecific resistances are the two main types of resistance to wheat rusts. Race-specific resistance is generally qualitatively inherited and often confers protection throughout the growth stages. Therefore, this type of resistance is also called all-stage resistance (Chen 2013). It is usually short-lived due to the high evolutionary potential of the pathogen (Huerta-Espino et al. 2011; Wellings 2011). In contrast, adequate levels of race-nonspecific resistance are usually quantitatively inherited and involve multiple genes, contributing minor to intermediate effects. Wheat lines carrying these resistance genes are usually susceptible at the seedling stage but express a slow-rusting type of resistance at the postseedling growth stages of wheat plants. This type of resistance is also often called adult plant resistance (APR) (Chen 2005;

Lagudah 2011; Lin and Chen 2007; Roelfs and Bushnell 1985). Numerous resistance genes to LR and YR have been identified and cataloged in wheat (McIntosh et al. 2014). Among these, the three pleiotropic APR genes reported in wheat—*Lr34/Yr18/Pm38/Sr57/Ltn1*, *Lr46/Yr29/Pm39/Sr58/Ltn2*, and *Lr67/Yr46/Pm46/Sr55/Ltn3*—confer resistance to LR, YR, powdery mildew, and stem rust, and are also associated with the morphological trait postflowering leaf tip necrosis (LTN) (Helguera et al. 2003; Herrera-Foessel et al. 2010, 2014; Lillemo et al. 2013; Singh et al. 2011; William et al. 2003). The effect of these pleiotropic APR genes is moderate when present alone but they play an important role in gene combinations and interactions with additive effects, resulting in high levels of durable resistance. In general, accumulating four to five APR genes into a single genotype can reach "near-immunity'' or a high level of resistance (Singh et al. 2000).

Closely linked molecular markers can be used to pyramid corresponding APR genes into elite wheat lines to achieve a high level of resistance (Chen 2013; Collard and Mackill 2008; Yang et al. 2012). In the last 30 years, several polymerase chain reaction-based marker systems such as amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA, resistance gene analog polymorphism, simple sequence repeat (SSR), and sequence-tagged site molecular marker systems have been implemented in wheat rust genetics and breeding research (Li et al. 2014; Rosewarne et al. 2013). However, next-generation sequencing (NGS) technologies provide better opportunities to unravel the genetic bases of quantitative traits by providing large amounts of data points in a cost- and time-effective manner (Elshire et al. 2011; Yang et al. 2012). Diversity arrays technology sequencing (DArT-Seq), a new approach based on traditional DArT-array and NGS techniques, was developed as a simple, rapid, and robust approach for reduced-representation sequencing of multiplexed samples that combines genome-wide molecular marker discovery and genotyping (http://www.diversityarrays.com/dart-application-dartseq). The advantages of DArT-Seq over the DArT-array version are the sequence of the genomic representations and the development of high-density linkage maps. Therefore, this technology accelerates high-resolution mapping and detailed genetic dissection of traits (Raman et al. 2014).

'Kundan' wheat (syn. 'DL153-2') has remained resistant to LR and YR for more than three decades (Dhanda and Sethi 2002). It is a semidwarf wheat cultivar released in 1985 in India under rainfed conditions by the Indian Agricultural Research Institute (Grewal et al. 2007).

Investigating the genetic basis of resistance in Kundan could be of importance to developing new resistant advanced wheat lines or varieties with higher yields and adaptation.

The objectives of our study were to (i) investigate the genetic basis of LR and YR resistance in an 'Avocet' × Kundan recombinant inbred line (RIL) population, (ii) identify co-located APR loci for LR and YR using molecular markers, and (iii) determine the additive effects between detected LR and YR quantitative trait loci (QTL).

Materials and Methods

Plant materials. In total, 148 F_5-derived F_6 RIL were developed from a cross between Kundan and the YrA-lacking selection Avocet-YrA (hereafter referred to as Avocet). Kundan (pedigree: Tanori 71/NP 890) shows susceptibility in seedlings and high levels of APR to current races of Mexican YR and LR fungi, whereas Avocet is susceptible to these races at all growth stages. The RIL population was developed using a single-head descent approach, as described by Basnet et al. (2013). After multiplication of the F_6 RIL and parents at El Batan, Mexico, the same source of seed was used for genotyping and all of the phenotyping experiments.

Field evaluations for characterizing LR and YR resistance. The RIL and parents were evaluated for APR to LR in Yaqui Valley near Ciudad Obregon, Mexico, in the 2012–13 and 2013–14 growing seasons (hereafter referred as LR2013Y and LR2014Y experiments, respectively) and at El Batan in 2014 (LR2014B). Similarly, three YR experiments were conducted at the Toluca, Mexico research station during the 2012, 2013, and 2014 crop seasons (YR2012, YR2013, and YR2014 experiments, respectively). Field plots consisted of 0.7-m paired rows with approximately 60 plants of each line. Avocet near-isolines (NIL) carrying $Yr24$ and $Yr26$ were used as spreaders in LR studies whereas a mixture of 'Morocco', Avocet NIL carrying $Yr31$, and six lines possessing the $Yr27$ gene derived from the cross Avocet × 'Attila' were used as YR spreaders. 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. An LR epidemic was initiated by spraying an equal mixture of *P. triticina* races MBJ/SP and MCJ/SP onto LR spreader, suspended in Soltrol 170. The equal mixture was prepared by suspending the same amount of each race inoculum (around 3 g/race) in 1 liter of Soltrol 170 oil. The avirulence and virulence formulas of MBJ/SP and MCJ/SP were described by Herrera-Foessel et al. (2012). Spreaders for YR studies were sprayed in a similar manner with a mixture of *P. striiformis* races Mex96.11 and Mex08.13. Although Mex96.11 is virulent to $Yr27$ and avirulent to $Yr31$ and Mex08.13 is avirulent to $Yr27$ and virulent to $Yr31$, both parents were susceptible to these isolates at the seedling stage in greenhouse experiments. Four races are predominantly prevalent in Mexico.

