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Karyotyping of *Brassica oleracea* L. based on Cot-1 and ribosomal DNAs

Wen-Hui WEI[1,2,*], Su-Feng ZHANG[1], Li-Jun WANG[2], Bo CHEN[2], Xiao-Ming WU[2], and Yun-Chun SONG[3]

[1] *College of Life Sciences, Xinyang Normal University, Xinyang Henan 464000, People's Republic of China*
[2] *Institute of Oil Crops, Chinese Academy of Agricultural Sciences, Wuhan Hubei 430062, People's Republic of China*
[3] *The Key Laboratory of MOE for Plant Developmental Biology, Wuhan University, Wuhan Hubei 430072, People's Republic of China*

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ABSTRACT. To explore a simple, reliable, and effective method of karyotyping *Brassica oleracea* L., Cot-1 DNA was isolated from its genome, labeled as probe with a Biotin-Nick Translation Mix kit, and *in situ* hybridized to mitotic spreads. Specific fluorescent bands appeared on each chromosome pair. 25S and 5S rDNAs were labeled as probes with a DIG-Nick Translation Mix kit and Biotin-Nick Translation Mix kit, respectively, and *in situ* hybridized to mitotic preparations. Signals could be detected on two chromosome pairs for 25S rDNA, and on only one for 5S rDNA. Cot-1 DNA contains rDNA. The site identity of Cot-1 DNA and 25S rDNA on the chromosome was determined by dual-colour fluorescence *in situ* hybridization (FISH). It showed that the karyotyping technique based on a combination of rDNA and Cot-1 DNA chromosome markers is a superior alternative. A more exact karyotype of *B. oleracea* has been developed based on rDNA locations and Cot-1 DNA fluorescent bands.

Keywords: *Brassica oleracea* L.; Cot-1 DNA; Karyotyping; Ribosomal DNA.

INTRODUCTION

The Multinational *Brassica* Genome Project, using diploid *Brassica rapa* L. (AA, 2*n*=20) and *Brassica oleracea* L. (CC, 2*n*=18) as two model species, is advancing rapidly in several laboratories (Rana et al., 2004; Park et al., 2005; Yang et al., 2005; Ayele et al., 2005; Katari et al., 2005). It has become another plant genome research hotspot. The genus *Brassica* includes many important oil and vegetable crops, and they play an increasing role in improving the lives of human beings. For *B. oleracea*, including a group of the most important vegetable crops, such as cauliflower, cabbage, calabrese and Brussels sprouts, the scientific community is anxious to perform cytogenetics research from an evolutionary or breeding point of view. However, karyotyping *B. oleracea* and exactly recognizing all individual chromosomes within a mitotic metaphase spread is very difficult. This is primarily due to the small size of the chromosomes as well as the similarity of chromosome lengths and/or arm ratios for some of the complement.

At present, distinguishing chromosomes from each other and the karyotyping of *B. oleracea* are mainly based on the number and position of the 45S (or 25S) and 5S rDNAs on the chromosome (Snowdon et al., 1997; Hasterok et al., 2001). FISH signals of 45S rDNA could be detected on two pairs of chromosomes of *B. oleracea*. The copy number of rDNA on the end of the short arm of chromosome 7 exceeds that on the end of the short arm of chromosome 4 (Cheng et al., 1995; Fukui et al., 1998). Sometimes, signals with a very low intensity are detected on the short arm end of chromosome 2 (Armstrong et al., 1998). For 5S rDNA, FISH signals with a very low intensity can detected on the long arms of chromosome 2 (Armstrong et al., 1998; Hasterok et al., 2001). Thus, rDNA sites are found in only three of nine chromosome pairs for *B. oleracea*. In *B. rapa*, another diploid species of *Brassica*, rDNA sites are found in six of ten chromosome pairs. Compared to *B. oleracea*, a more exact karyotyping was performed based on rDNA sites in *B. rapa* (Koo et al., 2004; Lim et al., 2005). Five chromosome pairs in *B. oleracea* were identified with cDNA and rDNA probes by Kamisugi et al. (1998), or with three repetitive sequences by Armstrong et al. (1998). How to exactly identify nine chromosome pairs of *B. oleracea* is a pending question, new markers are obviously needed for karyotyping.

