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A new family of dispersed repeats from *Brassica nigra*: characterization and localization

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Abstract The 459-bp *Hin*dIII (pBN-4) and the 1732-bp *Eco*RI (pBNE8) fragments from the *Brassica nigra* genome were cloned and shown to be members of a dispersed repeat family. Of the three major diploid *Brassica* species, the repeat pBN-4 was found to be highly specific for the *B. nigra* genome. The family also hybridized to *Sinapis arvensis* showing that *B. nigra* had a closer relationship with the *S. arvensis* genome than with *B. oleracea* or *B. campestris*. The clone pBNE8 showed homology to a number of tRNA species indicating that this family of repeats may have originated from a tRNA sequence. The species-specific 459-bp repeat pBN-4 was localized on the *B. nigra* chromosomes using monosomic addition lines. In addition to the localization of pBN-4, the chromosomal distribution of two other species-specific repeats, pBN34 and pBNBH35 (reported earlier), was studied. The dispersed repeats pBN-4 and pBNBH35 were found to be present on all of the chromosomes, whereas the tandem repeat pBN34 was localized on two chromosomes.

Key words *Brassica* species · Repetitive DNA sequences · Species-specific · Phylogenetic studies · Addition lines · *Sinapis arvensis*

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Introduction

Plant genomes, like those of other eukaryotes, contain different types of repeat sequences. Some are arranged in tandem (Hallden et al. 1987; Koukalova et al. 1989), whereas others are dispersed in the genome (Tsai and Strauss 1989; Tamhankar et al. 1990; Zhao and Kochert 1992; Mao et al. 1994). Of these repeated DNA sequences, many are common to genomes of related species whereas some are genome- or species-specific (Zhao et al. 1989; Fabijanski et al. 1990; Shepherd et al. 1990; Crowhurst and Gardner 1991; Gupta et al. 1992). The species-specific sequences have been used for analyzing interspecific and intergeneric hybrids (Schweizer et al. 1988; Pehu et al. 1990). Repeated DNA sequences localized in discrete regions of certain chromosomes have been used as chromosome-specific markers (Ganal et al. 1988; Visser et al. 1988; Francis et al. 1995) and also for analyzing chromosome addition lines (Hosaka et al. 1990; McGrath et al. 1990). Hellens et al. (1993) have shown that dispersed repeat sequences can be used to generate markers linked to a trait of interest.

Repetitive DNA sequences have been used to study phylogenetic relationships between species belonging to the same family. In the family *Brassicaceae*, the 177-bp repeat family has been extensively studied and been shown to be present in all *Brassica* species except *B. nigra* and *B. tournefortii* (Hallden et al. 1987; Lakshmikumaran and Ranade 1990). Similarly, a 496-bp dispersed repeat, pBNBH35, and the 348-bp tandem repeat, pBN34, from *B. nigra* have been shown to be species-specific (Gupta et al. 1990, 1992). Both these repeats do not hybridize with either *B. campestris* or *B. oleracea*, indicating that *B. nigra* is different from the other two diploid species in the *Brassica* (U) triangle (U 1935).

In this study, two members of a dispersed repeat family specific to the *B. nigra* genome were identified and characterized, and the repeats were then used to study phylogenetic relationships among crucifers. The chromosomal distribution of one of these repeats and two other previously characterized repeats, pBN34 and pBNBH35 (Gupta

et al., 1990, 1992), was studied using monosomic addition lines.

Materials and methods

DNA extraction

Leaf material of the various *Brassica* species and the wild relatives was collected from field-grown plants. For chromosomal localization of the B genome-specific markers, five of the eight possible addition lines corresponding to each of the *B. nigra* chromosomes, previously characterized by Chevre et al. (1991), were used. Total DNA was extracted by the method of Dellaporta et al. (1984). *B. nigra* cv 'IC257' nuclear DNA was prepared from frozen leaves according to the method of Malmberg et al. (1985). DNA samples were purified on a cesium chloride density gradient.

Construction of a partial *B. nigra* library in pUC19 vector

For cloning of the repetitive DNA sequences, nuclear DNA of *B. nigra* cv 'IC257' was restricted with *Hin*dIII and fractionated on a 0.8% low melting point agarose gel. Fragments in the range of 0.3 to 1 kb were eluted out from the gel and ligated to *Hin*dIII-linearized and dephosphorylated pUC19 plasmid. *E. coli* NM522-competent cells were transformed with the ligation mixture. Ampicillin resistant white colonies were selected at random. Plasmid DNAs were isolated according to the method of Birnboim (1983), and the clones were screened with nuclear DNA as a probe for the identification of repetitive DNA sequences.

