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Progress in marking scald resistance genes derived from wild barley

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Abstract

Genes for resistance to barley scald, a foliar disease caused by *Rhynchosporium secalis*, have been described in many cultivated and wild barley backgrounds. In wild barley, scald resistance genes occur in at least 4 separate chromosomal locations. To facilitate pyramiding of these resistance genes, which is likely to increase the durability of scald resistance, we are developing molecular markers for scald resistance genes from wild barley. We have previously used wild barley accessions as non-recurrent parents to generate a set of scald resistant third backcross (BC_3) lines in the susceptible barley cultivar Clipper, and a number of these lines have been used in the development of PCR-based markers for scald resistance genes on 1H, 7H and 3H. More recently, we have developed two co-dominant PCR-based markers flanking a scald resistance gene on 6H. In a further two lines, the chromosomal locations of the resistance genes are unknown, and we report here the progress in marker development for one of these resistance genes.

While wild barley is a source of desirable traits such as disease resistance, unwanted characters such as head shattering are also present. One wild barley-derived scald resistant line contains a shattering gene closely linked to the resistance locus; an isozyme marker, *Idh1*, also maps close to this resistance gene. We have used *Idh1* in a strategy to select non-shattering recombinant lines; field tests have yielded a number of non-shattering lines, which are described here.

Introduction

Scald is a foliar disease of barley that occurs throughout southern Australia, and causes annual yield losses of 10-15%. Individual losses can be as high as 45% (Brown, 1985). Scald also reduces grain malting quality due to reduced size and plumpness (Khan and Crosbie, 1988). The fungal pathogen that causes scald, *Rhynchosporium secalis*, is highly variable (Salamati and Tronsmo, 1997; McDonald et al., 1999), and rapidly overcomes singly deployed resistance genes. Cultivars with pyramided scald resistance genes are likely to show more durable resistance.

In the wild progenitor of barley (*Hordeum vulgare* ssp. *spontaneum*), scald resistance genes occur in at least 4 separate chromosomal locations (Abbott et al., 1992, 1995; Garvin et al., 1997, 2000). Deployment of these genes into commercial cultivars will be accelerated by the use of linked molecular markers, and these markers will be required to recognise the presence of multiple resistance genes if the genes are pyramided.

Materials and Methods

Plant material

Third backcross lines were generated from crosses between a range of *H. spontaneum* accessions and the scald susceptible cultivar Clipper as previously described (Brown et al., 1988; Garvin et al., 1997). Selected BC_3 individuals were selfed to produce segregating BC_3F_2 populations. BC_3F_2 plants were tested for their reaction to scald isolate 109.1.1 using the seedling assay described in Abbott et al. (1991). Progeny of the BC_3F_2 plants were tested for their scald reaction, in order to distinguish homozygous and heterozygous scald resistant BC_3F_2 individuals. Twelve progeny were tested for each scald resistant BC_3F_2 plant. Four progeny were tested for each scald susceptible BC_3F_2 individual, to confirm susceptibility.

PCR assays - line 30

PCR for the *Cxp3* assay was performed in a volume of 20 µl, containing 40 ng genomic DNA, 1X PCR buffer (Perkin Elmer), 2 mM $MgCl_2$, 200 µM each dNTP, 1 µM each primer, and 1 unit of *Taq* polymerase (Perkin Elmer). Conditions for the MWG916 PCR were the same, except that 100 ng of genomic DNA was used as a template, and the $MgCl_2$ concentration was 1.5 mM. Primers for *Cxp3* were AAC AAG TTC GGG TGG GAC AAG G (forward) and TGG GAC ATA ATC CAG GAG CTG AC (reverse); for MWG916, primers were CTG CAG ATG ACT TGG AGA GC (forward) and GAG GTG CTG GCG ATC TGC T (reverse). PCR conditions for *Cxp3* were one cycle of 94ºC for 2 minutes, 70ºC for 30 seconds and 72ºC for 30 seconds; 40 cycles of 94ºC for 30 seconds, 70ºC for 30 seconds and 72ºC for 30 seconds; and a final step of 72ºC for 5 minutes. For MWG916, conditions were one cycle of 94ºC for 2 minutes, 58.5ºC for 30 seconds and 72ºC for 45 seconds; 35 cycles of 94ºC for 30 seconds, 58.5ºC for 30 seconds and 72ºC for 45 seconds; and a final step of 72ºC for 5 minutes. MWG916 PCR products were digested with *Hin*6I before electrophoresis through 2% agarose; *Cxp3* products were also separated on 2% agarose.