The Cot-1 DNA is enriched with highly and moderately repetitive DNA sequences. In most eukaryote plant species, repetitive sequences comprise a large proportion of the genome (Flavell et al., 1974; Hake and Walbot, 1980; MCCouch and Tanksley, 1991). Class I transposable

*Corresponding author: E-mail: whwei@oilcrops.cn; Tel: +86-27-86722567; Fax: +86-27-86722567.

elements, or retrotransposons, represent a major fraction of all plant genomes (Kumar and Bennetzen, 1999; Alix et al., 2005) and are important factors in the generation of diversity because they can transpose from one genomic site to another. There is evidence that individual retroelement families have typical patterns of chromosomal localization (Presting et al., 1998). *In situ* hybridization has shown that *copia* group retrotransposons in *B. oleracea* are abundant and distributed along all chromosomes with higher density in some chromosomal regions (Brandes et al., 1997). Hence, it is conceivable to band individual chromosomes of *B. oleracea* with Cot-1 DNA. The karyotyping results presented in the present study are those obtained by the combination of a morphometric study and FISH with rDNA and Cot-1 DNA probes to *B. oleracea* chromosomes. The applied technique allowed more effective karyotype construction for this diploid *Brassica* species.

MATERIALS AND METHODS

Plant materials

Brassica oleracea var. *acephala* developed by the Institute of Oil Crops, Chinese Academy of Agricultural Sciences, was used in the present study. The first-born flower buds adopted from the field were used for chromosome preparation, and young plant leaves were used for genomic DNA extraction.

Preparation of rDNAs

The 25S and 5S rDNAs lodging bacteria supplied by Dr. Robert Hasterok (Department of Plant Anatomy and Cytology, The University of Silesia, Poland) were plated on solid LB medium, a single colony was selected for cloning in liquid medium, and plasmid DNA was isolated using Qiagen Mini Kit (Cat. No. 12125, supplied by Wuhan Boyer Bioengineering Co., Ltd; No. 40, Xudong Road, Wuhan City, China).

Chromosome preparation

The chromosome preparation method was developed using the technique described by Wei et al. (2001; 2005) with some modifications. Briefly, first-born full flower buds during mitosis were fixed in the mixture (95% ethanol : acetic acid glacial=3 : 1) at 4°C overnight after being treated in 4°C water for approximately 24 h. Flower buds were washed three to five times with distilled water, then digested in 1% (w/v) cellulase "Onozuka" R-10 (Yakult Honsha, Co., LTD, Japan) and 1% (w/v) pectolyase Y-23 (Yakult Honsha) dissolved in distilled water at 28°C for 2.5-3 h. Full flower buds, including tapetum and the wall of anther, were subjected to a hypotonic treatment in distilled water for 30 min before being squashed, and the preparations were dried with flame.

Genomic DNA extraction

The extraction of *B. oleracea* genomic DNA was performed according to the procedure described by Doyle and Doyle (1990). In brief, 5 g of young leaf tissue was homogenized in liquid nitrogen and mixed in 20 ml of preheated (65°C) DNA extraction buffer (0.1 M Tris-Cl, 20 mM Na_2 ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl and 20% [w/v] hexadecyltrimethylammonium bromide [CTAB] and 0.2% [v/v] β-mercaptoethanol [pH 8.0]) in sterile polypropylene centrifuge tubes and incubated at 65°C in a water bath for 1 h with occasional gentle swirling. The samples were mixed with chloroform-isoamylalcohol (24:1). The contents were centrifuged at 9000 *g* for 15 min at room temperature, and the aqueous phase was transferred to fresh sterile centrifuge tubes and mixed well with 0.67 vol (13.4 ml) of isopropanol by gently inverting the tubes 5-6 times. This mixture was centrifuged at 9000 *g* for 10 min to pellet the DNA. The pellet was washed with 70% (v/v) ethanol and air-dried. The DNA pellet was dissolved in Tris-EDTA (TE) buffer (pH 8.0).

Genomic DNA shearing and Cot-1 DNA isolation

Genomic DNA shearing and Cot-1 DNA isolation were performed as described by Zwick et al. (1997) and Wei et al. (2005). Briefly, the genomic DNA was diluted to a concentration of 300 ng/μL in 0.3 mol/L NaCl, samples were aliquot and autoclaved (121°C, 1.034×10^5 Pa) for 10 min to make about 100-1000 bp DNA fragments. The sample tube was then cooled on ice. The DNA was denatured by placing the tube in a 95°C water bath for 10 min. The tube was removed and cooled by swirling in ice water for 10 s before being incubated in a 65°C water bath for a re-annealing. The time needed for the reannealing reaction was calculated using the formula C_0t = DNA conc (Mole/L) × re-naturation time in sec (Ts), C_0t =1, Ts = 1/ DNA conc. C_0 = (0.300 g/L) / (339 g/mol, an average molecular weight for a deoxynucleotide monophosphate) = 8.85×10^{-4} mol/L, so T = 1 / (8.85×10^{-4}) = 1130 sec (Zwick et al., 1997).

After the time allotted for re-annealing had elapsed, the tube was removed from the 65°C water bath. An appropriate amount of 10 × S1 buffer (Promega, Cat. No. M5761) was added firstly, and the sample was mixed thoroughly. The S1 nuclease was then added, and the solution was mixed thoroughly, but gently. Immediately, the tube was placed in a 37°C water bath for 8 min. The reaction was stopped by immediate phenol extraction using equal volumes of Tris-equilibrated phenol, and the subsequent steps in the genomic DNA extraction method were performed until the Cot-1 DNA was resuspended in 20 μL TE. Samples were stored at -20°C after quantitative analysis.

