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Molecular and cytological characterization of repetitive DNA sequences in *Brassica*

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Summary. We isolated three different repetitive DNA sequences from *B. campestris* and determined their nucleotide sequences. In order to analyze organization of these repetitive sequences in *Brassica*, Southern blot hybridization and in situ hybridization with metaphase chromosomes were performed. The sequence cloned in the plasmid pCS1 represented a middle repetitive sequence present only in *B. campestris* and not detected in closely related *B. oleracea*. This sequence was localized at centromeric regions of six specific chromosomes of *B. campestris*. The second plasmid, pBT4, contained a part of the 25S ribosomal RNA gene, and its copy number was estimated to be 1,590 and 1,300 per haploid genome for *B. campestris* and *B. oleracea*, respectively. In situ hybridization with this sequence showed a clear signal at the NOR region found in the second largest chromosome of *B. campestris*. The third plasmid, pBT11, contained a 175-bp insert that belongs to a major family of tandem repeats found in all the *Brassica* species. This sequence was detected at centromeric regions of all the *B. campestris* chromosomes. Our study indicates that in situ hybridization with various types of repetitive sequences should give important information on the evolution of repetitive DNA in *Brassica* species.

Key words: *Brassica* – Repetitive DNA sequence – Nucleotide sequence – In situ hybridization

Introduction

Genomes of higher eukaryotes contain a large fraction of repetitive DNA sequences (Flavell 1980; Jelinek and Schmid 1982). In higher plants, a considerable variation in nuclear DNA contents among species is known (Bennet and Smith 1976) and the amount of repetitive DNA sequences is considered to contribute much of this variation (Flavell 1980; Flavell 1986). Types of repetitive DNA described in higher plants are highly repetitive DNA, which includes satellite DNA and middle repetitive DNA (Flavell 1980).

In situ hybridization of repetitive DNA sequences with metaphase chromosomes has been used to study the organization of these sequences at the chromosome level (Jones and Flavell 1982; Hutchinson and Lonsdale 1982; Jamieson et al. 1986; Ganal et al. 1988; Visser et al. 1988). These studies demonstrated that highly repetitive sequences are generally clustered at heterochromatic regions such as centromeres and telomeres. In contrast to highly repetitive sequences, chromosomal distribution of middle repetitive sequences, except for ribosomal RNA genes, has not been extensively studied in higher plants. In one instance, Ganal et al. (1988) showed that an interspersed middle repetitive sequence of tomato is present in most of the chromosomes throughout the entire length.

Repetitive DNA sequences of *Brassica* have not been extensively studied yet. A family of tandemly repeated sequences present in all the *Brassica* species has been identified (Halldén et al. 1987) and their nucleotide sequences have been recently reported (Lakshmikumaran and Ranade 1990). The basic monomeric unit of this sequence is 175–180 bp long and the estimated copy number in *B. campestris* is 1.45×10^5 per haploid genome (Halldén et al. 1987). No study dealing with chromosomal distribution of repetitive sequences in *Brassica* has been described, however. This is likely due to the small size of chromosomes in *Brassica*.

In this communication we report isolation and nucleotide sequences of three different repetitive DNA se-

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quences in *B. campestris*. To understand the organization of these sequences in *Brassica* genomes, we performed Southern blot analysis and in situ hybridization with these isolated repetitive sequences, and their results will be also described.

Materials and methods

Plant material

The *Brassica* species used in this study were *B. oleracea* var. *capitata* (cabbage), var. *italica* (broccoli), and var. *botrytis* (cauliflower); and *B. campestris* var. *pekinensis* (Chinese cabbage), var. *rapa* (turnip), and var. *perviridis* (Komatsuna). The *Arabidopsis thaliana* used was wild-type Columbia ecotype.

Isolation of plant DNA

Plant DNA was extracted from fresh leaves essentially by the procedure of Murray and Thompson (1980) and isolated by CsCl/EtBr centrifugation.

Isolation of repetitive DNA clones

The SalI- or TaqI-digested DNAs from *B. campestris* var. *pekinensis* were cloned into pUC19, and the ligated plasmids were transformed into *E. coli* JM109 or HB101. Recombinant plasmids were screened with the genomic DNA probes of *B. campestris* (Chinese cabbage) and *B. oleracea* (cabbage) by a standard colony hybridization procedure of Maniatis et al. (1982).

