Transfer of resistance to eyespot disease from Aegilops ventricosa to wheat

F.García-Olmedo, Angeles Delibes and Rosa Sánchez-Monge
E.T.S. Ingenieros Agrónomos
Universidad Politécnica de Madrid

Introduction

The extraspecific transfer of genes into cultivated plants is of considerable interest both in basic and applied terms (Sears, 1956; Riley and Kimber 1966). The wild grass Aegilops ventricosa has been recognised for almost 30 years as an important potential donor of genes that determine characters of agronomic interest. More specifically, Sprague (1936) discovered in this species a high level of resistance to the fungus Pseudocercosporella herpotrichoides, which causes eyespot disease. This disease is responsible for considerable lodging and reductions of yield in extensive areas of wheat cultivation. The level of resistance of wheat cultivars is too low, even among the less susceptible ones (Capelle Desprez and Cerco) and no single genes for resistance had been described in any species. Attemps to transfer resistance from Ae.ventricosa to wheat had been only partially successful. The purpose of this paper is to review work carried out in our laboratory for the past 12 years on the transfer of genes between these two species. This work has led to the recent demonstration of a major dominant gene for resistance, which confers a high level of resistance to cultivated wheat.

Transfer scheme

The breeding scheme used in the transfer is represented in Fig.1. Triticum turgidum H-1-1 (genomes AABB), used as the "bridge" species, was crossed with the donor (Ae.ventricosa, $D^{v}D^{v}M^{v}M^{v}$) and the resulting hybrid, which was male sterile, was rescued with polen from the recipient species (T.aestivum, AABBDD). Seeds obtained from these crosses were selfed repeatedly until a wide range of stable morphological types had been obtained (Delibes and García-Olmedo, 1973; Delibes, Sanchez-Monge and García-Olmedo, 1977). After stability had been achie-

ved, somatic chromosome numbers were counted in the root tips of germinating seeds, stained by the Feulgen procedure. Most of the lines had the euploid number of chromosomes (2n = 42) at that stage.

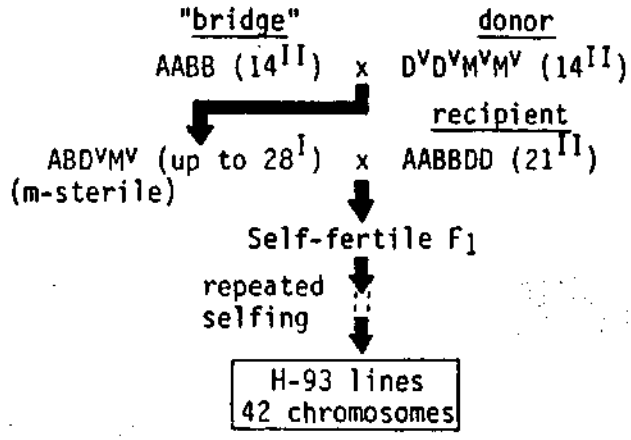

Figure 1. Breeding scheme

Transfer of genes encoding biochemical markers of genomes D^V and M^V from Aegilops ventricosa

Stable lines (H-93-1 to H-93-70), obtained as indicated, were used to investigate the transfer of a variety of markers (Delibes, Sanchez-Monge and García-Olmedo, 1977). Possible chromosomal locations in the genitors of the H-93- lines of the different types of genetic markers are represented in Fig.2: a) represents a genetic variant that is present in the A, B, or D genomes of only one of the genitors; b) idem, except that the marker is also present in the M^V genome; c) represents a locus which is occupied by the same allele in the A, B, or D genomes of two genitors; d) idem, except that the marker is also associated with the M^V genome; e) represents a marker that is present in more than one genitor, but in non homologous loci; and f) is a genetic marker that is only associated with the M^V genome of Ae.ventricosa.

If eggcells from the $ABDM^V$ hybrid carried the complete A, B, and D genomes, and only homologous transfers would have taken place, the expected frequencies of the different markers in the H-93 lines (42 chromosomes) would be as follows: 50% for types a and b, 100% for

types c and d, 75% for type e, and 0% for type f.

