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Identification of QTLs for Quantitative Resistance to Stripe Rust (*Puccinia striiformis* F. sp. *tritici*) in Bread Wheat

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Abstract: Quantitative resistance to stripe rust in wheat delays disease epidemic development and is often not controlled by race-specific resistance genes. To identify quantitative trait loci (QTLs) affecting quantitative resistance to stripe rust, 140 Doubled Haploid (DH) lines from a cross between adult plant resistant parent Cv. Otane and the susceptible parent Cv. Tiritea were developed. These DH lines were evaluated for Latent Period (LP), Pustule Density (PD) and Pustule Length (PL) at the adult plant stage in the glasshouse to pathotype 106E139A$^+$ of *Puccinia striiformis* f. sp. *tritici* and in the field for Area Under the Disease Progress Curve (AUDPC). Two genomic regions on chromosomes 7B and 7D were significantly associated with LP and one QTL (on chromosome 5D) was associated with PD only. A QTL on chromosome 3D was associated with PL. Favourable alleles contributed from Otane chromosomes 3D and 5D were associated with AUDPC in the field. Ten AFLP markers also showed significant association with these measures of resistance. The results suggested that the quantitative resistance of Otane is oligogenic and susceptible Tiritea also carrying QTLs for resistance. QTLs for AUDPC were also associated with the components of resistance LP, PD and PL. Therefore, calculating AUDPC can be an efficient way to estimate the joint effect of components of quantitative resistance.

Key words: Components of resistance, AUDPC, marker-assisted selection, quantitative trait loci

INTRODUCTION

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, is common in wheat growing areas where the climate is cool and moist. The disease can drastically reduce yield, especially if a severe epidemic develops before ear emergence. Losses in grain yield ranging from 20 to 100% in an extreme situation have been reported[1]. In New Zealand losses are especially severe in the south island reaching up to 60% yield reductions. Stripe rust continues to be an important constraint to wheat production due to continued appearances of new *Puccinia striiformis* races[2]. The use of resistant cultivars is an economic and effective means of reducing losses from the disease.

Rust resistance is generally classified into two types, qualitative and quantitative. Qualitative resistance is usually conferred by major genes, is nearly always race-specific, causes an hypersensitive or low infection type of response and is usually, although not always, short-lived due to appearance of new fungal races. However, in the absence of major genes, quantitative (usually polygenic) resistance can be observed. This type of resistance is generally considered to be a more promising measure for stripe rust control than qualitative resistance. Russell[3] stated that in the absence of qualitative resistance, several mechanisms contribute to quantitative resistance to stripe rust of wheat. These include reduced spore deposition, slower or reduced spore germination and reduced penetration and growth within the plant, as well as low spore production and long generation times.

The wheat cultivar Otane has shown a stable level of adult plant resistance since its release in 1984 in New Zealand[4]. However, despite the usefulness of this resistance, a genetic analysis of its resistance has not been performed. It has been difficult to characterise genes that control quantitative resistance accurately, but with the development of DNA markers and QTL mapping, such genes are now more accessible than previously. The objective of this study was to gain insight into the genetic basis of stripe rust resistance and identify chromosomal regions determining the durable resistance of Cv. Otane.

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MATERIALS AND METHODS

Development of Doubled Haploid (DH) lines: Otane, a NZ cultivar with durable quantitative stripe rust resistance, was used as a pollen parent in a cross with susceptible Cv. Tiritea and 140 DH lines were obtained through a wheat-maize system as described by Imtiaz *et al.*[4]. These 140 DH lines were grown in the glasshouse and field to record data for four measures of quantitative resistance.

Glasshouse evaluations: Plants grown from each of the 140 DH lines were inoculated with stripe rust pathotype 106E139A$^+$ (virulent to stripe rust resistance genes *Yr2*, *Yr3*, *Yr4*, *Yr7*, *YrA*) at the adult plant stage. One seed of each DH line was sown in each of three replicate pots, arranged in a randomized block design with benches being blocks. When the plants reached the flag leaf stage, they were inoculated sequentially according to flag leaf emergence. The inoculation methodology and experimental conditions were the same as reported by Imtiaz *et al.*[4]. Three components of resistance, Latent Period (LP), Pustule Length (PL) and Pustule Density (PD) were measured. LP (days from inoculation to the eruption of the first pustule) assessments on flag leaves of DH lines were started from the 5th day after inoculation by checking leaves for visible pustules. The date when the first pustule erupted on a DH line was recorded and this DH line was omitted from the next day's assessments. Daily assessments continued until pustules were present on almost all DH lines. At 18 days after inoculation of each DH line, the flag leaves were cut and preserved in jars containing lactophenol-ethanol (1:2 v/v) solution for laboratory assessment of pustule density (number of pustules per mm^2 of affected area) and pustule length (μm). The lengths of 15 randomly chosen pustules per leaf per replicate were measured using a microscope (magnification 100X) with a micrometer and the means calculated. Similarly, the number of pustules was measured under 3X magnification using a SZH 10 Olympus stereo microscope by placing a glass plate comprising grids of 2x2 mm^2 area, on the affected leaf and counting pustule numbers in a single grid (a pustule more than half inside the border of the grid was counted in); this was then divided by 4 to estimate urediosori mm^{-2}. Five readings per leaf per replicate were recorded.

