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Variation in Some *Lycopersicon esculentum* and *Capsicum annuum* Cultivars Revealed by RAPD and AFLP Markers
应用 RAPD和 AFLP标记的方法对甜 (辣)椒和番茄品种的多态性分析

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Abstract Twenty-five accessions of pepper (*Capsicum annuum*) and twenty-two accessions of tomato (*Lycopersicon esculentum*) cultivated in primary province where the main agricultural breeding insititutes breed the majority improved varieties of pepper and tomato in China were analysed. RAPD and AFLP markers were performed and compared for their effectiveness to discriminate near relatives. In pepper varieties, AFLP markers generated by two primer combinations distinguished every pepper accessions. The percentage of polymorphic markers for AFLP was lower than that for RAPD(9% and 35% respectively). However, the average numbers of polymorphic fragments were 2.2 per primer for RAPD and 5.1 per AFLP primer combination, respectively. Therefore AFLP primers were two times more efficient than RAPD primers in their ability to generate polymorphic markers in pepper varietes. In tomato varieties, the percentages of polymorphic markers were 5.5% for AFLP and 61% for RAPD single primer and 58% for RAPD two-primer, the percentage of polymorphic markers of AFLP was lower than that for RAPD. The average numbers of informative bands per primer were 4.2 for RAPD single primer and 4.4 for RAPD two-primer and 2.6 for AFLP primer combinations, respectively. The study demonstrated that RAPD markers were more useful than AFLP markers among nearly related tomato varieties. RAPD markers generated by one single primer and one two-primer reactions can discriminate 21 out of 22 tomato varieties. The effectiveness of various marker techniques for the two species *C. annuum* and *L. esculentum* is discussed.

Key words *C. annuum*, *L. esculentum*, RAPD, AFLP, polymorphic analysis

摘要 应用 RAPD和 AFLP的 DNA指纹图谱方法分析 25个甜(辣)椒和 22个番茄品种的真实性,并进一步比较 RAPD和 AFLP的 DNA指纹图谱在鉴定亲缘关系较近的品种之间的真实性时的有效性。对于甜(辣)椒品种,AFLP方法中,2个引物组合扩增反应的多态性片段即能将 25个品种完全分开。虽然,每个样品的 AFLP的扩增产物中多态性片段的百分率为 9%,低于 RAPD的 35%多态性片段的百分率。但是,AFLP的信息量远远大于 RAPD的信息量,它的每对引物组合扩增的平均多态标记为 5.1,而 RAPD仅为 2.2所以,在甜(辣)椒的指纹图谱中,AFLP的有效率是 RAPD的 2倍。对于番茄品种,每个样品的 AFLP的扩增产物中多态性片段的百分率为 5.5%,大大低于 RAPD的单引物 61%多态性片段和双引物 58%多态性片段的百分率。它的每对引物组合扩增反应的平均多态标记为 2.6,而 RAPD中,单引物扩增的平均多态标记为 4.2,双引物扩增的平均多态标记为 4.4 一个单引物和一个双引物的 RAPD扩增反应的多态性片段即能将 22个番茄品种中的 21个完全分开。所以,在番茄的指纹图谱中,RAPD的有效率是 AFLP的 2倍。因此,在应用分子标记辅助鉴定品种的真实性时,不同的作物所适用的方法是不同的。

关键词 甜(辣)椒 番茄 RAPD AFLP 多态性分析
中图法分类号 S641

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1 Introduction

Domesticated pepper and tomato cultivars are economically important vegetables in China. *Capsicum* species originated in Central and South America. The cultivated species of *Capsicum* which are widespread in China are introduced from Europe[1]. The lower diversity observed in the current cultivars may be a reflection of popular breeding methods (often single seed descent or pedigree selection) which promote genetic uniformity[2]. The relationships within the cultivated species of *Capsicum* have been investigated in several studies, using morphological, cytogenetical and molecular markers[3-10]. These studies demonstrated that the level of variation among domesticated peppers is lower than that among wild peppers.

