Molecular Markers and Pyramiding of Multiple Genes for Resistance to Scald in Barley

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Introduction

Scald incited by the fungus *Rhynchosporium secalis* is a serious foliar disease of barley. Under highly epiphytotic conditions, scald causes a yield loss of up to 67% on susceptible cultivars (Yitbarek *et al* 1998). The use of resistant cultivars is one control measure. During the last 6 decades, a number of sources for scald resistance have been exploited to breed scald resistant cultivars. As a result, a number of cultivars resistant to scald have been released for commercial cultivation. It has been seen that resistance derived from a single gene is not very durable (Houston, and Ashworth, 1957). The development of cultivars having durable resistance is quite unlikely as the pathogen causing scald is constantly evolving and is highly variable (Caldwell 1937, Dyck and Schaller 1961, Kajiwara and Iwata 1963, William and Owen 1973, Brown 1990, Raman *et al* 1998). However, combining the different scald resistance genes in a single cultivar may give medium to long-term control. A number of "pyramided" scald resistant lines carrying multiple genes (3) have been developed at CSIRO, Canberra and are being used to transfer these genes to elite breeding lines of barley at Wagga Wagga Agricultural Institute. It has been reported that the 'pyramided resistant lines' significantly outyield the susceptible cultivar, Clipper and had significantly higher grain size and grain weight (Brown *et al* 1996). However, it is very difficult to recognise the presence of different resistance genes in a cultivar under field conditions. Also, it is very difficult to analyse the effect of a specific gene (s) for resistance in the presence of others. To characterise these different resistance genes in the recombinant lines, we have used markers linked to scald resistance in AB35, AB200 and AB208 - lines derived from *H. spontaneum*. The linkage of scald resistance genes with isozymes, RFLPs and PCR based markers has been reported (Abbott *et al* 1992, 1995, Nesbitt *et al* 1997). In AB200, linkage between scald resistance and *Est 5* loci has been reported. However, the association of resistance with any molecular marker has not been reported so far. Herein we report the linkage of AFLP markers with scald resistance gene in AB200 along with marker aided selection of other scald resistances in the progenies derived from "pyramided" scald resistant lines.

Materials and Methods

Plant Materials

Different crosses were made between malting cultivars and pyramided scald resistant lines and their doubled haploids were produced at WWAI (Table 1).

Table 1: Crosses and parental lines used to pyramid scald resistance genes

Cross Number	Malting Parents	Number and Sources of Scald Genes	
XB2048	Arapiles, Franklin, ant28	4	30,35,53,200
XB2062	Sloop, Franklin	4	30,35,53,200
XB2069	Franklin, Wyalong, ant28	3	30,35,53
XB2072	Venture, Franklin	4	30,35,53,200
XB2101	Franklin	6	30,35,53,200,208,249

The doubled haploid population was screened for resistance using linked markers to scald. In this investigation, we have used UBC 807.12 and hordein (*Hor2*) markers to characterise gene for scald resistance in AB35 and AB208 respectively. Closely linked markers to resistance in other lines (AB30, AB53, AB249) are not yet available for routine marker assisted selection. Hence, the isozyme markers *Acid phosphatase* and *Est2* linked to scald resistance in AB35 and AB53 were used.

Isozyme analysis

The analysis was performed as described by Abbott *et al* (1992) using 5 per cent polyacrylamide gels.

DNA Extraction

About 8-10 cm of leaves collected from 2 wk-old glasshouse raised seedlings were used for DNA extraction in 2 ml eppendorf tubes. The frozen tubes containing tissues were ground into powder with mini-pestle and mortar (Eppendorf) and the DNA extraction was carried-out as described by Guidet *et al* (1991).

PCR Analysis and Gel electrophoresis

PCR was carried-out in reaction volume of 25 µl using 50 ηg of genomic DNA as template, 1X buffer (50mM KCl, 10mM Tris-HCl (pH 9.0) and 0.1 % Triton X-100), 0.5 units of Taq polymerase, 1.5mM $MgCl_2$, 100mM of each dNTP, 0.4µM of KV1 and KV2 primers for hordein gene analysis. For KV1 and KV2, amplifications were performed in Hybaid OMN-E thermocycler by following conditions described (Kanazin *et al* 1993). For UBC 807.12 primers, amplifications were performed using 50-100ηg of DNA, 1X buffer (Perkin-Elmer), 250µM dNTPs, 0.5U Taq polymerase (Perkin-Elmer), and 2µM of each primer. Amplifications were performed in the thermocycler following initial denaturing at 94 ^{0}C for 2min followed by 35 cycles at 94 ^{0}C for 45 seconds, 57 ^{0}C for 30 seconds, and 72 ^{0}C for 75 seconds and finally at 72 ^{0}C for 5 min. To discriminate the heterozygotes, PCR products were further digested with Hae III. The restricted digests were then separated on 2 per cent agarose gels. However, the PCR products amplified with KV1/KV2 primers were separated on polyacrylamide gels (5%).

Bulk Segregant Analysis

The BC_3F_2 population derived from Clipper x AB200 were screened for scald resistance under glasshouse conditions and scored for resistance at CSIRO. Bulk segregant analysis of resistant and susceptible plants was carried out by AFLP and inter simple sequence repeat (ISSR) analysis. Polymorphic loci were further analysed at single plant level. Linkage analysis was performed by using MAPMAKER 3.0.

AFLP and ISSR analysis

AFLP analysis was performed as described by Vos *et al* (1995) using Large Genome System (Gibco BRL). The denatured PCR products (3µl) were loaded on the gels (38 x 70 cm) and

electrophoresis was carried out at 120W at 50 ^{0}C for 2hr using 5 per cent polyacrylamide gels (0.4mm) containing 7.5M urea. The gels were then transferred on to Whatman 3mm filter papers and dried on a gel drier (Bio Rad). The dried gels were further used for autoradiography. Different ISSR primers (UBC) were also used for bulk segregant analysis to study polymorphism.

