Genetic Analysis of Adult Plant Resistance to Yellow Rust and Leaf Rust in Common Spring Wheat Quaiu 3

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Abstract

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Identifying and utilizing rust resistance genes in wheat has been hampered by the continuous and rapid emergence of new pathogen races. A major focus of many wheat breeding programs is achieving durable adult plant resistance (APR) to yellow (stripe) rust (YR) and leaf (brown) rust (LR), caused by *Puccinia striiformis* and *P. tritricina*, respectively. This study aimed to determine the genetic basis of resistance to YR and LR in the common spring wheat 'Quaiu 3'. To that end, we evaluated 198 F₅ recombinant inbred lines (RILs), derived from a cross of susceptible 'Avocet-YrA' with Quaiu 3, for APR to LR and YR in artificially inoculated field trials conducted in Mexico during the 2009 and 2010 growing seasons. High narrow-sense heritability

(h^2) estimates, ranging between 0.91 and 0.95, were obtained for both LR and YR disease severities for both years. The quantitative and qualitative approaches used to estimate gene numbers showed that, in addition to known resistance genes, there are at least two to three APR genes associated with LR and YR resistance in the RIL population. The moderately effective race-specific resistance gene *Lr42* and the pleiotropic slow-rusting APR gene *Lr46/Yr29* were found to interact with additional unidentified APR genes. The unidentified APR genes should be of particular interest for further characterization through molecular mapping, and for utilization by wheat breeding programs.

Yellow (stripe) rust (YR) and leaf (brown) rust (LR) diseases of wheat (*Triticum aestivum* L.), caused by *Puccinia striiformis* f. sp. *tritici* and *P. tritricina*, respectively, have been a significant threat in the majority of wheat-growing regions worldwide. Although the use of host resistance is the most economical and environmentally friendly method for controlling rust diseases in wheat, evolution of new pathogen races can easily overcome the resistance conferred by the existing race-specific genes, if deployed as the sole source of resistance. However, slow-rusting resistance genes are considered to be more durable, and their expression is marked by slow disease development, longer latent period, fewer and smaller uredinia, and lower spore production (3). This type of resistance is expressed at the adult stage despite seedling susceptibility (1,18,51). Thus, slow-rusting durable resistance genes are often called adult plant resistance (APR) genes and are effective against a broad range of pathogen races.

Though a single APR gene may not provide adequate protection against rust, especially under high disease pressure, studies have revealed that combinations of three to five slow-rusting genes usually result in "near-immunity," comparable with a high level of race-specific resistance (49). One of the most studied and utilized APR genes, which has provided protection against a broad range of LR and YR races for the last 60 years, is *Lr34/Yr18*, located on chromosome arm 7DS (9,45). This gene has recently been cloned (22), and is now being introgressed into elite lines in many countries using gene-specific DNA markers. Similarly, *Lr46/Yr29* is another important APR gene located in the 1BL chromosomal region (50,59), initially identified in an International Maize and Wheat Improvement Center (CIMMYT)-derived Mexican variety,

'Pavon 76'. It is considered to be widely distributed in CIMMYT-derived germplasm (47). Recently, a new and promising APR gene, located on chromosomal arm 4DL, was identified in Canadian wheat line 'RL6077' and designated as *Lr67/Yr46* (12,15). In order to meet the challenges of breeding for durable rust resistance, it is necessary to genetically characterize additional APR genes, which will then assist breeders in enhancing genetic diversity for APR in wheat. (61).

Slow-rusting resistance is quantitatively inherited; therefore, to successfully utilize it in breeding programs, it is important to understand the inheritance behavior, such as heritability of traits, segregation pattern, and the expected number of genes conferring the resistance. Knowledge of heritability and the number of genes controlling slow rusting helps breeders decide the right time to start selection and choose the optimum population sizes to be grown in various segregating generations (7). The phenotype-based quantitative and qualitative approaches of previous studies have led to the suggestion that a few to several minor genes are responsible for slow-rusting resistance (3,7,37,38,46,51,61). Recent molecular mapping studies have identified two to eight quantitative trait loci (QTLs) associated with slow-rusting resistance of YR and LR in different wheat genotypes (4,8,25,32,34,41). Similarly, several QTLs or genes associated with durable high-temperature APR to YR were identified in some wheat lines in different mapping experiments (11,30,39,44).

'Quaiu 3' is a high-yielding spring wheat line developed at CIMMYT that has displayed immunity to both YR and LR in the adult plant stage during field trials. A line of the same cross has been released as a variety by the national breeding programs of Afghanistan and Ethiopia, and is under testing for release in various other countries. This line is also being used extensively as a parent in crossing blocks of the CIMMYT Bread Wheat Improvement program. Quaiu 3 shows susceptibility to YR at the seedling stage and does not have any effective characterized race-specific resistance genes for YR. The race-specific resistance gene *Lr42* present in Quaiu 3, transferred to wheat from *Aegilops tauschii*, is known to confer intermediate seedling reactions and moderate levels of re-

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sistance to LR in the field. Therefore, we hypothesized that Quaiu 3 should possess additional resistance genes, most likely slow rusting, that contribute to the immunity against both LR and YR. The objective of this study was to analyze the inheritance of APR for both LR and YR, and to estimate the minimum number of genes present in Quaiu 3 using a recombinant inbred line (RIL) population developed by crossing it with the susceptible parent 'Avocet-YrA'.