The presence of a type f marker in a line would unequivocally indicate genetic transfer from the M^V genome. Absence of a type c or d marker from a line could be due to deletion, to incomplete homology between D genome chromosomes in Ae.ventricosa and T.aestivum, or to genetic transfer from the M^V genome. Markers of types a, b or e can be useful in the characterization of the genetic marker up of a particular line, but deviations from the expected frequencies are meaningless in our case because lines H-93-1 through -70 do not represent an unbiased sample of the progeny.

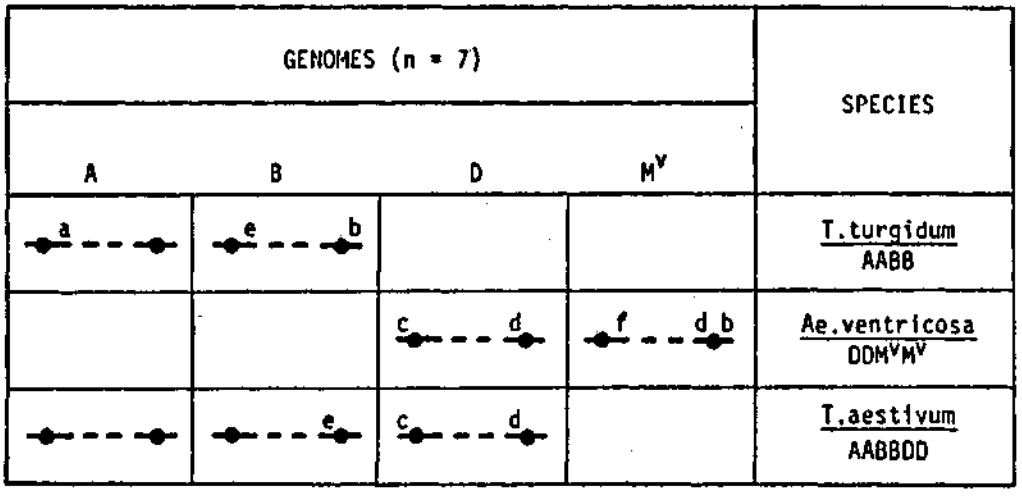

Figure 2. Types of biochemical markers used in this study

Thirteen biochemical systems, each representing a set of chemically or genetically related markers, were investigated. Their distribution in the H-93 lines and in the parental material is summarized in Table 1.

Biochemical markers controlled by the A or B genomes of one or both the wheat parents (types a, c, and d) are distributed in the H-93 lines as expected if the eggcells from the self-sterile ABD^VM^V hybrid, rescued by the ABD polen, carried the complete A and B genomes from T.turgidum. However, the distribution of D genome markers is only compatible with the presence of most of the D^V genome in most of the eggcells from the ABD^VM^V hybrid: three out of eight markers

TABLE 1. Distribution, type and genome assignment of biochemical markers

Biochemical system	Marker symbol	Distribution in genitors*						Marker type**	Genome assignment	Frequency (%) in H-93-lines
		ABD	AB	D	M^vD	M	M^u			
CM Proteins	CM1	+	-	+	+	+	+	d	D & M	93
	CM2	+	+	-	-	-	-	c	AB	100
NGE Proteins, 16, 17, 17v	NGE-16	+	+	-	-	-	-	c	AB	100
	NGE-17	+	-	+	-	-	-	a	D aest.	69
	NGE-17v	-	-	-	+	+	+	f	M^v	31
NGE Proteins, 5,6-7,14-15	NGE-5	+	-	+	+			c	D	98.5
	NGE-6-7	+	+	-	-			c	AB	100
	NGE-14-15	+	+	-	-			c	AB	100
NGE Proteins,I	NGE-I	+	-	+	+			c	D	100
NGE Proteins,II	NGE-II	+	-	+	+			c	D	98.5
U Proteins	U-1	-	-	-	+	+	+	f	M^v	4
	U-2	+	-	-	-	-	-	a	AB aest.	59
	U-3	-	-	-	+	+	+	f	M^v	0
Purothionins	α	+	+	+	+	-	-	c	B & D	α/β 2:1
	β	+	+	-	-	(+)	(+)	c	A	100 %
Gliadins	Gl-1	-	+	-	-	-	-	a	AB turg.	32
	Gl-2	+	-	-	+	-	-	c	D	75
	GL-3	+	-	-	-	-	-	a	ABD aest.	40
Athins	Ath-1-2	+	-	-	-	-	-	a	ABD aest.	72
Sterol esters	PL	+	-	+	+	+	+	d	D & M	100
Alkaline phosphatase	Aph-1-2	+	+	-	+	+	+	d	AB & M	100
	Aph-3	-	-	-	+	+	+	f	M^v	3
	Aph-5-6	+	-	+	+	+	+		D & M	100
Peroxidase	Px-a	+	-	+	+	-	-	c	D	100
	Px-m	-	-	-	+	+	+	f	M^v	0
	Px-d	+	+	-	-	-	-	c	AB	100
Esterase	Es-dv	-	-	+	+	-	-	a	D aest.	33