Labeling of probes, FISH, and detection of the signals

The Cot-1 DNA was labeled with Biotin-Nick Translation Mix Kit (catalogue no. 11745824910; Roche, Germany. Supplied by Wuhan Boyer Bioengineering Co., Ltd, China), 25S rDNA was labeled with DIG-Nick Translation Mix Kit (Cat. No. 11745816910; Roche), and 5S

rDNA was directly labeled with biotin-11-dUTP by PCR using forward primer 5′-GGATGGGTGACCTCCCGG GAAGTC-3′ and reverse primer 5′-CGCTTAACTGCG GAGTTCTGATGGG-3′ (Yang et al., 1998). DNA was amplified for 35 cycles of 1 min at 94°C, 45 s at 55°C, 1 min at 72°C, and a final period of 5 min at 72°C. FISH and hybridization signal detection were performed according to the procedures described by Wei et al. (2002; 2003; 2005). Briefly, chromosome preparations were pretreated with 100 μg/mL RNase (in 2 × SSC, 0.3 mol/L sodium chloride plus 0.03 mol/L sodium citrate) at 37°C for 1 h and then rinsed briefly in 2 × SSC. Chromosomal DNA was then denatured by immersing the slide in 70% deionized formamide in 2 × SSC at 70°C for 3 min. After dehydration of the preparation in an ice-cold 70%, 95%, and 100% ethanol series and air drying, 40 μL denatured probe cocktail (5 ng/μL labeled probe DNA, 0.5 μg/μL sheared salmon sperm DNA, 10% dextran sulphate, 50% deionized formamide, 0.1% sodium dodecyl sulfate (SDS), and 2 × SSC) was added to the slide and hybridization was performed at 37°C overnight. Post-hybridization washes included a stringent wash in 20% formamide and a wash in 2 × SSC at 42°C for 10 min to remove weakly bound probe; signals were detected with streptavidin-Cy3 (catalogue no. PA43001; Amersham Biosciences UK Limited, England) for the biotin labeled probe and with anti-digoxigenin-fluorescein (Roche) for the digoxigenin labeled probe; washing in phosphate-buffered saline (0.13 M NaCl, 0.007 M $Na_2HPO_4 \cdot 12H_2O$, 0.003M $NaH_2PO_4 \cdot 2H_2O$) was done for 10 min after each probe detection step. Slides were counterstained with 2 μg/mL 4′, 6′-diamidino-2-phenylindole (DAPI) and examined under a Leica DM IRB fluorescence microscope equipped with a DFC300 CCD camera.

RESULTS

Preparation and shearing of genomic DNA and isolation of Cot-1 DNA

The preparation and shearing of genomic DNA and the isolation of Cot-1 DNA results are shown in Figure 1. The size of genomic DNA is above 20 kb in size, and it became about 100-2000 bp, mostly under 1000 bp, after being autoclaved for 10 min. The isolated Cot-1 DNA was under 1000 bp in size.

FISH

25S rDNA was detected on chromosome pairs 4 and 7, a fact affirmed by published results (Howell et al., 2002). Chromosome 7 presented especially bright signals, and detecting them on these two chromosome pairs was easy (Figure 2A). 5S rDNA was detected on one chromosome pair, but the signal was weak (Figure 2B) and detection was difficult. Individual metaphase chromosomes showed bright Cot-1 DNA fluorescence bands (Figure 2C, D). The sites of 25S rDNA are in accord with those of Cot-1 DNA, identified by dual-colour FISH (Figure 3). The Cot-1 DNA fluorescence bands combined with rDNA loci sites and chromosome morphology should be powerful for karyotyping because recognition markers of the chromosomes contain not only specific banding patterns of Cot-1 DNA but also the rDNA signals and the chromosome morphology.

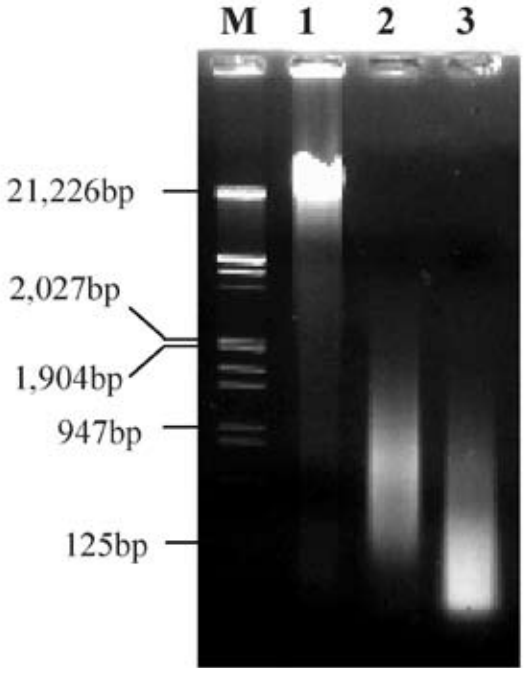

Figure 1. Preparation of *B. oleracea* Cot-1 DNA. M: λDNA/*Eco*RI+*Hin*dIII marker; 1: Total genomic DNA of *B. oleracea*; 2: Genomic DNA after autoclaved for 10 min; 3: Cot-1 DNA.