Bulked segregant analysis - line 245

Two pools of DNA were created for each line, one from ten plants homozygous for resistance to scald, and one from ten plants homozygous for susceptibility to scald. A set of 64 AFLP markers (Life Technologies AFLP Analysis System I) was used to screen for polymorphisms between the resistant and susceptible bulked DNA.

Screening was also performed with all possible primer combinations for the IRAP, REMAP and microsatellite primers reported in Kalendar et al. (1999).

Linkage analysis - line 245

Polymorphisms identified between resistant and susceptible bulks were scored on 57 BC_3F_2 individuals for line 245. The Mapmaker program (version 3.0) was used to assess the strength of linkage between the resistance loci and the polymorphic bands. Polymorphisms identified for line 245 were "aagcac2", generated by the AFLP primers *Eco*RI+AAG and *Mse*I+CAC; "aaccaa1", generated by the AFLP primers *Eco*RI+AAC and *Mse*I+CAA, and "R8081", generated by the REMAP primer "LTR reverse 7286" and the microsatellite primer "8081" (Kalendar et al., 1999).

Cloning and characterisation

The polymorphic band designated "aagcac2" was excised, eluted and reamplified using AFLP primers *Eco*RI+AAC and *Mse*I+CAC. Fragments were cloned using a TA cloning kit (Promega), and 12 separate clones were sequenced (Big Dye, Perkin Elmer) on an ABI Prism Model 377 DNA sequencer.

Idh1 isozyme assay

The genotype at the *Idh1* locus was assayed on imbibed half seeds; the embryo end of the seed was saved so that plants could be grown and assessed for shattering. The *Idh1* assay is described in Brown (1983).

Results and Discussion

Line 30

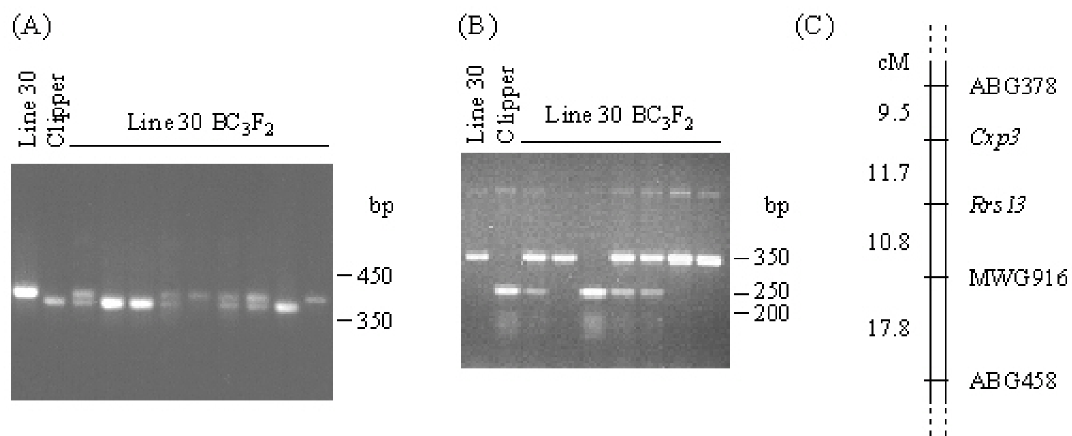
The scald resistance gene was previously mapped to the short arm of chromosome 6H, and an RFLP marker, *Cxp3*, 7.3 cM from the scald resistance gene, was developed (Abbott et al., 1995). The primers used to amplify the *Cxp3* fragment from wild barley (Abbott et al., 1995) were used to amplify *Cxp3* fragments from true-breeding BC_3F_3 plants of line 30 and from Clipper plants. The PCR products were sequenced, and the sequences were used to design PCR primers. These primers detect a size difference between the Clipper allele, linked to susceptibility, and the allele in scald resistant plants of line 30, providing a co-dominant assay for the scald resistance gene in line 30 (Figure 1a).

As the map location of the *Cxp3* marker is known, it was possible to select other markers, potentially linked to the scald resistance in line 30, from the genetic linkage map. A polymorphism between line 30 and Clipper was detected for the MWG916 RFLP marker, using *Eco*RV. This polymorphism was scored on a subset of plants from the line 30 BC_3F_2 population, and preliminary linkage estimates indicated that the marker was linked to resistance. The DNA fragment used as a probe was

sequenced, and PCR primers were designed. The product of these primers, after digestion with *Hin*6I, showed a size polymorphism between line 30 and Clipper, providing a second co-dominant assay (Figure 1b).