* ABD, T.aestivum, H-10-15; AB, T.turgidum, H-1-1; DM^v, Ae.ventricosa; D, Ae.squarrosa; M, Ae.comosa; M^u, Ae.uniaristata

** See Fig. 2

of the c or d type were present in the H-93 lines with frequencies close to but lower than the expected 100%, indicating incomplete homology between the two D genomes, non-homologous transfer, or deletion.

Biochemical characters present in Ae.ventricosa (D^VM^V), Ae.comosa (M), and Ae.uniaristata (M^U), and absent in T.aestivum (ADB), Ae.squarrosa (D) and T.turgidum (AB) were selected as M^V genome markers. Two of these markers were not transmitted to the H-93 lines, two were transmitted with low frequency and one with high frequency. The latter is NGE-17v, a marker which appears alternating with protein NGE-17, which is controlled by chromosome 4D in T.aestivum cv. Chinese Spring. This means either that the gene(s) for NGE-17v must have been located in the D^V genome of Ae.ventricosa prior to the performance of the hybridization under study, or, less probably, that the transfer took place during the meiosis of the ABD^VM^V hybrid.

It should be pointed out that in a separate study of wheat-Aegilops addition lines, in which a different accession of Ae.ventricosa was used, markers NGE-17v and Aph-3 (equivalent to Aph_V-a, -b) were associated with the same M^V chromosome (Delibes et al. 1981).

Resistance to Erysiphe graminis was determined by Doussinault at Rennes (France) and was found to be transmitted with low frequency. Furthermore, the three resistant lines are among the four lines carrying M^V genome markers (Fig. 3).

Meiotic studies

In order to further characterize the form of integration of the alien material in the H-93 lines, meiosis was studied in the following stocks: lines H-93-1, -8, -33, and -35, which carry M^V genome markers; lines H-93-10 and -51, which lack marker CM1; hybrids between all these lines and the T.aestivum H-10-15 genitor (Delibes, Sanchez-Monge and García-Olmedo, 1977). Also included in the study was line H-93-5 and its hybrid, which was not suspected of carrying non homologous transfers but was the line with the highest protein content. Meiotic observations are summarized in Table 2. Meiosis of all lines, except H-93-33, was rather regular and only the number of open bivalents was significantly higher than in the H-10-15 wheat. Line H-93-33 was signifi-

