Biochemical Markers Associated with Two M^V Chromosomes from *Aegilops ventricosa* in Wheat-*Aegilops* Addition Lines

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Summary. The distribution of three biochemical markers, U-1, CM-4 and Aph_v-a, -b, among wheat-*Aegilops* addition lines carrying M^V chromosomes from *Aegilops ventricosa* (genomes D^VM^V) has been investigated. Addition lines which had been previously grouped together on the basis of common non-biochemical characters carried marker U-1, a protein component from the 2M urea extract. The added chromosome, in the appropriate genetic background, seems to confer a high level of resistance to the eyespot disease, caused by the fungus *Cercosporella herpotrichoides*. The other two markers were concomitantly associated with another similarly formed group of addition lines. Both CM-4, a protein component from the chloroform:methanol extract, and Aph_v-a, -b, alkaline phosphate isozymes, have been previously shown to be associated with homoeologous chromosome group 4, which suggests that the added chromosome in the second group of addition lines is $4M^V$.

Key words: Wheat – *Aegilops ventricosa* – Addition lines – Biochemical markers

Introduction

Since Sprague (1936) discovered in *Aegilops ventricosa* (genomes D^VM^V) a high level of resistance to the fungus *Cercosporella herpotrichoides* (eyespot disease), a number of interspecific crosses involving this species have been carried out in order to transfer the genes for resistance into hexaploid wheat (*Triticum aestivum*, genomes ABD) (Simonet 1957; Maia 1967; Kimber 1967; Doussinault et al. 1974; Delibes et a. 1977; Dosba and Doussinault 1977, 1978).

Genes for eyespot resistance seem to be located in both the D^V and the M^V genomes from *Ae. ventricosa* (Dosba and Doussinault 1977). In general, genes from the D^V genome are more readily transferred through homoeologous recombination than those located in the M^V genome (Delibes et al. 1977).

Development of addition lines carrying chromosomes from the M^V genome was undertaken, both to facilitate the genetic analysis of resistance and its actual transfer to hexaploid wheat. Five different addition lines were identified on the basis of cytological, morphological and agronomical characters (Dosba et al. 1978).

The use of biochemical markers can be of help both in the identification of addition lines and in the further manipulation of these lines in breeding programmes. Delibes and García-Olmedo (1973) selected potential M^V genome markers based on their presence in accessions of *Ae. ventricosa* (D^VM^V), *Ae. comosa* (M) and *Ae. uniaristata* (M^u) and their absence in accessions from *T. aestivum* (ABD), *Ae. squarrosa* (D) and *T. turgidum* (AB). The association of these markers with M^V genome chromosomes was further investigated by their inheritance pattern in a cross (*T. turgidum* × *Ae. ventricosa*) × *T. aestivum* (Delibes et al. 1977). We report here the association of three of these biochemical markers with two M^V chromosomes.

Materials and Methods

Addition Lines

The progenitors of the addition lines were: *Aegilops ventricosa* n° 11, an accession supplied by Kihara in 1969,which is highly resistant to eyespot disease at the seedling stage; *Triticum aethiopicum* 1A, a tetraploid wheat used as bridge species, which is susceptible to eyespot; and *T. aestivum* cv. 'Moisson', a French hexaploid wheat susceptible to eyespot. Addition lines on *Aegilops* cytoplasm (v lines) were obtained by crossing (*Ae. ventricosa* n° 11 × *T. aethiopicum* 1A) × *T. aestivum* cv. 'Moisson^3', and self pollinating 5 or 6 times.

Lines on wheat cytoplasm (m lines) were derived by crossing (*T. aestivum* cv. 'Moisson' × *Ae. ventricosa* n° 11) × *T. aestivum* cv. 'Moisson^4' and self pollinating 4 or 5 times. The main charac-

teristics of these lines have been previously described (Dosba et al. 1978). In most cases, the self pollinated lines were analysed, but, when these were not available, open pollinated material was used. A total of 60 v lines and 60 m lines have been analysed since 1978.

