Theor Appl Genet (2003) 106:1248–1255
DOI 10.1007/s00122-002-1182-z

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A new Hessian fly resistance gene (*H*30) transferred from the wild grass *Aegilops triuncialis* to hexaploid wheat

Received: 5 April 2002 / Accepted: 21 October 2002 / Published online: 23 January 2003

Abstract A new Hessian fly (*Mayetiola destructor* Say) resistance gene from *Aegilops triuncialis* and its transfer to hexaploid wheat via interspecific hybridisation is described. The transfer line TR-3531 (42 chromosomes), derived from the cross [(*Triticum turgidum* × *Ae. triuncialis*) × *Triticum aestivum*] and carrying the *Heterodera avenae* resistance gene *Cre*7, showed a high level of resistance to the *M. destructor* biotype prevailing in the SW of Spain. A single dominant gene (*H*30) seems to determine the Hessian fly resistance in this introgression line, and its linkage with an isozyme marker (*Acph*-U1) has also been studied. It has been demonstrated that the resistance gene *H*30 in the TR-3531 line is non-allelic with respect to the genes *H*3, *H*6, *H*9, *H*11, *H*12, *H*13, *H*18 and *H*21, present in wheat cultivars from the Uniform Hessian Fly Nursery (UHFN), as well as to *H*27, carried by the introgression line H-93-33. Advanced lines with the *H*30 gene were obtained by backcrossing the transfer line and different commercial wheats as recurrent parents. Several of them showed a high yield in tests carried out in the infested field. Electronic Supplementary Material is available if you access this article at http://dx.doi.org/10.1007/s00122-002-1182-z. On that page (frame on the left side), a link takes you directly to the supplementary material.

Keywords *Aegilops triuncialis* · Hessian fly · *Mayetiola destructor* · Resistance gene · *Triticum aestivum*

Communicated by F. Salamini

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Introduction

The Hessian fly, *Mayetiola destructor* (Say), is a destructive pest of wheat and is widely distributed throughout most wheat-growing regions of Europe, North Africa, Asia and North America. A recent review of Hessian fly distribution, biology and ecology, host range, crop damage, losses and control methods was published by Ratcliffe and Hatchett (1997). Yield losses up to 35% have been ascribed to this pest in semiarid Morocco and in the South of the Iberian Peninsula (Amri et al. 1992; Arias and Bote 1992; Del Moral et al. 1994). Even if effective insecticide treatments exist, the most economical and environmentally friendly way for Hessian fly control remains the development of resistant cultivars. The biological interaction between *Triticum* spp. and Hessian fly is highly specific, with a gene-for-gene relationship between resistance genes (R) in wheat and avirulence genes (Avr) of the Hessian fly biotypes (Hatchett and Gallun 1970) that determine the virulence evolution of the insect. A number of resistance sources toward *M. destructor* were reported in the last few years and were used to obtain resistant wheats from current commercial cultivars in various breeding programmes. Twenty nine genes conditioning resistance to this pest were identified in *Triticum* species, to which the gene symbols *H*1 through *H*29 were assigned (McIntosh et al. 1998). All these genes, except h4, are dominant or partially dominant. However, the continuous evolution of virulent biotypes makes the identification of new resistance genes (RGs) from diverse origins necessary for wheat breeding. Although the optimum strategy to adopt in breeding for resistance to Hessian fly is still an open question, usually the single-gene method is selected. Sequential or simultaneous release of pure cultivars, each one with a single and different RG, was proposed by Cox and Hatchett (1986), whereas Gould (1986) suggested the accumulation of as many RGs as possible into individual cultivars (pyramiding) for a more durable resistance.

The identification of genetic resistance to Hessian fly in hexaploid and tetraploid wheats has been highly success-

