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ORIGINAL PAPER

Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an Avocet × Pastor wheat population

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Abstract Leaf rust and stripe rust are important diseases of wheat world-wide and deployment of cultivars with genetic resistance is an effective and environmentally sound control method. The use of minor, additive genes conferring adult plant resistance (APR) has been shown to provide resistance that is durable. The wheat cultivar 'Pastor' originated from the CIMMYT breeding program that focuses on minor gene-based APR to both diseases by selecting and advancing generations alternately under leaf rust and stripe rust pressures. As a consequence, Pastor has good resistance to both rusts and was used as the resistant parent to develop a mapping population by crossing with the susceptible 'Avocet'. All 148 F_5 recombinant inbred lines were evaluated under artificially inoculated epidemic environments for leaf rust (3 environments) and stripe rust (4 environments, 2 of which represent two evaluation dates in final year due to the late build-up of a new race virulent to *Yr31*) in Mexico. Map construction and QTL analysis were completed with 223 polymorphic markers on 84 randomly selected lines in the population. Pastor contributed *Yr31*, a moderately effective race-specific gene for stripe rust resistance, which was overcome during this study, and this was clearly shown in the statistical analysis. Linked or pleiotropic chromosomal regions contributing to resistance against both pathogens included *Lr46/Yr29* on 1BL, the *Yr31* region on 2BS, and additional minor genes on 5A, 6B and 7BL. Other minor genes for leaf rust resistance were located on 1B, 2A and 2D and for stripe rust on 1AL, 1B, 3A, 3B, 4D, 6A, 7AS and 7AL. The 1AL, 1BS and 7AL QTLs are in regions that were not identified previously as having QTLs for stripe rust resistance. The development of uniform and severe epidemics facilitated excellent phenotyping, and when combined with multi-environment analysis, resulted in the relatively large number of QTLs identified in this study.

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Introduction

Rust (*Puccinia* spp.) diseases are major constraints to wheat performance and productivity worldwide. They not only reduce photosynthetic area to limit photosynthesis, but also sequester assimilates for spore production, ultimately limiting growth and yield. Under extreme epidemic conditions, crops can be completely destroyed by any of the three wheat rust pathogens (Roelfs et al. 1992). These diseases can sometimes be economically controlled by the

use of foliar fungicides under high input agricultural production systems. However, fungicides may not be used for a number of reasons including financial constraints, chemical availability and inclement weather conditions. Accordingly, genetic control of rust diseases in the development of resistant cultivars is an attractive approach and can also have significant environmental benefits. Many wheat-breeding programs place considerable efforts and resources in breeding and screening for rust resistance in the development of improved cultivars.

In genetic control, the wheat plant recognises the fungal pathogen and initiates cellular responses that slow down or stop fungal development. Flor (1956) first used this interaction in the "flax–flax rust" pathogen interaction to describe the gene-for-gene hypothesis of resistance in the host and avirulence in the pathogen. Mechanisms which stop fungal development usually involve plant programmed cell death, where the host ablates its own cells as they become infected (Heath 2000). This hypersensitive response proves a very powerful mechanism through which fungal infections can be partially or completely stopped, and is observed as a reduction in infection type (Stakman et al. 1962). Lower infection types severely inhibit sporulation and place high selection pressures on the fungal pathogens to overcome resistance, leading to race-specificity. As a breeding strategy, incorporating such race-specific genetic resistance is commonly a short-term option.

Slow rusting describes resistance mechanisms that, under most circumstances, do not completely halt fungal infections, but slow the infection process, reduce the number and size of the lesions, consequently reducing the disease rate and final disease severity in the field. A number of these slow-rusting genes need to be pyramided together to achieve effective genetic control since a single gene usually does not have enough effect to significantly limit disease progress. For slow-rusting genes to be most effective, they also need to be durable. To date, there are only four well characterised, non-hypersensitive slow-rusting genes *Lr34/Yr18*, *Lr46/Yr29*, *Lr67/Yr46* and *Sr2/Yr30* that are effective in the field. Interestingly, these loci are pleiotropic in nature, in that they are effective against multiple pathogens and also confer phenotypic markers associated with non-rust related cell death. The first three mentioned above have a necrotic effect on the leaf tips commonly known as leaf tip necrosis (LTN), and the *Sr2* locus confers pseudo-black chaff (PBC), a darkening of the glumes and internodes.

Numerous QTL studies have identified genomic regions with large effects on rust diseases, many of them co-locating with the aforementioned genes. However, these studies have also highlighted other regions that have small but significant effects in reducing disease severity. These slow-rusting genes have additive effects and Singh et al. (2000a) showed that by combining 4–5 such genes, near immunity can be achieved against leaf rust and stripe rust.

In this study, we investigated genetic control of resistance to both leaf rust and stripe rust in a population of recombinant inbred lines (RILs) developed from the cross of 'Avocet' (susceptible) with 'Pastor' (resistant). Pastor also carries a partially effective race-specific resistance gene *Yr31* for stripe rust (Singh et al. 2003) that was effective against prevalent pathotypes in Mexico until 2008. However, a race change in 2009 defeated this gene and changes in QTL patterns are found in the analyses.

Materials and methods

Development and evaluation of Avocet × Pastor F_5 RILs

The population consisted of 148 F_5 RILs from a cross between Avocet (reselection 'Avocet S' that lacks resistance gene *YrA*) and Pastor (GID3587478, pedigree: Hork/Yamhill//Kalyansona/Bluebird/3/Seri M82/4/Aurora//Kalyansona/Bluebird/3/Woodpecker). Avocet is susceptible to current pathotypes of both leaf rust and stripe rust, whereas Pastor was immune in the field to stripe rust until 2008, due to the combination of the resistance gene *Yr31* and other slow-rusting genes. It also carried an adequate level of slow-rusting genes to leaf rust. The seed for all trials were sourced from a single, large scale multiplication with each line being derived from a single F_5 plant. The parents and RILs were evaluated under artificially inoculated field trials at CIMMYT research stations near Ciudad Obregon, Sonora State, for leaf rust, and near Toluca, Mexico State, for stripe rust for multiple seasons. Each line was sown as 1-m long plots on 75-cm wide raised beds in paired rows with 20 cm between the rows and a 50 cm pathway. Susceptible spreader rows of cv. Morocco were planted as hills on one side of the plots and inoculated approximately 4 and 8 weeks after sowing (stripe rust and leaf rust, respectively). Rust urediniospores were suspended in light weight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX, USA) and sprayed onto Morocco. *P. triticina* races MBJ/SP (avirulence/virulence formula: *Lr2a,2b,2c,3 ka,9,16,19,21,24,25,26,28,29,30,32,33/1,3,3bg,10,11,12,13,14a,14b,15,17,18,20,22b,23,27 + 31)* and MCJ/SP (as above but with virulence for *Lr26*) (Singh 1991) are virulent on race-specific resistance genes present in both parents and were used in field trials in Cd. Obregon. The *Yr31*-avirulent *P. striiformis* isolate Mex96.11 (Singh et al. 2000b) was used in field trials at Toluca during 2000 and 2002. A mixture of Mex96.11 and the *Yr31*-virulent isolate Mex08.13 (avir/vir formula not fully characterized) was