Analytical Methods

The markers used, U-1, NGE-1, and CM-4, have been described previously (Delibes and García-Olmedo 1973; Delibes et al. 1977) and will be only summarized here: marker U-1 was analysed in the 2M urea extract (2:1 v/w) by starch gel electrophoresis (aluminium lactate buffer pH 3.2, 3M urea, 10 v/cm, 3.5 h) and stained with 0.05% nigrosine. The 70% ethanol extract was fractionated by combined electrofocusing (pH 5-8, gel 2 × 150 mm, 480 v, 7 h) × electrophoresis (pH 3.2, 10 v/cm, 5 h) (Aragoncillo et al. 1975). Marker CM-4 was analysed in the chloroform:methanol (2:1 v/v) extract with the same electrophoretic technique used for U-1.

Phosphatase isozymes from wheat kernels were extracted, fractionated, and stained essentially as described for alkaline phosphatase of wheat kernels and leaves by Brewer (1970), except that 10% acrylamide was used for the electrophoretic separation, which markedly improved the resolution of isozyme bands, as compared with 7.5% acrylamide (Delibes and García-Olmedo 1973) or with starch (Brewer 1970).

Results

Description of Markers

The M^v biochemical markers used in this study are designated U-1, CM-4 (equivalent to NGE-17v) and Aph_v-a, -b (previously called Aph-3) (Delibes and García-Olmedo 1973; Delibes et al. 1977). Marker U-1 is a component of the 2M urea extract which is detected by electrophoresis as shown in Fig. 1. It can be also detected in the 70% ethanol extract when fractionated by combined electrofocusing × electrophoresis (Fig. 2). Marker CM-4 corresponds to a band in the one-dimensional electrophoretic pattern of the chlorofom:methanol (2:1 v/v) extract (Fig. 3) and to three spots (NGE-17v) in the electrofocusing × electrophoresis map of the 70% ethanol extract (Fig. 2a).

No adequate biochemical characterization of wheat kernel phosphatases has been carried out, so the designation of the isozymes studied here as alkaline phosphatases is an operative one, only based on the fact that staining conditions used are those previously reported for alkaline phosphatases (Brewer 1970). The previously described Aph-3 band (Delibes and García-Olmedo 1973) has been resolved by the improved electrophoretic separation into two components, Aph_v-a, -b (Fig. 4). This designation is kept because it is not yet known whether the electrophoretic bands correspond to single polypeptides.

Also shown in Fig. 2 are components NGE-1 and NGE-17, respectively associated with chromosomes 5D and 4D in *T. aestivum* cv. 'Chinese Spring' (Aragoncillo et al. 1975).

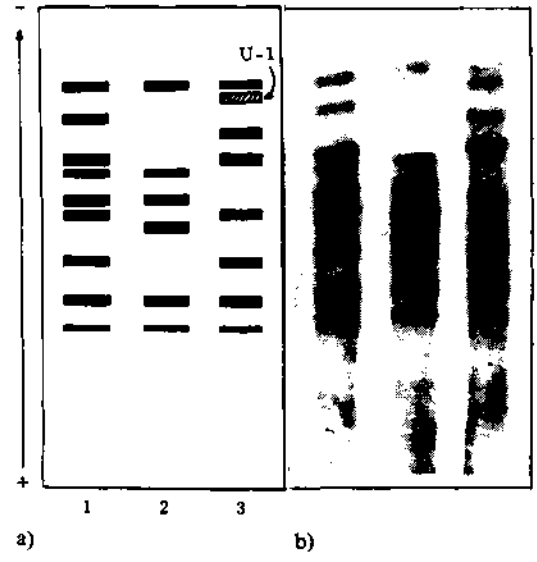

Fig. 1a and b. Electrophoretic patterns of 2M urea extracts from (*1*) *T. aestivum*, (*2*) *T. turgidum* and (*3*) *Ae. ventricosa*. a Schematic representations; b Picture of gel

