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Characterization of wheat/*Aegilops ventricosa* introgression and addition lines with respect to the M^v genome

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Summary. Stable wheat-*Aegilops* introgression lines with 42 chromosomes (H-93), derived by repeated selfing from a cross (*Triticum turgidum* × *Aegilops ventricosa*) × *T. aestivum*, have been characterized using the following DNA probes and isozyme markers: (1) single or low-copy DNA fragments from *Ae. ventricosa;* (2) known cDNA probes corresponding to α1-thionin, monomeric α-amylase inhibitor, the CM3 subunit of tetrameric α-amylase inhibitor, and sucrose synthase from wheat; (3) anonymous cDNA probes from wheat that have been mapped by Sharp et al. (1989); (4) isozyme markers corresponding to aconitase, shikimate dehydrogenase, adenylate kinase, and endopeptidase. Meiotic metaphases of appropriate hybrids involving selected H-93 lines have been investigated by the Giemsa C-banding technique. The substitution of whole chromosomes [(5A) $5M^v$; (4D) $4M^v$; (5D) $5M^v$; (7D) $7M^v$] and chromosomal segments ($1M^v$; $3M^v$; $5M^v$; $7M^v$) from the M^v genome of *Aegilops ventricosa* has been demonstrated. The distribution of selected markers among putative wheat-*Ae. ventricosa* addition lines has also been investigated. The $7M^v$ addition has been characterized for the first time, while the identity of the previously reported $5M^v$ and $6M^v$ additions has been confirmed.

Key words: *Aegilops ventricosa* – DNA probes – Introgression lines – Addition lines – *Triticum aestivum*

Introduction

The wild grass *Aegilops ventricosa* Tausch., an allotetraploid (2n = 28; genomes D^v D^v M^v M^v), has attracted considerable attention as a source of genes for resistance to pathogens such as the fungi *Pseudocercosporella herpotrichoides* (eyespot disease), *Erysiphe graminis* sp. *tritici* (powdery mildew), and *Puccinia recondita* (brown leaf rust; Sprague 1936; Dosba et al. 1980, 1982; and others); to insects, such as *Mayetiola destructor* (Jones 1938); and to the cyst nematode *Heterodera avenae* (Dosba and Rivoal 1981). The D^v genome of *Ae. ventricosa* is evolutionarily close to the D genome of hexaploid wheat, *Triticum aestivum* L. (genomes AABBDD), whereas the M^v genome is more distantly related (Dosba 1985). Interspecific hybrids between the two species can be obtained only with difficulty (Dosba and Cauderon 1972). In these hybrids, chromosomes from the D^v and the D genome pair at meiosis and recombination occurs, while those of the M^v genome do not pair or recombine, except under exceptional circumstances (Dosba 1974; Mena 1990).

Genetic material from *Ae. ventricosa* has been transferred to hexaploid wheat through an intermediate self-sterile hybrid between *T. turgidum* (AABB) and *Ae. ventricosa* (D^v D^v M^v M^v), which was backcrossed using pollen from hexaploid wheat (AABBDD), and the progeny were repeatedly selfed to obtain stable wheat lines with 42 chromosomes, designated H-93 lines (Delibes and García-Olmedo 1973; Delibes et al. 1977a, b; Doussinault et al. 1983).

Five potential wheat/*Ae. ventricosa* addition lines have already been characterized on the basis of cytological, morphological, and agronomic characters (Dosba and Doussinault 1978). Three of these have been further identified as corresponding to lines carrying chromosomes $4M^v$ (Delibes et al. 1981; Mena et al. 1989), $5M^v$ (Delibes et al. 1981; Dosba 1985) and $6M^v$ (Dosba 1985).

We have now further characterized these H-93 lines using DNA probes and biochemical markers, as well as cytological methods. Additionally, a disomic addition line carrying chromosome $7M^v$ has been identified.

