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Molecular and chromosomal characterization of repeated and single-copy DNA sequences in the genome of *Dasypyrum villosum*

C. DE PACE[1], V. DELRE[1], G. T. SCARASCIA MUGNOZZA[1], C. O. QUALSET[2], R. CREMONINI[3], M. FREDIANI[3] and P. G. CIONINI[4]

[1] *Dept. Agrobiology and Agrochemistry, University of Tuscia, 01100 Viterbo, Italy*
[2] *Dept. Agronomy & Range Science, University of California, Davis CA 95616, USA*
[3] *Dept. Botanical Science, University of Pisa, 56100 Pisa, Italy, and*
[4] *Dept. Plant Biology, University of Perugia, 06100 Perugia, Italy*

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Restriction fragment length polymorphism of ribosomal DNA repeated unit and single-copy DNA fragments and chromosomal distribution of a highly repeated sequence, have been studied to assess molecular markers and the extent of their heterogeneity in *Dasypyrum villosum*. Substantial variation has been found for the length of the intergenic spacer of ribosomal genes clustered in different alleles at *Nor-V1* locus of heterozygous individuals, but not within the cluster of rDNA of homozygous individuals. After Southern blots and hybridization to an intergenic spacer probe, each cluster of rDNA was detected as a single band with at least four variants differing for the number of 130 bp subrepeats in the intergenic spacer. One recombinant plasmid contained a 2270 bp DNA insert from the *D. villosum* genome that upon *Sph* I restriction endonuclease digestion was cleaved in three 380 bp repeat elements and one 1090 bp fragment. When Southern blots of *Sph* I digested *D. villosum* DNAs of different genotypes were hybridized to the ^{32}P-labelled 380 bp repeat, a distinct ladder consisting of multiples of a basic repeat unit of about 380 bp in length was revealed on autoradiograms. The in situ hybridization of the ^{3}H-labelled 380 bp repeat element showed that one chromosome pair (7V) was not labelled. In the other pairs, silver grains remained clustered at or near the telomeres. Dot-blot hybridization analysis of DNAs from a range of diploid, tetraploid, and hexaploid Triticeae species indicated that the 380 bp repeated element was a specific feature of the *D. villosum* genome. Other cloned DNA sequences of *D. villosum* showed a large restriction length polymorphism and one was located on V chromosomes.

C. De Pace, Dept. Agrobiology and Agrochemistry, University of Tuscia, 01100 Viterbo, Italy

Dasypyrum villosum (L.) CANDARGY is a widespread outcrossing diploid (2n = 14) annual species, which belongs to the Triticeae tribe (FREDERIKSEN 1991).

The genome of *D. villosum*, indicated V by SEARS (1953), is considered an important donor of genes to wheat for improving powdery mildew resistance (DE PACE et al. 1988a), take-all (SCOTT 1981), and plant and seed storage protein content (DELLA GATTA et al. 1984; SHEWRY et al. 1987; DE PACE et al. 1988a). The hybridization of *D. villosum* with diploid, tetraploid and hexaploid species of *Triticum* has been performed (SANDO 1935; SEARS 1953; HYDE 1953), and other intergeneric hybridizations involving *D. villosum* were tempted (NAKAJIMA 1962). Individual V chromosomes have been added to both durum (BLANCO et al. 1987) and bread wheat (SEARS 1953; HYDE 1953).

Morphological, isozyme and seed storage protein markers have been identified to tag each of the seven V chromosome pairs (MONTEBOVE et al. 1987; DE PACE et al. 1988; BENEDETTELLI and HART 1988) and, on average, two markers for each V chromosome are known. However, this situation is not satisfactory to monitor gene transfer from *D. villusum* to wheat through intergeneric hybridization. Moreover, information on the allelic polymorphism for gene markers in *D. villosum* is limited (DE PACE 1987; DE PACE et al. 1988b), although they are of paramount importance for a deliberate choice of *D. villosum* phenotypes that possess the desired alleles for genes that may improve given wheat characters. Another problem is the identification of V chromosomes following their introduction into wheat. The Giemsa banding may be helpful, but its potential is limited, since the banding pattern of the alien chromosomes may be non-reproducible when introduced into the wheat nucleus. *D. villosum* DNA markers obtained by restriction fragment length polymorphism (RFLP) or repeated sequence abundance polymorphism (RSAP) (DVOŘÁK et al. 1988) may be a valid means for the identification of defined, polymorphic, DNA sequences to be used for V chromosome identification and assessment of linkage between those sequences and other useful genes.

In this paper, we report on (i) the polymorphism for restriction sites of repeated and single-copy DNA sequences in the V genome, (ii) the molecular and chromosomal characterization of a species-specific highly repeated DNA sequence, and (iii) the feasibility of using single-copy sequences as markers for the V chromosomes.

Material and methods

Plant material

The species studied and their origin are listed in Table 1. Seeds (caryopses) were germinated in Petri dishes on wet paper at room temperature in the dark. Plantlets to be used for DNA or RNA extraction were washed, dried, quickly frozen in liquid nitrogen and stored at −80°C. The roots of other plantlets, to be used for cytological preparations, were treated with a 0.2 % aqueous solution of colchicine at room temperature and fixed in ethanol-acetic acid 3:1 (v/v).

DNA isolation

For DNA preparation, about 0.5 g of fresh tissues were pulverized with pestle and mortar in the presence of liquid nitrogen and homogenized in 5 ml of pH 8 buffer containing 0.1 M Tris, 0.05 M EDTA, 0.5 M NaCl, and 0.01 M mercaptoethanol.