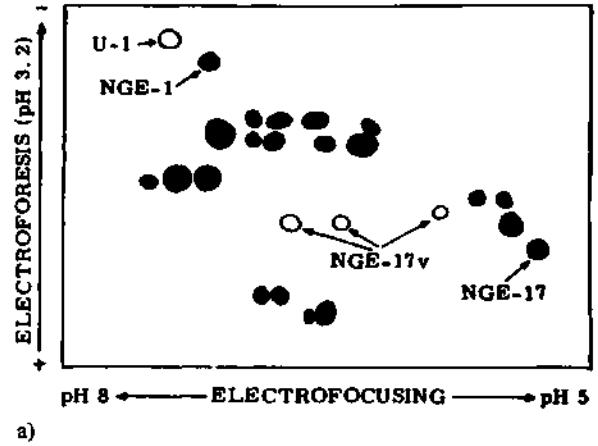

Fig. 2a and b. Combined electrofocusing × electrophoresis maps of NGE proteins from 70% ethanol extracts of *T. aestivum*. a Schematic representation; open spots show the relative positions of markers U-1 and NGE-17v with respect to the map components of hexaploid wheat (black spots); b Picture of gel corresponding to hexaploid wheat

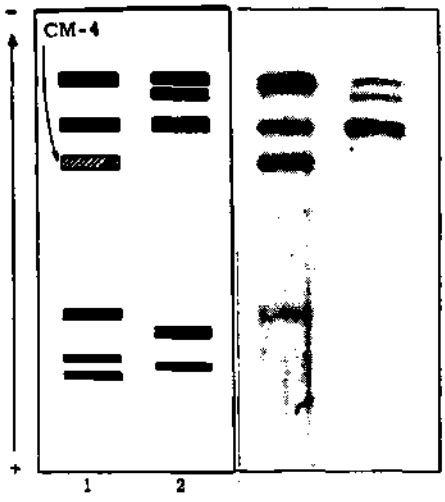

Fig. 3a and b. Electrophoretic patterns of chloroform:methanol (2:1) extracts from (*1*) *Ae. ventricosa* and (*2*) *T. aestivum*. a Schematic representation; b Picture of gel

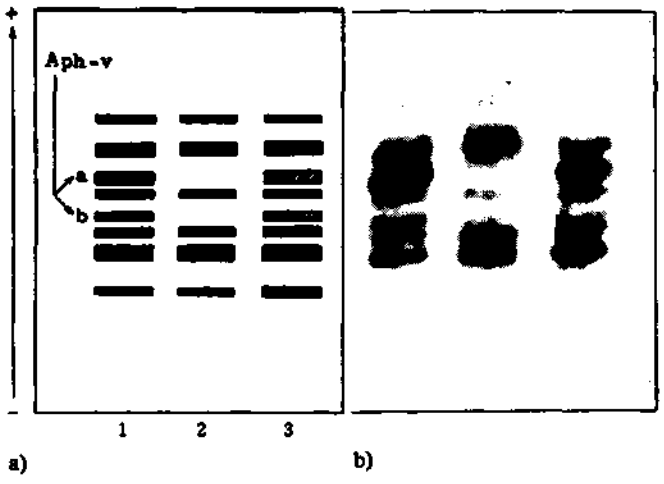

Fig. 4a and b. Electrophoretic patterns of alkaline phosphatase isozymes from (*1*) v113; (*2*) v115; and (*3*) v110. a Schematic representation; b Picture of gel

Distribution of Markers Among Addition Lines

The distribution of biochemical markers among addition lines with *Aegilops ventricosa* cytoplasm (v lines) and wheat cytoplasm (m lines) is summarized in Tables 1 and 2 respectively.

