Theor Appl Genet (1999) 99: 323–327

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Identification and mapping of a gene conferring resistance to the spot form of net blotch (*Pyrenophora teres* f *maculata*) in barley

Received: 24 September 1998 / Accepted: 19 December 1998

Abstract Spot form of net blotch (SFNB) (*Pyrenophora teres* f *maculata*) is an economically damaging foliar disease of barley in many of the world's cereal growing areas. The development of SFNB-resistant cultivars may be accelerated through the use of molecular markers. A screen for SFNB resistance in 96 lines identified four new sources of resistance, including a feed variety, 'Galleon', for which a fully mapped doubled haploid population was available. Segregation data indicated SFNB resistance was conferred by a single gene in the 'Galleon' × 'Haruna Nijo' cross, positioned on the long arm of chromosome 7H. This gene is designated *Rpt*4 and is flanked by the RFLP loci *Xpsr117(D)* and *Xcdo673* at distances of 6.9 cM and 25.9 cM, respectively. The marker *Xpsr117(D)* was validated using another population segregating for *Rpt4*, correctly predicting SFNB resistance with more than 90% accuracy.

Key words *Hordeum vulgare* · Disease resistance · Genetic mapping · RFLP · QTL

Introduction

The spot form of net blotch (SFNB), caused by the fungus *Pyrenophora teres* (Drechs.) forma *maculata* (Smedegaard-Petersen) is a foliar disease of barley found on crops grown in Scandinavia, Canada, South Africa and Australia (Tekauz and Buchannon 1977; Louw 1995; Khan and Tekauz 1982). The spot form was differentiated from the net form of net blotch (*P. teres* f *teres*) by Smedegaard-Petersen (1971) on the basis of leaf symptom type. The two pathotypes have different distributions, with the spot form being prevalent in Scandinavia (Makela 1972; Smedegaard-Petersen 1971), whereas in western Canada, Tekauz (1990) found that 82% of collected isolates were of the form producing net symptoms. SFNB has been responsible for severe disease outbreaks in Canada (Tekauz and Buchannon 1977) and in Western Australia, where up to 25% losses in grain yield were estimated by Khan and Tekauz (1982).

The use of resistant varieties is the most economic method of control of SFNB, although few sources of resistance to *P. teres* f *maculata* have been identified. In Canada, CI 5791 and CI 9214 have resistance to some but not all isolates (Tekauz and Buchannon 1977; Tekauz 1990) and are being used in breeding programs. There has not been any conclusive study of the genetics and chromosomal location of SFNB resistance (Ho et al. 1996). In Australia, barley breeding programs are using marker-assisted selection to rapidly introgress disease resistance genes into elite lines, and resistance to the spot form of net blotch is a high priority. This paper describes the identification of sources of resistance to SFNB, the mapping of a major locus controlling resistance and the development of molecular markers for use in marker-assisted selection.

Communicated by G. Wenzel

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Materials and methods

Plant materials and disease screening

A 'Galleon' (SFNB-resistant) × 'Haruna Nijo' (SFNB-susceptible) doubled-haploid (DH) population was used for mapping (Barr et al. 1998). The BC_1F_2-derived validation lines 'Sloop/(Sloop/(Triumph/Galleon))' were obtained from Dr. Andrew Barr (University of Adelaide).

P. teres f *maculata* inoculum was prepared according to Tekauz (1990). Five isolates (43/96/1;49/96/9;49/96/10;50/96/9;10/97, obtained from separate areas of South Australia) were used as mixed inoculum. Seedling resistance tests were performed in growth rooms with 12-h light at 24°C and 12-h dark at 14°C. Four replicates of five plant clumps from each line were inoculated, including parents and standards. Validation line assessments were carried out on single F_5 plants. Scoring for seedling SFNB phenotype was based on the numerical lesion-type scale developed by Tekauz (1985), where 1 indicates the most resistant reaction type and 9 the most susceptible reaction type. Field screening data came from four row plots naturally infected at Yeelanna, South Australia.

Genetic mapping

The 'Galleon' × 'Haruna Nijo' map has a length of 1584 cM, and the average distance between markers is 5.3 cM (Barr et al. 1998). The position of loci for SFNB resistance was determined using Q-GENE (Nelson 1997) and RI MANAGER (Manly 1993) with the Kosambi map unit function (Kosambi 1944). For marker validation, DNA extraction, restriction digestion and Southern blotting were carried out as described in Guidet et al. (1991), except that a solution of 0.4 *M* NaOH was used for DNA transfer onto membranes. BRGA probes were derived from resistance gene analogs (Seah et al. 1998).

