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Eyespot resistance gene *Pch-1* from *Aegilops ventricosa* is associated with a different chromosome in wheat line H-93-70 than the resistance factor in "Roazon" wheat

A. Delibes, G. Doussinault [1], M. Mena, I. López-Braña and F. García-Olmedo

Department of Biochemistry, E.T.S. Ingenieros Agrónomos-UPM, E-28040 Madrid, Spain
[1] INRA, Station d'Amelioration des Plantes, BP 29, F-35650 Le Rheu, France

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Summary. The hexaploid wheat line H-93-70 carries a gene (Pch-1) that has been transferred from the wild grass *Aegilops ventricosa* and confers a high degree of resistance to eyespot diesease, caused by the fungus *Pseudocercosporella herpotrichoides*. Crosses of the resistant line H-93-70 with the susceptible wheat Pané 247 and with a 7D/7Ag wheat/*Agropyron* substitution line were carried out and F2 kernels were obtained. The kernels were cut transversally and the halves carrying the embryos were used for the resistance test, while the distal halves were used for genetic typing. Biochemical markers were used to discriminate whether the transferred *Pch-1* gene was located in chromosome 7D, as is the case for a resistance factor present in "Roazon" wheat. In the crosses involving Pané 247, resistance was not associated with the 7D locus *Pln*, which determines sterol ester pattern (dominant allele in H-93-70). In the crosses with the 7D/7Ag substitution line, resistance was neither associated with protein NGE-11 (7D marker), nor alternatively inherited with respect to protein C-7 (7Ag marker). It is concluded that gene *Pch-1* represents a different locus and is not an allele of the resistance factor in "Roazon" wheat.

Key words: Wheat – *Aegilops ventricosa* – *Pseudocercosporella herpotrichoides* – Eyespot resistance – Chromosome markers

Introduction

Little resistance to eyespot (*Pseudocercosporella herpotrichoides*, Fron) exists within the species *Triticum aestivum* L., where only some degree of tolerance has been found in the French cv Capelle-Desprez that was incorporated into other cultivars. Ever since a high level of eyespot resistance was reported in the wild grass *Aegilops ventricosa* (Sprague 1936), the transfer of this resistance to cultivated wheat has been a standing breeding objective. Maja (1967) derived the resistant line VPM1 from a cross between the amphiploid (*Ae. ventricosa* × *Triticum persicum*) and *T. aestivum* cv Marne. Line VPM1 was used as the source of resistance in "Roazon" wheat, where the resistance factor was found to be associated with chromosome 7D by F2 monosomic analysis (Jahier et al. 1979). A different strategy, which involved an intermediate sterile hybrid between *T. turgidum* (genomes AABB) and *Ae. ventricosa* ($D^VD^VM^VM^V$) that was rescued with pollen from hexaploid wheat (AABBDD), led to the transfer and characterization of gene *Pch-1*, which confers a high degree of eyespot resistance (Delibes and García-Olmedo 1973; Delibes et al. 1977; Doussinault et al. 1983). We now present evidence that the gene *Pch-1* is not located in chromosome 7D and, therefore, is not an allele of the resistance factor in "Roazon" wheat.

Materials and methods

Biological materials

The previously described (Delibes et al. 1977; García-Olmedo et al. 1984) resistant hexaploid transfer line H-93-70 was crossed in the field with *T. aestivum* L. cv Pané 247 and with a 7D/7Ag *T. aestivum*/*Agropyron elongatum* substitution line, which was the kind gift of E. R. Sears (Columbia/MO, USA). Plants from the F1 were selfed and F2 seeds were collected and numbered. The F2 kernels were cut transversally into two halves and the halves containing embryos were planted to carry out the disease resistance tests, while the distal halves were used for biochemical analysis.

Determination of disease resistance

Plants with a given biochemical phenotype were tested in groups and the proportion of plants sensitive to eyespot in each group was determined by previously established criteria (Doussinault et al. 1983).

Biochemical analyses

The pattern of sterol esters was analysed by thin-layer chromatography essentially as described by García-Olmedo (1968). The half kernels were crushed between 2 metal plates and extracted in individual 1 ml glass tubes with 0.3 ml of petroleum ether (b.p. 60°–70 °C). The extracts were spotted in silica-gel thin-layer plates and chromatographed with carbon tetrachloride as solvent. After chromatography, the plates were exposed to iodine vapour and photographed.

