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Hessian fly-resistance gene transferred from chromosome 4M^v of *Aegilops ventricosa* to *Triticum aestivum*

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Abstract A new Hessian fly (*Mayetiola destructor*) resistance gene from *Aegilops ventricosa* and its transfer to hexaploid wheat is described. The 4D(4M^v) substitution line H-93-33 derived from the cross [(*Triticum turgidum* H-1-1 × *Aegilops ventricosa* no. 11) × *Triticum aestivum* H-10-15] was highly resistant to the Spanish population tested. Resistance seemed to be inherited as a single dominant factor in the F_2 generation resulting from a cross of H-93-33 with its susceptible parent (H-10-15). Resistance in *Ae. venticosa* no. 10 was located on chromosome 4M^v using M^v wheat/*Ae. ventricosa* addition lines. The resistance gene transferred from *Ae. ventricosa* no. 11 to H-93-33 (H27) is allelic with respect to that of *Ae. ventricosa* no. 10 and is non-allelic with respect to the genes H3 and H6 from Monon and Caldwell respectively. The assignment of H27 gene to chromosome 4M^v is further supported by its linkage to a gene encoding isozyme Acph-M^{v}1, previously located on chromosome 4M^v in the line H-93-33. A new marker from homoeologous chromosome group 4 (Amp-M^{v}2) present in H-93-33 and the 4M^v addition line is described.

Key words *Aegilops ventricosa* · *Triticum aestivum* · *Mayetiola destructor* · Hessian fly · Resistance gene

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Introduction

The Hessian fly *Mayetiola destructor* is one of the most destructive pests of wheat crops. This insect, first described in Spain in the 19th century (Herreros 1896), causes extensive damage to wheat in the Iberian peninsula and North Africa.

Losses caused by Hessian fly infestation have been reduced by using resistant cultivars or by sowing after the "fly-free" date. Sowing delay to avoid damage is not necessary when resistant cultivars are used. There is a gene-for-gene relationship between resistance genes in wheat and the avirulence of the Hessian fly biotypes, which determines the evolution of the virulence of the fly (Hachett and Gallun 1970). More than 25 resistance genes have been identified in *Triticum* species that can be used in breeding resistant cultivars (Friebe et al. 1991; Patterson et al. 1992; Raupp et al. 1993; Cox and Hachett 1994). All these genes, except h4, are dominant or partially dominant, and they cause antibiosis in larvae. However, the identification of new resistance genes is of great interest because deployed genes are periodically overcome by new virulent biotypes. Thus, the germ plasm of wheat and its relatives is being continually searched for more sources of resistance to the Hessian fly.

The wild grass *Aegilops ventricosa* has been recognized for almost 40 years as an important potential donor of genes that govern characteristics of agronomic interest. Gill et al. (1985), and Amri et al. (1992) described resistance to *M. destructor* in accessions of *Ae. ventricosa*, although the genetic basis of this resistance has not yet been investigated. *Ae. ventricosa* is an allotetraploid with the genomic constitution D^{v}D^{v}M^{v}M^v. It is partially homologous to *Triticum aestivum* (AABBDD); therefore, genes from the D^v genome are more easily transferred by recombination with the D genome than those from the homoeologous M^v genome (Delibes et al. 1977; Mena et al. 1993).

Thus, five Hessian fly resistance genes (H13, H22, H23, H24 and H26), located on chromosomes 6D, 1D, 6D, 3D and 4D respectively (Raupp et al. 1993; Cox and Hachett 1994), were transferred from the diploid *Triticum tauschii* (the D-genome donor of common wheat) into hexaploid wheat. Previously, we described the use of *Ae. ventricosa* no. 11 to derive 70 wheat lines (H-93 lines) with 42 chromosomes, carrying genetic material from both the D^v and the M^v genomes (Doussinault et al. 1983; Delibes et al. 1987; Mena et al. 1993; Delibes et al. 1993). Transfer line H-93-33 has been characterized by cytological methods and by RFLP/isozyme analysis, as carrying chromosome $4M^v$ from *Ae. ventricosa* (Mena et al. 1989, 1993). This line was resistant to the powdery mildew and eyespot disease caused by *Erysiphe graminis* f. sp. *tritici* and *Pseudocercosporella herpotrichoides* respectively (Delibes et al. 1987; Mena et al. 1992).