used at Toluca during 2009. The final disease severity for each plot was evaluated for three cropping seasons (2000, 2002 and 2004 for leaf rust and 2000, 2002 and 2009 for stripe rust) according to the modified Cobb Scale (Peterson et al. 1948). Infection type was also scored for stripe rust in 2000. Disease severity was evaluated when the flag leaf of Avocet showed between 80 and 100% leaf rust severities and between 70 and 90% stripe rust severities to capture maximum variation. In 2009, stripe rust was scored twice at a 6-day interval and are referred as environments 2009a and 2009b. LTN was scored as present, absent or segregating in the Obregon field site in 2004.

Greenhouse screening

The parents and the RIL population were screened in the greenhouse against two pathotypes of leaf rust (NCJ/BN and MCJ/QM). These pathotypes were avirulent to the race-specific resistance genes *Lr3*, *Lr13* (NCJ/BN) and *Lr23* (MCJ/QM) that were postulated to segregate in this population. Avocet carries *Lr13* and Pastor is postulated to carry *Lr3* and *Lr23*. Genes *Lr13*, *Lr23* and *Yr31* are known to be located in chromosome arm 2BS (Roelfs et al. 1992; Singh et al. 2003). Stripe rust isolate Mex96.11, which is avirulent to *Yr31*, was used to phenotype this population for seedling reaction conferred by *Yr31*. Six to eight seeds were sown in clumps in trays and grown in a glasshouse for 10 days. They were then sprayed with urediniospores suspended in Soltrol 170 oil using an atomizer and placed in a dew chamber for 16 h at 20°C or 40 h at 12°C for leaf rust and stripe rust, respectively. Plants were then placed in a greenhouse with night/day temperatures set at 14/24°C for leaf rust and 12/18°C for stripe rust. Reaction to leaf rust was scored 10-day post-inoculation on the 0–4 scale according to Roelfs et al. (1992). Reactions to stripe rust were scored 20-day post-inoculation on the 0–9 scale of McNeal et al. (1971). The seedling phenotyping experiments were repeated twice and the genotypes of RILs for various resistance genes were postulated based on infection types observed on the seedlings.

Molecular analysis

DNA was extracted from parents and each RIL from greenhouse grown material. About 10 seeds of each RIL were sown and plants grown in 10-cm pots for 5 weeks and the leaf material pooled. A CTAB (alkyltrimethyl-ammonium bromide) based extraction procedure (Hoisington et al. 1994) was used for DNA extraction. Mapping was conducted on 84 randomly sampled lines and the parents by genotyping with DArT (Diversity Array Technologies) markers by Triticarte Pty Ltd (Canberra, Australia) where 197 polymorphic markers were used. Further simple sequence repeat (SSR), markers (21) located on group 1 chromosomes were run on the complete population according to Hayden et al. (2008), as was the *csLV46* marker (unpublished, E.S. Lagudah personal communication) for the *Lr46/Yr29* locus and the GLUD1 protein marker. Seedling reactions for identifying the genes *Lr3*, *Lr13* and *Yr31* on the entire population were also used in generating the genetic map. In all, there were 223 informative polymorphic markers.

Statistical and genetic analyses

The phenotypic data were analysed statistically after first checking residuals for normality across environments. Residual plots revealed a random distribution for the percentage scores so data were left untransformed. Combined analyses of variance over environments were then performed for both rusts using the SAS mixed linear models procedure MIXED (Littell et al. 1996). Narrow-sense heritability (h^2) were calculated and expressed on a line-mean and single environment basis.

Predictions of the minimum numbers of genes contributing to resistance were estimated through χ^2 analysis for each environment and for the average of environments for each pathogen. For leaf rust, lines were scored for disease severity relative to severity in the parents (i.e. homozygous parental type resistant, HPTR, homozygous parental type susceptible, HPTS) or an intermediate level of resistance (Other). Two classes were used for stripe rust (HPTS and Other). χ^2 analyses of observed against expected frequencies for disease severity classes were calculated with the "ChiTest" function in Microsoft Office 12 Excel.

Genetic linkage groups were determined with Map Manager QTX Version 2.0 (Manly et al. 2001) using linkage criteria set at $P = 0.001$ and the Kosambi mapping function. Marker orders within linkage groups were then confirmed using the program "Record" (van Os et al. 2005). The QTL analysis was undertaken for each environment (year) separately and then across environments (years) using mixed linear composite interval mapping in QTLNetwork 2.0 (Yang et al. 2005). Multiple interval mapping (MIM) and phenotypic explained variance (PEV) were conducted using "MultiQTL" software for analysis of multi-environment data. This was undertaken using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05 and window size set at 10 cM. Significance thresholds for QTL detection were calculated for each dataset using 1,000 permutations (Churchill and Doerge 1994) and a genome-wide error rate (α) of 0.10 (suggestive) and 0.05 (significant). The resulting genetic model incorporated significant main additive and additive × additive epistatic genetic effects and their interactions with environment.

Results

Disease development, resistance expression and estimating number of resistance genes

The field studies achieved high levels of infections for both leaf rust and stripe rust. This is shown in Table 1 where leaf rust and stripe rust severities for the susceptible parent were >80 and >90%, respectively. The data across years was also highly repeatable as evidenced by the high line-mean heritabilities (Table 1) and with Pearson correlation coefficients generally exceeding 0.8 for rust severity across seasons (Table 2). However, correlation coefficients were lower when the *Yr31*-virulent *P. striiformis* pathotype was used in 2009b.