Disease severities (DS) on parents and RIL were recorded at least twice according to the modified Cobb's Scale (Peterson et al. 1948) and the host reaction was scored as described by Roelfs et al. (1992). In instances of repeated DS data, the first note was recorded when the susceptible parent Avocet displayed at least 70% severity at milk stage onward for Avocet and middle dough stage for Kundan, and was continued at weekly intervals until it reached 90 to 100%. For multiple disease readings, the area under the disease progress curve (AUDPC) value was calculated following Bjarko and Line (1988).

Genetic and statistical analysis. The RIL and their parents were evaluated with an augmented complete randomized design in all experiments (Federer 1956, 1961), where two parents and 28 RIL were replicated twice and randomly distributed across the experimental field. Based on DS and infection responses, RIL were classified into three different phenotypic categories according to Singh and Rajaram (1992): homozygous parental type resistant (HPTR), homozygous parental type susceptible (HPTS), and lines with different responses from the two parents (OTHER).

The number of LR and YR APR genes in the Avocet × Kundan 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 χ^2 analysis. Moreover, the minimum number of APR genes was also estimated using the quantitative approach described by Wright (1968) as $n = (GR)^2/4.27(\sigma^2 g)$, where GR (genotypic range) = phenotype range × narrow-sense heritability (h^2), $h^2 = \sigma^2 g/(\sigma^2 g + \sigma^2 e)$, $\sigma^2 g$ = genetic variance of the F_6 RIL in the present population, and $\sigma^2 e$ = error variance. Analysis of variance was carried out using SAS 9.2 (SAS Institute, Cary, NC) with a program from Scott and Milliken (1993) based on the final disease severity (FDS) in each environment.

Molecular markers, linkage map construction, and QTL analysis. The DNA of parents and RIL were extracted using the cetyltrimethylammonium bromide method described in the molecular breeding manual by the International Maize and Wheat Improvement Center (CIMMYT 2005). Two parents and the 131 RIL, selected for homozygosity of phenotypic responses, were analyzed with 50,000 DArT-Seq platforms by DArT Company in Australia and 176 polymorphic SSR markers in the International Maize and Wheat Improvement Center (CIMMYT) Biotech laboratory. Finally, 2,937 polymorphic markers without redundant alleles were used to construct the linkage maps with Joinmap 4.1 (Van Ooijen 2006). Linkage maps were graphically visualized with MapChart (Voorrips 2002). The order of markers and the chromosomal assignments of the linkage groups were based on a wheat consensus genetic map and physical bin location of DArT markers (Francki et al. 2009; Huang et al. 2012; Somers et al. 2004; Wilkinson et al. 2012). QTL mapping for FDS and AUDPC (namely, AYR2013Y is the AUDPC in Obregon in the 2013 crop season) from each experiment and mean of FDS for both LR and YR diseases (LRM and YRM, respectively) across experiments was carried out using inclusive composite interval mapping (ICIM) (Meng et al. 2015). The QTL results have also been confirmed by QTL Cartographer software (Wang et al. 2012). The logarithm of odds (LOD) threshold to declare significant QTL at the $P = 0.05$ for each trait was determined based on 1,000 permutation tests. Stepwise regression was used for detecting the percentages of phenotypic variance explained by individual QTL and additive effects at the LOD peaks. QTL designations were assigned following the recommended practices (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm).

Table 1. Adult plant disease responses in Mexico of parents and Avocet × Kundan recombinant inbred lines (RIL) when evaluated to leaf rust (LR) for two seasons in Ciudad Obregon (LR2013Y and LR2014Y) and one season in El Batan (LR2014B), and to stripe rust (YR) during three seasons (YR2012, YR2013, and YR2014) in Toluca, with estimated minimum number of resistance genes using the Wright method

Parent, parameter	LR2013Y	LR2014Y	LR2014B	YR2012	YR2013	YR2014
Avocet[a]	90 S	100 S	90 S	90 MSS	90 S	90 S
Kundan[a]	5 MSS	10 MSS	5 MSS	5 RMR	10 RMR	10 RMR
RIL mean	35.6	50.0	34.9	40.5	51.2	51.4
Range low	1.0	1.0	1.0	1.0	5.0	5.0
Range high	90.0	90.0	80.0	100.0	100.0	100.0
$\sigma^2 g$	407.3	429.9	307.6	566.6	597.6	479.8
$\sigma^2 e$	31.8	75.0	54.2	56.4	33.8	65.5
h^2	0.93	0.85	0.85	0.91	0.95	0.88
Genes (n)	3.7	3.1	3.4	3.4	3.2	3.4

[a] Parental severities are accompanied with corresponding infection responses: RMR = resistant to moderately resistant, MSS = moderately susceptible to susceptible, and S = susceptible.

Results

Phenotypic evaluation in the adult plant stage. The FDS (%) and reaction to LR were 90 to 100% with response of susceptible for Avocet and 5 to 10% with response of moderately susceptible to susceptible for Kundan during the three crop seasons. LRM of RIL ranged from 34.9 to 50.0% in the three experiments (Table 1). The frequency distribution of RIL for LR severities showed continuous and approximately normal distributions over 3 years (Supplementary Fig. S1A), indicating the quantitative inheritance of APR to LR. Approximately three to four LR APR genes were estimated based on the Wright's method (Table 1) and Mendelian segregation analysis (Table 2).

