Theor Appl Genet (2004) 108:1426–1433
DOI 10.1007/s00122-003-1567-7

ORIGINAL PAPER

V. P. Ramburan · Z. A. Pretorius · J. H. Louw · L. A. Boyd · P. H. Smith · W. H. P. Boshoff · R. Prins

A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega

Received: 11 July 2003 / Accepted: 4 December 2003 / Published online: 12 February 2004

Abstract The wheat cultivar Kariega expresses complete adult plant resistance to stripe rust in South Africa. The aim of this investigation was to determine the extent and nature of variability in stripe rust resistance in a population of 150 doubled haploid lines generated from a cross between Kariega and the susceptible cultivar Avocet S. Analysis of field data for adult plant stripe rust resistance identified two major QTLs and two minor QTLs in the resistant cultivar Kariega. The two major QTLs were located on chromosomes 7D (*QYr.sgi-7D*) and 2B (*QYr.sgi-2B.1*), contributing 29% and 30% to the phenotypic variance, respectively. *QYr.sgi-2B.1* is primarily associated with a chlorotic and/or necrotic response, unlike *QYr.sgi-7D*, which is believed to be the adult plant resistance gene *Yr18*. These two QTLs for adult plant resistance in Kariega appear to represent different forms of resistance, where *QYr.sgi-7D* may represent potentially more durable resistance than *QYr.sgi-2B.1*. Mixture model analysis of the field leaf infection scores suggested a genetic model involving two independent genes combining in a classical, epistatic manner. The results of the QTL analysis demonstrate its higher resolution power compared to the mixture model analysis by detecting the presence of minor QTLs.

Communicated by P. Langridge

V. P. Ramburan (✉) · R. Prins
ARC-Small Grain Institute,
P.O. Box 3507, Matieland 7602, South Africa
e-mail: viresh@infruit.agric.za
Tel.: +27-21-8093490
Fax: +27-21-8874171

Z. A. Pretorius
Department of Plant Sciences,
University of the Free State,
P.O. Box 339, Bloemfontein 9300, South Africa

V. P. Ramburan · J. H. Louw
Department of Genetics,
University of Stellenbosch,
Private Bag X1, Matieland 7602, South Africa

L. A. Boyd · P. H. Smith
Department of Disease and Stress Biology,
John Innes Centre,
Norwich, NR47UH, UK

W. H. P. Boshoff
ARC-Small Grain Institute,
Private Bag X29, Bethlehem 9700, South Africa

Introduction

Stripe (yellow) rust of wheat, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., was first detected in South Africa in 1996 (Pretorius et al. 1997). Since the initial detection in the Western Cape, the disease has spread to all wheat production areas, causing significant damage in epidemic years. Several locally bred cultivars are resistant to stripe rust, but resistance was incorporated inadvertently, as no breeding or selection for resistance was conducted prior to detection of the disease in 1996. The disease first occurred due to the introduction of pathotype 6E16A- (Pretorius et al. 1997; Boshoff et al. 2002a), but subsequent variants, 6E22A- (Boshoff and Pretorius 1999; Boshoff et al. 2002b) and 7E22A- (Boshoff, unpublished), were identified in 1998 and 2002, respectively.

Current indications are that resistance in many cultivars is monogenic (Bender and Pretorius 2001) and may have limited potential for durability. Evidence in this regard was provided by the early breakdown of monogenic resistance in the local cultivars Hugenoot and Carina during 1998 (Boshoff and Pretorius 1999). In Australia and New-Zealand, more than 20 *P. striiformis* f. sp. *tritici* pathotypes have been detected since the original single introduction in 1979 (Wellings and McIntosh 1990; Wellings et al. 2000). Stripe rust is also becoming increasingly important in the USA where 42 pathotypes, of which 21 are new virulence combinations, were detected (Chen et al. 2002).

Several APR genes have been identified, including their chromosomal location (http://www.cdl.umn.edu/). *Yr18* was mapped to chromosome 7D in a number of

different wheat cultivars (Singh et al. 2000, 2001; Bariana et al. 2001; Boukhatem et al. 2002). *Yrns-B1* was mapped to 3BS in the German breeding line LGst.79-74 (Börner et al. 2000), while Singh et al. (2000) also located a stripe rust APR gene, now designated *Yr30*, on chromosome 3BS in the cultivar Opata 85. The APR gene *Yr29*, from cultivar Lalbahadur, is located on 1B (William et al. 2003), whereas the recently designated *Yr31* gene from Pastor, which is associated with minor APR genes in this cultivar (Singh et al. 2003), has been placed on 2BS. An APR gene (*YrKat*) in the cultivar Katepwa, and a temperature-sensitive, seedling-resistance gene (*YrCk*) in the cultivar Sunco, has been found on chromosome 2DS (Bariana et al. 2001). Further, in the French cultivar Camp Remy, two QTLs for stripe rust APR have been located on chromosomes 2A and 2B (Boukhatem et al. 2002).

Statistical methods such as QTL analysis have often been used to predict the number of genes contributing to a trait showing quantitative variation. The power of molecular mapping now allows us to confirm these genetic predictions, to determine the proportion of the phenotype conferred by each QTL and locate the QTLs to specific chromosomes (Young 1996; Lynch and Walsh 1997).

