Eyespot resistance gene \textit{Pch-1} in H-93 wheat lines. Evidence of linkage to markers of chromosome group 7 and resolution from the endopeptidase locus \textit{Ep-D1b}

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Summary. Gene \textit{Pch-1}, which confers resistance to eyespot disease (\textit{Pseudocercosporella herpotrichoides} Fron), has been located on chromosome 7D in the H-93 wheat-\textit{Aegilops ventricosa} transfer lines using isozyme markers and DNA probes corresponding to group 7 chromosomes. Previous experiments had failed to ascertain this location. The lack of segregation of the resistance trait in progeny from reciprocal crosses between lines H-93-70 and VPM1 indicates that their respective resistance factors are allelic. Line H-93-51 carries the endopeptidase allele \textit{Ep-D1b} but is susceptible to eyespot, which indicates that resistance to eyespot is not a product of the \textit{Ep-D} locus, as had been proposed in a previous hypothesis.

Key words: Wheat - \textit{Aegilops ventricosa} - \textit{Pseudocercosporella herpotrichoides} - Eyespot disease - Resistance gene

Introduction

Two independent introductions of genetic resistance to eyespot disease (\textit{Pseudocercosporella herpotrichoides}) from \textit{Aegilops ventricosa} to hexaploid wheat have been carried out. Maia (1967) derived the resistant line VPM-1 from a cross between the amphiploid (\textit{Ae. ventricosa} \times \textit{Triticum persicum}) and \textit{T. aestivum} cv 'Marne'. Line VPM-1 was then used as the source of resistance to obtain 'Roazon' wheat; \(F_2\) monosomic analysis indicated that the resistance factor here was associated with chromosome 7D (Jahier et al. 1979). A different procedure was used to obtain the hexaploid wheat line H-93-70: an intermediate self-sterile hybrid between \textit{T. turgidum} (genomes AABB) and \textit{Ae. ventricosa} (\(D^vD^vM^mM^m\)) was rescued with pollen from hexaploid wheat (AABBD), and the progeny was repeatedly selfed to obtain stable wheat lines (H-93-1 through H-93-70) with 42 chromosomes (Delibes and Garcia-Olmedo 1973; Delibes et al. 1977a, b; Doussinault et al. 1983).

A close linkage between VPM-1-derived eyespot resistance and the endopeptidase locus \textit{Ep-D1b} located on the long arm of chromosome 7D has been reported (Koebner et al. 1988; Worland et al. 1988), and the hypothesis, proposed is that this resistance could be a product of the \textit{Ep-D1b} locus (Worland et al. 1988). However, the lack of linkage of resistance gene \textit{Pch-1} with a putative marker of chromosome 7D in a cross of H-93-70 with a 7D/7Ag wheat/Agropyron substitution line suggested a different location for this gene (Delibes et al. 1988). We have now re-examined this question using a number of biochemical markers and DNA probes and have concluded that gene \textit{Pch-1} is indeed on chromosome 7D. We further report that this gene was transferred from chromosome 7D of \textit{Ae. ventricosa} and that although \textit{Pch-1} is linked to \textit{Ep-D1b}, the two loci can be separated from each other.

Materials and methods

Biological material

The hexaploid H-93 lines, derived from the cross (\textit{T. turgidum} \(H-1-1 \times \textit{Ae. ventricosa} AP-1\)) \times \textit{T. aestivum} cv 'Almatense H-10-15' have been described previously (Delibes and Garcia-Olmedo 1973; Delibes et al. 1977b).

Tests for resistance to the eyespot disease

The tests were conducted according to Macer (1966). The coleoptiles were covered with a straw cylinder colonized by the
parasite. The seedlings were maintained in a growth chamber at 8°C under an 8 h/day illumination for 10 weeks. During this time the development of the parasite through the leaf sheaths of the seedlings was observed. The number of leaf sheaths penetrated indicates the level of resistance. Dependent on the specific host, the pathogen produces two types of mycelium: on susceptible plants the mycelium is black in colour, and abundant between the leaf sheaths, m-type; on resistant plants with the Pch1 gene, the mycelium is spotted and dark-brown, v-type (Doussinault et al. 1983).

