BIOCHEMICAL EVIDENCE OF GENE TRANSFER FROM THE M^v GENOME OF AEGILOPS VENTRICOSA TO HEXAPLOID WHEAT

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SUMMARY

Endosperm protein and isozyme patterns of lines derived from the cross [Triticum turgidum (AABB) x Aegilops ventricosa (DDM^vM^v)] x T. aestivum (AABBDD) were studied. Five biochemical characters present in Ae. ventricosa (DDM^vM^v), Ae. comosa (MM) and Ae. uniaristata (M^uM^u), but absent from Ae. squarrosa (DD), T. aestivum (AABBDD) and T. turgidum (AABB) were investigated. Three of these were identified in several lines, indicating gene transfer from the M^v genome of Ae. ventricosa into hexaploid wheat. The wide distribution of one of the markers (CM-4) suggests its previous transfer to the D genome of Ae. ventricosa.

INTRODUCTION

The introduction of extraspecific or extrageneric characters into cultivated plants has been long recognized of practical importance and of considerable genetic interest (SEARS, 1956; RILEY and KIMBER, 1966). The transfer of resistance to leaf-rust from *Aegilops umbellulata* (SEARS, 1956) and to eyespot *(Cercosporella herpotrichoides)* from *Ae. ventricosa* (KIMBER, 1967) into wheat are well-known examples of such genetic manipulation.

A *T. turgidum* x *Ae. ventricosa* amphiploid was obtained by KIMBER (1967) and then crossed with *T. aestivum*. Plants forming 21 bivalents at meiosis and resistant to eyespot were recovered in backcrosses to *T. aestivum*. Homologous transfer of a gene or genes from the D genome of *Ae. ventricosa* to wheat was rightly assumed by Kimber.

A somewhat different procedure for homologous transfer was proposed earlier by SIMONET (1952). It involved obtaining the hybrid (tetraploid wheat x *Ae. ventricosa*) x *T. aestivum* and screening the progeny for plants with *Ae. ventricosa* characters and 21 meiotic bivalents.

We report here biochemical evidence indicating non-homologous gene transfer from the M^v genome of *Ae. ventricosa* to hexaploid wheat by crossing (*T. turgidum* x *Ae. ventricosa*) x *T. aestivum*.

MATERIALS AND METHODS

Seventy F_{20} lines (H-93-1 through 70) derived from 10 seeds obtained in a cross (*T. turgidum* var. H-1-1 x *Ae. ventricosa*) x *T. aestivum* var. H-10-15 were the gift of M. Alonso Peña, who also kindly supplied us with the parental material and samples of *Ae. squarrosa*, *Ae. comosa* and *Ae. uniaristata*.

Somatic chromosome numbers of the H-93 lines were counted in the root tips from germinating seeds after staining by the Feulgen procedure.

Single seeds were crushed between two metal plates with a hammer and defatted with diethyl ether. Protein extractions were usually carried out with five volumes of one of the following solvents: Chloroform; methanol (CM) (2:1, v/v); 3M Urea (U); or 70% Ethanol (Et). Starch gel electrophoresis of the extracts was performed in 0.05M aluminum lactate buffer, pH 3.1, at 10 volts/cm for 3 hours to show albumins, globulins and proteins of similar mobility and for 7 hours to show gliadins and athins. Gels run for the shorter time were stained with nigrosine (0.05% in acetic acid:water, 1:1 v/v) for 12 hours. Gliadins and athins were stained with nigrosine (0.5% in acetic acid:water, 2:1, v/v) for 20 minutes. Destaining was achieved with 70% ethanol. Protein patterns for each line were ascertained in at least three repetitions, and the parental material was included in all the gels.

Electrophoresis and staining of alkaline phosphatase isozymes was carried out essentially as described by BREWER (1970), except that 7.5% acrylamide was used instead of starch.

Peroxidase isozymes were electrophoresed and stained according to MacDONALD and SMITH (1972).

Sterol esters patterns were screened as previously described (GARCÍA-OLMEDO, 1968).