Five wheat/*Ae. ventricosa* addition lines have already been characterized by cytological, morphological, and agronomic traits (Dosba and Doussinault 1978). Four of these have been further identified as corresponding to lines carrying chromosomes $4M^v$ (Delibes et al. 1981; Mena et al. 1989), $5M^v$ (Delibes et al. 1981), $6M^v$ (Dosba 1985) and $7M^v$ (Mena et al. 1993).

Our objective in this report was to establish the potential usefulness of *Ae. ventricosa* as a source for Hessian fly resistance in wheat breeding. We present evidence of Hessian fly resistance transference from *Ae. ventricosa* to hexaploid wheat and the inheritance of this resistance as a single Mendelian factor (H27). We further report that this gene is transferred linked to the Acph-M^v1 marker from $4M^v$ chromosome. Another marker from this chromosome is also described (Amp-M^v2).

Materials and methods

Biological materials

The hexaploid H-93 lines derived from the cross [(*Triticum turgidum* H-1-1 × *A. ventricosa* no.11) × *T. aestivum* cv "Almatense H-10-15"] have been described previously (Delibes et al. 1977; Doussinault et al. 1983 García-Olmedo et al. 1984). The crosses in the present work were carried out by standard manual procedures. Resistant line H-93-33 was crossed with hexaploid wheat (*T. aestivum*) cultivars "Almatense H-10-15", "Monon", "Caldwell" and "Abe", in order to study the inheritance of resistance derived from *Ae. ventricosa*. Also, the hybrid between *Ae. ventricosa* no. 10 and no. 11, the parents of the addition lines and H-93-33, respectively, were obtained. F_1 plants of all crosses were grown to maturity in a greenhouse to obtain F_2 seeds and all F_1 spikes were bagged before anthesis to prevent outcrossing. Some F_1 plants had low fertility and were discarded for F_2 testing. The wheat/*Ae. ventricosa* addition lines were a gift of F. Dosba (Dosba and Dousinault 1978; Dosba 1985). The *T. aestivum* cultivars with different resistance genes to Hessian fly, such as Monon, Caldwell and Abe, were supplied by Dr. H. E. Bockelman and F. Maas from the National Small Grains Collection of USDA-ARS.

Isozyme markers

Isozymes of aminopeptidase (AMP-2) were extracted from individual embryos with 35 µl of 10 mM DTT, and separated by isoelectric focussing in a 20-cm-length gel according to Koebner and Martin (1989), using L-lysine β-naphthylamide as a substrate. Phosphatase isozymes (ACPH) were extracted, fractionated and stained essentially as described by Brewer (1970), except that 10% acrylamide was used for the electrophoretic separation, which markedly improved the resolution of isozyme bands.

Tests for resistance to Hessian fly

Infestation under field conditions was investigated in a naturally infested plot at the Experimental Station in Azuaga (Badajoz, Spain). The infested plants were characterized by the presence of larvae as third instars inside puparia at the crown area of the plants. Tiller number, the number of infested tillers, and pupae number (indicative of previous larval infestation) were recorded for each sample in the second generation of the insect. Except in segregation studies, susceptibility scores were expressed as pupal number per tiller. We used "Astral" wheat as the susceptible cultivar to assess the level of infestation in all experiments. The study of the segregation of the resistance was carried out by the analysis of individual F_2 plants, and the parentals used for all crosses, and scores were expressed as pupae per plant. Cultivars were also sown in a plastic tunnel in standard greenhouse trays. When the plants were at the one-leaf stage, they were infested with the same biotype as the naturally infested plots, by the technique described by Carwright and LaHue (1944). Plants were classified as susceptible or resistant at 30 days (eggs) and at 90 days (pupae) after infestation.

Results

In Spain two Hessian fly generations per year occur in infested fields with the GP biotype present in this area (unpublished data). The temporal course of the infestation in the susceptible *T. aestivum* cv "Astral" was studied in Azuaga (Badajoz) and is shown in Fig. 1.