The resistance in Pastor was stable across environments and although the line-mean heritabilities were high, some lines grouping in the tails of the distribution did not show the same stability. Therefore, the χ^2 analysis was conducted on all single environments and on the average score for each line, with the later indicating that at least four genes conferred adult plant resistance to leaf rust (Table 3). In the first three stripe rust environments, segregation of the moderately effective race-specific resistance gene *Yr31* skewed disease severity scores in the progeny, as there were more HPTR than would have been expected. This was not the case in the 2009b score when *Yr31* had been overcome. To take account of this, χ^2 analysis for predicted gene number was completed on two groups (HPTS and all other lines) and this showed that the observed segregation ratios were not significantly different from what would be expected if a minimum of five genes conferred resistance (Table 4). Interestingly, the 2009b scoring also showed no statistical difference from the expected ratios for the presence of at least four genes.

Mapping and QTL analysis

The 223 markers formed 31 linkage groups (Supplementary Fig. 1) containing at least one representative on each chromosome, with the exception of 5D and 6D. There were multiple linkage groups on some chromosomes and a few chromosomes had limited coverage. Total physical distance of the map was 2,096 cM with an average marker distance of 9.4 cM.

Total PEV in each environment is given in Table 5. The PEV for leaf rust severity varied between 77 and 86%, while for stripe rust severity, it varied between 81 and 93%. The multi-environment analysis revealed numerous QTLs involved in reducing leaf rust and stripe rust severities (Table 6). In the case of leaf rust severity, the locus containing the slow-rusting gene *Lr46/Yr29* had a very large effect, with a cross environment logarithm of odds (LOD) of 31.2. The seven other detected leaf rust resistance QTLs had small but significant LOD scores ranging between 3.8 and 7.6, with three of these inherited from the susceptible parent. Stripe rust genetic control was more complex with 13 QTLs identified as having a significant ($P < 0.05$) effect across environments. The most pronounced of these was at the *Yr31* locus with a LOD of 60. Alleles at the *Lr46/Yr29* locus also had large effects on stripe rust severity (LOD 23), as did a 7B QTL (LOD 25.8). The other ten QTLs had LOD scores of between 2.7 and 16.2, with four of these derived from the susceptible parent Avocet. There was a substantial genotype × environment effect at the *Yr31* locus where a pathotype virulent to this gene was present in the field during 2009b. This pathotype had the effect of almost halving the single environment PEV for that locus in 2009b.

Table 1 Summary of the disease severity (% average leaf area covered by rust) for the Avocet × Pastor RIL population recorded in 2000 (environment 1), 2002 (environment 2), 2004 (environment 3 for leaf rust) and 2009a and 2009b (environment 3 and 4 for stripe rust recorded 6 days apart)

	Leaf rust			Stripe rust			
	2000	2002	2004	2000	2002	2009a	2009b
Avocet	90	100	80	90	90	90	100
Pastor	1	1	1	1	1	5	15
Population mean	39.1	41.5	26.7	19.7	13.5	30.1	52.5
Range low	1	1	1	1	1	5	5
Range high	100	100	90	100	100	100	100
$\sigma^2_{\text{Genotype}}$	739 ± 94**			402 ± 52**			
$\sigma^2_{\text{Genotype} \times \text{environment}}$	190 ± 16**			134 ± 10**			
h^2_{LM}	92 ± 8**			93 ± 9**			

** The variance component and heritability estimates are significantly different from zero at $P = 0.01$. Standard errors indicated on these components (±)

LTN was also measured in one environment and had an extremely high PEV (99.5%) which was solely due to the *Lr46/Yr29* locus (LOD 28).

Table 2 Comparison of leaf rust and stripe rust severity data for different environments (as described in Table 1) using the Pearson correlation coefficient (*r*)

Environments compared	*r* for leaf rust	*r* for stripe rust
Environment 1 and 2	0.83	0.89
Environment 1 and 3	0.74	0.85
Environment 1 and 4	Not applicable	0.58
Environment 2 and 3	0.89	0.89
Environment 2 and 4	Not applicable	0.51
Environment 3 and 4	Not applicable	0.70

Discussion

Number of resistance genes and their expression

The production of consistent severe disease epidemics resulted in high heritability across environments for both leaf rust and stripe rust (Table 1). In turn, high Pearson correlation coefficients (Table 2) reflected the strong among-line association for disease reaction to both rusts. Together, high repeatability arising from the multi-environment assessments increased power for detecting minor QTLs that might contribute to disease severity in the tested population.

The χ^2 analysis for leaf rust severity (Table 3) indicated that the observed ratios best conformed to the hypothesis that at least four genes were involved in the disease reaction. However, this type of analysis assumes that all genes involved have equal contributions to the disease reaction.

Table 3 Estimation of the number of additive, slow-rusting genes conferring resistance to leaf rust by grouping Avocet × Pastor RILs in 'homozygous parental type resistant' (HPTR), 'homozygous parental type susceptible' (HPTS) and 'other' (includes all RILs with intermediate severities)

	RILs (No.)			*P* values			
	HPTR	HPTS	Other	2000	2002	2004	Average
Observed no. of lines 2000	23	21	103				
Observed no. of lines 2002	23	35	89				
Observed no. of lines 2004	7	11	129				
Observed average all years	6	8	134				
Expected proportions, 2 genes	0.19	0.19	0.62	0.13	0.26	8.E−10*	7E−12*
Expected proportions, 3 genes	0.084	0.084	0.832	1E−4*	1E−13*	0.24	0.05
Expected proportions, 4 genes	0.036	0.036	0.928	1E−25*	3E−53*	0.03*	0.47

* Significantly different from expected ratio; *P* values were calculated from χ^2 statistic

Table 4 Estimation of the number of additive, slow-rusting genes conferring resistance to stripe rust by grouping Avocet × Pastor RILs in 'homozygous parental type susceptible' (HPTS) and 'other' (includes all intermediate and 'homozygous parental type resistant' phenotypes)