Marker U-1 appears independently from markers CM-4 and Aph_v-a, -b. All type 7 v and m lines carry U-1. Type 7 corresponds with the previously designated type B, which was defined on the basis of cytological morphological and agronomical characters (Dosba et al. 1978). Four v lines, not clearly classified as type 7 by these criteria, did carry U-1:v9 is a monotelosomic addition line; v27 was not controlled cytologically in 1975 or in 1976; v152 is not yet

Table 1. Distribution of markers among addition lines and their parental material (*T. aestivum* cv. 'Moisson' cytoplasm = m lines)

Type	Stock	2n	Year of analysis	Markers			
				U-1	CM-4 (NGE-17v)	Aph3 (Aph_v-a,-b)	NGE-1[a]
7	m 73 -(3-3-9)	43	79	+	–	–	+
	m 105-(12-12)	44	78	+	–	–	
	m 114-(2-6)	44	78	+	–	–	
	m 269-(25-6)	44	78	+	–	–	
	m 359-(15-9-8)	43	79	+	–	–	+
	m 372-(36-7-2)	44	79	+	–	–	+
9	m 128(20xM-7-2)	44	79	–	+	+	+
	m 175-(20-18)	44	78	–	+	+	
	m 328-(1-5)	42+t	78	–	+	+	
9?	m 370-(41-7-8)	44	79	–	–	–	–
	25 other plants	43 or 44	78	–	–	–	
	25 other plants	42 + 2t, 43, or 44	79	–	–	–	+
	T. aestivum, cv. 'Moisson'	42	78-79	–	–	–	+
	T. aethiopicum	28	78-79	–	–	–	–
	Ae. ventricosa no. 11	28	78-79	+	+	+	+

[a] Analysed only in stocks of 1979

Table 2. Distribution of markers among addition lines and their parental material (*Aegilops ventricosa* cytoplasm = v lines)

Type	Stock	2n	Year of analysis	Markers			
				U-1	CM-4 (NGE-17v)	Aph3 (Aph_v-a,-b)	NGE-1[a]
7	v 118-(6-32-3)	44	79	+	+f	–	+
	v 121-(24-2)	44	78	+	–	–	
	v 183-(1-2)	43	78	+	–	–	
	v 204-(12-43-7)	44	79	+	–	–	+
	v 205-(2-43-9)	44	79	+	–	–	+
	v 208-(11-15)	44	78	+	–	–	
	v 209-(52-51-7)	43	79	+	–	–	+
	v 215-(43-17-1)	43	79	+	–	–	+
	v 318-(10-22)	43	78	+	–	–	
	v 408-(24-12)	43	78	+	–	–	
7?	v 9 -(4-4-17)	42+t	79	+	–	–	–
	v 27 -(41-43-1)	44	79	+	–	–	–
	v 152-(23-13-4)	44	79	+	–	–	–
	v 308-(41-43-12)	44	79	+	–	–	–
9	v 110-(40-46)	?	79	–	+	+	+
	v 113-(12-32)	?	79	–	+	+	+
	v 115-(24-8-1)	44	79	–	+	+	+
	v 130-(1-7-10)	44	79	–	+	+f	+
	v 132-(35-2-8)	44	79	–	+	+f	+
	v 177-(8-2)	44	78	–	+	+	
	v 276-(14-8)	43	78	–	+	+	
	v 278-(2-14)	44	79	–	+	+	+
	v 404-(6-16)	44	78	–	+	+	
?	v 136-(43-18)	44	78	–	+	+	
	v 137-(18-3)	44	78	–	+	+	
	17 other plants	42+t 43 or 44	78	–	–	–	
	17 other plants	43 or 44	79	–	–	–	+
	T. aestivum cv. 'Moisson'	42	78-79	–	–	–	+
	T. aethiopicum 1A	28	78-79	–	–	–	–
	Ae. ventricosa no. 11	28	78-79	+	+	+	+

f = faint;
[a] Analysed only in stocks of 1979

fixed (1.2 univalents, 21.4 bivalents) and the added chromosomes of these two lines probably carry a D-M^v interchange; and v308 is not yet fixed but its phenotype is close to that of type 7 lines. Neither of those four lines expressed the 5D chromosome marker NGE-1, indicating that other modifications besides addition might have taken place in them. Line v118, in which marker CM-4 seems to be expressed at a low level, could have a substituted or recombined M^v chromosome besides the added one.