Construction of a partial *B. nigra* library in Lambda Zap II

Nuclear DNA of *B. nigra* cv 'IC257' was restricted with *Eco*RI and fractionated on a sucrose density gradient. Fractions containing DNA fragments in the size range of 1 to 6 kb were ligated to Lambda Zap II (Stratagene) *Eco*RI arms. The ligated DNA was packaged using standard protocols (Gigapack II Stratagene). The repeated DNA clone, isolated and characterized from the *Hin*dIII plasmid library, was used as a probe for screening the lambda Zap II library. Few positive plaques were polymerase chain reaction (PCR)-amplified using the forward and reverse primers following the protocol of Innes et al. (1990) with minor modifications. The amplified sample was purified and subcloned in the pGEM-7zf plasmid vector. The ligation mixture was used to transform *E. coli* NM522-competent cells.

Southern blotting and hybridization

Genomic DNA was digested with the various restriction enzymes as recommended by the manufacturers and according to Maniatis et al. (1982). The digested samples were fractionated by electrophoresis on 1.0% agarose gels. DNA was transferred onto nitrocellulose membranes according to the procedure of Southern (1975); hybridizations were carried out according to the methods of Lakshmikumaran et al. (1985). Prehybridization of the filters was done at 60°C for 6–14 h followed by hybridization to probes labelled with a-[^{32}P]dCTP (from Bhabha Atomic Research Centre, Bombay). High-stringency washings were done twice in 2×SSC followed by one wash with 3 m*M* TRIS; for low-stringency conditions only two washes of 2×SSC were done. All washings were carried out at room temperature for 20 min each. After washing, the filters were exposed at –70°C using intensifying screens.

Sequence analysis

Double-stranded plasmid DNA was sequenced by the dideoxy chain termination method modified by Kraft et al. (1988). Polyacrylamide gels (5%) were used to fractionate the reaction products. Exonuclease III deletion clones of pBNE8 were generated using the 'Erase-a-base' system (Promega). The sequence of the overlapping clones was merged together in order to obtain the complete sequence. Sequence analysis was carried out using the DNASIS software from LKB (Sweden). In order to check homology with reported sequences, we conducted searches using the EMBL sequence database.

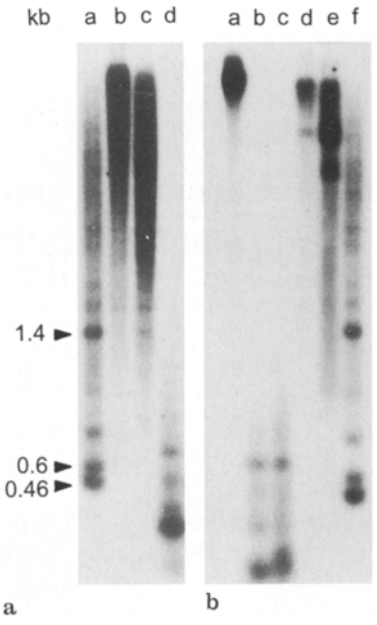

Fig. 1 Hybridization pattern of *B. nigra* clone pBN-4. **a** Five micrograms *B. nigra* DNA was digested with *Hin*dIII (*lane a*), *Bam*HI (*lane b*), *Eco*RI (*lane c*) and *Hinf*I (*lane d*). **b** Five micrograms *B. nigra* DNA was digested with *Dpn*I (*lane a*), *Mbo*I (*lane b*), *Sau*3AI (*lane c*), *Hpa*II (*lane d*), *Msp*I (*lane e*) and *Hin*dIII (*lane f*) and probed with pBN-4

Results

Isolation and characterization of the repeat from *B. nigra*

The *Hin*dIII library of *B. nigra* cv 'IC257' constructed in the plasmid vector pUC19 was screened using *B. nigra* nuclear DNA as a probe. Many of the recombinant clones produced strong hybridization signals which enabled us in identifying clones containing highly repeated DNA sequences (Saul and Potrykus 1984). A number of these clones were screened for their species-specificity by hybridizing with *B. campestris* and *B. oleracea* DNA. One clone, pBN-4, showed no cross-hybridization (discussed in later section). This clone was further characterized and sequenced.

The organization of the clone pBN-4 in the *B. nigra* genome was studied by digesting *B. nigra* total DNA with different restriction enzymes and then probing it with pBN-4. The hybridization pattern obtained is shown in Fig. 1a. The *Hin*dIII digest showed the presence of prominent bands at 0.46 kb, 0.60 kb and 1.40 kb (Fig. 1a, lane a). Smears were obtained in the *Bam*HI and *Eco*RI lanes