We report here on the QTL analysis of stripe rust APR in the Kariega × Avocet S doubled haploid mapping population (Prins et al., submitted), to determine the number and chromosomal location of QTLs for this trait. Early field trial observations also identified polymorphism for leaf-tip necrosis in the population, possibly due to the gene *Ltn* on chromosome 7D. To assess the feasibility of a more rapid detection of APR, field and growth chamber results were compared. We also report on an early analysis, fitting a mixture model to the disease scores, undertaken before construction of the linkage map. The model tests whether an observed, continuous (quantitative) distribution of phenotypic scores is consistent with two or more mixed underlying distributions, as can arise through joint segregations of two or more QTLs controlling the trait (Lynch and Walsh 1997).

Materials and methods

Plant material

A doubled haploid (DH) mapping population, consisting of 150 lines derived from the cross Kariega × Avocet S and the relevant parental lines were used in this study. Kariega {pedigree: SST44[CI13523(Agent)/3*T4 (Anza)]//K4500.2/Sapsucker S} is a hard, red spring wheat that exhibits flag leaf-tip necrosis, APR to stripe rust (0–10R for pathotypes 6E16A- and 6E22A-, Boshoff et al. 2002b) and leaf rust, suggesting the presence of the linked *Yr18*/*Lr34* rust-resistance genes (Dyck 1991; Singh 1992). Based on pedigree analysis, it can be assumed that some resistance was transferred from Anza, a variety in which the durable stripe rust resistance has been ascribed in part to the presence of *Yr18* (McIntosh 1992; Singh 1992). In addition, a second level expansion of the pedigree of Sapsucker reveals the occurrence of Frontana (Payne et al. 2002), a known source of the *Yr18*/*Lr34* genes (McIntosh et al. 1995). Kariega also carries the leaf rust genes *Lr1* and *Lr3a* for seedling resistance to *P. triticina* (Pretorius and Singh, unpublished). The cultivar has excellent baking quality, but is susceptible to stem rust. Avocet S is a white-seeded, stripe rust-susceptible selection (100S for pathotypes 6E16A- and 6E22A-; Boshoff et al. 2002b) from the Australian spring wheat cultivar Avocet. Avocet carries *Sr26* derived from *Thinopyrum elongatum*, *Lr13*, and is heterogeneous for the seedling resistance gene *YrA,* as well as its adult plant stripe rust reaction (Wellings et al. 1988; McIntosh et al. 1995). The presence of *Sr26* and *Lr13* has not been confirmed in the Avocet S selection (Wellings, personal communication), but *Sr26* was shown to be segregating in the DH population (Prins et al., submitted).

Disease evaluation

Field trial

The 150 DH lines were planted in a field experiment with four randomized complete blocks on 2 June 2000 at the PANNAR Research Station, Greytown, KwaZulu-Natal. Entries were planted in 1-m row plots spaced 90 cm apart. Each block contained six Kariega and two Avocet S control plots. The entire trial area was surrounded by two rows of a mixture of stripe rust-susceptible spreader wheats. Spreader rows were also sown in 1-m wide paths running perpendicular to all experimental plots. The field trial was infected with a spore suspension of pathotype 6E22A- of *P. striiformis* f. sp. *tritici* and was scored by three independent scorers. Severity of infection was scored on 11 September (early) and 28 September (final) using the modified Cobb scale (0–100% infected leaf area) as a quantitative measure of disease infection (Peterson et al. 1948; McIntosh et al. 1995, p. 11). Host reaction type was scored on the classical ordinal scale R (resistant), MR (moderately resistant), MS (moderately susceptible) and S (susceptible), augmented with three classes through practical experience of scoring this disease, viz, RMR between R and MR, MRMS between MR and MS and MSS between MS and S, corresponding to the order of the seven classes in the Plant Breeding Institute Cobbity (Australia) adaptation of the original ten-class scale of McNeal et al. (1971) (McIntosh et al. 1995, p. 10). These seven classes of reaction type were assigned a numerical value of 1 (resistant) to 7 (susceptible). One evaluator (Z.P.) undertook the early assessment in three of the four blocks, while the final assessment was undertaken by three independent evaluators (Z.P., W.B. and L.B.) in all four blocks. The assessment by L.B. was for percentage leaf area infected only.

Growth chamber

The DH population and control entries were also evaluated for resistance in a growth chamber, under controlled environmental conditions, as part of a broader, ongoing experimental programme investigating the feasibility of quick and early assessment of APR, with the aim of shortcutting extensive and costly field trials (Pretorius et al. 2000). The DH population and control entries were grown in a steam-sterilized, soil-peat mixture in 3.8×3.8×10-cm seedling cones. Each cone contained 5 to 10 plants per entry. Plants were grown at 25°C in a growth chamber where continuous light (200 μmol/m^2/s) was provided by fluorescent tubes and incandescent bulbs situated 70 cm above the chamber floor. Plants were inoculated 30 days after planting when most entries were heading (Zadoks growth stage 55, Zadoks et al. 1974) by spraying them with a fine mist of sterile, distilled water containing spores of *P. striiformis* f. sp. *tritici* pathotype 6E22A- and a surfactant. Following incubation at 6°C in a high humidity chamber for 48 h, plants were returned to the growth chamber where a temperature of 18°C and 14 h of light per day were maintained. Reactions were assessed 12 days after inoculation when stripe rust development on susceptible plants appeared optimal. Disease assessment was based on flag-leaf infection types (0–4 scale, McIntosh et al. 1995) and whole-plant, host-reaction type (R to S scale), converted to numerical values on the 1–7 ordinal scale as in the case of the field trial.