Southern blot hybridization

Restricted DNAs were electrophoresed in 0.7–1.0% agarose gels and blotted onto nylon membranes essentially by the procedure of Reed and Mann (1985). Hybridization was performed at 42 °C for 16 h in the solution containing 50% formamide, 5 × SSCP [1 × SSCP = 50 m*M* sodium phosphate (pH 6.8), 120 m*M* NaCl, 15 m*M* sodium citrate], 100 μg per milliliter of sheared and denatured salmon testis DNA, 0.5% nonfat dry milk (w/v), 10% dextran sulphate (w/v). For hybridization probes, inserts of the recombinant plasmids were isolated on a low-melting-point agarose gel and labeled by a random prime DNA labeling system (Amersham). The hybridized membrane was washed in 2 × SSC (1 × SSC = 150 m*M* NaCl, 15 m*M* sodium citrate) 0.1% SDS at 42 °C for 15 min three times and twice in 0.1 × SSC 0.1% SDS at 65 °C for 30 min.

Copy number estimation

Appropriate dilutions of genomic and plasmid DNA solutions were dot-blotted onto nylon membranes. Membranes were denatured in 0.5 *M* NaOH, 1.5 *M* NaCl, then neutralized in 1.5 *M* NaCl, 0.5 *M* Tris-HCl (pH 7.2), 1 m*M* Na_2EDTA. DNAs were fixed to membranes by UV irradiation. Conditions for hybridization and washing were as described for Southern blot hybridization. After development of an X-ray film, radioactivity of the hybridized DNA sequence was measured by a liquid scintillation counter. Comparison of the radioactivity between genomic DNA and plasmid DNA within a linear range of cpm/ng DNA was used to estimate the relative amount of hybridized DNA sequence per haploid genome. The genome size of *Brassica* species was taken from Bennet and Smith (1976).

DNA sequencing

All sequencing procedures followed the method described by Sanger et al. (1977). DNA sequences were analyzed using GENETYX program (Software Development).

Chromosome preparation

Plant material. Seedling roots of *B. oleracea* and *B. campestris* were used for chromosome preparation.

Pretreatment and fixation. Root tips (ca. 1 mm) were treated with a solution containing 0.02% colchicine and 7 μg/ml EtBr at 10 °C for 3.75 h, and the solution was replaced with ice-cold Farmer's solution (3:1 = ethanol:glacial acetic acid) and kept overnight at −20 °C.

Refixation. After removing Farmer's solution by a Pasteur pipette, root tips were washed with distilled water for 2–3 min. After three washes, the tissue was treated with an enzyme solution containing 4% cellulase "Onozuka" RS (Yakult), 1% pectolyase Y-23 (Seishin Pharmaceutical), 75 m*M* KCl, and 7.5 m*M* EDTA (Nishibayashi and Kaeriyama 1986) for 45 min at 37 °C. Dispersed root-tip cells were then washed with 0.05 *M* NaOH overnight for 1 day and spread on slides with forceps. Drops of Farmer's solution were occasionally added onto the slide to avoid desiccation of the cells. After air drying, the slides were kept for at least 2 weeks in a desiccator at room temperature before use for in situ hybridization. Approximately 50 slides were prepared for one experiment.

In situ hybridization

Denaturation of chromosomal DNA. A RNase solution (100 μg/ml RNaseA in 2 × SSC) was dropped (100 μl/slide) on a slide with dispersed root-tip cells, and a coverslip was placed on the slide and kept in a humid chamber for 1 h at 37 °C. Then the slide was washed twice in 2 × SSC for 5 min at room temperature, and chromosome preparations were dehydrated in an ethanol series (70%, 90%, and 100%) for 10 min each and air dried for at least 15 min. For denaturation of chromosomal DNA, the slides were placed in 70% (v/v) formamide in 2 × SSC at 70 °C. To determine an optimal length of time for DNA denaturation, several test slides were denatured for 1–4 min and then examined for the morphology of chromosomes. Immediately after denaturation of chromosomal DNA, chromosomal preparations were dehydrated in an ice-cold ethanol series (70%, 90%, 100%) and air dried for more than 15 min.