The genus *Lycopersicon* itself is highly variable, as there are some taxa within the genus[11-14]. The analysis based on RFLP[2] revealed two major dichotomies in the genus: one corresponding to mating behavior (self-compatible (SC) versus self-incompatible (SI) species) and the another corresponding to fruit colour (red versus green-fruited species). Furthermore, they described the fact that more genetic variation could be found within a single accession of one of the SI species (e.g. *L. peruvianum*) than among all accessions tested of any one of the SC species (e.g. *L. esculentum*). That is especially true for self-pollinated crops whose genetic bases trend to be narrow. The domesticated tomato varieties are originated from species *L. esculentum* where genetic diversity seems very limited[15].

It is essential to find out diagnostic molecular markers. Estimation of genetic variation between varieties of a cultivated crop is of interest in cultivar registration and identification. New techniques for DNA profiling are also a powerful tool for improving identification of varieties since morphological and resistance traits used for the identification of *Capsicum* and *Lycopersicon* varieties are not easy to judge. Furthermore, diagnostic markers could facilitate the decision for the seed quality distinguishing seed lots with high quality from lots with different varieties mingled purposely or unintentionally for commercial varities. Their use for distinctiveness purposes has been discussed by the UPOV (Union pour la Protection des Obtentions Vegetables). The using of molecular markers proposed by Sollar and Beckmann provided an additional tool for varietial description. DNA markers have the advantage of being independent of environmental effects and providing direct information on the genome of each individual. PCR-based markers are capable to be efficient in the routine assessment of seed purity of F1 hybrids. Nevertheless, there is few information available regarding the suitability of PCR-based markers to analyse genetic variation among commercial pepper and tomato varieties that are agronomically well-characterised and currently being highly produced.

BioGEVES has investigated molecular markers including RFLP (Restriction Fragment Length Polymorphism), RAPD, SSR (Simple Sequence Repeat) and AFLP used in identification of varieties and discrimination of seed quality since 1992. Comparing these methods AFLP generated much more fragments and stable reproducibility, which is capatable to be employed to a wide range size genome DNA. Nowadays, the investigations such as relationship between genetic markers and morphological traits and marker distances and phenotypic among horticultural crop varieies develop rapidly. But it is hardly see the reports that PCR-based markers´ techniques were directly applied in identification of commercial varieties. The aim of this study is to investigate the potential of RAPD (Random amplified polymorphic DNA) and AFLP (Amplified fragment length polymorphism) technology for generation of polymorphic DNA markers among commercial pepper (*C. annuum*) and tomato (*L. esculentum*) varieties cultivated in China. Up to now, RAPD's and AFLP's have never been compared in the same materials as well as among two various species of the family Solanaceae.

2 Materials and methods

2.1 Plant materials and DNA

Twenty-five accessions of *C. annuum* were collected which were commercial varieties of various types from different regions of China (Table 1). Sweet pepper is normally large-fruited type with thick wall. Chili pepper is a hot thin-fruited type. Nineteen varieties represent F1 hybrids, six are homozygous inbred varieties.

Table 1 Accessions of investigated *C. annuum* cultivars from various regions of China

Number	Name	Type	Region
P1	Weijiao No. 1[a]	Chili	Shandong
P2	Zhongjiao No. 10[a]	Chili	Beijing
P3	Zhongfeng No. 18	Bloky	Beijing
P4	Fudijianlajiao	Chili	Hunan
P5	Xingji No. 3[a]	Bloky	Liaoling
P6	Haihua No. 3	Chili	Beijing
P7	Haifeng No. 1[a]	Chili	Beijing
P8	Jinjiao 851	Bloky	Shaanxi
P9	Tongjiao No. 1[a]	Chili	Jiangsu
P10	Zhongjiao No 4[a]	Bloky	Beijing
P11	Zhongjiao No. 5[a]	Bloky	Beijing
P12	Zhongjiao No. 6[a]	Chili	Beijing
P13	Zhongjiao No. 7fe	Bloky	Beijing
P14	Zhongjiao No. 7ma	Chili	Beijing
P15	Zhongjiao No. 7[a]	Chili	Beijing
P16	Tianza No. 3[a]	Bloky	Beijing
P17	Chili 931[a]	Chili	Beijing
P18	Tianza No. 6[a]	Bloky	Beijing
P19	Tianza No. 4[a]	Bloky	Beijing
P20	Dujiao No. 1[a]	Chili	Beijing
P21	9387[a]	Chili	Beijing
P22	Xiangyan No. 1[a]	Chili	Hunan
P23	Xiangyan No. 2[a]	Chili	Hunan
P24	Xiangyan No. 9[a]	Chili	Hunan
P25	Xiangyan No. 19[a]	Chili	Hunan

The accessions are homozygous inbreds except those labeled by a are F1 hybrids.