Cytological procedures

After the authors had been fixed in acetic-ethanol 1:3 at 4°C for 2 months, the cells were squashed, then stained by the Feulgen technique, and the meiotic chromosomes were analysed.

Biochemical markers

Endopeptidase isozymes (E.C.3.4.21–24) were separated by isoelectric-focusing essentially as described by Koebner et al. (1988) using 250 x 125 x 0.4 mm gels and servalyt ampholyte pH 4–6. Adenylate kinase isozymes (E.C.2.7.4.3.) from wheat (1988) using 250 x 125 x 0.4 mm gels and servalyt ampholyte pH isoelectric-focusing essentially as described by Koebner et al. (1988) using 250 x 125 x 0.4 mm gels and servalyt ampholyte pH 4–6. Adenylate kinase isozymes (E.C.2.7.4.3.) from wheat leaves were extracted, fractionated, and stained as described (Benito et al. 1990). Electrophoresis was carried out at a constant voltage of 150 V for 3 h at 2°C–4°C.

DNA hybridization

Sources of the DNA probes are listed in Table 1; the designation of RFLP loci, alleles, and markers of these probes are presented according to Hart and Gale (1988), but with E for EcoRI, G for BglII, and H for HindIII. Total DNA was isolated from etiolated seedlings according to Taylor and Powell (1982). DNA was restricted with the appropriate endonuclease and separated on 0.75% agarose gels. Southern blottings to nylon membranes (Hybond-N, Amersham) were performed according to the manufacturer’s recommendations. The inserts from clones used as probes were labelled by random priming (Feinberg and Vogelstein 1983). Hybridizations were performed at 65°C and washed under stringent conditions.

Results

Description of biochemical and molecular markers

Those markers associated with chromosomes of homoeologous group 7 that were used in this study are listed in Table 1. Endopeptidase (EP) zymograms of the parental material of the H-93 lines are presented in Fig. 1A, and the alleles corresponding to chromosomes 7D^Y and 7D are indicated. A similar survey for adenylate kinase (ADK) is presented in Fig. 1B. As the allele associated with chromosome 7D^Y is different from that previously observed to be associated with chromosome 7D of both cv ‘Chinese Spring’ and cv ‘Almatense’ H-10-15, the nomenclature has been adapted accordingly. A wheat cDNA probe (Ss1-1) that encodes the enzyme sucrose synthase (Maraña et al. 1988) allowed us to identify a double locus on the short arm of chromosome 7D^Y from Ae. ventricosa (Fig. 1C). The patterns corresponding to the remaining probes for group 7 chromosomes listed in Table 1 are not shown.

Table 1. Biochemical and molecular genetic markers used in the present study

<table>
<thead>
<tr>
<th>Marker</th>
<th>Allele</th>
<th>Chromosome (arm)</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isozymes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endopeptidase (EP)</td>
<td>Ep-D1b</td>
<td>7D^Y (L)</td>
<td>1</td>
</tr>
<tr>
<td>Adenylate kinase (ADK)</td>
<td>Adk-D1b</td>
<td>7D^Y (L)</td>
<td>2, 3</td>
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<tr>
<td>cDNA probes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ss1-1</td>
<td>XsS1-E-7D^Y</td>
<td>7D^Y (S)</td>
<td>4, 5</td>
</tr>
<tr>
<td></td>
<td>XsS1-E-7M^Y</td>
<td>7M^Y (S)</td>
<td>4, 5</td>
</tr>
<tr>
<td>psr129</td>
<td>Xpsr129-H-7M^Y</td>
<td>7M^Y (L)</td>
<td>5, 6</td>
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<tr>
<td>gDNA probes</td>
<td>abm2</td>
<td>Xabm2-G-7M^V</td>
<td>7M^V</td>
</tr>
<tr>
<td>abm5</td>
<td>Xabm5-G-7M^V</td>
<td>7M^V</td>
<td>5</td>
</tr>
<tr>
<td>abm7</td>
<td>Xabm7-E-7M^V</td>
<td>7M^V</td>
<td>5</td>
</tr>
<tr>
<td>abm10</td>
<td>Xabm10-H-7M^V</td>
<td>7M^V</td>
<td>5</td>
</tr>
</tbody>
</table>