Purification of probe DNA. Probe DNA was separated by electrophoresis, electroeluted and purified by NenSorb 20 (Du Pont). Purified DNA was labeled with biotin-11-dUTP and biotin-7-dATP using a random prime labeling system (Amersham).

Hybridization. A solution containing biotin-labeled probe DNA (4 ng/μl biotinylated DNA, 50% formamide, 10% dextran sulphate, 0.6 μg/μl denatured carrier DNA in 2 × SSC) was dropped onto a slide (50 μl/slide) and a siliconized covership was placed on it. Hybridization was performed for 16–18 h at 37 °C, and the slide was washed as described by Rayburn and Gill (1985).

Detection of hybridization signals. Hybridization signals were detected by streptavidin-alkaline phosphatase conjugate by using NBT-BCIP as substrate (Blugene, BRL). A solution containing 1 μg/ml avidin-alkaline phosphatase in Buffer 1 as described in the protocol supplied by the manufacturer (150 m*M* NaCl, 100 m*M* Tris-HCl at pH 7.5) was dropped on the slides (150 μl/slide) and kept at room temperature for 10 min. Then the slides were washed twice in Buffer 1 for 15 min and washed in Buffer 3 (50 m*M* $MgCl_2$, 100 m*M* NaCl, 100 m*M* Tris-HCl pH 9.5) for 10 min. After the wash, 100 μl of a reaction solution containing the substrates was added onto the slide. After 1-h

incubation at room temperature, the slide was washed with Buffer 3, covered with a cover glass and observed by a phase contrast microscope (Nikon Optiphoto). For photographing, Fujichrome DX100 and Minicopy HRII were used.

Results

Isolation and sequence analysis of B. campestris repetitive DNA sequences

To isolate repetitive DNA sequences from *B. campestris*, approximately 1,100 recombinant plasmids were screened, and ca. 15% of the recombinants showed strong signals when total *B. campestris* nuclear DNA was used as a hybridization probe. We assumed that they contained middle or highly repetitive DNA sequences. First, we isolated those clones that gave an intense signal with *B. campestris* probe but showed a weak hybridization with *B. oleracea* probe. They were further tested for species specificity by Southern blot hybridization using total DNAs isolated from *B. campestris* and *B. oleracea* as probes. Thus, we isolated one clone, pCS1, and its insert was only present in *B. campestris*, but absent in *B. oleracea*. The size of the insert was 88 bp and its sequence is shown in Fig. 1 a. The sequence was 58% GC and consisted of two stretches of a repeated sequence (33 bp and 31 bp), and its related sequence and estimated copy number of the insert in haploid *B. campestris* genome was 1,680. Therefore, the *B. campestris*-specific repetitive sequence cloned in the plasmid pCS1 belongs to a middle repetitive sequence family. In addition to CS1, we isolated another sequence, CT10, which was 213 bp in length and contained three tandem repeats found in the CS1 sequence and unrelated flanking sequences (data not shown).

We cloned two other repetitive sequences, BT4 and BT11, which were hybridized with both *B. campestris* and *B. oleracea* nuclear DNAs. Sequence analysis of BT4 (Fig. 1 b) revealed that the 296-bp fragment showed 86% homology with a corresponding part of the 25S ribosomal RNA gene of rice (from base position 1,576 to 1,871; Takaiwa et al. 1985). Based on this sequence homology, we assumed that the plasmid pBT4 contained a part the *B. campestris* 25S ribosomal RNA gene. Our estimated copy number of this gene was approximately 1,600, 1,300, and 160 per haploid genome for *B. campestris*, *B. oleracea*, and *Arabidopsis thaliana*, respectively.