	RILs (No.)			*P* values			
	HPTS[†]	Other[†]	2000	2002	2009a	2009b	Average
Observed no. of lines 2000	2	145					
Observed no. of lines 2002	4	143					
Observed no. of lines 2009a	5	143					
Observed no. of lines 2009b	6	142					
Observed average all years	1	147					
Expected proportion, 2 genes	0.19	0.81	5E−8*	4E−7*	1E−6*	4E−6*	1E−8*
Expected proportion, 3 genes	0.084	0.916	2E−3*	0.01*	0.03*	0.057	7E−4*
Expected proportion, 4 genes	0.036	0.963	0.14	0.57	0.86	0.77	0.06
Expected proportion, 5 genes	0.016	0.984	0.81	0.27	0.08	0.02*	0.37

[†] Only two categories used in analysis due to the presence of *Yr31*

* Significantly different from expected ratio; *P* values were calculated from χ^2 statistic

Table 5 'Phenotypic explained variance' (PEV) for Avocet × Pastor RILs evaluated for leaf rust severities in three environments (2000, 2002 and 2004) for stripe rust severities in four environments (2000, 2002, 2009a and 2009b); seedling reaction to stripe rust in greenhouse following the 0–9 Scale (McNeal et al. 1971); and leaf tip necrosis (present or absent in leaf rust environment 3)

	Environment 1	Environment 2	Environment 3	Environment 4
Leaf rust severity	0.77	0.86	0.81	
Stripe rust severity	0.91	0.90	0.93	0.81
Stripe rust seedling reaction	0.79	–	–	–
Leaf tip necrosis	–	–	0.99	–

Table 6 QTLs identified in the Avocet × Pastor F_5 RIL population by estimating the multi-environment 'Logarithm of odds' (LOD) and the 'Phenotypic explained variance' (PEV) for each environment

Resistance source	Chromosome	Flanking markers	LOD LR	PEV			LOD YR	PEV			
				2000 LR	2002 LR	2004 YR		2000 YR	2002 YR	2009a YR	2009b
Pastor	1AL	*wPT-6005-wPT-4709*	–	–	–	–	4.9	4.1	–	3.6	–
Pastor	1BSa	*wPT-5580-wPT-3179*	4.4	4.1	6.7	6.1	–	–	–	–	–
Pastor	1BSb	*wPT-8168-wPT-6240*	–	–	–	–	6.6	5.1	5.0	–	–
Pastor	1BL	*Lr46/Yr29-Xgwm818*	31.2	17.5	25.4	16.7	23	15.1	16.4	16.3	22.1
Avocet	2A	*wPT-4419-wPT-8226*	3.8	–	−7.2	-5.8	–	–	–	–	–
Pastor	2BS	*Yr31*	6.3	5.2	9.6	5.4	60.0	17.9	13.8	15.2	8.2
Avocet	2D	*wPT-8319-wPT-3728*	4.8	−3.8	−9.8	−7.4	–	–	–	–	–
Avocet	3A	*wPT-6422-wPT-7890*	–	–	–	–	11.0	–	−2.4	–	−3.2
Pastor	3B	*wPT-2458-wPT-0036*	–	–	–	–	16.2	4.6	5.8	3.8	–
Pastor	4DS	*wPT-4572-wPT-6880*	–	–	–	–	11.8	4.9	–	2.8	–
Pastor	5AL	*wPT-0837-wPT-5231*	–	–	–	–	13.2	3.9	6.6	–	–
Avocet	5AL	*wPT-0373-wPT-0837*	5.5	−7.4	−6.8	−5.2	–	–	–	–	–
Avocet	6AS	*wPT-2573-wPT-0959*	–	–	–	–	7.4	–	2.3	−2.0	−7.1
Pastor	6BL	*wPT-6329-wPT-5176*	5.3	6.6	10.8	5.4	5.6	–	3.9	2.3	4.4
Avocet	7AS	*wPT-4172-wPT-8149*	–	–	–	–	5.9	−0.3	−0.8	−3.2	−3.3
Avocet	7AL	*wPT-2260-wPT-2501*	–	–	–	–	9.1	−3.2	–	−3.4	−6.3
Pastor	7BL	*wPT-4342-wPT-8921*	7.6	11.5	7.4	3.8	–	–	–	–	–
Pastor	7BL	*wPT-3190-wPT-1475*	–	–	–	–	25.8	6.3	7.8	5.4	4.2

– indicates scores were not significant, negative PEV values indicate resistance is derived from Avocet

We identified *Lr46/Yr29* as having a large effect across all environments along with a number of minor QTLs contributing to resistance. Clearly not all loci contributed to disease severity at the same level, partially explaining the discrepancy between the expected gene numbers from the χ^2 analysis and that observed in the QTL study. Furthermore, when one or two of these minor QTLs are present in a line without other supporting resistance loci, they do not have an observable phenotypic effect under severe epidemic conditions. Such lines appear as HPTS and result in an underestimation of gene number in the χ^2 analysis. The PEV was high for all three leaf rust environments, indicating that most of the QTLs that contributed to reduction in disease severity were captured.

The χ^2 analysis for stripe rust was complicated by the large phenotypic effect of *Yr31*. When only two classes of disease severity were analysed, a minimum of five genes conferred resistance (Table 4). This fitted well with the fourth environment (2009b) data where the effect of *Yr31* was greatly reduced and χ^2 analysis showed a minimum of four were involved. The PEV (Table 5) was very high in the first three environments for stripe rust severity but dropped marginally in the 2009b environment, most likely due to the increased abundance of the *Yr31* virulent pathotype in the field when data were recorded 6 days later. It is expected that an effective race-specific resistance gene would significantly contribute to a high PEV, and that this contribution would drop as the gene was overcome.

In this study, we have identified a surprisingly high number of QTL and believe this is partly a function of the use of multi-environment data analysed with the program QTL Network 2.0. This methodology has great sensitivity

and has previously been shown to identify high numbers of minor QTLs for drought resistance in tetraploid wheat (Peleg et al. 2009). Rosewarne et al. (2008) compared single and multi-environment analyses in a bulked segregant analysis study using the same program and found one leaf and two to three stripe rust loci in a single environment analysis. Yet the multi-environment analysis of the same dataset identified three leaf rust loci and six stripe rust loci. Many of the loci identified in the current study have small but consistent effects and are likely to be important in developing durably resistant varieties.

The remainder of the discussion will focus on summarising genetic effects in each chromosomal group.

Group 1 QTLs

There was a small QTL for stripe rust severity on chromosome 1AL (LOD of 4.9) but it was only significant in years 2000 and 2009a. This was an interesting locus, despite its small effect, as it is the only gene reported for stripe rust severity on this chromosome. It is possible that this locus has been under reported due to its small effect, with excellent phenotyping and multi-environment analysis being required to show its significance.