Linkage maps

Linkage maps, as described by Prins et al. (submitted), were used in the QTL analysis. The 'distribute' function of Map Manager was used to incorporate *Ltn* into the existing linkage map. Leaf-tip necrosis (*Ltn*) typing of the DH population was done in the field at the same time as the final stripe rust rating (28 September 2000), but in concurrence with William et al. (1997) and Messmer et al. (2000), it was found to be difficult. One scorer (L.B.) scored all entries for presence or absence of *Ltn*, while two scorers (Z.P., W.B.) scored for presence only, which resulted in a large number of missing values in the linkage analysis. Initially, the different *Ltn* scores were mapped separately. Despite the large numbers of missing values for the two scores referred to above, they all mapped to the same region, and therefore, only the most complete set of data (L.B.) was used in the final linkage analysis. The *Ltn* phenotype was mapped as a marker not a quantitative trait, as the pathologists scored the trait on an ordinal scale.

Data analysis

Disease scores

Three disease scores were computed:

1. Field scores on the percentage of leaf area infected were converted to proportions, p, and transformed to arcsin ($\sqrt{p}$) (angular transformation) as is appropriate for percentage data (Sokal and Rohlf 1995). Host reaction-type scores on the ordinal scale were assigned numerical values from 1 (resistant) to 7 (susceptible) for analytical purposes.
2. The mean host reaction type (field early and final) or
3. Host reaction type (growth chamber) was transformed to ln(score + 1) for the QTL mapping analysis on this trait. Repeatability of scores over replications by independent scorers was obtained by standard analysis of variance and expressed as intraclass correlation coefficients (Kempthorne 1957, pp. 228–230), and between growth chamber, early and final field scores by standard product-moment correlation.

QTL analysis

Field and growth chamber scores were compared in the QTL analysis based on: (1) the arcsin-transformed mean percentage leaf area infected (field early and final), (2) ln-transformed mean host reaction type (field early and final) and (3) ln-transformed host reaction type (growth chamber).

The program Map Manager QTX, version 15 (Manly and Olson 1999), was used for interval mapping (IM) and IM with marker cofactors (Zeng 1993). A permutation test (Doerge and Churchill 1996) set at 1,000 iterations was used to calculate the likelihood ratio statistic (Lrstat) threshold for declaring statistical significance.

Mixture model analysis

Statistical genetic properties of the distribution of disease scores were analysed initially using a standard mixture model method for detecting gene segregations as described by Lynch and Walsh (1997, p. 359), using the maximum likelihood mixture analysis program FINMIX written by J.H. Randall (Flury 1995). This application makes provision for fitting the model to the data assuming a common variance in the putative underlying distributions and assuming unequal variances as alternative models.

Results and discussion

Disease scores

A severe and uniformly distributed epidemic developed among plots in the field trial. Occurrence of pathotype 6E22A- was confirmed by infecting stripe rust differential cultivars with rust collections from the trial site (data not shown). At the early assessment, Avocet S displayed reactions of 70S to 80S, denoting susceptibility and 70–80% leaf area infected, while Kariega showed no signs of infection. At the final assessment Avocet S consistently scored 100S and Kariega tR to 10R, denoting resistant and trace to 10% leaf area infected. Extreme responses of the DH lines were similar, and scores recorded covered the full range between these extremes.

A similar range of variation was also found for growth chamber scores. Flag leaves of plants were small (5 cm to 10 cm in length) but provided adequate leaf tissue for scoring. Flag-leaf infection types (0–4 scale) of the DH lines ranged from flecking to large susceptible-type pustules, with many intermediate phenotypes. Low reactions were often associated with excessive chlorotic flecking. Z-reactions, where the leaf base supports more susceptible reactions than the tip, were commonly observed. Host reaction type (R to S rating scheme) appeared to be a more reliable estimate of stripe rust response, as several DH lines displaying a three-flag leaf infection type were rated in the R to MR range on a whole plant basis. Hence, the R to S rating scheme was used in the analysis of growth chamber results. Kariega produced a ;12-flag-leaf infection type and R whole-plant response, whereas Avocet S was rated as 3C and MSS, respectively.

Table 1 Coefficients of correlation measuring repeatability of disease scores and scorers in a wheat stripe rust field trial at two dates (early and final) and in a growth chamber

Scorers and scores[a]	Coefficient of correlation±standard error[b]	
	Reaction type	Transformed leaf area infected
Early score (Z.P.):		
Between blocks	t=0.911±0.012	t=0.929±0.010
Final score (Z.P., W.B., L.B.):		
Between scorers within blocks	t=0.799±0.023	t=0.942±0.007
Between blocks (averaged over scorers)	t=0.965±0.005	t=0.928±0.009
Early score-final score	r=0.865±0.021	r=0.886±0.017
Growth chamber-early field score	r=0.372±0.070	-
Growth chamber-final field score	r=0.482±0.063	-

[a] Z.P., W.B. and L.B. refer to individual independent scorers. Growth chamber scores were by Z.P. for reaction type only

[b] t intraclass, r product-moment

Table 2 Summary of interval mapping analysis of chromosomes involved with APR to stripe rust with the different disease scores