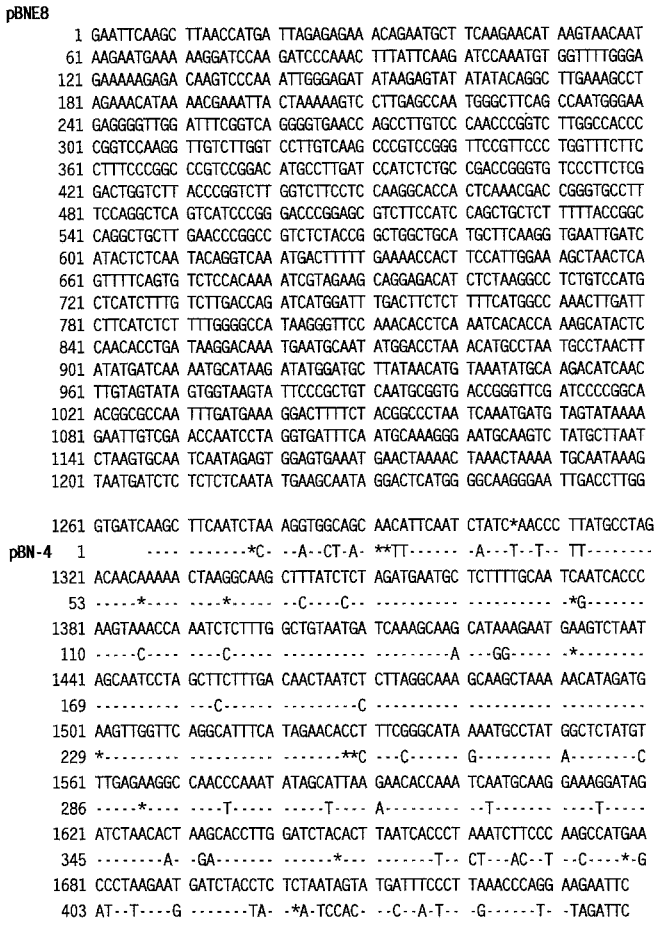

Fig. 2 The nucleotide sequences of the cloned repeats pBNE8 and pBN-4 from *B. nigra* in the 5′ to 3′orientation. A *dash* (-) indicates a similarity to the sequence of clone pBNE8, whereas an *asterisk* (*) denotes a deletion. The nucleotide sequences reported here appear in EMBL nucleotide sequence database under the accession number X89901 (pBNE8), X67835 (pBN-4)

in the high-molecular-weight region (Fig. 1a, lanes b, c), whereas with *Hin*fI the presence of a low-molecular-weight band (Fig. 1a, lane d) was revealed. The restriction pattern clearly indicated pBN-4 to be a dispersed repeat. In order to further confirm the dispersed nature of pBN-4, we carried out a partial time-course restriction of the nuclear DNA. Upon probing with pBN-4, the signals remained either at the top of the gel or were restricted to bands at 0.46 kb, 0.6 kb and 1.4 kb (not shown).

Isoschizomers were used to investigate the methylation pattern of pBN-4 repetitive DNA. Of the isoschizomers *Mbo*I, *Sau*3AI and *Dpn*I, the first two gave almost similar hybridization patterns, whereas with the latter most of the signal was localized at the top, indicating that *Dpn*I does not restrict at all. (Fig. 1b, lanes a, b and c, respectively). Isoschizomers *Msp*I and *Hpa*II showed different hybridization patterns. *Msp*I cut more frequently than *Hpa*II (Fig. 1b, lanes d, e), indicating methylation of some of the inner C residues of the site 5′-CCGG-3′.

In addition to the prominent bands obtained at 0.46 kb, 0.6 kb and 1.4 kb when pBN-4 was used as a probe on the *Hin*dIII digests of *B. nigra* DNA, faint bands also were obtained in the higher-molecular-weight region. This indicated that pBN-4 was also present as a larger-size repeat family. To isolate the larger-size family of the pBN-4 repeat, we constructed a partial library of *B. nigra* cv 'IC257' in lambda ZapII vector. This library was screened using pBN-4 as a probe. A few of the positive plaques obtained were PCR-amplified, and the inserts were subcloned in vector pGEM-7zf(+). One of the clones obtained, pBNE8, was selected for further characterization. When pBNE8 was used as a probe on nuclear DNA digested with different restriction enzymes, a pattern similar to that of pBN-4 was obtained, thereby indicating that both pBN-4 and pBNE8 may be members of the same repeat family.

Sequence analysis

The clone pBN-4 was sequenced in both orientations and was found to be 459 bp in length with an AT content of 61% (Fig. 2). The repeat pBN-4 contained a number of direct and inverted repeats with the longest direct repeat being 10 bp in size. The clone pBNE8 was also sequenced and was found to be 1732 bp in length (Fig. 2); it also showed the presence of a number of direct repeats. Comparisons of the pBN-4 sequence with the pBNE8 sequence showed a homology of 83.6% in the 475-bp region extending from nucleotide position 1267 to the nucleotide position 1732 in pBNE8 (Fig. 2). Upon comparison with sequences in the EMBL database pBN-4 did not reveal any homology with any of the sequences, suggesting that this sequence was a new family of dispersed repeats. On the other hand, pBNE8 showed 71% homology from nucleotide position 957 to nucleotide position 1029 with a putative Asp-tRNA from soybean (Waldron et al. 1985). The portion of pBNE8 showing homology to a putative Asp-tRNA lies upstream of the region that is homologous to pBN-4. Sequence comparison of pBNE8 with Asp-tRNA is shown in Fig. 3a. pBNE8 also showed high homologies with the Asp-tRNA from rat liver and a tRNA from the prokaryote *Bacillus subtilis*. Transfer RNAs are known to have secondary/folded structures. Similarly, pBNE8 also folded into a tRNA-like structure (Fig. 3B) to give homologous nucleotides located in similar regions as in the soybean Asp-tRNA (Waldron et al. 1985). The intragenic promoter residues (Box A and Box B, Fig. 3A) are conserved in the sequence pBNE8 and are located in the same region as in the putative Asp-tRNA. The invariant and semi-invariant residues typical of all tRNA molecules are encircled.