Materials and Methods

Plant materials. A set of 198 randomly advanced F_4 -derived F_5 RILs, derived from the cross Avocet-YrA/Quaiu 3, was used in this study. Quaiu 3 (pedigree: Babax/Lr42//Babax*2/3/Vivitsi) is a high-yielding CIMMYT line that has shown high APR to LR and YR in field conditions, based on international trials and nurseries. The source parent for resistance gene *Lr42* was a winter wheat germplasm line, 'KS91WGRC11' (pedigree: Century*3/TA2450 [A. *tauschii*]) developed by the United States Department of Agriculture–Agricultural Research Service and Kansas State University and kindly provided by S. Cox. The susceptible parent Avocet-YrA is a reselection from the original heterogeneous Australian cultivar, which lacks the race-specific resistance gene *YrA* and is also known as 'Avocet S'. For simplicity, the reselection will be designated as Avocet throughout this article, and Quaiu 3 as Quaiu.

To develop the population, a single spike from each F_2 plant, generated from three different F_1 plants, was randomly harvested under fungicide application and advanced to the F_4 generation by harvesting a single spike in each subsequent generation. The F_5 plots, derived from single F_4 spikes, were then harvested in bulk to obtain sufficient seed of the F_5 RILs.

Seedling testing. Parents were tested for YR, and the whole population for LR, as seedlings in a greenhouse at CIMMYT headquarters in 2010. For YR, 12 seeds of parental lines and a differential set, comprised of Avocet near-isogenic lines and other testers, were planted as hills in trays. Similarly, for LR, 8 to 10 seeds of all the lines, a set of LR differentials (48), and some CIMMYT tester lines were planted as hills. The young but fully expanded first leaves (about 10 days after planting) of three experimental sets of materials were inoculated with the *P. triticina* race MBJ/SP and *P. striiformis* isolates Mex96.11 and Mex08.13, respectively, by using an atomizer to spray urediniospores suspended in Soltrol 170 oil. The two races of *P. striiformis* were evaluated separately. The avirulence/virulence formula for both MBJ/SP and MCJ/SP is *Lr2a,2b,2c,3ka,9,16,19,21,24,25,28,29,30,32,33,36/1,3,3bg,10,11,12,13,14a,14b,15,17a,18,20,23, 26,27+31*. However, MBJ/SP is only partially virulent to *Lr26*. Postinoculation environmental conditions for both rusts were maintained as described by Herrera-Foessel et al. (12). The LR infection type for each entry was recorded 11 days after inoculation, based on a 0-to-4 scale (43), whereas the YR response was recorded based on a 0-to-9 scale (33), at 20 days postinoculation.

Field experiments and disease evaluation. Field experiments were carried out in Mexico during the 2008–09 and 2009–10 crop seasons for LR and 2009 and 2010 crop seasons for YR at CIMMYT research stations near Ciudad Obregon (recently named Campo Experimental Normal E. Borlaug, CENEB) and Toluca, respectively. The environment in Toluca, which averages monthly maximum temperatures of 19 to 23°C and monthly precipitation from 65 to 145 mm during the growing season, is very conducive to development of YR. Similarly, CENEB is considered to be a highly suitable place for LR development during the crop season, with average monthly maximum temperatures of 24 to 31°C and average monthly precipitation of 15 to 22 mm. In Toluca, planting begins in mid-May and the season ends in early October whereas, in CENEB, planting begins in late November and the season ends in early May.

About 4 to 5 g of seed (expected 60 to 70 mature plants) of the parents and 198 RILs were hand-sown in 1-m paired rows, spaced 10 cm apart, on top of 80-cm-wide raised beds. The CENEB experiments were planted in the third week of November during

the 2008–09 season and in the first week of December during 2009–10. To create homogenous rust epidemics, spreader rows of the highly susceptible 'Morocco' were planted around the experimental area and at one side of each plot in the middle of the 0.5-m-wide pathway. Artificial inoculations were carried out twice, approximately 8 weeks after sowing, with an equal mixture of prevalent Mexican *P. triticina* races MBJ/SP and MCJ/SP, using the method described by Herrera-Foessel et al. (12). The Toluca experiments were planted in the fourth week of May during both growing seasons. In Toluca, the spreaders consisted of a mixture of six susceptible wheat lines, derived from the cross Avocet × 'Attila', which possessed the defeated race-specific resistance gene *Yr27*. The varying maturities of these lines ensured a continuous production of inoculum during the critical crop growing period. An artificial epidemic was created by inoculating the spreaders with Mexican isolates Mex96.11 and Mex08.13 of *P. striiformis* f. sp. *tritici* at least three times, 4 to 5 weeks after planting. The avirulence or virulence characteristics of the races used in our studies were previously described by Herrera-Foessel et al. (12).