The FDS (%) and infection responses to YR for Avocet and Kundan were 90% with response of susceptible and 5 to 10% with response of resistant to moderately resistant, respectively, during the three crop seasons. YRM of RIL ranged from 40.5 to 51.4% across 3 years (Table 1). Quantitative inheritance of APR to YR was also observed according to the continuous and approximately normal distributions of RIL for YR severities over three environments (Supplementary Fig. S1B). In addition, genetic analysis showed that four YR genes contributed to APR in the Avocet × Kundan population based on both qualitative and quantitative estimates (Tables 1 and 2).

The Pearson correlation coefficients (*r*) of DS between 148 RIL were high among 3 years for LR (*r* = 0.79 to 0.83, *P* < 0.0001) and YR (*r* = 0.87 to 0.90, *P* < 0.0001) (Table 3). These results indicated that the experimental conditions and testing environments were well controlled and consistent with an insignificant experiment–genotype interaction. In addition, significant correlations were also found between LR and YR severities (*r* = 0.63 to 0.77, *P* < 0.0001; Table 3), indicating the presence of potential co-located APR genes to both rusts in Avocet × Kundan population.

Linkage map construction. Approximately 50,000 DArT-Seq markers covering all 21 chromosomes were used in genotyping the selected 131 RIL and parents. In total, 9,474 polymorphic markers (18.9%) were explicitly observed between the parents. Of these polymorphic markers, 6,595 (69.6%) redundant markers were excluded by the BIN tool of IciMapping 4.1. Finally, 2,879 (5.8% of total) DArT-Seq markers and 58 SSR markers were used in developing the whole genomic linkage maps. Forty-one linkage groups were created with 2,589 mapped markers, representing each wheat chromosome (Supplementary Table S1). The genetic linkage map covered 1,500.5, 1,519.0, and 308.8 centimorgans (cM) of genomes, A, B, and D, respectively, with an average marker distance of 1.29 cM/marker. Only the genetic linkage maps related to the location of detected QTL are reported herein.

QTL mapping for co-located resistance loci. Two co-located resistance loci for LR and YR were detected in Avocet × Kundan RIL using ICIM with 1,000 permutations. The most important QTL was located at the same chromosome position as the known pleiotropic APR gene *Lr46/Yr29* according to the tightly linked marker *csLV46G22*. It was detected in the resistant parent Kundan and explained 49.5 to 65.1 and 49.2 to 66.1% of LR and YR severities variations, respectively (Table 4). This locus was located in the interval of markers *1092272* and *102414* within 1.2 cM on chromosome 1BL (Fig. 1A) and had a major effect on both rusts across all field trials with high LOD scores (22.4 to 32.4 for LR and 24.8 to 36.5 for YR). The second co-located resistance QTL derived from susceptible parent Avocet, *QLr.cim-6AL/QYr.cim-6AL*, was closely linked to marker *1021176_60: T>G* on chromosome 6AL (Fig. 1B). It explained 8.5 to 10.9% of LR variations in LR2013Y and LR2014Y and 9.0 to 12.6% of YR variations in YR2013 and YR2014, respectively (Table 4).

QTL mapping for APR to LR. Two APR LR QTL, identified on chromosomes 2BL and 2DS based on ICIM analysis, were designated as *QLr.cim-2BL* (Fig. 1C) and *QLr.cim-2DS* (Fig. 1D), respectively. *QLr.cim-2BL* was located between *1237388* and *1081780_35:C>T*. This QTL was detected in the LR2013Y and LR2014Y and it explained 4.1 to 5.6% of the LR variation (Table 4). It was derived from Avocet. Similarly, *QLr.cim-2DS* was flanked by DArT-Seq markers *1020115* and *1242814* and explained 6.4 to 7.8% of LR variation in the LR2013Y (Table 4). The resistance allele of this QTL was contributed by Kundan.

QTL mapping for APR to YR. Three YR resistance QTL were detected in the Avocet × Kundan population. The first QTL for APR to YR, *QYr.cim-2AL*, was located in the interval of DArT-Seq markers *3064488_30:T>G* and *1106314_11:A>G* on chromosome 2AL (Fig. 1E). It explained 5.1 to 8.9% of the YR variation across all 3 years (Table 4). The second minor APR QTL, *QYr.cim-3DS*, flanked by molecular markers *3021242* and *2243560* on chromosome 3DS (Fig. 1F), explained 3.9 and 4.0% of YR variation in YR2012 and AYR2012, respectively (Table 4). Both YR resistance QTL were contributed by Kundan. In addition, a minor QTL derived from Avocet, *QYr.cim-5AS*, was located in the interval of markers *3025355_10:T>C* and *1067589* on chromosome 5AS (Fig. 1G). It was detected in YR2013, YR2014, and YRM and explained 4.0 to 5.1% of YR variation (Table 4).

Table 3. Phenotypic correlations for final leaf rust (Ciudad Obregon: LR2013Y and LR2014Y and El Batan: LR2014B) and stripe rust (Toluca: YR2012, YR2013, and YR2014) severities in the Avocet × Kundan recombinant inbred line population

Experiment	LR2013Y	LR2014Y	LR2014B	YR2012	YR2013
LR2014Y	0.83**[z]	…	…	…	…
LR2014B	0.82**	0.79**	…	…	…
YR2012	0.70**	0.71**	0.73**	…	…
YR2013	0.68**	0.72**	0.77**	0.90**	…
YR2014	0.63**	0.67**	0.74**	0.87**	0.90**

[z] Two asterisks (**) indicate *P* < 0.0001.