ful, and also several RGs (*H*13, *H*22, *H*23 and *H*26) have been transferred from *Aegilops tauschii* to the D chromosomes of bread wheat (Gill et al. 1987; Raupp et al. 1993; Cox and Hatchett 1994). Several diploid and tetraploid *Aegilops* species belonging to the section Cylindropyrum (C genome) and Polylides (U genome) including *Aegilops triuncialis* (UC genomes) showed a resistance reaction to Hessian fly (Gill et al. 1985; El Bouhssini et al. 1998). The wild grass *Ae. triuncialis* is an important source of diseases and pest resistance genes for cultivated wheat improvement. A cereal cyst nematode (CCN) resistance gene (*Cre*7) was transferred from *Ae. triuncialis* to the TR-353 wheat line (Romero et al. 1998). *Ae. triuncialis* is an allotetraploid species whose UC genomes are homoeologous to those of *Triticum aestivum* (AABBDD). It is known as the ability conferred by a gene(s) on the C genome, to suppress the Ph diploidization mechanism of *T. aestivum* and *Triticum turgidum*, which normally prevents homoeologous pairing and recombination in polyploid wheats and their hybrids (Sears 1976). Previous work from our laboratory has shown pairing among chromosomes of the U and C genomes of *Ae. triuncialis* A-1 and those of *T. aestivum* H-10-15 (Romero et al. 1998).

When interspecific hybridization between the donor and recipient species is used as a transfer strategy, the introgression of resistance genes from alien species into breeding material often results in a dramatically reduced agronomic performance. To get rid of the negative traits of the donor plant, the progeny is backcrossed several times with the elite breeding line. This is a time-consuming, and not always successful, strategy. Biochemical markers linked to the RGs can improve the efficiency selection through such backcrosses, and the use of MAS (marker-assisted selection) to pyramid major resistance genes in wheat is nowadays largely applied. DNA markers associated with *H*3, *H*5, *H*6, *H*9–*H*17, *H*21 and *H*23–*H*25 wheat resistance genes were identified (Ma et al. 1993; Ohm et al. 1995; Dweikat et al. 1997; Seo et al. 1997; Yencho et al. 2000). The *Acph*-M^v1 isozyme marker from the $4M^v$ chromosome of *Ae. ventricosa* was found to be linked to the *H*27 gene in the wheat/*Ae. ventricosa* introgression line, H-93-33 (Delibes et al. 1997).

Our objective in the present work was to establish the potential usefulness of *Ae. triuncialis* as a new source for Hessian fly resistance in wheat breeding. We present evidence on the introgression of Hessian fly resistance from *Ae. triuncialis* to wheat lines (TR-353, TR-3531 and backcrossed lines) and of its inheritance as a single Mendelian factor (*H*30), non-allelic with respect to the *H*3, *H*6, *H*9, *H*11, *H*12, *H*13, *H*18, *H*21 and *H*27 genes. The resistance was transferred to wheat lines together with the *Acph*-U1 associated marker of *Ae. triuncialis*.

Materials and methods

Biological materials

The TR lines derived from the cross [(*T. turgidum* ssp. *turgidum* cv Rubroatrum, H-1-1 × *Ae. triuncialis* A-1) × *T. aestivum* cv Almatense, H-10-15] have been described previously (Delibes et al. 1988; Romero et al. 1998). Hereafter the abbreviations *T. turgidum* H-1-1, *Ae. triuncialis* A-1 and *T. aestivum* H-10–15 will be used. One of the TR lines (TR-353, with 41 chromosomes) was employed as a donor to obtain Hessian fly resistant lines in a hexaploid wheat background using different commercial wheats (*T. aestivum* cvs Anza, Betres, Cajeme, Cartaya, Marius, Osona and Rinconada) as recurrent parents. Several advanced lines, carriers of the resistance from *Ae. triuncialis* A-1, were tested for agronomic performance, in a Hessian fly infested field in the SW of Spain. Some traits such as yield (grams/m^2), spike fertility (kernels/spikelet) and kernel weight (thousand kernel weight) were evaluated. Sixteen entries were tested in a randomized block design with four replications; the experimental plot was 0.35 m × 0.35 m. In the trial, three wheat cultivars, used in this wheat-growing area, were employed as checks.

The 4D/$4M^v$ wheat/*Ae. ventricosa* substitution line, H-93-33 (resistant to Hessian fly), derived from the cross [(*T. turgidum* H-1-1 × *Ae. ventricosa* AP-1) × *T. aestivum* H-10-15] was described previously (Mena et al. 1989, 1993; Delibes et al. 1997). Hexaploid wheat (*T. aestivum*) cultivars from ''The Uniform Hessian fly Nursery'' (UHFN), with resistance to different biotypes of *M. destructor*, were kindly supplied by Dr. Bockelman and F. Maas from USDA-ARS. The disomic addition lines *T. aestivum* cv Chinese Spring/*Aegilops umbellulata* and *T. aestivum* cv Alcedo/*Aegilops caudata*, and their corresponding parents and amphiploids, were a gift of Dr. Raupp from the ''Wheat Genetics Resource Center'' (Kansas University, USA). The 3U and 5C addition lines are not available. The *Aegilops* species from Cylindropyrum (C genome) and Polylides (U genome) sections were from the collection at INIA (Madrid, Spain), kindly provided by Dr. E. Sánchez-Monge Parellada.