Proteins NGE-11 and C-7 were analysed by two-dimensional electrophoresis by a modification of the method described by Rodriguez-Loperena et al. (1975). Shorter gel columns (0.2 × 5.5 cm) and only 2 h of focusing time were used in the first dimension. Under these conditions, the alkaline end of the pH 5–8 gradient was better preserved and better resolution for the proteins of interest was achieved, at the expense of greater overlapping of components in the rest of the two-dimensional gel.

Results and discussion

Gene *Pln* is located in the short arm of wheat chromosome 7D and its dominant allele determines a drastic decrease in the free sterols present at the end of endosperm development, just prior to kernel dessication, with a concomitant increase of their palmitate esters (García-Olmedo 1968; Torres and García-Olmedo 1974, 1975). The sterol-esters patterns of the eyespot-resistant line H-93-70, the susceptible wheat cv Pané 247 and several F2 half-kernels are shown in Fig. 1. The F2 kernel halves that carried the embryos were planted and tested for eyespot resistance. Results of these tests are summarized in Table 1. Resistance was not linked with the dominant *Pln* allele present in the resistant line, either in combination with the *T. aestivum* cytoplasm from cv Pané 247 or with the *T. turgidum* cytoplasm of the H-93-70 transfer line (Doussinault et al. 1983).

Since hybrids between the H-93-70 line and *T. aestivum* have a regular 21-II meiosis and free recombination was probably possible between the 7D chromosomes from the resistant and the susceptible parents, a different linkage experiment was carried out in which the susceptible parent carried chromosome 7Ag from *Agropyron elongatum*, instead of chromosome 7D, and recombination was not expected (Sears 1977). Biochemical typing

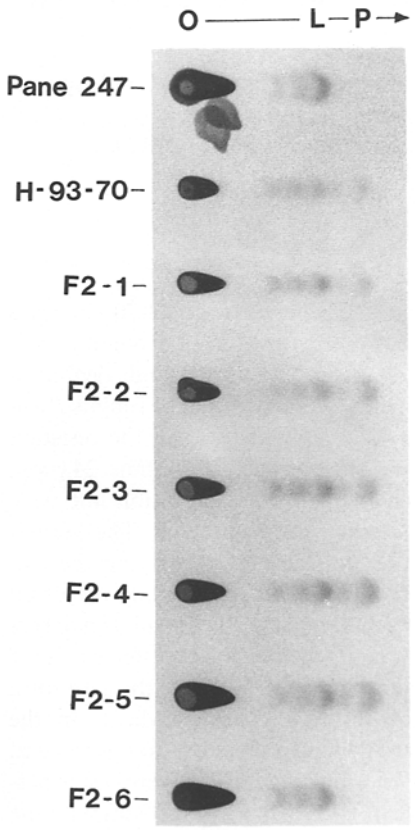

Fig. 1. Thin-layer chromatography of sterol esters from half-kernels from *T. aestivum* cv Pané 247, resistant transfer line H-93-70, and F2 (Pané 247 × H-93-70); *O* application point; *L* migration of sterol linoleate; *P* migration of sterol palmitate. *Arrow* indicates direction of migration

Table 1. Non-linkage between genes *Pch*-1 and *Pln*

	Proportion of susceptible plants										
Repetitions →	1	2	3	4	5	6	7	8	9	10	Total
T. aestivum cv. Pané 247	4/4	5/5	4/4	4/4	5/5	3/3	5/5	5/5			35/35
Transfer line H-93-70	0/5	0/5	0/4	0/5	0/5	0/5	0/5	0/4			0/38
F2 (Pané 247 × H-93-70)											
P-L phenotype	0/10	1/9	2/9	1/10	1/10	2/10	3/10	1/10	1/10		12/88
L phenotype	0/5	0/5	1/5	3/5	3/5	0/4	0/2				7/31
F2 (H-93-70 × Pané 247)											
P-L phenotype	0/10	2/10	2/10	2/10	1/10	0/10	2/10	0/8	3/10	2/9	14/97
L phenotype	2/5	1/5	2/5	0/4	1/5	0/5	1/5				7/34

