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Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis

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Abstract Rust diseases are a major cause of yield loss in wheat worldwide, and are often controlled through the incorporation of resistance genes using conventional phenotypic selection methods. Slow-rusting resistance genes are expressed quantitatively and are typically small in genetic effect thereby requiring multiple genes to provide adequate protection against pathogens. These effects are valuable and are generally considered to confer durable resistance. Therefore an understanding of the chromosomal locations of such genes and their biological effects are important in order to ensure they are suitably deployed in elite germplasm. Attila is an important wheat grown throughout the world and is used as a slow-rusting donor in international spring wheat breeding programs. This study identified chromosomal regions associated with leaf rust and stripe rust resistances in a cross between Attila and a susceptible parent, Avocet-S, evaluated over 3 years in the field. Genotypic variation for both rusts was large and repeatable with line-mean heritabilities of 94% for leaf rust resistance and 87% for stripe rust. Three loci, including *Lr46/Yr29* on chromosome 1BL, were shown to provide resistance to leaf rust whereas six loci with small effects conferred stripe rust resistance, with a seventh locus having an effect only by epistasis. Disease scoring over three different years enabled inferences to be made relating to stripe rust pathogen strains that predominated in different years.

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Introduction

Leaf rust and stripe rust caused by *Puccinia triticina* and *P. striiformis* f. sp. tritiici, respectively, cause major yield losses in wheat worldwide. The most cost effective and environmentally safe means by which these diseases can be controlled is through the use of resistance in commercial cultivars. The complementary gene-for-gene interaction described by Flor (1956) enhanced the understanding of race-specific rust resistance genes and subsequently their utilization in controlling rust pathogens. These genes elicit a hypersensitive response in the host upon infection by a pathogen race that possesses the corresponding avirulence allele. However, this kind of interaction also imposes a strong selection pressure on the pathogens to overcome resistance. In contrast, horizontal, slow-rusting or partial resistance mechanisms are considered to be long lasting or durable (Van der Plank 1963; Caldwell 1968; Johnson 1988; Parlevliet 1975). Slow-rusting resistance functions by increasing latent period and reducing uredinial size, infection frequency and spore production (Caldwell 1968; Ohm and Shaner 1976; Parlevliet 1975).

Identification of multigenic resistance against the wheat rusts goes back to the late nineteenth century, when Farrer (1898) observed transgressive segregation of resistance against rusts in progeny of certain crosses. Caldwell et al. (1957) and Johnson (1988) described slow rusting or

durable resistance against leaf rust and stripe rusts, respectively. Slow-rusting resistances to both diseases were also identified in spring bread wheat (*Triticum aestivum* L.) germplasm of CIMMYT (Singh and Rajaram 1991, 1994), where such resistance has been a major target for selection for over 30 years. Singh et al. (2000) developed wheat lines that have near-immune levels of adult-plant resistance based on 4–5 slow-rusting genes that have small to intermediate, but cumulative, effects.

To more effectively develop and deploy resistance based on diverse slow-rusting genes, it is important to determine their chromosomal locations and develop diagnostic markers for marker-assisted selection. Two independent loci, *Lr34/Yr18* on chromosome 7DS (Dyck 1987) and *Lr46/Yr29* on 1BL (Singh et al. 1998) confer slow-rusting resistance to both leaf and stripe rusts. As these and other slow-rusting genes function additively, quantitative trait locus (QTL) analysis has been employed to identify at least 18 loci with slow-rusting effects against leaf rust on all wheat chromosomes except 1A, 3D, 6B, 6D and 7A (Faris et al. 1999; Messmer et al. 2000; Navabi et al. 2005; Schnurbusch et al. 2004; Singh et al. 2005; Suenaga et al. 2003; William et al. 1997; Xu et al. 2005a, b). Similarly, at least 15 loci for stripe rust resistance have been reported (Bariana et al. 2001; Borner et al. 2000; Boukhtem et al. 2002; Mallard et al. 2005; Navabi et al. 2005; Ramburan et al. 2004; Singh et al. 2000, 2005; Suenaga et al. 2003; William et al. 2003), illustrating the diversity for these types of resistance genes in wheat germplasm.