The crosses were carried out in a greenhouse by standard manual procedures, obtaining two generations per year. Plants of the F_1 generation were allowed to self-pollinate inside glassine bags to obtain the F_2 generation. The genetic control of the resistance from *Ae. triuncialis* A-1 was studied in the F_2 generation from the cross (TR-3531 × H-10-15). Bread wheat cultivars, carriers of different resistance genes (RGs), including Monon (*H*3), Howell (*H*3), Abe (*H*5), Caldwell (*H*6), Ella (*H*9), Kay (*H*11), 841453 H15 (*H*12), 86925.RA1-16 (*H*13), Brule (*H*18), KS86 HF012 (*H*21) and the H-93-33 line (*H*27), were crossed as females with the TR-3531 resistant line, in order to verify if the new resistance gene, described here, was allelic with any of the previously identified genes.

Tests for resistance to Hessian fly

A screening for Hessian fly resistance of TR lines and their parents, UHFN cultivars, the *Aegilops* set, wheat/*Aegilops* addition lines, their parents and amphiploids was performed in naturally infested field in Azuaga (SW of Spain) as described in Delibes et al. (1997). Similarly, tests for allelism among UHFN cultivars, carrying different Hessian fly RGs, and the TR-3531 line were carried out by planting individual F_1 and F_2 plants into plastic cylinders (6-cm diameter, 20-cm high) buried in the same soil as before. A visual inspection of the pupae inside the leaf sheath was conducted as described by Delibes et al. (1997). Studies about the genetic control of the resistance trait in the TR-3531 line, the linkage to the *Acph*-U1 marker, as well as the putative allelism between the gene(s) conferring resistance in the TR-3531 and H-93-33 introgression lines, were conducted in the greenhouse, using a heterogeneous Hessian fly population collected from the same experimental station. Individual plants were sown in a plastic tunnel in standard greenhouse trays as described by Delibes et al. (1997).

Scores were expressed as the number of pupae per plant. The χ^2 tests were conducted on F_2 data to determine the goodness of fit to the hypothesised ratios. Data of agronomic traits from advanced lines were analyzed with an analysis of variance for a randomized block design, and means were separated using the least significant difference (LSD) test.

Linkage analysis

Linkage between resistance to Hessian fly and a phosphatase isozyme marker was determined by the analysis of individual (TR-3531 × H-10-15)F_2 plants. The F_2 kernels were cut transversally and the embryo halves were used for the resistance test, while the distal halves were used for biochemical analysis. Acid phosphatase isozymes (ACPH-1) were extracted, fractionated and stained as described by Delibes et al. (1997).

Results and discussion

Hessian fly resistance to the Spanish biotype

Nine TR lines, obtained by seven rounds of selfing from the cross [(*T. turgidum* H-1-1 × *Ae. triuncialis* A-1) × *T. aestivum* H-10-15], were screened for Hessian fly resistance in a naturally infested field. In addition to the TR lines and their parents, the susceptible wheat cv Adalid was also included as a control in the test, whose results are summarised in Fig. 1. The TR lines 5 and 9 exhibited little or no infestation, while other TR lines showed a low number of pupae, below that of the *T. aestivum* H-10-15 parent, but in the range of that of *T. turgidum* H-1-1. The TR-9 line (also named TR-353), with 41 chromosomes, was selected for further studies because of its better performance in the tests for resistance in all the selfing rounds. This line was subjected to four additional self-fertilisations and selection for resistance, to derive a stable resistant line with 42 chromosomes (TR-3531).