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Materials and methods

Plant materials

The H-93 lines used in this study, all of which had been derived from the cross (*Triticum turgidum* H-1-1 × *Aegilops ventricosa* AP-1) × *T. aestivum* cv "Almatense" H-10-15, have been previously described (Delibes and García-Olmedo 1973; Delibes et al. 1977b). Appropriate H-93 lines were hybridized to the H-10-15 parent or to ditelosomic stocks of *T. aestivum* cv "Chinese Spring". Wheat/*Ae. ventricosa* addition lines were the gift of F. Dosba (Dosba and Doussinault 1978; Dosba 1985), and a (6D) $6M^v$ substitution line, a gift from J. Jahier.

Construction of a gDNA library and selection of gDNA probes

Total DNA from *Ae. ventricosa* was extracted from dark-grown seedlings as described by Murray and Thompson (1980) with modifications (Rogers and Bendich 1988). The DNA was digested to completion with the *Sau*3AI restriction endonuclease, and fragments between 0.5 kb and 1 kb were selected by preparative electrophoresis on 0.8% agarose gels and cloned into the *Bam*HI site of plasmid pUC18. DNAs from an ordered collection of clones were hybridized with total DNAs from *Ae. ventricosa* and *T. aestivum* that had been [^{32}P]-labelled by the random primer procedure (Feinberg and Vogelstein 1983) under experimental procedures described by Landry and Michelmore (1985).

cDNA probes

Inserts from cDNA clones corresponding to the following proteins were used in this study: α1-thionin (WT1; probe pTT1), sucrose synthase (Ss1; probe P1), monomeric α-amylase inhibitor (IAM, syn. WMAI-1; probe pUP28) from wheat, gift from C. Maraña, and wheat tetrameric α-amylase inhibitor, (subunit CM3, syn. WTAI-3; probe pCT1), the gift of F. García-Maroto. Ten anonymous cDNA probes corresponding to previously located homoeology sets (Sharp et al. 1989) were kindly provided by M. Gale (Norwich, UK).

Isozyme markers

Isozymes of endopeptidase (EP) were extracted from individual embryos and separated by isoelectric focusing using the technique described by Koebner et al. (1988) and modified by Mena (1990). Adenylate kinase (ADK), aconitase (ACO) and shikimate dehydrogenase (SKDH) isozymes were analyzed using extracts from leaves collected from 12-day-old green plants. The leaves were extracted with water (1:1; w:v), and electrophoresis was performed in horizontal electrostarch gels (12% w:v) at a constant voltage 150 V and at a temperature of 2°–4 °C for 4 h. The gels were stained for adenylate kinase according to Benito et al. (1990), for aconitase according to Chenicek and Hart (1987), and for shikimate dehydrogenase following Koebner and Shepherd (1982).

RFLP analysis

DNAs were extracted as described above and subjected to digestion by restriction endonucleases and electrophoresis in 0.75% agarose gels. Southern blotting and UV cross-linking to nylon membranes (Hybond-N, Amersham) were carried out by standard procedures (Sambrook et al. 1989). The probes were [^{32}P]-labelled according to Feinberg and Vogelstein (1983).

Cytological procedures

Meiotic analysis was carried out after fixation of the anthers in acetic acid:ethanol (1:3) at 4 °C for 2 months following the Giemsa C-banding technique described by Giraldez et al. (1979).

Table 1. Sizes of cloned DNA fragments from *Ae. ventricosa* used as probes

Probe	kb	Probe	kb
abm1	0.77	abm 6	0.56
abm2	0.45	abm 7	0.35
abm3	0.82	abm 8	0.42
abm4	0.75	abm 9	0.50
abm5	0.46	abm10	0.65

Results

Selection of markers

A genomic library of *Ae. ventricosa* DNA digested with the *Sau*3AI restriction endonuclease was obtained in plasmid pUC18. An ordered collection of clones from this library were blotted onto filters and sequentially hybridized with radioactively labelled DNA from *Ae. ventricosa* and from *T. aestivum* in order to select inserts corresponding to low- and single-copy sequences. The selected inserts were used as probes to investigate polymorphism among the DNAs of *Ae. ventricosa, T. turgidum,* and *T. aestivum*, the parental material used to derive the H-93 lines. The DNAs were separately digested with four restriction endonucleases: *Bam*HI, *Bgl*II, *Hin*dIII, and *Eco*RI. Out of the 23 inserts tested 10 gave differential bands for *Ae. ventricosa* in the Southern blot analysis with at least one of the enzymes. Designations and sizes of the inserts are presented in Table 1, and the most informative pattern for each of those probes is presented in Fig. 1. Fragments corresponding to the M^v genome of *Ae. ventricosa* were assigned on the basis of their low frequency of appearance in the H-93 lines (<3%), whereas those corresponding to the D^v genome appeared at a high frequency (18%–80%). In the case of probes abm3 and abm9 (not shown), the differential (M^v) fragment did not appear in any of the H-93 lines. Only in those cases in which M^v fragment(s) substituted for D fragment(s) are the latter indicated.