Determination of species-specificity

In order to determine the species-specificity of pBN-4, several species of *Brassica* and related crucifers were restricted with *Hin*dIII, Southern blotted and hybridized with pBN-4 (Fig. 4). Among the different *Brassica* species, the repeat pBN-4 hybridized to *B. nigra* cv 'IC257' but not to

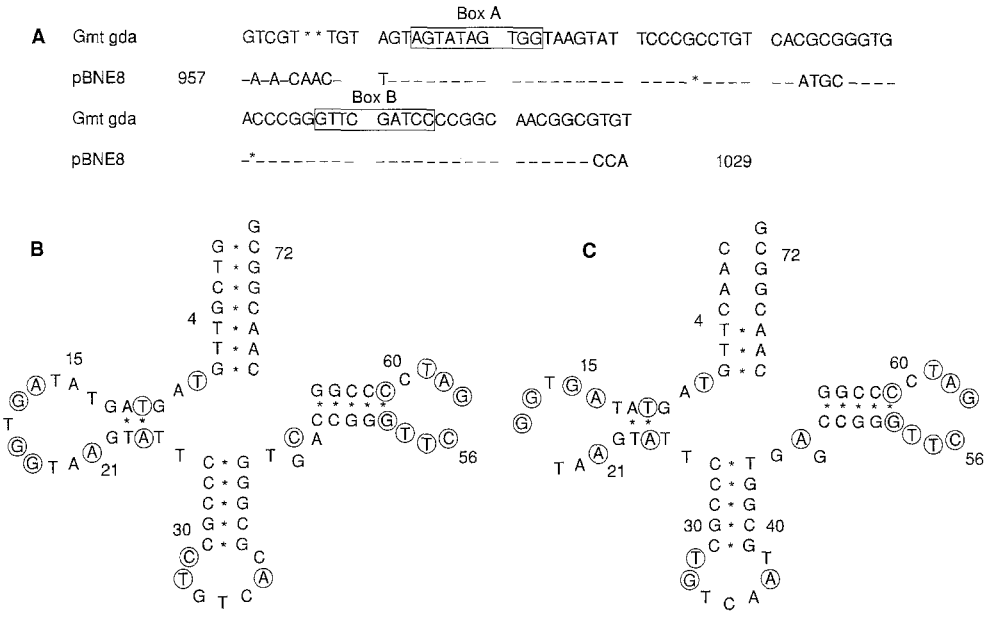

Fig. 3 **A** Sequence comparison of clone pBNE8 with the Asp-tRNA from soybean represented as Gmt gda (Waldron et al. 1985). An *asterisk* (*) denotes a deletion and a *dash* (–) a nucleotide homologous to that in the Asp-tRNA sequence. **B, C** Secondary structures of Asp-tRNA (**B**) and pBNE8 (**C**). Semi- invariant and invariant residues are *encircled*

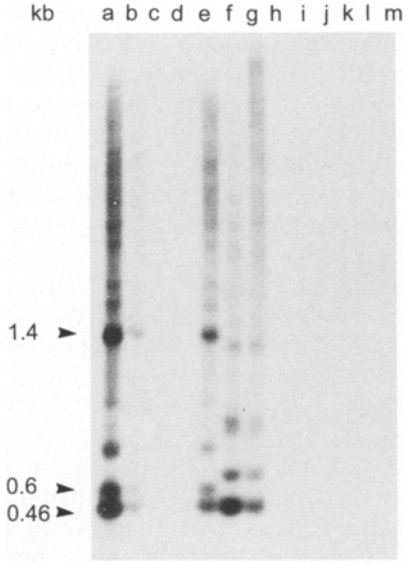

Fig. 4 Hybridization of pBN-4 with different *Brassica* species and related crucifers. Five micrograms of total DNA of *B. nigra* cv 'IC257' (*lane a*), *B. nigra* cv 'Junius' (*lane b*), *B. campestris* (*lane c*), *B. oleracea* (*lane d*), *B. juncea* (*lane e*), *S. arvensis* cv '1' (*lane f*), *S. arvensis* cv '2' (*lane g*), *S. alba* (*lane h*), *D. erucoides* (*lane i*), *E. gallicum* (*lane j*), *E. sativum* (*lane k*), *B. tournefortii* (*lane l*) and *R. sativus* (*lane m*) were restricted with *Hin*dIII, Southern blotted and hybridized with the probe pBN-4

B. campestris and *B. oleracea* (Fig. 4, lanes a, c and d). It also hybridized to *B. nigra* cv 'Junius' (Fig. 4, lane b), a European cultivar of *B. nigra*, and to *B. juncea* (Fig. 4, lane e), a naturally occurring amphidiploid that contains the *B. nigra* genome.