In the same conditions, *Aegilops* species belonging to the section Cylindropyrum and Polylides were evaluated for resistance. All the diploid and tetraploid *Aegilops* species appeared uninfested as is shown in the upper part of Table 1. Previous works have described resistance against other Hessian fly biotypes, in *Aegilops* species carrying U and C genomes. El Bouhssini et al. (1998) found resistance against the Moroccan biotype in several accessions of *Ae. triuncialis, Ae. neglecta, Ae. geniculata, Ae. cylindrica* and *Ae. caudata*; however, the two accessions of *Ae. umbellulata* tested were susceptible. Diploid, including *Ae. umbellulata*, and tetraploid *Aegilops* species containing U and C genomes were also resistant against the biotype D from the USA (Gill et al. 1985). The wheat parents (*T. aestivum* cvs Chinese Spring and Alcedo) used to obtain the disomic addition lines with the U and C chromosomes, respectively, showed a low infestation level, in comparison to the susceptible control cv Adalid (Table 1). Neither in the available addition lines Chinese spring/*Ae. umbellulata* and Alcedo/*Ae. caudata*, nor in the corresponding amphiploids, was found full resistance as in their *Aegilops* parents (0 pupae). This result could be explained by (1) Rearrangements or losses of genetic material generated during the production of addition lines and amphiploids (Friebe et al. 1995; Schubert and Blüthner 1995). (2) The expression of RGs may be modified o suppressed in a new wheat genetic background (Kerber 1983). It is also known that the expression of resistance is reduced when RGs are transferred from lower to a higher ploidy level (Gill et al. 1986; Hanušová et al. 1996) and, in our case, the resistance was transferred from the diploid to octoploid (amphiploids) and hexaploid (addition lines) level. (3) Absence of Hessian fly RGs in the tested addition lines (3U and 5C are not available). This does not explain the susceptibility of the two amphiploids.

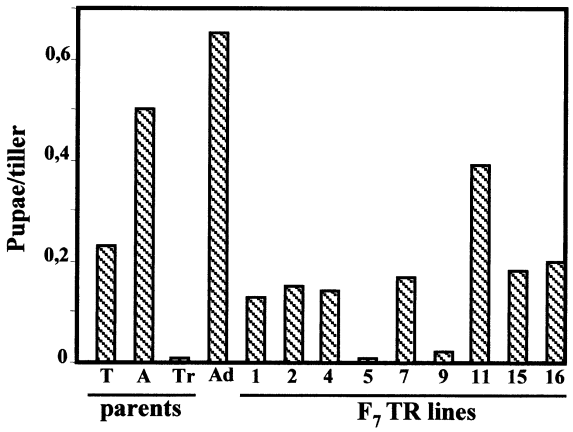

Fig. 1 Susceptibility evaluation, on a naturally infested field, to Hessian fly *M. destructor* of the offspring of nine F_7 TR lines (1, 2, 4, 5, 7, 9, 11, 15 and 16) derived from the cross [(*T. turgidum* × *Ae. triuncialis*) × *T. aestivum*], their parents [*T. turgidum* H-1-1 (*T*), *Ae. triuncialis* A-1 (*Tr*) and *T. aestivum* H-10-15 (*A*)] and the cultivar Adalid (*Ad*) as the susceptible control. The average of 15 plants per stock is shown, except for the progeny of the TR lines F_7 4 and F_7 5, because most of the plants died in the field in both cases

Although, the 4U and 6C addition lines showed the lowest number of infested tillers, the differences with respect to their parents, amphiploids and the other available addition lines were not statistically significant.

Therefore, from all the data shown in Table 1, it was not possible to determine whether the resistance transferred from *Ae. triuncialis* A-1 to the TR lines came from C, U or both genomes, or to determine the chromosome carrying the Hessian fly resistance.

Inheritance of resistance transferred from *Ae. triuncialis*

One F_2 population (93 plants) obtained from the cross between the resistant line TR-3531 and its parent *T. aestivum* H-10-15 (susceptible) was used, in the greenhouse, to study the segregation of the resistance trait (Fig. 2A). Using as a discrimination limit between resistance (R) and susceptibility (S) the lower limit of the confidence interval of the mean (P = 99%) for the susceptible con-

Table 1 Infestation degree by the Hessian fly of different *Aegilops* carrying the U and C genomes, of their amphiploids with wheat, as well as of single chromosome addition lines