Fourteen anonymous cDNA probes from *T. aestivum* that had been previously characterized by Southern blot analysis of DNAs from cv "Chinese Spring" and its aneuploids (Sharp et al. 1989) were similarly screened for *Ae. ventricosa* differential patterns, and only 10 gave informative patterns with the enzymes used. Thus, 30 out of the 42 chromosome arms in hexaploid wheat were covered. Four of the identified cDNAs were also used as probes. In Fig. 2 patterns obtained for the parental species of the H-93 lines are compared with those of wheat cv "Chinese Spring"; criteria for the identification of the M^v fragments were as in Fig. 1. Only in those cases in which M^v fragment(s) substituted for fragment(s) from other genomes are the latter indicated on the basis of previous mapping

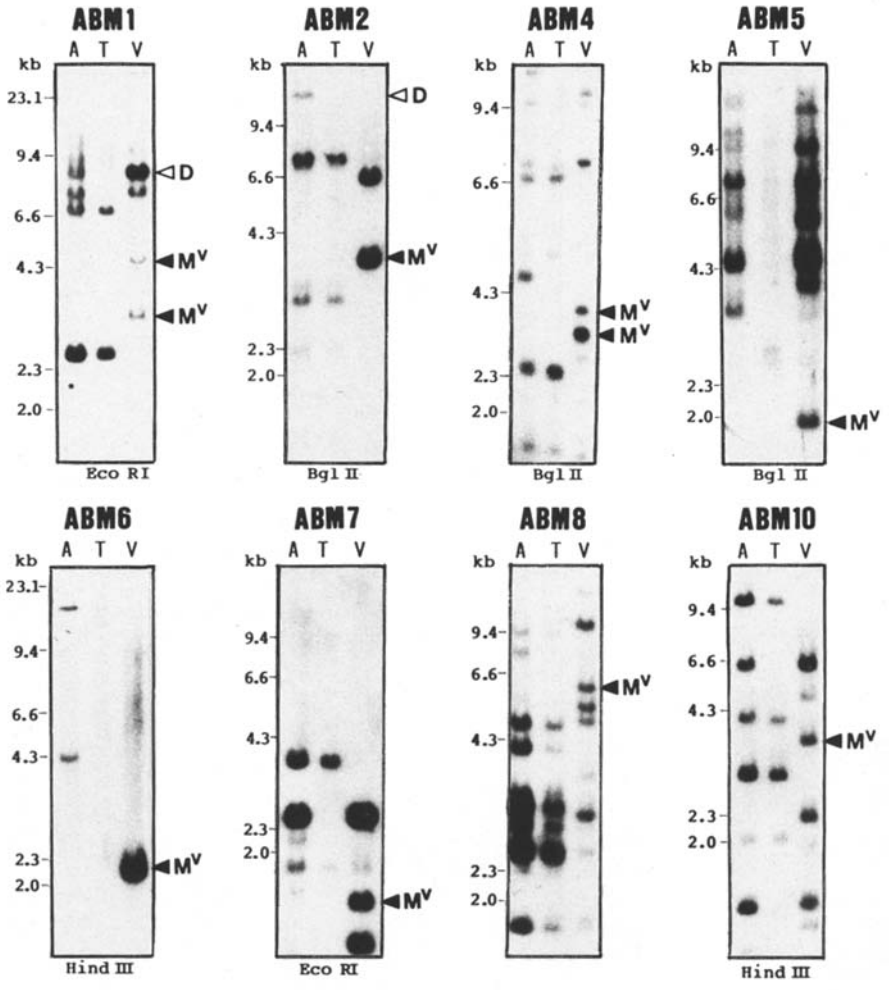

Fig. 1. Southern blot hybridization patterns of DNAs from *Triticum aestivum* cv "Almatense" H-10-15 (*A*), *T. turgidum* H-1-1 (*T*), and *Aegilops ventricosa* AP-1 (*V*) using cloned segments of genomic DNA from *Ae. ventricosa* as probes. Diagnostic bands corresponding to the M^v and the D genomes are indicated with *arrowheads*

in cv "Chinese Spring" or on the present patterns (i.e., bands present both in the tetraploid and the hexaploid wheat denoted as A/B).