Of the other crucifers, pBN-4 hybridized to two cultivars of *Sinapis arvensis*, giving bands at 0.46 kb and 0.65 kb (Fig. 4, lanes f and g). However, the repeat pBN-4 did not hybridize to *S. alba*, *Diplotaxis erucoides*, *Erucastrum gallicum*, *Eruca sativa*, *B. tournefortii* and *Raphanus sativus* (Fig. 4). No change in the hybridization pattern was obtained upon lowering the stringency conditions, indicating that pBN-4 was highly species-specific. The repeat pBNE8 showed a hybridization pattern similar to that of pBN-4 when used as a probe on different species of *Brassica* and the related crucifers. In addition, pBNE8 hybridized to most of the crucifers producing smears.

Localization of *B. nigra*-specific repeats on chromosomes

With a view to localize pBN-4 on the respective chromosomes in the *B. nigra* genome, we restricted and hybridized DNA from the five monosomic addition lines of *B. nigra* in *B. napus* background to species-specific repeat pBN-4. The repeat was found to be present in all of the addition lines with prominent bands at 0.46 kb, 0.6 kb and 1.40 kb (Fig. 5). However, the intensity of the signal varied among the addition lines. The most intense signal was obtained in addition line 5, whereas in addition lines 3 and 4 the intensity of the signal was weak (Fig. 5, lanes g, e and f, respectively). Since pBN-4 hybridized to all five addition lines we then became interested in studying the localization of some of the other *B. nigra* repeats earlier characterized in our laboratory (Gupta et al. 1990, 1992).

Species-specific repeats pBNBH35 and pBN34, previously characterized in our laboratory (Gupta et al. 1990, 1992), were also used for the chromosomal localization studies. The repeat pBNBH35 is dispersed whereas pBN34 is tandem in nature. Hybridization of pBNBH35 to *Bam*HI-restricted DNA from the addition lines showed that the dispersed repeat pBNBH35 was present on all the addition

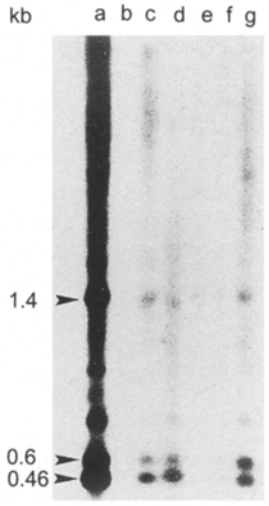

Fig. 5 Localization of the dispersed species-specific repeat on the *B. nigra* chromosomes. Total DNA of *B. nigra* (*lane a*), *B. napus* (*lane b*) and addition lines 1–5 (*lanes c–g*) were restricted with *Hin*dIII and probed with pBN-4

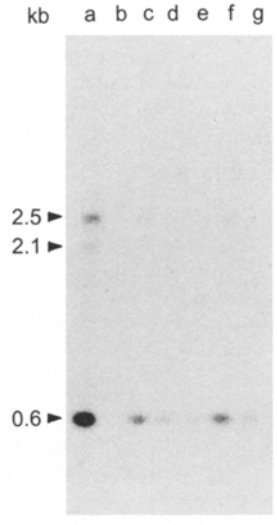

Fig. 6 Localization of the dispersed species-specific repeat pBNBH35 on the *B. nigra* chromosomes. Total DNA of *B. nigra* (*lane a*), *B. napus* (*lane b*) and addition lines 1–5 (*lane c–g*) were restricted with *Bam*HI and probed with pBNBH35

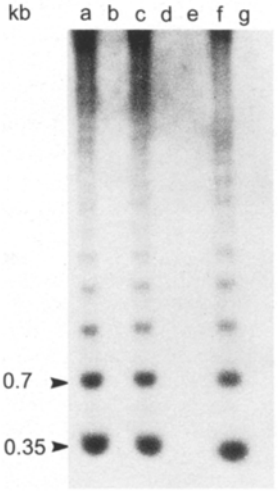

Fig. 7 Localization of the species-specific tandem repeat pBN34. Total DNA of *B. nigra* (*lane a*), *B. napus* (*lane b*) and the five alien addition lines (*lanes c–g*). All the DNAs were restricted with *Hin*dIII and probed with pBNH34

lines. The dispersed repeat, pBNBH35, revealed distinct bands of sizes 0.6 kb, 2.1 kb and 2.5 kb (Gupta et al. 1992). The intensity of the signals was maximum in addition lines 1 and 4 and minimum in 3 (Fig. 6, lanes c, f and e respectively). The localization of tandem repeat pBN34 was studied by restricting the DNA from the addition lines with *Hin*dIII and then hybridizing it with pBN34. The tandem repeat pBN34 gave a ladder-like pattern of hybridization having a monomeric unit size of 350 bp (Gupta et al. 1990). The tandem repeat pBN34 was found to be present in only two of the five addition lines. The intensity of the signal obtained was the same for both addition lines (Fig. 7, lanes c and f).