Genotypes			Genomes	Hessian-fly reaction	
				Tested tillers	% Infested tillers
Aegilops	Cylindropyrum section	*Ae. caudata*	C	100	0
		Ae. cylindrica	DC	100	0
	Polylides section	*Ae. umbellulata*	U	100	0
		Ae. geniculata	UM^o	100	0
		Ae. neglecta	UM^t	100	0
		Ae. columnaris	UM^c	100	0
		Ae. biuncialis	UM^b	100	0
		Ae. variabilis	US^1	100	0
		Ae. Kotschyi	US^1	100	0
T. aestivum	cv Adalid		ABD	100	80
	cv Chinese Spring			100	4
	cv Alcedo			100	10
Ae. umbellulata/Chinese Spring	Addition lines	1U	ABD+1U	74	8
		2U	ABD+2U	38	8
		4U	ABD+4U	100	3
		5U	ABD+5U	100	6
		6U	ABD+6U	100	12
		7U	ABD+7U	100	6
	Amphiploid		ABDU	100	9
Ae. caudata/Alcedo	Addition lines	1C	ABD+1C	59	10
		2C	ABD+2C	71	24
		3C	ABD+3C	100	24
		4C	ABD+4C	93	21
		6C	ABD+6C	100	5
		7C	ABD+7C	100	23
	Amphiploid		ABDC	100	5

trol *T. aestivum* H-10-15, all the F_1 plants were resistant and the F_2 distribution fitted to a 3R:1S ratio [$\chi^2_{1:df} = 1.89$ ($0.1 < P < 0.2$)]. This result indicates the presence in the TR-3531 line of a single dominant gene responsible for the resistance observed, which we propose to nominate as *H*30. In order to ascertain the resistance of each F_2 plant, F_3 progeny, obtained in the same conditions, were analysed. Segregation ratios of F_3 plants and derived advanced lines also supported the single-factor resistance hypothesis. In order to know if the genes *H*27 in H-93-33 and *H*30 in TR-3531 were allelic, one F_2 population (55 plants) derived from the cross between the introgression resistant line H-93-33, carrying the *H*27 gene from *Ae. ventricosa*, and the TR-3531 line was tested for resistance in the same greenhouse conditions. Using the same criterion, some susceptible plants were found in the segregation of the resistance trait of the F_2 progeny from this cross (Fig. 2B). Therefore, the hypothesis that *H*27 and *H*30 genes are allelic in the H-93-33 and TR-3531 lines, must be rejected. The 50R:5S proportion of F_2 plants obtained, fitted to a 15R:1S ratio [$\chi^2_{1:df} = 0.76$ ($0.3 < P < 0.5$)] and, in consequence, confirms the presence of one dominant gene conferring resistance to the Hessian fly in each introgression line.

F_2 progeny, derived from crosses between different wheat cultivars, carrying other resistance sources, and TR-3531, were also tested for resistance under field conditions, in order to know if the new resistance gene was allelic with the *H*3, *H*5, *H*6, *H*9, *H*11, *H*12, *H*13, *H*18, and *H*21 genes. Although the cultivars from UHFN are

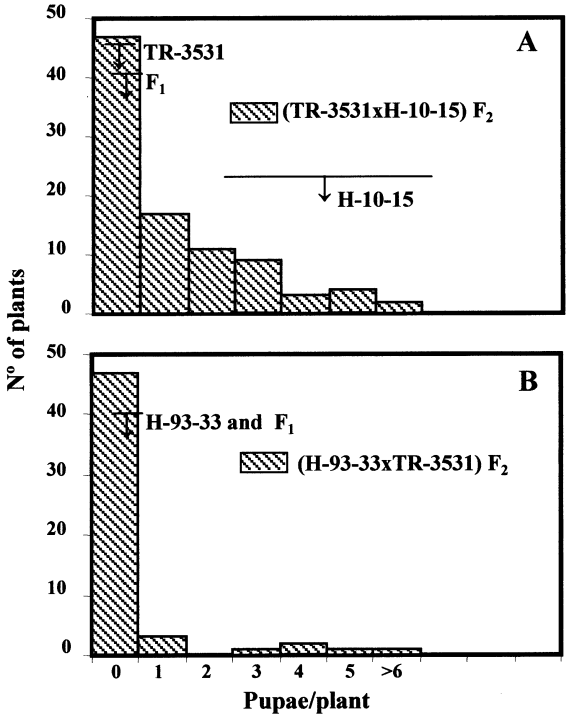

Fig. 2 Distribution of Hessian fly infestation under greenhouse conditions of **A**: (TR-3531 × H-10-15)F_2 (93 plants) and **B**: (H-93-33 × TR-3531)F_2 (55 plants). In the upper part of these panels the average (*vertical arrow*) and the 99% confidence interval (*horizontal line*) is shown for TR-3531, H-10-15 and H-93-33 (15 plants of each parent); (TR-3531 × H-10-15) F_1 and (H-93-33 × TR-3531) F_1 (six plants of each cross)