Twenty-two accessions of *L. esculentum* were collected which were mostly commercial varities of various types from different regions of China (Table 2). One accession, Hadahuang (T7), produces yellow fruits. Another accession, Jingdan No. 1 (T26), represents cherry tomato type. All of the other accessions belong to the red fruit type. All of the accessions are F1 hybrids, which were provided by P. R. China.

The young plants grew in the greenhouse for about forty days. Three fresh young leaves were collected from five single plants of every accession, respectively. The total weight was about 30 to 40 mg. DNA from each single plant was isolated by a rapid method according to Dorokhov and Klocke[17]. The concentration of DNA was estimated by Pharmacia Biotech (Biochrom) Ltd. Spectrophotometer Ultrospec 2000. We bulked a pool from five single plants´ DNA of each accession to ensure the uniformity of accessions. The concentration of pooled DNA samples made from DNA´s of single plants was the same as that of each single plant DNA (10ng/μl).

Table 2 Accessions of the investigated *L. esculentum* cultivars from various regions of China

Number	Name	Type (Fruit color)	Region
T1	Zhongsu No. 4	Red	Beijing
T2	Zhongsu No. 5	Red	Beijing
T3	Zhongsu No. 6	Red	Beijing
T4	Zhongza No. 9	Red	Beijing
T5	L 402	Red	Liaoning
T6	Dongnong 704	Red	Heilongjiang
T7	Hadahuang	Yellow	Heilongjiang
T8	Hashi No. 2	Red	Heilongjiang
T9	Taiyuan 823	Red	Shanxi
T10	Hongkang 218	Red	Shanxi
T11	Shukang No. 11	Red	Zhejiang
T12	Guocui	Red	Shaanxi
T13	Texuan 28	Red	Shanxi
T14	Jinhong No. 1	Red	Shanxi
T15	Lufanqie No. 4	Red	Shandong
T16	Zhongfen No. 1	Red	Beijing
T17	Jiafen No. 15	Red	Beijing
T18	Jiafen No. 10	Red	Beijing
T19	Maofen 802	Red	Shaanxi
T22	Jiafen No. 17	Red	Beijing
T26	Jingdan No. 1	Cherry fruit	Beijing
T29	Chunguang No. 19	Red	Beijing

Table 3 RAPD primers employed and discussed in this study

Primer	Sequence	Usage
OPG-09	CTGACGTCAC	P, T
OPG-12	CAGCTCACGA	P, T
OPH-09	TGTAGCTGGG	T
OPH-13	GACGCCACAC	T
OPI-02	GGAGGAGAGG	T
OPI-09	TGGAGAGCAG	T
OPJ-17	ACGCCAGTTC	T
OPK-11	AATGCCCCAG	P, T
OPK-12	TGGCCCTCAC	P
OPK-13	GGTTGTACCC	P, T
OPK-14	CCCGCTACAC	P, T
OPL-11	ACGATGAGCC	T
OPV-14	AGATCCCGCC	P
OPX-04	CCGCTACCGA	P, T
OPX-08	CAGGGGTGGA	P, T
OPX-09	GGTCTGGTTG	P, T
OPX-12	TCGCCAGCCA	P
OPX-13	ACGGGAGCAA	P
OPX-14	ACAGGTGCTG	P

P: primer used for *Capsicum* cultivars; T: primer used for *Lycopersicon* cultivars