QTL interval and location		Field: percentage leaf area infected[a]		Field: host reaction type[b]		Growth chamber: host reaction type[c]
		Early	Final	Early	Final	
QYr.sgi-7D	LrStat[d]	46.2***	53.1***	30.4***	14.9**	22.1***
Xgwm295-Ltn	% Var[e]	27	29	18	9	16
QYr.sgi-2B.1	LrStat	28.6***	55.2***	60.2***	94.7***	NS[f]
Xgwm148-s12m60A	% Var	17	30	33	46	NS
QYr.sgi-1A	LrStat	8.3*	11.8*	10.2**	18.5**	NS
s15m19D-s23m18E	% Var	6	7	7	12	NS
QYr.sgi-4A.1	LrStat	23.7***	NS	7.8*	NS	NS
s21m40A-s22m55A	% Var	15	NS	6	NS	NS
QYr.sgi-4A.2	LrStat	NS	NS	NS	NS	40.3***
s13m94B-s12m53B	% Var	NS	NS	NS	NS	24
QYr.sgi-7A	LrStat	NS	NS	NS	NS	18**
s19m89C-s18m47B	% Var	NS	NS	NS	NS	11
QYr.sgi-2B.2	LrStat	NS	NS	NS	NS	11.1*
Xpsp3030-s16m40A	% Var	NS	NS	NS	NS	7

[a] Mean percentage leaf area infected converted to proportions (p) and transformed to arcsin ($\sqrt{p}$)
[b] Mean host reaction type (field) transformed to ln(score+1)
[c] Host reaction type (growth chamber) transformed to ln(score+1)
[d] Indicates the maximum likelihood ratio statistic score obtained on the chromosome in question. Permutation tests were done to determine the likelihood-ratio statistic (*Lrstat*) threshold values for each disease score used and correspond to genome-wide α levels of: 0.63 (*suggestive), 0.05 (**significant) and 0.001 (***highly significant)
[e] Percentage phenotypic variance explained (R^2)
[f] Indicates not significant

The repeatability of disease scoring is statistically measurable in terms of coefficients of correlation (Table 1), where it should be recalled that the percentage leaf area infected was calculated from means averaged over three scorers (Z.P., W.B. and L.B.) and the host reaction type over two scorers (Z.P. and W.B.). This repetition of scoring, and the highly positive correlation between scorers (Table 1), made a major contribution to the power of resolution of the subsequent QTL analyses. The correlations between replications (blocks) for reaction type (0.965) and transformed leaf area infected (0.928), averaged over scores, relate directly to relative magnitudes of genetic variance in these traits, being the heritability (broad sense) of the traits averaged over scorers in this set of lines. This translates to field heritabilities of 0.932 for reaction types and 0.811 for transformed leaf area infected, on a single-score basis within replications (Falconer and Mackay 1996). Estimation of heritability under growth chamber conditions was not possible because there was no replication of scoring. However, we note that correlations between growth chamber and field scores for reaction type are low and perhaps not inconsistent with results of the QTL analyses, which follow.

QTL analysis

IM analysis on field data detected QTLs on chromosomes 7D (*QYr.sgi-7D*) and 2B (*QYr.sgi-2B.1*) (Table 2). SSR locus *Xgwm295-7D* was located closest to the *QYr.sgi-7D* peak (~10 cM) (Fig. 1). The paucity of polymorphism on the 7D chromosome prevented construction of a dense linkage map for this chromosome, resulting in flanking markers 34 cM apart for *QYr.sgi-7D*.

It is known that the *Lr34/Yr18* complex is present on chromosome 7D (Singh 1992; Singh et al. 2000) and is reportedly linked to the gene *Ltn*. In the present study, *Ltn* was mapped at a position 12 cM away from the marker closest to the *QYr.sgi-7D* peak (*Xgwm295–7D*, Fig. 1). It was not possible to map *Lr34* in the population because of the presence and confounding effects of other *Lr* genes. However, *Lr34* and *Yr18* have been mapped to a region on chromosome 7DS flanked by the RFLP marker loci *Xbcd1872-7D* and *Xbcd1438/Xrz2-7D* in Opata 85 (Nelson et al. 1997; Singh et al. 2000), and Röder et al. (1998) mapped *Xgwm295-7D* close to *Xbcd1438-7D* in the same population (~3 cM). This marker evidence, with the occurrence of Frontana (probable donor of *Yr18* in Opata 85, Payne et al. 2002), via Anza or Sapsucker S, in Kariega's pedigree, makes it highly likely that *QYr.sgi-7D* is linked to the *Yr18* gene. QTLs for stripe rust APR on chromosome 7D, believed to be *Yr18*, have also been detected in the CIMMYT cultivar Parula (Singh et al. 2001) and in the Australian breeding line, CD87 (Bariana et al. 2001).

QYr.sgi-2B.1 is detectable in a region spanning 25 cM on chromosome 2B, with *Xgwm148-2B* as the closest marker (Fig. 1). Börner et al. (2002) identified QTLs for APR to stripe rust in Opata 85 in the marker interval *Xbcd152-2B* and *Xcdo405-2B*, and Röder et al. (1998) mapped *Xgwm148-2B* close to this same interval (~4 cM). QTLs for stripe rust APR have also been detected on chromosome 2B in Opata 85 in the *Xbcd152-2B* and *Xcdo405-2B* interval (Boukhatem et al. 2002). Stripe rust resistance genes on 2BS include *Yr27* (Selkirk), *Yr31*(Pastor) and *Yr32* (Carstens V) (McIntosh et al. 2003).

