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RAPD Analysis for Varietal Identification in *Brassica*

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ABSTRACT

The concentration of DNA extracted from leaves of Brassica seedlings was amplified by RAPD (Random Amplified Polymorphic DNA) using 35 single 10-base oligonucleotide primers to analyzed genetic relationships among *Brassica species*. Amplified products were then analyzed by polyacrylamide gel electrophoresis. Each species expressed specific banding pattern. RAPD products were then used to (a) prove genetic basis of an interspecific hybrid between *Brassica campestris* and *B. juncia*, and (b) examine differences both within and among parents and hybrids in *B. campestris* var. *pekinensis*.

Keywords: RAPD, PCR, *Brassica*, cultivar identification

INTRODUCTION

Plant variety identification is a critical activity in breeding programs, germplasm preservation, and seed marketing to control propagation and quality of products. Phenotypic description in the field is time- and labor- consuming and often provides ambiguous descriptors. Isozymes and protein electrophoresis have been developed and used for genotypic description recently (Gupta and Robbelen, 1986; Cooper, 1987; and Glazmann, 1988). These analyses allow detection of a limited amount of polymorphism among closely related genotypes but their efficiency is influenced by age, specific organ and environmental factors (Stegman *et al.*, 1973; Hosaka *et al.*, 1985; Temiesak and Hasdiseve, 1987). Another technique, restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980; Soller and Beckmann 1983), which is based on DNA and is not influenced by internal and environmental factors, is powerful to detect many polymorphic markers but the procedure is costly and time consuming. Williams *et al.* (1990) and Welsh and McCleland (1990) introduced another method based on amplification of random DNA sequence using polymerase chain reaction (PCR) with a single short oligonucleotide arbitrary primer. This technique, called random amplified polymorphic DNA (RAPD), is simple, rapid and needs only nanogram amount of genomic DNA and thus allows the analysis to be performed using a single seedling alone. This technique does not involve the step of using specific restriction enzyme and DNA probe for hybridization and only primer change primer RAPD could give a wide polymorphism. RAPD has been used for taxonomic classification in several contexts, ranging from individuals, cultivars, and to species in several crops [(Cocoa (Wilde *et al.*, 1992), rice (Fukuoka *et al.*, 1992), apple (Koller *et al.*, 1993) and potato (Baird *et al.*, 1992; and Mori *et al.*, 1993)]. In this report, application of RAPD to examine the genetic basis of differences in *Brassica* is described.

MATERIALS AND METHODS

1. Plant materials

Different Brassica species and cultivars collected from difference sources (Table 1) were used.

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Table 1 Sources of plant material used for DNA extraction.

Plant species and cultivars	Sources
B. campestris var. *pekinensis* -E 7 (male), B 18 (female) and ASVERG 1 (F1 hybrid) *B. campestris* var. *chinensis* *B. juncea* *B. oleracea* var. *italica* *B. oleracea* var. *alboglabra*	Asian Vegetable Research and Development Center, Nakhon Pathom
Interspecific hybrids of *B. campestris* var. *parachinensis campestris* var. *chinensis* as female and *B. juncea* var. *rogusa* as male plants	Central Laboratory and Green House Complex Kasetsart University

2. Preparation of genomic DNA (template DNA) :

Genomic DNA from fresh young leaves of 5-day old seedling was extracted according to a modified method of Doyle and Doyle (1987) as follows:

- 50 mg young leaf was put into liquid nitrogen then ground.
- The powdered leaf was put into an eppendorf tube containing 500 ul extraction buffer, 7 ul 2-mercaptoethanol and 100 ul SDS and incubated at 65°C for 15 min
- 17 ul 5 M potassium acetate (pH 5.2) was added and kept at -40°C for 20 minutes or longer before centrifuging at 15,000 rpm for 10 min.
- 450 ul of supernatant was taken and added to 350 ul cold isopropanol and kept at -40°C for 10 minutes before centrifuging at 15,000 rpm for 10 min and drying up in vacuum suction.
- The pellet was dissolved in 140 ul T_{50} E_{10} buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA) and centrifuge at 15,000 rpm for 10 minutes.
- The supernatant was added to 15 ul of 3 M sodium acetate and 300 ul cold-isopropanol and spinned for 3 min.
- The pellet was washed with 80% ethanol and short spin before dry up in vacuum.
- DNA was dissolved in 100 ul $T_{10}E_1$ (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and was then kept at low temperature but not lower than -20°C.
- DNA concentration was determined by spectophotometer at a wavelength of 260 nm and adjusted concentration to 25 ng/ul with $T_{10}E_1$ buffer.