Karyotyping

Karyotyping of *B. oleracea* was performed according to the method published by Armstrong et al. (1998) and Howell et al. (2002). The *B. oleracea* karyotype obtained in the present study on the basis of Cot-1 DNA hybridization bands combined with 25S rDNA sites and morphometric analysis is shown in Figure 4. The green 25S rDNA hybridization signals were located on chromosome pairs 4 and 7. The red or pinkish white Cot-1 bands are shown on the long arms of chromosome pairs 6 and 8, the short arms of chromosome pairs 2, 4, and 7, and the pericentromeric sites of chromosome pairs 1, 3, 5 and 9. Different chromosome pairs could be recognized reliably based on Cot-1 DNA banding patterns and rDNA locations.

DISCUSSION

In the present study, 25S rDNA was detected steadily on two chromosome pairs, and 5S rDNA accidentally on one chromosome pair. The numbers of chromosomes with detectable rDNAs were coincident with former reports (Cheng et al., 1995; Fukui et al., 1998). However, the number of chromosome pairs with detectable 25S rDNA was one less than the result reported by Howell et al. (2002), and the detection frequency of the 5S rDNA site

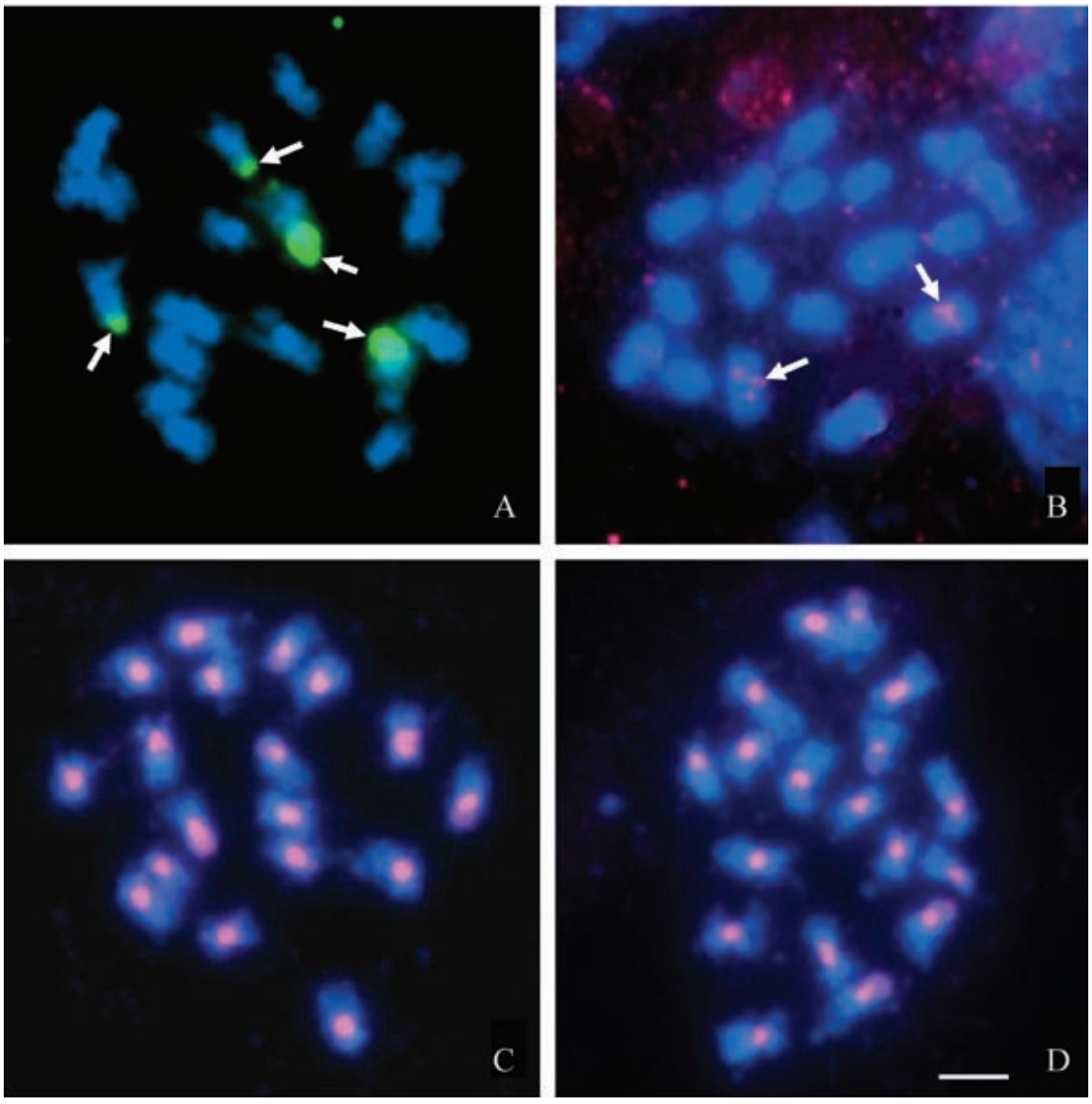

Figure 2. 25S rDNA, 5S rDNA and Cot-1 DNA FISH to the metaphases of *B. oleracea*. A, 25S rDNA showed green fluorescence, labeled with Digoxigenin-11-dUTP and detected with anti-digoxigenin-fluorescein; B, 5S rDNA showed red fluorescence, labeled with Biotin-11-dUTP and detected with streptavidin-Cy3; C and D, Cot-1 DNA FISH. Bar, 5 μm.