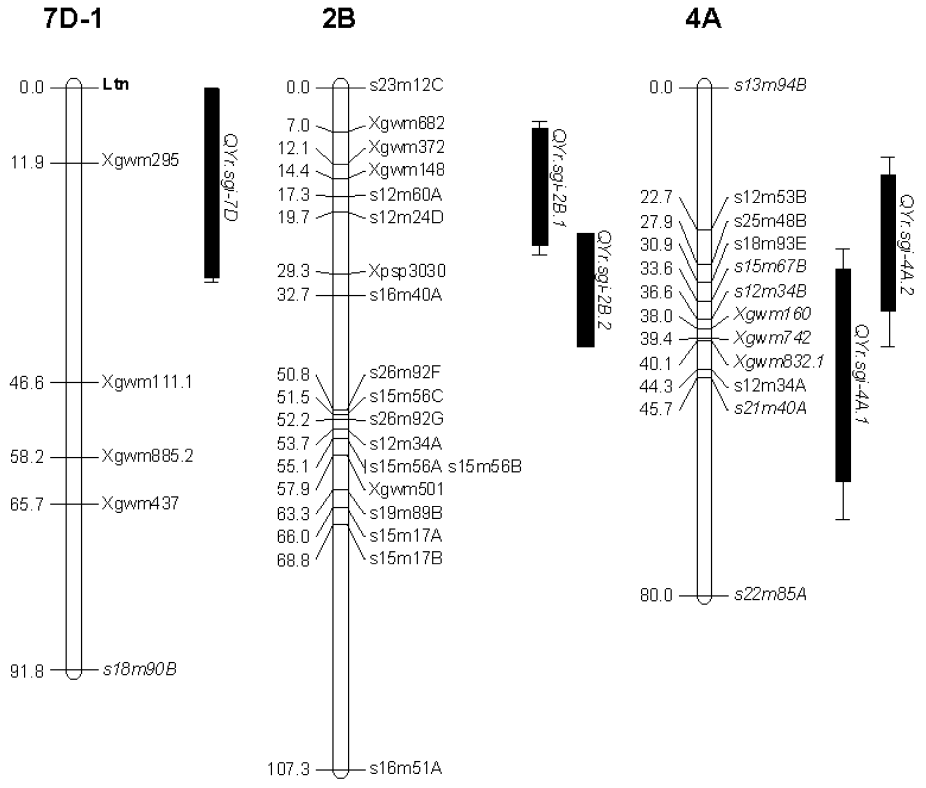

Fig. 1 QTLs for stripe rust resistance identified on wheat chromosomes 2B, 7D, and 4A. Likelihood ratio statistic values were converted to LOD scores, and the standard 1 LOD (*shaded region*) and/or 2 LOD support intervals are represented

Pedigree analysis reveals that Selkirk and Pastor share some commonalities in ancestry with the Anza, Sapsucker and K4500.2 parents of Kariega (Payne et al. 2002). The relationship between *QYr.sgi-2B, Yr27, Yr31* and *Yr32* thus needs to be determined.

It is clear from the present results that *QYr.sgi-7D* explains a higher proportion of the variance in percentage leaf area infected (27–29%) than in host reaction type (9–18%) (Table 2). Assuming that the *QYr.sgi-7D* effect is at least partly influenced by *Yr18*, it would then seem that the gene is expressed as a partial resistance reaction under the conditions that prevailed in the field, rather than as a chlorotic or necrotic reaction. Although the effect of *Yr18* is known to be influenced by genetic background and environment, stripe rust development in wheat containing this gene is consistently less than in susceptible control lines (Singh 1992; Johnson et al. 2000).

On the other hand, *QYr.sgi-2B.1* explains a higher proportion of the variance in host reaction type (33–46%) than percentage leaf area infected (17–30%). In this case, the reaction was more clearly detectable in the final evaluation, by which time the full chlorotic/necrotic reaction phenotype had developed, presumably because warmer environmental conditions were more conducive to the MR–MS range of reactions. Qayoum and Line (1985) described the expression of high-temperature APR to stripe rust, and it is possible that one or more genes in Kariega function in a similar way, thus exhibiting a more pronounced manifestation of the resistance response as the season progresses and the temperature increases. Working with leaf rust of wheat, Singh et al. (1998) reported that early and late-disease ratings are required for accurate ranking of lines. The present study also emphasized the importance of monitoring the progression of the disease by scoring the disease reaction at more than one time during the growing season.

QYr.sgi-2B.1 appears to condition resistance expressed by a combination of necrotic, chlorotic and sporulating stripes, whereas *QYr.sgi-7D* appears to confer a partial resistance with more limited host-cell death. The latter QTL may therefore represent a non-race-specific form of resistance, and may have greater durability. If *QYr.sgi-7D* is the *Yr18* gene postulated from Kariega's pedigree analysis, then this stripe rust resistance gene has already been shown to confer a durable source of resistance (Singh 1992).

QTLs that contribute less than 10% to the phenotypic variance are particularly susceptible to environmental variances and are difficult to detect across years and environments (Boukhatem et al. 2002). The four minor QTLs found in this study were not consistently detected by the different disease scores used to measure stripe rust APR, or between field and growth chamber tests (Table 2). IM with the aid of marker cofactors did not identify any additional QTL(s) to those in Table 2. *QYr.sgi-1A* was consistently detected by the field test scores, but not by the growth chamber test. *QYr.sgi-4A.1* was detected early in the field test, but was poorly expressed when final scores were taken. As the disease progressed, the resistance conferred by *QYr.sgi-4A.1* may have been ineffective and, therefore, was not detected by the final disease scores.