Table 2. Estimated number of resistance genes that conferred adult plant resistance to leaf rust (LR) and stripe rust (YR) in 148 Avocet × Kundan recombinant inbred lines (RIL) based on Mendelian segregation analysis

Response	Number of RIL[v]					
	LR2013Y	LR2014Y	LR2014B	YR2012	YR2013	YR2014
HPTS[w]	4	12	11	6	7	6
HPTR[x]	3	8	9	5	2	4
OTHER[y]	134	121	121	130	132	131
Missing	7	7	7	7	7	7
Total	148	148	148	148	148	148
Genes (*n*)	4	3	3	4	4	4
P value[z]	0.52	0.51	0.65	0.94	0.27	0.82

[v] Based on disease severity and host response for three seasons to LR: Ciudad Obregon in 2012–13 (LR2013Y) and 2013–14 (LR2014Y) and El Batan in 2014 (LR2014B); and to YR: Toluca in 2012 (YR2012), 2013 (YR2013), and 2014 (YR2014).

[w] Homozygous parental type susceptible.

[x] Homozygous parental type resistant.

[y] Lines with responses different from the two parents.

[z] *P* values are for the χ^2 test. The expected ratio of RIL grouped under HPTR, HPTS, and OTHER are 0.084:0.084:0.832 and 0.037:0.037:0.926 for segregation of three and four independently inherited additive genes, respectively, in the F_5-derived F_6 generation.

Additive interaction between detected resistance loci. The Avocet × Kundan RIL were divided into 16 and 20 genotypes based on the presence of four LR and five YR APR QTL (Tables 5 and 6). The flanking markers for each QTL were used in determining the presence of parental alleles in the RIL. Three lines in the population possessed all four LR QTL and their mean DS (LRM) was 14.6% (Table 5). Lines with three QTL combinations showed LRM of 16.0 to 47.8%. Generally, more QTL imparted higher LR resistance in the RIL, although most of the differences in mean severities were not significant for each minor QTL individual (Table 5). Similarly, a mean of YR severity was 24.5% for the three RILs carrying all five YR resistance QTL, whereas four RILs carrying resistance QTL combination 1BL + 2AL + 6AL showed the lowest YR severities in each environment (Table 6). The pleiotropic APR locus *Lr46/Yr29* played the most important role in conferring resistance to both rusts in this population. Mean LR and YR severity reductions of 28.8 and 33.8%, respectively, were obtained for RILs with *Lr46/Yr29*, compared with RILs without it across 3 years (Table 5, 6).

Discussion

Kundan is an Indian spring wheat cultivar released in 1985, and has remained resistant to LR and YR at the adult plant stage in Mexico and India. The qualitative and quantitative genetic approaches estimated that there were at least three and four APR genes for LR and YR, respectively, that segregated in the RIL population, which is consistent with the QTL mapping results that identified two co-located resistance QTL, two LR QTL, and three YR QTL in the Avocet × Kundan population. However, previous studies have reported that qualitative and quantitative methods normally underestimate the number of resistance genes in comparison with the number of QTL detected in mapping population. This may be because of assumptions such as additive effects of polygenes and their equal contributions to phenotypic variation for disease resistance (Basnet et al. 2013; Lan et al. 2014; Wright 1968).

Co-located resistance QTL to LR and YR. Two co-located resistance QTL, *Lr46/Yr29* and *QLr.cim-6AL/QYr.cim-6AL*, were detected in the Avocet × Kundan RIL population, which was expected based on high correlation between LR and YR severities ($r = 0.63$ to 0.77). The 1BL co-located resistance QTL was identified as the known pleotropic APR gene *Lr46/Yr29* based on the tightly linked marker *csLV46G22*. *Lr46/Yr29*, which was first identified in 'Pavon 76' (Singh et al. 1998; William et al. 2003), and provides partial APR to the three rust diseases and powdery mildew (Herrera-Foessel et al. 2014; Lillemo et al. 2008, 2013) in wheat. 1BL is also associated with the LTN gene, *Ltn2* (Rosewarne et al. 2006; William et al. 2003). Kundan derived from the

Table 4. Position and effect of quantitative trait loci (QTL) that were detected for adult plant resistance to leaf rust (LR) and stripe rust (YR), area under the disease progress curve (namely, ALR2013Y), and mean of final disease severities over all environments (LRM and YRM) using inclusive composite interval mapping by IciMapping 4.1 using 131 selected recombinant inbred lines from the Avocet × Kundan population

QTL[u]	Environment	Pos[v]	Marker interval	LOD[w]	PVE (%)[x]	Add[y]
Lr46[z]	LR2013Y	22	csLV46G22-1102414	26.9	49.5	14.5
	ALR2013Y	21	1092272-1164928	25.2	52.5	188.7
	LR2014Y	21	1092272-1164928	24.4	57.2	18.8
	ALR2014Y	21	1092272-1164928	28	61.9	72.8
	LR2014B	21	1092272-1164928	23.6	64.5	15.1
	ALR2014B	21	1092272-1164928	22.4	62.2	115.3
	LRM	21	1092272-1164928	32.4	65.1	15.8
Yr29	YR2012	21	1092272-1164928	31.8	66.1	19.3
	AYR2012	21	1092272-1164928	31.2	65.6	239.7
	YR2013	21	1092272-1164928	30.5	57	18
	AYR2013	21	1092272-1164928	28.2	55	96.9
	YR2014	21	1092272-1164928	24.8	49.2	16.1
	AYR2014	21	1092272-1164928	27.5	53.9	114.1
	YRM	21	1092272-1164928	36.5	63.1	18
QLr.cim-6AL	LR2013Y	22	1021176_60:T>G-2259361	2.4	8.5	−3.7
	LR2014Y	28	1076263_18:C>T-1101578	3.2	10.9	−6.6
	YR2013	22	1021176_60:T>G-2259361	3.5	12.4	−8.1
	AYR2013	22	1021176_60:T>G-2259361	3.5	12.6	−52
QYr.cim-6AL	YR2014	22	1021176_60:T>G-2259361	2.9	10.2	−7.9
	AYR2014	28	1076263_18:C>T-1101578	2.5	9	−49
	YRM	22	1021176_60:T>G-2259361	2.6	9.3	−7.7
	LR2013Y	459	1218181-1081780_35:C>T	3.5	4.1	−4.4
QLr.cim-2BL	LR2014Y	455	1237388-1265455	3.6	5.6	−5.9
	ALR2014Y	455	1237388-1265455	3.7	4.9	−20.6
QLr.cim-2DS	LR2013Y	1	1020115-1242814	6.6	7.8	5.7
	ALR2013Y	1	1020115-1242814	4.5	6.4	65.7
QYr.cim-2AL	YR2012	87	1089750_12:A>G-1106314_11:A>G	7.2	8.9	7.1
	AYR2012	87	1089750_12:A>G-1106314_11:A>G	6.4	8.1	83.9
	AYR2013	84	998788_19:T>C-3959527	6.6	8.4	37.8
	YR2014	86	3064488_30:T>G-1089750_12:A>G	4	5.1	5.2
	AYR2014	86	3064488_30:T>G-1089750_12:A>G	4.8	6	37.8
	YRM	87	1089750_12:A>G-1106314_11:A>G	6	5.5	5.3
QYr.cim-3DS	YR2012	55	3021242-2243560	3.4	4	4.8
	AYR2012	55	3021242-2243560	3.3	3.9	59.5
QYr.cim-5AS	YR2013	168	3025355_10:T>C-2262017_48:A>G	3.8	4.1	−4.9
	YR2014	174	3938058-1067589	4	5.1	−5.2
	YRM	168	3025355_10:T>C-2262017_48:A>G	4.4	4	−4.7