was very low. The reasons may be that the copy numbers of 5S rDNA repetitive sequences are very low at these sites and more difficult to detect, or the rDNAs sites vary by cultivars (Weiss and Maluszynska, 2000).

The Cot-1 DNA is enriched with highly and moderately repetitive DNA sequences. rDNA, a moderately repetitive DNA sequence, is included in Cot-1 DNA. Hence, the Cot-1 DNA isolated from a species should contain rDNA. Theoretically speaking, the sites that present Cot-1 DNA signals should include the sites of rDNAs loci in FISH, which has been proved partially by dual-color FISH of 25S rDNA and Cot-1 DNA. This is also the reason that 25S rDNA and Cot-1 DNA were used as chromosome co-markers. In this study, individual metaphase chromosome pairs of *B. oleracea* could be stably banded by Cot-1 DNA. Based on steady 25S rDNA and Cot-1 DNA chromosome markers, individual chromosome pairs of *B. oleracea* could be identified reliably by dual-color FISH. These two co-markers exceed any one used alone in chromosome identification. This technique is a simple, fast, and credible means of identifying the chromosome set of a species.

The results reported in the present study demonstrate that Cot-1 DNA FISH can successfully show bands on individual chromosome pairs in *B. oleracea*. Moreover, the Cot-1 DNA FISH banding patterns possessed some important features. For example, non-homologous chromosomes are different, and two members of each chromosome pair are analogous in the banding patterns. Therefore, based on

