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Identification of RFLP markers linked to the cereal cyst nematode resistance gene (*Cre*) in wheat

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Abstract The cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) is an economically damaging pest of wheat in many of the worlds cereal growing areas. The development of CCN-resistant cultivars may be accelerated by the use of molecular markers. The *Cre* gene of the wheat line "AUS10894" confers resistance to CCN. Using a pair of near-isogenic lines (NILs) that should differ only in a small chromosome segment containing the *Cre* locus, we screened 58 group-2 probes and found two (Tag605 and CDO588) that detect polymorphism between the NILs. Nulli-tetrasomic and ditelosomic lines confirmed that the restriction fragment length polymorphism (RFLP) markers identified were derived from the long arm of wheat chromosome 2. Crosses between "AUS10894" and "Spear" and the NIL "AP" and its recurrent parent "Prins" were used to produce F_2 populations that gave the expected 3:1 segregation ratio for the resistance gene. Linkage analysis identified two RFLP markers flanking the resistance gene. *Xglk605* and *Xcdo588* mapped 7.3 cM (LOD=6.0) and 8.4 cM (LOD=6.7), respectively, from the *Cre* locus.

Key words Restriction fragment length polymorphism CCN · Genetic mapping · *Triticum aestivum* *Heterodera avenae*

Introduction

The cereal cyst nematode (CCN) (*Heterodera avenae* Woll.) attacks cereals worldwide (Meagher 1977) and causes serious economic loss to wheat growers in many countries. Yield losses in an intolerant cultivar may be up to 30% (Fisher 1982a). Host resistance is the only economically viable and environmentally acceptable method of keeping nematode numbers below damage thresholds. Resistance to CCN has been identified in *Triticum tauschii* (Eastwood et al. 1991), *Aegilops* spp. (Brown 1973; Dosba et al. 1978; Eastwood et al. 1991), and *Secale cereale* (Asiedu et al. 1990), although to-date there has only been one resistance gene reported in hexaploid wheat (Nielsen 1966; O'Brien and Fisher 1974). This dominant allele at the locus *Cre* is located on chromosome 2B in the lines "Loros" (Slootmaker 1974) and "AUS10894" (O'Brien et al. 1980; Nielsen 1982). The gene *Cre* confers resistance to *H.avenae* by arresting female development at about 15 days by causing the degeneration of feeding sites in the root stele (Williams and Fisher 1993). The introgression of *Cre* into commercial cultivars is currently constrained by the test for resistance, a bioassay which is time-consuming and expensive. An improved asssay based on molecular markers is necessary.

RFLP markers linked to genes conferring nematode resistance have previously been identified in potato (Gebhardt et al. 1993), sugarbeet (Jung et al. 1990), tomato (Messeguer et al. 1991) and *T. tauschii* (Eastwood et al. 1994). Near-isogenic lines (NILs) carrying different alleles for disease resistance can facilitate the identification of closely linked RFLP markers, as reported by Schuller et al. (1992) for the *Mla* locus in barley, Klein-Lankhorst et al. (1991) for the *Mi* locus in tomato, and Hartl et al. (1993) for the *Pm3* locus in wheat.

In this paper we present the idenification of *Cre*-linked RFLP markers using near-isogenic lines.

Materials and methods

Plant material

A set of near-isogenic lines was used to screen for probes that produced RFLPs. The NIL "AP" was developed by Prof. James MacK-

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ey (Upsalla, Sweden) after seven backcross generations with "Prins" as the recurrent parent and "AUS10894" as the donor line. "Chinese Spring" nulli-tetrasomic (Sears 1966) and ditelosomic lines (Sears and Sears 1978) were used to localise RFLPs to chromosomes and chromosome arms. For linkage analysis, F_2 progeny of the crosses "AUS10894" × "Spear", and "AP" × "Prins", were kindly supplied by F. Green.