DNA extraction was performed as described by DELLAPORTA et al. (1983) and the isolated DNA was suspended in 0.01 M Tris plus 0.001 M EDTA pH 8, and then stored at 4°C.

D. villosum genomic DNA to be cloned was further purified at 55×10^3 rpm overnight on a CsCl gradient in a Beckman L8 70M ultracentrifuge using the Ti 65 rotor.

Table 1. Plant materials used for preparation of genomic DNAs. The *D. villosum* chromosomes in disomic addition lines are identified by their homologous relationships to the chromosomes of wheat (MONTEBOVE et al. 1987; BENEDETTELLI and HART 1988; DE PACE et al. 1988a)

Species and accessions	Origin or supplier	Genome
D. villosum		
3/1	Puglia, Alberobello, Italy	VV
3/3	Puglia, Alberobello, Italy	VV
3/9	Puglia, Alberobello, Italy	VV
8/1	Puglia, Castellaneta Marina, Italy	VV
16b/24	Lazio, Bomarzo, Italy	VV
16b/27	Lazio, Bomarzo, Italy	VV
120/2	Calabria, Castrovillari, Italy	VV
120/7	Calabria, Castrovillari, Italy	VV
136/3	Puglia, Vieste, Italy	VV
145/4	Toscana, Casone, Italy	VV
197/17	Sardinia, Nuoro, Italy	VV
T. monococcum G4284	Germplasm Inst., CNR, Bari Italy	AA
T. boeoticum G1724	Germplasm Inst., CNR, Bari Italy	AA
T. urartu G1545	Germplasm Inst., CNR, Bari Italy	AA
T. searsii	Germplasm Inst., CNR, Bari Italy	related to B genome
Ae. speltoides G250	P. E. McGuire, Davis, CA, USA	related to B genome
Ae. speltoides 72	Germplasm Inst., CNR, Bari Italy	related to B genome
Ae. squarrosa 2111 (= *T. tauschii*)	Germplasm Inst., CNR, Bari Italy	DD
Secale cereale	Lazio, Italy	RR
T. araraticum 2542	Germplasm Inst., CNR, Bari Italy	AABB
T. dicoccum MG4375	Germplasm Inst., CNR, Bari Italy	AABB
T. turgidum var. *durum* cv 'Modoc'	C. O. Qualset, Davis, CA, USA	AABB
T. aestivum cv 'Chinese Spring' (CS)	C. O. Qualset, Davis, CA, USA	AABBDD
T. aestivum cv 'Chinese Spring' × *D. villosum*, (CS × DV)	Senior Author	AABBDDVV
CS + DV disomic addition lines 1V, 2V, 4V, 5V, 6V, 7V	E. R. Sears, Columbia, MO, USA	

DNA cloning

Purified DNA was cleaved with *Bam* HI plus *Pst* I restriction enzymes and ligated into the *Bam* HI/*Pst* I site of pUC8. *Escherichia coli DH5 α* competent cells were transformed with the ligated hybrid plasmids and plated on LB medium containing 100 ng/ml ampicillin and 2 % of Bluo-Gal (BRL). White colonies were transferred to a similar medium and colony hybridization was performed according to GRUNSTEIN and OGNESS (1975) using Hybond-C (Amersham) filters and *D. villosum* genomic DNA which was ^{32}P-labelled by nick-translation according to RIGBY et al. (1977). After autoradiography, the colonies were divided in two groups: group I comprised colonies giving very low hybridization signal; group II comprised those giving very strong signals. From 200 colonies of group I, 5 were chosen for further characterization and were indicated as pDVA7, pDVA9, pDV11, pDV17, and pDV18 (henceforth pA7, pA9, p11, p17, and p18, respectively). From five colonies of group II, 1 (p2270) was chosen for further characterization.

Probe preparation

DNA probe. — Plasmids from the chosen colonies were isolated using the mini-preparation boiling method (SAMBROOK et al. 1989), and digested with *Bam* HI plus *Pst* I. The DNA was fractionated in 0.8 % low melting point agarose gel and gel portions containing the *D. villosum* inserts were excised from the gels, melted at 65°C for five minutes, and the DNA concentration was adjusted at approximately 25 ng/μl. Random sequence hexanucleotides were used to obtain ^{32}P-labelled probes with a specific activity of about 1×10^9 cpm/μg. The labelling was performed using the "Multiprime" Kit (Amersham). A plasmid containing a 2.7 kb portion of the nontranscribed intergenic spacer of *Triticum aestivum* ribosomal DNA (pTA250.4) was kindly provided by R. Appels (C.S.I.R.O., Canberra, Australia).

RNA probe. — *Vicia faba* 18S and 25S rRNA were prepared according to CARMONA et al. (1984) and labelled, using T4 polynucleotide kinase and ^{32}P (g)-ATP (Amersham) as described by MAIZELS (1976). The specific activities were 7×10^7 cpm/μg and 5×10^7 cpm/μg, respectively.