The other sequence, BT11, was 175 bp in length and its nucleotide sequence (Fig. 1 c) was highly homologous (90%) to a family of highly repetitive DNA described

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a
CS1  GTGGGTTGTC TGTTCAGTAC ACACAGGACG TCAGTGGGTT TCCGCCAGCA CACACAGGAC

     GTCTGTGGCT GTCCATGCTG TCCGTCAG

b
BT4  TCGAAAGGGG ATCCGGGTAA -AATTCCAGA ACCGGGACGT GGCGGTTGAC GGCAACGTTT
     -********A ***G**T*** G*T****C** G********* ********** ***G*****A

     GGGAGTTCCG GAGACGTTCG GCGGGAATTC CGGAAAGAGT TATCTTTTCT GTTTAACAGC
     **A**-**** ******-C** *****GGCCT ***G****** ********** *C*****G**

     CTGCCCACCC TGGAAACGGT TCAGCCGGAG GTAGGGTCCA GCTGCTGGAA GAGCACCGCA
     *C***A**** ********** ********** ********** **G**C**** **********

     CGTCGCGTGG TGTCCGGTGC ATTCCCGGCG G-CCTTGAAA ATCCGGAGGA CCGAGT-GCG
     *******C** ********** GCC******* *C******** ********** ******AC**

     CTCACG-CCG GTCGTACTCA TAACCGCATC AGGTCTCCAA GGTCAACAGC CTCTGGTCGA
     TC****C*** ********** ********** ********** ***G***GA* ******-*C*

c
BT11 ---------- ---------- ---------T CGATTGGAAC GACGAAGAAG -TGTCATATT
     AAGCTTCTTA CAAAGTGATT CATCCTGGT* T********* ********** C***GC****

     CCCAAACTGG GAAACTGGAA TCAACTGATT TGAAAGTGGG ATAAC-TCTT C-TTCCAA--
     ********** ********** ***C****** ********** *****T**** *A*C****CT

     TCTATGAGAT TTATTAAACT TCCTGGTCAT TCTCCACCAC TTTATGTATC CATGTCAAGC
     C********* *****C**** *******G** ********** ********** **AA******

     TTGTTACAAA GTTATTCATC CTGGTTCGA
     **-------- ---------- ---------
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Fig. 1 a–c. Nucleotide sequences of cloned repetitive DNA sequences isolated from *B. campestris*. **a** CS1, a repeated sequence, is underlined with a *bold line* and a related sequence is underlined with a *dotted line*. **b** BT4 sequence (upper) aligned with the nucleotide sequence of the rice 25S rRNA gene (lower; Takaiwa et al. 1985). **c** BT11 (upper) sequence aligned with a consensus sequence (lower) reported for a family of highly repetitive sequences from *B. campestris* (Lakshmikumaran and Ranade 1990). Homologous bases are represented by an *asterisk* and gaps with a *dash*

previously (Lakshmikumaran and Ranade (1990). Therefore, it is likely that we cloned a sequence that belongs to this predominant class of highly repetitive sequences. According to Halldén et al. (1987), its estimated copy number is 1.45×10^5 and 0.9×10^5 per haploid genome for *B. campestris* and *B. oleracea*, respectively.

Southern blot hybridization and in situ hybridization of B. campestris repetitive sequences

In order to understand the organization of the three repetitive sequences isolated, Southern blot analysis was performed with TaqI-digested DNAs isolated from three varieties of *B. campestris* (Chinese cabbage, turnip, Komatsuna) and three varieties of *B. oleracea* (cauliflower, broccoli, cabbage). *A. thaliana* was also included to examine differences in the sequence organization at the level of genus in Cruciferae. In situ hybridization was performed with metaphase chromosomes of *B. campestris* using each cloned repetitive sequence.

CS1: B. campestris-specific middle repetitive sequence

As our preliminary results suggested, the sequence in pCS1 was only hybridized with *B. campestris*, but not with *B. oleracea* or *A. thaliana* nuclear DNAs (Fig. 2a). Furthermore, minor differences in the hybridization pattern were detected between three varieties of *B. campestris* examined, suggesting that some degree of differentiation in this sequence family had taken place after each variety was generated.

Prior to the analysis of in situ hybridization, ten pairs of *B. campestris* chromosomes were classified into six groups according to their size after Giemsa-staining. In situ hybridization of CS1 with metaphase chromosome of *B. campestris* var. *pekinensis* (Chinese cabbage) exhibited strong hybridization signals at centromeric regions of three pairs of chromosomes (Fig. 2b). Because those three pairs of chromosomes with signals are in the different size groups, we concluded that CS1 was localized in three separate chromosomes of *B. campestris*. With respect to the chromosomal distribution, CS1 is distinct from a middle repetitive sequence (TGRIII) of tomato, which is shown to be localized at all the chromosomes along the entire length (Ganal et al. 1988). Furthermore, two pairs of chromosomes in groups III and IV that were not distinguished by their size could be differentiated by in situ hybridization.