Disease severity (DS) scores were recorded following the 0-to-100% visual ratings based on the modified Cobb Scale (40). For both LR and YR, the first disease severity readings were taken when the susceptible parent (Avocet) showed at least 70% disease severity, followed by second and sometimes third readings at weekly intervals. Similarly, the host response to infection data was recorded based on visual criteria (17,43,51), with some modification to the scale, where R = resistant (necrotic tissue or yellow stripes with or without tiny uredinia), R-MR = resistant to moderately resistant (necrotic tissues or stripes with few small uredinia), MR = moderately resistant (necrotic or chlorotic tissues or stripes with smaller to medium sized uredinia), M (or MR-MS) = moderately resistant to moderately susceptible (necrotic or chlorotic tissues or stripes with medium-sized to large uredinia and intermediate sporulation), MS = moderately susceptible (medium-sized uredinia or stripes without chlorosis and necrosis and abundant sporulation), MS-S = moderately susceptible to susceptible (medium-sized to large uredinia or stripes without chlorosis or necrosis, and abundant sporulation), and S = susceptible (large uredinia or stripes without chlorosis or necrosis, and abundant sporulation). Further, for the repeated measurements, the area under disease progress curve (AUDPC) values for DS were calculated using the following equation (3):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left(\frac{x_i + x_{i+1}}{2} \right) t_i$$

where x_i = the rust severity scores on date i , t_i = time interval (in days) between date i and date $i + 1$, and n = number of readings.

Molecular marker analysis for selected rust resistance genes. Based on the pedigree record and selection history of Quaiu, molecular marker analyses were carried out to test for the presence of potential LR and YR resistance genes. The parents Quaiu and Avocet were tested with 10 molecular markers (*csGS*, *Xgwm533*, *Xgwm192*, *Xcmwg682*, *Xwmc432*, *Sr24#12*, *csLV46G22*, *csLV34*, *+Lr34sp*, and *-Lr34sp*) to analyze eight genomic loci associated with 14 named rust resistance genes of bread wheat (Table 1). Markers that showed polymorphism between the two parents were further analyzed in all the RILs, in addition to the parental lines. The source genotypes or lines reported to have resistance genes were used as positive controls in the marker analysis (Table 1). Young leaf tissue from parents and RILs was harvested from 10 randomly selected plants of each line planted at the Toluca field station. The DNA was extracted using the cetyltrimethylammonium bromide method and, subsequently, polymerase chain reactions, gel electrophoresis, and visualization of amplified products were performed using standard CIMMYT laboratory protocols (5).

Statistical analysis. All statistical analyses, including phenotypic distribution, correlation coefficients, analysis of variance (ANOVA), and marker-phenotype regression, were performed using SAS 9.2 (SAS Institute). ANOVA was conducted to deter-

mine the differences in DS scores among 198 RILs. Because the two experiments were treated as replicates, the interaction effect between experiment and genotype could not be tested (2). However, the *F* test for genotypes can be effectively carried out with interaction mean square as an error term. The disease severity scores were highly correlated between 2 years of experiments; therefore, the interaction effect was relatively unimportant (J. Crossa, CIMMYT, *personal communication*).

Calculation of narrow-sense heritability (h^2) was carried out using the variance component method as $h^2 = \sigma_g^2 / \sigma_p^2$, where $\sigma_g^2 = (MS_g - MS_{ge})/r$ and $\sigma_p^2 = \sigma_g^2 + \sigma_{ge}^2/r$; in this formula, σ_g^2 = genetic variance, σ_p^2 = phenotypic variance, σ_{ge}^2 = variance attributed to interaction between experiments and genotypes (equivalently, total error variance in this analysis), r = number of replicates (equivalent to number of experiments in this analysis), MS_g = mean square of lines, and MS_{ge} = mean square of line-experiment interaction. The 90% confidence interval of heritability was estimated by using the equation given by Knapp et al. (20). This calculation of heritability is considered narrow-sense heritability because the $F_{4,5}$ RILs are very close to complete homozygosity, resulting in the dominance variance being close to zero, and additive-additive interactions can be accounted as additive components of variance (28).