Discussion

The isolation and characterization of two dispersed DNA sequences, pBN-4 and pBNE8, belonging to the same family of repeats is reported in this paper. Hybridization patterns obtained for both repeats indicated that they may be members of the same repeat family of the *B. nigra* genome. This was confirmed by sequence comparisons, which showed more than 80% homology between pBN-4 and the 475-bp region at the 3′ end of pBNE8. Digestion of *B. nigra* genomic DNA with various restriction enzymes and hybridization to either pBN-4 or pBNE8 revealed different hybridization patterns. The partial restriction digests also did not reveal a ladder-like pattern upon hybridization with either pBN-4 or pBNE8. These results indicated that the repeats are dispersed in nature. The hybridization patterns obtained with isoschizomers *Mbo*I, *Sau*3AI and *Dpn*I, which recognize the sequence 5′-GATC-3′, indicated that neither the 'A' nor 'C' residues in 5′-GATC-3′ are methylated. However, methylation studies with isoschizomers *Msp*I and *Hpa*II indicated that the internal C is more methylated than the external C in 5′-CCGG-3′.

pBN-4 was found to be species-specific as no hybridization signals were obtained with the diploid species of either the A (*B. campestris*) or the C (*B. oleracea*) genome when pBN-4 was used as a probe. This clearly established the presence of this repeat only in the B (*B. nigra*) genome. This distinction at the DNA sequence level between the pBN-4 repetitive sequence from the B genome and repeats present in both the A and C genomes may have arisen due to the elimination of this repeat from both the A and C genomes or its extensive modification during the speciation process. The repeat pBN-4 may thus be considered to be species-specific for *B. nigra* (B genome). On the other hand, the repeat pBNE8 produced a smear with most of the other crucifers, indicating that the region of pBNE8 upstream of the pBN-4-like region is not species-specific. This was clear as the 5′ upstream sequence of pBNE8 shows homology to a putative Asp-tRNA (Waldron et al. 1985).

pBN-4 did not hybridize with any of the wild relatives of family *Brassicaceae* used in the study except *S. arvensis*. This shows that *B. nigra* is closer to the *S. arvensis* genome than to *B. campestris* and *B. oleracea*. This had

been established earlier using other repeat families of *B. nigra* (Gupta et al. 1990, 1992). On the basis of chloroplast DNA analysis (Yanagino et al. 1987; Pradhan et al., 1992), *S. arvensis* and *B. nigra* have been grouped together. Mizhushima (1980) and Song et al. (1988) have suggested that *S. arvensis* may be the progenitor of *B. nigra*. Based on sequence comparison of the 5S rDNA non-coding spacer regions Bhatia et al. (1993), Capesius (1993) and Lakshmikumaran (unpublished) showed that *B. nigra* is closer to the *Sinapis* species than to *B. campestris*.

Repeated DNA families showing homology to tRNA sequences have been reported earlier (Benslimane et al. 1986; Endoh et al. 1990). This homology extends to specific-sequence motifs such as the tRNA intragenic promoters, direct and inverted subrepeats within the sequence and the secondary structure. The 177-bp tandem repeat from *B. oleracea* showed 64% and 60% homologies with Lys- and His-tRNA, respectively, from yeast mitochondria (Benslimane et al. 1986). The 350-bp repeat from rice showed homology to both 5S RNA and tRNA (Wu and Wu 1987). A family of SINE retroposons from *B. napus* showed 57–59% homology to several tRNA species (Deragon et al. 1994). A highly repetitive and transcribable sequence in the tortoise genome, the 6.5S rRNA, showed 68–70% similarity with Lys- and Thr-tRNAs from rabbit and mouse genome, respectively (Endoh et al. 1990). Recently, Batistani et al. (1995) reported a tandemly repeated DNA family in European salamanders originally derived by retroposition of tRNA intermediates. The clone pBNE8 reported here also showed homology to a putative Asp-tRNA from *Glycine max* (Waldron et al. 1985) and other tRNAs. The tRNA-like secondary structure of pBNE8 is a computer structure obtained by the RNA secondary structure prediction program developed by Zuker (1989). The biological importance of the tRNA-like structure is unknown, but there is strong evidence that similar to the other repetitive sequences, pBNE8 may have evolved from a tRNA.

Genome-specific markers have been assigned to their respective chromosomes in the B genome with the aid of *B. napus – nigra* alien addition lines (Quiros et al. 1991). Kianian and Quiros (1992) mapped the rDNA intergenic spacer sequences in *B. oleracea* to three independent chromosomes. A family of long interspersed repetitive elements specific to genus *Zea* was mapped on chromosomes by *in situ* hybridization (Aledo et al. 1995). Wong et al. 1995 showed chromosomal localization of a chromosome 5-specific repetitive DNA sequence. The dispersed repeat family characterized in this study proved to be a strict marker for the B genome. Since the location of this family of repeats in the genome of *B. nigra* was not known, monosomic addition lines were used for the same and the repeat was found to be present on all chromosomes.