Hessian fly resistance in lines carrying chromosome $4M^v$

A preliminary screening for Hessian fly resistance among introgression lines H-93-1 to H-93-70, obtained from the cross [(*T. turgidum* H-1-1 × *Ae. ventricosa* no. 11) × *T. aestivum* H-10-15], was carried out in a naturally infested field by a visual inspection of the pupae inside the leaf sheath at the bottom of the plants. Line H-93-33 appeared with little or no infestation, while several other lines showed a low number of pupae. These H-93 lines, as well as their progenitors and *T. aestivum* cv "Astral" as a susceptible control, were then subjected to a more quantitative test in the same field. The results of this test are summarized in Fig. 2. A high level of resistance was found in line H-93-33, while the susceptibility of the other H-93 lines tested was in the same range as that of the parental wheat H-10-15. These results were confirmed in field tests with H-93-33

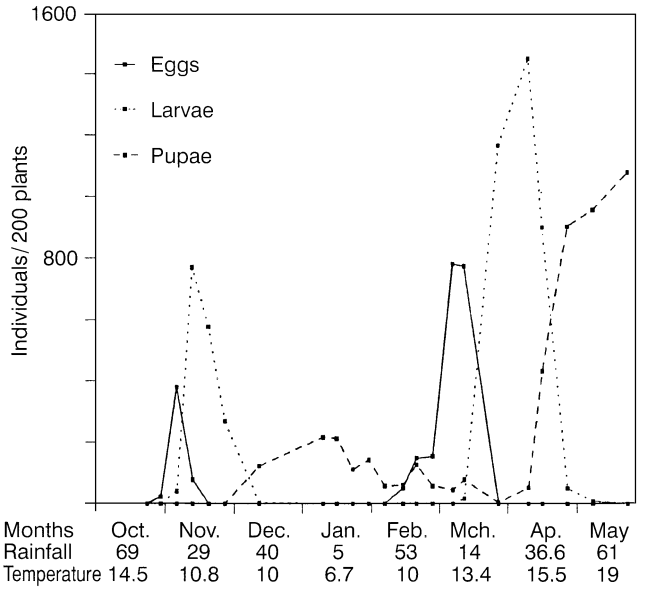

Fig. 1 Time course of infestation by Hessian fly of *T. aestivum* cv "Astral" on a naturally infested field, from October 1990 to May 1991. Each *point* represents the number of individuals per 200 plants. At the bottom, whole rainfall (in mm^3) and average temperature (°C) in each month

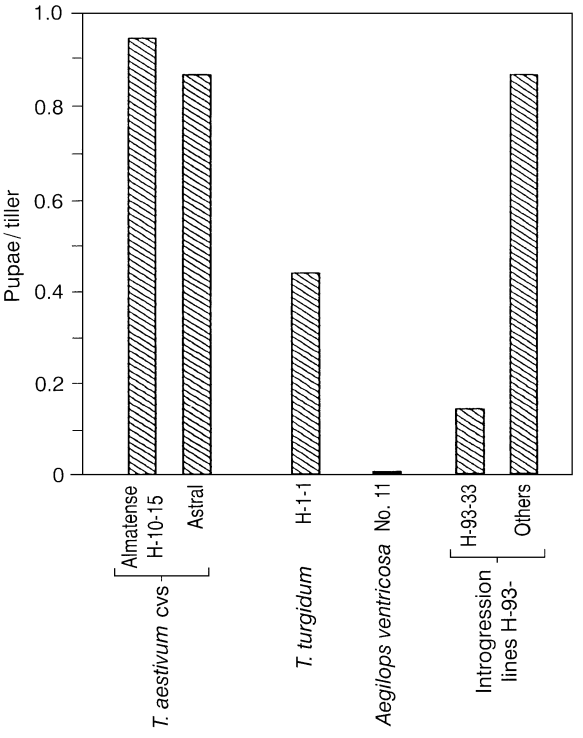

Fig. 2 Evaluation of the susceptibility to the Hessian fly *M. destructor* of the introgression lines H-93, their parental and the cultivar Astral as a control susceptible, on a naturally infested field. The average of ten plants (from 150 to 300 tillers) per stock is shown, except for the seven H-93 lines, with intermediate resistance, whose overall average is shown

and *T. aestivum* cv "Astral" as a susceptible control, which were analyzed for 4 consecutive years (Fig. 3). In all cases, a lower infestation level was observed in H-93-33. *T. aestivum* cv "Moisson"/*Ae. ventricosa* no. 10 addition lines were used to investigate the chromosome location of the Hessian fly resistance gene in *Ae. ventricosa* no.10. The $4M^v$ addition line was the most-resistant line of those tested (Fig. 4 A), with a similar level of resistance to that of line H-93-33. The addition lines $4M^v$, $7M^v$, which showed a low number of pupae, as well as line H-93-33 and *T. aestivum* cv "Astral", were subjected to a new test in the greenhouse. The results obtained in these conditions were similar, but with higher infestation levels in both resistant and susceptible plants (Fig. 4 B).