2.2 RAPD analysis

We have screened 19 single primers and 9 two-primers combinations. Decamer primers were purchased from Roth Company (Germany). The

primer name and their sequence for single primer reactions are given in Table 3. Additionally, there were performed RAPD reactions with the following two-primer combinations: OPK-11-OPG-12, OPG-09-OPK-13, OPG-09-OPK-14, OPG-12-OPX-04, OPI-09-OPH-13, OPI-02-OPJ-17, OPJ-17-OPL-11, OPI-09-OPH-09, OPX-04-OPK-13. Each reaction was amplified in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Germany). The reactions were carried out in a 12.5 μl solution containing Appligene 1x buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1% Triton X-100) 1.5 mM $MgCl_2$, 100 μM of each nucleotide, 0.2 μM of each primer, 0.25 Unit Taq DNA polymerase (Appligene, Germany) as well as 20 ng DNA. The reactions employed single primer were incubated for one cycle of 2 min at 94℃, 45 cycles of 0.2 min. at 94℃, 1 min. at 36℃ (down-ramp rates were 50%) 1 min. at 72℃ (up-ramp rates were 50%), 9 min. at 72℃, then kept at 4℃. For amplification reactions using two decamer primers the cycler was programmed for one cycle of 0.5 min. at 94℃, 45 cycles of 0.5 min. at 94℃, 1 min. at 35℃ (down-ramp rates were 50%), 1 min. at 72℃ (up-ramp rates were 50%), 4 min. at 72℃, finally kept at 4℃. After amplification, 5 μl of loading buffer (0.25% bromphenol blue, 40% sucrose in sterile bidest water) were added to each sample which was loaded into a 2.0% agarose gel in 0.5x TBE buffer (pH value 8.0), (0.044 M Tris, 1.25 mM EDTA-Na_2, 0.044 M boric acid) submitted to electrophoresis at 3V/cm. The amplified fragments were visualized by ethidium bromide staining. The gels were saved using an image system with videocamera and computer software KS 400 (Zeiss Germany). The amplifications were repeated at least two times. Only reproducible bands, i.e., present or absent in both reactions were calculated in the analysis.

2.3 AFLP analysis

The AFLP reactions were performed with the AFLP Core Reagent kit and AFLP Starter Primer kit (Life Technologies™, Germany), following to manufacturer's protocol with a modification after the step of ligation of adapters. The ligation mix was used directly for preamplification without preliminary dilution. Generally 250 ng DNA were digested with 2.5 U *Eco*RI/*Mse*I, mixing with 5 μl 5x reaction buffer that the total volume was 25 μl, following to ligation *Eco*RI/*Mse*I adapter and amplification in two successive steps. For preamplication the primers used contained the respective restriction enzyme adaptor sequence plus recognition site and one base extension (A for *Eco*RI primer and C for *Mse*I primer). For selective amplification, the complexity of the amplification products was further reduced by using primers with extensions of additionally two bases. The preamplification reactions were performed in Autogene II (Grant U.K.), and the selective amplification reactions in GeneAmp PCR System 9700 (Applied Biosystems, Germany). The same DNA preparations that were used for RAPD were also applied for AFLP analysis. Thirteen primer combinations were employed in this study for analysis of *Capsicum* and *Lycopersicon* cultivars (Table 4).

Table 4 Primer combinations used for selective amplification for AFLP of various *Capsicum* and *Lycopersicon* varieties

Mse I-Primer	*Eco* RI-Primer						
	AAG	ACA	ACC	ACG	ACT	AGC	AGG
CAA		T	T		T	P	
CAC		T	P	P	T	P	
CAG				P, T			
CAT		T	T		T		
CTA				P, T		P	
CTC				P, T	P, T		T
CTG	P, T	P, T	T		P	T	
CTT		T				T	P, T

P: primer combination used for *Capsicum* cultivars; T: primer combination used for *Lycopersicon* cultivars

The amplification products were analysed in 4% denatured polyacrylamide gels, visualized with the DNA silver staining system (Promega U.S.A.) according to the manufacturer's protocol.

3 Results

Out of the 13 RAPD primers performed for amplification of the 25 pepper accessions, ten (77%) primers generated polymorphic products. All primers have given intensely amplified products. A total of 81 amplification fragments were scored (an average of 6.2 fragments per primer), of which, twenty-eight bands (35%) were polymorphic. The fragment size was among 400 to 1500 nucleotides. The maximum number of polymorphic products revealed by one primer was four. This was true for primer OPX-04 (Fig. 1) OPX-09, OPK-11. The average of polymorphic fragments per RAPD primer amounted 2.2.