There were a number of reported QTLs on 1B, the most significant of which was the *Lr46/Yr29* locus (Singh et al. 1998). This locus provided relatively high levels of protection against both leaf rust and stripe rust in all environments (Table 6), and accounted for LTN in the population. Similar phenotypic characteristics were also conferred by the cloned resistance gene *Lr34/Yr18* (Krattinger et al. 2009). They used mutation studies to show that a single gene has effects against leaf rust and stripe rust, as well as coding for LTN. It seems likely that the *Lr46/Yr29* locus contains a single gene. However, cloning and mutation studies are required to confirm this.

There were two other QTLs on 1B. The first QTL gave a LOD of 4.4 against leaf rust severity, and the second was for stripe rust severity and gave a LOD of 6.6. These loci correspond to a region that had previously been identified as containing a QTL for leaf rust severity in the winter wheat "Forno" (Messmer et al. 2000; Schnurbusch et al. 2004). Lin and Chen (2009) also identified a 1B QTL that contributed a small degree of resistance to stripe rust but they could not conclude whether this locus was *Lr46/Yr29*.

There are clearly multiple QTLs contributing to leaf rust and stripe rust resistance on chromosome 1B. To further clarify the relative locations of all published 1B QTLs (Suenaga et al. 2003; Lillemo et al. 2008; Schnurbusch et al. 2004; Messmer et al. 2000; Melichar et al. 2008; Lin and Chen 2009; Rosewarne et al. 2006; William et al. 2006; and this study), map positions of QTL-flanking markers were investigated on consensus maps (Fig. 1) sourced from the CMAP website (http://ccg.murdoch.edu.au/cmap/ccg-live/). As Pastor contained DArT markers not present in many of the maps, SSR markers closely linked to the DArT markers in other maps were used as reference points. Three consensus maps shown in Fig. 1 contained the majority of the markers. There are two clear clusters of markers. The first cluster is situated on chromosome 1BS and contains the flanking markers for leaf rust QTL from the winter wheat "Forno" and SSR markers linked to the DArT markers on QTL 1BSa and 1BSb (Table 6) in Pastor. The second cluster contains all markers that have previously been associated with the *Lr46/Yr29* locus. This consistent and clear differentiation indicates that there are at least two important loci on chromosome 1B for both diseases and that Pastor has important alleles in both of these regions. It seems likely that the HTAP gene described in Express (Lin and Chen 2009) is in a different position than the above-mentioned regions.

Group 2 QTLs

The most significant QTL in the group 2 chromosomes was associated with the moderately effective race-specific stripe rust resistance gene *Yr31* on 2BS. This had very high PEV scores, as *Yr31* was an effective resistance gene against stripe rust for most of this study and gave a seedling infection type (IT) (effective in all growth stages) ranging between 3 and 6 depending on the genetic background of RILs. Lines carrying this gene had rust scores of 0–40 in the years 2000, 2002 and 2009a. The observed variation depended on whether the individual lines had other genes complementing *Yr31*. When *Yr31* became ineffective (in the 2009b assessment), the final disease severity for the *Yr31* containing RILs increased by 30% on average, whereas disease severity increased only 14% for lines not carrying this gene. The PEV for the *Yr31* locus in 2009b was reduced by approximately 50% when compared to the other three environments indicating that this locus still had a minor effect despite the gene being overcome. This locus highlights the issue of working with mixed pathogen populations that can make effects of race-specific resistance genes appear like minor QTL.

The *Yr31* defined interval also had a small but significant effect on leaf rust severity (Table 6). It is known that in Pastor and Avocet, this region contains the resistance genes *Lr23* and *Lr13*, respectively. Glasshouse-based seedling tests were used to map these genes and in our study they mapped on two sides of *Yr31*. The field pathotypes used are completely virulent to both of these leaf rust resistance genes in seedling tests and in the adult plant stage. The minor leaf rust QTL observed in this region is therefore likely to be due to another resistance locus close to the aforementioned genes.

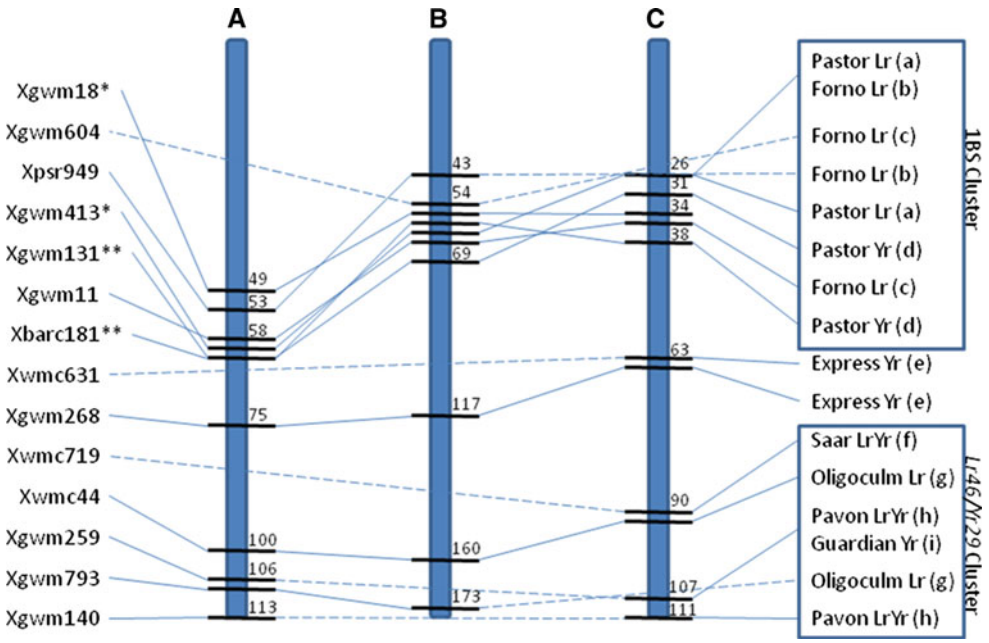

Fig. 1 Relative positions on consensus maps of flanking markers associated with leaf rust and stripe rust severity QTLs on chromosome 1B. Maps shown are **a** Consensus map August 11 2003, **b** Consensus Map July 2010 and **c** Somers Consensus Map (Somers et al. 2004). All positions are in cM and were taken from http://ccg.murdoch.edu.au/cmap/ccg-live/. Markers are shown to the *left* and QTL source, disease and reference are shown to the *right*. References are *a* this publication, *b* Messmer et al. (2000), *c* Schnurbusch et al. (2004), *d* this publication, *e* Lin and Chen (2009), *f* Lillemo et al. (2008), *g* Suenaga et al. (2003), *h* William et al. (2006), *i* Melichar et al. (2008). *Asterisk* indicates SSR markers that were used as reference points for *wPT-5580* (1BSa QTL). *Double asterisk* indicates SSR markers that were used as reference points for *wPT-6240* (1BSb QTL)