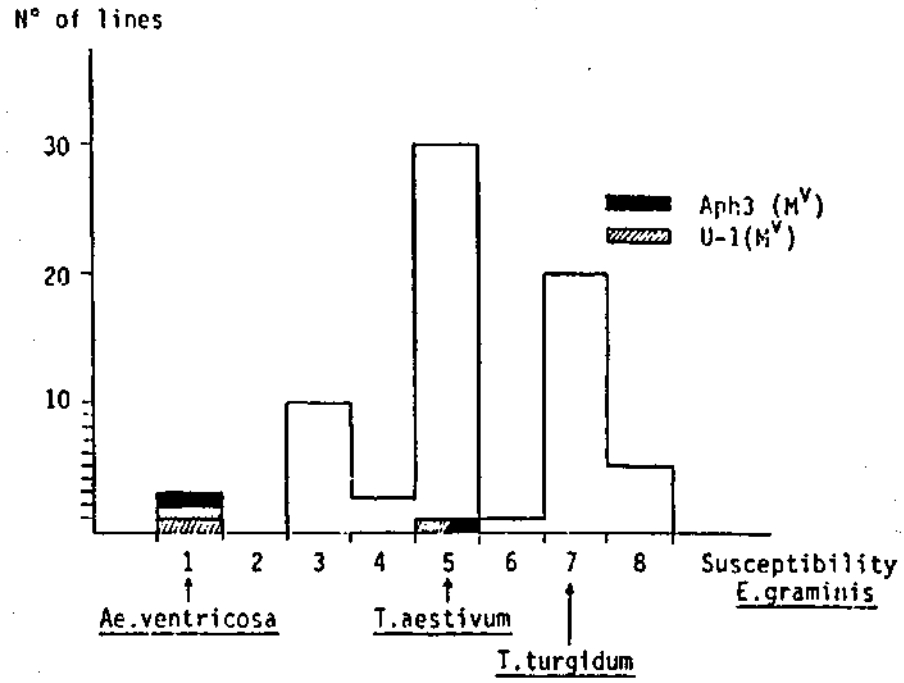

Figure 3. Susceptibility of H-93 lines to Erysiphe graminis

cantly more irregular than H-10-15 at the three meiotic phases studied. Meiosis of the hybrids involving the selected lines were significantly more irregular (2-4 univalents) than those of the corresponding genitors, except in the case of H-93-5 x H-10-15, which was quite regular.

The distribution of biochemical markers and the maximum number of chromosomes from the M^V genome in the selected lines are registered in Table 3. The latter is deduced from the number of univalents at MI observed in the hybrid and represent an overestimation for two reasons: i) because it has been repeatedly observed that there is incomplete homology between the D genomes of T.aestivum and Ae.ventricosa, and ii) because the transfer of a terminal segment of a chromosome from the M^V genome to a wheat chromosome would suffice to drastically reduce its meiotic pairing with the original wheat chromosome. It can be concluded, from the joint consideration of the biochemical and the cytological data, that the genetic transfer from the M^V genome of Ae.ventricosa to hexaploid wheat has taken place not only by chromosome substitution but also by recombination. In other words, lines carrying, at most, one whole M^V genome chromosome also carry two independently inherited M^V genome markers.

TABLE 2. Meiosis of select H-93- lines and their hybrids with T.aestivum (H-10-15)

Material	Metaphase I*		Anaphase I	%
	Univs/cell	Open bivs/cell	Laggards/cell	Micronuclei
10-15	0.04±0.04 (0-2)	2.52±0.25 (0-7)	0.16±0.10 (0-4)	0.01±0.01 (0-1)
93-1	0.28±0.10 (0-2)	4.28±0.31 (1-9)	0.14±0.06 (0-2)	0.02±0.01 (0-1)
93-1x10-15	2.92±0.27 (0-8)	5.82±0.40 (1-14)	1.70±0.30 (0-8)	0.58±0.08 (0-4)
93-5	0.00	2.70±0.54 (0-6)	0.39±0.07 (0-4)	0.55±0.08 (0-4)
93-5x10-15	0.42±0.09 (0-4)	5.14±0.38 (0-12)	0.58±0.09 (0-4)	0.43±0.13 (0-8)
93-8	0.28±0.10 (0-2)	5.60±0.35 (1-11)	0.20±0.11 (0-4)	0.01±0.01 (0-1)
93-8x10-15	4.32±0.19 (2-8)	4.12±0.29 (0-9)	2.98±0.27 (0-8)	1.01±0.09 (0-3)
93-35	0.00	4.64±0.33 (1-12)	0.10±0.06 (0-2)	0.04±0.02 (0-2)
93-35x10-15	2.56±0.22 (0-8)	4.12±0.32 (0-10)	1.10±0.17 (0-4)	0.94±0.07 (0-3)
93-10	0.56±0.15 (0-4)	3.46±0.32 (0-9)	0.32±0.08 (0-5)	0.11±0.04 (0-3)
93-10x10-15	1.86±0.15 (0-5)	4.40±0.30 (0-10)	2.19±0.15 (0-6)	0.78±0.08 (0-3)
93-33	1.00±0.18 (0-4)	4.98±0.31 (0-12)	0.59±0.09 (0-5)	0.12±0.03 (0-2)
93-33x10-15	3.74±0.21 (2-8)	6.17±0.33 (0-11)	2.90±0.17 (0-8)	0.69±0.07 (0-3)
93-51	0.00	2.72±0.28 (0-8)	0.26±0.07 (0-4)	0.02±0.01 (0-1)
(93-51x10-15	1.6 ±0.18 (0-4)	3.32±0.27 (0-8)	1.8 ±0.12 (0-6)	0.54±0.06 (0-2)