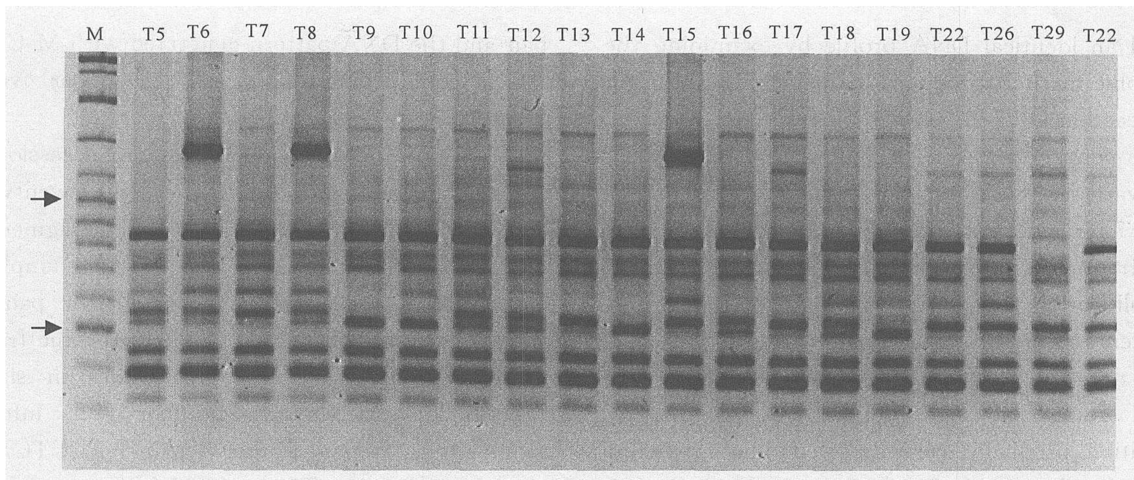

Fig. 1 An electropherogram of amplification pattern with primer OPX-04. P1-P19: Various pepper accessions. M: GeneRulerTM DNA Ladder Mix (Fermentas, Germany). The arrows indicate molecular weight 500 and 1031 bp

Out of the 14 primers performed for amplification of the 22 tomato accessions, all (100%) primers amplified polymorphic fragments. A total of 95 bands from 14 single primers (an average of 6.8 fragments per primer) were obtained. Of which 58 bands (61%) were polymorphic bands with size varying from 300 to 1500 nucleotides. For tomato, the maximum number, nine, of polymorphic bands scored after one primer RAPD reaction was detected with primer OPX-08. Six polymorphic bands were found after amplification performed with primer OPI-09 (Fig. 2). On average 4.2 informative fragments per used primer were found.

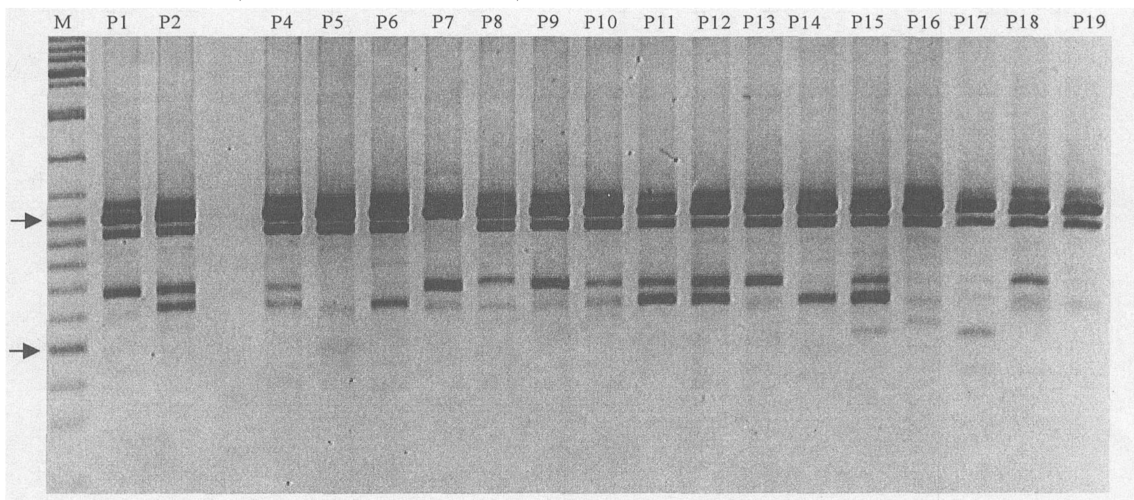

Fig. 2 An electropherogram of amplification pattern with primer OPI-09. T5-T29: pooled DNA from various tomato accessions. M: GeneRulerTM DNA Ladder Mix (Fermentas, Germany)