Two other leaf rust resistance QTLs, contributed by the susceptible parent Avocet, were identified in the group two chromosomes. The 2A and 2D QTLs had LOD scores of 3.8 and 4.8, respectively. Leaf rust resistance QTLs on 2AL have previously been identified by Schnurbusch et al. (2004) and on 2D by Xu et al. (2005). It is currently unknown if these are in the same region as the Pastor QTL.

Group 3 QTLs

The QTLs on 3A and 3B gave moderate LOD scores against stripe rust severity. The 3A was derived from Avocet and was inconsistent as it was only significant in two environments. The 3B QTL was derived from Pastor and was more consistent. The DArT markers defining the 3B QTL were *wPT-2458* and *wPT-0036*. Current consensus maps of the DArT markers could not directly associate either marker with known SSR markers. However, this chromosome does contain the slow-rusting gene *Yr30* that is linked, or pleiotropic, to the slow-rusting stem rust resistance gene *Sr2* and PBC phenotype. Because Pastor displays PBC, it is possible that we have identified *Yr30*. There are a number of previous studies identifying QTLs on both 3BS and 3BL. QTLs on 3BS have been identified by Singh et al. (2000b), Suenaga et al. (2003) and Khlestkina et al. (2007) and on 3BL by Lin and Chen (2009). It seems highly likely that the 3BS QTL of Khlestkina et al. (2007) and Singh et al. (2000b) are at the same locus as they are flanked by a common SSR marker locus (*Xgwm533.2*). Furthermore, all of the other markers defining these QTLs have close linkage with each other across many genetic maps. For example, in the ITMI mapping population, the marker locus *Xfba190* in Singh et al. (2000b) maps closely to marker loci (*Xgwm389* and *Xgwm533*) defining the (Suenaga et al. 2003) QTL, as well as the marker loci (*Xgwm493* and *Xgwm533*) of the Khlestkina et al. (2007) QTL. The markers defining the 3BL locus of Lin and Chen (2009) are not associated in any maps with the 3BS markers. At this stage, we are unable to determine if our QTL is in a similar location as those described above.

Group 4 QTL

There was only one QTL in the group 4 chromosomes and it was located on chromosome 4DS. The resistance allele was derived from Pastor and affected stripe rust severity (LOD 11.8). The QTL peak was situated on the DArT marker *wPT-6880*, and although this marker has been mapped in very few populations, the linked marker of *wPT-4572* (27 cM) had been loosely associated with *Rht-D1b* (*Rht2*) in the Ajana × 2074 population (http://ccg.murdoch.edu.au/cmap), suggesting a QTL location near the centromere of

chromosome 4D. Three studies have found genes for leaf and/or stripe rust resistance in this region. Singh et al. (2000b) identified an *Aegilops tauschii* Coss. derived race-specific gene *Yr28* near the centromere of 4DS, Suenaga et al. (2003) identified a leaf rust QTL and Herrera-Foessel et al. (2011) identified the dual resistance locus of *Lr67/Yr46* near the centromere of 4DL. The current data does not allow the Pastor QTL to be differentiated from these loci on the basis of chromosomal location, however, it is clearly not the (Suenaga et al. 2003) QTL as that was for leaf rust. Also the Pastor population did not have dual QTL for both leaf and stripe rust at this locus so it is unlikely to be *Lr67/Yr46* that has relatively large effects on both leaf rust and yellow rust resistance and is linked to strong LTN (Herrera-Foessel et al. 2011). Finally, the Pastor locus is unlikely to be *Yr28* as this is derived from *Ae. tauschii* and confers race-specific resistance effective in seedlings.

Group 5 QTLs

Chromosome 5A had a QTL for leaf rust severity (LOD 5.5) derived from Avocet and one for stripe rust severity (LOD 13.2) from Pastor. They were on the same linkage group with the QTL peaks mapping 88.6 cM apart. The Avocet allele had a LOD peak near *wPT-0837* and was flanked (within 30 cM) by *wPT-5231*. The latter marker has been reported to be tightly linked (2 cM) with *VrnA1* on 5AL in the Berkut/Krichauff population (Huynh et al. 2008). Maturity can have minor effects on rust scoring and it cannot be ruled out that the QTL we identified for leaf rust severity may be a result of altered maturity.

Group 6 QTLs

Chromosome 6AS contained a QTL affecting stripe rust severity, while 6BL contained a QTL affecting severity against both pathogens. The resistance allele for the QTL on 6AS was derived from Avocet and had been previously described (William et al. 2006; Lillemo et al. 2008). It is most likely located on the *Thinopyrum elongatum* translocation that carries the stem rust resistance gene *Sr26*.

The 6BL resistance allele was derived from Pastor and had LOD scores that were relatively low (5.3 and 5.6 for leaf rust and stripe rust, respectively). William et al. (2006) identified a QTL in Pavon that was effective against both leaf rust and stripe rust on 6BL and further investigation of the location of the DArT markers in Pastor suggest they were also on 6BL. One of the interval defining markers, *wPT-6329*, has been mapped in a Kukri × Janz population on 6BL along with a number of closely linked DArT markers and *Xgwm219* (http://ccg.murdoch.edu.au/cmap/ccg-live/cgi-bin/cmap).