BT4: 25S ribosomal RNA gene

In contrast to the Southern blot with CS1 probe, BT4 was hybridized with one major band (296 bp) in all three

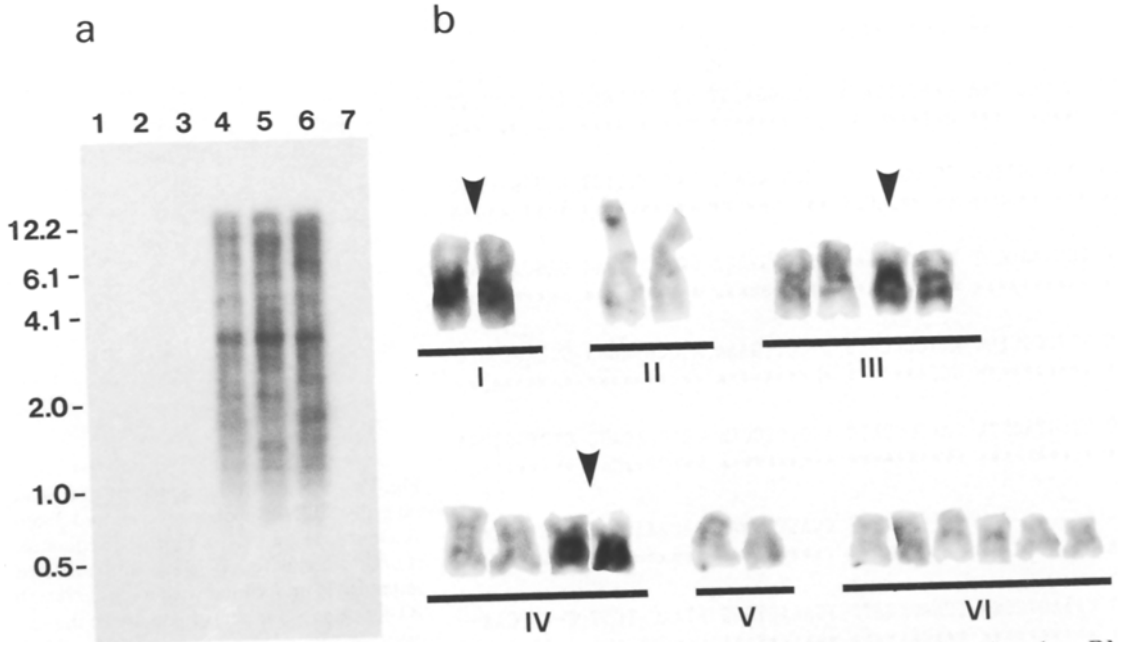

Fig. 2a and b. Southern blot analysis and in situ hybridization with CS1. **a** A Southern blot of nuclear DNAs isolated from *B. oleracea*, *B. campestris*, and *Arabidopsis thaliana*. *Lane 1: B. oleracea* var. *botrytis* (cauliflower); *lane 2: B. oleracea* var. *italica* (broccoli); *lane 3: B. oleracea* var. *capitata* (cabbage); *lane 4: B. campestris* var. *pekinensis* (Chinese cabbage); *lane 5: B. campestris* var *rapa* (turnip); *lane 6: B. campestris* var. *perviridis* (Komatsuna); *lane 7: A. thaliana*. Nuclear DNAs were digested with TaqI and hybridized with CS1. Numbers at the *left* are kilobase. The 1-kb ladder (BRL) was used as molecular weight markers. **b** In situ hybridization of *B. campestris* chromosomes with CS1. *Arrows* indicate hybridizing signals. Chromosome groups I–VI were determined according to the size of Giemsa-stained chromosomes