Southern blot hybridization

Genomic DNAs were digested with restriction endonucleases, fractionated in 1% agarose gel, and blotted onto Zeta-probe membrane(Biorad). The membranes were prehybridized in 5 × SSC, 6 × Denhardt, 0.05 M Tris, 0.4 % SDS, 10 mM EDTA, and 0.4 mg/ml heat-denatured salmon sperm DNA, at 62°C for 4 h. Heat-denatured ^{32}P labelled DNA was then added (1×10^8 cpm per ml of hybridization mixture) and allowed to hybridize for 12 h at 62°C. The membranes were then washed in 2 × SSC, 0.1 % SDS (2 × 5′, RT), 0.1 × SSC (1 × 5′, RT), 0.1 % SDS (1 × 5′, RT), 0.1 × SSC, 0.1 % SDS (1 × 15′, 62°C), and exposed to X-OMAT film for autoradiography. Probe stripping was performed soon after autoradiography by washing once in a large volume of 0.1 × SSC/0.5 % SDS at 95°C for 20 min. Filters were reprobed at least five times without losing hybridization efficiency.

Dot blot hybridization

One μg of DNA was spotted on Zeta-probe (Biorad) filters using the Minifold apparatus (Schleicher and Schuell). Filters were then dried at 80°C for two hours in a vacuum oven and hybridized as described for Southern blot hybridization.

In situ hybridization

Tips of colchicine-treated and fixed roots were squashed after treatment with a 5 % aqueous solution of pectinase (Sigma) as already described (CIONINI et al. 1985). Cytological hybridization was performed according to MACGREGOR and MIZUNO (1976). The DNA of the nuclei and chromosomes was denatured in 0.07 N NaOH for 3 min at room temperature and a highly repeated DNA sequence, which had been ^{3}H-labelled by nick-translation (RIGBY et al. 1977) to a specific activity of 3.5×10^6 cpm/μg, was used as hybridization at a concentration of 1 μg/ml. After incubation, unbound labelled DNA was removed by stringent washings (HENNEN et al. 1975) and the preparations were covered with Ilford L_4 emulsion. After exposure times ranging from one week to three months, the slides were developed and stained with Giemsa (Merck).

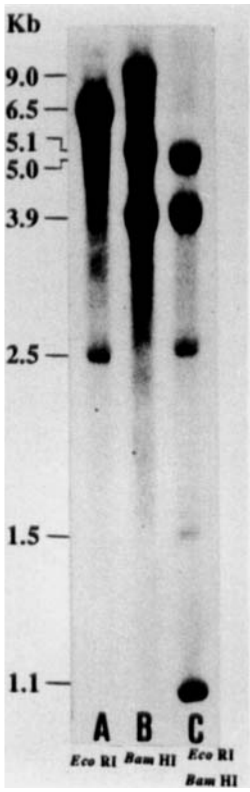

Fig. 1. Southern blots showing the rDNA restriction pattern observed after *D. villosum* DNA digestion with *Eco* RI (A), *Bam* HI (B), and *Eco* RI–*Bam* HI (C) and hybridization to ^{32}P-rRNA 25S. Size markers were lambda DNA cleaved with *Hind* III and *Eco* RI.

Results and discussion

Ribosomal DNA

When cleaved with *Eco* RI, *D. villosum* genomic DNA released two fragments of 6.5 kb and 2.5 kb, respectively, that hybridized with ^{32}P-labelled 25S rRNA (Fig. 1A). The same DNA produced three fragments of 9.0 kb, 5.1 kb, and 3.9 kb that hybridized to 25S rRNA probe upon cleavage with *Bam* H (Fig. 1B). After double digestion with *Eco* RI plus *Bam* HI, rDNA fragments of 5.0 kb, 3.9 kb, 2.5 kb, 1.5 kb, and 1.1 kb in size were found (Fig. 1C). The 1.5 kb fragment was very faint in Fig. 1C because it is an 18S rRNA fragment that cross-hybridizes to the 25S rRNA probe due to common sequence they may share as observed in other higher plants by MIASSOD and CECCHINI (1976). The 1.5 kb fragment gave a strong hybridization signal when the 18S rRNA was used as probe (Figure not shown).

On the basis of these results, and of those obtained after hybridization of the same digests with ^{32}P-18S RNA and ^{32}P-labelled pT A250.4 (not shown), the restriction map of the rDNA repeat of *D. villosum* was constructed (Fig. 2). It can be seen that each rDNA repeat has: (i) an average length of 9.0 kb; (ii) two *Eco* RI restriction sites, one located in the intergenic spacer (igs), the other in the 25S transcribed region, and (iii) one *Bam* HI restriction site in both 18S and 25S transcribed regions, which divide the rDNA repeat into two fragments (A and B, respectively). The appearance of a band matching the 9.0 kb rDNA fragment indicated that a *Bam* HI restriction site is methylated in about 50 % of the rDNA repeats. The double digestion with *Eco* RI plus *Bam* HI indicated that the methylated *Bam* HI site must be in the 25S rDNA region.

The restriction fragment pattern observed for *D. villosum* rDNA repeats is in accordance with that observed in *D. villosum* and other Triticeae species for the position of the *Eco* RI restriction sites and the length of the igs subrepeats (130 bp) (GILL and APPELS 1989).

However, the occurrence of the methylated *Bam* HI site was not reported previously.

Southern blots of the *Bam* HI digested genomic DNA of *D. villosum* extracted from single plantlets belonging to different populations followed by hybridization with ^{32}P-pTA 250.4 (Fig. 3), clearly showed that the length of the rDNA fragment A might differ between individuals, due to the variation in number of 130 bp subrepeats in the igs. Fragment A appeared as a single bond in certain individuals (phenotypes A, B, C, and D); or as two bands in other individuals (phenotypes G, H, I, and J). The double-banded phenotypes contained different combinations of fragment A in the single-banded phenotypes (Fig. 4).

