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

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Summary. Gene *Pch1*, which confers resistance to eyespot disease (*Pseudocercosporella herpotrichoides* Fron), has been located on chromosome 7D in the H-93 wheat-*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 *Ep-D1b* but is susceptible to eyespot, which indicates that resistance to eyespot is not a product of the *Ep-D* locus, as had been proposed in a previous hypothesis.

Key words: Wheat – *Aegilops ventricosa* – *Pseudocercosporella herpotrichoides* – Eyespot disease – Resistance gene

Introduction

Two independent introductions of genetic resistance to eyespot disease (*Pseudocercosporella herpotrichoides*) from *Aegilops ventricosa* to hexaploid wheat have been carried out. Maia (1967) derived the resistant line VPM-1 from a cross between the amphiploid (*Ae. ventricosa* × *Triticum persicum*) and *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 *T. turgidum* (genomes AABB) and *Ae. ventricosa* ($D^vD^vM^vM^v$) was rescued with pollen from hexaploid wheat (AABBDD), 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 *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 *Ep-D1b* locus (Worland et al. 1988). However, the lack of linkage of resistance gene *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 *Pch-1* is indeed on chromosome 7D. We further report that this gene was transferred from chromosome $7D^v$ of *Ae. ventricosa* and that although *Pch-1* is linked to *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 (T. *turgidum* H-1-1 × *Ae. ventricosa* AP-1) × *T. aestivum* cv 'Almatense H-10-15' have been described previously (Delibes and García-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 anthers 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 × 125 × 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°–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 $7D^V$ and 7D are indicated. A similar survey for adenylate kinase (ADK) is presented in Fig. 1B. As the allele associated with chromosome $7D^V$ 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 $7D^V$ 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

Marker	Allele	Chromosome (arm)	References[a]
Isozymes			
Endopeptidase (EP)	*Ep-D1b*	$7D^V$ (L)	1
Adenylate kinase (ADK)	*Adk-D1b*	$7D^V$ (L)	2, 3
cDNA probes			
Ss1-1	*XSs1-E-7D*^{V}	$7D^V$ (S)	4, 5
	XSs1-E-7M^V	$7M^V$ (S)	4, 5
psr129	*Xpsr129-H-7M*^{V}	$7M^V$ (L)	5, 6
gDNA probes			
abm2	*Xabm2-G-7M*^{V}	$7M^V$	5
abm5	*Xabm5-G-7M*^{V}	$7M^V$	5
abm7	*Xabm7-E-7M*^{V}	$7M^V$	5
abm10	*Xabm10-H-7M*^{V}	$7M^V$	5

[a] References: 1, Koebner et al. 1988; 2, Benito et al. 1990; 3, this report; 4, Maraña et al. 1988; 5, Mena 1990; 6, Sharp et al. 1989

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 $7D^V$, 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 $7D^V$, long arm) and *XSs1-E-7D*^{V} (chromosome $7D^V$, short arm) shown in Fig. 2 is less close, which is consistent with the occurrence of recombination between chromosome $7D^V$ and 7D. None of the markers of chromosome $7M^V$ listed in Table 1 were detected in the resistant H-93 lines, indicating that the transfer of resistance occurred exclusively from chromosome $7D^V$. Reciprocal crosses between lines VPM-1 and H-93-70 were carried out, and the corresponding F_2 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 $7D^V$ (long arm), *Xpsr129-H-7M*^{V} from chromosome $7M^V$ (proximal part of long arm), absence of protein compo-