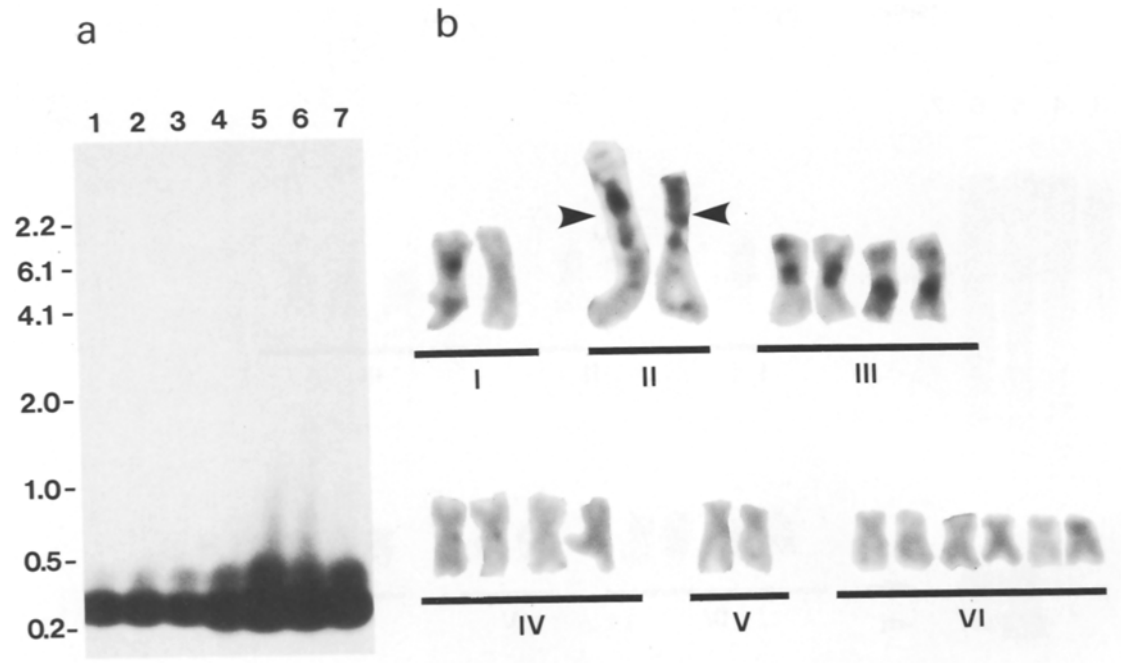

Fig. 3a and b. Southern blot analysis and in situ hybridization with BT4. **a** A Southern blot of nuclear DNAs isolated from *B. oleracea*, *B. campestris*, and *Arabidopsis thaliana*. *Lane 1: B. oleracea* var. *botrytis* (cauliflower); *lane 2: B. oleracea* var. *italica* (broccoli); *lane 3: B. oleracea* var. *capitata* (cabbage); *lane 4: B. campestris* var. *pekinensis* (Chinese cabbage); *lane 5: B. campestris* var *rapa* (turnip); *lane 6: B. campestris* var. *perviridis* (Komatsuna); *lane 7: A. thaliana*. Nuclear DNAs were digested with TaqI and hybridized with BT4. Numbers at the *left* are kilobase. The 1-kb ladder (BRL) was used as molecular weight markers. **b** In situ hybridization of *B. campestris* chromosomes with BT4. *Arrows* indicate hybridizing signals. Chromosome groups I–VI were determined by the size of Giemsa-stained chromosomes

species examined (Fig. 3a). In addition to the 296-bp band, a larger fragment was also hybridized with the BT4 probe. The relative intensity of this additional band to the main 296-bp band varied among the three species. In *B. oleracea* this band was faint, while a stronger signal was found in *B. campestris* and *A. thaliana*. The results of DNA blot confirmed that rDNA is strongly conserved in Cruciferae species.

In situ hybridization with the sequence in the plasmid pBT4 revealed a clear hybridization at the NOR region found in the second largest chromosome of *B. campestris* (Fig. 3b). Faint signals were also found in three other chromosomes. Because these signals were repeatedly observed in our experiments, it appeared that those chromosomes contained some sequences related to the 25S ribosomal RNA gene.