Table 2 Hessian fly reactions of parents, F_1 and F_2 populations from crosses between wheats with different resistance genes and the resistant line TR-3531, carrier of the *H*30 gene

Cultivar (cv) UHFN	Gene	Chromosome	Hessian fly reaction				
			cv UHFN R:S[b]	Crosses ♀ cv UHFN × ♂ TR-3531[a]			
				No. F_1 plants R:S	No. F_2 plants R:S	Chi-square$_{(1:df)}$ 15:1 ratio	
						Value	Probability (*P*)
Howell	*H*3	5A	18:0	7:0	154:8	0.46	0.5
Monon	*H*3	5A	19:0	9:0	187:6	3.25	$0.05 < P < 0.1$
Caldwell	*H*6	5A	29:0	8:1	218:14	0.02	0.9
Ella	*H*9	5A	20:0	10:0	178:16	1.32	$0.2 < P < 0.3$
Kay	*H*11	1A	39:0	10:0	230:9	2.52	$0.1 < P < 0.2$
841453 H15-1-1-2-5-2	*H*12	5A	26:1	2:0	95:1	4.45	$0.01 < P < 0.05$
86925 RA1-16	*H*13	6DL	18:2	3:0	140:7	0.55	$0.3 < P < 0.5$
Brule	*H*18	–	15:4	7:0	141:2	5.74	$0.01 < P < 0.05$
KS86 HF012-23-6	*H*21	2BS	19:0	7:0	243:6	6.27	$0.01 < P < 0.05$

[a] TR-3531 Hessian fly reaction; 25R:0S
[b] R=resistant and S=susceptible to Hessian fly. cv = cultivar. UHFN = Uniform Hessian Fly Nursery

effective against Hessian fly in the United States, there is no evidence that the selected genes confer resistance to the biotype present in Azuaga (SW of Spain). The results, summarised in Table 2, showed that all the UHFN cultivars tested, carrying different genes, were resistant to this biotype, except the cv Abe, with the gene *H*5, which showed an inconsistent reaction. This result agrees with Ratanatham and Gallun (1986), which revealed that the expression of the *H*5 gene in Abe, against different biotypes, is reduced when this cultivar is grown at high temperatures. In the field tests carried out in the SW of Spain over 30 °C is not an unusual temperature. As a consequence, the segregation data obtained from the cross (Abe × TR-3531)F_2 were not included in Table 2.

In the test for allelism, the F_1 and F_2 plants of all crosses were classified as resistant or susceptible using as discrimination limit the 20% of the maximum infestation level within the segregating population, which is the most common criterion used in crosses between resistant cultivars. All the F_1, as well as the most of the F_2 plants, showed a resistance level similar to those of their resistant parents, but in each cross there were a few F_2 susceptible plants. The classification of the F_2 plants, in most of the crosses, was consistent with the independent segregation of two dominant genes, fitting a 15R:1S ratio (see Table 2). The shift towards the resistance of several F_2 distributions could be explained in terms of infestation failure, or by the presence of an additional RG to the Spanish biotype in some of the parents used as female (cv UHFN). The resistance, in all UHFN cultivars, was conferred by dominant or partially dominant genes, therefore these results support the hypothesis the presence of two different loci, with two alleles in each cross. So, the resistance in the TR-3531 line, would be determined by one different locus with respect to those of the genes *H*3, *H*6, *H*9 and *H*12 (on chromosome 5A), *H*11 (on chromosome 1A), *H*13 (on chromosome 6DL), *H*18 and *H*21 (on chromosome 2BS). The high proportion of F_2 plants from all crosses that appeared with null infestation (data no shown) could suggest that they could be carriers of two resistance genes, one from each parent. Pyramiding different resistance genes into a genotype is one way of achieving breeding durable resistance to pathogens and pests (Pedersen and Leath 1988; Keller et al. 2000).