A limited number of isozyme markers were also used (Fig. 3). Adenylate kinase (E.C. 2.7.4.3) isozymes have been shown previously to be associated with the long arms of homologous group-7 chromosomes (Benito et al. 1990; Mena et al. 1992). Endopeptidase (E.C. 3.4.21-24.) isozymes have been assigned to the same chromosome arm of group-7 chromosomes (Koebner et al. 1988; Mena et al. 1992). The pattern of aconitase (E.C. 4.2.1.3) has bands associated with group-6 chromosomes (Aco-1; Chenicek and Hart 1987) an that of shikimate dehydrogenase (E.C. 1.1.1.25) is controlled by group-5 chromosomes (Koebner and Shepherd 1982).

Distribution of markers among H-93 lines

The distribution of the complete set of markers corresponding to the M^v genome among 40 H-93 lines is summarized in Table 2. Also included in Table 2 are previously reported data relating to protein U1 and ACPH-1 isozymes (Delibes et al. 1981; 1987) and to ADH-1 isozymes (Mena et al. 1989). From these data, the following conclusions were drawn. (1) Line H-93-18 carried a segment of the long arm from chromosome $1M^v$, but not the complete chromosome, as it lacked another marker from the same arm and a marker from the short arm. Because of the overlapping of fragment(s) corresponding to the D genome of hexaploid wheat with those of the A/B genomes, the concomitant disappearence of the D genome marker could not be ascertained. (2) At least a segment of the long arm of chromosome $3M^v$ had been exchanged with chromosome 3D of line H-93-3, but substitution of the whole chromosome is also possible. (3) At least a segment of the short arm of chromosome $4M^v$ had been exchanged with chromosome 4D of line H-93-33. (4) Markers of both the long and the short arms of chromosome 5 M^v were present in lines H-93-8 and -35, substituting for chromosome 5A in the first case and chromosome 5D in the second. (5) Markers from both the long and the short arms of chromosome $7M^v$ were present in lines H-93-1, -8, -10, and -22 substituting for chromosome 7D markers, whereas line -51 had all of the $7M^v$