Furthermore, a total of 60 bands were scored from eight different two-primer reactions (an average of 7.5 fragments per primer) for tomato varieties. Thirty-five bands (58%) were polymorphic fragments ranging from 300 to 1200 nucleotides in size. It was noticeable that the DNA profiles from two-primer reactions had fragments with a lower molecular weight on average than those from reactions with only one primer. The maximum number of polymorphic bands of a two-decamer primer reaction was six produced by primer combination OPX-04-OPG-12 and OPX-04-OPK-14, respectively. On average, the number of informative fragments was 4.4, which was quite similar to that from one-primer reactions.

The presence and absence of the amplification products were used to compare among the 25 pepper accessions and 22 tomato accessions and to distinguish the varieties. In pepper accessions, the polymorphic bands generated by OPX-04, OPK-12, OPX-14, OPX-09, OPK-11 reactions recognized 24 pepper accessions (Table 5). Only P10 and P15 showed the same DNA profile over these five primers.

For tomato, two RAPD analyses were enough to discriminate 21 accessions. Only varieties T6 and T8

exhibited an identical DNA profile by examining the polymorphic markers produced by primer I09 and two-primer reaction OPG-12-OPX-04 (Table 6).

The pepper accessions including three hot small-fruited varieties were further analyzed with AFLP markers. Thirteen primer combinations were used to amplify fragments from the pepper genome. A total of 788 amplification fragments were generated. The size of fragments ranged from 270 to 800 nucleotides. The average number of products per primer pair was 60.6. A total of 81 (10.3%) polymorphic amplification products were detected. The maximum number of polymorphic bands from one primer pair was fourteen (with M-CAC/E-ACC) (Table 7). Moreover, the DNA pattern revealed by this primer pair and the DNA pattern generated with M-CAG/E-ACG could distinguish all 25-pepper varieties, respectively.

All of the twenty-two tomato accessions were further analyzed with AFLP markers; twenty primer combinations were used to amplify the fragments from the tomato genome. A total of 930 amplification fragments (46.5 fragments per primer pair) were generated. Fifty-one (5.5%) polymorphic fragments ranging from 95 to 400 nucleotides in size were scored. The maximum number of informative fragments from primer pair M-CTC/E-ACT amounted to nine. The used 20 primer combinations were insufficient for discrimination among the tomato varieties.

Table 5 Variation in twenty-five pepper (*C. annuum*) accessions revealed by RAPD polymorphic markers produced by five single primers X04, K12, X14, X09, and K11 reactions