3. Polymerase chain reaction (PCR)

3.1 Primers

The primers used were OPERON 10-mer kits (Operon Technologies, Inc.) as follows:

3.2 Amplified DNA reaction

OERON 10-mer KITS (OPERON TECHNOLOGIES. INC.)

KIT A

OPA-01	CAGGCCCTTC	06	GGTCCCTGAC	11	CAATCGCCGT	16	AGCCAGCGAA
02	TGCCGAGCTG	07	GAAACGGGTG	12	TCGGCGATAG	17	GACCGCTTGT
03	AGTCAGCCAC	08	GTGACGTAGG	13	CAGCACCCAC	18	AGGTGACCGT
04	AATCGGGCTG	09	GGGTAACGCC	14	TCTGTGCTGG	19	CAAACGTCGG
05	AGGGGTCTTG	10	GTGATCGCAG	15	TTCCGAACCC	20	GTTGCGATCC

KIT B

OPB-01	GTTTCGCTCC	06	TGCTCTGCCC	11	GTAGACCCGT	16	TTTGCCCGGA
02	TGATCCCTGG	07	GGTGACGCAG	12	CCTTGACCCA	17	AGGGAACCAG
03	CATCCCCCTG	08	GTCCACACGG	13	TTCCCCCCCT	18	CCACAGCAGT
04	GGACTGGAGT	09	TGGGGGACTC	14	TCCGCTGTGG	19	ACCCCCGAAG
05	TGCGCCCTTC	10	CTGCTGGGAC	15	GGAGGGTGTT	20	GGACCCTTAC

KIT T

OPT-01	GGGCCACTCA	06	CAAGGGCAGA	11	TTCCCCGCGA	16	GGTGAACGCT
02	GGAGAGACTC	07	GGCAGGCTGT	12	GGGTGTGTAG	17	CCAACGTCGT
03	TCCACTCCTG	08	AACGGCGACA	13	AGGACTGCCA	18	GATGCCAGAC
04	CACAGAGGGA	09	CACCCCTGAG	14	AATGCCGCAG	19	GTCCGTATGG
05	GGGTTTGGCA	10	CCTTCGGAAG	15	GGATGCCACT	20	GACCAATGCC

Amplified DNA reaction was performed in volume of 25 ul including 25 ng genomic DNA, 15 ng primer DNA, 0.5 unit Taq polymerase (Perkine), 2 mM $MgCl_2$ and 100 μM dNTP (dATP, dCTP, dGTP and dTTP).

3.3 Thermal cycle for PCR reaction

Thermal cycle (Perkin) was set for 1 min at 94° C, 1 min at 37°C and 2 min at 72°C for 44 cycles and terminated at 72°C for 2 min. The PCR reaction was repeated at least four times to test reproducible of the results.

4. Electrophoresis

Amplified DNA was roughly screened by a mini Mupid) agarose gel (mixed with 4 ul of 10 mg/ml ethidium bromide) electrophoresis. Only polymorphic amplified products were further analysed by 5% polyacrylamide gel electrophoresis. Silver staining of DNA on polyacrylamide gel was performed according to a modified method of Basam *et al.*, 1991.