Markers CM-4 and Aph_v-a, -b appear concomitantly in v and m lines of type 9 (type D in Dosba et al. 1978). Non-biochemical characters discriminating type 9 are less clearly expressed in wheat than in *Aegilops* cytoplasm. Line m175 is the most similar to type 9 v lines. Line m128 is unstable. Monotelosomic addition line m328 carries both markers, which implies that their corresponding genes are probably located in the same chromosome arm. Line m370, which lacks both markers, is unstable and also lacks marker NGE-1 associated with chromosome 5D, indicating that other modifications might have ocurred in it.

Two v lines (v136 and v137) morphologically different from type 9, possess markers CM-4 and Aph_v-a, -b. The meiotic behaviour of an F_1 between v137 and a type 9 line (Table 3) clearly shows lack of complete homology, indicating that either a chromosome or chromosomal seg-

Table 3. Mean meiotic behaviour of hybrids (2n = 44) between stocks belonging to the same type or different types

F_1	No. of different crosses	No. of plants observed	No. of cells analysed	I	II	III	IV
type 7 × type 7	3	6	200	0,50	21,75		
type 9 × type 9	1	1	70	0,22	21,88	0,01	
type 7 × type 9	2	5	260	2,22	20,89		
v 137 × type 7	2	5	190	1,70	20,32	0,54	0,01
v 137 × type 9	1	3	90	1,79	20,18	0,59	0,02

I = univalents; II = bivalents; III = trivalents; IV = quadrivalents

ment has been substituted besides the added one, or that the added chromosome in v137 carries an extensive translocation not affecting the region carrying the markers.

It should be noted that the expression of marker NGE-17 (chromosome 4D) is totally or partially blocked in all lines carrying CM-4 (NGE-17v; Fig. 2).

Discussion

The fact that marker U-1 appears independently of CM-4 and Aph_v-a, -b is in agreement with previous observations seen in the cross (*T. turgidum* × *Ae. ventricosa*) × *T. aestivum* involving a different *Ae. ventricosa* stock from that used in the present study (Delibes et al. 1977).

The association of U-1 with the added chromosome of type 7 addition lines is of interest because the v lines of this type are highly resistant to eyespot disease provided certain D^v genome genes are also present (lines v205, v208). In v209, the resistance was significantly higher in the monosomic addition line (2n = 43) than in the disomic addition line (2n = 44). On the wheat cytoplasm, the effect of this added chromosome on resistance has not yet been investigated (Dosba et al. 1978; Dosba and Doussinault unpublished results).

It should be pointed out that in the previously alluded cross (Delibes et al. 1977) CM-4 and Aph_v-a, -b were independently inherited, whereas in the present case they appear associated, and CM-4 (NGE-17v) was alternatively inherited with respect to NGE-17 (chromosome 4D). It was then assumed that the gene(s) corresponding to CM-4 had been transferred from the M^v to the D^v genome prior to the experimental cross, because although CM-4 had been selected as a putative M^v genome marker, it was actually inherited as a D^v genome gene, presumably located in chromosome $4D^v$. It is also known that alkaline phosphatase isozymes are associated with group 4 chromosomes in *T. aestivum* cv. 'Chinese Spring' (Brewer et al. 1969). These observations and the partial or total block of NGE-17 (chromosome 4D) expression reported here for type 9 addition lines, suggest that the added chromosome is also of the same homoeology group ($4M^v$). It is, however, desirable to further verify this hypothesis by other methods, including optimal substitution lines and additional biochemical markers.

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