Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in barley (*Hordeum vulgare* L.)

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Abstract. Identification and deployment of disease resistance genes are key objectives of Australian barley breeding programs. Two doubled haploid (DH) populations derived from Tallon × Kaputar (TK) and VB9524 × ND11231 (VN) crosses were used to identify markers for net type net blotch (NTNB) (*Pyrenophora teres* f. *teres*). The maps included 263 and 250 markers for TK and VN populations, respectively. The TK population was screened with 5 pathotypes and the VN population with 1 pathotype of NTNB as seedlings in the glasshouse. In addition, the TK population was subjected to natural infection in the field at Hermitage Research Station, Qld. Analyses of the markers were performed using the software packages MapManager and Qgene. One region on chromosome 6H was strongly associated with resistance to NTNB in both populations ($R^2 = 83\%$ for TK and 66\% for VN). In the TK population, 2 more quantitative trait loci (QTLs) were identified on chromosomes 2H and 3H, with $R^2$ values of 30\% and 31\%, respectively. These associations were consistent over all pathotypes studied during the seedling stage. The same QTL on chromosome 6H was also found to be highly significantly associated ($R^2 = 65\%$) with the adult plant (field) response in the TK population. There are several very closely linked markers showing strong associations in these regions. Association of the 4 markers on chromosome 6H QTL with resistance to the NTNB has been validated in 2 other DH populations derived from barley crosses Pompadour × Stirling and WPG8412 × Stirling. These markers present an opportunity for marker assisted selection of lines resistant to NTNB in barley breeding programs.

Additional keywords: SSR, AFLP, genetic mapping, net type net blotch.

Introduction

Net blotch, caused by *Pyrenophora teres* Drechs. (anamorph: *Drechslera teres* (Sacc.) Shoemaker), is a major disease in most barley-growing areas in Australia and around the world. *Pyrenophora teres* f. *teres* causes a net type lesion, which is characterised by dark brown blotches with a net-like pattern, sometimes accompanied by chlorosis (Smidegard-Petersen 1971; Khan and Tekauz 1982). Host reaction is influenced by the genotype and the infection environment (Khan and Boyd 1969).

*Pyrenophora teres* is a highly variable pathogen (Pon 1949; Afanasenko and Levitin 1979; Tekauz 1990; Steffenson and Webster 1992). Thirteen pathotypes of net type net blotch have been identified in Australia (Platz et al. 2000). Due to high variability of the pathogen and reduced tillage farming practices, the incidence of net type net blotch (NTNB) in Australia has increased over the years, with estimated yield losses of >30\% (Khan 1987). Consequently, a major objective of Australian barley breeding programs is to increase resistance to this disease in local barley cultivars.

Several studies have explored the inheritance of resistance to NTNB. In most cases, 1–3 genes have been identified against different pathotypes of the fungus. In Canada and Egypt, one dominant gene for resistance was detected
(Buchannon and McDonald 1965), whereas in Australia, Khan and Boyd (1969) identified 2 dominant resistance genes. More recently, numerous genes were identified in different barley lines from around the world (Wilcoxson et al. 1992; Douyiissi et al. 1996; Ho et al. 1996; Afanasenko et al. 1999; Jonsson et al. 1999). A current study of barley genotypes using NTNB isolates from Western Australia, Queensland, and South Australia, has detected more than 6 genes for resistance (Gupta et al. 2002).

Molecular markers were used to determine the number of quantitative trait loci (QTLs) involved in resistance to NTNB. Steffenson et al. (1999) mapped 3 QTLs for seedling resistance and 7 for adult plant resistance. The seedling and adult plant resistances were located on different chromosomes. In a doubled haploid (DH) population from the cross of Igri × Franka, a single dominant gene for resistance was located on chromosome 3H (Graner et al. 1996). Richter et al. (1998) found 12 QTLs conditioning seedling resistance in an F2 population derived from the susceptible barley cultivar Arena and the resistant Ethiopian landrace Hor 9088. Spanel et al. (1998) mapped 3 QTLs on chromosomes 4H, 5H, and 6H in a DH population of the cross Harrington × TR306. Manninen et al. (2000) used retrotransposon-based molecular markers (retrotransposon-microsatellite amplified polymorphism and inter-retrotransposon amplified polymorphism) and identified a major locus on chromosome 6H accounting for 65% of the response variation in a cross between the resistant line CI9819 and the susceptible cultivar Rolfi. These studies indicate that many genes have been mapped for resistance to Pyrenophora teres f. teres pathotypes prevalent around the world.