Attila is an important parent in the rust-resistance breeding program of CIMMYT and is currently grown on millions of hectares throughout India under the pseudonym PBW343. Attila was previously shown to contain at least two and three genes that conferred resistance to leaf rust and stripe rust, respectively (Rosewarne et al. 2006). Initial molecular analysis identified a highly significant QTL on chromosome 1BL in the region corresponding to *Lr46/Yr29* and the linked phenotype of leaf tip necrosis (LTN).

The objective of our study was to identify genomic regions of other minor, slow-rusting resistance genes associated with leaf rust and stripe rust reactions in a mapping population developed from the slow-rusting resistant Attila and susceptible Avocet-S wheat varieties.

Materials and methods

Field analyses

Development of the Avocet-S × Attila population was described in Rosewarne et al. (2006). This population contains 148 F_2-derived, F_5 recombinant inbred lines (RILs). In Mexico, Avocet-S is susceptible to both leaf rust and stripe rust, whereas Attila shows moderate levels of slow rusting resistance to both rusts. The parents and RILs were evaluated in the field for reaction to stripe and leaf rusts for 3 years under artificial epidemics as described in Rosewarne et al. (2006). Briefly, stripe rust and leaf rust assessments were made at CIMMYT research stations in Toluca near Mexico City and Ciudad Obregon in Sonora State, Mexico, respectively. The parents and RILs were sown on 75-cm-wide raised beds in paired-row plots, 1 m in length, with 20 cm between rows and with a 50-cm pathway. Rust epidemics were initiated about 4 weeks and 8 weeks after planting (stripe rust and leaf rust, respectively) by inoculating susceptible spreader rows of cv. Morocco planted as hills adjacent to the pathway. To initiate the epidemics, Morocco was sprayed with a suspension of rust urediniospores in the lightweight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX, USA). The leaf rust and stripe rust strains used were virulent for all common seedling resistance genes in CIMMYT germplasm. The percent rust severity for each plot was evaluated for three crop seasons (2000, 2002 and 2003 for stripe rust and 1999–2000, 2001–2002 and 2003–2004 for leaf rust) according to the modified Cobb Scale (Peterson et al. 1948).

Molecular analysis

Details of DNA extraction, amplified fragment length polymorphism (AFLP) and microsatellite analysis are also described in Rosewarne et al. (2006). For bulked segregant analysis (BSA), three distinct bulks were obtained by pooling DNA from 12 leaf rust resistant RILs, 10 stripe rust resistant RILs, and 10 susceptible (both leaf rust and stripe rust) RILs, respectively. Initially, 208 AFLP primer combinations using *Pst1/Mse1* restriction enzyme sites were applied to bulks to identify polymorphisms. Rosewarne et al. (2006) identified the *Lr46/Yr29* locus by this procedure. In the present study, the work was expanded further using a genetically subtracted bulk to identify any other leaf rust resistance loci. Stripe rust resistance was not investigated in the genetic subtraction due to the higher level of genetic complexity of this trait (Rosewarne et al. 2006). The genetically subtracted leaf rust resistance bulk was developed by the removal of all RILs containing the flanking markers for the *Lr46/Yr29* locus. Of the remaining population, the nine lines most resistant to leaf rust (average 61% leaf area infected) were reselected as the genetically subtracted bulk. The susceptible bulk consisted of 15 lines highly susceptible to leaf rust. A further 178 *Pst1/Mse1* AFLP primer combinations were applied to these bulks. When primer combinations gave different amplification products between those bulks, the reactions were run on the individual lines making up those bulks, and subsequently on

the entire population. AFLP bands were named as defined by KeyGene, http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html and primer sequences can be obtained from this web site.

