中山大学学报 (自然科学版)
第37卷 第2期 ACTA SCIENTIARUM NATURALIUM Vol. 37 No. 2
1998年 3月 UNIVERSITATIS SUNYATSENI Mar. 1998

Identification of Mustard(*Brassica juncea* Coss.) Cultivars with RAPD Markers*

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Abstract Random amplified polymorphic DNA(RAPD) is a new molecular marker technology developed by Williams and Welsh in 1990. Sixteen mustard(*Brassica juncea* Coss.) cultivars of eight varieties were identified with RAPD markers. Seven 10-mer arbitrary primers were selected out of sixty one, and genomic DNA fingerprints of sixteen mustard cultivars were obtained. One or two characteristic DNA bands were shared by each primer among sixteen cultivars. RAPD markers between two mustard cultivars of one variety were rich and two cultivars could be distinguished through comparing the different DNA bands between them. No single primer out of seven could distinguish all the eight pair cultivars on one analysis while combination of two or more primers could do it. The results showed that identification of mustard cultivars with RAPD markers is a sensitive and rapid approach technology which can be applied widely in seed scientific researches and seed production.

Keywords Cultivar identification, Mustard(*Brassica junce* Coss.), RAPD marker
Classification number Q 7

Cultivar identification is important not only for genetics and breeding, but also for germplasm banks and seed scientific researches and seed production. In the past, morphological markers, isozyme markers, electrophoresis of seed storage proteins and high performance liquid chromatography of seed proteins were applied to cultivar identification. But the common weakness of the afore mentioned markers is that there are insufficient polymorphic markers when closely related genomes are to be identified. Restriction fragment length polymorphism(RFLP) was developed with the advancement of molecular genetics and molecular biology as well as the perfection of molecular cloning and DNA combination. RFLP has been successfully used as genetic marker to detect DNA polymorphisms of plant genomes[1]. But there exist weakness such as complicated in technology, high cost, time consuming, and labor intensive in RFLP application.

Random amplified polymorphic DNA(RAPD) is a new molecular marker technology based on PCR(Polymerase Chain Reaction), which was devoloped by Williams and Welsh in 1990. RAPD has been applied to nearly all fields of life sciences, especially in taxonomic researches[2], cultivar identification[3], construction of genetic linkage maps[4] and gene localization[5]. In this paper, RAPD markers were used to distinguish sixteen mustard(*Brassica juncea* Coss.) cultivars of eight varieties. The purpose was to figure out whether RAPD markers could be applied in the identification of mustard cultivars.

* 广东省博士后基金资助项目
收稿日期: 1997-07-17 乔爱民,男,32岁,博士后.

1 Materials and methods

(1) **Seeds and seed germination** Seeds of 16 mustard cultivars were collected. The 16 cultivars belonged to 8 varieties, and each variety included 2 cultivars(Tab. 1). One hundred seeds of each cultivar were chosenfor germination. Germination was performed at 25℃ (dark) for 5 days. 2.5 g seedling was used to extract genomic DNA.

(2) **Gernomic DNA extraction** Extraction of genomic DNA see McCouch[6]. Purity of extracted genomic DNA was determined by both UV-spectrophotometer at 260 nm and 280 nm, and 1.5% agar.

(3) **Primer screening** Sixty 10-mer arbitrary primers from Operon Tech. were screened to choose suitable primers for identification of mustard cultivars. Seven primers were selected(Tab. 2).

(4) **RAPD protocal** RAPD reactions were performed in 25μL reaction mixture containing *Taq* DNA polymerase(5μg/μL) 0.15μL, genomic DNA 2μL(10 ng/μL), dNTPs (2.5 mmol/L) 1μL, MgCl2 (25 mmol/L) 2μL, 10× buffer 2.5μL, supper-purified water 16.85μL. 20μL mineral oil was added on the top of the reaction mixture. RAPD reactions were performed in" PCR-90AD" manufactured by the Institute of Genetics, China Academy of Sciences. With thirty-five cycles of 94℃ for 50 seconds, 38℃ for 70 seconds, 72℃ for 120 seconds, followed by another 10 min at 72℃ to fill in all the ends.

(5) **Electrophoresis and polymorphism observation** Amplified products of genomic DNAs were separated though 1.5% agar, stained with ethidium bromide(EB). DNA polymorphisms were observed under UV-light, and photographed.