RESULTS AND DISCUSSION

Composite diagrams of protein patterns observed for the different extracts are shown in Figure 1. The shorter electrophoretic run of the 3M urea extract shows the proteins generally considered as belonging to the albumins + globulins fraction (Fig. 1a). The chloroform:methanol extract shows, under the same conditions of electrophoresis and staining, 2 to 4 bands of uncertain classification (ARAGONCILLO and GARCÍA-OLMEDO, unpublished). Bands CM1 and CM2 of this extract have been shown to be controlled by chromosomes 7D and 7B, respectively (GARCÍA-OLMEDO and CARBONERO, 1970). Bands of similar mobility to the CM's and slower are present in the 70% ethanol extract. Classical gliadins (Fig. 1b) are included in the 3 types of extracts, but with 70% ethanol a clearer pattern is obtained for the slower moving athins.

Composite alkaline phosphatase and peroxidase isozymograms are presented in Figure 2.

The palmitate-linoleate β-sitosterol esters phenotype (PL) is controlled by a single mendelian factor located in the D genome of *T. aestivum* (GARCIA-OLMEDO, 1968).

All H-93 lines have 42 chromosomes with the exception of line H-93-1 which showed 56.

The distribution of the selected biochemical markers in H-93 lines as well as in their parental and related species is summarized in Tables 1 and 2.

Markers of AB or D genomes are present in a high proportion of the lines as expected (Table 1). Those present in the homologous genomes of two parental species should show in all lines. This is the case for PL and CM-2, but not for CM-1 and Gl-2. The D genomes of

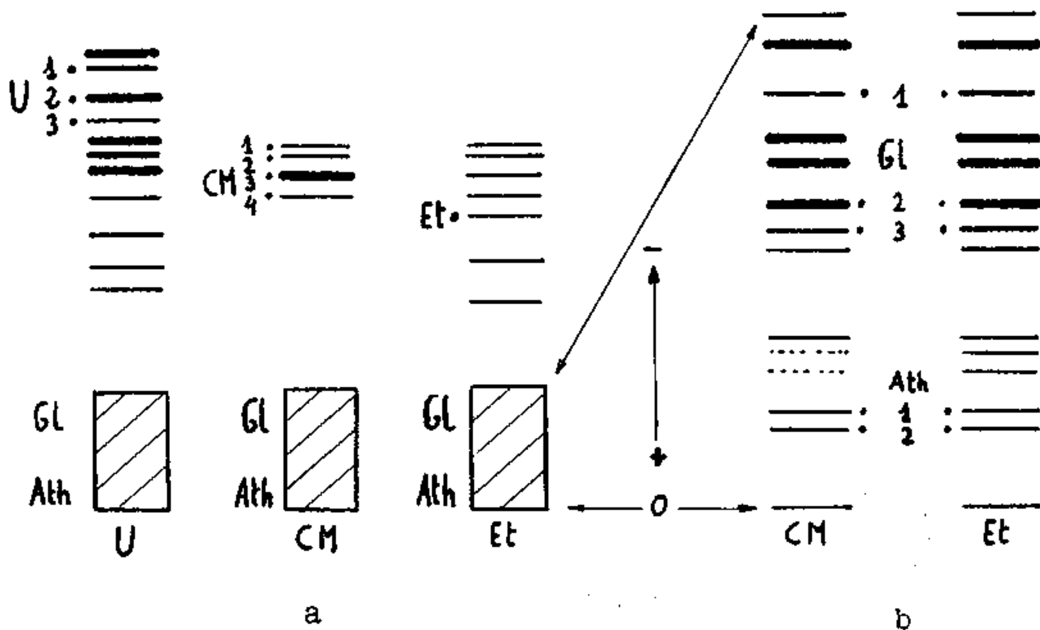

Figure. 1. Composite diagrams of protein electrophoretic patterns with (a) 3M Urea (U); Chloroform:Methanol (CM), and 70% Ethanol (Et) extracts; 3 hours at 10 volts/cm; nigrosine 0.05%, 12 hours. (b) CM and Et extracts; 7 hours at 10 volts/cm; nigrosine 0.5%, 20 minutes.