To localize important markers to chromosomes, the AFLP primer combinations were run on several densely mapped populations including the Opata × Synthetic (Röder et al. 1998), Oligoculm × Fukuho-komugi (Suenaga et al. 2005), Frontana × Inia 66 (unpublished, but see Ayala et al. 2002) and Cranbrook × Halberd (Chalmers et al. 2001) populations. Microsatellite markers were then selected from associated regions and run on the Avocet-S × Attila population to confirm the location of the AFLP markers.

Simple sequence repeat (SSR) markers were amplified from approximately 50 ng of genomic DNA in PCR amplifications using the recommended annealing temperatures for the respective SSR markers. Visualization of the amplified SSR products was by using agarose gel electrophoresis (3%) coupled with ethidium bromide staining.

Statistical and genetical analyses

The 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 heritabilities (h^2) were calculated and expressed on a line-mean and single environment basis.

Genetic linkage maps and single locus associations were determined with Map Manager QTX Version 20 using linkage criteria set at $P = 0.001$ and the Kosambi mapping function. QTL analysis was undertaken for each environment separately and then across environments using mixed linear composite interval mapping in QTLNetwork 2.0 (Yang et al. 2005). Composite interval analysis 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. Significant 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

Analysis of field rust reactions

Variation among lines in this population showed a continuous distribution in the field for leaf rust and stripe rust reactions (Rosewarne et al. 2006). As field inoculated races were virulent to all major seedling resistances, low rust reactions were likely to result from slow-rusting resistance genes. The disease scores for the parents, population means, population maxima and minima for all environments are listed in Table 1. Leaf rust or stripe rust scores were taken from similar locations in different years and observed differences could be interpreted as genotype × year interactions. However, in keeping with standard statistical nomenclature, we described this study as a multi-environment analysis, with environments representing results obtained in a similar location but from different years.

Table 1 Summary of rust reactions for the Avocet-S × Attila RIL population (% average leaf area covered by rust)

	Leaf rust			Stripe rust		
	2000	2002	2004	2000	2002	2003
Avocet-S	100	100	80	90	90	100
Attila	10	5	1	15	10	10
Population mean	49	52	30	52	37	54
Range low	1	1	1	5	1	5
Range high	100	100	90	100	100	100
$\sigma^2_{\text{Genotype}}$	878 ± 109**		344 ± 46**			
$\sigma^2_{\text{Genotype} \times \text{environment}}$	170 ± 14**		157 ± 13**			
h^2_{LM} (h^2_{SE})	94 (84)		87 (69)			

Scores are given for the parents, population means, and highest and lowest scoring lines in each environment. Genetic and genotype × environment interaction variances (± standard errors), and narrow-sense heritabilities on a line-mean (h^2_{LM}) and single-environment (h^2_{SE}) basis are included. $^{**}P < 0.01$

Attila, the resistant parent, consistently scored very low for both leaf rust and stripe rust, and Avocet-S scored high. Table 1 also shows that the genotype and genotype × environment interaction variances were significantly different from zero. The genotype × environment variance was approximately one-half the genotypic variance for stripe rust, indicating strong interaction of genotype with environment. Indeed the correlation (r_p) of stripe rust scores across years ranged between 0.46 and 0.79. In contrast, genotype × environment interaction was approximately 20% as large as the genotypic variance for leaf rust indicating a strong correlation ($r_p = 0.91$–0.93) of genotype performance across environments.

Lines selected for the initial bulked segregant analysis had average rust scores of 6.1 and 11.7% for the two resistant bulks (leaf rust and stripe rust, respectively). The single susceptible bulk was highly susceptible to both diseases (96% for leaf rust and 81% for stripe rust). The genetically subtracted bulk (from lines that did not contain *Lr46/Yr29*) scored an average of 61% for leaf rust in the resistant bulk and 96% for leaf rust in the susceptible bulk.