Inheritance of Hessian fly resistance

Reciprocal crosses and their F_2 generations were obtained from line H-93-33 and the recipient *T. aestivum* cv "Almatense" H-10-15 (hereafter called H-10-15). A total of 319 F_2 (H-10-15 × H-93-33) plants were scored for susceptibility to *M. destructor* as shown in Fig. 5 A. When the (H-10-15 × H-93-33) F_2 plants are classified into resistant and susceptible, using the lower limit of the confidence interval of the mean (P = 99%) for the susceptible parent as a demarcation point, a 3 : 1 segregation of resistant versus susceptible plants is obtained $[\chi^2 = 0.037 \ll \chi^2 \quad (gl = 1; \quad P = 0.05) = 3.84]$. A similar result was obtained with the reciprocal cross (data not shown).

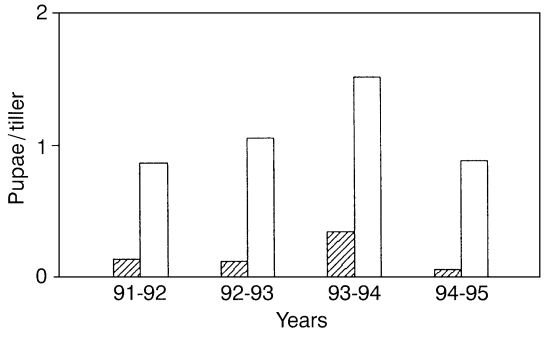

Fig. 3 Infestation from 1991 to 1995 in a plot at the Experimental Station in Azuaga (Badajoz, Spain) of line H-93-33 (*shaded bars*) and *T. aestivum* cv "Astral" (*open bars*). Each *bar* represents the average of a minimum 100 tillers per stock

The *T. aestivum* cvs "Monon", "Abe" and "Caldwell", which carried H3, H5 and H6 resistance genes, respectively, and showed a high level of resistance to the GP biotype present in Azuaga, were crossed with resistant line H-93-33. The degree of infestation of 116 and 350 individual F_2 plants from the crosses with "Monon" and "Caldwell", respectively, was determined (Fig. 5 B, C). In both crosses most of the plants fell into the resistant class, but there were also a few plants that

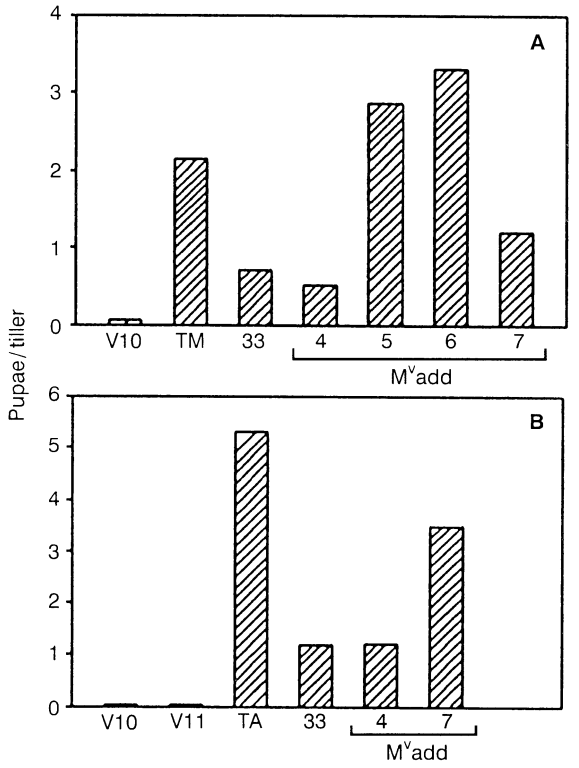

Fig. 4 Evaluation of the susceptibility to the Hessian fly under field (**A**) and greenhouse (**B**) conditions of the stocks: *Aegilops ventricosa* no.10 (*V10*) and no. 11 (*V11*); *Triticum aestivum* cvs "Moisson" (*TM*) and "Astral" (*TA*); H-93-33 line (*33*); addition lines *Triticum aestivum* cv "Moisson"/*Aegilops ventricosa* no.10: 4M^v (*4*), 5M^v (*5*), 6M^v (*6*) and 7M^v (*7*). Each *bar* represents the average of from 16 to 25 plants per stock (172–341 tillers)

