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Cloning, identification and characterization of a repetitive sequence flanking telomere and homologous to *canrep* in *Brassica napus*

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ABSTRACT. Satellite DNAs consist of long tandem arrays of short or large repeated sequences that form the centromeric and sometimes also subtelomeric regions of all higher eukaryote chromosomes. Telomeres and centromeres are the most important elements involved in the structure and function of chromosome. Telomeric DNA sequences are conserved among nearly all plant species. Although centromere-specific DNA sequences have been isolated in a wide range of plant species, almost no conservation was found in their DNA sequences. Here, we utilized the cassette-ligation-mediated PCR approach for cloning the sequence near the telomeric repeats of *Brassica napus*. Sequences analysis showed that one sequence was highly homologous to the centromeric satellite DNA sequence reported. FISH revealed that this satellite was located primarily at centromeric regions of most chromosomes, and also at some chromosome ends. The sequence was a subtelomeric satellite DNA which possessed variability in the evolution of chromosomes of *Brassica* species. The phylogenetic tree analysis of the satellite showed its sequence was conservative in *Brassica*. We discussed why the centromeric satellites appeared in the subtelomeric region.

Keywords: *Brassica napus*; Centromere; Satellite DNA; Subtelomere; Telomeric repeat.

INTRODUCTION

The majority of genomic DNA in most plant species is made up of repetitive elements including satellites and retrotransposons. Satellite DNAs consist of long tandem arrays of short or large repeated sequences that form the centromeric regions of all higher eukaryote chromosomes. They are sometimes also found in subtelomeric or other chromosomal locations. Satellite DNAs are implicated in centromeric functions, such as segregation in mitosis and meiosis, recognition and pairing of homologous chromosomes, sister chromatid attachment, and formation of kinetochore structures (Willard, 1998). Telomeres and centromeres are the most important functional elements in plant chromosomes, as in other eukaryotic chromosomes. The two elements are in general composed of repetitive DNA sequences and binding or associated proteins (Murata, 2002). In most eukaryotic chromosomes, telomeres are composed of variable numbers of simple repeat sequences characterized by clusters of G residues on the 3′-end of each strand of chromosomal DNA. The telomeric and centromeric DNA, RNA, and protein components have been analyzed in recent years. Although our understanding of their functions remains elusive, recent findings show that they have some similarities. For instance, telomeric-like sequences are present in centromeric regions in *Arabidopsis thaliana* (Richards et al., 1991), maize (Alfenito et al., 1993), and potato (Tek and Jiang, 2004). There is a common telomeric-like secondary structure in Drosophila centromeric DNA (Abad et al., 2000). The centromeric and subtelomeric regions of eukaryotic chromosomes consist of mosaics of repeats and retrotransposons structured in a remarkably similar way (Pryde et al., 1997; Nagaki et al., 2004).

The subtelomeric regions of most organisms are dynamic with frequent turnover and exchange of sequences. In general, their structures are conservative from yeast to humans (Pryde et al., 1997). In plant, there are large tracts of tandem repeats in subtelomeric regions, often with spacer sequences between them and TRs (Ganal et al., 1992). The spacer sequences are called telomere-associated sequences (TASs). Telomere-associated regions represent boundaries between the relatively homogeneous telomeres and the subtelomeres, which show much greater heterogeneity in chromatin structure

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and DNA composition (Sykorova et al., 2003). Sometimes we can not differentiate TASs and subtelomeres, the DNA sequences proximal to the TRs. In this study, we found there were no spacer sequences TASs in some *Brassica napus* chromosomes and the TRs directly combined with the subtelomeric repeats. Subtelomeric dynamics result in a gene duplication rate significantly higher than the genome average. The subtelomeres are hot spots of interchromosomal recombination and segmental duplication which may invent new gene during the evolution (Linardopoulou et al., 2005). So it is not only a structural element but also an important functional element.

Satellite DNAs consist of long arrays of tandemly repeated DNA families in eukaryotic genomes. Variability concerns monomer size, nucleotide sequence, long-range periodicity, quantity and chromosomal location. The function of satellite DNA has been associated to heterochromatic regions, whose biological role itself is not fully understood. Satellite DNA evolves in a concerted manner. Unequal crossing over, unequal sister chromatid exchange, gene conversion and transposition are the most important molecular processes which lead to sequence homogenization within and between different arrays in a species (Charlesworth et al., 1994). Satellites might play a role in the speciation of organisms with the evolution of large new clusters being responsible for a considerable lack of chromosome homology between species.

In this study, we cloned the *Brassica napus* subtelomeric sequences which attached to the TRs and sequenced them. Sequences analysis by BLAST approach of NCBI showed that one sequence was a satellite DNA sequence, which was mainly clustered at centromeric regions of chromosomes in the previous study results (Xia et al., 1993; Harrison et al., 1995). FISH revealed that the satellites appeared in both subtelomeric and centromeric regions.