Molecular mapping of the Avocet-S × Attila RIL population

The initial BSA identified 31 AFLPs associated with resistance or susceptibility by their near co-segregation with lines making up the bulks. A further eight markers were identified with the genetically subtracted BSA. However many of these were shown to be false positives when analysed against the entire population. Single marker regression showed that 17 of these markers were significantly associated with stripe rust or leaf rust reactions in one or more environments. A number of these markers mapped into two main linkage groups on chromosomes 1BL and 2BS (Fig. 1). The identification of AFLP markers on chromosome 1BL was described in Rosewarne et al. (2006). The 2BS linkage group contained AFLP markers from both the original BSA and the genetically subtracted BSA (*XP32/M62*) along with five chromosome 2BS SSR markers (Fig. 1). The AFLP marker *XP34/M59* from the 2BS linkage group and a third unlinked marker, *XP32/M59,* were localized by mapping in the Opata × Synthetic mapping population. Localizations to 2BS and 7BL, respectively was confirmed through the application of appropriate SSR markers to the Avocet-S × Attila population (Fig. 1). There were five other small linkage groups, but none showed a significant association with leaf rust or stripe rust reaction. Five AFLP markers could not be mapped and two of them showed an association with stripe rust reaction.

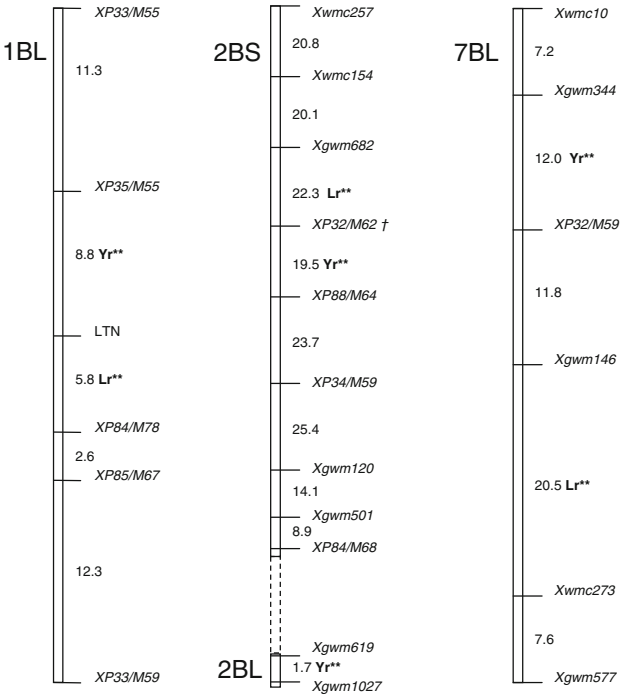

Fig. 1 Linkage maps of loci identified through bulked-segregant analysis and genetic subtraction (†). Intervals with significant leaf rust (Lr) and stripe rust (Yr) reducing effects are marked with * for one or two environments or ** for all environments

QTL analysis of leaf rust reaction

An initial single environment QTL analysis using leaf rust scores from the years 2000, 2002 and 2004 identified only a single QTL associated with variation in leaf rust score (Table 2). This locus was on chromosome 1BL and was shown to be *Lr46/Yr29* (Rosewarne et al. 2006). However, a multi-environment analysis for leaf rust reactions identified two other loci as having small but consistent effects (Table 3). A locus on chromosome 2BS was identified on the interval *Xgwm682-XP32/M62* and gave an additive effect of +4.4% for leaf rust and the effect was repeated across environments. Another locus on chromosome 7BL, defined by the SSR markers *Xwmc273-Xgwm146*, gave an additive effect of +3.0% for leaf rust, and was significant in all years.