Gene number estimates. A qualitative approach to gene number estimation was carried out using the expected and observed F_5 segregation ratio. All 198 RILs were classified into three phenotypic classes based on DS and host responses: homozygous for parental type resistant (HPTR), homozygous for parental type susceptible (HPTS), and intermediate types (Others), as described by Singh and Rajaram (51). In this classification, all the lines in HPTR and HPTS are assumed to have resistant and susceptible alleles, respectively, in the homozygous state. The classification was based on consistent expression of near immunity or complete susceptibility in the RILs across repeated measurements. Based on this qualitative classification of RILs, χ^2 tests were carried out for two-, three-, or four-gene segregation ratios with two degrees of freedom. Similarly, χ^2 tests were also carried out by classifying the RILs into two phenotypic classes (i.e., HPTR and Others), with one degree of freedom.

A quantitative assessment of the minimum number of genes controlling APR to LR and YR in wheat was carried out using Wright's formula (60), with some modification to correct the level of inbreeding (3,6,13,36): $N = (GR)^2 / 4.57 \times \sigma_g^2$, where N = minimum number of genes present in the segregating population, GR = genotypic range of lines at a given generation, and σ_g^2 = genetic variance of the population. Generally, genotypic range is estimated by subtracting the phenotypic means of two parents or two extreme observations in the segregating population, assuming that there is no dominant gene action, linkage, or epistasis within or among the genes. Moreover, when two parents are used to calculate the genotypic range, it is assumed that all the contributing alleles are from one parent (i.e., there is no transgressive segregation). In this

study, the gene number estimate was performed using both types of genotypic range (methods I and II). Additionally, another genotypic range was calculated as the phenotypic range between two parents, multiplied by corresponding heritability estimates (method III). This third approach, which gives better estimates of genotypic range because it accounts for environmental influence (36), has been effectively used to estimate the number of genes in previous studies (7,28,52). Finally, the average of estimates from methods I and II was also calculated as method IV.

Results

Seedling studies. Both parents (Avocet and Quaiu) showed susceptible reactions (with seedling infection types of 8 and 7 on the 0-to-9 scale for Avocet and Quaiu, respectively) in seedling tests in the greenhouse with the two *P. striiformis* isolates. In contrast, only Avocet seedlings were susceptible to *P. triticina* race MJB/SP, with a seedling score of 3+ on the 0-to-4 infection-type scale, whereas Quaiu seedlings were scored ;1. This observation indicates that Quaiu possibly possesses a race-specific resistance gene. This was verified by a χ^2 test for single-gene segregation of RILs in the Avocet/Quaiu population, where the observed segregation ratio (71:97:21 resistant/susceptible/segregating lines) conformed to the expected single-gene segregation ratio of 0.4375:0.4375:0.125 ($\chi^2 = 4.42$, $P = 0.11$). Lines with poor germination and ambiguous disease readings were omitted from analysis. Furthermore, the seedling resistance locus was found to be linked with marker *Xwmc432* by a recombination frequency of 0.0342 between two loci (equivalently, 3.43 centimorgans [cM] of genetic distance between the resistance gene and marker locus, based on the Kosambi mapping function). In the mapping study by Sun et al. (57), *Xwmc432* was reported to be the closest marker linked to *Lr42* at the 0.8-cM distance. Results of this study indicate that the seedling leaf rust resistance gene in Quaiu is most likely *Lr42*.

Field studies. Excellent YR development occurred in the Toluca trials during both seasons, and the susceptible parent Avocet displayed 70 to 90% severity at the flowering stage when the first evaluations were conducted. Average YR severities of RILs were 41 to 62% for two experiments at different readings. Mid-parental (MP) DS values were comparable with that of population averages (PA) for YR (MP = 45%, PA = 41% and MP = 35%, PA = 46% in the 2009 and 2010 growing seasons, respectively). The distribution of RILs for YR severity and AUDPC scores were continuous but not normal across experiments (Fig. 1). Relatively higher disease pressure was observed during the 2010 season, resulting in a higher average DS of RILs compared with 2009.

Similarly, LR development was also excellent during both seasons at CENEB, and the susceptible parent displayed 100% severity in both experiments at early grain-filling stage. Average LR severities of RILs were less than YR severities and were 22 to 33% across two experiments and different readings. Larger differences between MP and PA were observed for LR severities (MP = 50%, PA = 33% and MP = 50%, PA = 22% in the 2009 and

Table 1. Molecular marker analysis of wheat parents 'Avocet' and 'Quaiu' for selected rust resistance genes

Gene	Marker	Marker analysis ^a		Positive control	Reference
		Avocet	Quaiu		
<i>Lr68</i>	<i>csGS</i>	–	–	Avocet/Prl	14
<i>Sr2/Yr30</i>	<i>Xgwm533</i>	–	+	Parula	54
<i>Lr67/Yr46</i>	<i>Xgwm192</i>	–	–	RL6077	12
<i>Yr17/Sr38</i>	VENTRIUP/LN2	–	–	VPM1	42
<i>Lr24/Sr24</i>	<i>Sr24#12</i>	–	–	Krichauf	31
<i>Lr42</i>	<i>Xwmc432</i>	–	+	<i>Lr42</i> ^b	56
<i>Lr46/Yr29</i>	<i>csLV46G22</i>	–	+	Parula	E. Lagudah ^c
<i>Lr34/Yr18</i>	<i>csLV34</i>	–	–	Parula	24
<i>Lr34/Yr18</i>	<i>+Lr34sp</i>	–	–	Parula	22
<i>Lr34/Yr18</i>	<i>–Lr34sp</i>	–	–	Parula	22

^a Presence (+) or absence (–) of the genes based on corresponding marker alleles. Avocet is the negative control for all the markers under study.