Nemotode assay

F_2 progeny were tested for their resistance to *H.avenae* using the bioassay developed by Fisher (1982b). Seedlings were planted in 3-cm tubes filled with sterile soil, and inoculated five times at 3-day intervals with 100 second-stage juveniles. After 12 weeks at 15°C, the number of cysts formed on roots were counted. To confirm the resistance score, six to eight F_3 progeny of each F_2 individual were also assayed by the same procedure.

DNA clones

Clones were obtained through the Australian Triticeae Mapping Initiative. Group-2 cDNA and genomic clones were selected from maps of barley (Heun et al. 1991) and wheat (Liu and Tsunewaki 1991).

RFLP analysis

DNA extraction, restriction digestion, Southern blotting, and hybridization were carried out as described by Guidet et al. (1991). NIL leaf DNA was digested with *Hin*DIII, *Eco*RI, *Eco*RV, *Dra*I, *Bam*HI and *Xba*I. DNA membranes of F_2 progeny were screened with RFLP probes and the results analysed with Mapmaker (Lander et al. 1987) and Joinmap (Stam 1993) software using the Kosambi map unit function (Kosambi 1944).

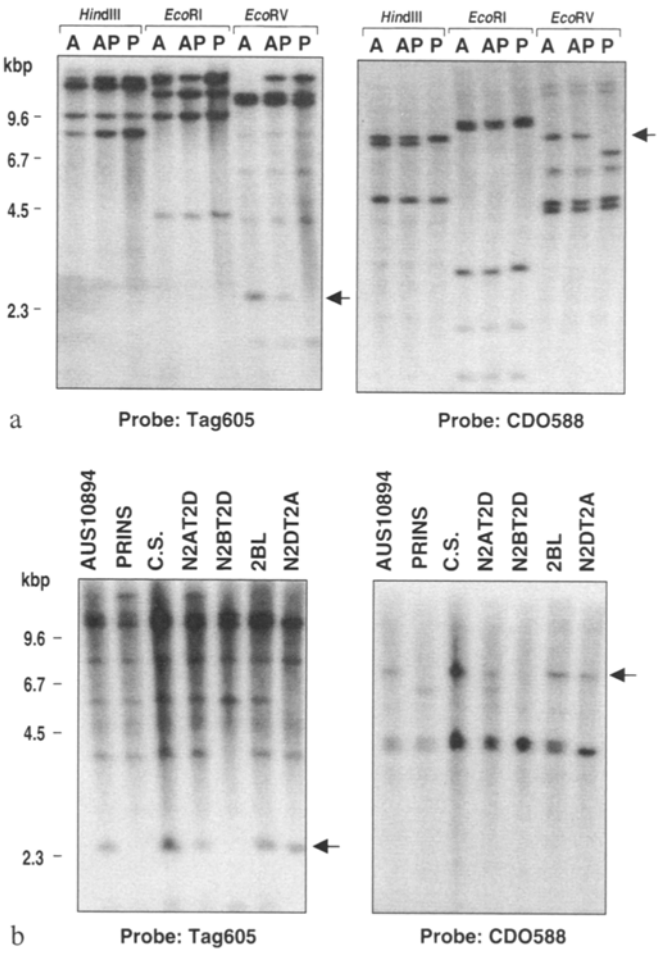

Fig. 1a Southern analysis of NILs. DNA was digested with three restriction enzymes, and hybridised to Tag605 and CDO588. The *arrow* indicates the polymorphic band. *A* AUS10894, *AP* AUS10894 × Prins[7], *P* Prins. **b** Chromosomal localisation of RFPLs. Southern blot of nulli-tetrasomic (N2AT2D - nulli 2A tetra 2D) and ditelosomic ($2B_L$) lines digested with *Eco*RV and hybridised to Tag605 and CDO588. Absence of polymorphic bands (*arrow*) in N2BT2D and their presence in $2B_L$ indicates that the RFLP is derived from $2B_L$

Results

Segregation of the nematode resistance gene

A total of 89 F_2 progeny were tested for resistance to *H. avenae*. The segregation of individuals from the cross "AUS10894" × "Spear" was 34 resistant : 18 susceptible (^{2}=2.56) and that of "AP × Prins" was 30 resistant : 7 susceptible (χ^2=0.42). The χ^2 values indicate that the segregations fit the expected 3:1 ratio for a single dominant gene for resistance to CCN.