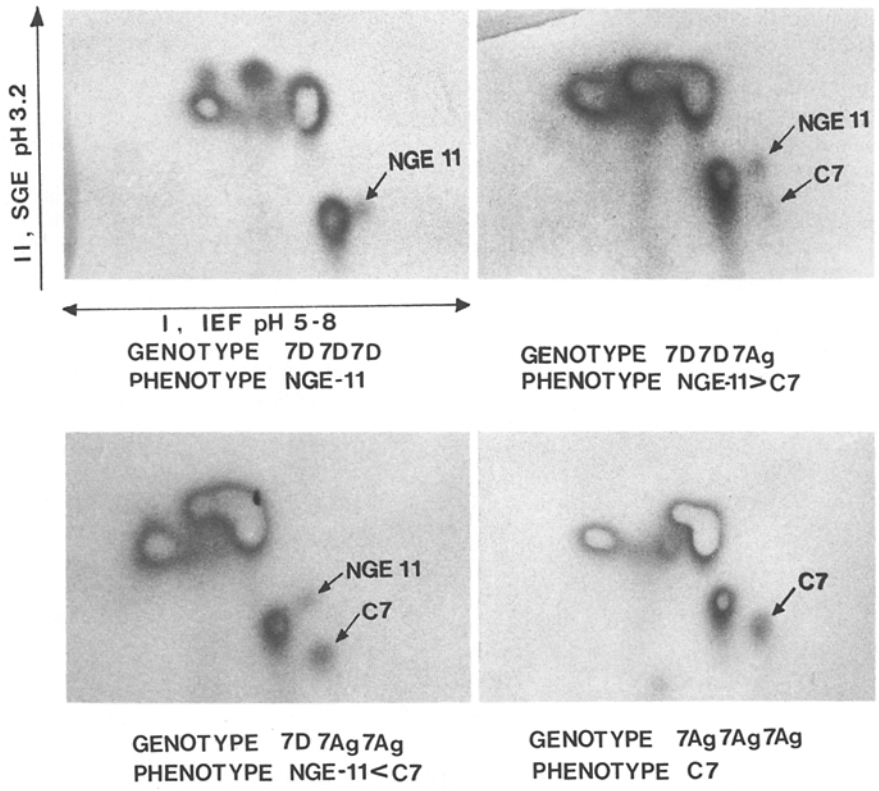

Fig. 2. Separation by two-dimensional electrophoresis of protein NGE-11, marker of chromosome 7D, and protein C7, marker of chromosome 7Ag. First dimension was isoelectric focusing (IEF), pH range 5–8, and second dimension was starch-gel-electrophoresis (SGE), pH 3.2

Table 2. Lack of association of gene *Pch-1* with chromosome 7D

	Proportion of susceptible plants							
Repetitions →	1	2	3	4	5	6	7	Total
Wheat/Agropyron 7D/7Ag subst.	9/10	10/10	7/9	8/9				34/38
Transfer line H-93-70	0/10	0/10	0/10	0/10				0/40
F2 (7D/7Ag subst. × H-93-70)								
7D7D7D	2/10	1/9	0/9	3/8				6/36
7D7D7Ag+7D7Ag7Ag	3/9	5/9	2/10	4/10	3/9	2/9	1/9	20/65
7Ag7Ag7Ag	4/10	3/9	1/10	1/9				9/38

of the F2 half-kernels was carried out as indicated in Fig. 2, where phenotypes corresponding to the four possible chromosome combinations (AgAgAg; AgAgD; AgDD; DDD) can be discerned on the basis of the presence and relative amounts of proteins NGE-11 (7D marker) and C-7 (7Ag marker), based on the previous observations of Rodriguez-Loperena et al. (1975). The F2 half-kernels that carried the embryos were planted and tested for eyespot resistance (Table 2). Again, resistance was found not to be associated with chromosome 7D. This is in contrast with the finding of Jahier et al. (1979) concerning the location in that chromosome of a factor for eyespot resistance present in wheat cv, "Roazon", where it had been introduced from line VPM1. It can be concluded that the alluded factor and gene *Pch-1* are not alleles of the same locus and therefore could be combined in the same phenotype.

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