Progeny testing of individuals showing different *Bam* HI phenotypes (not shown) suggested that the variability for rDNA fragment A was governed by one locus (*Nor-V1*), with four codominant alleles indicated as *Nor-V1a*, *Nor-V1b*, *Nor-V1c*, and *Nor-V1d* (Fig. 4). Each allele can be visualized as a cluster of highly homogeneous repeats, all having the same number of subrepeats in the igs. Eight out of ten phenotypes resulting from all the possible combinations of the four alleles have been actually observed (phenotypes A, B, C, D, G, H, I, and J of Fig. 3).

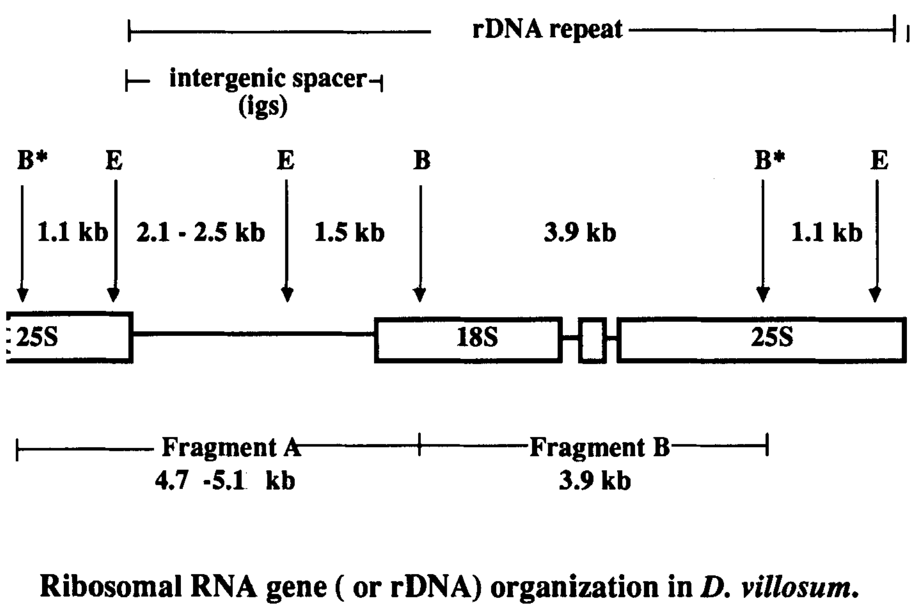

Fig. 2. rDNA organization in *D. villosum*. B = *Bam* HI; E = *Eco* RI. *: restriction site which may undergo cytosine methylation.

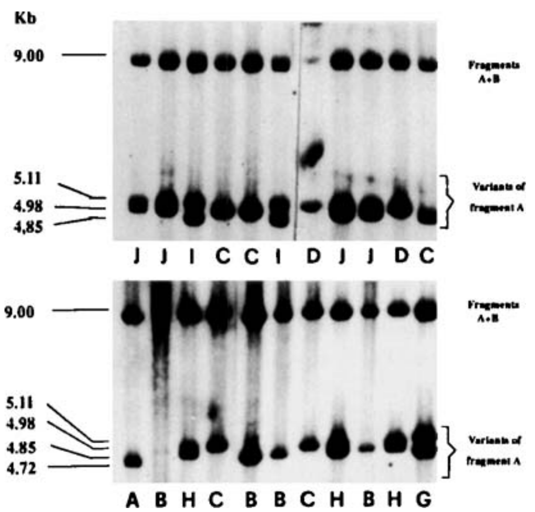

Fig. 3. Southern blots showing 8 (A, B, C, D, G, H, I, and J) different rDNA igs phenotypes observed in different *D. villosum* plants belonging to different populations. DNAs were digested with *Bam* HI restriction enzyme and hybridized to ^{32}P-pTA250.4. Molecular sizes are determined in relation to the size markers in Fig. 1.

A highly repeated DNA sequence

From a *D. villosum* genomic library (see Materials and methods), a clone was chosen which gave the strongest hybridization signal among those detected after colony hybridization to total *D. villosum* labelled DNA; hence, this clone was expected to contain a highly repeated DNA sequence. The insert was 2270 bp long (not shown) and *Sph* I and *Sac* I restriction enzymes were chosen to construct its restriction map. Agarose gel electrophoresis indicated that, upon *Sph* I cleavage, two bands containing fragments of 1090 bp and 380 bp, respectively, were released (Fig. 5). These two fragments were subcloned in T3/T7α 18 plasmid, and the chimeric constructs obtained were indicated as p1090 and p380, respectively. The *Sac* I digestion of the 2270 bp insert released four fragments of 760 bp, 510 bp, 450 bp, and 390 bp, respectively. The *Sph* I–*Sac* I double restriction released five fragments of 510 bp, 450 bp, 380 bp, 260 bp, and 120 bp (this last fragment was barely visible in ethidium bromide stained agarose gels). Southern blot hybridizations using the ^{32}P-labelled 2270 bp insert gave autoradiographic signal on all the restriction fragments; however, the 510 bp, 450 bp, and 390 bp *Sac* I fragments and the *Sac* I–*Sph* I 120 bp fragment gave very faint hybridization signals. Partially digested *Sac* I and *Sac* I–*Sph* I fragments were also revealed (dark dots in Fig. 5). When the same Southern blot was reprobed using ^{32}P-labelled 380 bp unit, the 510 bp and 450 bp fragments did not show any hybridization signal.

