Detection of molecular markers linked to the durable adult plant stripe rust resistance gene \(Yr18\) in bread wheat (\textit{Triticum aestivum} L.)

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Abstract
Striped rust of wheat caused by \textit{Puccinia striiformis} West, f. sp. tritici presents a serious problem for wheat production worldwide, and identification and deployment of resistance sources to it are key objectives for many wheat breeders. Here we report the detection of simple sequence repeat (SSR) markers linked to the durable adult plant resistance of cv. ‘Otane’, which has conferred this resistance since its release in New Zealand in 1984. A double haploid population from a cross between ‘Otane’ and the susceptible cv. Tiritea was visually assessed for adult plant infection types (IT) in the glasshouse and field, and for final disease severity in the field against stripe rust pathotype 106E139A\(^{-}\). At least three resistance loci controlled adult plant resistance to stripe rust in this population. Quantitative trait loci (QTL) mapping results revealed that two of these, one on chromosome 7DS corresponds to the durable adult plant resistance gene \(Yr18\) and other on chromosome 5DL were contributed from ‘Otane’; while the remaining one on chromosome 7BL, was contributed from the susceptible ‘Tiritea’. Interval mapping placed the ‘Otane’-resistant segment near the centromere of chromosome 7DS at a distance of 7 cM from the SSR marker \(gwm44\). The stability of QTL in the two environments is discussed. SSR \(gwm44\) is potentially a candidate marker for identifying the durable resistance gene \(Yr18\) in breeding programmes.

Key words: \textit{Puccinia striiformis} — \textit{Triticum aestivum} — bulk segregant analysis — disease resistance — gene mapping — molecular markers — \(Yr18\)

Of the three rust diseases of bread wheat, stripe rust is the most damaging to grain yields in cool, moist environments (Singh et al. 2000). While fungicides can control the disease, economic and environmental considerations favour the use of resistant cultivars. The development of molecular markers linked to a resistance gene is useful in plant breeding as the presence of the gene can be detected without waiting for the phenotypic expression of the gene of interest. A number of genes (\(Yr1\)–\(Yr28\)) conferring resistance to stripe rust have been identified in hexaploid wheat, as well as others provisionally designated (McIntosh et al. 1998), but several of these genes are now ineffective due to the occurrence of pathogen races that have overcome their resistance. However, some genes that confer adult plant resistance have remained effective. McIntosh (1992) and Singh (1992) attributed the durable resistance of several wheat cultivars to the adult plant resistance gene \(Yr18\). Later, they noted a genetic association between \(Lr34\) and \(Yr18\), which is recently mapped to the short arm of chromosome 7D (Suene et al. 2003).

Most stripe rust resistance genes have been mapped using aneuploid stocks (McIntosh et al. 1995) but some were recently molecularly tagged. These include \(Yr15\) (Chague et al. 1999), \(YrH52\) (Peng et al. 1999), \(Yr17\) (Robert et al. 1999), \(Yrns-B1\) (Börner et al. 2000) and \(Yr28\) (Singh et al. 2000). In New Zealand stripe rust resistance genes such as \(Yr1\), \(Yr6\), \(Yr7\), \(Yr9\), \(Yr14\), \(Yr17\), \(Yr27\) and \(YrA\) have broken down because of the variability in \textit{Puccinia striiformis} (Cromey 2000). Some 28 different pathotypes have now been reported in New Zealand (Cromey 2000), the first one being detected in 1980 (Beresford 1982). This indicates depletion in effective resistance genes with increase in pathogen virulence, and thus there is a need to search for new genes and characterize the phenotype and genotype of the durable resistance available in wheat. This paper therefore describes the detection of molecular markers linked to the adult plant resistance of the New Zealand wheat ‘Otane’, which has conferred this moderate but durable resistance since its release in 1984 (Cromey and Munro 1992). Once the markers for the resistance are identified, they may potentially be used in marker-assisted breeding programmes to develop resistant cultivars.

Materials and Methods

Plant materials: A total of 140 double haploid (DH) lines of wheat, \textit{Triticum aestivum} L., derived from the \(F_1\) of the cross Tiritea × Otane through the wheat–maize technique (Laurie and Bennett 1988) were provided by Bill Griffin, Crop and Food Research Ltd, Lincoln, New Zealand. The moderately but durably resistant ‘Otane’ and the susceptible ‘Tiritea’ were included as controls in both the glasshouse and field experiments.