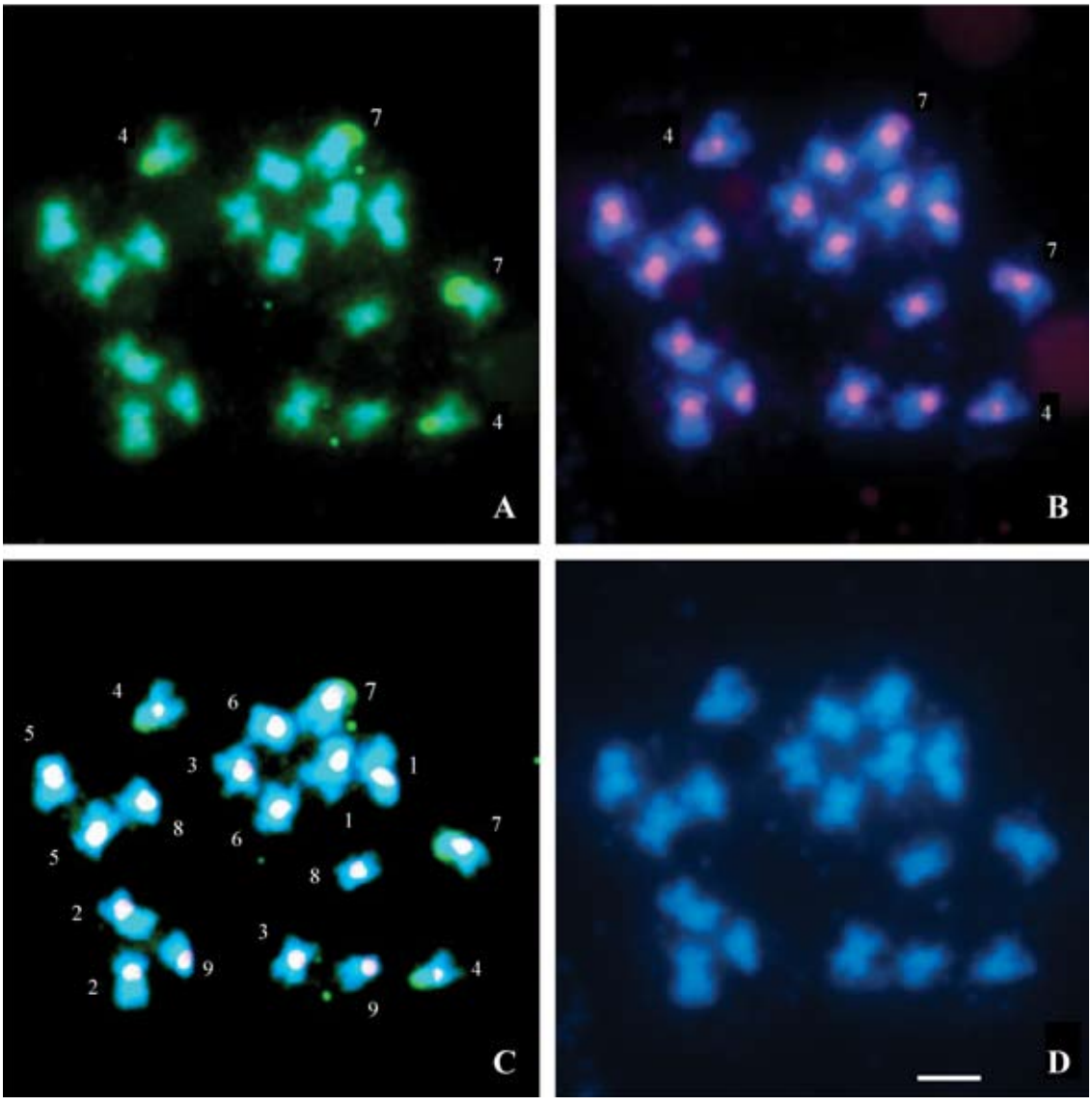

Figure 3. Dual-colour FISH of 25S rDNA and Cot-1 DNA to the mitotic spreads of *B. oleracea*. A, 25S rDNA labeled with Digoxigenin-11-dUTP and detected with anti-digoxigenin-fluorescein showed green fluorescence; B, Cot-1 DNA labeled with Biotin-11-dUTP and detected with streptavidin-Cy3 showed red fluorescence; C, An overlapping of green fluorescence for 25 rDNA and red fluorescence for Cot-1 DNA; D, Metaphase of DAPI dyeing corresponding to the spread in C. Bar, 5 μm.

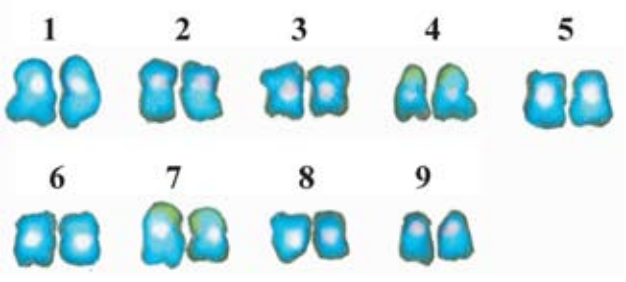

Figure 4. Ideogram of *Brassica oleracea* somatic chromosomes based on the metaphase plate shown in Figure 3C. Numbers show the general numbering of chromosomes, which are arranged in descending order according to total length taking no account of the length of chromosome 7 satellite.

the features of the banding patterns, pairing between two members of each chromosome pair and recognition among non-homologs over the entire genome could be performed easily and effectively. The Cot-1 DNA banding has been used previously in humans (Wang et al., 1995) and in *Brassica napus* (Wei et al., 2005). However, combining it with rDNA markers in karyotyping has never been attempted. We have successfully completed the karyotyping of *B. oleracea* by rDNA locating and Cot-1 DNA banding for the first time. Obviously, Cot-1 DNA banding could be further applied to those plants in which karyotyping has been performed based solely on rDNA locating.

Although Cot-1 bands were confirmed on the long arms of chromosome pairs 6 and 8, the short arms of chromo-

some pairs 2, 4 and 7, and the pericentromeric sites of chromosome pairs 1, 3, 5 and 9; they are all located in a domain near the centromere. Sometimes it is difficult to affirm their sites on long arms, short arms or centromeres. This question will be probably solved with additional FISH of the centromere markers. Even now, the specific Cot-1 band patterns of each chromosome pair provide a fine maker for chromosome pair identification as rDNA.

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LITERATURE CITED

Alix, K., C.D. Ryder, J. Moore, G.J. King, and J.S. Heslop-Harrison. 2005. The genomic organization of retrotransposons in *Brassica oleracea*. Plant Mol. Biol. **59:** 839-851.