Tab. 1 Varieties and cultivars for identification

variety number	variety	cultivar number	cultivar	source
1	Da Tou Jie	1^1	Da Chongqing, Tou Jie	Chong Qing
		1^2	Qing Ye Da Tou Jie	Zi Gong, Si Chuan
2	Feng Nie Jie	2^1	Da Kexue, Li Hong	Nan Jing, Jiang Su
		2^2	Jiu Tou, Niao Xue, Li Hong	He Fei, An Hui
3	Juan Xinjie	3^1	Bao Bao, Qing Cai	Da Xian, Si Chuan
		3^2	Guan Ercai	Dian Jiang, Si Chuan
4	Feng Weijie	4^1	Feng Wei, Qing Cai	Zi Gong, Si Chuan
		4^2	Yan Jiwei, La Cai	Xi Chang, Si Chuan
5	Chang Bingjie	5^1	Xian Cai	Feng Du, Si Chuan
		5^2	Xian Cai	Liang Ping, Si Chuan
6	Xiao Yejie	6^1	Bai Gan, Qing Cai	Lu Zhou, Si Chuan
		6^2	Jian Yetian, Qing Cai	Ma Bian, Si Chuan
7	Da Yejie	7^1	Niu Leba, Qing Cai	Pan Zanhua, Si Chuan
		7^2	Ji Bazi, Qing Cai	Xian Chong, Si Chuan
8	Bai Huajie	8^1	Bai hua, Qing Cai	Lu Xian, Si Chuan
		8^2	Bai Gan, Qing Cai	Lu Xian, Si Chuan

2 Results and discussion

RAPD amplifications of 16 mustard cultivars from 8 varieties were performed. Genomic DNA fingerprints of sixteen mustard cultivars were obtained(Fig. 1-a). From the fingerprints, models were obtained(Fig. 1-b). Cultivar identification could be performed through comparing the different DNA bands between/among cultivars. From Fig. 2-a, cultivars 1^1 and 1^2 of Da Tou Jie could be distinguished because their DNA band patterns

Tab. 2　Primer selected for cultivar identification

primer number	primer sequence(5'—3')	primer number	primer sequence(5'—3')
OPH-05	AGTCGTCCCC	OPJ-18	TGGTCGCAGA
OPH-12	ACGCGCATGT	OPS-07	TCCGATGCTG
OPH-16	TCTCAGATGG	OPS-19	GAGTCAGCAG
OPH-19	CTGACCAGCC		

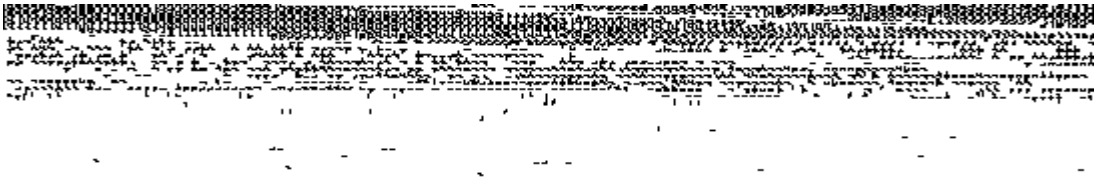

Fig. 1　Genomic DNA fingerprints of 16 mustard cultivars amplified by OPS-19(a) and OPS-07(b)
(1^1~ 8^2 are cultivar numbers, see Tab. 1　M: λDNA/*Hind*Ⅲ)　　(a) (b)

were different. Bands OPS-19-2 and OPS-19-8 were present in cultivar 1^1, but were absent in cultivar 1^2. For the same reason, cultivars 2^1 and 2^2 of Feng Nie Jie could be distinguished by the different bands OPS-19-2, OPS-19-7 and OPS-19-8. Cultivars 3^1 and 3^2 of Juan Xinjie could not be distinguished for there was no different band between them. Cultivars 4^1 and 4^2 of Feng Weijie were distiguished by their different bands OPS-19-5, OPS-19-6 and OPS-19-7. Cultivars 5^1 and 5^2 of Chang Bingjie were ditinguished by their different bands OPS-19-3 and OPS-19-7. Cultivars 6^1 and 6^2 of Xiao Yejie could be distin-