Adult plants in the field: Seeds of 140 DHLs and their parents, Tiritea and Otane were sown on 3 August 2000 at Crop and Food Research Lincoln, Canterbury New Zealand. Field design and inoculation with pathotype 106E139A$^+$ were the same as reported by Imtiaz *et al.*[4]. To calculate Area Under the Disease Progress Curve (AUDPC), the DH population was divided into two groups based on flag leaf emergence, since Tititea is 7 to 10 days later in heading than Otane. Group I consisted of DH lines which had completed flag leaf emergence by 11 November 2000 and Group II those DH lines with later flag leaf emergence. Disease assessments measuring percentage leaf area infected based on the modified Cobb scale[5] were made 4 times during the growing season on flag leaves starting at GS 37-39 (flag leaf and flag leaf ligule just visible) and continuing untill GS 61-69[6]. The AUDPC was calculated for each replicate of each DH line using the formulae described by Viljanen-Rollinson and Cromey[7] and mean values from three replicates were used in QTL analyses.

Genotyping: To provide maximum coverage of the A, B and D genomes, 139 (4 to 6 per chromosome) mostly single locus SSR markers were selected from the mapped SSR markers of wheat[8]. 60 AFLP primer combinations were also used for polymorphism tests using two parents of the mapping population. Two different enzyme combinations EcoRI x MseI and PstI x MseI were used to generate template DNA samples[9]. Six SSRs and six AFLP primer combinations showed associations with measures of resistance in selective genotyping of 50 DH lines and those were extended to the DH population as reported by Lander and Botstein[10]. DNA extraction, PCR conditions for microsatellite markers, electrophoresis and AFLP protocol were performed as previously reported[9].

Statistical analysis: Analyses of variance (ANOVA) were performed to determine the genetic variation among lines of the DH population for all the parameters measured. The results of the ANOVA were used to obtain an estimate of the broad sense heritability (h^2) of the four measures of resistance, namely LP, PL, PD and AUDPC. The broad sense heritability (h^2) estimate described by Toojinda *et al.*[11] was calculated as follow:

$$h^2 = \sigma^2 g/(\sigma^2 g + \sigma^2 e/r)$$

where, $\sigma^2 g$ is the genetic variance among DH lines, $\sigma^2 e$ is the error variance and r is the number of replicates.

QTL analysis: Quantitative resistance to stripe rust assessed through different measures of resistance was analysed with marker data as reported by Lander and Botstein[10]. Analyses of QTL's were performed using Map Manager QTX version b11[12]. The estimates of the additive effects (co-efficient of regression) of a single allele substitution and R^2 for each QTL were also computed. The R^2 value was also used as a measure of the total phenotypic variance accounted for by each marker and by the joint analyses of multiple markers, the genotypic co-efficient of variation was therefore calculated as R^2p/h^2[11], where, R^2p was the proportion of phenotypic variance accounted for by a marker and h^2 was the heritability.

RESULTS

Disease phenotypes: The analysis of variance indicated significant ($p<0.001$) differences among the DH lines tested for all four measures of quantitative resistance. In both cultivars cuticle rupturing occurred after 10 days. The fungus produced 1.2 pustules mm^{-2} in Otane compared with 2.1 mm^{-2} on Tiritea. The pustules were longer in Tiritea (0.55 mm) than Otane (0.37 mm). The frequencies of the components for quantitative resistance were approximately normally distributed while distribution was skewed toward Otane in the case of AUDPC (Fig. 1).

Broad-sense heritabilities of the four measures of quantitative resistance were estimated. AUDPC had the highest heritability (0.93) whereas LP and PL had the lowest (0.61). PD was correlated with LP ($r = -0.53$) and AUDPC (0.37) but there was no other significant correlation.