^b Germplasm line KS91WGRC11, which is represented as line *Lr42* in International Maize and Wheat Improvement Center pedigrees.

^c E. Lagudah, *personal communication*.

2010 growing seasons, respectively), with significantly skewed distribution toward the resistant side (Fig. 1).

The Pearson's correlation coefficient (r) between experiments and diseases was highly significant (Table 2). For YR, it was 0.90 to 0.92 whereas, for LR, it was 0.86 to 0.88 between the two experiments, based on DS and AUDPC values. The coefficients of correlation between YR and LR disease scores were 0.22 to 0.32 across experiments. ANOVA showed that both the experiments and the genotypes had highly significant effects on DS. The narrow-sense heritability estimates (with 90% confidence intervals) for YR and LR severity scores were 0.95 (0.96 and 0.93) and 0.92 (0.94 and 0.90), respectively. Similarly, after removing the lines with the

Lr42 gene, the heritability estimate for LR severity scores was 0.91 (0.93 and 0.87). These high levels of heritability estimates indicate high stability of resistance or less environmental influence on DS and reactions of RILs.

Gene number estimates. The qualitative approach of gene number estimates suggested that at least three to four genes segregated in the RIL population for YR in the 2009 experiment (Table 3). However, in the 2010 experiment, observed phenotypic ratios were consistent with segregation of three genes. The reason for lower gene number estimates in the 2010 experiment may be due to some transgressive segregants, which showed higher DS than the susceptible parent Avocet, resulting in more lines in the HPTS

Fig. 1. Phenotypic distributions of 'Avocet' x 'Quaiu' F₅ wheat recombinant inbred lines (RILs). **A**, yellow rust disease severity (DS), 2009; **B**, yellow rust area under disease progress curve (AUDPC), 2009; **C**, yellow rust DS, 2010; **D**, yellow rust AUDPC, 2010; **E**, leaf rust DS, 2009; **F**, leaf rust DS, 2010. DS scores for both yellow and leaf rust were obtained from the first stage of evaluation.

category. However, by combining the 2 years of YR data, the qualitative classification of lines agreed with a three- or four-gene segregation ratio.

For LR, gene number estimates were carried out in the population after excluding lines with the *Lr42* gene. Using a qualitative approach, three to four genes were estimated based on LR disease scores across experiments (Table 3). We observed relatively lower numbers of resistant-type lines for LR severity in 2009 compared with 2010. Fewer lines in the HPTR category caused significant deviation from the expected three- or four-gene ratios, based on three phenotypic classes. However, by combining 2 years of LR data, the number of lines in HPTR and HPTS were found to be in agreement with both three- and four-gene segregation ratios.

Similarly, gene number estimates based on a quantitative approach were found to be relatively lower than qualitative estimates. The quantitative approach estimated a minimum of two to three genes in the population for both LR and YR (Table 4). For YR, the gene-number estimate of the 2009 experiment was slightly lower than that of 2010, when parental DS scores were used for a genotypic range calculation (method I) that did not account for the transgressive segregation. However, for LR, equal numbers of genes were estimated by both methods I and II (i.e., using extreme phenotypic values as genotypic range) in both experiments. After controlling for environmental effect (method III), the gene-number estimates were lower in all the LR and YR experiments.

Effect of known genes. Quaiu showed a positive allele for genes *Lr42*, *Lr46/Yr29*, and *Sr2/Yr30* based on markers *Xwmc432*, *csLV46G22*, and *Xgwm533*, respectively, but showed negative results for the other genes tested (Table 1). Therefore, these three markers were used to further screen the whole population. As expected, the *Lr42* gene alone conferred a moderate level of resistance, with DS reaching 40% (Fig. 2). Further, the presence of

additional slow-rusting genes in conjunction with *Lr42* imparted near-immunity, as indicated by the disease severity ranges of 0 to 40 and 10 to 100% in the lines with and without the *Lr42* gene, respectively (Fig. 2).

The marker *csLV46G22* was regressed with DS data for both LR and YR. Regression analysis showed a significant association of marker with both YR and LR severities in all experiments (Table 5). Because the dominance effect is absent in RILs, the average effect of allele substitution represents the additive effect of the genes. The additive effect of *Lr46/Yr29* on YR severities was relatively small compared with its effects on LR severities.