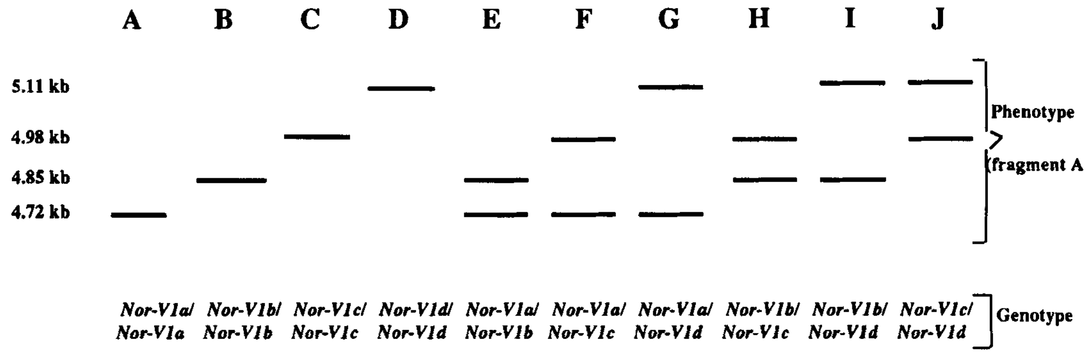

Fig. 4. Diagram of the phenotypes of rDNA fragment A and inferred genotypes at the *Nor-V1* locus of the *D. villosum* when all possible combinations of the *Nor-V1a*, *Nor-V1b*, *Nor-V1c*, and *Nor-V1d* alleles are considered.

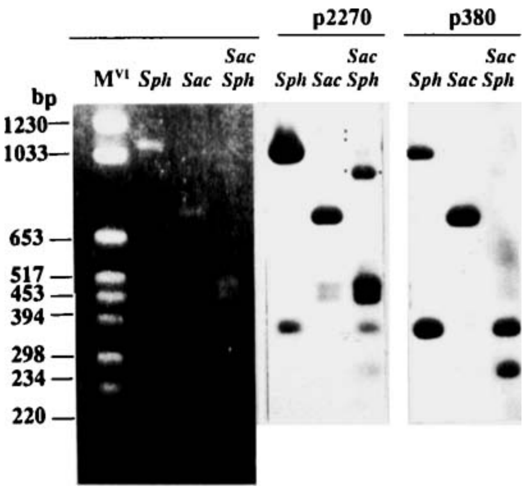

Fig. 5. Electrophoresis of the 2270 bp DNA fragment cleaved with *Sph* I, *Sac* I, and *Sph* I plus *Sac* I restriction enzymes and related Southern blots hybridized to ^{32}P-labelled p2270 or p380 clones. M^{VI} = pBR328 cleaved with *Bgl* I and *Hinf* I.

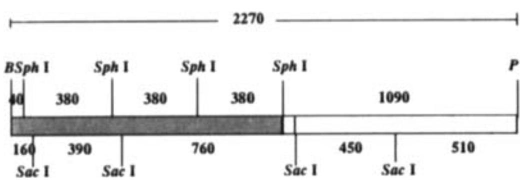

Fig. 6. Restriction map of the *D. villosum* insert in p2270 clone. B = *Bam* HI; P = *Pst* I; Numerals indicate fragment length in base pairs. ■: location of the 380 bp elements; □: region with partial homology to the 380 bp units.

On the basis of these findings, the restriction map reported in Fig. 6 was constructed. About half of the 2270 bp DNA element has an evident repeated structure, since it is composed by three 380 bp units on its left side ending with the *Bam* HI restriction site; the other half is represented by the *Sph* I–*Pst* I 1090 bp fragment.

Chromosomal distribution of the 380 bp repeated unit

Southern blots of partially digested genomic DNAs with *Sph* I were hybridized, using 380 bp or 1090 bp DNA elements (Fig. 7). Different *D. villosum* individuals showed difference in the length and abundance of the stretches of subrepeated 380 bp units. The same finding was obtained through the analysis of the presence of the 380 bp element in each *D. villosum* chromosome, which was possible using CS + DV disomic addition lines (because the 380 bp unit is virtually absent from the wheat genome). The 380 bp unit was absent in the chromosome 7V. In the octoploid amphiploid CS × DV the number of bands hybridizing to the 380 bp unit is lower than those in *D. villosum*. Using the 1090 bp fragment as a probe, hybridization on the DNA in the lower portion of the Southern blot was similar, though lighter than that observed using the 380 bp unit. This result indicates that the two DNA elements share partial nucleotide sequence homology. Additional hybridization signals (that were absent when the 380 bp unit was used) were observed for the DNA fragments of 1.60 kb and 3.22 kb, and very high molecular weight genomic DNA in both *D. villosum* and CS. This observation indicates that nucleotide sequences