The objectives of this study were to identify markers closely linked to resistance to NTNB in 2 Australian barley mapping populations derived from crosses Tallon × Kaputar and VB9524 × ND11231, and to validate the markers in 2 other barley DH populations.

Materials and methods

Mapping populations

Mapping population Tallon/Kaputar (Cakir et al. 2003a, this issue) is described in detail elsewhere in this issue. The population VB9524/ND11231 was developed by the barley breeding program of the Victorian Institute for Dryland Agriculture (L. C. Emebiri, unpublished data). The population was constructed by doubled haploidy using anther culture technique and included 180 lines.

Disease response tests

The DH lines and parents were grown in pots in a replicated experiment in the glasshouse. Ten plants of a single line were grown in each pot. Potting mix consisted of loam, peat, and vermiculite in the ratio 2:1:1 (by vol.) to which was added a basal fertiliser of GF306N (Grow Force Australia) at 2.5 kg/m3. After emergence, plants were fertilised weekly with Aquasol (Yates Australia) solution at ~75 mL/pot. Seedlings were raised in a glasshouse where temperatures ranged from 10 to 25°C with a daylength around 12 h.

Five isolates [NB50 from Gatton, Qld; NB54 from Biloela Research Station, Qld; NB81 from Mt Rascal (near Toowoomba), Qld; NB97 from Byee (north of Kingaroy), Qld; and NB52B from South Australia] were used to screen the TK DH population. The VN population was screened with isolate NB77 (from Chinchilla, Qld). Each is a distinct isolate representing virulences from Australia (Gupta et al. 2003, this issue).

To prepare inoculum, single conidial cultures of each strain were increased on peanut oatmeal agar (Speakman and Pommer 1986) at 19°C under cool white and NUV light on a 12-h cycle. After 9 days in culture, conidia were washed from the agar surface, filtered through a 330-µm strainer, and made up into an aqueous suspension containing 12 500 conidia/mL. Approximately 1.125 mL of this suspension was applied per pot with a Krebs airless paint sprayer (Oldfields Pty Ltd) when plants were at an average growth stage of 13.5 (Zadoks et al. 1974). Inoculated plants were immediately placed in a fogging chamber and held at 100% relative humidity for 24 h (14 h dark, 10 h light) at 19°C, then returned to the glasshouse for disease development. Notes on infection types (IT) were scored 9 days after inoculation using a scale developed by Tekauz (1985).

Field trial

Adult plant resistance in the TK population was assessed in a field nursery of 65 DH lines and parents, sown in 2 replicates in a randomised block design at Hermitage Research Station, Qld, in 1999. The trial site carried barley stubble infected with P. teres and a moderate epidemic developed. Disease levels were scored 10 days before anthesis, based on disease severity on a 1–9 scale where 1 was highly resistant and 9 was very susceptible.

Marker analysis

The genetic maps of the TK (Cakir et al. 2003a) and VN (L. C. Emebiri, unpublished data) populations utilising 263 and 250 DNA markers, respectively, were used to identify marker loci associated with resistance to NTNB. QTL analyses were performed using software packages MapManager QTX (Manly et al. 2001) and Qgene (Nelson 1997). A threshold LOD (logarithm of odds ratio) score of 3.0 was chosen for declaring the existence of a QTL. Wherever appropriate, simple regression and interval mapping analyses were used to identify the associations. Data for each isolate or site were analysed separately and a joint analysis over all strains/sites was performed for each trait.

Validation of the markers

Two DH populations, each comprising 300 lines, were constructed by anther culture. One population was derived from a cross between barley varieties Pompadour (FDO192/Patty) and Stiriling (Dampier/Priot/Ymer/3/Piroline) and the other from a cross between WPG8412 (Bowman/Ellice/TR451) and Stiriling. These populations were screened with NTNB pathotypes from Western Australia (95NB100 and 97NB1) and Queensland (NB81) at the seedling stage using the screening procedure of Gupta and Loughman (2001). For the marker validation, bulk segregant analysis (BSA) was employed (Michelmore et al. 1991). Statistical analysis of the segregation data had identified the presence of a single resistance gene operative against all 3 isolates in both populations (S. Gupta, unpublished data). Based on this gene, DNA samples from 11 resistant and 11 susceptible lines for each population were mixed in equal proportions. The bulks and the parents from both crosses were assayed with a set of simple sequence repeat (SSR) markers and amplified fragment length polymorphism (AFLP) markers that had been mapped in the TK population. Genotyping conditions were the same as those reported by Cakir et al. (2003a) except that the genotyping of individual plants with SSR markers was carried out according to the method of Rampling et al. (2001).
Results

Tallon × Kaputar population

Resistance at the seedling stage

The parent lines differed widely in their response to pathotypes NB54 and NB52B of NTNB (Table 1). The DH population showed a wide range of segregation for response to all pathotypes. Frequency distributions of infection types against NB54, NB52B, and NB97 indicated the presence of one major gene in the population (Fig. 1a). The presence of DH lines with intermediate ITs suggests the existence of additional genes with smaller effects. For all of the NTNB pathotypes, transgressive segregation was observed (Fig. 1a, Table 1).