Linkage of eyespot resistance with markers in the H-93 lines

All resistant H-93 lines, including H-93-70, carried the marker Ep-D1b which corresponds to the long arm of chromosome 7D^Y, while except in H-93-51 this marker was absent from susceptible lines (Fig. 2A). Line H-93-51 clearly showed m-type mycelium, and the number of leaf sheaths penetrated by the fungus did not differ from the susceptible control, which indicates that gene Pch1 was absent (Fig. 3). The association of resistance with markers Adk-D1b (chromosome 7D^Y, long arm) and XsS1-E-7D^Y (chromosome 7D^Y, short arm) shown in Fig. 2 is less close, which is consistent with the occurrence of recombination between chromosome 7D^Y and 7D. None of the markers of chromosome 7M^Y listed in Table 1 were detected in the resistant H-93 lines, indicating that the transfer of resistance occurred exclusively from chromosome 7D^Y. Reciprocal crosses between lines VPM-1 and H-93-70 were carried out, and the corresponding F2 generations were obtained. No susceptible plant was found among 100 plants screened for resistance at the seedling stage, and the distribution of the plants according to the number of leaf sheaths attacked by the fungus was the same as that of the parents.

Line H-93-51 was crossed to the parental wheat cv ‘Almatense H-10-15’, and the meiosis of the hybrid was studied (Table 2). Results were consistent with one low-pairing chromosome in the D genome complement of line H-93-51. The genotype of H-93-51 was found to be the following: Adk-D1a and pch1 from chromosome 7D (distal part of long arm), Ep-D1b from chromosome 7D^Y (long arm), Xpsr129-H-7M^Y from chromosome 7M^Y (proximal part of long arm), absence of protein compo-
Table 2. Number of meiotic configurations observed at metaphase I in *T. aestivum* H-10-15, H-93-51 and the hybrid (H-93-51 × H-10-15)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Number of cells</th>
<th>R</th>
<th>O</th>
<th>U</th>
<th>bound arms/cell.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-10-15</td>
<td>50</td>
<td>18.46</td>
<td>2.52</td>
<td>0.04</td>
<td>39.44</td>
</tr>
<tr>
<td>H-93-51</td>
<td>50</td>
<td>18.28</td>
<td>2.72</td>
<td>0.00</td>
<td>39.28</td>
</tr>
<tr>
<td>H-93-51 × H-10-15</td>
<td>50</td>
<td>16.88</td>
<td>3.32</td>
<td>1.60</td>
<td>37.08</td>
</tr>
</tbody>
</table>

R, Ring bivalents; O, open bivalents; U, univalents
References

Maia V (1967) Obtenção de bôs tendres resistentes ao Pétiole (Cercosporella herpotrichoides) por crocements interspécifiques. CR Acad Agric Fr 53:149-154
Taylor B, Powell A (1982) Isolation of plant DNA and RNA. Focus 4:4-6

Discussion

It can be concluded from the present evidence that gene Pch-I in line H-93-70 is indeed located on the long arm of chromosome 7D and is allelic to the resistance factor in VPM-1. The conclusion was based on the lack of linkage of a putative protein marker of chromosome 7D in a cross with a wheat substitution line carrying chromosome 7Ag from Agropyron elongatum, which was not expected to recombine with chromosome 7D. The apparent contradiction probably means that the gene encoding the protein marker was located differently in H-93-70 than in cv 'Chinese Spring' where the chromosome assignment had been made, or, less likely, that recombination had occurred between the 7D and 7Ag chromosomes.

The tight linkage of gene Pch-I to Ep-D1b clearly shows that chromosome 7D from Ae. ventricosa was the donor of gene Pch-I to the H-93 lines. It had been previously speculated that resistance to eyespot was located in the M genome of Ae. ventricosa (Delibes et al. 1977). The recovery of a line, H-93-51, which carries the Ep-D1b allele but is susceptible, contradicts the hypothesis proposed by Worland et al. (1988) that the resistance could be a product of the Ep-D locus.

Acknowledgements. We gratefully acknowledge technical assistance from D. Lamoneda and J. Garcia. The work was supported by Grant PBT87-0012 from the Comision Interministerial de Ciencia y Tecnologia, Spain.