Armstrong, S.J., P. Fransz, D.F. Marshall, and G.H. Jones. 1998. Physical mapping of DNA repetitive sequences to mitotic and meiotic chromosomes of *Brassica oleracea* var. *alboglabra* by fluorescence in situ hybridization. Heredity **81:** 666-673.

Ayele, M., B.J. Haas, N. Kumar, H. Wu, Y. Xiao, S.V. Aken, T.R. Utterback, J.R. Wortman, O.R. White, and C.D. Town. 2005. Whole genome shotgun sequencing of *Brassica oleracea* and its application to gene discovery and annotation in *Arabidopsis*. Genome Res. **15:** 487-495.

Brandes, A., J.S. Heslop-Harrison, A. Kamm, S. Kubis, R.L. Doudrick, and T. Schmidt. 1997. Comparative analysis of the chromosomal and genomic organization of Ty1-*copia* like retrotransposons in pteridophytes, gymnosperms and angiosperms. Plant Mol. Biol. **33:** 11-21.

Cheng, B.F., W.K. Heneen, and B.Y. Chen. 1995. Mitotic karyotypes of *Brassica campestris* and *Brassica alboglabra* and identification of the *B. alboglabra* chromosome in an addition line. Genome **38:** 313-319.

Doyle, J.J. and J.I. Doyle. 1990. Isolation of plant DNA from fress tissue. Focus **12:** 13-15.

Flavell, R.B., M.D. Bennett, J.B. Smith, and D.B. Smith. 1974. Genome size and the proportion of repeated sequence DNA in plants. Biochem. Genet. **12:** 257-269.

Fukui, K., S. Nakayama, N. Ohmido, H. Yoshiaki, and M. Yamabe. 1998. Quantitative karyotyping of three diploid *Brassica* species by imaging methods and localization of 45S rDNA loci on the identified chromosomes. Theor. Appl. Genet. **96:** 325-330.

Hake, S. and V. Walbot. 1980. The genome of *Zea mays*, its oeganization and homology to related grasses. Chromosoma **79:** 251-270.

Hasterok, R., G. Jenkins, T. Langdon, R.N. Jones, and J. Maluszynska. 2001. Ribosomal DNA is an effective marker of *Brassica* chromosomes. Theor. Appl. Genet. **103:** 486-490.

Howell, E.C., G.C. Barker, G.H. Jones, M.J. Kearsey, G.J. King, E.P. Kop, C.D. Ryder, G.R. Teakle, J.G. Vicente, and S.J. Armstrong. 2002. Integration of the cytogenetic linkage maps of *Brassica oleracea*. Genetics **161:** 1225-1234.

Kamisugi, Y., S. Nakayama, C.M. O'Neill, R.J. Mathias, M. Trick, and K. Fukui. 1998. Visualization of the *Brassica* self-incompatibility *S*-locus on identified oilseed rape chromosomes. Plant Mol. Biol. **38:** 1081-1087.

Katari, M.S., V. Balija, R.K. Wilson, R.A. Martienssen, and W.R. McCombie. 2005. Comparing low coverage random shotgun sequence data from *Brassica oleracea* and *Oryza sativa* genome sequence for their ability to add to the annotation of *Arabidopsis thaliana*. Genome Res. **15:** 496-504.

Koo, D.H., P. Plaha, Y.P. Lim, Y. Hur, and J.W. Bang. 2004. A high-resolution karyotype of *Brassica rapa* ssp. *pekinensis* revealed by pachytene analysis and multicolor fluorescence in situ hybridization. Theor. Appl. Genet. **109:** 1346-1352.

Kumar, A. and J.L. Bennetzen. 1999. Plant retrotransposons. Annu. Rev. Genet. **33:** 479-532.

Lim, K.B., H. Jong, T.J. Yang, J.Y. Park, S.J. Kwon, J.S. Kim, M.H. Lim, J.A. Kim, M. Jin, Y.M. Jin, S.H. Kim, Y.P. Lim, J.W. Bang, H. Kim, and B.S. Park. 2005. Characterization of rDNA and tandem repeats in the heterochromatin of *Brassica rapa*. Mol. Cells **19:** 436-444.

McCouch, S.R. and S.D. Tanskley. 1991. The world rice economy: Challenges ahead. *In* G.S. Khush and G.H. Toenniessen (eds.), Rice Biotechnology: Biotechnology in Agriculture No. 6. Commonwealth Agricultural Bureaux International, Wallingford, UK.

Park, J.Y., D.H. Koo, C.P. Hong, S.J. Lee, J.W. Jeon, S.H. Lee, P.Y. Yun, B.S. Park, H.R. Kim, J.W. Bang, P. Plaha, I. Bancroft, and Y.P. Lim. 2005. Physical mapping and microsynteny of *Brassica rapa* ssp. *pekinensis* genome corresponding to a 222 kbp gene-rich region of *Arabidopsis* chromosome 4 and partially duplicated on chromosome 5. Mol. Genet. Genom. **9:** 1-10.