Simple regression analysis identified markers highly significantly associated with each pathotype on chromosomes 2H, 3H, and 6H. For example, for pathotype NB97 there were 14 markers with a LOD score >3.0 on

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![Chart](chart.png)

**Fig. 1.** Net type net blotch disease distributions for the pathotypes in TK population: (a) B50, (b) NB52B, (c) NB54, (d) NB81, (e) NB97, (f) adult plant scores, (g) Disease distribution in the VN population for the pathotype NB77. Disease infection types at seedling stage were taken according to Tekauz (1985) using a 1–10 scale (1, resistant; 10, susceptible). Adult plant scoring was taken based on the disease severity using a 1–9 scale (1, resistant; 9, susceptible). Arrows indicate the means of the DH lines.
chromosome 6H and this result was consistent for all pathotypes. Overall, $R^2$ values for the significant markers on this chromosome ranged between 46% and 83%, depending on the pathotype (Table 2). In particular the SSR marker Bmag0381 had the highest LOD score and $R^2$ values for the pathotypes NB52B, NB54, NB81, and NB97. $R^2$ and LOD score for pathotype NB97 were 83% and 22.79, respectively (Table 2). For NB50, M61P12K116 was the most significant marker, with an $R^2$ of 65%.

Genetic data for SSR marker Bmag0381 showed a complete co-segregation with the disease data in the Table 2. Markers associated with resistance to net type net blotch (NB97 for the TK population and NB77 for the VN population) on chromosomes 2H, 3H, and 6H, their values for $R^2$, LOD scores, and probability in Tallon $\times$ Kaputar (TK) and VB9524 $\times$ ND11231 (VN) populations

<table>
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<tr>
<th>Marker</th>
<th>Chrom.</th>
<th>Pop.</th>
<th>$R^2$</th>
<th>LOD</th>
<th>$P$</th>
<th>AA</th>
<th>aa</th>
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$^A$Phenotypic variation explained by each marker.  
$^B$Logarithm of odds ratio.  
$^C$Disease severity mean of the DH lines within each genotypic class; AA and aa indicate the first and the second parents, respectively.  
$^D$Additive effect of replacing one allele (a) with the other (A).
Mapping and validation of the genes for NTNB

Mapping population (Fig. 2), indicating a very tight linkage to the NTNB resistance gene on chromosome 6H. AFLP marker M61P12K116 and SSR marker Bmag0173 had only 3 recombinant lines with the disease resistance gene, indicating tight linkage with this gene (results not shown).

The second QTL region near the centromere of chromosome 2H was found to be linked to a gene for resistance to NTNB. This QTL accounted for 20–29% of the variation in disease reaction, depending on the pathotype, with the highest $R^2$ of 29% for NB97 (Table 2). For pathotypes NB52B, NB54, NB81, and NB97, the SSR marker Bmag0114 was the most significant. AFLP marker M47P13T109 had the highest $R^2$ value for the pathotype NB50 (results not shown). The third QTL was located on the short arm of chromosome 3H. The region between markers p11m47TK118 and p13m47KT191 showed a high level of association with low disease response from the pathotype NB97, with an $R^2$ value of 31% (Table 3). $R^2$ values of disease response data from other pathotypes ranged from 24% to 30% (results not shown).

Resistance at the adult plant stage

Analysis of the adult plant data revealed the same 6H QTL to be the most significant region. In this QTL region, EBmac0874 SSR marker had the highest $R^2$ value ($R^2 = 65\%$). The second most important marker was M61P12K116 ($R^2 = 56\%$) (Table 3).

