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Prospection for Using RAPD Analysis for Varietal Identification in Tomato and Cucumber

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ABSTRACT

DNA extracted from leaves of seedlings was assessed using polymerase chain reaction (PCR) with single 10- base oligonucleotide random primers to analyse genetic differences among varieties of tomato and cucumber. Electrophoresis of amplified product revealed polymorphism among genetically related varieties. The RAPD (random amplified polymorphic DNA) analysis indicated a higher similarlity between Seeda Tip 93 to Seeda the two Tip 92 than Seeda Yak tomato variety, and higher similarlity between C4 cucumber improved varieties than the two local variety.

Keywords: RAPD, tomato, cucumber, variety, identification

INTRODUCTION

Advances in plant varietal identification using RFLP (Restriction Fragment Length Polymorphism) (Botstein, *et al.* 1980) are considered updated. New methods, independent of restriction sites, are being developed for detection of polymorphism, by using polymerase chain reaction (PCR) technology in developing a set of random amplified polymorphic DNA (RAPD) (Welsh and McCleland 1990). RAPD using single primers appears to be an effective and highly sensitive method to identify varieties in breeding program. RAPD assay avoids many of the technical limitations of RFLP to map triats and to fingerprint individuals of crop improvement (Rafalsik, *et al.* 1991; Carlson *et al.* 1991; Oliver, 1990; Klein-Lankhorst *et al.* 1991; Waugh and Powell 1992). RAPD is powerful tool for identification and monitoring pedigree breeding record inbred parents or varieties, evaluation in test crosses (Struss, *et al.* 1992; Echt, 1992; and Baird, et al. 1992) and determing genetic relationships among genotype (Vierling and Nguyen, 1992). In this report, we describe an application of RAPD to examine the polymorphism in varieties of tomato and cucumber.

MATERIALS AND METHODS

1. Plant materials.

The plant materials were collected from difference sources (Tabel 1) were used.

2. Preparation of genomic DNA (template DNA):

Genomic DNA from fresh young leaves of 7 - 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 and 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

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Table 1 Sources of plant material used for DNA extraction.

Plant material	Sources
KU porter tomato	
- Seeda Tip 2	Asian Vegetable Research and Development Center, Nakhon Pathom
- Seeda Tip 93	
- Seeda Yak	Central Laboratory and Green House Complex (CLGC), Kasetsart University,
Cucumber	CLGC, Kasetsart University
- C#4 (short green Type)	
- C#4 (short pale green Type)	
- local variety (short type)	
- local variety (long type)	

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 below -20°C.
- DNA concentration was determined by spectophotometer at wavelength 260 nm and concentration adjusted to 25 ng/ul with $T_{10}E_1$ buffer.

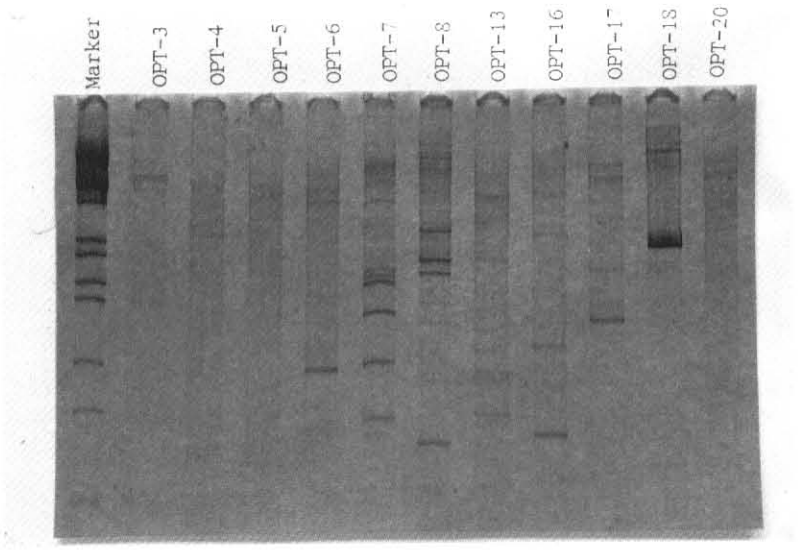

Figure 1 Differences in RAPD band patterns of genomic DNA extracted from tomato (variety name: Seeda Yak) and amplified with difference primers.