RFLP analysis

Fifty-eight probes were screened using the NILs and the donor parent "AUS10894" in order to identify those showing polymorphisms. Thirty-eight of the clones detected RFLPs between the parental lines. Two clones, CDO588 and Tag605, identified RFPLs between the NIL "AP" and its recurrent parent "Prins" (Fig. 1a). A third clone, ABC451, gave an identical restriction pattern to CDO588. Partial sequencing of these clones showed 79% homology in 530 bases, indicating that they may be products of identical loci, although ABC451 is a cDNA clone from barley while CDO588 is from oats.

To verify that the RFLPs produced by these informative probes were derived from chromosome 2B, the clones were hybridised to DNA membranes of nulli-tetrasomic and ditelosomic lines of Chinese spring wheat. The restriction patterns indicated that these probes identify loci on chromosomes 2B and 2D, and that the RFLBs observed were derived from the long arm of chromosome 2B (Fig. 1b).

Linkage analysis of the RFLP loci relative to the *Cre* locus was conducted on 52 F_2 individuals of the cross "AUS10894" × "Spear". CDO588 linkage was also measured on 37 F_2 progeny of a "AP" × "Prins" cross and the results integrated into a consensus map derived from both populations.

A linkage group containing the *Cre* locus was identified, which positioned the markers *Xcdo588*/*Xabc451* and *Xglk605* 8.4 cM and 7.3 cM, respectvely, from the resistance locus (Fig. 2). Two other flanking markers were also

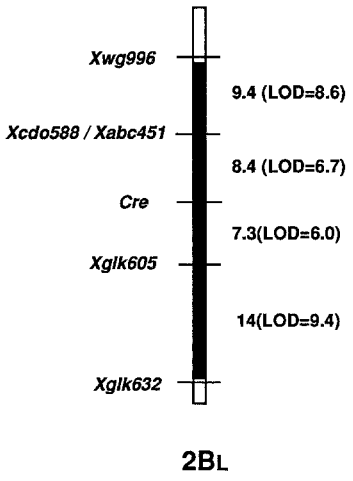

Fig. 2 Partial linkage map of the long arm of wheat chromosome 2B derived from linkage analyses of progeny from crosses between "AUS10894" and "Spear", and "AP" and "Prins". Map distances, in centimorgans (Kosambi 1944) were determined using Mapmaker and Joinmap software. The *shaded area* represents the estimated maximum size of the "AUS10894" chromosome segment that was introgressed into the "AP" backcross line

contained in the linkage group, but were not polymorphic between the NILs, giving an indication of the size of the DNA segment introgressed from "AUS10894" into "AP"(Fig. 2).

Discussion

RFLP markers can provide powerful tools for indirect selection of an agronomically important gene in a breeding program (Beckman and Soller 1983; Tanksley 1983). This study has used NILs to identify RFLP markers linked to the cereal cyst nematode resistance gene *Cre*. As the near-isogenic lines should be identical apart from a segment of DNA containing the gene selected for, any RFLPs between the NILs should be, and are, closely linked to *Cre*. *Cre* and its closest linked markers reside on a small segment of "AUS10894" that has been integrated into "AP" chromosome 2B.

The RFLP markers *Xglk605* and *Xcdo588/Xabc451* can be used in a selection program in place of the bioassay currently used. Unlike this bioassay, the RFLP markers can detect homozygous resistant individuals and can also be used in a breeding program to pyramid CCN resistance genes, such as the *T. tauschii* gene (Eastwood et al. 1994) and the *Aegilops ventricosa* gene which has recently been transferred into wheat (Delibes et al. 1993).

Work is currently underway to convert the RFLP markers reported here into PCR markers, to further reduce the cost and time required to develop new CCN-resistant wheat cultivars.

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