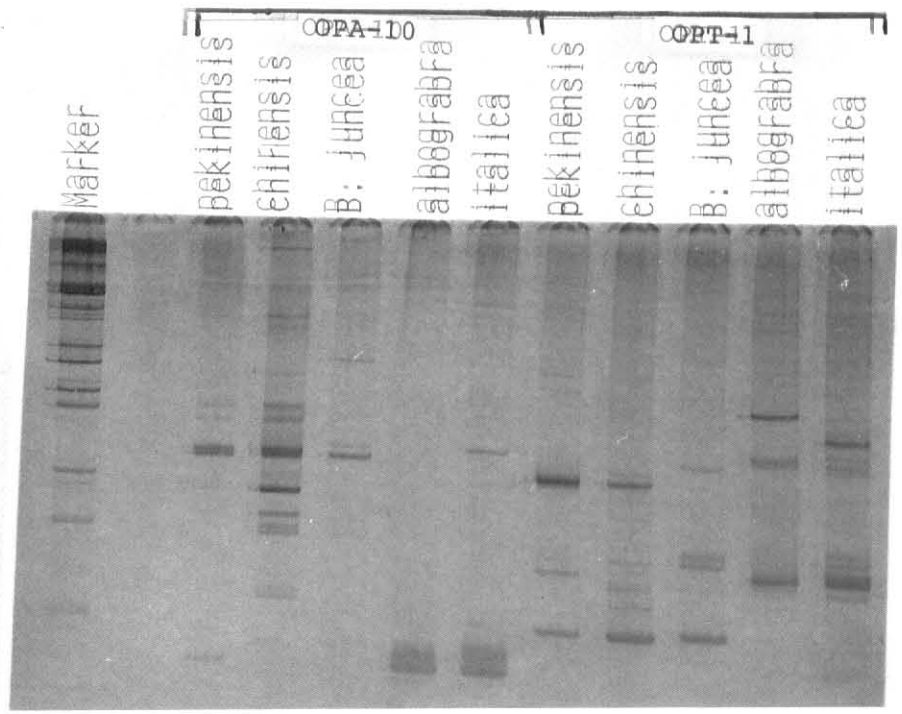

Figure 1 **Differences in RAPD band patterns of genomic DNA extracted from various Brassica species OPA-10 (lanes 3-7) and OPT-1 (lanes 8-12) primers.**

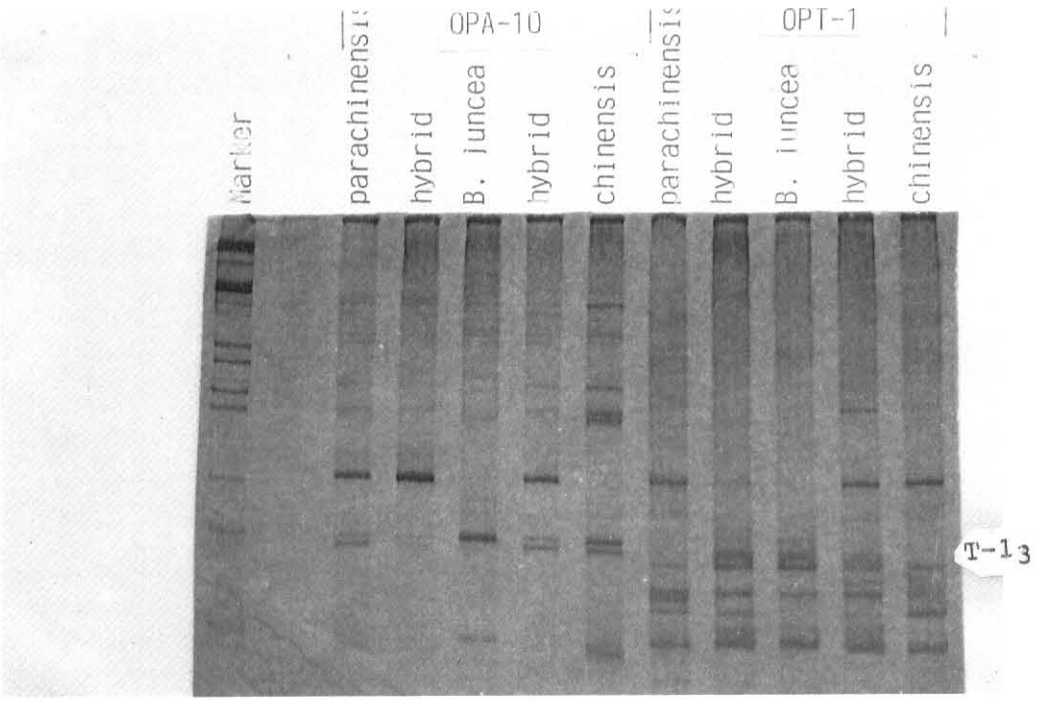

Figure 2 RAPD band patterns of interspecific hybrids of Brassica and their parents using OPA-10 (lanes 3-7) and OPT-1 (lane 8-12) primers.