* One cell with one trivalent was observed in H-93-10 x H-10-15 and one cell with one quadrivalent in H-93-5 x H-10-15. No associations above bivalent was otherwise observed.

TABLE 3. Distribution of genetic markers and maximum number of chromosomes from the M^v genome in selected H-93 lines

Genomes	Markers	Lines H-93-					
		1	8	10	33	35	51
M^v	Aph-3	+	-	-	+	-	-
	U-1	+	+	-	-	+	-
	Pm*	-	+	-	+	+	-
D^v/D^a	NGE-3 y -4	-	-	-	+	+	-
	NG-11	-	+	+	+	+	+
D^v	NGE-17v	+	+	+	+	-	+
	Es-dv	+	+	+	-	-	-
D^A	NGE-17	-	-	-	-	+	-
	Ath-1 y -2	-	+	-	-	+	-
Maximum number of M^v chromosomes		1	2	1	2	1	1

* resistance to E.graminis

Transfer of resistance to Pseudocercosporella herpotrichoides

Resistance of the H-93 lines to the eyespot disease was tested both at the seedling and at the adult stage (Delibes et al., 1977). A high proportion of the lines did not differ significantly from Aegilops ventricosa in their level of resistance. These results strongly suggested that a single mendelian factor was involved in the transfer of resistance (Delibes et al., 1977). In order to test this hypothesis, line H-93-70 was selected because of its good performance, both as seedling and as adult plant (Doussinault et al., 1983). Reciprocal crosses (F_1 and F_2 generations) and backcrosses were obtained between this line and the recipient T.aestivum cv. Almatense H-10-15 (hereafter designated H-10-15)and tested for resistance at the seedling stage. Two types of observations were made: a quantitative one, the

number of leaf sheaths affected per plant, and a qualitative one, the type of mycelium. The pathogen, which is relatively nonspecific, produces two types of mycelium, depending on the host: on susceptible plants, the mycelium is abundant between the leaf sheaths, and is coloured black (m type); on resistant plants with Ae.ventricosa cytoplasm the mycelium is spotted and dark-brown (v type). Leaf sheaths of resistant plants are invaded more slowly than those of susceptible ones and often they are not invaded beyond the first leaf, so that no stroma can be observed. Sometimes the distinction between the two types of mycelium is not clear-cut.

Table 4. Inheritance of resistance to eyespot disease in crosses of line H-93-70 with cv. Almaténse H-10-15

Sample	Phenotypes*
H-93-70	R
H-10-15	S
F_1 H-93-70 x H-10-15	R
F_1 H-10-15 x H-93-70	R
F_2 H-93-70 x H-10-15	3R:1S
F_2 H-10-15 x H-93-70	3R:1S
Backcross (H-93-70 x H-10-15) x H-10-15	1R:1S
Backcross (H-93-70 x H-10-15) x H-93-70	R
Backcross (H-10-15 x H-93-70) x H-10-15	1R:1S
Backcross (H-10-15 x H-93-70) x H-93-70	R

* Plants were classified into resistant (R, two or less leaf sheaths penetrated) and susceptible (S, three to five leaf sheaths penetrated). The observed segregations did not differ significantly from those expected for a single dominant gene ($\chi^2 \leq \chi^2_{df=1,P=0.05} = 3.84$).