[u] QTL that extend across single one-log support confidence intervals were assigned the same symbol.
[v] Peak position in centimorgans from the first linked marker of the relevant linkage group.
[w] Logarithm of odds score based on 1,000 permutations.
[x] Percentage of phenotypic variance explained by the QTL.
[y] Additive effect of each QTL on disease severities.
[z] Y and B indicate Ciudad Obregon and El Batan, respectively.

cross of Tanori 71/NP 890, whereas 'Tanori 71' has the pedigree Sonora 64//Ciano-67/Inia 66 and 'NP 890' was C281//C281/Gaza. All of these lines carried the positive allele of *Lr46/Yr29* based on the closely linked marker *csLV46G22*, indicating that all parents possibly possessed the resistance gene or, more likely, the lack of diagnostic ability of the marker, because 'Sonora 64' and 'Inia 66' are known to be susceptible to LR. *Lr46/Yr29* is known to be present in many wheat lines developed at CIMMYT, because it continues to play a key role in breeding for durable rust resistance against LR and YR (Basnet et al. 2013; Lan et al. 2014; Lillemo et al. 2008; Ren et al. 2012b; Rosewarne et al. 2006; Singh et al. 2011; Yang et al. 2013). Previous studies reported that *Lr46/Yr29* explained 16 to 55% and 11 to 43% of total phenotypic variation for LR and YR, respectively, and the additive effect of *Lr46/Yr29* was much less in the presence of *Lr34/Yr18* or *Yr54* (Basnet et al. 2014; Lan et al. 2014; Lillemo et al. 2008; Rosewarne et al. 2012). In our study, *Lr46/Yr29* explained 49.5 to 65.1% and 49.2 to 66.1% of LR and YR severity variations, respectively. This was in accordance with the previously observed results that this gene reduced LR and YR severities up to 40 to 50% when it was present alone (Lan et al. 2014; Rosewarne et al. 2012; Singh et al. 1998), indicating that a single APR gene does not provide acceptable levels of resistance. Therefore, it is necessary to combine this gene with other APR genes to achieve adequate levels of resistance in the field as in Kundan.

The second co-located resistance QTL, *QLr.cim-6AL/QYr.cim-6AL*, was found to be inherited from the susceptible parent Avocet. This QTL was located on chromosome arm 6AL and flanked by DArT-seq markers *1021176_60* and *2259361*. These markers have been mapped in the vicinity of DArT-array markers *wPt-4229*, *wPt-6829*, and *wPt-8954* and SSR markers *Xgwm999* and *Xgwm1089* at the distal end of chromosome 6AL (Marone et al. 2012). Two LR APR QTL, *QLr.cim-6AL* (William et al. 2006) and *QLr.Hbau-6AL* (Zhang et al. 2009), have been reported on chromosome 6AL from Avocet. Both of them were located in the middle of chromosome 6AL and linked to SSR marker *Xgwm617* within genetic distances of 11.5 and 16.0 cM, respectively. William et al. (2006) also mapped a YR resistance QTL *QYr6A.2* in the same region in Avocet. It was flanked by SSR markers *Xgwm617* and *Xgwm427*. In addition, Lillemo et al. (2008) detected a YR resistance QTL close to the centromere on the long arm of chromosome 6A, which was linked to *Xbarc3* with a genetic distance of 3.3 cM and explained 14% of the YR variance in Avocet. These QTL were located in the same genomic region as *QLr.cim-6AL/QYr.cim-6AL* based on an integrated linkage map of diploid wheat (Singh et al. 2007). Although this locus was not detected in all tested environments, it is likely to be a useful locus in combination with other genes.