MATERIALS AND METHODS

Seeds of *Brassica napus* (AACC, 2n=38), *B. oleracea* (CC, 2n=18), *B. rapa* (AA, 2n=20) and Chinese Xinjiang wild mustard (*Sinapis arvensis*) were conserved by our lab. Seeds were germinated on moist paper at 23-25°C for 5-6 days or until seedlings reached 5 to 6 cm length.

DNA extraction

Genomic DNAs were isolated from the seedlings according to the method described by Aldrich (Murray and Thompson, 1980). Digest of the isolation of genomic DNA with restriction endonucleases *Hin*dIII. The nuclear DNA and restriction fragments were electrophoresed through 0.8% agarose gels to check their quality.

Cloning of the sequence near the TRs from *Brassica napus*

Escherichia coli strain DH5α was used as a bacterial host. We used the TaKaRa LA PCR in vitro Cloning Kit to clone the sequence near the TRs from *Brassica napus*. For PCR amplification of the sequence near the TRs, we used the telomeric repeats primer (TELO 5'- AACCC TAAAC CCTAA ACCCT AAACC C-3') and the cassette primers (C1 5'- GTACA TATTG TCGTT AGAAC GCGTA ATACG ACTCA-3', C2 5'- CGTTA GAACG CGTAA TACGA CTCAC TATAG GGAGA-3'). The telomeric repeats primer is based on the sequence of *A. thaliana* TRs (Richards and Ausubel, 1988), which is very conserved in plants.

About 5 μg *Brassica napus* DNA was digested with *Hin*dIII and then was recovered by precipitation with ethanol. Half of the obtained DNA was ligated with 50 ng of the *Hin*dIII cassette and then the ligation product was recovered by precipitation with ethanol. One-fifth of the obtained product was used as a template for the first amplification. The template was denatured for 10 min at 94°C. PCR amplification reaction contained the template, 0.4 mM dNTP, LA Taq, PCR Buffer, 0.2 μM primer C1 and TELO in a 50 μl volume. Amplification was carried out for 30 cycles of 30 sec at 94°C, 2 min at 55°C, 1 min at 72°C. The diluted amplification product was used as a template for the second amplification with the primer C2 and TELO. The amplification product was recovered by precipitation with ethanol and analyzed on a 2% agarose gel. The recovered product was ligated to the pMD18-T vector (TaKaRa). The ligation product was used to transform competent *E. coli* cell, and clones carrying inserts were selected.

DNA sequencing and southern hybridization

Sequencing reactions of the cloned fragments were performed by Sangon Company. The obtained DNA Sequences were aligned and analyzed by the BLAST to GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). Related sequences were aligned by using the program DNAMAN. A phylogenetic tree was constructed using the DNAMAN program and 1000 trials of bootstrap analyses were used to provide confidence estimates for phylogenetic tree topologies. Exonuclease digestion of genomic DNA was performed using 6 U/ml of Bal31 nuclease at 30°C at a DNA concentration of 27 μg/ml. The reaction was stopped by heating to 65°C for 10 min. Southern blots were prepared using positively charged nylon membranes (Millipore). Southern Hybridization was performed using the DIG DNA Labeling and Detection Kit (Roche).

Chromosome preparation and FISH

The Chromosome preparation technique described by Wei et al. (2007) was used with some modifications. Briefly, flower buds were fixed in a mixture of ethanol:acetic acid glacial (3:1) at 4°C over night. They were washed three to five times with distilled water, then digested in 1% (W/V) cellulase "Onozuka" R-10 (Yakult Honsha Co., Ltd) and 1% (W/V) pectolyase Y-23 (Yakult Honsha Co., Ltd) dissolved in distilled water at 28°C for

2.5-3 h. Then they were subjected to a hypotonic treatment in distilled water for 30 min before preparing spreads by the flame drying method.

Probes were generated by PCR with the primer TEL3-SENSE and TEL3-ANTISENSE according to the method, labeling of probe with digoxigenin described by Lion et al. (1990). Amplification was carried out for 35 cycles of 30 sec at 94°C, 45 sec at 55°C, 1min at 72°C. The labelling results were evaluated by means of dot blots and gel electrophoresis. Chromosome preparations were pretreated with 100 µg/ml RNase (in 2×SSC) at 37°C for 1 h, rinsed briefly in 2×SSC. Chromosomal DNA was then denatured by immersing the slide in 70% deionized formamide at 70°C for 3 min. After dehydration of the preparation in an ice-cold 70%, 95% and 100% ethanol series and air drying, 60 µl of denatured probe cocktail (10 ng/µl labelled probe DNA, 0.5 µg/µl sheared salmon sperm DNA, 10% dextran sulphate, 50% deionized formamide, 0.1% SDS, 2×SSC) was added to the slide and hybridization was carried out at 37°C overnight. Posthybridization washes included a stringent wash in 20% formamide, a wash in 2×SSC and a wash in 0.1×SSC at 42°C for 10 min, respectively, to remove weakly-bound probe. Signals were detected with Anti-Digoxigenin-Fluorescein (Roche, Cat. No. 1207741), washed in PBS for 10 min. Slides were counterstained with 2 µg/ml PI (propidium iodide) and examined under a Leica DM IRB fluorescence microscope assembled with DFC300 CCD and FW4000 software.