Table 4 summarizes the results of these tests. In our experimental conditions, close to 90% of the H-93-70 plants tested had two or less leaf sheaths infected, whereas a similar proportion of plants from H-10-15 had from three to five leaf sheaths infected. Both the pattern of segregation and the average number of leaf sheaths penetrated in F_1, F_2 and backcross generations indicated that resistance was inherited as though determined by a single mendelian factor (Pch1),

which showed almost complete dominance.

In F_1, F_2 and backcross generations, the proportion of plants with black mycelia (m type) was greater when the cytoplasmic genetic determinants (mitochondrial and chloroplast genomes), which are maternally inherited, were contributed by T.aestivum cv. Almatense H-10-15 than when the source was H-93-70, whose mitochondrial and chloroplast genomes were donated by T.turgidum H-1-1, the bridge species used as a female in the transfer scheme (Fig. 4). The proportions of mycelium types seem to be more variable in response to environmental conditions, or in relation to plant vigour, when the Pch1 gene is expressed in the H-10-15 cytoplasmic genetic background. This effect is less conspicuous when resistance is assessed by the number of leaves penetrated.

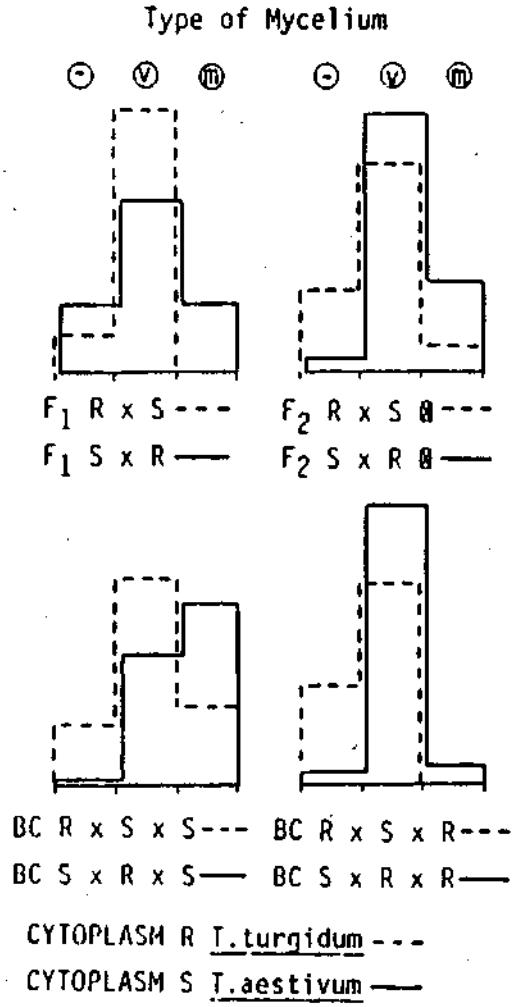

Figure 4. Effect of cytoplasm on type of mycelium observed in F_1, F_2, and backcrosses. T.turgidum H-1-1 and T.aestivum cv. Almatense H-10-15.

To investigate whether the Pch1 gene in H-93-70 was integrated in a chromosome capable of pairing and recombination with wheat chromosomes, meiosis of the hybrid H-93-70 x H-10-15 was studied and was found to be quite regular: 20.70±0.02 bivalents at metaphase I in the hybrid, versus 20.82±0.05 bivalents in H-93-70 and 20.98±0.02 bivalents in H-10-15 (at least 5 plants and 50 cells were analysed in each case; differences between the hybrid and the progenitors were not significant). These observations exclude the presence in H-93-70 of whole chromosomes or large chromosomal segments non-homologous to wheat chromosomes and indicate that the Pch1 gene can be introduced by recombination into other wheats.

It is not possible to discriminate whether the Pch1 gene has been transferred from the M^v or D^v genomes of Ae.ventricosa because, although resistance was transferred with the high frequency expected of a D^v gene, we have already shown that, as in the case of marker NGE-17v, recombination between the D^v and M^v chromosomes might have taken place either during or before the transfer.

The higher level of resistance attained in the present case, as compared with previous attempts, can be explained in terms of the monogenic nature of the resistance and of the nature of the genetic background, nuclear as well as cytoplasmic, into which the transfer was made: T.aestivum cv. Almatense H-10-15 is almost as tolerant as cv. Capelle Desprez, and T.turgidum H-1-1, the cytoplasm donor, is even more resistant.