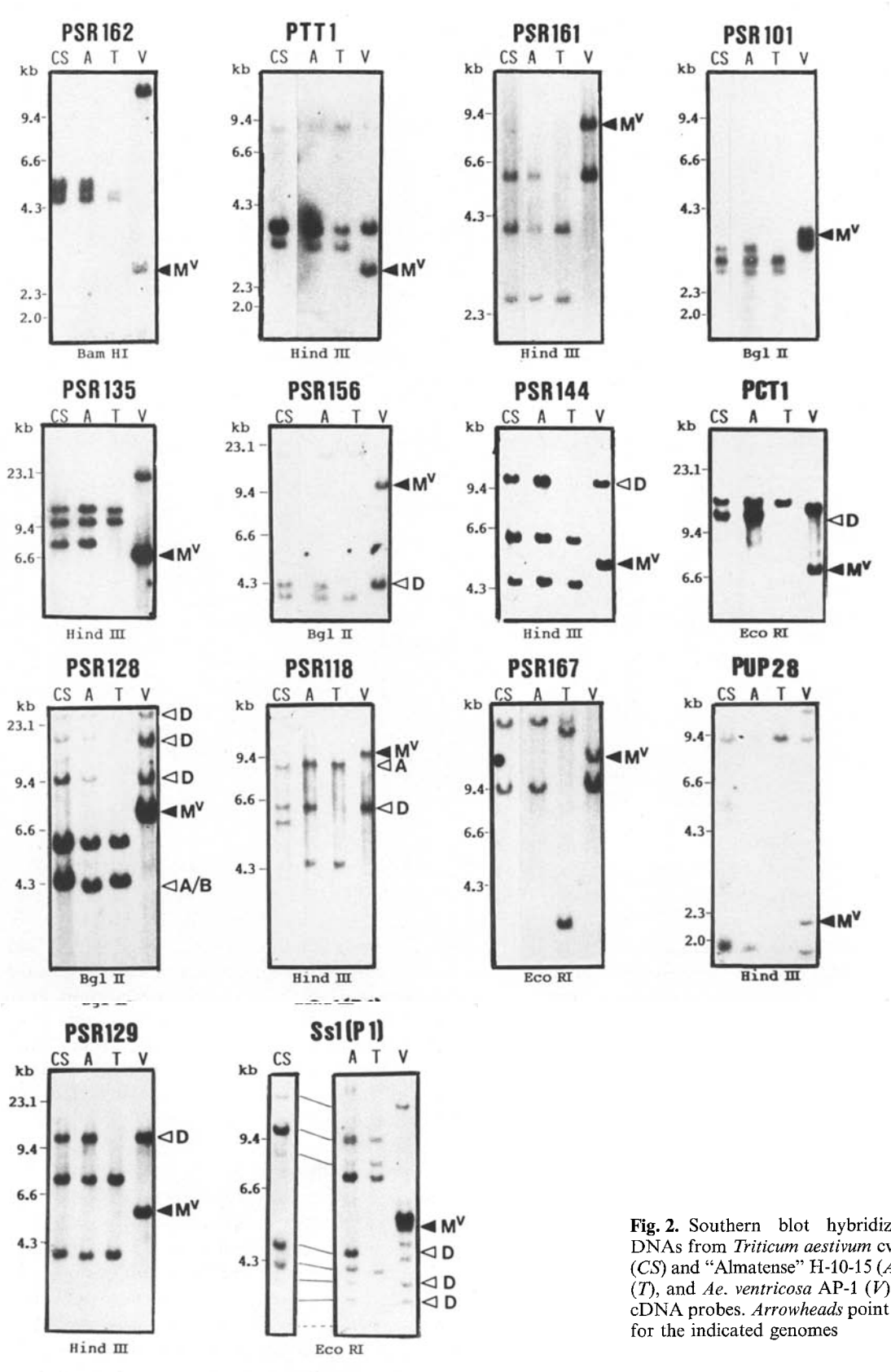

Fig. 2. Southern blot hybridization patterns of DNAs from *Triticum aestivum* cvs "Chinese Spring" (*CS*) and "Almatense" H-10-15 (*A*), *T. turgidum* H-1-1 (*T*), and *Ae. ventricosa* AP-1 (*V*) using the indicated cDNA probes. *Arrowheads* point to diagnostic bands for the indicated genomes

Table 2. Distribution of markers from the M^v genome among H-93 lines

Marker set	Chromosome group (arm)	Distribution		
		M^v Marker		Marker(s) missing from genome
		Designation [b]	H-93 lines with marker	
psr161	1(S)	Xpsr161-H-1M^v	–	–
ptt1	1(L)	Xptt1-H-1M^v	–	–
psr162	1(L)	Xpsr162-B-1M^v	18	–
abm6		Xabm6-H-1M^v	18	–
psr135	2(S)	Xpsr135-H-2M^v	–	–
psr101	2(L)	Xpsr101-G-2M^v	–	–
psr156	3(L)	Xpsr156-G-3M^v	3	D
psr144	4(S)	Xpsr144-H-4M^v	33	D
pct1	4(S)	Xpct1-E-4M^v	33	D
ACPH-1[a]	4	Acph-M^v1	33	D
ADH-1[a]	4	Adh-M^v1	33	D
abm1		Xabm1-E-4M^v	33	–
psr118	5(S)	Xpsr118-H-5M^v	8, 35	A(8); D(35)
SKDH-1	5(S)	Skdh-M^v1	8, 35	A(8); D/B(35)
psr128	5(L)	Xpsr128-G-5M^v	8, 35	A/B(8); D(35)
ACO-2	5(L)	Aco-M^v2	8, 35	A(8); D/B(35)
U1[a]	5	U-M^v1	1, 8, 35	–
abm4		Xabm4-G-5M^v	8, 35	–
abm8		Xabm8-H-5M^v	8, 35	–
psr167	6(S)	Xpsr167-E-6M^v	–	–
pup28	6(S)	Xpup28-H-6M^v	–	–
ACO-1	6(L)	Aco-M^v1	–	–
Ss1(P1)	7(S)	XSs1-E-7M^v	1, 8, 10, 22, 51	D
ADK-1	7(L)	Adk-M^v1	1, 8, 10, 22	D
EP-1	7(L)	Ep-M^{v1}	1, 8, 10, 22	D
psr129	7(L)	Xpsr129-H-7M^v	1, 8, 10, 22, 51	D
abm2		Xabm2-G-7M^v	1, 8, 10, 22, 51	D
amb5		Xabm5-G-7M^v	1, 8, 10, 22, 51	–
amb7		Xabm7-E-7M^v	1, 8, 10, 22, 51	–
abm10		Xabm10-H-7M^v	1, 8, 10, 22, 51	–