In addition to pBN-4, two repeats, pBN34 and pBNBH35, characterized earlier in our laboratory (Gupta et al. 1990, 1992), were also localized on the *B. nigra* chromosomes using the *B. napus-nigra* alien addition lines. The dispersed repeat pBNBH35, like pBN-4, is present on all chromosomes. The difference in the intensity of the signals obtained with both pBN-4 and pBNBH35 may be due to variations in the copy number of the repeats present on each chromosome. Tandem repeats generally occur in clusters and may thus be present on a number of chromosomes. The tandem repeat pBN34 was localized to only two of the chromosomes. A satellite DNA from rice has been shown to be present on only some of the chromosomes (Wu et al. 1991). Similarly, the 177-bp repeat family of *Brassicacea* is not located on all the chromosomes (Harrison and Heslop-Harrison 1995). Since both the dispersed repeats pBN-4 and pBNBH35 are present on all chromosomes it will be interesting to study the organization of these repeats with respect to each other in the *B. nigra* genome. Work involving the use of the *B. nigra*-specific repeat described here and other species-specific repeats for the screening of wide/somatic hybrids and synthetic *B. juncea* is now in progress in our laboratory.

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References

Aledo R, Raz R, Monfort A, Vicient CM, Puigdomenech P, Martinez-Izquierdo JA (1995) Chromosome localization and characterization of a family of long interspersed repetitive DNA elements from the genus *Zea*. Theor Appl Genet 90:1094–1100

Batistoni R, Pesole G, Marracci S, Nardi I (1995) A tandemly repeated DNA family originated from SINE – related elements in the European Plethodontid Salamanders (Amphibia, Urodela). J Mol Evol 40:608–615

Benslimane A, Dron M, Hartmann C, Rode A (1986) Small tandemly repeated DNA sequences of higher plants likely originate from a tRNA gene ancestor. Nucleic Acids Res 14:8111–8119

Bhatia S, Singh K, Jagannathan V, Lakshmikumaran M (1993) Organization and sequence analysis of the 5S rRNA genes in *Brassica campestris*. Plant Sci 92:44–55

Birnboim HC (1983) A rapid alkaline extraction procedure for screening of plasmid DNA. Methods Enzymol 100:243–255

Capesius I (1993) Sequence of the 5S rRNA gene from *Brassica nigra* and its relation to other 5S rRNA genes from *Brassicaceae*. J Plant Physiol 142:112–114

Chevre AM, Thip P, Eber F, Deschamps M, Renard M, Delseny M, Quiros CF (1991) Characterization of disomic addition lines *Brassica napus- Brassica nigra* by isozyme, fatty acid, and RFLP markers. Theor Appl Genet 81:43–49

Crowhurst R, Gardner R (1991) A genome-specific repeat sequence from Kiwi fruit (*Actinidia deliciosa* var. 'deliciosa'). Theor Appl Genet 81:71–78

Dellaporta SL, Wood J, Hicks JB (1984) Molecular biology of plants: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp 36–37

Deragon JM, Landry BS, Pelissier T, Tutois S, Tourmente S, Picard G (1994) An analysis of retroposition in plants based on a family of SINEs from *Brassica napus*. J Mol Evol 39:378–386

Endoh H, Nagahashi S, Okada N (1990) A highly repetitive and transcribable sequence in the tortoise genome is probably a retroposon. Eur J Biochem 189:25–31

Fabijanski S, Fedak G, Armstrong K, Altosaar I (1990) A repeated sequence probe for the C genome in *Avena* (Oats). Theor Appl Genet 79:1–7

Francis HA, Leitch AR, Koebner RMD (1995) Conversion of a RAPD-generated PCR product, containing a novel dispersed repetitive element, into a fast and robust assay for the presence of rye chromatic in wheat. Theor Appl Genet 90:636–642

Ganal MW, Lapitan NLV, Tanksley SD (1988) A molecular and cytogenetic survey of major repeated DNA sequences in tomato *(Lycopersicon esculentum)*. Mol Gen Genet 213:262–268

Gupta V, Jagannathan V, Lakshmikumaran M (1990) A novel AT-rich tandem repeat of *Brassica nigra*. Plant Sci 68:223–229

Gupta V, LakshmiSita G, Shaila MS, Jagannathan V, Lakshmikumaran M (1992) Characterisation of species-specific repeated DNA sequences from *B. nigra*. Theor Appl Genet 84:397–402

Hallden C, Bryngelsson T, Sail T, Gustafsson M (1987) Distribution and evolution of a tandemly repeated DNA sequence in the family *Brassicaceae*. J Mol Evol 25:318–323

Harrison G, Heslop-Harrison J (1995) Centromeric repetitive DNA sequences in the genus *Brassica*. Theor Appl Genet 90:157–165

Hellens RP, Ellis THN, Lee D, Turner L (1993) Repeated sequences as genetic markers in pooled tissue samples. Plant Mol Biol 22:153–157

Hosaka K, Kianian SF, McGrath JM, Quiros CF (1990) Development and chromosomal localization of genome-specific DNA markers of *Brassica* and the evolution of amphidiploids and *n*=9 diploid species. Genome 33:131–142