Group 7 QTLs

Two QTLs for stripe rust severity were identified on different linkage groups and were located on chromosome arms 7AS and 7AL with both originating from Avocet. Both Zwart et al. (2010) and Dedryver et al. (2009) have identified QTLs for stripe rust severity on 7AS. The former had linked marker loci that indicate it is near the centromere (*Xwmc283*, *Xbarc108*) while the later appears to be more centrally located on 7AS (*Xfba127*). A consensus map "7A Consensus June 2011" (http://ccg.murdoch.edu.au/cmap/ccg-live/) indicates the Avocet 7AS marker loci are at the terminus of 7AS. It seems likely that it is a new QTL but finer mapping would be required to differentiate it from that of Dedryver et al. (2009). The 7AL QTL from Avocet was defined by the marker locus *wPT-2260*. Two maps (Ajana/WAWHT2074 and Cadoux/Reeves) described by Francki et al. (2009) indicate that *wPT-2260* is at the distal end of 7AL, differentiating this from the centromeric QTL of Zwart et al. (2010).

A 7BL linkage group had QTLs for both leaf rust and stripe rust severity and was inherited from Pastor. The QTLs were 21 cM apart, suggesting they are two closely linked loci. This close linkage is supported by several published genetic maps that anchor the two loci with common markers. For example, one of the DArT markers (*wPT-8921*) defining the leaf rust QTL, has been mapped to within 6 cM of *Xgwm577* (Arina × NK93604). Similarly, one of the stripe rust QTL defining DArT markers, *wPT-3190*, has been mapped to within 6 cM of the same SSR marker in a Cranbrook × Halberd population (Chalmers et al. 2001). The stripe rust locus was large in its effect on the disease, while the PEV for the leaf rust locus showed a minor effect on disease severity (Table 6).

Slow-rusting QTLs have previously been identified on 7BL for stripe rust severity (Suenaga et al. 2003; Lin and Chen 2007), leaf rust severity (Xu et al. 2005; Schnurbusch et al. 2004; Nelson et al. 1997) and both diseases (Rosewarne et al. 2008). The map positions of flanking markers in these studies are summarised in Fig. 2. Map data from two consensus maps and the ITMI Opata × synthetic population obtained from the CMAP website (http://ccg.murdoch.edu.au/cmap/ccg-live/) contained the majority of markers. There appears to be two clusters of markers in different genomic regions. On 7BS, there are the 'Oligoculm' stripe rust QTL (Suenaga et al. 2003) and one of the 'Forno' leaf rust markers (Messmer et al. 2000). The second cluster contains the linked leaf rust and stripe rust QTLs of Attila (Rosewarne et al. 2008) and Pastor (this study), as well as the leaf rust QTL of Xu et al. (2005). Interestingly there is another 'Forno' marker (Schnurbusch et al. 2004) between these clusters, as well as the HTAP of Alpowa (Lin and Chen 2007).

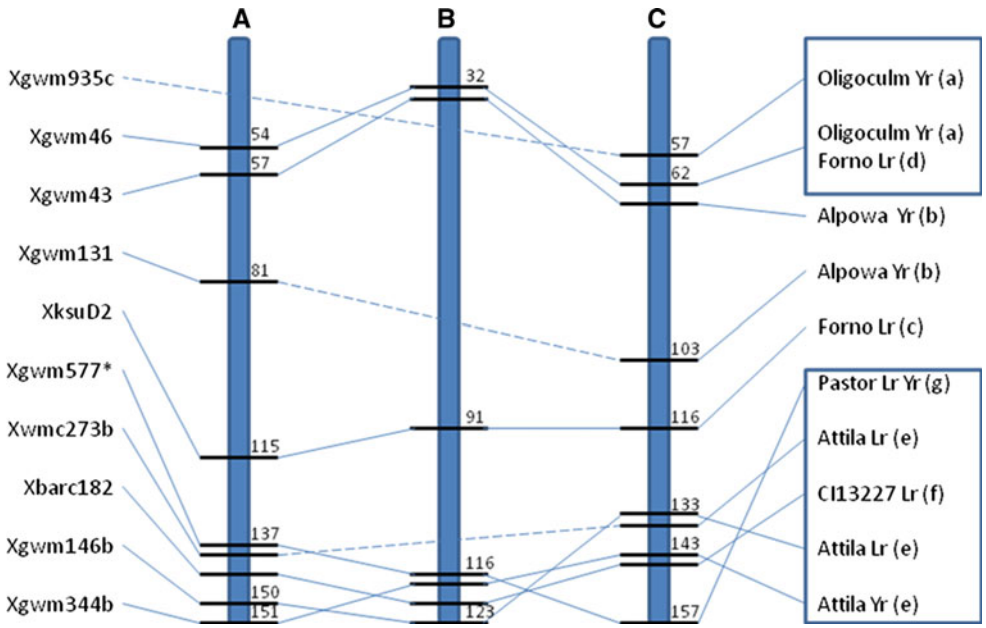

Fig. 2 Relative map positions of flanking markers of QTL associated with leaf rust and stripe rust severity on chromosome 7B. Maps shown are **a** Somers Consensus Map (Somers et al. 2004), **b** Synthetic x Opata, and **c** Consensus Map 2010. All positions are in cM and were taken from http://ccg.murdoch.edu.au/cmap/ccg-live/. Markers are shown to the left and QTL source, disease and reference are shown to the right. References are *a* Suenaga et al. (2003), *b* Lin and Chen (2007), *c* Schnurbusch et al. (2004), *d* Messmer (2000), *e* Rosewarne et al. (2008) *f* Xu et al. (2005) and *g* this publication. *Asterisk* indicates SSR marker that were used as reference points for *wPT-4342*, *wPT-8921* and *wPT-3190* (7BL QTLs)

Both Pastor and Attila (Rosewarne et al. 2008) contain closely linked leaf rust and stripe rust severity QTLs (11.8 cM in Attila, 19.2 cM in Pastor) and as shown in Fig. 2, they are in similar chromosomal locations. Both Attila and Pastor have a common parent 'Seri M82' that has pedigree Kavkaz/Buho//Kalyansona/Bluebird. Kalyansona/Bluebird is a known slow-rusting parent and it seems likely that the 7BL QTL may have been inherited from this source in both varieties through Seri M82. Furthermore, the CIMMYT breeding program selects for leaf rust and stripe rust resistance in alternate segregating generations, a process that enhances selection for chromosomal regions with dual-linked resistances.

