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*

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SUMMARY

There are not good intraspecific sources of resistance to the eyespot disease of wheat, caused by Cercosporella herpotrichoides Fron . The interspecific transfer of genes for resistance from Aegilops ventricosa into hexaploid wheat has been only partially achieved, because the degree of resistance attained is not as high as that of the donor. We report here on the transfer of resistance in a double cross (Triticum turgidum var. rubroatrum H-1-1 x Ae.ventricosa AP-1) x T.aestivum cv. Almatense H-10-15.

The high level of resistance in a high proportion of the lines strongly suggests a simple genetic control for this character (possibly by one major gene).

The gene(s) responsible for resistance in the selected lines must be associated with the D genome of Aegilops ventricosa on the basis of a detailed study of the distribution of biochemical markers in the H-93 lines.

These results do not exclude that genes with similar effects might be located in the M^v genome.

INTRODUCTION

The incorporation of genes for resistance to the eyespot disease, caused by the fungus *Cercosporella herpotrichoides* Fron. is a recognised objective in the breeding of hexaploid wheat, *Triticum aestivum* L., because present cultivated varieties are all more or less susceptible.

In 1936, Sprague (1) found a high level of resistance in *Aegilops ventricosa* and, in 1952, Simonet (2) reported a series of interspecific crosses involving this species and different wheats, and pointed to their possible use in the transfer of resistance. Maia (3) derived the variety VPM1 from *T.aestivum* cv. Marne and an amphiploid (*Ae.ventricosa* x *T.persicum*) obtained by Simonet (4). This line was more resistant than Capelle, the most resistant wheat variety known, but less resistant than the *Ae.ventricosa* genitor. At the same time, Kimber (5) published the results of a similar transfer experiment, but, in this case, the level of resistance attained was no higher than that of Capelle.

The use of VPM1 in wheat varietal improvement has been investigated (6,7) and different approaches have been proposed to obtain lines more resistant than VPM1. Some results of these studies will be reported elsewhere in these Proceedings by Dosba and Doussinault (8).

We report here on the transfer of resistance in a double cross (*T. turgidum* x *Ae. ventricosa*) x *T. aestivum*. The cross was performed in 1950 by M. Alonso Peña in Cuenca (Spain) and lines with 42 chromosomes were derived from it. These have been studied by biochemical and cytological methods (9,10).

MATERIALS AND METHODS

Biological material

The following stocks were used in this study: Lines H-93-1 through 70 (42 chromosomes), derived from a cross (*Triticum turgidum* var. rubroatrum H-1-1 x *Aegilops ventricosa* AP-1) x *T. aestivum* cv. Almatense H-10-15 and the genitors of these lines; *Ae. ventricosa* n°11 and the amphiploid (*Ae. ventricosa* n°11 x *T. aethiopicum* 1A); *T. aestivum* cvs. Capelle, Moisson, Rex and transfer line VPM-1112-R4.

Tests for resistance to the eyespot disease

Resistance at the seedling stage was measured in terms of the number of leaf-sheaths attacked per plant. Three replicates, of 50 seeds each, were sown in a plastic tunnel in order to maintain the required humidity and to slightly increase the temperature. Artificial inoculation was carried out with mycelium obtained by *in vitro* culture and ground into a powder, according to Ponchet (11).

Resistance at the adult stage was expressed in terms of the proportion of stems with less than 50% of their section attacked by the fungus. Three replicates, of 50 seeds each, were sown in the field. Inoculation was carried out by spraying the fungus previously cultivated on oat grains. Stems are cut at The base and classified into two classes (>50% and <50% cross section attacked). In these experimental conditions, when the notation is taken late in the season (end of May and June), plants with thin straw are disadvantaged.

Biochemical markers

Fourteen biochemical systems, each representing a set of related markers, as judged from chemical and/or genetic data, were investigated in the H-93- lines and their genitors as described elsewhere in this Proceedings (10).