[a] Data for protein U1 and ACPH-1 (formerly Aph-v) are from Delibes et al. (1981; 1987); data for ADH-1 (formerly ADHμ) are from Mena et al. (1989); data for pct1 are from García-Maroto et al. (1990); data for Ss1(P1) are from Maraña et al. (1988)
[b] The following letters indicate the restriction endonuclease used: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII

markers except two. (6) Markers of chromosomes 2M^v and 6M^v were not detected in the H-93 lines investigated.

Distribution of selected markers among wheat/Ae. ventricosa addition lines

Selected markers from each of the groups appearing in Table 2 were investigated in the addition lines available that carried M^v chromosomes. The 4M^v addition had been previously identified on the basis of the Adh-M^v1 and Acph-M^v1 (formerly Aph-v) markers (Delibes et al. 1981; Mena et al. 1989). As expected from their grouping in Table 2, markers Aco-M^v1 appeared in both the 6M^v addition and the (6D)6M^v substitution, and Skdh-M^v1 in the 5M^v addition (Fig. 3A, B). The putative 7M^v marker Adk-M^v1 appeared in the unidentified addition line v172 (Fig. 3C) and so did markers XSs1-E-7M^v (Fig. 3D), Ep-M^v1 (Fig. 3E), and Xpsr129-H-7M^v (Fig. 3F). Because of insufficient backcrosses the addition lines and the (6D)6M^vsubstitution do not have a uniform ABD background. Additionally, the accession of *T. aethiopicum* used as an intermediate to obtain these lines is no longer available, so the observed background variability, which is not only due to introgression from the AB genomes from this species but also to introgression from the D^v genome, is difficult to analyze in full, although M^v bands are readily identified. For example, addition line 6M^v has the 7D^v allele of endopeptidase instead of that corresponding to chromosome 7D, which explains its high resistance to eyespot disease (Mena et al. 1992) and indicates that this resistance is not due to the added chromosome as previously thought (Dosba and Doussinault 1981). The same explanation would be valid in the case of the Aco-1 pattern of addition line 6M^v. Another example of background variability is represented by the sucrose synthase Ss1 (P1) pattern of the 4M^v addition, which shows an additional fragment also present in *Ae. ventricosa*. This fragment could represent either a second