Fungal materials: Culture WYR 93/5 of \textit{Puccinia striiformis} from the rust culture collection of Crop and Food Research Ltd was used for both glasshouse and field experiments. This culture (pathotype 106E139A\(^{-}\)) was derived from cv. ‘Karamu’, which possesses the stripe rust resistance gene \(YrA\). This pathotype is virulent to \(Yr2\), \(Yr3\), \(Yr4\), \(Yr7\) and \(YrA\) stripe rust resistance genes (Welling 1995).

Disease resistance phenotyping: The 140 DH lines and their parents were assessed for adult plant resistance in the glasshouse and field at Crop and Food Research Ltd, at Lincoln in the year 2000–01. The test
and inoculation conditions for both experiments have been described elsewhere (Imtiaz et al. 2003). Plants were scored on a 0–9 scale (Fig. 1) of Line et al. (1974), which is based on infection types (IT). Disease severity, based on the modified Cobb scale (Peterson et al. 1948), was recorded on the flag leaf of each DH line at adult stage (GS 61–69) (Tottman and Makepeace 1979).

**Bulk segregant analysis:** Based on the IT at the adult plant stage, the DH population was divided into three classes, resistant (R), moderately resistant (MR) and susceptible (S). The DNA samples from DH lines representing these three groups were pooled into three categories for bulk segregant analysis. The DNA pools were constructed by mixing equal aliquots of DNA extracted from each of the DH lines.

**Microsatellites (SSR) and AFLP assay:** DNA was extracted from 3–4-week-old seedlings of the two parents and 140 DH lines as described by Dellaporta et al. (1983). To provide maximum coverage of the A, B and D genomes, 139 (four to six per chromosome) mostly single locus SSR markers were selected from the mapped SSR markers of wheat (Röder et al. 1998). These markers were chosen for polymorphism tests using the two parents of the mapping population and three bulks, R, MR and S. Polymerase chain reaction amplifications were conducted as described by Röder et al. (1998). Amplification products were resolved by the silver staining method as described by Tixier et al. (1997).

To further enhance the genome coverage and supplement SSR markers, 60 amplified fragment length polymorphism (AFLP) primer combinations were used for polymorphism tests using two parents of the mapping population and the three bulks. The AFLP protocol was followed as described by Vos et al. (1995). Two different enzyme combinations EcoRI × MseI and PsiI × MseI were used (Table 1) to generate template DNA samples.

**Statistical analysis:** Genetic linkage analyses were performed using Map Manager QTX Version b11 (Manly et al. 2000). Multiple loci detected by single markers had a suffix a, b, c, d, added following the regular marker name. These loci were assigned to a specific chromosome by using well-mapped loci in different groups as anchor markers (Röder et al. 1998).

Both the mean IT and disease severity data were regressed on the marker alleles to determine any significant association. Interval mapping was used to assess the position of the QTL. For interval mapping, a nominal genome wide significance level of LOD corresponding to P = 0.05 was established by a permutation test (Churchill and Doerge 1994).

**Results**

**Genetic mapping**

From the 137 microsatellites markers tested on ‘Tiritea’, ‘Otane’ and the three bulks (R, MR and S), 11 SSR were polymorphic among the bulks and these were tested on the DH population. Similarly six AFLP primer combinations (Table 1) that identified polymorphisms among the bulks were screened on the DH population. This allowed the building of linkage groups involving loci amplified by both microsatellites and different AFLP primer combinations. Linkage groups and single unlinked loci were assigned to a respective chromosome using published microsatellite maps (Röder et al. 1998). However, the multiple loci designated by b, c and d were allowed to join one of these chromosomes through the ‘Distribute’ command of the programme.

The six AFLP primer combinations produced 25 polymorphic bands in total. However, only three AFLP markers joined chromosome 7D of the base maps constructed with microsatellite markers and the remaining did not form any linkage with these SSR maps.

**Adult plant resistance**

Of the 139 microsatellites tested on ‘Tiritea’, ‘Otane’ and on the three bulks R, MR, and S, 81 microsatellites did not reveal polymorphism between ‘Tiritea’ and ‘Otane’, 47 revealed polymorphism between the parents but not among the bulks, and 11 were polymorphic between the parents and among the bulks. Among these 11 microsatellites, gwm44, gwm611, gwm853, gwm332c and gwm340 showed association with IT and disease severity when tested on the DH population.