QTL analysis of stripe rust reaction

The single environment analysis (Table 2) of stripe rust reaction in each tested environment also identified *Lr46/Yr29* as described in Rosewarne et al (2006). A QTL on chromosome 2BS accounted for significant levels of resistance in 2002, but was not present in the other 2 years. Conversely, a locus on chromosome 2BL derived from Avocet-S, was observed in the stripe rust data from 2000 and 2003. A fourth locus conferred by an unmapped and

Table 2 Single environment QTL analysis for leaf (LR) and stripe (YR) rust reaction in the Avocet-S × Attila RIL population

QTL interval/marker	Chromosome location	Lr 2000	Lr 2002	Lr 2004	Yr 2000	Yr 2002	Yr 2003
LTN-*XP35/M55*	1BL	26.8	30.1	25.7	13.8	12.4	17.4
XP88/M64-XP32/M62	2BS	ns	ns	ns	ns	7.7	ns
Xgwm1027-Xgwm619	2BL	ns	ns	ns	−5.2	ns	−5.8
XP33/M61	nd	ns	ns	ns	5.0	ns	ns

Estimated additive effects are given for significant QTL. Negative values indicate the resistance allele was derived from the susceptible parent

nd not determined, *ns* not significant

Table 3 Multi-environment QTL analysis for leaf rust (LR) reaction in the Avocet-S × Attila population

QTL interval	Chromosome location	Lr All	Lr 2000	Lr 2002	Lr 2004
XP84/M78-LTN	1BL	27.2	−0.5	1.5	−1.1
Xgwm682-XP32/M62	2BS	4.4	0.0	0.0	0.0
Xwmc273-Xgwm146	7BL	3.0	−0.4	1.4	−1.0

Estimated additive effects are given for significant leaf rust reaction QTL in all environments and deviations from this value for individual environments

Table 4 Multi-environment QTL analysis for stripe rust (YR) reaction in the Avocet-S × Attila RIL population

QTL Interval/marker	Location	Yr All	Yr 2000	Yr 2002	Yr 2003
LTN-*XP35/M55*	1BL	13.2	−0.7	−1.1	1.8
XP32/M62-XP88/M64	2BS	6.5	−1.8	2.8*	−1.0
Xgwm1027-Xgwm619	2BL	−4.5	−0.6	1.5	−1.0
XP32/M59-Xgwm344	7BL	3.1	0.0	0.0	0.0
XP87/M68b-XP85/M67b	nd	−3.1	0.0	0.0	0.0
XP33/M61	nd	4.8	2.3*	−3.3**	1.0

Estimated additive effects are given for significant stripe rust reaction QTL in all environments and deviations from this value for individual environments. Negative values for "Yr all" indicate the resistance allele is derived from the susceptible parent.

* significantly different from mean additive effect at $P < 0.1$ and

** at $P < 0.01$

nd not determined

Table 5 Pearson-moment correlations for presence of alleles between flanking markers for two putatively epistatic loci affecting leaf rust reaction in the Avocet-S × Attila RIL population

	XP35/M55	*XP33/M55*	*XP38/M49b*	*XP38/M49c*
XP35/M55	1	0.68	0.06	0.02
XP33/M55		1	0.10	0.10
XP38/M49b			1	0.97
XP38/M49c				1

unlinked AFLP marker was shown to be effective in year 2000. The multi-environment analysis (Table 4) also identified the above loci but showed that they generally had effects across all seasons. Two extra loci that had small but consistent effects against stripe rust were identified in all environments. A chromosome 7BL allele derived from Attila gave an additive effect of +3.1 and another minor QTL came from a small linkage group of AFLP markers that could not be localized. The latter QTL was derived from Avocet-S. The multi-environment analysis also showed that the single, unmapped AFLP marker *XP33/M61*, had pronounced effects against stripe rust in 2000 and 2003.

There was evidence for an epistatic interaction between an interval on chromosome 1BL defined by the marker interval *XP35/M55-XP33/M55* and an unmapped AFLP linkage group *XP38/M49b-XP38/M49c*. This interaction accounted for about 7% of the phenotypic variance for stripe rust reaction, had a negative additive effect (decreased the infection level) and was derived from the Avocet-S parent. It was repeatable over all three environments. Table 5 shows the correlation of the four markers making up to two intervals, indicating that the linked markers were highly correlated with each other while unlinked markers were poorly correlated.