Selection of Desirable Recombinants
The derivatives exhibiting all the desired markers linked to scald resistance were selected and are being used to validate the results under field conditions.

Results and Discussion

Marker Assisted Selection of Scald Resistance Genes:
Isozyme loci linked with scald resistance in AB35, AB53 and AB200 lines were not resolved well in complex crosses. Both the molecular markers *Hor2* and UBC 807.12 linked with the resistance genes in AB208 and AB35 respectively were polymorphic between all the parents. The polymorphic loci segregated in the BC_3F_2 population of Clipper x AB 208 and in the derivatives of multiple scald resistance genes (Fig 1). The PCR products amplified with UBC 807 primers, upon digestion with HaeIII, also showed polymorphism within the resistant individuals and hence discriminated heterozygotes from homozygotes resistant to scald.

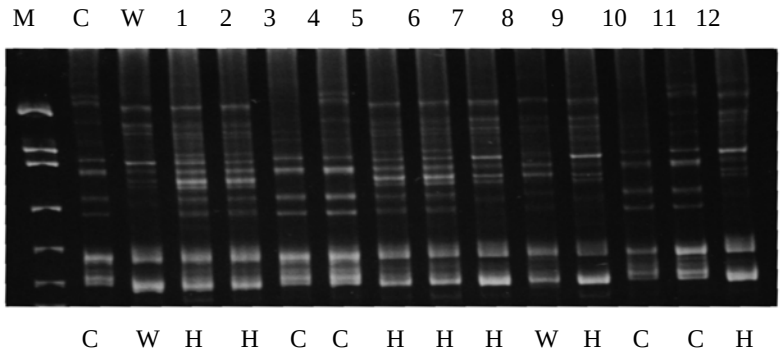

Figure 1. Segregation of Hordein Marker Linked with Resistance to Scald *Rrs14* gene in Barley. C: Clipper, W: AB208, 1-12: BC_3F_2 Plants of Clipper x AB208, M: pGEM marker

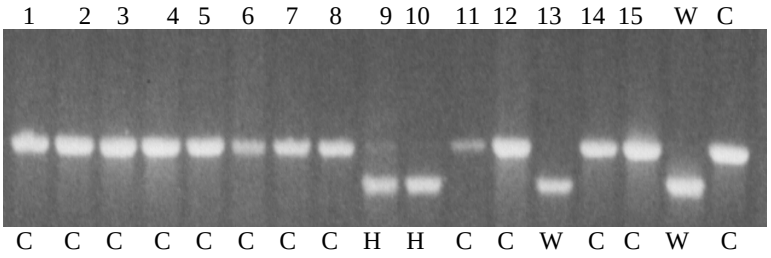

Figure 2. Electrophoretic Patterns of Restriction Digests of PCR Products Amplified Using UBC 807.12 Primers. C: Sloop and Franklin (bulk DNA) W: AB35, 1-15: DH Plants of Cross 2062. C: Clipper type (Susceptible), W: AB35 type (Resistant) and H: Heterozygotes

Bulk Segregant Analysis
This was performed using AFLP and ISSR analyses. Among different 64 combination of E- and M- primers used to study polymorphism, ACA/CAC was found to be closely linked with

scald resistance (5 cM). However, none of the ISSR primers were found to be closely linked with resistance to scald in AB200.

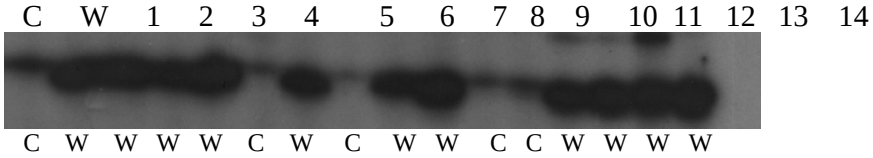

Figure 3. Autoradiograph Showing Segregating Loci Linked With Scald Resistance in AB 200. C: Clipper, W: AB200, 1-14: BC_3F_2 derived single plants of Clipper x AB200

AFLP based marker closely linked to scald resistance in AB200 was not present in other sources of scald resistance such as AB6, AB11, AB30, AB35, AB53, AB208. AB249, Atlas46, Halcyon, Forrest, Sultan, Osiris and CI3515, but was present in Turk (Fig 4). Hence the marker can be used in the populations derived from above scald resistant lines. Gene targeting is being carried-out to map this marker on the barley molecular map.

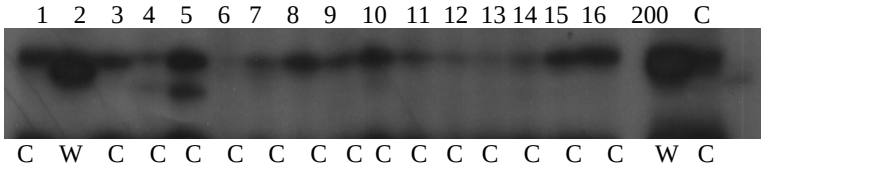

Figure 4. Autoradiograph Showing Segregation of Linked Marker With Scald Resistance Gene in AB200. C: Clipper, W: AB200, 1: Osiris, 2: Turk, 3: AB53, 4: Sultan, 5: Forrest, 6: Hudson, 7: Halcyon, 8: AB249, 9: AB6, 10: AB 30, 11 AB35, 12: Waveney, 13: Atlas 46, 14: Hudson, 15: AB280, and 16: AB11.

Acknowledgments

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