RESULTS

Isolation of the sequence near the TRs

The sequence flanking the TRs was cloned as outlined in Figure 1. The first amplification had no amplified bands and the second had diffuse bands. The second amplification products were ligated to the pMD18-T vector (TaKaRa) and the ligation products were used to transform competent *E. coli* cells. Seven selected clones, which had the obvious amplified bands by PCR with the primer C2 and TELO, were sequenced. The cloned sequences contained the telomere repeats with different lengths, from 26 to over 150 nucleotides. The obtained DNA sequences without the telomere repeat were aligned and analyzed with the BLAST. BLAST analysis indicated that the third clone named TEL-3 shared 99% and 95% identity with a satellite DNA sequence, *canrep* (Xia et al., 1993) and *B. nigra* tandem repeat DNA (Harrison et al., 1995). The clone is a representative of a family that has been sequenced many times and it is a satellite DNA, *canrep* (176 bp). Large clusters of these satellites appear to be located primarily at centrometic regions of most chromosomes. The telomere repetitive sequence in this clone was about 150 nucleotides which consisted of 21 repeat units of seven-nucleotide, minisatellite DNA (CCCTAAA) (Figure 2). The clone TEL-3 describes two kinds of different repetitive sequences linking with each other without any spacer sequences.

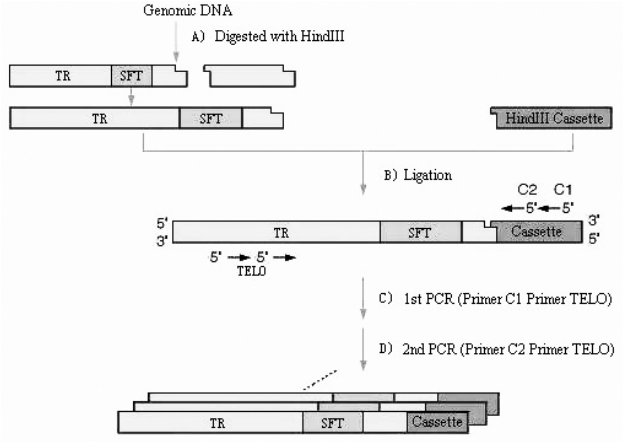

Figure 1. Strategy for cloning of the sequence flanking the TRs of *Brassica napus*. (SFT: sequence flanking telomere)

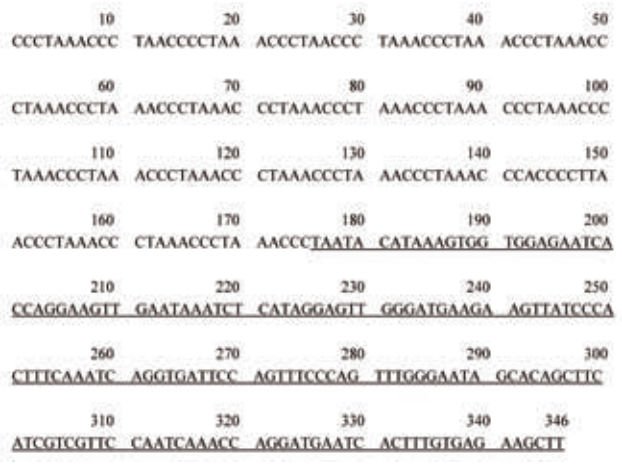

Figure 2. The sequence of TEL-3. The underlined indicate the *canrep* motif and the minisatellite DNA (CCCTAAA) is the telomeric repeated unit.

Verifying of the TEL-3 derived from the chromosomal end

Because there are also telomeric-like sequences in centromeric regions (Richards et al., 1991; Alfenito et al., 1993; Tek and Jiang, 2004), we need to verify of the sequence derived from the chromosomal end. To determine whether this clone TEL-3 is derived from the chromosomal end, we performed Southern hybridization of *Bal*31-treated *B. napus* genomic DNA with this clone. The genomic DNA was treated with *Bal*31 nuclease, subsequently digested with *Hin*dIII (Figure 3). Because the telomeric repetitive sequence (CCCTAAA) may cross-hybridize to the genomic TRs and may interfere with specific pattern of hybridization with the nearby sequences, the probe was generated by PCR removing this telomeric repetitive sequence. According to the sequenced TEL-3, the following pair of PCR primers was designed: TEL3-SENSE, 5'-TACATAAAGTGGTGGAGAAT-3' and TEL3-