Discussion

Genetic analysis of the Avocet-S × Attila RIL population indicated that there were at least two additive genes involved in slow-rusting resistance to leaf rust and three for stripe rust reaction (Rosewarne et al. 2006) and that the *Lr46/Yr29* locus was the main contributor to this resistance. In addition to the *Lr46/Yr29* locus, the initial BSA identified linkage groups in chromosomes 2BS, 2BL and 7BL that had small but significant effects on reaction to either or both diseases (Fig. 1). Furthermore, another AFLP marker was identified through the genetic subtraction approach (see "Results") that mapped to the 2BS linkage group. Two unlinked and unmapped loci were also identified that

were likely to have an effect on stripe rust reaction. Each of these loci are discussed below.

Leaf rust reaction

AFLP markers from one Avocet-S × Attila linkage group was mapped to 2B using the Opata × Synthetic population. We then identified microsatellite markers that were polymorphic in the slow-rusting population and widely distributed across the 2B chromosome. These markers formed two distinct linkage groups, one of which had effects on responses to both diseases and the other only against stripe rust. The first QTL, located on 2BS, had a significant effect against leaf rust in all three environments. This locus could not be any of the previously named 2BS leaf rust resistance genes, *Lr13*, *Lr16*, *Lr23* and *Lr35* (McIntosh et al. 1995), as either the rust races used were virulent to these genes (*Lr13, Lr16, Lr23*) or Attila did not carry the genes (*Lr35*).

The QTL *QLrlp.ous-2B* was designated on the basis that it extended latent period of leaf rust infection (Xu et al. 2005a) and decreased the area under disease progress curve (AUDPC), final severity and infection frequency (Xu et al. 2005b). Messmer et al. (2000) identified a 2BS QTL derived from the winter wheat variety Forno from field studies and suggested it may be allelic to *Lr13*. Our studies suggest that the 2BS QTL from Attila is in the region of the *Lr13* locus; however, a lack of molecular polymorphisms prevented finer mapping. Our study further confirms that the 2BS region is important in quantitative resistance to leaf rust.

The chromosome 7B locus was identified through a single AFLP marker and confirmed with microsatellite markers located on 7BL. This locus was derived from the Attila parent and significantly reduced disease severity in all three leaf rust environments. A number of QTL studies have identified loci on 7B giving resistance to leaf rust [William et al. (1997); Messmer et al. (2000); Xu et al. (2005a, b); Farris et al. (1999); Nelson et al. (1997)] as well as the *Lr14ab* complex (McIntosh et al. 1995), indicating the importance of this region in leaf rust resistance.

Stripe rust reaction

The single environment analysis identified a 2BS interval that gave a highly significant QTL for stripe rust in 2002. Field notes show that in 2002, an endemic stripe rust race with avirulence to *Yr27* initially infected the field, and this was followed by the inoculated race that was virulent to *Yr27*. The 2002 stripe rust QTL was attributed to *Yr27* as this gene is located on chromosome 2BS and is present in Attila. Furthermore, the multi-environment analysis indicated that the 2BS interval had a significant effect across all environments, although the effect in 2002 was stronger. The most likely reason for the *Yr27* region having small effects on stripe rust in 2000 and 2003 is that the endemic *Yr27* avirulent pathotype was present at low levels and the multi-enviroment analysis was able to detect avirulence in a small portion of the mixed rust population.