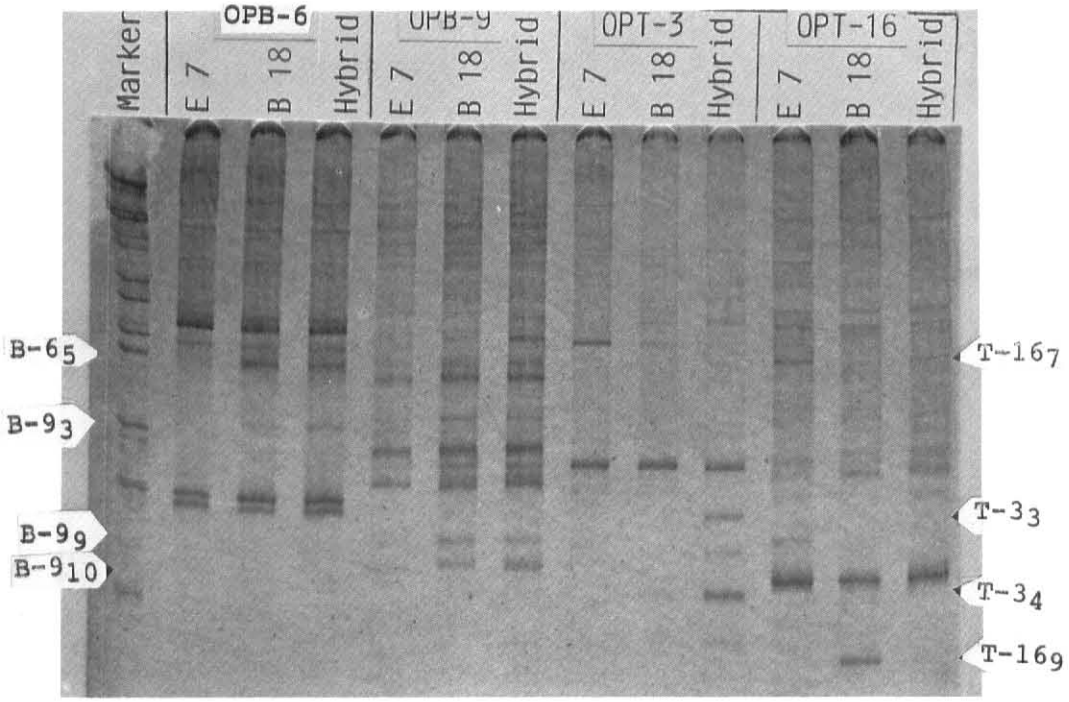

Figure 3 RAPD band patterns of F1-hybrid and their parents Brassica campestris var. pekinensis using OPB-6, OPB-9, OPT-3 and OPT-16 primers.

RESULTS AND DISCUSSION

1. RAPD band pattern of various Brassica species

Each *Brassica* species expressed specific banding pattern (figure 1). However, only a few differences were detected between closely related crops such as chinese kale (*B. oleracea* var. *alboglabra*) and broccoli (*B. oleracea* var. *italica*), both having CC genome set (U 1935). Similarly RAPD banding pattern of *B. juncea* (AABB), which was developed from *B. campestris* (AA) and *B. nigra* (BB), (U 1935 and Sunberg *et al.* 1987), was largely similar to that of chinese cabbage (*B. campestris* var. *pekinensis*). Demke *et al.* (1992), based on analysis of 284 RAPD bands, revealed the classical U triangle relationship between diploid and amphidiploid *Brassica* taxa. *Raphanus sativas* and *Synapsis. alba*. They reported that at least ten primers with approximately 100 total bands, were needed to adequately portray these relationships. In our experiments 60 primers were examined and finally 35 of them were used to analyse genetic relationships among species. Of these 35 primers, two representative primers (OPA-10 and OPT-1) show clear differences between species, as shown in figure 1. RAPD was thus powerful to examine various polymorphic on genetic on genetic diversity among Brassica species.

2. RAPD band patterns of interspecific hybrids and their parent

Through amplification with OPA-10 primer, it was found that RAPD band patterns of both interspecific hybrids were similar to those of female parent (*B. campestris* var. *parachinensis*/var. chinensis) as shown in figure 2. With OPT-1 primer however, it was found that there were some similarities in RAPD banding pattern of hybrids with that of male parents (*B. juncea*) at band mark T-13. The results showed that RAPD could be used successfully to examine genetic relationship of interspecific hybrids, obtained by embryo culture and their parents.

3. RAPD band patterns of F1-hybrid and its parents in chinese cabbage

The 60 primers were examined and 4 of them (OPB-6, OPB-9, OPT-3 and OPT-16) were finally used to analyse genetic relationships among F1-hybrid and its parents (figure 3). The results were entirely reproducible in different replications. Through amplication with OPB-6 or OPB-9 primers, F1-hybrid showed similar band pattern to its female parent (B 18). However, distinct differences in band patterns of hybrid and male plant (E7) were observed at marker of of B-65, B-93, B-99 and B-910. Amplication with OPT-3 show differences of F1-hybrid from male and female plants at T-33, T-34. Through amplication with OPT-16, distinguish differences of F1-hybrid from its female parent at T-167 and T-169 could be observed. According to the fact that these varieties were closed in genetic relationship, only 4 from 60 primers, i.e.; OPB-6, OPB-9, OPT-3 or OPT-16 could distinguish F1-hybrid. Hu and Quires 1991 reported that larger differences were found between cultivars from different seed companies than within the same company. The results from our experiments suggested that RAPD with a single 10 base oligonucleotide primers was powerful tool to distinguish F1-hybrid.

Huand Quiros (1991) concluded that RAPD markers produce a quick and reliable alternative to identify broccoli and cauliflower cultivars.. Our results (with various Brassica species) in this study confirm their observations.

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