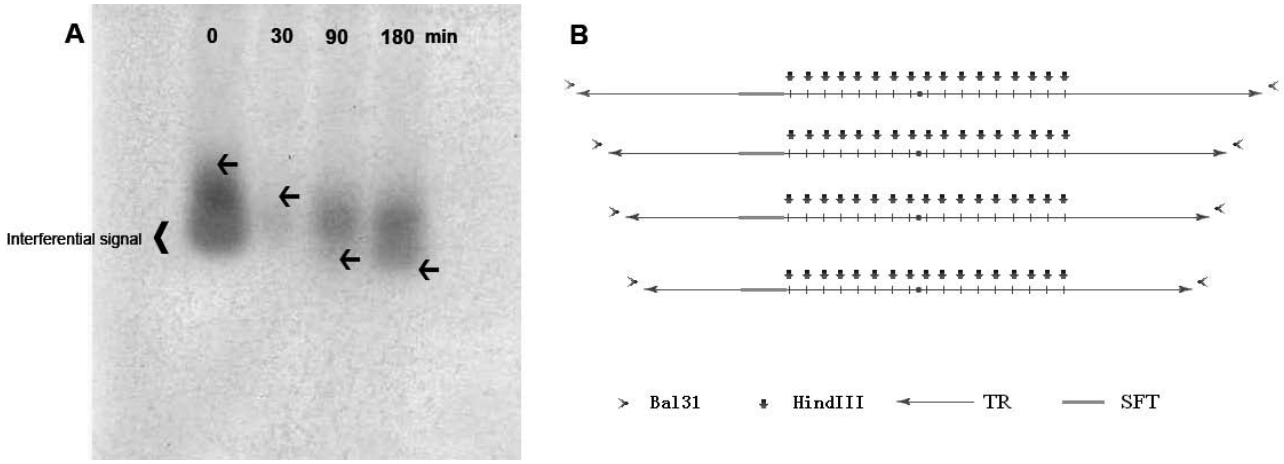

Figure 3. Hybridization patterns of the clone TEL-3 to *Bal*31-treated genomic DNA. *Brassica napus* genomic DNA was digested with *Bal*31 for 0, 30, 90, 180 min and then digested with *Hin*dIII. The DNA was separated on a 1% agarose gel. Arrowhead indicates the location change of hybridization signals (A). Strategy for genomic DNA digested with *Bal*31 and *Hin*dIII (B).

ANTISENSE, 5'-GTTTGATTGGAACGACGATG-3'. Because the *canrep* is a satellite DNA, the probe will cross-hybridize to the *canrep* and result in interferential signal. But the *canrep* and their multimer are more shorter than it with the telomeric repetitive sequences, so we use the 1% agarose gel to separate the digested DNA. The interferential signal will concentrate. We found that this clone hybridized to *Bal*31 and *Hin*dIII treated genomic DNA and detected diffuse bands which were progressively shortened with different *Bal*31 treating time.

In addition, we used the *B. napus* BAC library to test whether TEL-3 is derived from the chromosomal end. Our BAC library had a high coverage and provided a 99.82% probability of finding any specific genes or DNA sequences (Chen et al., 2008). Because the genomic DNA was digested with *Hin*dIII, ligated to the vector and TRs had no restriction sites and couldn't ligated to the BAC vector, the BAC clones could not contain TRs and partial TASs. So the BAC inserts (Mixture of all BAC clones DNA in the library) and genomic DNAs were respectively used as a template to amplify the TRs and TASs region with the primer TELO and TEL3-ANTISENSE. If this clone TEL-3 is derived from the chromosomal end, the genomic DNA should have an amplified band about 200 bp, and the BAC DNA did not have. The PCR result confirmed our deduction (Figure 4). The arrowed band was expected amplified product. These Southern and PCR results demonstrated that the TEL-3 sequence was derived from the chromosome end and it was a subtelomeric element.

The speciality of TEL-3 and the conservation of this satellite

To determine whether there is TEL-3 in *B. oleracea* and *B. rapa* chromosome ends, we performed PCR the same as in *B. napus* genomic DNA with the primer TELO and TEL3-ANTISENSE. As a result, there was not the band which was specific in *B. napus* genomic DNA (Figure 4). This result demonstrated there were not the same subtelomeric regions in *B. oleracea* and *B. rapa* as in *B. napus*. Perhaps there are no subtelomeric repetitive satellites *canrep* or different *canrep* composition forms in subtelomere of *B. oleracea* and *B. rapa*. Continued molecular biologic and cytological examinations are needed to validate that. But the assay can confirm the ideas that there are different structures in the subtelomere of *Brassica* and the subtelomeric satellite DNAs possess