BT11: a major class of highly repetitive DNA sequence

Southern blot hybridization with the sequence cloned in the plasmid pBT11 exhibited a ladder pattern in all the *Brassica* materials tested, which is typical of a tandemly repeated sequence family (4a). Our result of Southern hybridization was similar to that of the DNA blot of *Brassica* species probed with the corresponding repetitive DNA isolated from *B. napus* (Halldén et al. 1987). However, a different hybridization pattern was obvious at low-molecular-weight DNA between *B. campestris* and *B. oleracea*. *B. campestris* showed two prominent bands of approximately 350 bp and 200 bp in length, while such hybridization was completely absent in *B. oleracea*. This suggested that there is loss of restriction sites in *B. oleracea* DNA, which gives rise to these two fragments of discrete size. This sequence was not, however, detected in *A. thaliana,* which belongs to a different genus in Cruciferae.

In situ hybridization showed that this repetitive sequence is distributed in all the chromosomes of *B. campestris* (Fig. 4b). In large chromosomes (group I–III), the sequence appeared to be mainly localized at the centromeric regions of the chromosomes, while in smaller chromosomes (group IV–VI), end of the chromosomes were also hybridized with the sequence. Intensity of the signal in individual chromosomes was relatively uniform. This might suggest that a similar number of repeats is present in each chromosome whose size varies considerably.

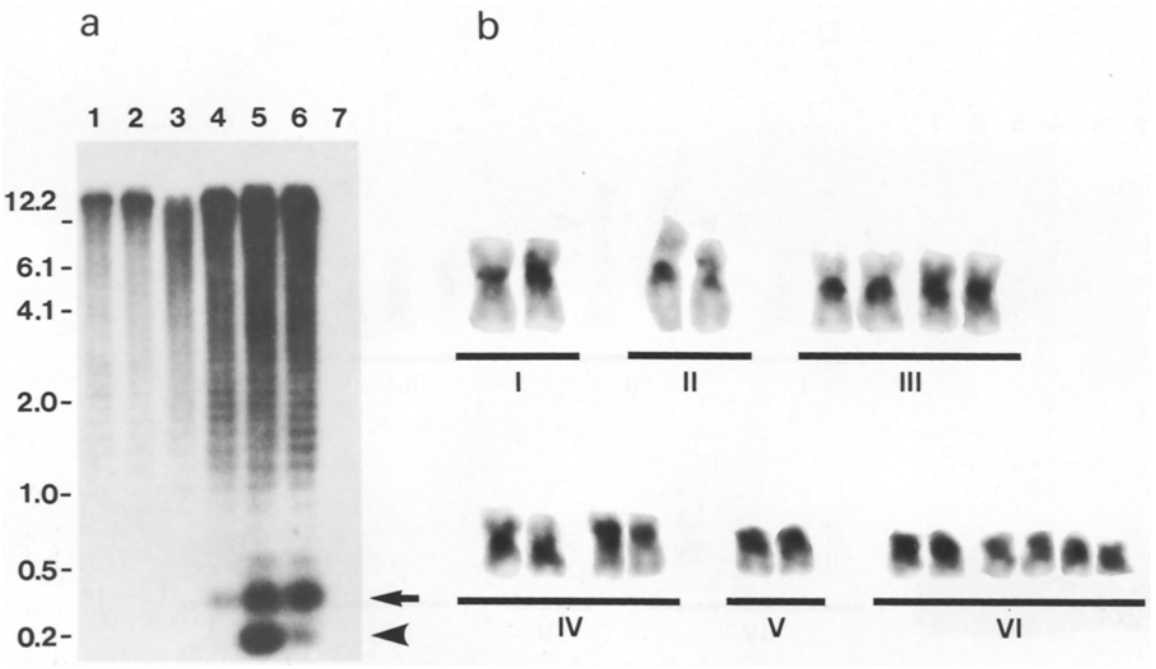

Fig. 4a and b. Southern blot analysis and in situ hybridization with BT11. **a** A Southern blot of nuclear DNAs isolated from *B. oleracea*, *B. campestris*, and *Arabidopsis thaliana*. *Lane 1: B. oleracea* var. *botrytis* (cauliflower); *lane 2:* B. oleracea var. *italica* (broccoli); *lane 3: B. oleracea* var. *capitata* (cabbage); *lane 4: B. campestris* var. *pekinensis* (Chinese cabbage); *lane 5: B. campestris* var *rapa* (turnip); *lane 6: B. campestris* var. *perviridis* (Komatsuna); *lane 7: A. thaliana*. Nuclear DNAs were digested with TaqI and hybridized with BT11. Numbers at the *left* are kilobase. The 1-kb ladder (BRL) was used as molecular weight markers. An *arrow* indicates a fragment of 350 bp and an *arrowhead* shows a fragment of 200 bp. **b** In situ hybridization of *B. campestris* chromosomes with BT11. Chromosome groups I–VI were determined by the size of Giemsa-stained chromosomes