Table 3 Estimates of the proportion, mean and variance of three proposed underlying distributions (I, II and III, see Fig. 1) from a mixture model analysis using the unequal variance option of transformed percentage leaf area infected (field-final score) of doubled haploid lines and proposed diploid homozygous genotypes accounting for proportions in the ratio 1:2:1. Log-likelihood = −40.43

Distribution	Proportion	Mean	Variance	Proposed genotype(s)
I	0.293±0.061	0.313±0.020	0.0037±0.0015	*AABB*
II	0.437±0.070	0.637±0.077	0.0394±0.0152	*AAbb, aaBB*
III	0.270±0.072	1.327±0.090	0.0349±0.0199	*aabb*

The low correlation between the field host-reaction type (early and final) and the growth chamber reaction type scores (Table 1), and the inconsistencies in the appearance of *QYr.sgi-1A, QYr.sgi-4A.1, QYr.sgi-4A.2, QYr.sgi-7A* and *QYr.sgi-2B.2* (Table 2) indicate that the growth chamber tests are not a reliable reflection of the results obtained from field tests. *QYr.sgi-2B.1* was consistently detected as a major QTL in the field test, but remained undetected in the growth chamber test, whilst *QYr.sgi-4A.2* and *QYr.sgi-7A* were only detected in the growth chamber test (Table 2). An additional QTL on chromosome 2B, *QYr.sgi-2B.2*, that lay close to *QYr.sgi-2B.1*, was also detected under growth chamber conditions. The results obtained indicate that a different set of QTLs are expressed in the growth chamber environment, suggesting possible genotype-environment interaction for the trait. However, it is promising that at least one major field QTL (*QYr.sgi-7D*) was detected in the APR growth chamber tests.

It is of interest that one of the significant QTLs detected in the growth chamber analysis (*QYr.sgi-4A.2*, Table 2) appears to be derived from the Avocet S susceptible parent. A minor QTL for stripe rust APR was contributed by Avocet S in a cross with Pavon 76, but this QTL was located on chromosome 6A (Singh et al. 2001). Several cases have been reported where components of APR to powdery mildew in wheat have been contributed by the susceptible parents and have been ascribed to effects of defeated seedling-resistance genes (Nelson 1978; Chantret et al. 1999; Keller et al. 1999; Paillard et al. 2000). It is therefore possible that some APR to stripe rust in Avocet might have been retained under the conditions in which the susceptible selection of Avocet S was made. In studying *Yr18* resistance to different pathotypes in various environments, Johnson et al. (2000) reported that Avocet S, one of the control genotypes in that particular study, had significantly lower stripe rust scores than Avocet R in four out of five comparisons in New Zealand and the United Kingdom. This indicates that some form of reduced susceptibility is detectable in Avocet S under certain conditions, e.g. the growth chamber, and in several subsequent tests under similar controlled conditions (Pretorius, unpublished). In Mexico, however, the two Avocet selections were equally susceptible (Johnson et al. 2000).

Overall, the genetic variance accounted for by the different field disease scores explains some 65–67% of the total variance in the trait. Variation not accounted for may be attributed to the presence of undetected minor QTLs in regions of the genome not adequately covered by markers, epistasis and QTL interactions, QTL-environment interactions or error variation. This highlights the need for linkage maps with more complete genome coverage, as well as adequately replicated field trials over many environments, and effective QTL-mapping software to detect QTL-interaction effects.

The results of this study will enable further characterization of major QTL regions and the development of genetic material for breeding programmes. Germplasm containing each of the major QTLs and a combination of them should be developed, using a marker-assisted backcross programme. These can be used as reference gene/QTL stocks, as crossing parents in breeding programmes, as comparison of the effectiveness of the major QTL in different genetic backgrounds and may aid further characterization of the QTL regions with more markers.

QTL mixture model analysis

The transformed percentage leaf area infected scores for the Kariega × Avocet S DH population have the frequency distribution shown in Fig. 2, where the smooth curve is the result of fitting a mixture model postulating three underlying and overlapping normal distributions. The analysis estimates the mean and variance of each of the putative underlying distributions and their proportional contribution to the total observed variance. The log-likelihood of the model fitted, using the unequal variance option, as suggested by the results (Table 3, Fig. 2), shows a markedly smaller variance in the underlying distribution I. Residual variation is ascribable to minimal environmental effects, measurement errors and minor QTLs as indicated by the high heritability of the scores for the stripe rust APR in this DH population (broad-sense heritability, final field scores; percentage leaf area infected, 0.93; and host reaction. 0.81). The results suggest a digenic epistatic model with DH genotypes in the ratio 1**:**2**:**1, and where (1) *AABB* denotes the resistant genotype of Kariega, (2) *aabb* denotes the susceptible genotype of Avocet S and (3) recombinant genotypes *AAbb* and *aaBB* confer partial resistance. This translates to an expected F_2 ratio of 9**:**6**:**1 in this cross, a classical epistatic modification of the Mendelian di-hybrid ratio, 9**:**3**:**3**:**1, referred to as ‘duplicate genes with cumulative effect’.