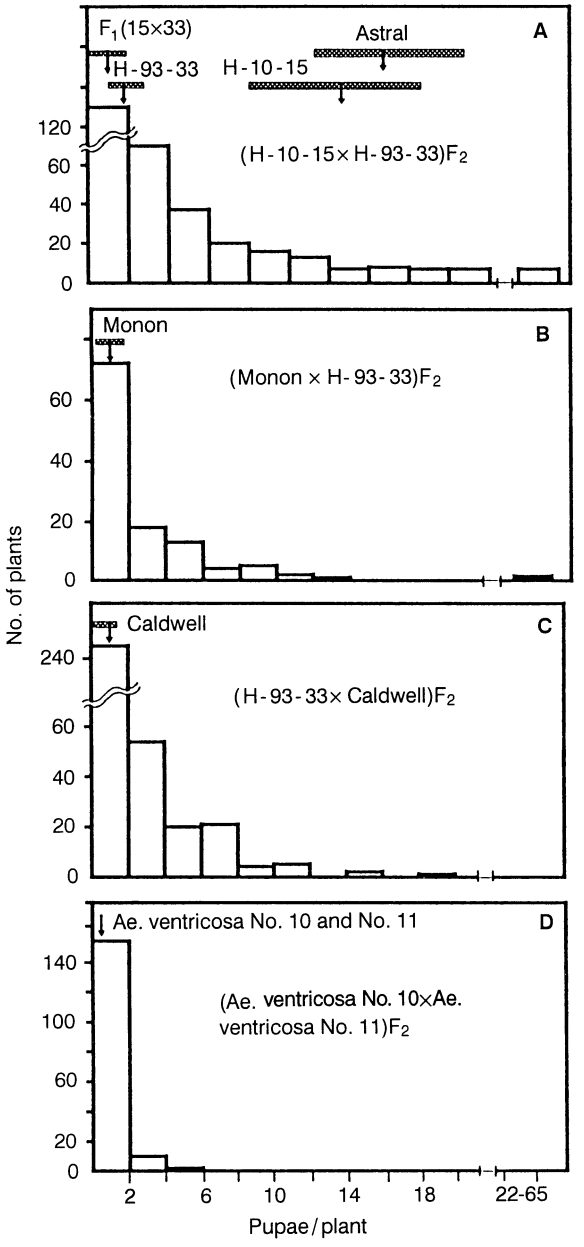

Fig. 5 The distribution of Hessian fly infestation under field conditions of **A**: (H-10-15 × H-93-33)F_2, **B**: (Monon × H-93-33)F_2, **C**: (H-93-33 × Caldwell)F_2, and **D**: (*Aegilops ventricosa* no. 10 × *Aegilops ventricosa* no. 11) F_2 plants. In the upper part of each panel the average (*vertical arrow*) and the 99% confidence interval (*shaded*) is shown for H-93-33 (30 plants); (H-10-15 × H-93-33)F_1 (6 plants); H-10-15 (24 plants); Astral (26 plants); Monon (25 plants); Caldwell (29 plants); *Aegilops ventricosa* no. 10 (15 plants) and *Aegilops ventricosa* no.11 (15 plants)

were clearly susceptible. The low fertility of the (H-93-33 × Abe) F_1 did not allow us to test F_2 plants from this cross because only a small quantity of F_2 seeds was available.

The resistance of 166 F_2 individual plants of the cross (*Ae. ventricosa* no. 10 × *Ae. ventricosa* no. 11) was tested to determine whether the resistance genes in both accessions were allelic. All F_2 plants fell into the resistant class (Fig. 5 D)

Distribution of markers in the H-93-33 line and the description of a new isoenzymatic marker (Amp-M^{v}2)