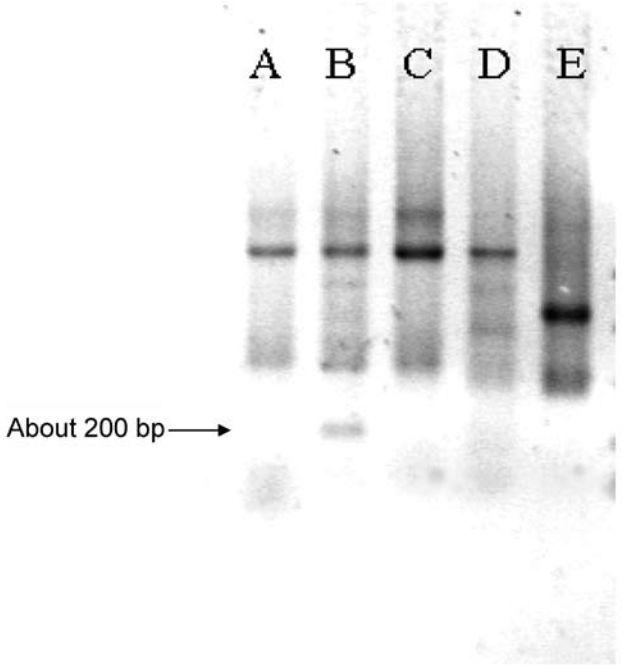

Figure 4. PCR amplification products obtained with the primer TELO and TEL3-ANTISENSE and the templates are the *Brassica napus* BAC library (lane A), *Brassica napus* (lane B), *B. oleracea* (lane C), *B. chinensis* (lane D) and *Sinapis arvensis* (lane E) genomic DNAs.

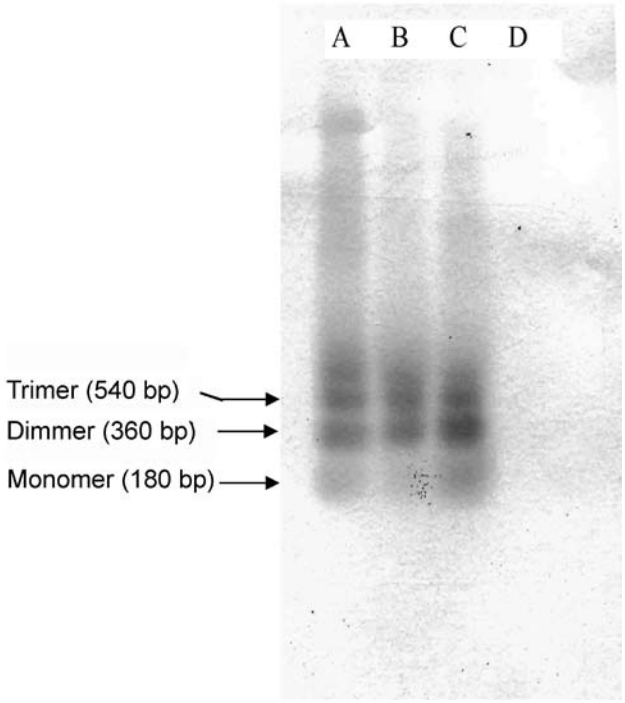

Figure 5. Hybridization patterns of the *canrep* to *Hin*dIII-treated *Brassica napus* (lane A), *B. oleracea* (lane B), *B. chinensis* (lane C) and *Sinapis arvensis* (lane D) genomic DNAs. Three lanes showed ladder patterns, indicating a tandem repeat nature of this element in these genomes. The DNA was separated on a 2% agarose gel.

variability in evolution.

Southern hybridization demonstrated that there were *canrep* satellite DNA sequences in *B. napus*, *B. oleracea* and *B. rapa* but not in *S. arvensis* (Figure 5). It is shown from the phylogenetic analytic results for sequences retrieved from GenBank database that the satellite of *S. arvensis* share only about 70% homology with characteristic sequences in *Brassica*. The satellite DNA *canrep* are distributed in genomes of *Brassica.* Southern hybridization showed ladder patterns, indicating a tandem repeat nature of this element in these genomes. The *canrep* units in genome include monomers, dimers and trimers. The dimers, trimers, and probably other multimeric forms of the *canrep* repeat arose predominantly from mutations affecting some of the *Hind*III sites within tandem arrays.

Detailed analysis of sequence diversity in repetitive sequences can offer interesting insight into the evolutionary relationships among and between *Brassica* species and their crucifer relatives, and may give useful information regarding the potential for intergeneric gene transfer to brassicas from more distant relatives via homoeologous recombination. Additionally, we wonder the origin and significance of this repetitive sequence *canrep* in the evolution of the genome especially in the subtelomere. We compared these repetitive sequences *canrep* in the Brassicaceae selected from the GenBank and the TEL-3 (Figure 6). Phylogenetic analysis revealed