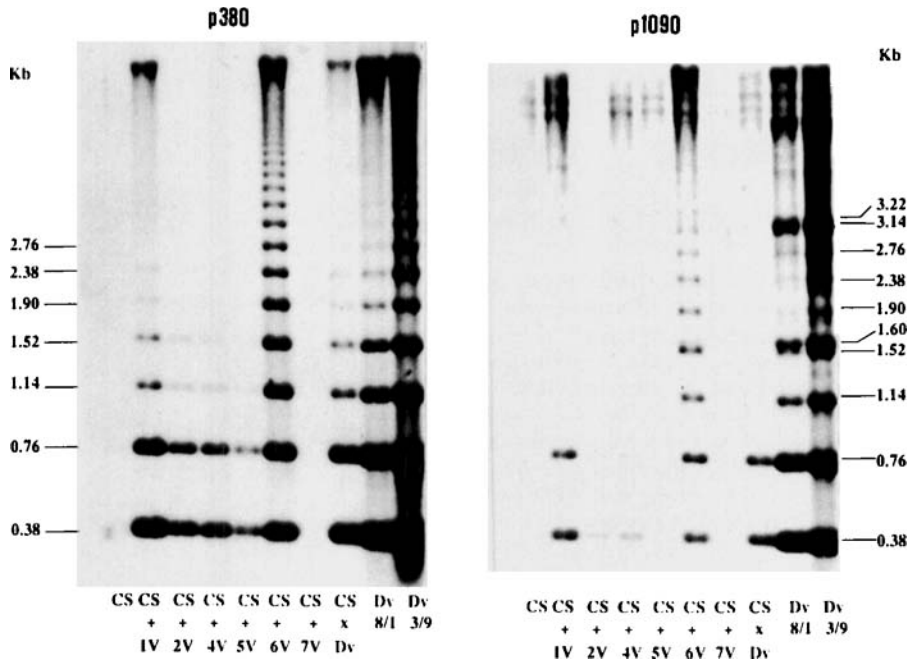

Fig. 7. Southern blots of *Sph* I digested genomic DNAs (1 g per lane) probed with ^{32}P-labelled p380 (a) or p1090 (b). Explanation for abbreviations and symbols as in Table 1. Molecular sizes were determined in relation to the markers in Fig. 5.

which share homology with the 1090 bp element of *D. villosum*, are present in CS.

The results obtained in Southern blots of the DNAs from CS + DV disomic addition lines (Fig. 7) were confirmed through cytological hybridization experiments. A metaphase plate after in situ hybridization with ^{3}H-labelled 380 bp DNA sequences is shown in Fig. 8. All chromosome pairs but one are substantially labelled at or near the telomeres. Though the seven chromosome pairs of *D. villosum* are rather similar to each other in length and shape and the chromosome morphology is unavoidably affected by the hybridization procedure, a careful analysis of a number of metaphase plates allows to state that the nonlabelled chromosome pair was the shorter one of the two subtelocentric pairs. In labelled pairs, silver grains occurred at both the telomeres with the exception of the longer subtelocentric pair: in its longer arm, the telomere was not labelled, whereas silver grains occurred at an intercalary position. Other labelling sites were observed, always near the telomeres, in the three submetacentric pairs, in both arms of the metacentric pair and in the longer, not satellited arm of the satellited pair. In this pair, also the satellite was labelled. Clear labelling over the background was not observed at or near the centromere in any chromosome. Apart from the nonlabelled pair and the centromeric or pericentromeric regions, the distribution of silver grains was similar to that of heterocrhomatin as

Fig. 8. Metaphase plate of *D. villosum* after in situ hybridization with ^{3}H-labelled 380 bp DNA sequences. Giemsa. × 1500.

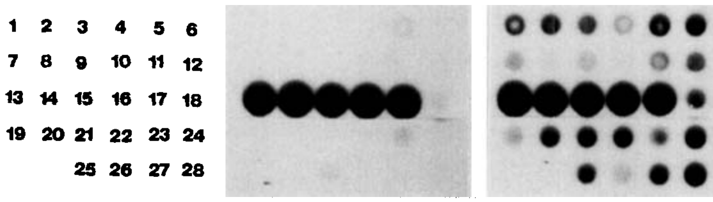

Fig. 9. Dot blot hybridization of genomic DNAs (0.5 µg per dot) from different Triticeae species to p380 and p1090 ^{32}P-labelled probes. Species with A genome: 1 through 3. *Triticum urartu* G1545; 4 and 5. *T. boeoticum* G1724; 6. *T. monococcum*. Species with D genome: 7 and 8. *Aegilops squarrosa*. Species with B genome: 9. *Ae. speltoides* 72; 10. *Ae. speltoides* G250; 11. and 12. *T. searsii*. Species with V genome: 13 through 17. *Dasypyrum villosum*. Species with R genome: 18. *Secale cereale*. Species with AB genomes: 19. *T. turgidum* var. *durum* cv 'Modoc'; 20 through 23. *T. araraticum* 2542; 24. *T. timopheevii* 1730; 27 and 28. *T. dicoccum* G375. Species with ABD genomes: 26. *T. aestivum* cv 'Chinese Spring'; 25. pTA 250.4 plasmid.

disclosed after C-banding (DONG et al. 1985; FRIEBE et al. 1987).

The same labelling pattern over the V chromosome was observed in the hexaploid amphiploid *D. villosum* × *Triticum turgidum* var. *durum* cv 'Modoc' (not shown). No clear localization of labelling was detectable, even after long times of exposure to photographic emulsion, when the 380 bp sequence was hybridized in situ to the chromosomes of rye and wheat.

^{32}P-labelled 1090 bp and 380 bp sequences were used as probes in dot blot hybridizations to genomic DNA from different Triticeae species with genomes closely (*T. boeoticum, T. urartu, T. monococcum, Ae. speltoides, T. searsii, Ae. squarrosa, T. araraticum, T. timopheevii, T. dicoccum, T. turgidum* var. *durum*, and *T. aestivum*) or distantly (*D. villosum* and *Secale cereale*) related to the A, B, and D genomes of cultivated wheats (Fig. 9). It was found that the 380 bp sequence is highly specific to the V genome, since it did not hybridize to the DNA of any species except *D. villosum*. On the other hand, the 1090 bp element gave hybridization signal on the DNA of all the species tested. The hybridization signal was very strong for *D. villosum*, as expected, and for *T. boeoticum, T. monococcum, T. timopheevii*, and *T. dicoccum*. Unlike the 380 bp subrepeat, the 1090 bp element seems to share nucleotide sequence homology with the wheat ribosomal igs in pTA 250.4.