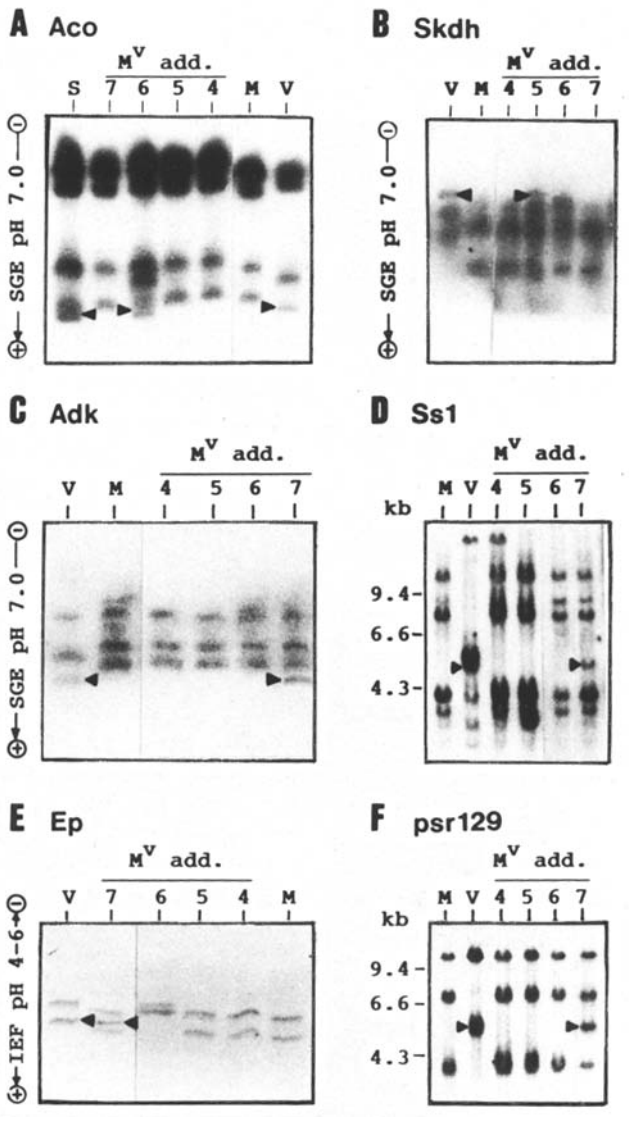

Fig. 3A–F. Distribution of selected markers among wheat-*Aegilops ventricosa* addition lines. The following stocks were analyzed: *Triticum aestivum* cv "Moisson" (*M*); *Ae. ventricosa* no. 11 (*V*), addition lines 4M^v (v177), 5M^v (v208), 6M^v (v260), and 7M^v (v172); and substitution line (6D)6M^v (*S*). The identities of the addition lines in parentheses correspond to Dosba (1985). Markers analyzed were: **A** aconitase isozyme (*arrowhead* indicates *Aco*-M^{v}1); **B** shikimate dehydrogenase isozymes (*arrowhead* indicates Skdh-M^{v}1); **C** adenilate kinase isozymes (*arrowhead* indicates Adk-M^{v}1); **D** sucrose synthase (Ss1 probe) RFLP patterns (*Eco*RI; arrowhead indicates XSs1-E-7M^v); **E** endopeptidase isozymes (*arrowhead* indicates Ep-M^{v}1); **F** RFLP pattern obtained with cDNA probe psr129 (*Hin*dIII; *arrowhead* indicates Xpsr129-H-7M^v)

Ss1-type locus or background variability due to *T. aethiopicum*.

Cytogenetic study of selected H-93 lines

The existence of chromosome substitutions in lines H-93-1, -8, -10, -33, and -51 has been investigated previ-