QTLs for quantitative resistance: Genetic mapping and chromosomal locations of the SSR and AFLP loci amplified in this study were reported elsewhere[8,9]. In the present study we emphasized the association of different genomic regions (Table 1) with measure of resistance. The individual markers that were significant determinants of LP, PD, PL and AUDPC are shown in Table 1.

Table 1: Chromosomal location and R^2 for SSR and AFLP markers significantly associated with measures of stripe rust resistance in a single locus regression. Chromosomal locations in bold are putative while X represents unknown location

Marker	LP R^2 (%)	PD R^2 (%)	PL R^2 (%)	AUDPC R^2 (%)
Xgwm52-(3D)	-	-	14	18^{**}
Xgwm611-(7B)	19^{**a}	13^{*}	-	14^{*}
Xgwm44-(7D)	20^{**}	-	-	-
*Xgwm340*a-(3BL)	12^{*}	-	-	-
Xgwm297-(7B)	-	10^{*}	-	-
Xgwm583-(5D)	-	20^{**}	-	14^{*}
Total	${}^{e}25$	${}^{e}48$	-	${}^{e}40$
XeAGTmCGA15-X	25^{**}	22^{*}	-	-
XeAGTmCCC5-X	38^{**}	-	-	-
XeAGTmCTC1-X	22^{*}	-	-	-
XeAGTmCGA8-7D	18^{*}	24^{*}	-	-
XeACCmCTA12-X	-	41^{**}	-	-
XeACCmCTA16-X	-	22^{*}	-	-
XeAGTmCGA19-X	-	-	30^{*}	-
*X*eAGTmCGA10-X	-	-	32^{*}	-
XeACAmCAG13-X	-	-	-	19^{*}
XeACCmCTA16-X	-	-	-	42^{**}

${}^{a**}p<0.01$; ${}^{*}p<0.05$. ^{e}Estimate obtained from a multiple QTL model

Latent period: Three SSR markers on three different chromosomes were identified as contributing to the latent period, explained 25% of the phenotypic and 40% of the genotypic variation. Four AFLP markers also showed significant association with LP. Among them, only *eAGTmCGA8* was mapped to chromosome 7D[9]. The effects for AFLP markers *eAGTmCGA15, eAGTmCCC5* and *eAGTmCTC13* were positive, indicating that Cv. Otane contributed alleles for prolonged latent period. The effects for the remaining 4 markers were negative, indicated Cv. Tiritea contributed alleles for longer latent period.

Pustule density: 3 SSR and 4 AFLP markers were determinants of pustule density (Table 1). Cv. Otane contributed alleles at loci *Xgwm583*-5D, *XeACCmCTA12* and *XeACCmCTA15*, which were involved in decreasing the number of pustules per mm^2. Alleles at loci *Xgwm611*, *Xgwm297*, *XeAGTmCGA8* and *XeACCmCTA16* were contributed by Cv. Tiritea. Markers *gwm611*, *eACCmCTA15* and *eAGTmCGA8* were common to both LP and PD (Table 1).

Pustule length: Pustule length was associated with microsatellite locus *gwm52*-3D and AFLP loci *XeAGTmCGA19* and *XeAGTmCGA10* (Table 1). The *Xgwm52* locus accounted for 14% of the variation in pustule length and the Otane alleles at locus *Xgwm52* of chromosome 3D and at *XeAGTmCGA19* were involved in reducing pustule length. Favourable alleles for reducing PL at locus *XeAGTmCGA10* were contributed from Cv. Tiritea. The amount of genetic variation accounted for by the marker *gwm52* on chromosome 3D was estimated at 23%.

AUDPC: The microsatellite markers identified three QTLs on chromosomes 7B, 3D and 5D associated with AUDPC (Table 1). The QTLs on chromosome 3D and 5D were associated with the decreasing effect of the Otane genotype on AUDPC. The QTL on loci *Xgwm611*-7B, *XeACAmCAG13* and *XeACCmCTA16* were associated with increasing effect from the Otane allele. Among AUDPC associated QTLs, only the one on chromosome 7B was common to LP and PD. Those on chromosome 3D and 5D were common for the PL and PD, respectively.