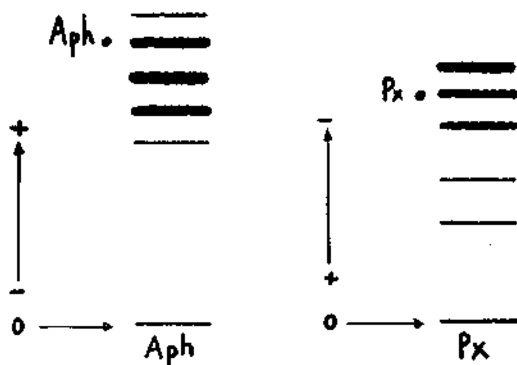

Figure 2. Composite alkaline phosphatase (Aph) and peroxidase (Px) isozymograms.

Table 1. Appearance of biochemical markers from AB and D genomes in H-93 lines. (ABD) *T. aestivum* var. H-10-15; (AB) *T. turgidum* var. H-1-1; (DM^v) *Ae. ventricosa*; (D) *Ae. squarrosa*; (M) *Ae. comosa*; (M^u) *Ae. uniaristata.*

Marker	ABD	AB	D	DM^v	M	M^u	Assignment	H-93 lines with marker (%)
PL[1]	+	–	+	+	+	+	D, M	100
U-2	+	–	–	–	–	–	ABD *T. aestivum*	59
CM-1	+	–	+	+	+	+	D, M	96
CM-2	+	+	–	–	–	–	AB	100
Et	+	–	+	–	–	–	D *T. aestivum*	71
Gl-1	–	+	–	–	–	–	AB *T. turgidum*	32
Gl-2	+	–	–	+	–	–	D	75
Gl-3	+	–	–	–	–	–	ABD *T. aestivum*	40
Ath-1 & 2	+	–	–	–	–	–	ABD *T. aestivum*	72

[1]β-sitosterol palmitate-linoleate phenotype

Table 2. Apperance of biochemical markers from M genome in species and H-93 lines.

Marker	ABD	AB	D	DM^v	M	M^u	$ABDM^v$*	Marker in H-93 lines No.
Aph	–	–	–	+	+	+	+	33
Px	–	–	–	+	+	+	–	–
U-1	–	–	–	+	+	+	+	8, 35
U-3	–	–	–	+	+	+	–	–
CM-4	–	–	–	+	+	+	+	2, 5-12, 14, 17, 19, 22, 24, 26-7, 31, 35, 50-3, 56, 63-4

*H-93-1, 56 chromosomes, presumed to be the amphiploid (*T. turgidum* x *Ae. ventricosa*) ($AABBDDM^vM^v$).

T. aestivum and *Ae. ventricosa* are involved in both exceptions and their incomplete homology (SEARS, 1968; SIMONET, 1952; SIDDIQUI and JONES, 1967) could supply a reasonable explanation of the results.

Biochemical characters present in *Ae. ventricosa* (DDM^vM^v), *Ae. comosa* (MM) and *Ae. uniaristata* (M^uM^u) and absent from *T. aestivum* (AABBDD), *Ae squarrosa* (DD) and *T. turgidum* (AABB) were selected as M^v genome markers. The distribution of five such markers is summarized in Table 2. A much more restricted distribution of these characters in the H-93 lines is to be expected, and indeed this is the case. Only three of the markers, Aph, U-1 and CM-4, were detected both in line H-93-1 (56 chromosomes) and in several of the 69 lines with 42 chromosomes. The other two markers, Px and U-3 were not identified either in H-93-1 or in the other lines.

The wider distribution of CM-4 (in 25 out of 69 lines), similar to that of markers present in the A, B, or D genomes of only one of the parental species, suggests that a previous transfer of this character from the M^v to the D genome of *Ae ventricosa* had taken place before the cross was performed.

Our results seem to indicate that gene transfer from the M^v genome of *Ae. ventricosa* into hexaploid wheat can be achieved without the use of irradiation or 5B-breaking systems. Further cytogenetic and biochemical evidence to confirm this possibility is being sought at present.

ACKNOWLEDGEMENT

We dedicate this communication to M. Alonso Peña upon retirement.

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