Conclusion

Our study has identified multiple QTLs associated with APR to leaf rust and stripe rust. Genetic analysis initially indicated that there would be at least four and five loci involved with leaf rust and stripe rust resistance, respectively. However, this is probably an underestimation as extreme epidemics as used in this study can make differentiation of truly susceptible lines difficult. The uniform epidemic conditions facilitated the identification of eight and 13 QTLs that reduced leaf rust and stripe rust severities, respectively. Many of these QTLs had quite minor effects but it is presumed they were identified due to consistently heavy epidemic conditions optimising power in the multi-environment analysis. Several chromosomal regions identified dual resistances that had effects against both diseases. *Lr46/Yr29* was highly significant on both diseases. The *Yr31* region also had extremely high LOD scores for stripe rust severity, however, there was genotype × environment interaction at this locus when a new virulent pathotype was used in the field study of 2009b and disease data were recorded when the leaves of the susceptible parent were almost necrotic. Furthermore, this locus also identified a QTL for leaf rust severity. The 7BL locus was particularly interesting as it contained closely linked loci to both diseases, and is probably identical to that identified by Rosewarne et al. (2008) in a study with Attila. Finally, amongst the minor QTLs, three new regions for stripe rust severity (1A, 2BS and 7AL) were identified.

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References

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A, Henschke PH, Pierens S, Harker N, Pallotta M, Cornish GB, Shariflou R, Rampling LR, McLauchlan A, Daggard G, Sharp PJ, Holton TA, Sutherland MW, Appels R, Langridge P (2001) Construction of

three linkage maps in bread wheat, *Triticum aestivum*. Aust J Agr Res 52:1089–1119

Churchill DA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M, Nègre S, Verplancke G, Jahier J (2009) Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. Phytopathology 99:968–973

Flor HH (1956) The complementary genic systems in flax and flax rust. Adv Genet 8:29–54

Francki MG, Walker E, Crawford AC, Broughton S, Ohm HW, Barclay I, Wilson RE, McLean R (2009) Comparison of genetic and cytogenetic maps of hexaploid wheat (*Triticum aestivum* L.) using SSR and DArT markers. Mol Genet Genomics 281:181–191

Hayden MJ, Nguyen TM, Waterman A, McMichael GL, Chalmers KJ (2008) Application of multiplex-ready PCR for fluorescence-based SSR genotyping in barley and wheat. Mol Breeding 21:271–281

Heath M (2000) Hypersensitive response-related death. Plant Mol Biol 44:321–334

Herrera-Foessel SA, Lagudah ES, Huerta-Espino J, Hayden MJ, Bariana HS, Singh D, Singh RP (2011) New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor Appl Genet 122:239–249

Hoisington D, Khairallah M, Gonzalez-de-Leon D (1994) Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory, 2nd edn. CIMMYT, Mexico

Huynh B-L, Wallwork H, Stangoulis JCR, Graham RD, Willsmore KL, Olson S, Mather DE (2008) Quantitative trait analysis for grain fructan concentration in wheat (*Triticum aestivum* L.). Theor Appl Genet 117:701–709

Khlestkina EK, Röder MS, Unger O, Meinel A, Börner A (2007) More precise map position and origin of a durable non-specific adult plant disease resistance against stripe rust (*Puccinina striiformis*) in wheat. Euphytica 153:1–10

Krattinger SG, Lagudah ES, Speilmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjørnstad Å (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155–1166

Lin F, Chen XM (2007) Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor Appl Genet 114:1277–1287

Lin F, Chen XM (2009) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 118:631–642

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute Inc., Cary

Manly KF, Cudmore RH Jr, Meer JM (2001) Map manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS (1971) A uniform system for recording and processing cereal research data. USDA-ARS Bull, Washington, pp 34–121

Melichar JPE, Berry S, Newell C, MacCormack R, Boyd LA (2008) QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian. Theor Appl Genet 117:391–399

Messmer MM, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet C, Keller B (2000) Genetic analysis of durable leaf rust resistance in winter wheat. Theor Appl Genet 100:419–431

Nelson JC, Singh RP, Autrique JE, Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928–1935

Peleg Z, Fahima T, Krugman T, Abbo S, Yakir D, Korol AB, Yehoshua S (2009) Genomic dissection of drought resistance in durum wheat × wild emmer wheat recombinant inbreed line population. Plant Cell Environ 32:758–779

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale of estimating rust severity on leaves and stems of cereals. Can J Res Sec C 26:496–500

Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico

Rosewarne GM, Singh RP, Huerta-Espino J, William HM, Bouchet S, Cloutier S, McFadden H, Lagudah ES (2006) Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor Appl Genet 112:500–508

Rosewarne GM, Singh RP, Huerta-Espino J, Rebetzke GJ (2008) Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. Theor Appl Genet 116:1027–1034

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B (2004) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major QTL in the Lr34 chromosomal region. Theor Appl Genet 108:477–484

Singh RP (1991) Pathogenicity variations of *Puccinia recondite* f. sp. *tritici* and *P. graminis* f. sp. *tritici* in wheat-growing areas of Mexico during 1988 and 1989. Plant Dis 75:790–794

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890–894

Singh RP, Huerta-Espino J, Rajaram S (2000a) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathol Hun 35:133–139

Singh RP, Nelson JC, Sorrells ME (2000b) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155

Singh RP, William HM, Huerta-Espino J, Crosby M (2003) Identification and mapping of gene *Yr31* for resistance to stripe rust in *Triticum aestivum* cultivar Pastor vol 1. In: Pogna NE, Romana M, Pogna EA, Galterio G (eds) Proceedings of the 10th International Wheat Genet Symp, Instituto Sperimentale per la Cerealicoltura, Rome, Italy, pp 411–413

Somers DJ et al (2004) A high-density wheat microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Stakman EC, Stewart DM, Loegering WQ (1962) Identification of physiological races of *Puccinia graminis* var. *Tritici*. US. Agric Res Serv ARS E617:1–53

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890

Van Os H, Stam P, Visser RGF, Van Eck HJ (2005) RECORD: a novel method for ordering genetic loci on a genetic linkage map. Theor Appl Genet 112:30–40

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterisation of genetic loci conferring adult plant resistance to leaf and stripe rust in spring wheat. Genome 49:977–990

Xu XY, Bai GH, Carver BF, Shaner GE, Hunger RM (2005) Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat. Theor Appl Genet 110:244–251

Yang J, Hu CC, Ye XZ, Zhu J (2005) QTLNetwork 2.0. Institute of Bioinformatics, Zhejiang University, Hangzhou, China (http://ibi.zju.edu.cn/software/qtlnetwork)

Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. Mol Breeding 26:107–124