RESULTS AND DISCUSSION

The distribution of susceptibility scores at the seedling stage of the seventy H-93 lines is represented in Fig. 1. Scores of 33 of the lines did not differ significantly from that of the *Ae. ventricosa* AP-1 genitor and were significantly lower than that of *T. turgidum* H-1-1, the most resistant of the two wheat genitors. The high level of resistance in a high proportion of the lines, as well as the shape of the distribution, strongly suggest a simple genetic control for this trait, possibly by one major gene.

To discern whether the gene(s) responsible for the enhanced resistance of these lines were originally located in the D or in the M^V genomes of *Ae. ventricosa*, consideration of the available biochemical data is warranted (10). The distribution of markers associated with the D and M^V genomes is summarized in Table 1. The following observations support the notion that the transfer of resistance has taken place, in general, from the D genome:

a) Biochemical markers from the D genome are transferred with high frequency.

b) Biochemical markers from the M^V genome are transferred with low frequen-

cy or not transferred at all.

c) The mode of inheritance of D genome markers (Table 1) is incompatible with high frequency gene transfer from the M^V genome into those chromosomes with which the markers are associated. The markers represent six different D genome chromosomes in *T.aestivum* cv. Chinese Spring. If the organization of the D genome in cv. Chinese Spring is completely homologous with that of cv. Almatense H-10-15, a matter which is being investigated at present, the above observation would exclude six M^V genome chromosomes as the potential donors of resistance genes for most of the resistant H-93 lines.

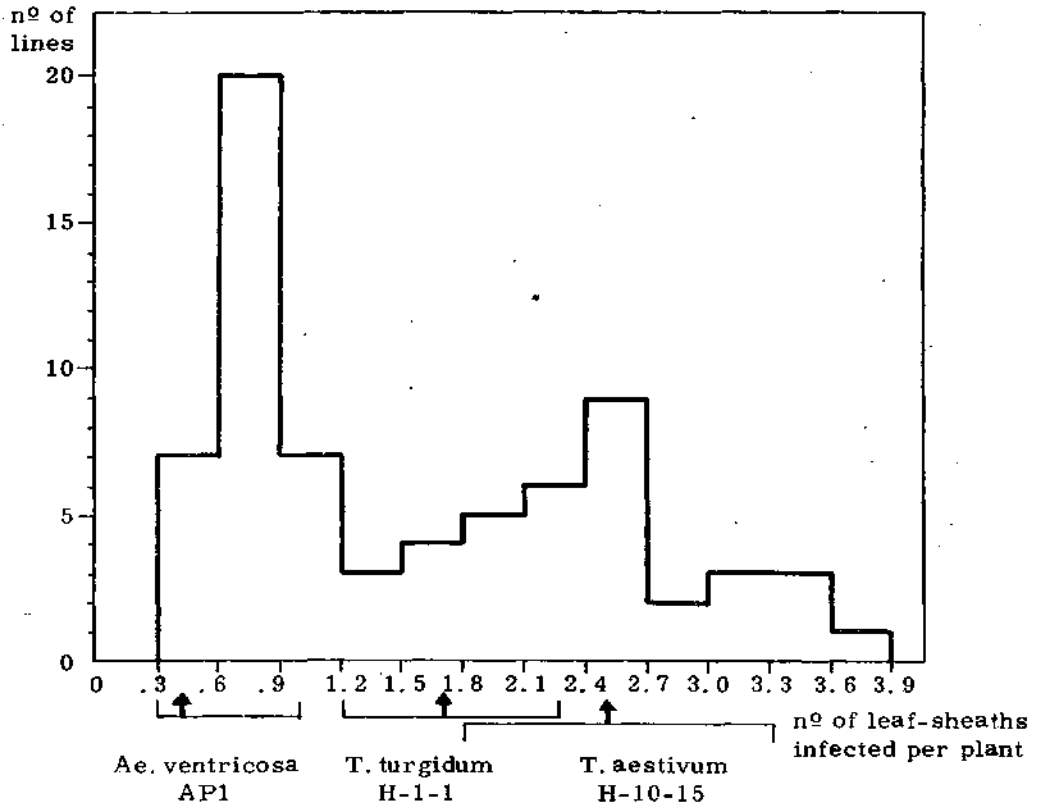

FIGURE 1. Susceptibility of H-93 lines to the eyespot disease (*Cercosporella herpotrichoides*) at the seedling stage.