It is worth noting the different hybridization signal from the DNA of different individuals of *Ae. squarrosa* or *Ae. speltoides*. This result suggests that the redundancy of the 1090 bp sequences may change in the genome of different individuals within a species.

Other DNA sequences

Five clones (pA7, pA9, p11, p17, and p18), randomly selected from our genomic *D. villosum* DNA library, were tested as potential RFLP probes.

The *D. villosum* genomic DNA of individual plantlets belonging to different populations was digested with *Eco* RI, *Hind* III, and *Taq* I restriction enzymes, and hybridized to the ^{32}P-labelled probes (Fig. 10).

A simple pattern with one or two bands per lane was observed when the genomic DNA was digested with *Eco* RI (Fig. 10A). These prominent bands showed polymorphism when the p11 probe was used.

The p18 probe revealed polymorphism for several minor bands; the presence of these bands indicates that this probe may have a core sequence related to moderately repeated elements.

The *Hind* III restriction enzyme left the majority of the genomic *D. villosum* DNA uncut (Fig. 10B). The fragments that hybridized to the pA9 and p11 probes were cut and a simple hybridization pattern with two prominent bands were revealed. The probe p11 revealed the same type of polymorphism detected in the genomic DNA digested with *Eco* RI, but the polymorphic fragments were shorter than those detected using *Eco* RI.

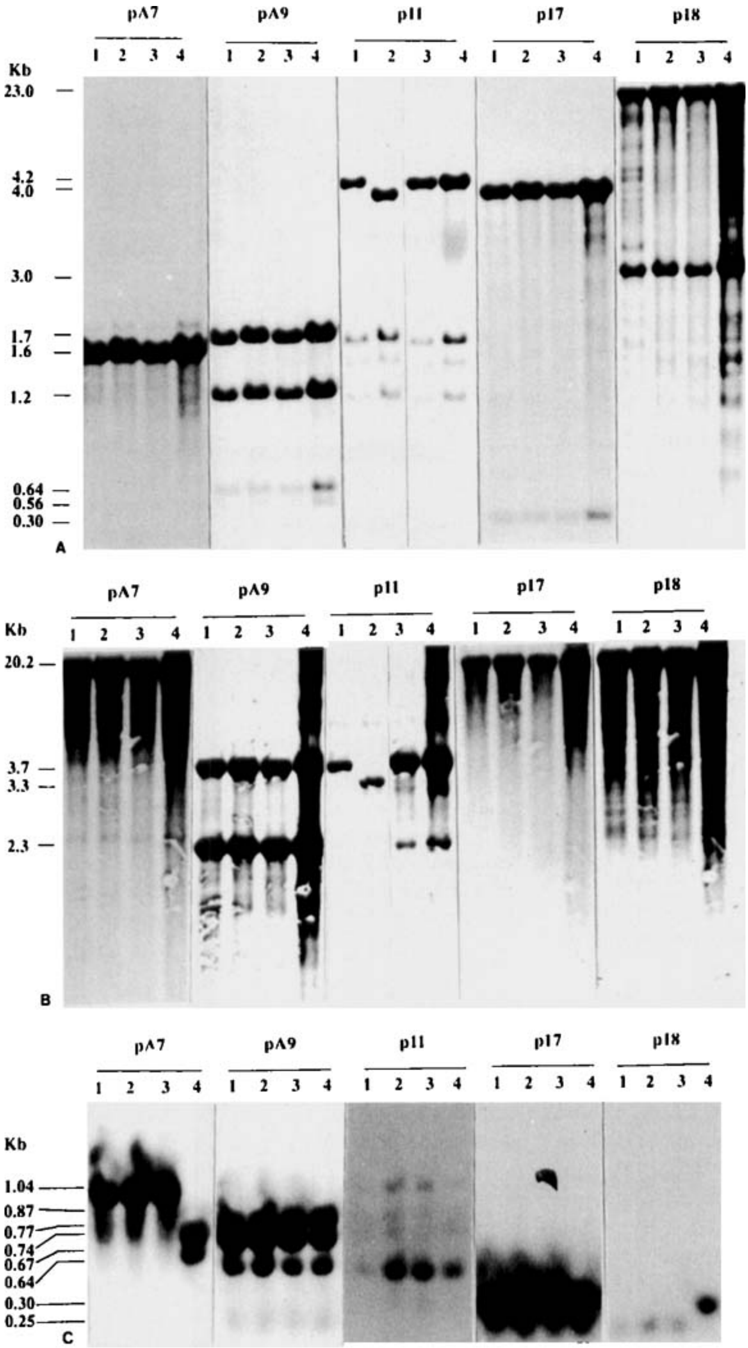

Fig. 10 A–C. Southern blots of digests with *Eco* RI (**A**), *Hind* III (**B**), and *Taq* I (**C**) of genomic DNAs (4 μg per lane) of selected individuals (1 = 3/1; 2 = 3/8; 3 = 3/9; 4 = 120/2; see Table 1) from different *D. villosum* populations. Hybridization probes were ^{32}P-labelled pA7, pA9, p11, p17, and p18.

Taq I restriction enzyme (Fig. 10C) was efficient in cleaving the *D. villosum* genomic DNA and produced fragments 250 to 1100 bp in length. A simple hybridization pattern with one to three prominent bands was observed. RFLP was revealed by pA7 and p18 probes, and in both cases the hybridizing genomic DNA fragments of an individual (120/2) were different in size from those of the other individuals indicated in Fig. 10C.