VB9524 × ND11231 population

Infection types of parental lines tested differed markedly with all pathotypes, except NB81, where VB9524 was not fully susceptible (Table 1). Population ITs revealed a distribution that was somewhat bimodal, indicating the presence of 1 major gene (Fig. 1g). Marker analysis

![Fig. 2. Frequency distributions for the NTNB infection type scores of pathotype NB97 in the DH lines carrying alleles from (a) Tallon, and (b) Kaputar at the SSR locus Bmag0381. The arrows indicate the means of each genotypic class.](image_url)

Table 3. The most significant marker intervals linked to resistance to net type net blotch (NB97 isolate for the TK population and NB77 isolate for the VN population), their chromosomal locations, interval sizes (cM), values for $R^2$, and LOD scores in Tallon × Kaputar (TK) and VB9524 × ND11231 (VN) populations

<table>
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<tr>
<th>Marker</th>
<th>Population</th>
<th>Chrom.</th>
<th>Interval size (cM)</th>
<th>$R^2$</th>
<th>LOD</th>
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<td>p13m47K1191- p11m47TK118</td>
<td>TK</td>
<td>3H</td>
<td>2.0</td>
<td>0.31</td>
<td>5.04</td>
</tr>
<tr>
<td>Bmac0381- EBmac0874</td>
<td>TK</td>
<td>6H</td>
<td>1.7</td>
<td>0.83</td>
<td>22.78</td>
</tr>
<tr>
<td>EBmac0874- p12m61K116</td>
<td>TK</td>
<td>6H</td>
<td>3.4</td>
<td>0.65</td>
<td>13.71</td>
</tr>
<tr>
<td>P11M48_160- p11m53_88</td>
<td>(adult plant)</td>
<td>VN</td>
<td>4.8</td>
<td>0.66</td>
<td>41.31</td>
</tr>
</tbody>
</table>

$^a$Phenotypic variation explained by the most significant marker.

$^b$Logarithm of odds ratio.
indicated the presence of a major gene resulting in only 1 strong QTL on chromosome 6H, which corresponded to the same region identified in the TK population (Fig. 3a, b). Single marker analysis revealed 12 markers with a LOD score >3.0 (Table 2). The most significant marker (p11m48_160) in this QTL explained 66% of the phenotypic variation for NTNB response in this population.

Validation of 6H QTL

Two AFLP and 2 SSR markers (M61P12K116, M55P13T311, Bmag0173, and EBmac0874) known to be located on 6H showed clear polymorphisms between parents and the bulks of Pompadour × Stirling and WPG8412 × Stirling populations (results not shown). Three of these markers were then assayed for the individual lines within these bulks. All of the individuals within resistant bulks and susceptible bulks exhibited complete association with the markers M55P13T311 and Bmag0173. For the AFLP marker M61P12K116, all but 3 individuals from the susceptible bulk showed the expected banding pattern. This validation analysis further confirmed the presence of a major QTL for the NTNB resistance on chromosome 6H.

Discussion

Plants with IT of 5 and below were considered resistant, whereas those with IT >5 were considered susceptible. There was distinct segregation within the TK-population for response to NB97, NB52B, and NB54 pathotypes. Pathotypes NB97 and NB52B showed a near 1:1 segregation for the resistant and susceptible lines, indicating the presence of one major gene. The presence of transgressive segregants and the DH lines with intermediate infection
types for all pathotypes suggest the presence of additional minor genes for resistance in the parents. Steffenson et al. (1996) also observed transgressive segregation for net blotch severity in a Steptoe × Morex DH population. Additional studies need to be done to identify the location of these minor genes.

None of the lines in the population was resistant to pathotype NB50 because both parents (Tallon and Kaputar) were susceptible to this pathotype. In contrast, only 4 lines were susceptible to the NB81 pathotype. The resistant reaction of both parents and most of the lines to NB81 indicates that there is either a common resistance gene in Tallon and Kaputar or additional gene(s) for resistance to this pathotype in Tallon. The resistance gene, located on 6H chromosome and confirmed in validation populations, appears to be from Tallon. Molecular genetic analysis of screening data with pathotype NB81 in the validation populations confirmed that resistance in Tallon is the same as in Pompadour and WPG8412 parental lines. Resistance to other pathotypes used in tests on the TK population were also mapped on the same region of chromosome 6H; a resistance locus from the parent Kaputar is also located on chromosome 6H. Further experiments are needed to determine if the resistance loci in the parental lines Tallon and Kaputar are the same loci for the NB81 pathotype.

Five of the 6 isolates used in this study were from Queensland and represented 5 different pathotypes (Platz et al. 2000). Two isolates used in the validation experiment were from Western Australia (Gupta and Loughman 2001) and one from Queensland. Our data indicated that one common resistance gene was effective against all of these pathotypes. In a similar experiment, Afanasenko et al. (2000) used NTNB isolates collected from different countries around the world to study resistance. Their findings in an F₂ population suggested the presence of one common dominant gene operating against all isolates and additive resistance genes specific to individual isolates. These studies indicated that common genes can be effective against a wide range of pathotypes of P. teres f. teres (Tekauz, 1990; Afanasenko et al. 2000; Platz et al. 2000). Molecular work is currently underway to find markers linked to the additional resistance genes in our validation populations.