Thirty markers from the seven chromosomes of the M^v genome had been used in the characterization of line H-93-33 (Mena et al. 1993). Only the 4M^v markers, from the short and the long arm, are present in line H-93-33, replacing those of chromosome 4D (Table 1). The aminopeptidase-2 (AMP-2) isozyme pattern, controlled by group-4 chromosomes in wheat (Koebner and Martin 1989), was analyzed in the wheat/*Ae. ventricosa* addition lines, as well as in the H-93 lines and in their parents. The AMP-2 isozyme pattern (Fig. 6 A) of *Ae. ventricosa* no. 10 and no. 11 showed two bands, one with the same mobility as that of chromosome 4D in wheat (Amp-D2), putatively assigned to 4D^{v}s. This band was present in the 4M^v addition line but absent in line H-93-33. The second band (Amp-M^{v}2) would correspond to chromosome 4M^v and was, as expected,

Table 1 Biochemical and molecular genetic markers from homoeologus chromosome group 4 used in the characterization of line H-93-33

Marker set	Chromosome group (arm)	M^v marker designation	Marker(s) missing from chromosome	Ref.[a]
psr 144	4(s)	Xpsr-144-H-4M^v	4Ds	1
pct 1	4(s)	Xpct1-E-4M^v	4Ds	1
ACPH	4	Acph-M^v1	4Dl	2
ADH	4	XAdh-H-4M^v	4Ds	3
ADH	4	Adh-M^v1	4Ds	3
abm 1	–	Xabm1-E-4M^v	–	1
AMP-2	4	Amp-M^v2	4Ds	4

[a]References:1, Mena et al. (1993); 2, Delibes et al. (1981); 3, Mena et al. (1993); 4, this report

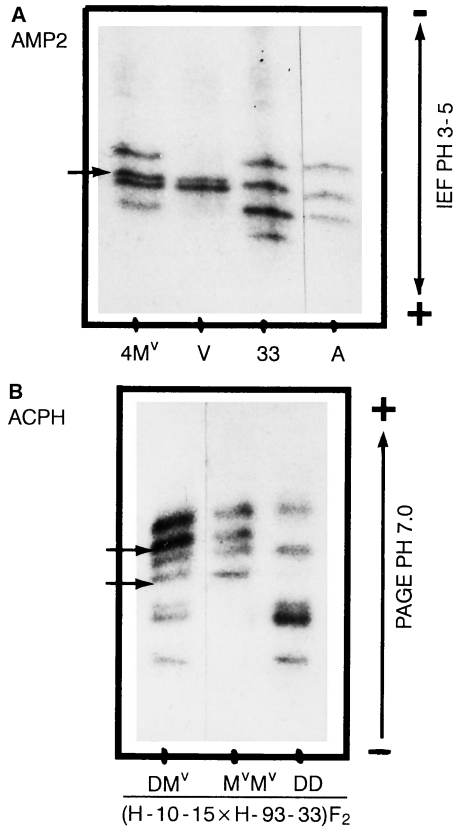

Fig. 6 **A** Aminopeptidase-2 zymogram obtained by isoelectric focussing as described by Koebner and Martin (1989) of: 4M^v addition line (*4M^v*); *Aegilops ventricosa* no. 11 (*V*); H-93-33 transfer line (*33*) and *Triticum aestivum* H-10-15 (*A*). The arrow indicates Amp-M^v2. **B** Phosphatase zymogram obtained, following the procedure of Delibes et al. (1981), from the three different patterns obtained by the cross (H-10-15 × H-93-33)F_2. *Arrows* show Acph-M^v1

exclusively found in the 4M^v addition line and in H-93-33, while the isozyme pattern of the remaining H-93 and addition lines was similar to that of parental wheat (Fig. 6 A).

Co-segregation of resistance to Hessian fly and the Acph-M^v1 marker in line H-93-33

The linkage between markers and resistance genes simplifies the screening of resistance in breeding populations. From the markers associated with chromosome 4M^v in line H-93-33, shown in Table 1, Acph-M^v1 was selected for linkage studies. This marker, which has been resolved into two components (Delibes et al. 1981), is more valuable than the others because it can be analyzed using a small quantity of seed endosperm. Linkage between resistance to Hessian fly and the Acph-M^v1 marker from chromosome 4M^v was determined by the analysis of 287 individual (H-10-15 × H-93-33)F_2 plants. Kernels were cut transversally and the halves carrying the embryos were used for the resistant test, while the distal halves were used for biochemical analysis. Phosphatase zymograms (ACPH) gave three different patterns (DM^v, M^{v}M^v, DD) in F_2 plants, as shown in Fig. 6 B, where the two bands corresponding to chromosome 4M^v are arrowed. It was not always possible to distinguish between hemizygous (Acph-M^v1 Acph-D1) and homozygous (Acph-M^v1 Acph-M^v1) types, so only two classes of plants (with and without the marker) were established. Evidence for linkage is presented in Fig. 7, and the likelihood ratio test was carried out (Sokal and Rohlf 1981). The G-test of independence value was $90.138 \gg \chi^2_{.0.5[1]} = 3.841$, and consequently the null hypothesis of independence between Hessian fly resistance and the Acph-M^v1 marker could be rejected.