DISCUSSION

Quantitative resistance is characterised by continuous variation ranging from highly susceptible to good levels of resistance and is often assumed to be durable[13]. In most studies, infection type has been used to assess quantitative resistance to stripe rust at different growth stages[14] but very little is known about other

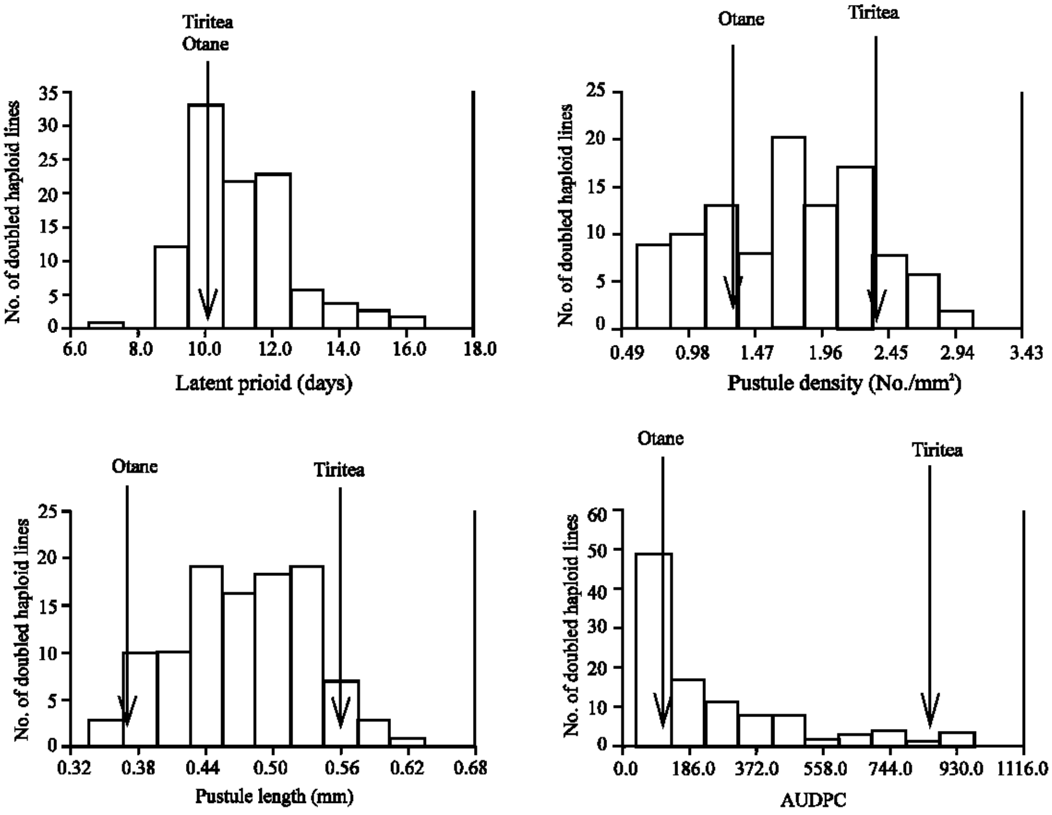

Fig. 1: Frequency distribution of phenotypes for the four measures of stripe rust resistance in double haploid lines derived form a cross Tiritea x Otane.

components of quantitative resistance such as latent period, pustule (lesion) length and density. There are no published reports where these components of quantitative resistance to stripe rust have been studied for their association with genomic regions. However, Boukhatem *et al.*[15] mapped only QTLs linked with AUDPC. Therefore, this would be a first study toward the identification of genomic regions contributing to the different components of quantitative resistance in addition to AUDPC.

Phenotypic variation: Latent Period (LP), Pustule Density (PD) and Pustule Length (PL) are components of quantitative resistance of wheat to stripe rust, while the severity of rust in the field as measured by AUDPC reflects the joint effects of all components[13]. The two parents did not differ in LP against pathotype 106E139A$^+$ but transgressions (longer and shorter LP) were observed in the DH progeny (Fig. 1). This indicates that neither parent carried all the positive or negative alleles and this was further confirmed by QTL analysis. The presence of DH lines with shorter and longer LP than both parents indicates additive gene effects, as reported in wheat stripe rust[16]. However, Van de Gaag and Jacobs[17] reported transgressive segregation with a non-additive effect to leaf rust. Although Tiritea and Otane differed for PD and PL, again the accumulation of favourable and unfavourable alleles in certain DH lines meant that some out-performed their parents (Fig. 1) and indicated that both parents contributed to the PD and PL in both directions. As a general trend in the cereal rust pathosystem, longer LP, lower infection frequency and smaller and slower growing lesions are thought to be associated with increasing quantitative resistance[13], however, this does not indicate that a single resistance mechanism is affecting all components. In this study LP was associated with PD, suggesting the involvement of some common or closely linked genes controlling these two components. Similarly, LP and AUDPC share some common genetic mechanism as they were correlated. Other components were not significantly correlated and hence may be affected by different genes as also suggested by Broers[18].