Table 1. Characteristics of repetitive DNA sequences isolated from *B. campestris*

Clone	Insert size (bp)	Estimated copy no. in *B. campestris*	Characteristics	No. *B. campestris* chromosomes with signals
CS1	88	1,680	*B. campestris*-specific middle repetitive DNA	6
BT4	296	1,590	A part of 25S rRNA gene	2
BT11	175	1.45×10^5 [a]	Tandemly repeated [a, b] sequence family	20

[a] Halldén et al. (1987)
[b] Lakshmikumaran and Ranade (1990)

Discussion

We have isolated three different types of repetitive DNA sequences from *B. campestris* and have studied their sequences, copy number, patterns in Southern hybridization, and chromosomal distribution by in situ hybridization. The results are summarized in Table 1.

The repetitive sequence CS1 is uniquely present in *B. campestris*. This is a middle repetitive sequence and its sequence contained repeats of 29 bp. Species-specific repetitive sequences were used to identify hybrids produced by cell fusion (Saul and Potrykus 1984). In most cell fusion studies, the two parents used are remotely related. Hence, it is rather easy to identify such sequences that are present in one species but absent in the other parent. In our study, the species specificity of CS1 was demonstrated by its absence in a closely related species, *B. oleracea*. These two species are very closely related (Palmer et al. 1983). For instance, the nucleotide sequence of a highly repetitive sequence belonging to a major class of repetitive sequence family in *B. campestris* shows 98% homology with that of *B. oleracea* (Lakshmikumaran and Ranade 1990). In view of the close relationship between these two species, CS1 is likely to be present only in the *B. campestris* genome among *Brassica* species, except for *B. napus*, which is an amphidiploid consisting of *B. campestris* and *B. oleracea*. It is interesting to note that this *B. campestris*-specific repetitive sequence be-

longs to a family of middle repetitive sequences and is localized in centromeric regions of several specific chromosomes (Fig. 2b). We applied in situ hybridization with somatic hybrids of *B. campestris* and *B. oleracea* using the *B. campestris*-specific DNA probes, and demonstrated that some *B. campestris* chromosomes are missing in the hybrids (Itoh et al. 1990), which otherwise cannot be studied.

Three lines of evidence indicate that BT4 is a part of the 25S ribosomal RNA gene. First, its nucleotide sequence is 86% homologous to the sequence of rice 25S ribosomal RNA gene (Takaiwa et al. 1985). Second, it exhibited a hybridization pattern on Southern blots characteristic of rDNA (Kavanagh and Timmis 1986). Third, the sequence was hybridized with the NOR region of the chromosome (Fig. 3b). Our estimated copy number of BT4, hence of the rRNA gene, was ca. 1,600 per haploid *B. campestris* genome. This number is relatively small among plant species, but is comparable to those in species that have a small genome size, such as soybean and mung bean (Rogers and Bendich 1987).

The sequence analysis showed that one of the repetitive sequences we isolated (BT11) is actually a family of a highly repetitive sequence that makes up a considerable part of the genome in most of the species belonging to the family Cruciferae (Halldén et al. 1987; Lakshmikumaran and Ranade 1990). For example, this sequence family makes up 4.2% and 2.3% of the genome of *B. campestris* and *B. oleracea*, respectively (Halldén et al. 1987). However, our Southern blot analysis indicated that this sequence is not present in *A. thaliana*. In situ hybridization revealed that this sequence family is localized in all the chromosomes, specifically at the centromeric region (Fig. 4b).

In situ hybridization with repetitive DNA sequences described in this investigation should be useful in studying evolutionary changes that occur in various *Brassica* species at the chromosome level. For instance, the repetitive sequence cloned in pCS1 is unique to *B. campestris* and is likely to be found in *B. napus* because one of its progenitor species is *B. campestris*. In situ hybridization of *B. napus* chromosomes with CS1 should yield interesting information concerning possible changes that have taken place after *B. napus* was synthesized.

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