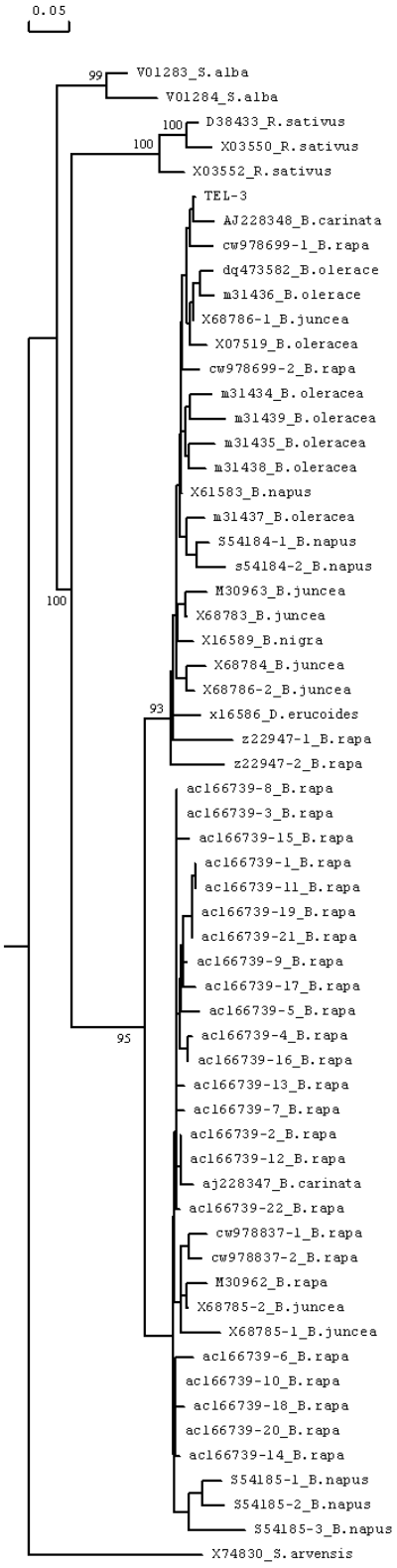

Figure 6. Phylogenetic analysis of *canrep* from different species in Brassicaceae retrieved from GenBank database including *Brassica rapa*, *Brassica carinata*, *Brassica juncea*, *Brassica napus*, *Brassica oleracea*, *Sinapis arvensis*, *Sinapis alba*, *Diplotaxis erucoides* and *Raphanus sativus*. The tree was displayed as a phylogram in which branch lengths are proportional to distance.Bootstrap values for the most robust groupings are shown.

that the 176 bp repeat motifs *canrep* have about *ca.* 85% sequence similarity within the selected sequences from Brassicaceae, while the members of the same species showed a higher degree of sequence homology. We can't find any homologous sequences in *A. thaliana* which is regarded as the model organism having closer phylogenetic relationship to *Brassica*.

Fluorescence in situ hybridization

FISH was used to locate the TEL-3 satellite DNA sequence on the *B. napus* chromosomes. Xia et al. (1993) and Harrison et al. (1995) have reported that large clusters of these satellites appear to be located primarily at centrometic regions of most chromosomes, and we have the similar results. Different cells analysis showed that nearly all of the 38 chromosomes had the hybridization signals and the signals mainly appeared in centromeric regions (Figure 7A). Also we found there were signals in subtelomeric regions (Figure 7D). The signals in subtelomeric region revealed that the *canrep* is the subtelomeric repeat monomer, the end of this chromosome had no TAS, the TRs (CCCTAAA) directly combined with the subtelomeric repeats just like the TEL-3.

DISCUSSION

The majority of genomic DNA in most plant species is made up of repetitive elements including satellites and retrotransposons. Telomeres and centromeres are in general composed of repetitive DNA sequences. In *B. napus*, we cloned the subtelomeric satellite DNAs and confirmed that these satellites located at the *B. napus* chromosome end through PCR and the Southern blot analysis. TRs were in combination with these satellites just as the clone TEL-3. FISH revealed that large clusters of these satellites appeared to be located primarily at centromeric regions of most chromosomes, and also at some chromosome ends.

The Brassicaceae comprises approximately 340 genera and 3,350 species, including the economically important *Brassica* crops and the model organism *A. thaliana*. The Brassicaceae are a particularly interesting group in which to study the diversity and evolution of retroelements. Studies of the phylogenetic relationships within the *Brassica* species have been mainly based on nuclear RFLPs (Song et al., 1990), chloroplast and mitochondrial sequences and restriction site data (Warwick