Even though the number of probes and enzymes used in the RFLP analysis was not high, three probes out of five (60 %) detected polymorphism, and every restriction enzyme used produced RFLP that was detected by at least one probe. Four out of 15 (27 %) of the probe-restriction enzyme combinations studied revealed polymorphism. The RFLP analysis of the disomic addition lines did not show any additional DNA fragments or fragments which were variable in length when compared to CS (Fig. 11). This situation occurred even though in CS × DV amphiploid there is a release of DNA fragments not present in the parental species. This may be explained recalling that the *D. villosum* genome used for producing the CS × DV amphiploids was different from that used for producing disomic addition lines. Therefore, the probes used here hybridized with defined DNA fragments of CS, CS + DV disomic addition lines, and *D. villosum*; the only difference being the intensity of the hybridization signal. The obscuring effect of DNA fragments in CS that are similar in size and hybridization ability to those of *D. villosum*, prevented the chromosomal location of the unique or moderately repeated *D. villosum* fragments inserted in our probes.

As shown in Fig. 12, a significant diversity among CS + DV disomic addition lines was evidenced for the intensity of the hybridization signal when the *Hind* III–pA9 combination was used. The disomic addition line CS + 1V, CS + 2V, and CS + 6V showed an autoradiographic signal as strong as that in *D. villosum* and CS × DV amphiploid for the two hybridizing DNA fragments. The other disomic addition lines (CS + 4V, CS + 5V, and CS + 7V) gave a faint autoradiographic signal as that in CS. As already noted for the p18 probe (Fig. 11), the probe pA9 most likely shares nucleotide sequence homology with repeated elements in DNA fragments that are particularly abundant on certain V chromosomes. This probe may be used to distinguish chromosome 1V, 2V, and 6V from the others.

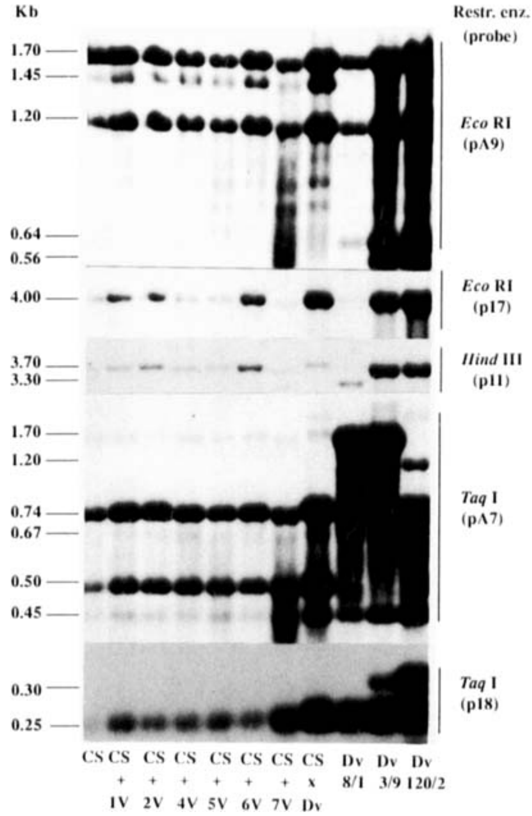

Fig. 11. Southern blots of genomic DNAs (4 μg per lane) cleaved with *Eco* RI, *Hind* III, and *Taq* I restriction enzymes and hybridized to probes that were ^{32}P-labelled pA7, pA9, p11, p17, and p18. Explanations for abbreviations and symbols are in Table 1. Molecular sizes are as in Fig. 7.

Conclusions

Our results increase the knowledge on molecular markers of the V genome of *D. villosum*. The characterization of the 380 bp species-specific repeats is reported for the first time in *D. villosum* and may not be related to other repeated DNA sequences already described in *D. villosum* (McIntyre et al. 1988). The 380 bp repeated unit, together with the repeated DNA element contained in the pA9 clone, may be used for chromosomal tagging. The use of these two units as probes can allow easy and simultaneous identification of chromosomes 1V, 2V, 6V, and 7V in different Triticeae genomic backgrounds. The restriction fragment length polymorphism found in the rDNA of *D. villosum* and for pA7 (using *Taq* I), p11 (using

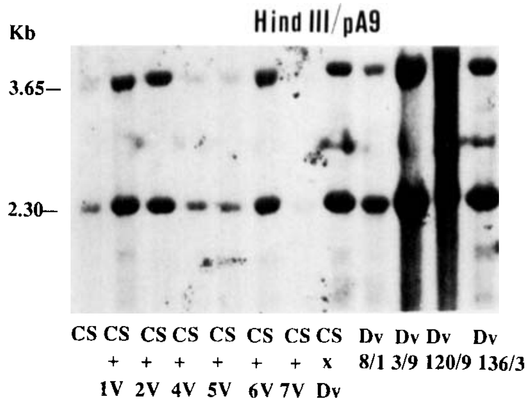

Fig. 12. Southern blots of genomic DNAs (4 μg per lane) cleaved with *Hind* III restriction enzyme and hybridized to ^{32}P-labelled pA9. Explanation for abbreviations and symbols are in Table 1. Molecular sizes are as in Fig. 7.

both *Eco* RI and *Hind* III) and p18 (using *Taq* I), may be helpful in population genetic studies. On the contrary, these probes cannot be used as markers in genetic linkage studies through disomic addition lines.

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