‘Otane’ contributed resistance alleles at QTL on chromosome 7D, while ‘Tiritea’ contributed resistance on chromosomes 7B in both the glasshouse and field (Table 2). The microsatellite gwm340 accounted for 6% of the variation in the phenotypic expression under glasshouse conditions but under field conditions it did not contribute (Table 2). The QTL with the largest effect was on the long arm of chromosome 7B (gwm611) and it was significant under both environments. This QTL accounted for 42 and 37% of the variation in adult plant resistance under glasshouse and field conditions, respectively (Table 2). Locus Xgwm44 on chromosome 7D explained 23

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**Table 1:** The sequence of adapters, primers and six primer combinations selected from 60 combinations to screen a population of DH lines for polymorphism associated with stripe rust resistance (selective bases in bold)

<table>
<thead>
<tr>
<th>Sequence of EcoRI and MseI adapters</th>
<th>Sequence of EcoRI and MseI primers</th>
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<tbody>
<tr>
<td>EcoRI adapter</td>
<td>MseI adapter</td>
</tr>
<tr>
<td>5’-CTCGTAGCTGCGTACC-3’</td>
<td>5’-GAGCATCAGTCTGAG-3’</td>
</tr>
<tr>
<td>3’-CTGACGCATGTTAA-5’</td>
<td>3’-TACTCAGACTCAT-5’</td>
</tr>
</tbody>
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**Primer overhangs for selectivity of specific bands**

<table>
<thead>
<tr>
<th>EcoRI</th>
<th>MseI</th>
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<tbody>
<tr>
<td>E1</td>
<td>M5</td>
</tr>
<tr>
<td>E2</td>
<td>M6</td>
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<tr>
<td>E3</td>
<td>M7</td>
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<tr>
<td>E4</td>
<td>M8</td>
</tr>
<tr>
<td>E5</td>
<td>M9</td>
</tr>
<tr>
<td>E6</td>
<td>M10</td>
</tr>
</tbody>
</table>

**The six primer combinations tested on the sub-population**

<table>
<thead>
<tr>
<th>E1/M6</th>
<th>E1/M8</th>
<th>E1/M9</th>
<th>E2/M5</th>
<th>E3/M7</th>
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**Fig. 1:** Infection type distribution of Tiritea/Otane-derived wheat double haploid lines tested with pathotype 106E139A− of *Puccinia striiformis*, where 0 = resistant, 9 = susceptible
Table 2: Chromosomal location and $R^2$ for microsatellite markers significantly associated with glasshouse and field infection types (IT) and stripe rust severity recorded at adult plant stage in a single-locus regression. Chromosomal locations in bold are putative marker regression. Chromosomal locations in bold are putative

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chromosomal location</th>
<th>Glasshouse $R^2$ (%)</th>
<th>Field $R^2$ (%)</th>
<th>Disease severity $R^2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gwm611</td>
<td>7B</td>
<td>42***</td>
<td>37***</td>
<td>26***</td>
</tr>
<tr>
<td>gwm44</td>
<td>7D</td>
<td>23***</td>
<td>17***</td>
<td>13**</td>
</tr>
<tr>
<td>gwm332c</td>
<td>7D</td>
<td>12**</td>
<td>7*</td>
<td>6*</td>
</tr>
<tr>
<td>gwm340d</td>
<td>7D</td>
<td>6*</td>
<td>–</td>
<td>6*</td>
</tr>
<tr>
<td>gwm583a</td>
<td>5DL</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>gwm340a</td>
<td>3BL</td>
<td>–</td>
<td>–</td>
<td>6*</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>45*</td>
<td>37*</td>
<td>29*</td>
</tr>
</tbody>
</table>

*** Significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$, respectively.

* Estimate obtained from a multiple QTL model.

Fig. 2: Interval mapping of mean infection types at the adult plant stage in double haploid wheat lines from Tiritea × Otane cross. The vertical lines show a significance threshold (LRS) value. Peak A detected by simple interval mapping and 17% of the phenotypic variation expression under the glasshouse and field conditions, respectively. Two other loci – gwm332c and gwm282c – also showed association with glasshouse and field IT in a single locus regression (Table 2). However, SSR marker gwm44 is linked with gwm340d and gwm332c (Fig. 2), there is therefore a possibility that a single QTL exists in this area detected by linked markers, which was confirmed by multiple regression analysis. All together, these two QTL explained 45 and 37% of the total variation under glasshouse and field conditions, respectively (Table 2).

Interval mapping revealed significant QTL peak at gwm44 on chromosome 7D, 7 cM away from the resistance gene contributed by ‘Otane’ (Fig. 2, peak A). There was no significant shift in the location of QTL between glasshouse and field conditions. Interval mapping was not performed for markers gwm297 and gwm611 of chromosome 7B as both markers were distantly linked (73 cM) and none of the AFLP markers showed linkage with these markers of group 7B.