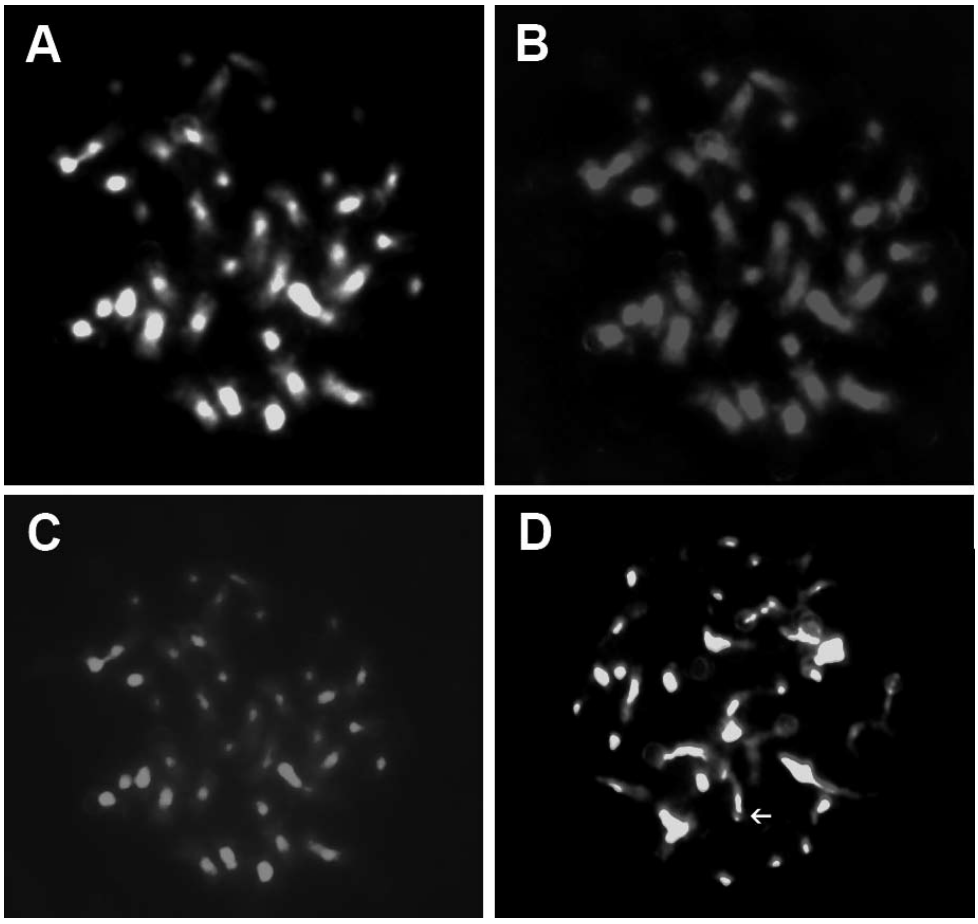

Figure 7. The location of TEL-3 in chromosomes. Arrowhead indicates the subtelomeric signals. The hybridization signals mainly appeared in centromeric regions (A) and also appeared in subtelomeric regions (D). PI counterstaining (B, red) and the hybridization signals (C, green) in (A) were showed respectively. There are both subtelomeric and centromeric signals in a chromosome (D).

and Black, 1991; Pradhan et al., 1992) and SINE insertion sites polymorphism (Tatout et al., 1999). These different analyses led to the division of the *Brassica* species into two evolutionary lineages: the *nigra* (B genome) lineage and the *rapa* (A)/*oleracea* (C) lineage (Yang et al., 2002). We constructed the phylogenetic tree chart based on the genetic distances among selected sequences from the Brassicaceae and the cloned sequence TEL-3.

In the phylogenetic tree, the main conclusions had been summarized as follows (Figure 6). First, the centromeric satellite DNAs in *B. rapa* had been divided into two classes by Lim et al. (2005) including the well-known centromeric repeats *canrep* of *B. napus* (GenBank accession No. X61583) and of *B. juncea* (GenBank accession No. X68785). We had the similar result and the TEL-3 belongs to class I. Second, it seemed that the centromeric repeats of *Brassica* species irregularly distributed within two classes. However, all the 8 analysed sequences of *B. oleracea* belonged to class.We don't know the reason unless we have much more sequences to analyse. Third, the connective sequences from a clone in the same species showed highly homologous with each other. For example, 22 monomers in ac166739 from *B. rapa* belonged to class II, while other monomers from *B. rapa* irregularly distributed within two classes. 3 monomers in s54185 from *B. napus* and 2 monomers in x68785 from *B. juncea* were similar. Last, the monomer from *Diplotaxis erucoides* showed high homology with that of *Brassica* species.

The *canrep* unit is conservative in *Brassica*, that is, there are similar sequences in *Brassica* species. But also they are diverse in any species. In their evolution process, the mutation was accumulated because they do not code for proteins. Distribution change often occurred due to inversion, rearrangement and so on. Evolution of the satellite DNA *canrep* mainly embodies the sequence diversity and distribution change.

There is similarity among the satellite DNAs in the Brassicaceae. It implies that the satellite DNA *canrep* appeared before species differentiation in the Brassicaceae. The A, B and C genomes might originate from an ancient genome which has been rich in the satellite DNAs.

The process of unequal crossing-over during meiosis and unequal sister chromatid exchange at mitosis is widely viewed as responsible for the creation of satellite DNA. Additionally, replication slippage can result in short but abundant repeats (Levinson and Gutman, 1987). It is also possible that microsatellites originate from proto-microsatellites in transposable elements, as with the microsatellite initiating mobile elements of *Drosophila* (Wilder and Hollocher, 2001).