A significant QTL at the distal end of chromosome 2BL had an allele derived from the susceptible parent and contributed a significant resistance effect in 2000 and 2003 in the single-environment analysis. This appeared to be a race-specific gene conferring resistance to the race inoculated into the field in those years. However, the multi-environment analysis showed this locus to be significant in all environments, probably reflecting the mixed nature of field pathogens that occurred late in 2002. The only stripe rust resistance genes on 2BL are the possibly allelic *Yr5* and *Yr7* (Bariana et al. 2001; Mallard et al. 2005) but as these are not present in Avocet-S, we have identified a new race-specific QTL for stripe rust. Slow-rusting mechanisms are often assumed to be non-race specific; however, this is not always the case. For example, McIntosh et al. (1995) and references therein, described the adult plant stripe rust resistance genes, *Yr11*, *Yr13* and *Yr14* as having variable responses to different pathotypes.

The 2BL locus was the only one that was chromosomally localised and shown to be derived from the susceptible parent. However, Singh et al. (2005) identified a minor QTL for both leaf rust and stripe rust resistances on 6A in Avocet-S but not on 2BL. As both studies utilized partial linkage mapping, the unidentified loci in each of the corresponding studies could be a reflection of limitations of this approach in identifying all minor QTL.

We have hypothesised that fluctuations in the 2BL QTL are due to race-specificity. Another explanation could be that less than optimal conditions for stripe rust infections prevailed in 2002 leading to a loss of significance for the 2BL QTL during 2002 in the single environment analysis. Although this cannot be ruled out, there are multiple lines of evidence to suggest that this was not the case. For example, Table 1 showed consistent levels of stripe rust infection of Avocet-S and Attila across the different years. The population means were also shown in this table and although the population mean for 2002 was significantly lower that in 2000 and 2004, this difference was entirely accounted for by the additive effect of the 2BS locus in 2002 (7.7% in Table 2). Furthermore, plots of near-isogenic Avocet containing *Yr27* indicated a *Yr27* avirulent pathogenic incursion early in 2002 which was followed by the spread of the inoculated, *Yr27* virulent stripe rust pathotype. Finally, the more sensitive multi-environment analysis identified significant QTL for both the 2BS and the 2BL loci across all environments, but the 2002 effect was significantly higher for the 2BS (*Yr27*) locus.

The multi-environment analysis also identified a small but significant QTL for stripe rust reaction flanked by *XP32/M59* and *Xgwm344* on chromosome 7BL. The same region of chromosome 7BL was previously identified by Suenaga et al. (2003) as having a stripe rust QTL, suggesting it is important in slow stripe rusting.

Two further stripe rust resistance loci were identified with AFLP markers, but the associated markers were not polymorphic in any of the mapping populations. The interval identified by the AFLP markers *XP87/M68b* and *XP85/M67* gave a small but highly consistent effect against stripe rust and was derived from the Avocet-S parent. This pattern was quite different from the 2BL locus that was also derived from the susceptible parent in that the 2BL effect varied substantially across environments. Another single AFLP marker, *XP33/M61* gave a resistance effect that was similar to the pattern for the chromosome 2BL QTL, in that resistance was most significant in the years 2000 and 2003. However, the single AFLP marker was derived from the Attila parent. As this was only a single marker, with no supporting linkage group, it is difficult to assess its importance.

An epistatic interaction for stripe rust resistance between the region around the *Lr46/Yr29* locus and another unmapped region was identified. In the statistical analysis, it is possible to confuse loosely linked markers as having epistatic interactions. This is of particular importance here as we were unable to map one of the intervals. Table 5 shows the poor correlation between markers from the two intervals involved in the interaction. This indicates that genetic control is not through simple additive genes, but that the resistance effects of one allele may be contingent on the presence of an appropriate allele at a second locus, that in itself does not contribute to resistance.

The stripe rust QTL analysis presented here shows the very complicated nature of slow-rusting resistance to stripe rust. Seven loci appeared to be involved, some of which were race-specific and some being derived from the susceptible parent, with the added complication of an epistatic effect of a locus with no phenotypic effect alone. Most of these QTL had relatively small effects on disease response, highlighting the value of phenotypic selection under epidemic field conditions and making marker development difficult.

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