Table 4. Quantitative approach to gene-number estimation in ‘Avocet’ × ‘Quaiu’ F₅ wheat recombinant inbred line (RIL) population using modified Wright’s formula based on rust severity scores in the first evaluation of 2009 and 2010 experiments

Experiment ^b	Estimated number of genes ^a			
	Method I	Method II	Method III	Method IV
YR-2009	2.16	2.66	1.95	2.41
YR-2010	1.27	2.66	1.14	1.97
LR-2009	2.41	2.41	1.99	2.41
LR-2010	2.41	2.41	1.99	2.41

^a Method I: genotypic range (GR) is the difference of disease severity between two parents; Method II: GR is the difference of disease severity between two extreme observations; Method III: GR is between two parents multiplied by respective heritability estimates; and Method IV: average number of gene number estimates from methods I and II. Gene number calculation is based on Wright’s formula adjusted for F₅ generation: $(GR)^2/4.57 \times \sigma_g^2$.

^b Year of experiment for disease (YR = yellow rust or LR = leaf rust) evaluation.

Table 2. Pairwise correlation coefficients (*r*) among yellow rust (YR) and leaf rust (LR) disease scores for ‘Avocet’ × ‘Quaiu’ F₅ wheat recombinant inbred lines in 2009 and 2010^a

Experiment	Disease scores	Experiment					
		YR-2009		YR-2010		LR-2009	LR-2010
		DS (%)	AUDPC	DS (%)	AUDPC	DS (%)	DS (%)
YR-2009	AUDPC	0.98	...	...	...	...	...
YR-2010	DS	0.90	0.91	...	...	...	...
	AUDPC	0.90	0.92	0.99	...	...	...
LR-2009	DS	0.29	0.28	0.22	0.21	...	...
LR-2010	DS	0.32	0.31	0.23	0.22	0.86	...
	AUDPC	0.32	0.31	0.23	0.23	0.88	0.99

^a DS = disease severity. All coefficient of correlation (*r*) values are significant at *P* = 0.01. AUDPC = area under disease progress curve scores for repeated DS readings.

Table 3. Classification of ‘Avocet’ × ‘Quaiu’ F₅ wheat recombinant inbred lines (RILs) into different phenotypic classes based on disease scores, and χ^2 test for Mendelian segregation for different numbers of genes^a

Experiment	χ^2 Test for three phenotypic classes ^b					χ^2 Test for two phenotypic classes ^c			
	Number of RILs			χ^2 values		Number of RILs		χ^2 values	
	HPTR	HPTS	Others	3 genes	4 genes	HPTR	Others	3 genes	4 genes
YR									
2009	11	12	173	3.33 ^{NS}	5.88 ^{NS}	11	185	1.94 ^{NS}	2.11 ^{NS}
2010	14	18	164	0.52 ^{NS}	24.87 ^{**}	14	182	0.38 ^{NS}	6.72 ^{**}
Combined	10	12	174	4.15 ^{NS}	4.85 ^{NS}	10	186	2.73 ^{NS}	1.15 ^{NS}
LR ^e									
2009	2	13	82	7.57 [*]	26.48 ^{**}	2	95	5.03 [*]	0.71 ^{NS}
2010	6	8	83	0.62 ^{NS}	7.77 [*]	6	91	0.60 ^{NS}	1.75 ^{NS}
Combined	5	5	87	2.88 ^{NS}	1.27 ^{NS}	5	92	1.31 ^{NS}	0.61 ^{NS}

^a RILs = recombinant inbred lines; ** and * indicate significant at *P* = 0.01 and 0.05, respectively; and NS = not statistically significant.

^b HPTR = homozygous parental type resistant; HPTS = homozygous parental type susceptible; Others = lines with intermediate type of disease scores. For χ^2 test, expected segregation ratios are HPTR/HPTS/Others = 0.0837:0.0837:0.8326 (3 genes) and HPTR/HPTS/Others = 0.03664:0.03664:0.92672 (4 genes).

^c Others = lines which do not belong to HPTR category. Expected segregation ratios for χ^2 test are HPTR/Others = 0.0837:0.9163 (3 genes) and HPTR/Others = 0.03664:0.96336 (4 genes).

^d Yellow (stripe) rust (YR) and leaf (brown) rust (LR) evaluations.

^e Lines which were resistant in seedling test for LR were excluded from the analyses.

Similarly, the variance explained by the gene was about 8% for YR, whereas it explained up to 47% of total phenotypic variation for LR. This could also be observed in the phenotypic distribution of lines with and without *Lr46/Yr29* (Fig. 2). For LR, DS of lines with *Lr46/Yr29* was 15 to 80% whereas, for YR, it was 0 to 100%. Similarly, after removing the lines that carried *Lr46/Yr29*, the distribution curve shifted more toward the susceptible side even though quite a few lines had a moderate level of resistance to LR. On the other hand, for the YR reaction, the presence of *Lr46/Yr29* did not appear to affect the distribution pattern of RILs.