Presting, G.G., L. Malysheva, J. Fuchs, and I. Schubert. 1998. A Ty3/Gypsy retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes. Plant J. **16:** 721-728.

Rana, D., T. van den Boogaart, C.M. O'Neill, L. Hynes, E. Bent, L. Macpherson, J.Y. Park, Y.P. Lim, and I. Bancroft. 2004. Conservation of the microstructure of genome segments in *Brassica napus* and its diploid relatives. Plant J. **40:** 725-733.

Snowdon, R.J., W. Kohler, and A. Kohler. 1997. Chromosomal localization and characterization of rDNA loci in the *Brassica* A and C genomes. Genome **40:** 582-587.

Wang, Y., S. Minoshima, and N. Shimizu. 1995. C_0t-1 banding of human chromosomes using fluorescence *in situ* hybridization with Cy3 labeling. Jpn. J. Human Genet. **40:** 243-252.

Wei, W.H., R. Qin, Y.C. Song, L.Q. Guo, and M.G. Gu. 2001. Comparative analyses of disease resistant and nonresistant lines from maize × *Zea diploperennis* by GISH. Bot. Bull. Acad. Sin. **42:** 109-114.

Wei, W.H., R. Qin, Y.C. Song, S.B. Ning, L.Q. Guo, and M.G. Gu. 2002. Location and analysis of introgressed segments in the parthenogenetic progenies of *Zea mays* × *Z. diploperennis* by GISH. Acta Bot. Sin. **44:** 373-376.

Wei, W.H., W.P. Zhao, Y.C. Song, L.H. Liu, L.Q. Guo, and M.G. Gu. 2003. Genomic *in situ* hybridization analysis for identification of introgressed segments in alloplasmic lines from *Zea mays* × *Zea diploperennis*. Hereditas **138:** 21-26.

Wei, W.H., W.P. Zhao, L.J. Wang, B. Chen, Y.C. Li, and Y.C. Song. 2005. Karyotyping of *Brassica napus* L. based on *Cot*-1 DNA banding by fluorescence in situ hybridization. J. Integ. Plant Biol. **47:** 1479-1484.

Weiss, H. and J. Maluszynska. 2000. Chromosomal rearrangement in autotetraploid plants of *Arabidopsis thaliana*. Hereditas **133:** 255-261.

Yang, T.J., J.S. Kim, K.B. Lim, S.J. Kwon, J.A. Kim, M. Jin, J.Y. Park, M.H. Lim, H. Kim, S.H. Kim, Y.P. Lim, and B.S. Park. 2005. The Korea *Brassica* genome project: A glimpse of the *Brassica* genome based on comparative genome analysis with *Arabidopsis*. Comp. Funct. Genom. **6:** 138-146.

Yang, Y.W., P.F. Tseng, P.Y. Tai, and C.J. Chang. 1998. Phylogenetic position of *Raphanus* in relation to *Brassica* species based on 5S rDNA spacer sequence data. Bot. Bull. Acad. Sin. **39:** 153-160.

Zwick, M.S., R.E. Hanson, T.D. McKnight, M.H. Islam-Faridi, D.M. Stelly, R.A. Wing, and H.J. Price. 1997. A rapid procedure for the isolation of C_0t-1 DNA from plants. Genome **40:** 138-142.

甘藍基於 Cot-1 DNA 及 rDNA 的核型分析

魏文輝[1,2]　張蘇鋒[1]　王力軍[2]　陳　波[2]　伍曉明[2]　宋運淳[3]

[1] 中國信陽師範學院生命科學學院

[2] 中國農業科學院油料作物研究所

[3] 中國武漢大學植物發育生物學教育部重點實驗室

為了探索一種簡單、可靠、有效的甘藍核型分析新方法，我們用 rDNA 結合 Cot-1 DNA 作為甘藍染色體識別標記，成功地對甘藍進行了準確的核型分析。甘藍基因組 DNA 用生物素切刻平移試劑盒標記後作探針，原位雜交到有絲分裂中期相，每對染色體均顯示出特定的螢光帶型。25S 及5S rDNAs 分別用毛地黃素及生物素切刻平移試劑盒標記後作探針，各自原位雜交到有絲分裂中期相，有2對染色體檢測出 25S rDNA 雜交信號，只有 1 對染色體檢測出較弱的 5S rDNA 雜交信號。Cot-1 DNA 包含 rDNA，雙色螢光原位雜交顯示，25S rDNA 與 Cot-1 DNA 雜交信號位置是一致的，表明基於 rDNA 及 Cot-1 DNA 的核型分析技術優於單獨使用一種標記技術。基於 rDNA 位置及 Cot-1 DNA 螢光帶，本研究對甘藍進行了精確的核型分析。

關鍵詞：核型分析；甘藍；Cot-1 DNA；rDNA。