QTL identification: A total of 6 SSR and 10 AFLP marker loci were identified that showed linkage with different measures of quantitative resistance (Table 1).The QTL analyses demonstrated the involvement of seven genomic regions of different chromosomes in the control of LP (Table 1). Out of these Otane contributed toward long LP from three unknown parts of the genome with which AFLP markers showed association while the susceptible Tiritea contributed favourable alleles on chromosomes 7B, 7D and 3B. This supports the observed transgressive segregation (Fig. 1) in the DH population. The low phenotypic (25%) and genotypic (40%) variation among DH lines are probably attributed partly to recombination between marker loci and the target QTL and also to the effects of additional loci that contribute to the expression of LP, as Parlevliet[19] reported six minor genes for longer LP in barley leaf rust.

The finding of QTLs on chromosomes 7B (*Xgwm611*) and 5D (*Xgwm583*) associated with PD supports the two genes model proposed by Imtiaz[20] for adult plant stage PD. In comparison with the seedling stage PD (data not shown), only locus *Xgwm611* of chromosome 7B was common, as supported by the loose association (r = 0. 36) between seedling and adult PD. On the other hand, although adult PD and adult LP were moderately associated (r = 0.57) only locus *Xgwm611* was common to both components. This association is in agreement with the results of Broers[21] and indicates that genes located in the same chromosomal regions may affect both components.

The QTL on chromosome 3D of Otane was involved in shortening the PL. This QTL accounted for 14% of the phenotypic and 23% of the genotypic variation in PL. It is therefore likely that there are additional QTL not detected in the present study. Of three known chromosomal regions associated with AUDPC, the Otane alleles at *Xgwm52*-3D and *Xgwm583*-5D decreased AUDPC. Together these three markers accounted for 40% of the phenotypic and 43% of the genotypic variation in the AUDPC. Therefore, it is still possible that some QTLs with smaller effects were not identified, possibly due to the small population size and/or large environmental noise. Parlevliet[13] was of the view that AUDPC reflects the joint effect of components of quantitative resistance such as LP, PD, spore production and pustule size. The QTL mapping in this study also provided evidence for this concept. Comparing the AUDPC linked QTLs with the other components of quantitative resistance, the loci *Xgwm52*, *Xgwm611* and *Xgwm583* were associated with PL, LP and PD, respectively. In this way AUDPC reflects the joint effects of all components of quantitative resistance measured in the present study. Therefore, it could be presumed that some or all of the genes that influence AUDPC, pleiotrophically also govern other components of quantitative resistance, or that genes located in the same chromosomal regions may affect all the measures of quantitative resistance. These results are consistent with previous observations in barley that the components are correlated[22].

In this study 4 AFLP markers were associated with different measures of quantitative resistance but the genome location for most of them is not known, as there was no linkage with genome specific microsatellite markers. Overall the AFLP marker loci also confirmed the multi-locus control of different measures of quantitative resistance to stripe rust. Although the AFLP technique has the potential to assay a much greater number of loci and is suitable for detailed mapping, extra effort is required for locating the position of AFLP loci in a genome.

A QTL with an effect opposite to that predicted by the parents could be responsible for the occurrence of individuals with transgressive phenotypes in a population. The QTL data in this study confirm the multi-locus control of the different measures of quantitative resistance, which could be responsible for the observed transgressions in DH population (Fig. 1). Similar multi-locus control has been reported for stripe rust in wheat[15]. This study suggests that durable resistance in Otane is conferred by a complex of genes associated with its quantitative resistance, but the study of this resistance is complicated by resistance effects found in Tiritea. Furthermore, the present study has clearly demonstrated that QTLs for AUDPC were associated with components of quantitative resistance, namely LP, PD and PL. Therefore, calculating AUDPC at the adult plant stage in the field could be an efficient way to select for quantitative resistance in the progenies. Thus by using marker-assisted selection, plant breeders could apply molecular markers associated with favourable QTL alleles for quantitative resistance at the early stage of plant development and as a result improve the efficiency of selecting for quantitative resistance to stripe rust in breeding programmes.

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