Based on the marker-phenotype regression analysis, the *Sr2/Yr30* gene, as represented by *Xgwm533*, also showed significant effect on YR severity by explaining up to 9.7% of total phenotypic variation (Table 5). Average YR severity among the lines with *Sr2/Yr30* alone was found to be significantly different from the lines without *Sr2/Yr30* and *Lr46/Yr29* genes in both experiments (Fig. 3). Similarly, lines with both *Sr2/Yr30* and *Lr46/Yr29* showed significantly lower DS than those with *Sr2/Yr30* only. Average YR severity among the lines with *Lr46/Yr29* was also found to be significantly different from the lines without *Sr2/Yr30* and *Lr46/Yr29* in both experiments. Though the resistant parent was immune to YR, two APR genes (*Sr2/Yr30* and *Lr46/Yr29*) together reduced the DS by 60 to 75%. These observations indicate that, in addition to *Yr29* and *Yr30*, some unknown genes must be present in the population.

Discussion

In the presence of substantial disease pressure, the resistant parent Quaiu remained immune to both LR and YR in all the field experiments. Subsequent marker analyses supported the presence of two APR genes (*Lr46/Yr29* and *Yr30*) and one major gene (*Lr42*) in Quaiu. Both the qualitative and quantitative approaches to gene-number estimation suggested the presence of at least three genes responsible for APR to LR and YR in the RILs. Additionally, the combined effects of known APR genes were not adequate in explaining near-immunity in the RILs. These observations indicate that there is at least one more undiscovered APR gene in Quaiu for each species of rust.

The presence of the pleiotropic slow-rusting resistance gene *Lr46/Yr29* in Quaiu was confirmed based on the association of marker *csLV46G22* with YR and LR disease scores in the RILs. Marker *csLV46G22* has been successfully used by Lillemo et al. (29) to assess the effects of *Lr46* with various additive YR resistance genes in an Avocet/Saar mapping population. Marker-phenotype regression showed a relatively smaller effect of the gene *Lr46/Yr29* on slow-rusting resistance in YR compared with its effect on LR. This observation was consistent with a previous study by Lillemo et al. (27).

Presence of the moderately effective race-specific resistance gene *Lr42* in Quaiu was confirmed based on the linkage analysis between seedling resistance and molecular marker *Xwmc432*. *Lr42* is considered to be effective against LR races in most of the world's wheat-growing regions. A previous report on the presence of *Lr42* virulence in the United States (21) was found to be misinterpreted because of the use of a poor differential set (J. Kolmer, personal communication). Similarly, a report of *Lr42* virulence in China lacks enough evidence (16). In this study, *Lr42* alone showed a moderate degree of resistance, with DS reaching about 40% in the RILs. However, it was observed that *Lr42* interacted with other APR genes, causing the phenotypic distribution to be skewed toward resistance.

A significant association of marker *Xgwm533* with YR severities in the RILs indicated the presence of the gene *Sr2/Yr30* in Quaiu. Past studies have suggested that the marker *Xgwm533* is closely linked with the pleiotropic gene *Sr2/Yr30* (53,55,56). Moreover, the Quaiu allele fragment amplified with marker *Xgwm533* was found to be similar to that of 'Parula', which has been reported to carry the *Sr2* gene for stem rust resistance (29). In addition, "pseudo black chaff" was also observed on Quaiu in the field, potentially indicating the presence of *Sr2*.

RILs were continuously distributed for LR and YR severities in field trials; however, the normality assumption was not met in all experiments for both diseases. The lack of a normal distribution may be due to the presence of epistasis or linkage between genes (3), as well as their unequal effects on disease resistance. Several genetic studies have reported non-normality as a common phenomenon of quantitatively inherited disease resistance traits (3,23,28,37,38,51,61).

Fig. 2. Phenotypic distributions of 'Avocet' x 'Quaiu' F₅ recombinant inbred lines (RILs) with different gene combinations for average disease severity (DS) across experiments. **A**, Leaf rust DS with and without *Lr42* gene; **B**, leaf rust DS with and without *Lr46* genes after excluding *Lr42* containing lines; and **C**, yellow rust DS in the lines with and without *Yr29* gene.

Table 5. Marker-phenotype regression for *Lr46/Yr29* and *Sr2/Yr30* on disease severity scores in the first evaluation of 'Avocet' × 'Quaiu' F₅ wheat recombinant inbred lines (RILs) in 2009 and 2010 experiments

Gene, experiment ^b	Intercept (±SE)	Slope ^a		R ² (%) ^c	Additive ^d
		Estimate (±SE)	P value		
<i>Lr46/Yr29</i>					
YR-2009	50.76 ± 3.4	-17.95 ± 5.1	0.0005	8.04	-8.97
YR-2010	55.16 ± 3.3	-17.58 ± 4.9	0.0005	8.13	-8.79
LR-2009	69.31 ± 4.1	-40.33 ± 5.6	<0.0001	41.17	-20.17
LR-2010	58.89 ± 4.3	-47.32 ± 5.9	<0.0001	46.85	-23.66
<i>Sr2/Yr30</i>					
YR-2009	53.68 ± 3.9	-19.91 ± 5.4	0.0003	9.69	-9.95
YR-2010	53.98 ± 3.9	-11.72 ± 5.5	0.0336	3.56	-5.86

^a Coefficients from marker-phenotype regression; SE = standard error. The negative sign represents the effects from resistant parent (Quaiu) allele.