The QTL analysis detected two major QTLs, which is supported by the mixture model analysis. In addition, it also detected minor QTLs using the field data demon-

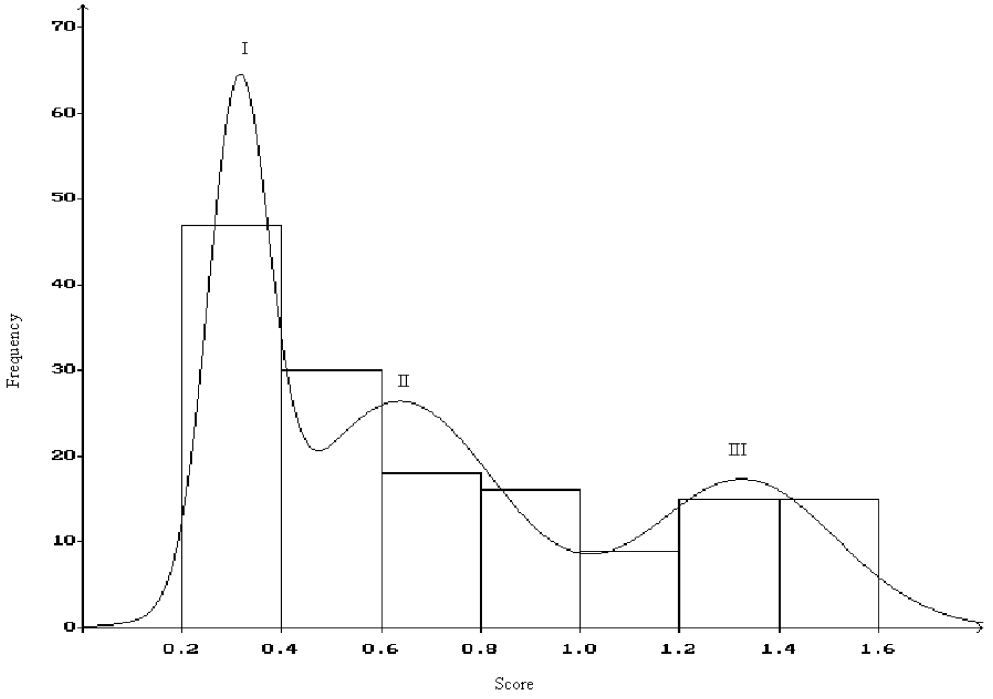

Fig. 2 Frequency distribution of transformed percentage leaf area infected (score) of 150 DH lines. *I*, *II* and *III* refer to underlying distributions in the mixture model analysis using the unequal variance option (see text and Table 3)

strating the higher resolution power of marker QTL linkage analysis. Mixture model analysis may, however, be useful in material for which no linkage maps have yet been constructed. However, there is no way of matching the pair of QTLs indicated by the mixture model analysis with QTLs detected in the IM analysis.

Conclusions

With the developments made in molecular marker technologies and QTL statistical analysis of traits in wheat, several studies of APR to stripe rust are beginning to emerge (Singh et al. 2000, 2001; Bariana et al. 2001; Börner et al. 2002; Boukhatem et al. 2002). QTL analysis will enable us to determine the genetic diversity present in wheat for this trait and the percentage of the phenotypic variance contributed by each QTL under different environmental conditions. As in this study, the resistance phenotype associated with each QTL can be determined by scoring not only the percentage of leaf area affected, but by separating the different responses of the plant to the pathogen. Mixture model analysis predicted the presence of two major QTLs in Kariega for stripe rust APR, but the increased sensitivity of the QTL analysis enabled additional QTLs with smaller effects and/or expressed at different stages in the development of the disease or under different environmental conditions, to be detected.

The high correlation between scorers and blocks indicates that costs can be minimized by reducing the number of scorers and replicates, and rather taking repeat scorings over a longer period during disease development. To manipulate stripe rust APR effectively in wheat breeding, it is important to understand the genetics behind the phenotype, and the interaction with the environment. The importance of chromosomes 2B and 7D in stripe rust APR in Kariega was confirmed, and the results suggested that *QYr.sgi-7D* (7D) is probably the durable stripe rust resistance gene, *Yr18*.

Acknowledgements We thank C.M. Bender (UFS) for assistance in planting and F.J. Kloppers (PANNAR) for the trial site and field plot care. Financial assistance by the South African Winter Cereal Research Trust, Agricultural Research Council and National Research Foundation is also acknowledged. L.B. would like to thank the British Society of Plant Pathology for a fellowship supporting this collaboration.

References

Bariana HS, Hayden MJ, Ahmed NU, Bell JA, Sharp PJ, McIntosh RA (2001) Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust J Agric Res 52:1247–1255

Bender CM, Pretorius ZA (2001) Assessing the vulnerability of South African wheat cultivars to pathogenic adaptation in *Puccinia striiformis* f. sp. *tritici* (abstract). S Afr J Sci 97

Börner A, Röder M, Unger O, Meinel A (2000) The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) of wheat. Theor Appl Genet 100:1095–1099

Börner A, Schumann E, Furste A, Cöster H, Leithold B, Röder MS, Weber WE (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105:921–936

Boshoff WHP, Pretorius ZA (1999) A new pathotype of *Puccinia striiformis* f. sp. *tritici* on wheat in South Africa. Plant Dis 83:591

Boshoff WHP, Pretorius ZA, Van Niekerk BD (2002a) Establishment, distribution and pathogenicity of *Puccinia striiformis* f. sp. *tritici* in South Africa. Plant Dis 86:485–492

Boshoff WHP, Pretorius ZA, Van Niekerk BD (2002b) Resistance in South African and foreign wheat cultivars to pathotypes 6E16A- and 6E22A- of *Puccinia striiformis* f. sp. *tritici.* S Afr J Plant Soil 19:27–36

Boukhatem N, Baret PV, Mingeot D, Jacquemin JM (2002) Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. Theor Appl Genet 104:111–118