APR QTL for LR. A QTL for APR to LR in Avocet, *QLr.cim-2BL*, was mapped to the proximal region of chromosome arm 2BL and flanked by DArT-Seq markers *1237388* and *1265455*. These markers have been mapped near DArT-array marker *wPt-5736* and SSR marker *Xbarc101* (Marone et al. 2012). Two LR resistance QTL, *QLr.sfrs-2BL* and *QLr.osu-2B*, were located on chromosome 2BL (Messmer et al. 2000; Xu et al. 2005). *QLr.sfrs-2BL* was derived from Swiss winter spelt (*T. spelta* L.) 'Oberkulmer', whereas *QLr.osu-2B* derived from common wheat 'CI 13227' was located on the centromere region of chromosome
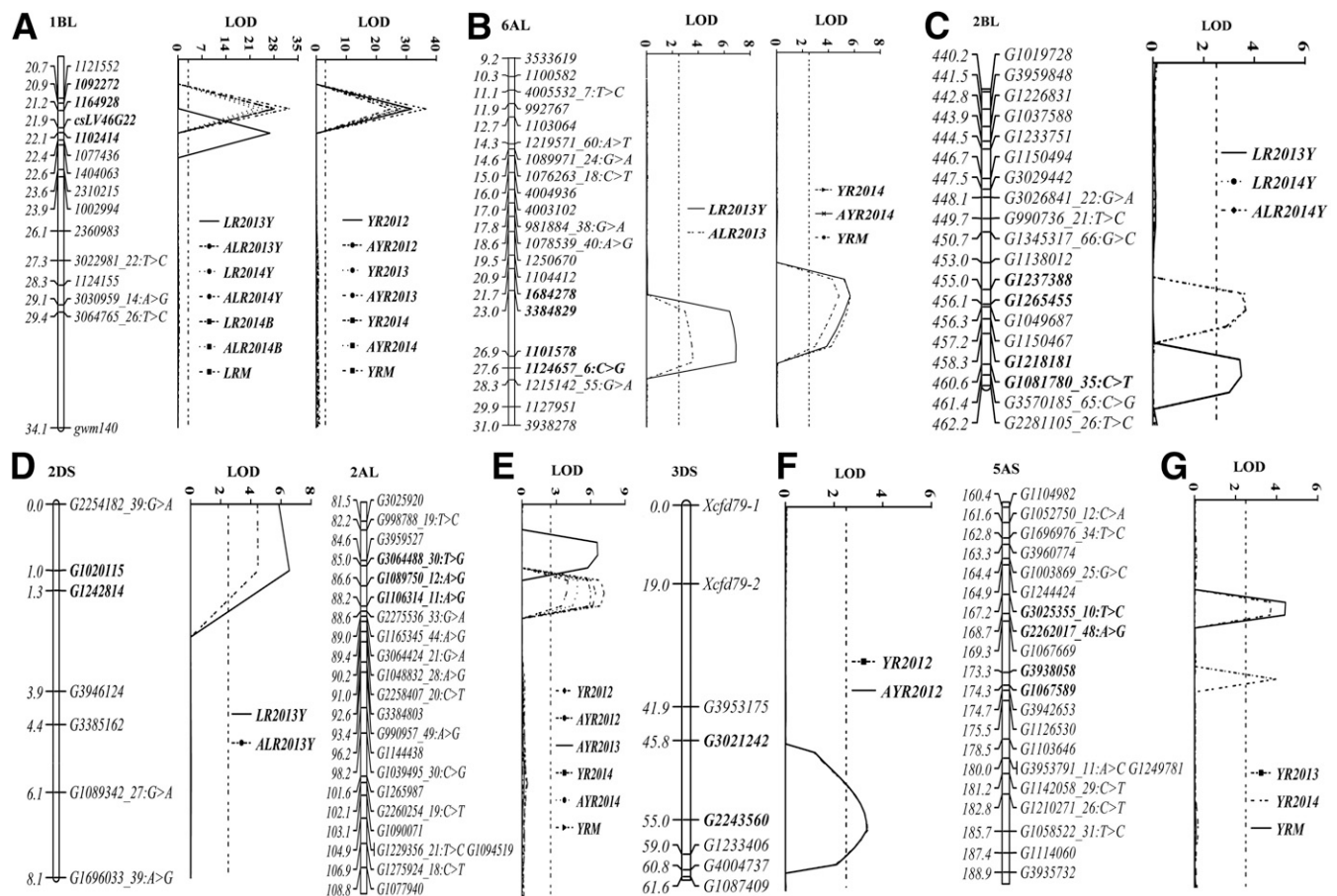

Fig. 1. Likelihood plots of quantitative trait loci for adult plant resistance to leaf rust (LR) on chromosomes **A,** 1BL; **B,** 6AL; **C,** 2BL; and **D,** 2DS; and to stripe rust (YR) on chromosomes **A,** 1BL; **B,** 6AL; **E,** 3DS; **F,** 2AL; and **G,** 5AS identified by IciMapping 4.1 in the Avocet × Kundan recombinant inbred line population. The significant logarithm of odds threshold was detected based on 1,000 permutations. Positions (in centimorgans) of cumulated genetic distances of linkage group along chromosomes are shown on the left axes and for molecular markers on the right. LR2013Y, LR2014Y, and LR2014B = final LR severity at Ciudad Obregon during 2012–13 and 2013–14 and El Batan in 2014, respectively; YR2012, YR2013, and YR2014 = final YR severity in Toluca in 2012, 2013, and 2014, respectively; ALR2013Y, ALR2014Y, ALR2014B, AYR2012, AYR2013, and AYR2014 = area under the disease progress curve for LR in Obregon 2012–13 and 2013–14 and El Batan 2014, and for YR in 2012, 2013, and 2014 in Toluca, respectively; LRM and YRM = the mean of final disease severity over LR2013Y, LR2014Y, and LR2014B and over YR2012, YR2013, and YR2014 for LR and YR, respectively.

2B and linked to SSR markers *Xbarc18*, *Xbarc167*, and *Xwmc314*, with a genetic distance around 20 cM away from *QLr.cim-2BL* based on the wheat consensus map (Somers et al. 2004). Therefore, it is likely that *QLr.cim-2BL* is a new LR resistance locus in common wheat.