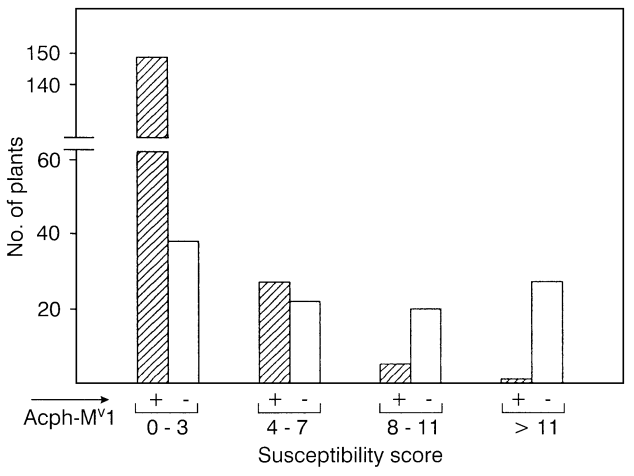

Fig. 7 The distribution of susceptibility scores in pupae per plant in plants from the (H-10-15 × H-93-33)F_2 cross with (1 or 2 doses) and without (0 doses) of the Acph-M^v1 marker. The genotypes were established by electrophoretic analysis of 287 F_2 kernels. The kernel halves that carried the embryos were planted and tested for Hessian fly resistance. The plants obtained were divided into four categories according to their susceptibility scores. Isozyme ACPH was analysed in each F_2 kernel half without embryos. Two different classes were established: + (Acph-M^v1/Acph-M^v1 and Acph-M^v1/Acph-D1, *shaded bars*); and − (Acph-D1/Acph-D1, *open bars*)

Discussion

The two accessions of *Ae. ventricosa*, parentals of the H-93 lines and the addition lines wheat/*Ae. ventricosa*, showed no symptoms of infestation by the GP biotype either in field trials or in the greenhouse. The resistance to Hessian fly from *Ae. ventricosa* no.11 ($D^vD^vM^vM^v$) was transferred to only one H-93 introgression line out of 70 tested. This low transfer frequency has been previously shown to be characteristic for genes from the M^v genome with the transfer procedure used (Delibes et al. 1977, Delibes et al. 1993; Mena et al. 1993). Under field conditions, the level of resistance in the line H-93-33 transferred from *Ae. ventricosa* no. 11 was high, showing little or no infestation.

A resistance similar to that of the substitution line 4D(4M^v)H-93-33 was transferred from *Ae. ventricosa* no.10 to the 4M^v addition line, which suggests that it is the same allele. The 7M^v addition line showed intermediate resistance, which was clearly different from that described for the 4M^v addition line.

In all cases, as expected, a higher level of infestation was found under greenhouse conditions, where the quantity of inoculum and the temperature were higher. Nevertheless, the infestation resistant/susceptible ratios were maintained.

The shift toward resistance of the (H-10-15 × H-93-33)F_2 distribution could be explained by a few susceptible plants that escaped infestation in the naturally infested field. No maternal effects were found in the reciprocal crosses. Resistance was apparently completely dominant and was not affected by the genetic background.