Chantret N, Pavoine MT, Doussinault G (1999) The race-specific resistance gene to powdery mildew, *MIRE*, has a residual effect on adult plant resistance of winter wheat line RE714. Phytopathology 89:533–539

Chen X, Moore M, Milus E, Long DL, Line RF, Marshall D, Jackson L (2002) Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant Dis 86:39–46

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Dyck PL (1991) Genetics of adult-plant leaf rust resistance in 'Chinese Spring' and 'Sturdy' wheats. Crop Sci 31:309–311

Falconer DS, Mackay T (1996) Introduction to quantitative genetics, 4th edn. Longman Group, Essex

Flury B (1995) Introduction to finite mixture analysis. Short course presented at the 4th Meeting of the East, Central and Southern African Network of the International Biometric Society, Stellenbosch, South Africa, 5–7 December 1995

Johnson R, Cromey MG, Viljanen-Rollinson SLH, Singh RP (2000) Resistance to yellow (stripe) rust in wheats possessing *Yr18* in England, New Zealand and Mexico. Acta Phytopathol Entomol Hung 35:3–9

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999) Quantitative trait loci for resistance against powdery mildew in a segregating wheat × spelt population. Theor Appl Genet 98:903–912

Kempthorne O (1957) An introduction to genetic statistics. Wiley, New York

Lynch MA, Walsh B (1997) Genetics and analysis of quantitative traits. Sinauer, Sunderland, Mass., pp 319–532

Manly KF, Olson JM (1999) Overview of QTL mapping software and introduction to Map Manager QT. Mamm Genome 10:327–334

McIntosh RA (1992) Close genetic linkage of genes conferring adult plant resistance to leaf rust and stripe rust in wheat. Plant Pathol 41:523–527

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO, Australia, pp 1–200

McIntosh RA, Yamazaki Y, Devos KM, Dubcovsky J, Rogers J, Appels R (2003) Macgene catalogue of gene symbols for wheat. 10th International Wheat Genetics Symposium, Paestum, Italy

McNeal FH, Konzak CF, Smith EP, Tate W, Russel TS (1971) A uniform system for recording and processing cereal research data. US Agric Res Serv 42:34–121

Messmer MM, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, Feuillet, 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

Nelson RR (1978) Genetics of horizontal resistance to plant diseases. Annu Rev Phytopathol 16:359–378

Paillard S, Goldringer I, Enjalbert J, Trottet M, David J, de Vallavieille-Pope C, Brabant P (2000) Evolution of resistance against powdery mildew in winter wheat populations conducted under dynamic management. II. Adult plant resistance. Theor Appl Genet 101:457–462

Payne TS, Skovmand B, Lopez CG, Brandon E, McNab A (eds) (2002) The International Wheat Information System (IWIS), ver. 4, 2001 on compact disk. CIMMYT, Mexico, D.F.

Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stems of cereals. Can J Res Sect C26:496–500

Pretorius ZA, Boshoff WHP, Kema GHJ (1997) First report of *Puccinia striiformis* f. sp. *tritici* on wheat in South Africa. Plant Dis 81:424

Pretorius ZA, Park RF, Wellings CR (2000) An accelerated method for evaluating adult-plant resistance to leaf and stripe rust in spring wheat. Acta Phytopathol Entomol Hung 35:359–364

Qayoum A, Line RF (1985) High-temperature, adult-plant resistance to stripe rust of wheat. Phytopathology 75:1121–1125

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Singh RP (1992) Genetic association of leaf rust resistance gene *Lr34* with adult plant resistance to stripe rust in bread wheat. Phytopathology 82:835–838

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

Singh RP, Huerta-Espino J, William M (2001) Slow rusting gene based resistance to leaf and yellow rusts in wheat: genetics and breeding at CIMMYT. 10th Assembly Proceedings of the Wheat Breeding Society of Australia Inc., Mildura, Australia,16–21 September 2001, pp 103–108

Singh RP, William HM, Huerto-Espino J, Crosby M (2003) Identification and mapping of gene *Yr31* for resistance to stripe rust in *Triticum aestivum* cultivar Pastor. In: Proceedings of the 10th International Wheat Genetics Symposium, Paestum, Italy, pp 411–413

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. Freeman, New York

Wellings CR, McIntosh RA (1990) *Puccinia striiformis* f. sp. *tritici* in Australasia: pathogenic changes during the first 10 years. Plant Pathol 39:316–325

Wellings CR, McIntosh RA, Hussain M (1988) A new source of resistance to *Puccinia striiformis* f. sp. *tritici* in spring wheats (*Triticum aestivum*). Plant Breed 100:88–96

Wellings CR, Singh RP, McIntosh RA, Yayhaoui A (2000) The assessment and significance of pathogenic variability in *Puccinia striiformis* in breeding for resistance to stripe (yellow) rust: Australian and international studies. In: Proceedings of the 11th Regional Wheat Workshop for Eastern, Central and Southern Africa, Addis Ababa, Ethiopia, pp 134–143

William HM, Hoisington D, Singh RP, González-de León D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253–260

William M, Singh RP, Huerta-Espino J, Ortiz Islas S, Hoisington D (2003) Molecular marker mapping of leaf rust resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93:153–159

Young ND (1996) QTL mapping and quantitative disease resistance in plants. Annu Rev Phytopathol 34:479–501

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zeng ZB (1993) Theoretical basis for the separation of multiple linked gene effects in mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976