Three amphidiploids, *B. juncea* (AABB, 2n =36), *B. napus* (AACC, 2n = 38), and *B. carinata* (BBCC, 2n = 34) were synthesized by the natural allopolyploidization of the three basic *Brassica* species fewer than 10,000 years ago (Rana et al., 2004). There is not any the same structure of *B. oleracea* (CC, 2n=18) and *B. rapa* (AA, 2n=20) chromosome ends as the TEL-3 in the *B. napus* (AACC) chromosome ends, that is, there are no subtelomeric repetitive satellites *canrep* or different *canrep* composition forms in subtelomere of *B. oleracea* and *B. rapa*. This result indicated that the *B. napus* subtelomeric sequence *canrep* next to the telomere appeared after allopolyploidization events that genome A and C combined with each other. The subtelomeric satellite DNA are evolving rapidly, on the contrary, the telomeric repeats are very conserved in plants with conservative function. The DNA and protein components specific to centromeric chromatin are evolving rapidly, but the chromosome segregation machinery is highly conserved across all eukaryotes (Henikoff et al., 2001). The subtelomere and centromere consist of rapidly evolving mosaics of repeats structured in a similar way.

As we know the maize knob satellite, first described as neocentromeres by Rhoades and Vilkomerson (1942), is a subtelomeric element (Lamb et al., 2007). Our research has the similar result that a *B. napus* satellite DNA sequence, clustered at centromeric regions of *B. napus* chromosomes, is a subtelomeric element.

Why do the centromeric and subtelomeric regions have the same repetitive elements? There are two possible hypotheses. One hypothesis is that the same repetitive elements originated from the generation of linear chromosome. Alfredo (Villasante et al., 2007a) have recently hypothesized that the centromeres originated from telomeres. In the origin of centromere, subtelomeric regions became the first centromeres after their recognition as new cargo by the tubulin-based cytoskeleton. The subtelomeric elements were the residues in the centromeric formation. Then the same repetitive sequences appeared in both centromere and subtelomere. In *D. melanogaster* three non-LTR retrotransposons, HeT-A, TART, and TAHRE (Mason et al., 1995; Pardue et al., 1996; Abad et al., 2004; Villasante et al., 2007b), maintain telomeres by occasional transposition to the chromosome ends. The tandem repeat sequences may form in the continuous transposition to the chromosome ends in the early time of the generation of linear chromosome. However, we can't find out the significant diversity through the sequence alignment between centromeric and subtelomeric satellite DNAs and the subtelomeric satellite DNA is not more primordial.

The other is that the same repetitive elements originated from the evolution of linear chromosome. Robertsonian rearrangements (Holmquist et al., 1979) and transposition (Dooner et al., 2008) may have an effect on this phenomenon. Robertsonian rearrangements demonstrate one-break chromosome rearrangement and the reversible appearance and disappearance of telomeres and centromeres. Telomere-like sequences are present in centromeric regions in plant (Richards et al., 1991; Alfenito et al., 1993; Tek and Jiang, 2004). These results could suggest a centromeric role for these sequences or be simply a reflection of Robertsonian fusions, however,

in no case has it been technically possible to assay a centromeric function of these telomere-like sequences nor has it been proposed that Robertsonian fusions could be a way to generate centromeric activity by modification of previous telomeric functions (Agudo et al., 1999). This dynamic process contact telomeres and centromeres and transposition may be found everywhere in the chromosome. They modify the linear chromosome and then the appearance of the same repetitive sequences is occasional. It has been known that clusters of repetitive sequences in maize are highly variable from one line to another. Mechanisms must exist to change the copy number and must operate on a regular basis to generate the observed variation (Birchler et al., 2008). In this process the telomere and centromere have the same repetitive sequences by accident. Virtually we do not know these exact processes. This process needs much more research. To clarify this point, and to complete the knowledge of karyotype evolution in *Brassica* regarding this repeat, it would be interesting to complete the FISH analysis and to study the sequences in other species of the genus *Brassica*.

Continued sequencing efforts and cytological examination of *B. napus* and additional species will improve understanding of the role of repetitive elements in genome evolution. The different repetitive sequences in telomeric, subtelomeric and centromeric regions need our more research including their structures and origins.

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甘藍型油菜端粒旁側 *canrep* 重複序列的選殖、鑒定與分析

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衛星 DNA 是由重複序列串聯形成，它是高等真核生物染色體的著絲粒有時也包括亞端粒區域的主要結構。端粒和著絲粒對於染色體的結構和功能至關重要。端粒 DNA 幾乎在所有植物中都是保守的。可是目前在很多植物中選殖的著絲粒序列幾乎沒有發現保守性。我們利用連接接頭進行巢式 PCR 的方法，通過選殖端粒旁側序列獲得了甘藍型油菜亞端粒序列。NCBI 的 BLAST 比對結果顯示其中選殖的一個序列與以前報導的甘藍型油菜著絲粒衛星 DNA 高度同源。通過螢光原位雜交，這一衛星 DNA 主要位於染色體的著絲粒區域，另外也出現在一些染色體的末端。這一亞端粒衛星 DNA 在芸苔屬的染色體進化中較為活躍。系統發生數的分析表明了它序列的保守性。最後，我們討論了這一著絲粒衛星 DNA 出現在亞端粒區域的原因。

關鍵詞：甘藍型油菜；亞端粒；端粒重複；著絲粒；衛星 DNA。