The second APR QTL for LR derived from Kundan, *QLr.cim-2DS*, was located in the interval of markers *1020115* and *1242814* on the distal region of chromosome 2DS-5, based on the DArT physical map (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/dart_chromosome.php?chromosome=2D). These markers are closely linked to DArT-array marker *wPt-2014* and SSR markers *Xcfd51*, *Xcfd56*, *Xcfd36*, and *Xbarc124* in this genomic region (Francki et al. 2009). Zhang et al. (2009) and Xu et al. (2005) detected two minor LR QTL, *QLr.hbau-2DS* and *QLrid.osu-2DS*, respectively, in chromosome 2DS. *QLr.hbau-2DS* was linked to *Xbarc124* and *Xgwm296* and explained 12.2 to 12.5% of the LR severity in CIMMYT 'Saar' spring wheat. *QLrid.osu-2DS* derived from common wheat CI 13227 was located in the interval of SSR marker *Xgwm261* and AFLP marker *XGCTG.CGCT118* within 12 cM of *QLr.cim-2DS* based on the wheat consensus map (Somers et al. 2004; Xu et al. 2005). In addition, two minor LR QTL, *QLr.cim-2DS* and *QLr.sfr-2DS*, were also mapped in this region in chromosome 2DS in common wheat Avocet and 'Forno', respectively (Rosewarne et al. 2012; Schnurbusch et al.

2004). Therefore, *QLr.cim-2DS* might be the same LR resistance locus as QTL in Saar, CI 13227, and Forno but different from the QTL in Avocet because we would not have detected this locus in the present population if they are the same.

APR QTL for YR. The first QTL for APR to YR in Kundan, *QYr.cim-2AL*, was located in the interval of DArT-seq markers *3064488_30:T>G* and *1106314_11:A>G* near the centromere of chromosome 2AL. These markers were also mapped in the vicinity of DArT-array marker *wPt-7306* and SSR markers *Xbarc220* and *Xgwm71* (Zhang et al. 2008). Two minor YR resistance QTL, *QYr.inra-2AL* (Dedryver et al. 2009) and QYR2 (Boukhatem et al. 2002), were mapped on chromosome 2AL in common wheat 'Récital' and 'Camp Remy', respectively, and closely linked to the same SSR marker *Xgwm382* in the distal region of 2AL (Boukhatem et al. 2002; Dedryver et al. 2009). However, *QYr.cim-2AL* was near the centromere of chromosome 2AL within 86 cM of *QYr.inra-2AL* and QYR2 based on the wheat consensus map (Francki et al. 2009; Somers et al. 2004), indicating the novelty of this consistent YR resistance locus in Kundan.

The second minor QTL, *QYr.cim-3DS*, was located on the short arm of chromosome 3D and was effective only in 2012 at Toluca. *QYr.inra-3DS* was located in the interval of SSR markers *Xbarc125* and

Table 5. Mean leaf rust (LR) severities of Avocet × Kundan recombinant inbred lines (RIL) carrying different quantitative trait loci (QTL) combinations[y]

QTL combination	RIL (*n*)	LR2013Y	LR2014Y	LR2014B	LRM[z]
None	15	60.3 a	73.0 a	55.0 a	63.0 a
6AL	3	60.0 a	66.7 a	50.0 a	58.9 a
2BL	8	59.4 a	60.0 b	50.0 a	56.5 a
2DS	5	56.0 a	68.0 a	50.0 a	58.0 a
2BL + 2DS	11	46.4 b	69.1 a	48.2 a	54.6 b
2BL + 6AL	2	40.0 b	60.0 b	40.0 b	46.7 b
2BL + 2DS + 6AL	3	36.7 b	66.7 a	40.0 b	47.8 b
2DS + 6AL	2	35.0 b	75.0 a	35.0 b	48.4 b
1BL + 6AL	6	33.3 c	49.2 c	28.3 c	36.9 b
1BL	6	30.0 c	41.3 c	31.3 c	34.2 c
1BL + 2BL	9	24.4 c	36.1 c	30.0 c	30.2 c
1BL + 2DS	16	23.8 c	35.9 c	22.6 c	27.4 c
1BL + 2DS + 6AL	6	15.8 d	30.8 d	15.8 d	20.8 d
1BL + 2BL + 2DS	10	12.5 d	26.6 d	15.1 d	18.1 e
1BL + 2BL + 6AL	4	13.8 d	23.1 d	11.3 e	16.0 e
1BL + 2BL + 2DS + 6AL	3	12.7 d	25.8 d	5.3 e	14.6 e

[y] Different letters within a column following the mean indicate significant differences based on a *t* test (*P* < 0.05).
[z] Mean of final disease severities over all environments.

Table 6. Mean stripe rust (YR) severities of Avocet × Kundan recombinant inbred lines (RIL) carrying different quantitative trait loci (QTL) combinations[y]

QTL combination	RIL (*n*)	YR2012	YR2013	YR2014	YRM[z]
None	9	78.3 a	86.1 a	85.0 a	83.2 a
3DS + 6AL	2	72.5 a	75.0 a	85.0 a	77.5 a
5AS	2	62.5 b	75.0 a	67.5 a	68.4 b
2AL + 5AS	6	62.5 b	71.7 b	74.0 a	68.6 b
2AL + 3DS	3	60.0 b	68.3 b	62.5 b	63.9 b
2AL + 6AL	4	52.5 b	62.5 b	62.5 b	59.2 b
2AL	2	50.0 b	55.0 b	65.0 a	56.7 b
2AL + 3DS + 5AS	9	43.3 c	55.0 b	58.1 b	52.2 c
1BL	9	37.8 c	52.8 c	57.8 b	49.4 c
1BL + 6AL	6	29.2 d	35.8 c	46.7 c	37.2 d
1BL + 2AL	3	26.7 d	53.3 c	56.7 b	45.6 c
1BL + 5AS	2	25.0 d	40.0 c	45.0 c	36.7 d
1BL + 3DS	4	20.0 d	37.5 c	45.0 c	34.2 d
1BL + 2AL + 3DS	6	18.0 d	27.9 d	38.8 c	28.2 d
1BL + 2AL + 5AS	4	17.5 d	22.5 d	24.5 d	21.5 e
1BL + 2AL + 3DS + 5AS + 6AL	3	16.7 d	23.3 d	33.3 c	24.5 d
1BL + 3DS + 5AS	2	12.5 d	25.0 d	35.0 c	24.2 d
1BL + 3DS + 5AS + 6AL	2	12.5 d	30.0 d	21.5 d	21.3 e
1BL + 2AL + 6AL	4	9.0 e	20.0 d	18.8 d	15.9 e