Data from the tested crosses, (Monon × H-93-33)F_2, (H-93-33 × Caldwell)F_2 and (*Ae. ventricosa* no. 10 × *Ae. ventricosa* no. 11)F_2, were analyzed in order to establish whether the resistance gene of H-93-33, derived from *Ae. ventricosa* no. 11, segregates independently to that of Monon, Caldwell and *Ae. ventricosa* no. 10. The resistance factor of Monon (H3 gene) is at 9.0 map units from that of Caldwell (H6 gene) on chromosome 5A (Patterson and Gallun 1977), and both are inherited independently from the resistance gene in H-93-33. The hypothesis of two dominant genes should lead to a 15 resistant : 1 susceptible ratio in the F_2 generation. The small proportion of susceptible plants found in the F_2 populations in both crosses supports this model and would also be consistent with the hypothesis of a dominant resistance gene in H-93-33. It can be concluded that the resistance genes in Monon and Caldwell (H3 and H6) and in H-93-33, which we propose to call H27, are not alleles of the same locus and therefore can be combined in the same genotype. The lack of segregation of the resistance trait in the F_2 population of the hybrid between *Ae. ventricosa* no. 10 and *Ae. ventricosa* no. 11 supports the hypothesis of a locus in *Ae. ventricosa* that is involved in the resistance to the *M. destructor* GP biotype, and which is localized in chromosome 4M^v, occupied by the H27 allele, in the two accessions of *Ae. ventricosa* (nos 10 and 11) studied. However, we cannot rule out the existence of other loci with an effect on resistance to the same biotype. This would explain the partial resistance shown by the 7M^v addition line. The level of infestation of individual plants of this line was, however, clearly higher than that shown by individual plants of the 4M^v addition line.

The use of markers linked to the resistance simplifies the selection process for obtaining cultivars resistant to the Hessian fly. Furthermore, as the expression of Hessian fly resistance genes is influenced by the environment, the use of closely linked markers could overcome this limitation.

A linkage analysis of genes for the isozyme Acph-M^v1 markers and resistance to the Hessian fly was carried out in (H-10-15 × H-93-33)F_2 plants. The distribution of susceptibility scores for the two classes of plants (with and without marker) indicated that the linkage between the two traits (Fig. 7) is not very tight. However, evidence of linkage was found in the clearly susceptible plants (with a pupal number higher than 11) without the marker. Only one of these susceptible plants carried the Acph-M^v1 marker, which would be consistent with recombination between chromosome 4M^v, carrying both genes, and a wheat chromosome. This result agrees with our previous work showing recombination between chromosomes of the M^v and D genomes in some H-93 lines (H-93-1, H-93-3, H-93-18, H-93-33 and H-93-51; Mena et al. 1993). The unexpected number of resistant plants without the marker could also be explained by the lack of infestation under the naturally infested field conditions described in this paper.

Aminopeptidase-2 isozymes (AMP-2) have been associated with group-4 chromosomes (Koebner and Martin 1989). The gene encoding Amp-M^v2 can be tentatively assigned to the M^v genome, as could be deduced from its presence in *Ae. ventricosa* (DM^v), *Ae. comosa* (M) and *Ae. uniaristata* (M^u), and its absence in *Ae. squarrosa* (D) (data not shown). Indeed, this gene was found in the H-93 lines at the expected low frecuency, being present in only one line (H-93-33). The distribution of the Amp-M^v2 marker supports the previous characterization of line H-93-33 as a 4D(4M^v) substitution line (Mena et al. 1993) and confirms the identity of the previously reported 4M^v addition line (Delibes et al. 1981; Mena et al. 1989).

Hessian fly resistance loci are widely distributed in the D genome, and have been found on chromosomes 1D, 3D, 4D, 5D and 6D (Raupp et al. 1993; Gill et al. 1987; Amri et al. 1990; Cox and Hatchett 1994). In the present report, a resistance gene (H27) was located on chromosome 4M^v in two different accessions of *Ae. ventricosa* and was introduced, together with other markers from this chromosome, into the 4D/4M^v substitution line (H-93-33) and the 4M^v addition line. If the

H26 gene (associated with chromosome 4D) and H27 from *Ae. ventricosa* were non-allelic genes, it should be possible to develop recombinant lines having both resistance sources in one linkage block. Recombination between the chromosomes $4M^v$ and 4D has been previously described in the transfer line H-93-33 (Mena et al. 1989). A combination of the resistance gene of chromosome $4M^v$ with that from other sources may be desirable to improve the maintainance of resistance in wheat. We have crossed line H-93-33 with several wheat cultivars and breeding lines (unpublished), showing that it is possible to produce a sufficient number of viable, fertile, backcrossed progeny for efficient gene transfer.

In conclusion, the single resistance factor derived from *Ae. ventricosa* may represent a new source of genetic resistance to the Hessian fly available to wheat breeders. The gene in question, called H27, is the first one to be mapped on an M-genome chromosome that has been introduced into wheat. The development of wheat cultivars having *Ae. ventricosa* resistance will provide a broader range of genetic resistance to the Hessian fly.

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