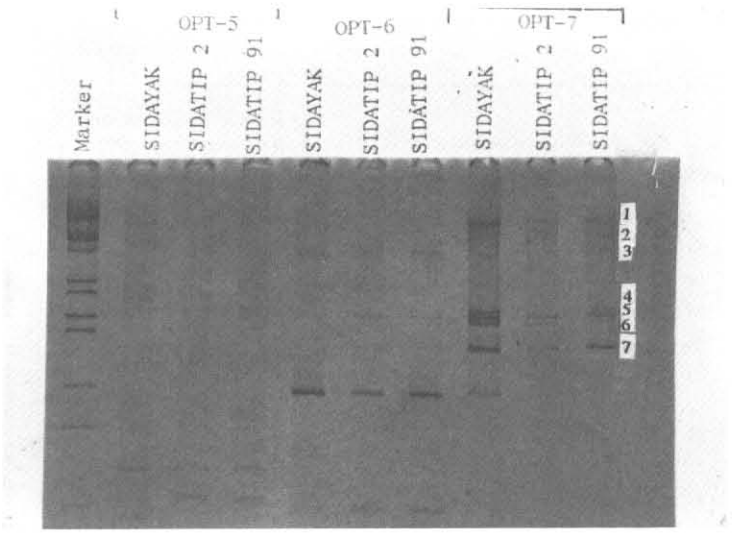

Figure 2 Differences in RAPD band patterns of genomic DNA extracted from three varieties of tomato (Seeda yak, Seeda Tip 2 and Seeda Tip 93) and amplified with three primers (OPT-5, OPT-6 and OPT-7).

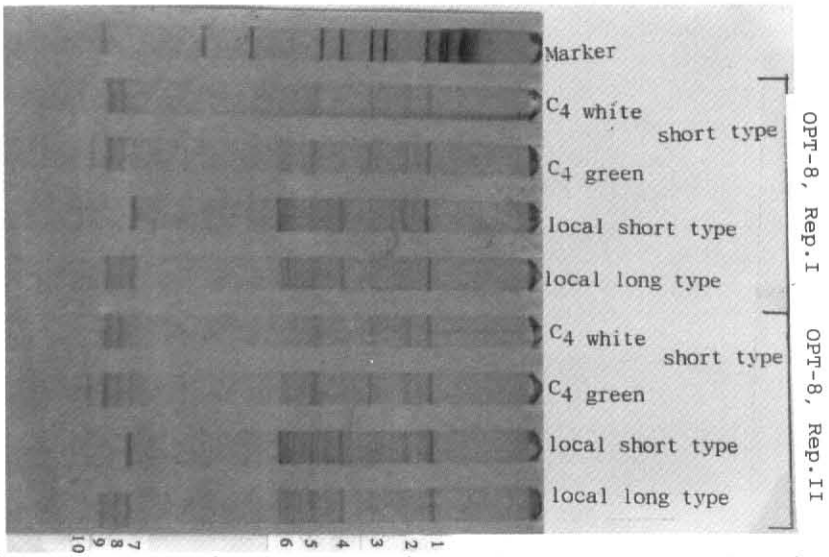

Figure 3 Differences in RAPD band patterns of genomic DNA extracted from four varieties of cucumber (C_4 white, C_4 green, local short type and local long type), which were amplified with OPT-8 primer.

3. Polymerase chain reaction (PCR)

3.1 Primers

The following 20 primers were (OPERON 10-mer kits, Operon Technologies, Inc.) :

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	CACCCCTGAT	14	AATGCCGCAG	19	GTCCGTATGG
05	GGGTTTGGCA	10	CCTTCGGAAG	15	GGATGCCACT	20	GACCAATGCC

3.2 Amplified DNA reaction

Amplified DNA reaction was performed in volume of 25 ul including 25 ng genomic DNA, 15 ng primer DNA, 0.5 unit Taq polymerase (Perkin), 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 Bassam *et al.*, 1991

RESULTS AND DISCUSSION

1. Tomato

Of the 20 primers only 11 were successfully tested with tomato DNA. Each primer expressed specific a RAPD band pattern (Figure 1). These 11 primers were selected to examine with 3 varieties of tomato; Seeda Tip 2 and Seeda Tip 93, which were developed from the same genetic source. OPT-5, OPT-6 and OPT-7 could distinguish different among these closely varieties. However, results were reproducible only with one primer, OPT-7. Seeda Yak was different from the other two cultivars at band marker 7. Seeda Tip 2 was different from the others at marker 4 (Figure 2).

Klein-Lankhorst, et al. 1991 were successful to construct RAPD markers for genetic mapping in tomato. They also found that the polymorphic DNA markers were increased by further combing two primers in a single PCR. Our results further confirm that RAPD can be successfully employed to distinguish closly related genotypes.

2. Cucumber

Twenty primers have been tested and only OPT-8 was useful for varietal identification in cucumber. C_4 varieties were different from local varieties at band markers 4 and 6. C_4 white and green varieties were different from each other at band marker 7 and 10. Local variety short type was different from local variety long type at band marker 2, 8 and 9.

As per our knowledge, this is the first report to apply RAPD for varietal identification in cucumber. Since only one primer was identified in this study to recognize some varietal differences, it seems desirable to test more diverse primers than used in this study.

The results from our experiments supported that RAPD is a useful tool to commercial applications, including its usage breeding program in determining relatedness of pedigree verification in tomato and cucumber. However, there is currently no requirement that DNA fingerprinting be submitted in supported of a patent application or plant variety protection (Robert,1992). It seems that RAPD for DNA fingerprinting will find initial use in plant property right to prove ownership or derivation of plant lines as well as to prove infingerprint of patent and plant variety protection or misappropiation of trade secrets in future. Until now 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), potato (Mori *et al.*, 1993), Brassica (Hu and Quires; 1991 Demeke *et al.* 1992, and Temisak *et al.* 1994)]

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