Co-segregation of Hessian fly resistance and the *Acph*-U1 marker in the TR-3531 line

The search of closely linked markers to genes of interest is useful in wheat breeding programs, especially for backcross assisted selection. Resistant plants can be selected without the requirement of culture and the inoculation process. A phosphatase marker, resolved into two components, is present in the TR-3531 line, *Ae. triuncialis* (UC), *Ae. umbellulata* (U), amphiploid Chinese Spring/*Ae. umbellulata* (ABDU) and is absent in *Ae. caudata* (C), as is shown in Fig. 3A. Therefore, this marker, pointed out by full arrowheads in Fig. 3, would be associated with the U genome, although it was absent in all the available Chinese Spring/*Ae. umbellulata* addition lines (data not shown). This absence could be due to what was present in the untested 3U addition line, or by losses of genetic material in the production of addition lines. The *Acph*-D1 component (pointed out by empty arrowheads in Fig. 3), which has previously been located in chromosome 4D of *T. aestivum* H-10-15 (Delibes et al. 1997), was absent in the TR-3531 line. On the other hand, Benito et al. (1987) described structural genes for phosphatases in the E chromosome of *Ae. umbellulata*, which is partially homoeologous to the wheat chromosomes of groups 7 and 4. For all these reasons, the new marker, named *Acph*-U1, was provisionally associated with the 4U chromosome of *Ae. triuncialis*. This chromosome could be different from that of *Ae. umbellulata*,

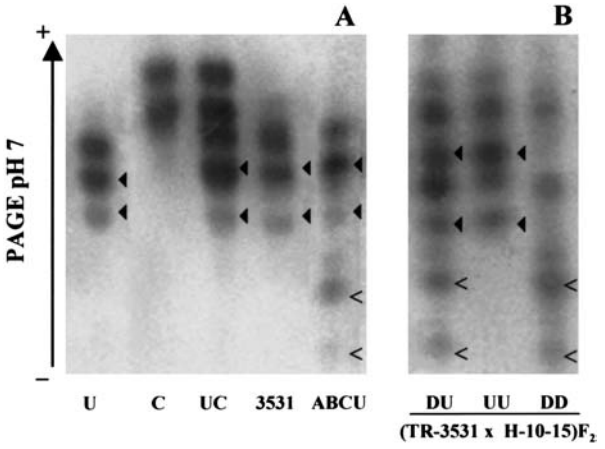

Fig. 3A, B Phosphatase zymogram obtained following the procedure described by Delibes et al. (1997). **A**: *Ae. umbellulata* (*U*), *Ae. caudata* (*C*), *Ae. triuncialis* (*UC*), the TR-3531 line (*3531*) and the amphiploid *T. aestivum* cv Chinese Spring/*Ae. umbellulata* (ABCU). **B**: The three different patterns obtained by the (TR-3531 × H-10-15)F_2. *Full and empty arrowheads* show the *Acph*-U1 and *Acph*-D1 isoforms, respectively

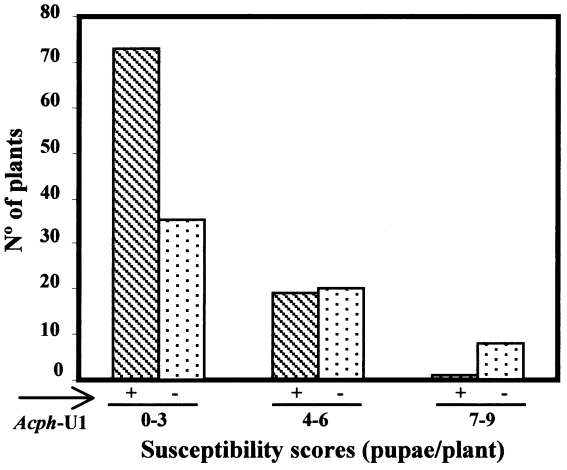

Fig. 4 Distribution of the susceptibility scores (pupae/plant) in F_2 individual plants from the (TR-3531 × H-10-15) cross with (1 or 2 doses) and without (0 doses) the *Acph*-U1 marker. The genotypes were established by electrophoresis analysis of 126 F_2 kernels. The embryo halves were sown in the greenhouse and tested for Hessian fly resistance. The plants obtained were divided into three categories according to their susceptibility scores. Isozyme ACPH was analysed in each F_2 half kernel without the embryo. Two different classes were established: **+**(*Acph*-U1/*Acph*-U1 and *Acph*-U1/*Acph*-D1, *shaded bars*); and **–** (*Acph*-D1/*Acph*-D1, *dotted bars*)

although Kimber and Yen (1989) demonstrated that the U genomes of both *Aegilops* species are very closely related.