Although, line H-93-70 should be immediately useful in converting susceptible wheat cultivars to this major disease into resistant ones, further studies are under way concerning the mode of integration and location of the Pch1 gene.

Summary

A method of genetic transfer from Ae.ventricosa into hexaploid wheat has been investigated using biochemical markers. The method involves the rescue of the sterile hybrid [T.turgidum (AB) x Ae.ventricosa (D^vM^v)] with polen from T.aestivum (ABD). After repeated selfing, lines with 42-chromosomes were selected.

Seventy F_{20} lines (H-93-1 through 70) derived as described were

analysed for 13 biochemical systems, each representing a set of up to 4 homoeologous loci. Markers of D genome chromosomes from Ae.ventricosa were transferred at the high frequencies expected for homologous recombination. Biochemical characters present in Ae.ventricosa (D^vM^v), Ae.comosa (M), and Ae.uniaristata (M^u), but absent in T.aestivum (ABD), Ae.squarrosa (D) and T.turgidum (AB) were selected as putative M^v genome markers. These markers were transmitted with a low frequency (0-4%), with the exception of a protein marker (NGE17v) that we transmitted at high frequency (31%), indicating prior transfer from the M^v to the D^v genome in Ae.ventricosa. Meiosis was studied in lines carrying M^v genome markers and in their hybrids with the T.aestivum parent and evidence was found that both whole chromosome substitutions and homoeologous recombination had taken place.

This method of gene transfer has allowed the introduction into wheat of alien characters of agronomic importance, such as resistance to Erysiphe graminis and to Pseudocercosporella herpotrichoides. The latter case is an important breakthrough as no intraspecific source of resistance is available against this fungus, which causes eyespot disease in wheat.

Grant n° PR83-2046 from the Comisión Asesora de Investigación Científica y Técnica is gratefully acknowledged.

Literature

Delibes, A., Dosba, F., Doussinault, G., Sanchez-Monge, R., and García-Olmedo, F., 1977: Resistance to eyespot (Cercosporella herpotrichoides) and distribution of biochemical markers in hexaploid lines derived from a double cross (Triticum turgidum x Aegilops ventricosa) x T.aestivum. In: Proc. 8th Congress of EUCARPIA, "Interspecific hybridization in plant breeding" 1977, Madrid. EUCARPIA. Pp. 91-97.

Delibes, A., and García-Olmedo, F., 1973: Biochemical evidence of gene transfer from the M^v genome of Aegilops ventricosa to hexaploid wheat. In: Proc. 4th Intern.Wheat Genet.Symp., 1978. Missouri. Agr. Exp.Sta., Columbia, Mo. Pp. 161-165.

Delibes, A., Otero, C., García-Olmedo, F., and Dosba, F., 1981: Biochemical markers associated with two M^v chromosomes from Aegilops ventricosa in wheat-Aegilops addition lines. Theor.Appl.Genet. 60, 5-10.

Delibes, A., Sanchez-Monge, R., and García-Olmedo, F., 1977: Biochemical and cytological studies of genetic transfer from the M^v genome of Ae.ventricosa into hexaploid wheat. A progress report. In: Proc. 8th Congress of EUCARPIA, "Interspecific hybridization in plant breeding", 1977, Madrid. EUCARPIA. Pp. 81-89.

Doussinault, G., Delibes, A., Sanchez-Monge, R., and García-Olmedo, F. 1983: Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature 303, 698-700.

Riley, R., and Kimber, G., 1966: The transfer of alien genetic variation to wheat. Rep.Pl.Breed.Inst. 1964-65: 3-36.

Sears, E.R., 1956: The transfer of leaf-rust resistance from Aegilops umbellulata to wheat. Brookhaven Symposia in Biology 9, 1-22

Sprague, R., 1936: Relative susceptibility of certain species of gramineae to Cercosporella herpotrichoides. J.Agric.Res. 53, 659-670.

Address of authors:

Departamento de Bioquímica
E.T.S.Ingenieros Agrónomos, UPM
Ciudad Universitaria
Madrid-3, Spain