Innes MA, Gelfand DH, Sninsky JJ, White TJ (1990) PCR protocols. Academic Press, San Diego, pp 134–135

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84: 544–554

Koukalova B, Reich J, Matyasek R, Kuhrova V, Bezdek M (1989) A *Bam*HI family of highly repeated DNA sequences of *Nicotiana tabacum*. Theor Appl Genet 78:77–80

Kraft R, Tardiff J, Krauter KS, Leinwand LA (1988) Using miniprep plasmid DNA for sequencing double-stranded templates with Sequanase. Biotechniques 6:544–546

Lakshmikumaran M, Ranade S (1990) Isolation and characterization of a highly repetitive DNA of *Brassica campestris*. Plant Mol Biol 14:447–448

Lakshmikumaran M, D'Ambrosio E, Laimins L, Lin D, Furano AV (1985) Long interspersed repeated DNA (LINE) causes of polymorphism at the rat insulin I locus. Mol Cell Biol 5:2197–2203

Malmberg R, Messing J, Sussex I (1985) Molecular biology of plants: a laboratory course manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Mao L, Zhai W, Hu H, Zhu L (1994) Cloning and characterization of a repetitive sequence in rye (*Secale cereale*). Plant Sci 100: 51–57

McGrath JM, Quiros CF, Harada JJ, Landry BS (1990) Identification of *Brassica oleracea* monosomic alien chromosome addition lines with molecular markers reveals extensive gene duplication. Mol Gen Genet 223:198–204

Mizushima V (1980) Genome analysis in *Brassica* and allied genera. In: Tsunoda S, Hinata K, Gomez-Campok (eds) *Brassica* crops and wild allies. Japanese Science Society Press, Tokyo, pp 89–105

Pehu E, Thomas M, Poutala T, Karp A, Jones MGK (1990) Species-specific sequences in the genus *Solanum*: identification, characterization, and application to study somatic hybrids of *S. brevidens* and *S. tuberosum*. Theor Appl Genet 80:693–698

Pradhan A, Prakash S, Mukhopadhyay A, Pental D (1992) Phylogeny of *Brassica* and allied genera based on variation in chloroplast and mitochondrial DNA patterns: molecular and taxonomic classification are incongruous. Theor Appl Genet 85:331–340

Quiros CF, Hu J, This P, Chevre AM, Delseny M (1991) Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82:627–632

Saul MW, Potrykus I (1984) Species-specific repetitive DNA used to identify interspecific somatic hybrids. Plant Cell Rep 3:65–67

Schweizer S, Ganal M, Ninnemann H, Hemleben V (1988) Species-specific DNA sequences for identification of somatic hybrids between *Lycopersicon esculentum* and *Solanum acaule*. Theor Appl Genet 75:679–684

Shephard AL, Anderson S, Smith SM (1990) Species-specific repeated DNA sequences from *Petunia*. Plant Sci 67:57–62

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theor Appl Genet 75:497–506

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Tamhankar, Gupta V, Joshi K, Ranjekar P (1990) Occurrence and characterization of a dispersed *Mbo*I repeat family in french bean (*Phaseolus vulgaris*) seedling DNA. Plant Sci 68:203–211

Tsai C, Strauss S (1989) Dispersed repetitive sequences in the chloroplast genome of Douglas-fir. Curr Genet 16:211–218

U.N.: (1935) Genomic analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Visser RGF, Hoekstra R, Leij FR Vander, Pijnacker LP, Witholt B, Feenstra WJ (1988) *In situ* hybridization to somatic metaphase chromosomes of potato. Theor Appl Genet 76:420–424

Waldron C, Wills N, Gesteland RF (1985) Plant tRNA genes: putative soybean genes for tRNA-asp and tRNA-met. J Mol Appl Genet 3:71–17

Wong ZX, Kurata N, Saji S, Katayose Y, Minobe Y (1995) A chromosome 5-specific repetitive DNA sequence in rice (*Oryza sativa* L.). Theor Appl Genet 90:907–913

Wu HK, Wu T (1987) A new rice repetitive DNA shows sequence homology to both 5S RNA and tRNA. Nucleic Acids Res 15: 5913–5923

Wu HK, Chung MC, Wu T, Ning CN, Wu R (1991) Localization of specific repetitive DNA sequences in individual rice chromosomes. Chromosoma 100:330–338

Yanagino T, Takahata Y, Hinata K (1987) Chloroplast DNA variation among diploid species in *Brassica* and allied genera. Jpn J Genet 62:119–125

Zhao X, Kochert G (1992) Characterization and genetic mapping of a short, highly repeated, interspersed DNA sequence from rice (*Oryza sativa* L.). Mol Gen Genet 231:353–359

Zhao X, Wu T, Xie Y, Wu R (1989) Genome-specific repetitive sequences in the genus *Oryza*. Theor Appl Genet 78:201–209

Zuker M (1989) Computer prediction of RNA structure. Methods Enzymol. 180:262–288