d) It is reasonable to assume that the five M^V genome markers could represent at least four different chromosomes, because three of them are inherited independently, two are not transferred, and, as previously concluded (10), homoeologous recombination occurs, at best, with low frequency. Thus, these chromosomes could not be responsible for the enhanced resistance of the H-93 lines. Among the lines for which there is direct evidence of genetic transfer from the M^V genome (H-93-1, -8, -33, -35), only H-93-33 is resistant to eyespot at the

TABLE 1. Distribution of biochemical markers for the D and M^V genomes in H-93-lines.

Biochemical system	Marker symbol	Genome assignment	Frequency (%) in H-93		Other observations
			Expected	Found	
CM Proteins	CM1	D & M^V	100	93	marker absent in H-93-1, -8, -10, -22, -51
NGE Proteins, 16, 17, 17v	NGE-17	D aest.	50	69	alternatively inherited segregate independent of eyespot resistance
	NGE-17v	D vent.*	50	31	
NGE Proteins, 5,6-7,14-15	NGE-5	D	100	98.5	marker absent in H-93-3
NGE Proteins,1	NGE-1	D	100	100	
NGE Proteins,11	NGE-11	D	100	98.5	marker absent in H-93-1
Gliadins	G1-2	D	100(75)**	75	marker independent eyespot resistance
U Proteins	U-1	M^V	0	4	marker present in H-93-1, -8, -35
	U-3	M^V	0	0	
Sterol esters	PL	D & M^V	100	100	
Alkaline phosphatase	Aph-3	M^V	0	3	marker present in H-93-1, -33
	Aph-5-6	D & M^V	100	100	
Peroxidase	Px-a	D	100	100	
	Px-m	M^V	0	0	
Esterase	Es-dv	D vent.	50	33	marker independent of eyespot resistance
Alcohol dehydrogenase	Adh-μ	M^V	0	1.5	marker present in H-93-33

* See ref. 10 for a discussion of this assignment.

** The expected frequency if the locus in one genitor is non-homologous with that in the other genitor.

seedling stage.

On the basis of these considerations, it is very unlikely that the M^V genome donated the gene(s) for resistance in most of the H-93 lines. Dosba and Doussinault (8) will present evidence of gene(s) for resistance located in the M^V genome but it does not seem that such gene(s) have been generally involved in the present transfer.

Susceptibility scores at the seedling and at the adult stages of selected H-93 lines are present in Table 2. Resistance to eyespot depends on three main

TABLE 2. Susceptibility to eyespot (*Cercosporella herpotrichoides*) of selected H-93 lines at the seedling and at the adult stages.