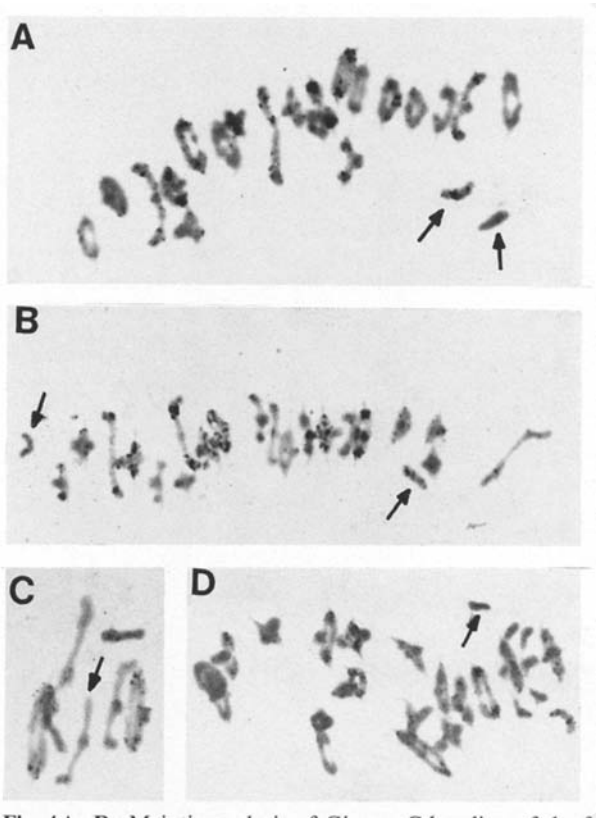

Fig. 4A–D. Meiotic analysis of Giemsa C-banding of the following hybrids: **A** *T. aestivum* cv "Almatense" H-10-15 × H-93-1; **B** H-93-1 × H-93-8; **C** *T. aestivum* cv "Chinese Spring", ditelosomic 5DL × H-93-8; **D** *T. aestivum* cv "Chinese Spring", ditelosomic 5DL × H-93-35. **A, B** *Arrows* point to unpaired chromosomes; **C, D** *arrows* point to 5D telosome

ously in routine analysis of the number of univalents at meiosis in hybrids with *T. aestivum* cv "Almatense" H-10-15 (Delibes et al. 1977a, b, 1987; Mena et al. 1989, 1992). Hybrids between lines H-93-1, -8, -35 and *T. aestivum* showed two, four, and two univalents, respectively.

In order to confirm presence the M^v chromosomes in these lines a further cytogenetic analysis using the Giemsa C-banding technique was carried out. As expected in H-10-15 × H-93-1 hybrids, two univalents were found at metaphase I, one with dispersed and scattered heterochromatin characteristic of the M^v genome and the other without any C-band, like chromosomes of the D genome (Fig. 4A). This result is consistent with the existence of the 7D(7M^v) substitution detected by molecular markers.

Two univalents were also observed in the H-93-1 × H-93-8 hybrid one highly C-banded and the other without C-heterochromatin. However, in this case the heavy banded chromosome had a different C-banding pattern than that in the previous hybrid (Fig. 4B). The existence of two chromosome substitutions, probably 7D(7M^v)

Table 3. Substitution of genetic material from the M^v genome in H-93 lines

Line H-93	Chromosome	Chromosomal segment
1	7D/7M^v	5D?/5M^v
3		3D/3M^v [a]
8	5A/5M^v; 7D/7M^v	
10, 22	7D/7M^v	
18		1D?/1M^v
33	4D/4M^v	
35	5D/5M^v	
51		7D/7M^v/7D^v [b]

[a] Could be a substitution of the whole chromosome 3D
[b] Further details in Mena et al. (1992)

and 5A(5M^v) in H-93-8, was confirmed by the presence of four univalents in its hybrid with "Chinese Spring" 5DL, where telosome 5DL was always paired (Fig. 4C), and excluded the possibility of a 5D(5M^v) substitution. Therefore, lines H-93-1 and H-93-8 have a substitution in common, 7D(7M^v), and differ in a second one, 5A(5M^v), that is present only in H-93-8 (see Table 2). The results shown in Table 2 indicate that line H-93-35 has a 5D(5M^v) substitution. This was confirmed in hybrid Dt5DL × H-93-35, where telosome 5DL was never paired (Fig. 4D).

These results seem to confirm predictions based on the distribution of molecular markers.

Discussion

Joint consideration of the distribution of chromosomal markers and the cytological data allowed us to conclude that the breeding scheme had resulted in the incorporation of genetic material from the M^v genome of *Ae. ventricosa* into the H-93 lines both by the introgression of chromosomal segments and by chromosome substitution. Thus, lines H-93-1, -18, and -51, and possibly line H-93-3, had integrated segments from M^v chromosomes, while lines H-93-1, -8, -10, -22, 33, and -35 had substitutions. Table 3 summarizes our conclusions concerning these lines. A more detailed account of H-93-51 has been recently published (Mena et al. 1992). The present data obtained with probe psr144 further supports the previous characterization of line H-93-33 as a (4D)4M^v substitution (Mena et al. 1989).

The distribution of markers among the addition lines confirms the identification of those corresponding to chromosomes 5M^v and 6M^v and allows the identification for the first time of that carrying chromosome 7M^v.

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