Linkage between Hessian fly resistance and the putative *Acph*-U1 marker from chromosome 4U was determined by the analysis of 126 individual (TR-3531 × H-10-15) F_2 plants. The kernels were cut transversally and the halves without embryos were used to obtain phosphatase zymograms (ACPH). Three different electrophoretic patterns (DU, UU and DD) were observed in F_2 plants, as is shown in Fig. 3B. It was not always possible to distinguish between hemizygous (*Acph*-U1/*Acph*-D1) and homozygous (*Acph*-U1/*Acph*-U1) types, so only two classes of F_2 plants (with and without the marker) were established. The evidence for linkage is presented in Fig. 4 and the likelihood ratio test was carried out (Sokal and Rohlf 1981). The G-test of the independence value was $14.08 \gg \chi^2_{1:df;\ 0.05:P} = 3.84$ and, consequently, the null hypothesis of independence between Hessian fly resistance and the *Acph*-U1 marker must be rejected. This result suggests that the TR-3531 line carries a putative 4U chromosome segment with both genes, *Acph*-U1 and *H*30, into the 4D chromosome. With regard to this subject, we would like to emphasise that the 4U addition line showed the lowest % of infested tillers among the available U addition lines. The linkage in this cross is not very tight, indicating that the introgressed *Ae. triuncialis* A-1 segment in the TR-3531 line is relatively large. The absence of the whole chromosomes of the U and C genomes in the TR-3531 line, detected in our laboratory by in situ hybridisation and confirmed by isozyme markers (unpublished data) together with the above result, would be consistent with the homoeologous recombination expected by the ability of the C genome to suppress the Ph diploidization mechanism of wheat (Sears 1976; Romero et al. 1998).

Transfer of resistance to commercial wheat

Introduction of the resistance from line TR-353 into commercial wheat was carried out by backcrossing and selection in the greenhouse. The crosses of the TR-353 line (41 chromosomes) with several wheat cultivars and breeding lines showed that it is possible to produce a sufficient number of viable and fertile progeny for efficient gene transfer. Several advanced lines were obtained using the TR-353 line as a donor and different commercial wheats (*T. aestivum* cvs Anza, Betres, Cajeme, Cartaya, Marius, Rinconada and Osona) as recurrent parents. In all advanced lines the infestation level was higher, but in the same range than the donor. Several agronomic characteristics were studied in 16 advanced lines and the results of three of them are summarised in Table 3. The best results were achieved with the Ma-6 line, which displayed good agronomic characteristics, in comparison to the susceptible controls, in the three traits studied. The other fact that increases the importance of this line is that it also carries the CCN resistance gene *Cre*7. However, the *Cre*7 gene was absent in some of the 16 advanced lines, indicating that two different genes confer resistance to the two pathogens.

On the basis of the current knowledge of genetic relationships among wheat genes for resistance to the Hessian fly, *Ae. triuncialis* appears to be a new source of

Table 3 Agronomic characteristics of three advanced lines with the *H*30 resistance gene in comparison to three bread wheat cultivars Ma: advanced lines; TR: TR-353 line; AZ: Anza; BT: Betres; CJ: Cajeme; CY: Cartaya; MA: Marius; OS: Osona; RN: Rinconada; ⊗: selfing

Cultivar or line	Yield (g/m^2)	Kernels/spikelet	1,000 kernels weight (g)
T. aestivum cv Osona	1,240.57	2.71	29.49
T. aestivum cv Astral	897.55	1.64	30.17
T. aestivum cv Adalid	1,687.51	2.65	33.14
Ma6: TR/OS⊗//OS/3/RN/4/OS/5/RN/6/AZ 3⊗	1,914.12	3.00	43.37
Ma4: TR/BT⊗//AL/3/MA/4/3*BT 2⊗	1,336.08	2.84	27.65
Ma3: TR/3*OS//4*CY⊗/3/CJ 4⊗	1,486.86	2.64	32.50
Least significant difference ($LSD_{P<0.05}$)	409.16	0.47	5.05

resistance that can be readily transferred to hexaploid wheat, and provides an opportunity to study the effectiveness of pyramiding genes for resistance to the Hessian fly.

Acknowledgements We thank C. Martínez, A. Mejías, M. Senero, F. Pérez and L. Barragán for their technical assistance. This work was supported by grants AGl2001-3824-CO4 and PTR95-0496-OP from the Comision Interministerial de Ciencia y Tecnología of Spain.

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