**Final disease severity**

Stripe rust severity of ‘Tiritea’ was rated as 77% and of ‘Otane’ as 8% at GS 61–69 (Tottman and Makepeace 1979). The responses of the DH population were distributed in a continuous manner from low to high severities (Imtiaz et al. 2003). Linear regression analysis identified four different genetic regions contributing to stripe rust severity (Table 2). The QTL detected at chromosome 7B explained 26% of the phenotypic variation in disease severity and the favourable allele was contributed by ‘Tiritea’. The locus Xgwm340a of ‘Tiritea’ also explained 6% of the variation in the resistance. ‘Otane’ contributed the resistant alleles at chromosomes 7D and 5D. The 7D second largest QTL explained 13% of the total variation while 5D explained 6%. All together these markers explained 29% (Table 2) of the total phenotypic variation in the disease severity. No AFLP marker was found to be the significant determinant of stripe rust severity in the single locus regression analysis. Interval mapping of disease severity on 7D revealed a significant QTL peak corresponding to the same region where the peak for IT was recorded (Fig. 2, peak A).

**Discussion**

‘Tiritea’ is susceptible while ‘Otane’ is moderately resistant to stripe rust (Imtiaz et al. 2003) at the adult plant stage. The continuous distribution of IT and final disease severity recorded is probably due to the quantitative expression of the genetic determinants of resistance in this DH population. The occurrence of transgressive segregation (Fig. 1) indicated the presence of complementary alleles in the two parents, confirmed by QTL data.

The single-locus regression for IT in the glasshouse and the field (Table 2) underscores the importance of the resistance QTL on chromosome 7D and 7B that were contributed from ‘Otane’ and (Tiritea) respectively. The QTL on chromosomes 7B and 7D were consistent across the glasshouse and the field, except that the $R^2$ decreased from 42 to 37% in the case of ‘Tiritea’ locus Xgwm611 and from 23 to 17% in the case of ‘Otane’ locus Xgwm44. ‘Otane’ consistently provided resistance at locus Xgwm44 on the short arm of chromosome 7D which corresponds to gene Yr18, the only identified stripe rust-resistant gene on the short arm of chromosome 7D. Singh et al. (2000) found that the region Yr18 + 7DS of bread wheat cv. ‘Opata 85’ was involved in both seedling and adult plant resistance. In this study, locus Xgwm44 on chromosome 7DS is also involved in the adult plant and seedling resistance of ‘Otane’ (Imtiaz 2002). This region therefore might be responsible for the durable resistance of ‘Otane’, which also expresses moderate resistance to leaf rust (Cromley 2000). Furthermore, the presence of Yr18 in ‘Otane’ is very likely because pedigree analysis revealed that ‘Otane’ had six parental cultivars where the presence of this gene has been reported (Imtiaz 2002). Bariana et al. (2001) and Suenaga et al. (2003) mapped the adult plant resistance of ‘CD87’ and ‘Fukuho-komugi’ to a region near the centromere in the chromosome arm 7DS, respectively.

The distribution of disease severity in the replicated field trials revealed the involvement of multiple loci in the resistance of stripe rust for which the DH population was segregating. The hypothesized genetic model described by Imtiaz et al. (2003), based on the grouping of disease severity into classes, revealed the involvement of three genes when three classes were made, and two genes when two classes were formed. In the single-locus regression, four chromosomes were contributed towards disease severity, in contrast to two chromosomes for glasshouse and field IT. The two main QTL (7B and 7D) were the same as for IT, but there was also one on chromosome arm 3BL (gwm340a) and another on chromosome arm 5DL (gwm583), each contributing 6% to final disease severity. Although no stripe rust resistance gene has
been reported on chromosome arm 5DL, recently a QTL for powdery mildew resistance was reported on this arm (Chantret et al. 2001). Disease resistance genes are not randomly distributed over the genome of a species; rather they frequently occur in clusters on a particular chromosome (Islam et al. 1989). Therefore, it is possible that there are clusters of resistance genes in this region as the other coincidence was the association of the same microsatellite marker (gwm583) with stripe rust as reported here, and with powdery mildew, as reported by Chantret et al. (2001).

Two major regions, one on chromosome 7B and another on chromosome 7D, were stable in both the glasshouse and field experiments. These QTL may correspond to the genes or clusters of genes efficient in both environments. The regions on chromosome arms 3BL and 5DL varied between the two measures of resistance (Table 2). The SSR marker gwm44, which detected the race non-specific adult plant resistance of ‘Otane’, could be a potential marker for use in marker-assisted selection. These findings support the durability of resistance conferred by the gene Yr18 in ‘Otane’. Although the SSR marker gwm611 was also consistently involved in both environments, and explained a high proportion of the variability, it was linked with race-specific factors of the susceptible ‘Tirita’ (Imtiaz 2002) and thus may not be suitable for selection of race non-specific component of resistance.

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References