[y] Different letters within a column following the mean indicate significant differences based on a *t* test (*P* < 0.05).
[z] Mean of final disease severities over all environments.

Xgwm456a on chromosome 3DS and explained 4.7 to 8.3% of the phenotypic variation in common wheat Récital (Dedryver et al. 2009). *QYr.cim-3DL* was flanked by SSR markers *Xgdm8* and *Xgdm128* and explained 3.6 to 13.5% of the YR variation in 'Chapio' (Yang et al. 2013). *QYr.Tam-3D* was located in the interval of markers *wPt-672034* and *Xbarc125* and explained 4 to 5% of the YR variation in CIMMYT line 'Quaiu 3' (Basnet et al. 2014). The genetic distance between *QYr.cim-3DL*, *QYr.inra-3DS*, and *QYr.Tam-3D* was estimated to be about 15 to 19 cM based on the consensus map (Francki et al. 2009; Somers et al. 2004). However, we cannot rule out the relationship between *QYr.cim-3DS* and QTL in Récital, Chapio, and Quaiu 3 due to the unavailable genetic map of flanked DArT-seq markers *3021242* and *2243560*. Despite the observed minor effect of these loci on YR and their inability to be detected in all environments, they possibly play an important role in CIMMYT wheat germplasms for breeding resistant lines.

QTL *QYr.cim-5AS*, derived from Avocet had significant effects on YR in YR2013, YR2014, and YRM. It was located in the interval of DArT-seq markers *3025355, 2262017, 3938058*, and *1067589* and mapped near the DArT-array markers *wPt-4131, wPt-0605*, and *wPt-742925* and SSR markers *Xcfa2104, Xbarc186*, and *Xwmc418* based on a DArT and SSR consensus map from a multiparent advanced generation intercross wheat population (Huang et al. 2012). Five YR resistance QTL were mapped in *T. boeoticum* and wheat lines 'PAU5088' (Chhuneja et al. 2008), 'Opata 85' (Boukhatem et al. 2002), 'Pingyuan 50' (Lan et al. 2010), 'Pastor' (Rosewarne et al. 2012), and 'Shanghai 3' × 'Catbird' (Ren et al. 2012a) in chromosome 5AL. *QYrtb.pau-5A* from *T. boeoticum* mapped on 5AL in the 8.9-cM interval between *Xbarc151* and *Xcfd12*. The Opata 85 QTL was observed in seedling tests. The Pingyuan 50 QTL was flanked by *Xwmc410* and *Xbarc261* and located in the same region as QTL in Pastor and Shanghai 3 × Catbird. Because no other YR resistance QTL has been reported on chromosome 5AS thus far, it is possible that *QYr.cim-5AS* is a new APR QTL to YR.

Additive effect between detected QTL. The mean LR severities of RIL combining *Lr46/Yr29* with two or three LR resistance QTL were significantly lower than the lines with *Lr46/Yr29* alone. Similar additive interaction between QTL was also detected in the resistance to YR. Lan et al. (2014) also analyzed the additive interaction between *Lr46/Yr29* and other detected QTL for LR and YR resistance in the Avocet × 'Francolin 1' population and concluded that there were no significant additive interactions between minor QTL in the background of strong-effect gene *Lr46/Yr29* for LR. However, significant YR reductions were observed with RIL carrying *Lr46/Yr29* and other two or more minor QTL, together with the exceptions of *QYr.cim-3DS* and *QLr.cim-6AL/QYr.cim-6AL*. Of the 70 and 80% of the phenotypic variations explained by detected LR and YR QTL, respectively, in the present population, *Lr46/Yr29* alone contributed a large proportion of the variation (65.1 and 66.1%, respectively). As far as the resistance loci derived from the susceptible parent Avocet are concerned, two RILs carrying both resistance loci (2BL and 6AL) showed lower LR severity (40 to 60%) than the susceptible parent Avocet (90 to 100%). This difference could be due to the other undetected minor LR resistance QTL in the population.

Although the durability of effective race-specific resistance genes can be prolonged by using gene combinations, an alternative approach is to develop varieties that possess APR based on combinations of pleiotropic and other minor, slow-rusting genes. Kundan, with good agronomic characteristics and a high level of APR to LR and YR, can be used in wheat breeding programs as a source of slow-rusting APR genes. Resistance loci *Lr46/Yr29, QLr.cim-2DS, QYr.cim-2AL*, and *QYr.cim-3DS* were derived from Kundan. The genetic interval between flanking markers of these QTL ranged from 0.3 to 4.2 cM. With the availability of defined molecular markers for each resistance locus, pyramiding the APR gene from the single resistance source Kundan should be easier and more efficient in the future. However, all of the closely linked markers are DArT-seq and need to be converted into breeder-friendly kompetitive allele specific PCR (KASP) markers and validated in a broader set of germplasm before being used in marker-assisted selection for improving resistance to LR and YR in wheat breeding.

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