Comparative mapping, based on common markers among genetic maps, allows comparison of QTLs for a given trait among different populations. The QTL reported on chromosome 6H in this study appears to be located at the same region of chromosome 6H in at least 2 other studies (Steffenson et al. 1996; Manninen et al. 2000). However, in the absence of markers common among these studies it cannot be concluded that these QTL regions represent the same resistance genes.

The use of BSA with polymorphic markers from a genetic map in a different population allowed us to comparatively map a trait and validate the chromosomal location of a gene in barley populations without a saturated genetic map of the subsequent population. BSA was first used to identify markers for disease resistance genes in lettuce species (Michelmore et al. 1991). Since then the technique has been used in many studies for identification of markers in different crop species such as apple (Yang et al. 1997), grapevine (Lahogue et al. 1998), and maize (Quarrie et al. 1999). A similar approach to ours identified 4 markers closely linked to the Rsv1 locus of soybean mosaic virus (Hayes and Saghai Maroof 2000). They first identified the markers by BSA then mapped it on a segregating population to find the location of the markers.

This study indicated that the QTLs identified on 2H, 3H, and 6H gave effective seedling resistance and that the 6H QTL also conferred resistance to a field isolate at the adult plant stage. This result agrees with the inheritance studies of Gupta et al. (2002). They found that the gene on chromosome 6H conferred resistance to NTNB in both seedling and adult plant stages in 3 different barley DH populations. It has been reported that some genes function only in adult plants (Tekauz 1990; Douiyssi et al. 1998). We have observed in another barley DH population that some lines that were susceptible to pathotype NB321 (which is prevalent in Qld) at the seedling stage showed high levels of resistance at the adult plant stage (G. J. Platz, unpublished data). Similarly, Steffenson et al. (1996) identified different QTLs for resistance to P. teres during the seedling stage and adult plant stage. Richter et al. (1998) detected different numbers of QTLs from disease response data taken on the first and second seedling leaves. They found that different genes were expressed, depending on the period from inoculation to observation of disease response. From these studies it appears that some genes are expressed during both the seedling and adult plant stages, whereas other genes are expressed at only the seedling or the adult plant stage. It is also possible that differences in resistance between seedlings and adults may be pathotype-specific.

The QTL region detected on chromosome 6H spanned a large chromosomal segment in both populations (Fig. 3). In this region, there were 6 SSRs and 11 AFLP markers in the TK population and 12 markers (1 SSR, 10 AFLP, and 1 expressed sequence tag) in the VN population. Bmag0173 was the common marker between both populations. Strong linkage between the disease resistance gene and marker Bmag0173 in both populations indicates that both populations have the same resistance gene on chromosome 6H. SSR marker Bmag0381 completely co-segregated with the disease data in the TK population (Fig. 2) and we believe that the NTNB resistance gene must be tightly linked to this marker. The parents used in the validation populations and VN population were not polymorphic for marker Bmag0381, and we were therefore unable to test it in those populations. Among the markers that were validated, Ebmag0874 was 1.7 cM away from Bmag0381. Bmag0173 and
M61P12K116 markers, which were completely linked to each other, were 5.1 cM from Bmag0381.

Having multiple markers linked to low disease response in each QTL region offers a good opportunity for plant breeders to use markers to select for resistance to NTNB. This increases the chances of having an ample number of polymorphisms in a wide range of parental germplasm. SSR markers used in this study generally had high levels of polymorphic information content in a study of European barley germplasm (Macaulay et al. 2001). For this reason, SSR markers Bmag0173 and Bmag0381 may prove to be very useful markers for breeding programs. We are also currently testing other SSR markers and the conversion of AFLP markers into sequence characterised amplified region markers, with the ultimate aim of developing single nucleotide polymorphism markers for high throughput screening in breeding programs.

DNA markers are being used as selection tools in breeding programs throughout Australia (Barr et al. 2000, Cakir 2003b). This work has identified several markers that are associated with resistance to NTNB. The chromosomal regions in which these markers are located will be focal points of further research and the validation and implementation of markers for routine selection in breeding programs. The markers located in these regions could also be used in pedigree-based association mapping studies using diverse barley genetic resources.

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