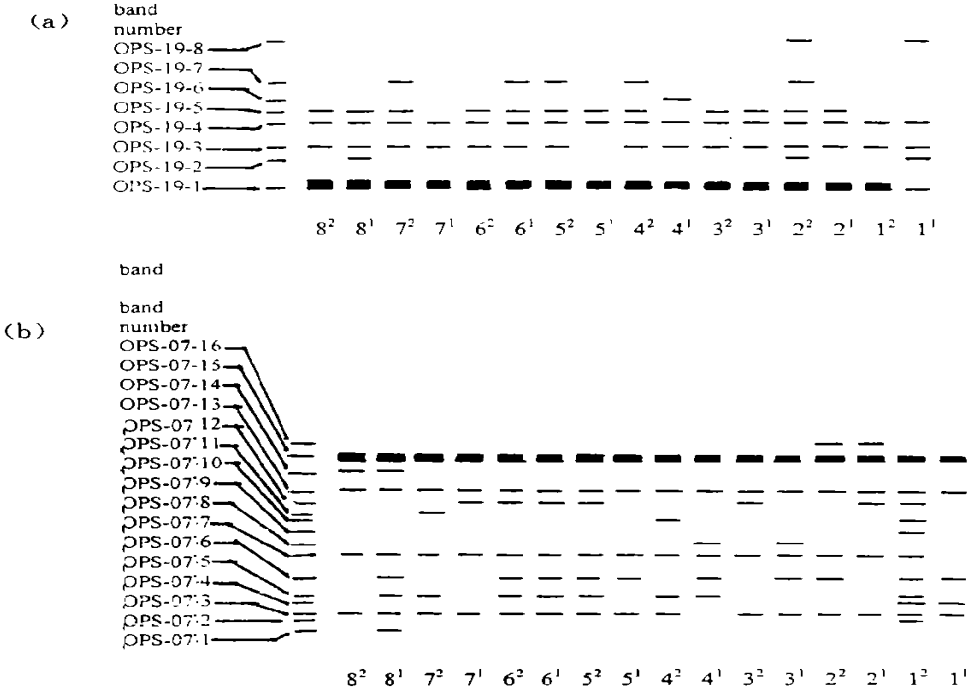

Fig. 2　Model of DNA fingerprints of 16 cultivars, OPS-19 (a) and OPS-07 (b)
(1^1~ 8^2 are cultivar numbers, see Tab. 1　M: λDNA/*Hind*Ⅲ)　　(a) (b)

guished by their different band OPS-19-7. Cultivars 7^1 and 7^2 of Da Yejie could be distinguished by their different bands OPS-19-5 and OPS-19-7. Cultivars 8^1 and 8^2 of Bai Huajie could be distinguished by their different band OPS-19-2. Primer OPS-19 could distinguish 7 cultivar pairs out of 8. Primer OPS-07 could also distinguish 7 cultivar pairs out of 8 (Fig. 2-b). The two cultivars which could not be distinguished by OPS-07 were cultivars 6^1 and 6^2 of Xiao Yejie.

RAPD markers have been applied to cultivar identification[3, 7, 8]. The results show that different primers produced different DNA band patterns. RAPD markers between 2 cultivars of 1 variety were rich, and the 2 cultivars could be distinguished through comparing their different DNA bands. Among the 7 primers chosen. No single primer could distinguish all the 8 cultivar pairs, but combination of 2 or more primers could do it. Out of the 7 primers, 4 could distinguish 7 cultivar pairs, 2 could distinguish 6 cultivar pairs, and 1 could distinguish 4 cultivar pairs. It is believed that RAPD marker is a sensitive, rapid method for identification of mustard cultivars, and can be applied widely in seed scientific researches and seed productions.

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利用 RAPD标记鉴定芥菜 (*Brassica juncea* Coss.) 品种

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摘 要 从60种 10 bp随机引物中筛选出 7种引物, 对 8个芥菜变种的 16个品种 (每个变种有 2个品种) 进行了随机扩增多态性 DNA (RAPD) 鉴定, 能快速、灵敏、较为简便获得了 16个芥菜品种的基因组 DNA指纹图谱. 结果表明, 不同引物在 16个品种上都有其特征 DNA带, 一般只有 1条, 少数有 2条. 在同 1个变种的 2个品种之间存在着丰富的 RAPD标记, 通过比较 2个品种各自独具的 RAPD标记, 就可明确地将 2个品种区分. 在所用的 7种引物中, 没有 1种能同时将 8对芥菜品种全部区分开, 但用 2种或 2种以上的引物就完全可以将 8对芥菜品种全部区分开.

关键词 芥菜, 随机扩增多态性 DNA (RAPD) 标记, 品种鉴定
分类号 Q 7

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