Seedling stage		Adult stage	
Stocks	Score*	Stocks	Score**
Ae.ventricosa n°11	0.366	H-93-19	0.117
H-93-70	0.367	H-93-44	0.142
Ae.ventricosa AP1	0.404	H-93-29	0.205
H-93-30	0.509	H-93-68	0.215
H-93-20	0.521	H-93-57	0.219
H-93-58	0.525	H-93-21	0.220
H-93-19	0.534	H-93-8	0.220
H-93-21	0.570	H-93-70	0.221
H-93-45	0.570	H-93-55	0.225
H-93-14	0.603	H-93-40	0.241
H-93-54	0.668	H-93-36	0.244
H-93-31	0.669	H-93-20	0.254
H-93-55	0.671	H-92-31	0.259
H-93-32	0.703	H-93-32	0.259
H-93-18	0.708	H-93-47	0.280
H-93-23	0.711	H-93-9	0.283
H-93-41	0.722	H-93-23	0.288
H-93-38	0.764	H-93-35	0.294
H-93-49	0.772	H-93-54	0.296
H-93-17	0.780	H-93-25	0.300
H-93-68	0.782	H-93-41	0.305
H-93-44	0.782	H-93-58	0.306
H-93-33	0.790	H-93-45	0.307
H-93-4	0.802	H-93-46	0.309
VPM-1112-R4	0.808	H-93-11	0.312
H-93-57	0.830	H-93-17	0.313
H-93-65	0.877	H-93-65	0.321
H-94-46	0.881	H-93-33	0.322
H-93-47	0.896	H-93-14	0.340
H-93-26	0.896	H-93-38	0.343
H-93-9	0.914	H-93-30	0.344
H-93-25	0.926	H-93-18	0.349
H-93-36	0.965	*Ae.ventricosa* n°11	0.356
H-93-40	0.997	*Ae.ventricosa* AP1	0.373
H-93-11	0.998	H-93-49	0.399
H-93-29	1.011	VPM-1112-R4	0.399
T.turgidum H-1-1	1.713	H-93-26	0.408
T.aestivum Cappelle	2.220	H-93-4	0.449
T.aestivum H-10-15	2.540	*T.turgidum* H-1-1	0.453
T.aestivum Rex	2.584	922 (*Ae.vent.* x *T.aeth.* 1A)	0.495
T.aestivum Moisson	2.584	*T.aestivum* Cappelle	0.503
		T.aestivum Rex	0.569
		T.aestivum H-10-15	0.585
		T.aestivum Moisson	0.632
Variation coefficient	22%	Variation coefficient	24.8%

* Number of leaf-sheaths attacked per plant.

** Proportion of stems with more than 50% section attacked.

factors: i) probability of plant infection, ii) resistance of the leaf-sheaths to penetration, and iii) resistance of the stems to the attack. These factors do not seem to be entirely linked (12) and thus complementary information is acquired with the two types of tests. Lines that were resistant at the seedling stage were also resistant at the adult stage, with few exceptions. The fact that the two *Ae.ventricosa* lines tested did not perform as well at the adult stage, seems to be the result of taking the notation late in the season (end of May and June). Differences in morphology and tillering do no allow a proper comparison between the wheat lines and the Aegilops.

Law et al. (12), studying substitution lines and F_2 monosomic families of appropriate hybrids, demonstrated that more than one chromosome (gene) was involved in the differences in susceptibility between Capelle and other *T.aestivum* cultivars. They detected both positive and negative effects that were background dependent. Th- higher level of resistance attained in the present case, as compared with previous attempts (3,5), can be explained in terms of the genetic background into which the transfer was made: *T.aestivum* cv. Almatense H-10-15 is almost as resistant as Capelle and *T.turgidum* H-1-1 is even more resistant.

Dosba and Doussinault (unpublished) have shown that the differences in susceptibility between *Ae.ventricosa* n°11 and VPM-1112-R4 are greater at lower accumulated day-degrees above 0°C. Thus, further experiments are needed to confirm that the level of resistance attained in the H-93 lines is equal to that of *Ae.ventricosa* AP-1 under all conditions.

From the present data, it can be concluded that the major part of the resistance to eyespot can be simply transmitted from *Ae.ventricosa* to an appropriate hexaploid wheat background. As to the problem of how many genes might be required to attain the same level of resistance of *Ae.ventricosa*, the following citation from A. Robertson (13) is pertinent: "Because there may be many loci affecting a particular measurement to a very small extent, the question *How many genes affect this character* is rather a meaningless one, but we may ask the same kind of question in a more useful way as *Taking the loci in order of importance, how many must we include to explain, say, 80% of the difference between extreme selected lines?*". It seems that in our case the answer could be one locus.

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