Results

Screening for resistance

Seedling tests for resistance were carried out on 96 Australian and overseas lines. Resistance was identified in two Australian lines: 'Galleon', a widely grown feed barley, and a breeding line, WI2976 (Table 1). The progenitors of these lines were tested, and the results indicated that CI3576 and 'California Mariout', respectively, were the probable sources of SFNB resistance, although they themselves only exhibited moderate levels of resistance in the seedling test (Table 1).

Table 1 Seedling and field reaction to *P. teres* f *maculata*

Line	Seedling reaction[a]	Field reaction[b]
OK82850	1.5	MR
Dairokkaku	2.0	MR
WI2976	2.0	MR
Schooner	2.5[c]	MS
Galleon	2.7	MR
CI9214	3.5	MR
California Mariout	3.5	–[d]
CI 3576	4.3	–
Arapiles	5.1	MS
Sloop	5.5	S
Franklin	6.5	S
Haruna Nijo	7.0	S

[a] Scale: 1, most resistant; 9, most susceptible
[b] R, Resistant; S, susceptible; M, moderately (S or R)
[c] Resistance only observed at seedling stage
[d] Not tested

Two further lines, OK82850, from Oklahoma, and 'Dairokkaku', from Japan, also had good resistance. All other lines tested were found to be susceptible. The 4 lines exhibiting resistance to SFNB in seedling tests also had good resistance in the field (B. Read, personal communication). The malting barley 'Schooner' consistently showed resistance in seedling tests but was always moderately susceptible in the field (Table 1).

The four sources of seedling and adult plant resistance identified in this study were more effective than CI9214, a resistant line identified in Canada (Tekauz and Buchannon 1977).

Genetic mapping

Seedling tests were used to score the reaction phenotype of 95 'Galleon' × 'Haruna Nijo' DH lines inoculated with *P. teres* f *maculata*. When a reaction score of 4 was used to delimit the resistant and susceptible classes, segregation of the progeny fitted the 1 : 1 ratio for a single gene for resistance to SFNB ($\chi^2 = 5.4$, $P < 0.05$) (Fig. 1). F_1 tests had indicated that the 'Galleon' SFNB resistance gene is dominant (data not shown). The existence of DHs with transgressive phenotypes showing reduced symptom development compared to 'Galleon' may indicate the effects of other minor genes (Fig. 1). To locate the position of the resistance loci, we compared the DH SFNB reaction phenotypes to those of previously mapped restriction fragment length polymorphism (RFLP) probes using quantitative trait locus (QTL) analysis. A major locus, explaining 80% (Rsq = 0.797, $P = 0.0001$) of the SFNB reaction variation observed was located on the long arm of chromosome 7H in cv 'Galleon' (Fig. 2); this gene was designated *Rpt*4 (reaction to *Pyrenophora teres*). The closest marker to *Rpt*4 is the RFLP loci *Xpsr117(D)* (6.9 ± 2.72 cM, LOD = 16.7). Four apparent double recombinants that were observed around *Rpt*4 in the data set were included in the analysis. If the double recombinants were excluded from the analysis,

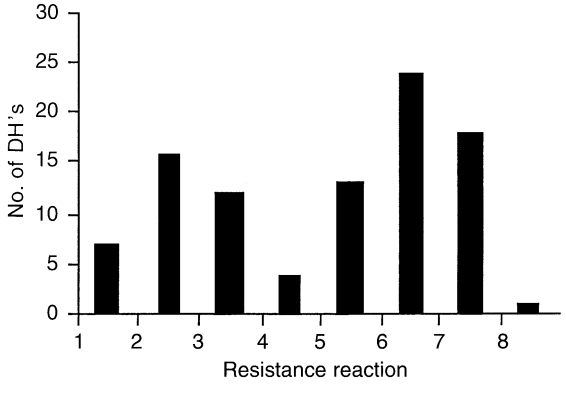

Fig. 1 Reaction to *P. teres* f *maculata* in 'Galleon' × 'Haruna Nijo' doubled haploids ('Galleon' = 3.1, 'Haruna Nijo' = 6.6)