^b Experimental year and disease evaluation (YR = yellow rust and LR = leaf rust).

^c Variation explained by the model (in this case, percentage of total phenotypic variance explained by *Lr46/Yr29* and *Sr2/Yr30* genes).

^d Additive effect of genes based on linked markers (i.e., average effect of allele substitution, assuming no dominance effect).

Fig. 3. Yellow rust (YR) severities of 'Avocet' × 'Quaiu' F₅ wheat recombinant inbred lines with *Lr46/Yr29* and *Sr2/Yr30* genes, and their interaction in the first evaluation of 2009 (YR2009) and 2010 (YR2010) experiments. Bars not represented by the same letter are significantly different. Least significant difference values at $P = 0.05$ are 15.73 and 15.30 for YR2009 and YR2010 experiments, respectively.

Transgressive segregation toward higher susceptibility was evident during both years for YR but the difference in disease severities between Avocet and the most susceptible line was larger in the 2010 experiment (70 and 100% of DS, respectively) than in 2009, based on the first date of disease evaluation (Fig. 1). This observation indicated that the susceptible parent Avocet also contributed some level of slow-rusting resistance in the RIL population. This observation is further supported by a QTL mapping study by William et al. (58), who reported two Avocet-derived resistance QTLs that explained 5.8 to 13.1% and 2.4 to 6.1% of total phenotypic variation for YR and LR severities, respectively.

In our study, the heritability estimates of LR and YR severity scores were very high, which concurs with previous studies, especially when associated with low estimates of gene numbers (3,10,37,38,54). The highly significant correlation coefficients among the experiments suggest that the experimental conditions were well controlled and consistent with little experiment–genotype interaction. Further, relatively smaller but significant correlation between LR and YR scores supports the presence of a dual APR gene, *Lr46/Yr29*.

Based on DS scores and AUDPC values, χ^2 tests were carried out to determine the best fit between the observed and expected number of lines in phenotypic classes for different numbers of genes. When evaluating APR, the timing of disease observation is a crucial factor. Proper disease development is affected by local environment conditions, from flag-leaf development to physiological maturity. Therefore, in order to capture all possible information regarding differential responses of RILs on rust diseases, we took multiple readings wherever possible. From the YR experiments, it was evident that we could capture the susceptible transgressive segregants only at early stages of evaluation, whereas the true resistant types can be diagnosed in later stages. This is because

the χ^2 tests were performed using both three (HPTR, HPTS, and Others) and two (HPTR and Others) phenotypic classes of RILs. In order to avoid complexity in gene-number estimate, the phenotypic classification of each RIL was carried out based on consistent expression of resistance or susceptibility in repeated measurements of each experiment (2009 and 2010) or across experiments.

A relatively lower number of genes was estimated in the quantitative approach compared with the estimates using qualitative methods. This is probably due to high genetic variance associated with DS values in the RILs. Similarly, when transgressive segregation was observed, parental phenotypic range resulted in fewer genes. The gene-number estimates in our study were in accordance with previous reports in terms of phenotypic distribution pattern and heritability estimates of slow-rusting resistance (7,10,19,35,37,38,46,51,54,61,62). Most molecular mapping studies have shown a large number of QTLs or genes in segregating populations, compared with the corresponding gene-number estimates based on phenotypic segregation ratios and quantitative methods (26–28,37).

Because visual DS scores were highly heritable in RILs for both LR and YR, transferring resistance from Quaiu to other genetic backgrounds should be relatively simple via visual selection in segregating populations subjected to high disease pressure. Virulence to *Lr42* has not been explored in North American *P. triticea* populations; therefore, its utilization in conjunction with *Lr46/Yr29* and other unidentified slow-rusting genes is a promising strategy for achieving near-immunity to LR. The near-immunity to YR in Quaiu, based on the presence of at least three APR genes, further increases its breeding value as a parent for achieving durable rust resistance. Despite the known APR genes *Lr46/Yr29* and *Sr2/Yr30*, a large proportion of variation on LR and YR resistance still remains unexplained in the Quaiu/Avocet population. This observation, along with the gene-number estimates, indicates the presence of additional APR genes in Quaiu. Further molecular mapping studies will help to determine the genomic locations of the uncharacterized resistance genes in order to utilize them in breeding programs.

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