Both markers were scored on 58 individuals from the line 30 BC_3F_2 population; linkage analysis showed that the markers flank the resistance gene. The *Cxp3* marker maps 11.7 cM from the scald resistance gene, while MWG916 is 10.8 cM from the resistance gene (Figure 1c).

Figure 1



Line 245

As yet, the chromosomal location of the scald resistance gene in line 245 is unknown. Previously we reported that bulk segregant analysis with a set of 64 *Eco*RI-*Mse*I AFLP markers did not yield any polymorphisms closely linked to the resistance gene (Genger et al., 1999). Two polymorphic bands, when scored on 57 BC_3F_2 individuals, showed loose linkage to the resistance gene. The first of these, "aagcac2", maps 20.2 cM from the resistance gene. The second, "aaccaa1", maps 24.9 cM from the resistance gene.

In the hope of finding markers that were more closely linked, we used the retrotransposon-based techniques, IRAP and REMAP for bulk segregant analysis. All possible combinations of IRAP, REMAP and microsatellite primers were used (Kalendar et al., 1999). Few polymorphisms were seen between the resistant and susceptible bulks, and of those seen, only one proved to be linked to the resistance gene. This polymorphism, "R8081", maps 28 cM from the resistance gene.

As none of the markers so far identified are tightly linked to the resistance gene in line 245, we decided to clone the closest marker, "aagcac2" in order to use it to determine the chromosomal location of the resistance gene. The polymorphic band, which lay

between the 260 bp and 270 bp marker bands, was excised from the gel and cloned (as described in Materials and Methods). Twelve separate clones were sequenced, and from these, six different sequences were identified. One of these sequences can be discarded, as it is only 249 bp long. The other sequences are in the correct size range; the most common (5 clones) is 263 bp long.

To identify the correct sequence, primers have been designed to the ends of the five sequences. These primers will be used on the bulked DNA samples; polymorphisms between resistant and susceptible bulks should indicate the correct sequence. Once the correct DNA fragment has been identified, the diagnostic primers will be used on the BC_3F_2 plants to confirm the linkage estimate. We plan to use these primers on a set of disomic and ditelosomic addition lines (from Betzes barley into Chinese Spring) in order to identify the chromosomal location of the marker, and thus of the resistance gene.

Line 249

Previous work on line 249 showed that a gene that causes head shattering is closely linked to the scald resistance gene on chromosome 3H. This limits the use that can be made of the scald resistance from line 249. An isozyme marker, *Idh1*, also maps to this region, 3.8 cM from the resistance gene (Garvin et al., 1997); the order of the three loci is not known. We used the *Idh1* marker as a tool in selection for non-shattering, scald resistant recombinants within an F2 population generated from a cross between line 242 (non-shattering, scald susceptible) and line 249 (shattering, scald resistant).

As the shattering character is dominant, we selected F2 plants that were heterozygous for *Idh1*. These plants were grown in glasshouse conditions, and at maturity, were assessed for head shattering relative to Clipper, line 242 and line 249 controls. Preliminary assessments in the glasshouse gave equivocal results; seed was collected from putatively non-shattering plants, and phenotyping was repeated in the field. F2 plants homozygous for the Clipper allele of *Idh1* were also selected; a small number of scald resistant plants were identified, and these plants were also assessed for shattering in the field. Field trials identified a number of non-shattering families originating from both *Idh1* heterozygotes and Clipper homozygotes; these families will be tested to confirm scald resistance.

Conclusion

If the scald resistance from wild barley is to be fully utilized, markers closely linked to the resistance loci are needed, and detrimental wild characters must be eliminated from breeding stocks. We have previously developed markers for several scald resistance genes from wild barley, and we are continuing to develop better markers for these loci, and also markers for genes from novel scald resistant lines. Where close markers cannot be quickly identified, more loosely linked markers may be used to identify the chromosomal location of the scald resistance gene; once this is known, potentially close markers can be selected from various genetic linkage maps and

screened on our material. Markers closely linked to scald resistance can also be used to select against deleterious wild characters; our preliminary work selecting against head shattering has given positive results, which should lead to useful scald resistant breeding material.

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