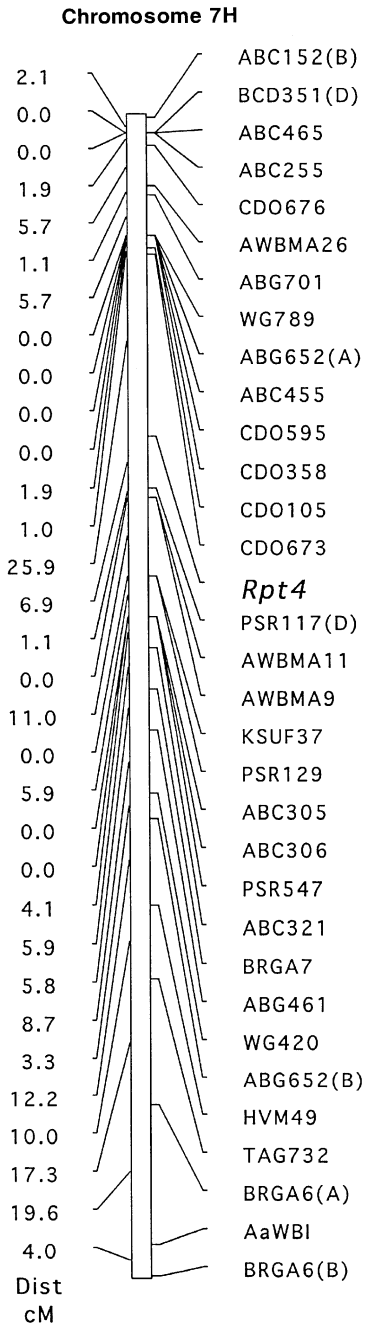

Fig. 2 RFLP map of chromosome 7H showing the map location of the *Rpt*4 locus for resistance to *P. teres* f *maculata*

the order of loci was unchanged, although the distance between *Rpt*4 and *Xpsr117(D)* was reduced to 3.6 ± 2.0 cM.

Marker development

*Rpt*4-linked markers were assessed for their ability to reveal useable polymorphisms between varieties. When the probe/enzyme combinations PSR129/*Bam*HI, PSR117(D)/*Eco*RI and AWBMA11/*Hin*dIII were used, polymorphisms were found between the *Rpt*4 donor 'Galleon' and 102 other barley varieties or breeder's lines. The full list of polymorphic markers is available on the graingenes website at: http://greengenes.cit.cornell.edu:80/WaiteQTL/

Marker validation

The effectiveness of marker-assisted selection (MAS) using *Rpt*4-linked markers was investigated in the progeny of a 'Galleon'-derived cross. The marker PSR117(D)/*Eco*RI correctly predicted the SFNB seedling phenotype in 22 out of 24 F_5 progeny from the backcross 'Sloop/(Sloop/(Triumph/Galleon))' (Fig. 3).

Discussion

This study reports the identification of resistance to four Australian isolates of *P. teres* f *maculata* in two Australian barley lines, 'Galleon' and WI 2976, as well as two other lines, OK82850 and 'Dairokkaku'. The SFNB resistance of the Australian lines probably comes from CI3576 and 'California Mariout', respectively, although these lines had only intermediate resistance in our seedling test.

We have shown that the SFNB resistance gene from 'Galleon' gives a similar level of resistance in adult plants as in seedlings. This is in agreement with Tekauz (1986) who found that genotypes having seedling net blotch resistance also had adult plant resistance, although Steffenson et al. (1995) identified different seedling and adult resistance genes to *P. teres* f *teres*. In our screening, we have found one variety, 'Schooner', that has seedling resistance but is moderately susceptible as an adult plant.

The 'Galleon' SFNB resistance locus was located on chromosome 7H using QTL analysis of reaction phenotypes, a powerful technique for mapping Mendelian trait loci (Lander and Botstein 1989; Steffenson et al. 1995). Using QTL analysis of phenotypic scores obviates the need to create arbitrary resistance reaction classes, which may be skewed by differences in symptom expression under screening conditions. The study of the genetics of net blotch resistance has previously been complicated by this inconsistency of host reactions due to environmental conditions (Shipton et al. 1973). This study is the first to definitively map a SFNB resistance locus. In their mapping study, Ho et al. (1996) investigated the genetics of resistance to a spot form isolate of net blotch but were not able to determine if one or two genes were responsible for resistance due to inconsistencies between DH and F_2 progeny with respect to SFNB segregation.