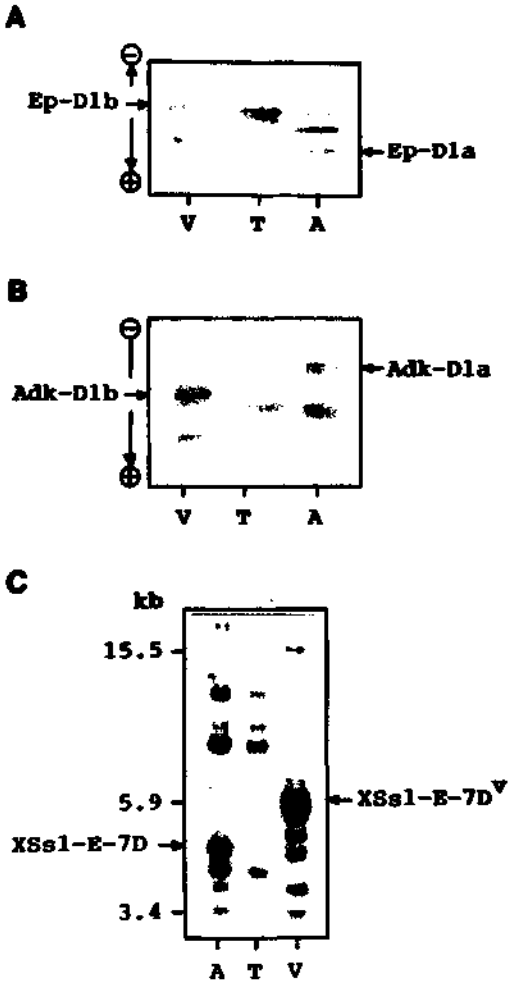

Fig. 1 A–C. Characterization of the three genitors of the H-93 lines, *Aegilops ventricosa* AP-1 (*V*), *Triticum turgidum* H-1-1 (*T*), *T. aestivum* cv 'Almatense' H-10-15 (*A*). **A** Endopeptidase zymograms obtained by electrofocusing as described by Koebner et al. (1988). **B** Adenylate kinase zymograms following Benito et al. (1990). **C** RFLP patterns obtained with a probe corresponding to the sucrose synthase gene *Ss1* (Maraña et al. 1988)

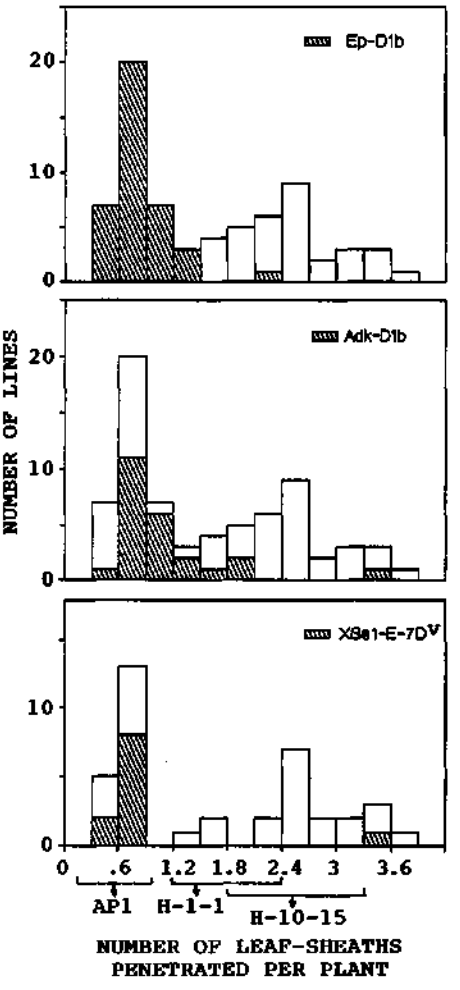

Fig. 2. Distribution of the indicated markers among H-93 lines having different levels of resistance to eyespot disease at the seedling stage

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)

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

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

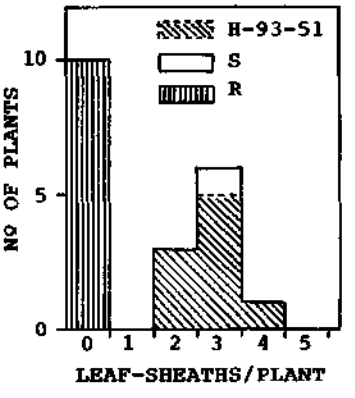

Fig. 3. Susceptibility of line H-93-51 to eyespot disease. Resistant control, VPM-1 (*R*); susceptible control, 'Pané 247' (*S*)

nent CM1 encoded by the short arm of chromosomes 7D and $7D^V$ and the absence of the $7D^V$ bands detected by the Ss1 probe, bands which also correspond to the short arm. This genotype and the cytological data indicate that line H-93-51 has chromosome 7D substituted for by a $7M^V$-$7D^V$-7D composite.

Discussion

It can be concluded from the present evidence that gene *Pch-1* 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 drawn by Delibes et al. (1988) to the contrary 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-1* to *Ep-D1b* clearly shows that chromosome $7D^V$ from *Ae. ventricosa* was the donor of gene *Pch-1* to the H-93 lines. It had been previously speculated that resistance to eyespot was located in the M^V genome of *Ae. ventricosa* (Delibes et al. 1977a).

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.

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