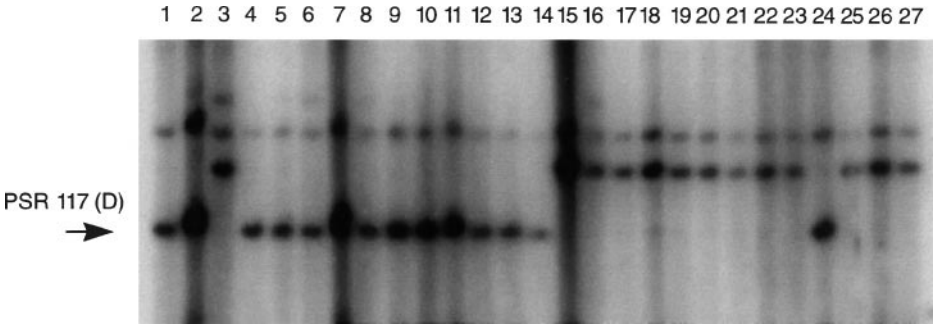

Fig. 3 Southern analysis of marker verification lines. DNA from individual F_5 plants with known scores for reaction to *P. teres* f *maculata* from the cross 'Sloop/(Sloop/(Triumph/Galleon))' was digested with *Eco*RI and probed with the *Rpt*4-linked marker PSR117. *Lanes*: *1* 'Galleon', *2* 'Triumph/Galleon', *3* 'Sloop', *4–15* resistant F_5 plants, *16–27* susceptible F_5 plants

RFLP and other markers are powerful tools for indirect selection of agronomically important genes (Beckman and Soller, 1983; Tanksley 1983). However, the identification of an RFLP marker that shows close linkage to a trait of interest is only the first stage in developing a marker that can be used in pragmatic breeding. Before a marker can be actively used in a breeding program several issues must be addressed. Specifically, it is important to determine how the marker will perform in a breeding program. In the case of the marker described here, it is important to determine if the RFLP markers closely linked to the *Rpt*4 locus detect different alleles in resistant compared to susceptible barley lines. This will give an indication of the extent to which the marker can be used in tracking the resistance across different genetic backgrounds. It is also important to test the reliability of the marker in predicting resistance in additional crosses other than the one used for the original mapping. Armed with this information it is then possible to give the barley breeder clear information on how to implement the marker.

In our experience, a limiting factor in developing markers for use in MAS is the lack of polymorphism observed between breeder's lines. In the example presented here, three *Rpt*4-linked RFLP markers were shown to detect a polymorphism between the resistance source, 'Galleon', and 102 barley varieties and breeder's lines. Until they are replaced with marker systems that reveal equal or greater levels of polymorphism for lower cost, RFLP markers are valuable tools for MAS and are the primary method for indirect selection of a number of traits in the South Australian barley improvement program.

After mapping a trait in a segregating population, a marker must be tested in a subsequent cross, both to verify the accuracy of the original interval mapping and also to determine the functionality of linked markers in different genetic backgrounds. In this study, one of the *Rpt*4-linked markers identified, PSR117(D)/*Eco*RI, was shown to be about 92% reliable in predicting resistance in an advanced cross, thereby verifying the original mapping results. If we wish to relate this level of accuracy to practical breeding; after three rounds of backcrossing with selection based on this marker, about 78% of the lines would show resistance compared to only 12.5% that would be resistant in the absence of MAS. To produce ten resistant lines, this reduces the number of lines to be carried through a backcrossing program from 80 with no selection to 13 with selection based on a marker at 8 cM. In our judgement, a marker at this distance is therefore very acceptable, as the development costs of closer markers must be carefully compared with their advantages. In the above example, a marker at 4 cM would only decrease the number of plants required by 1, to 12.

By following a process of mapping, verification and validation, breeders can now directly use the *Rpt*4-linked markers identified in this study in breeding programs to transfer the spot form of net blotch resistance to barley cultivars by backcrossing or other breeding strategies.

Acknowledgements Thanks to Dr. Andrew Barr for critical reading of the manuscript. This work was funded in part by the Australian Grains Research and Development Corporation. The experiments described in this publication, to the best of our knowledge, comply with the laws of Australia.

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