b. p.	X04					K12					X14		X09		K11	
	1100	940	700	690	610	1200	1100	950	800	750	1300	450	1750	1300	1031	550
P1	+	+	−	+	−	+	+	−	+	−	−	−	+	+	+	−
P2	+	+	−	+	+	+	+	+	−	−	+	−	+	+	−	−
P3	+	+	−	+	−	+	+	+	−	−	+	−	+	+	+	−
P4	+	+	−	+	+	+	−	+	−	−	+	−	−	+	−	−
P5	+	+	−	−	−	+	+	+	−	−	+	−	+	+	+	+
P6	+	+	−	−	+	+	+	+	−	−	−	−	+	+	+	−
P7	+	−	−	+	+	+	+	+	−	+	−	−	−	−	−	−
P8	+	+	+	−	+	+	+	+	−	−	−	−	+	+	+	−
P9	+	+	−	+	−	+	+	+	−	−	−	−	−	+	+	−
P10	+	+	−	+	+	+	+	+	−	−	+	−	+	+	+	−
P11	+	+	−	+	+	+	+	+	−	−	+	−	+	−	+	−
P12	+	+	−	+	+	+	+	+	−	+	−	−	+	+	+	−
P13	+	+	−	+	−	+	+	+	−	−	−	−	+	+	+	−
P14	+	+	−	−	+	+	+	+	−	−	+	−	+	−	+	−
P15	+	+	−	+	+	+	+	+	−	−	+	−	+	+	+	−
P16	+	+	−	−	−	+	+	+	−	−	+	−	+	+	+	−
P17	−	+	−	−	−	−	+	−	−	−	−	+	−	+	−	−
P18	+	+	−	+	−	+	+	−	+	−	+	+	+	+	+	−
P19	+	+	−	−	+	+	+	−	−	−	+	−	+	+	+	−
P20	+	+	−	+	+	+	+	−	−	−	−	+	−	+	−	−
P21	+	+	−	−	+	+	+	+	−	−	+	−	+	+	+	−
P22	+	+	−	+	+	+	+	+	−	−	+	+	+	−	+	−
P23	−	+	−	−	+	−	−	−	−	−	+	−	−	+	−	−
P24	+	+	−	+	+	+	+	+	−	+	+	−	−	+	+	−
P25	+	+	−	+	−	+	+	+	−	+	+	−	−	+	+	−

Table 6 Variation in twenty-two tomato (*Lycopersicon esculentum*) accessions revealed by RAPD polymorphic markers produced by one single primer I09 and two-primer G12-X04 reactions

b. p.	I09							G12-X04				
	1400	1300	850	610	550	540	520	1400	1200	610	430	200
T1	−	−	+	−	−	−	−	+	+	−	+	−
T2	−	−	+	−	−	−	−	−	+	−	−	−
T3	−	−	+	−	−	−	−	+	+	−	+	+
T4	−	−	+	−	+	−	+	−	−	+	−	−
T5	−	−	+	+	+	−	+	−	−	+	−	−
T6	+	−	+	+	+	−	+	−	+	+	−	−
T7	−	−	+	+	−	+	−	−	+	−	+	−
T8	+	−	+	+	+	−	+	−	+	+	−	−
T9	−	−	+	−	−	−	+	−	+	−	+	−
T10	−	−	+	+	−	−	+	+	+	−	+	−
T11	−	−	+	+	+	−	+	+	+	+	−	−
T12	−	+	+	−	+	−	+	+	+	+	+	−
T13	−	−	+	−	−	+	−	−	+	+	+	−
T14	−	−	+	−	−	−	+	−	+	+	+	−
T15	+	−	+	+	−	+	−	+	+	−	−	−
T16	−	−	+	−	+	−	+	−	+	+	−	+
T17	−	+	+	−	−	+	−	−	+	+	−	−
T18	−	−	+	+	−	−	+	+	+	+	−	−
T19	−	−	+	−	−	−	+	−	+	+	−	−
T22	−	−	+	−	−	+	−	−	−	+	−	−
T26	−	−	+	+	−	+	−	−	−	+	−	−
T29	−	+	−	−	−	+	−	+	+	+	−	−

Table 7 Variation in twenty-five pepper (*Capsicum annuum*) accessions revealed by AFLP polymorphic markers produced by primer pairs for selective amplification M-CAG/E-ACG and M-CAC/E-ACC, respectively

b. p.	M-CAG/E-ACG											M-CAC/E-ACC													
	270	290	325	360	470	490	540	590	600	700	800	280	290	295	300	315	320	325	330	360	390	405	565	700	750
P1	−	+	+	+	+	−	−	+	+	−	+	−	−	+	−	−	−	−	+	+	+	+	−	−	+
P2	+	+	+	−	+	−	+	+	−	−	+	−	−	+	−	−	−	+	+	+	−	−	−	−	+
P3	+	+	+	+	+	−	−	+	+	−	+	−	−	+	−	+	−	+	+	+	+	−	−	−	−
P4	+	+	+	−	−	−	+	+	−	−	−	+	+	+	+	−	+	+	+	−	−	+	+	+	+
P5	−	+	+	+	+	−	+	−	−	−	+	+	−	+	−	−	+	−	+	+	+	−	−	−	−
P6	−	+	+	−	+	−	+	−	−	−	−	−	−	+	−	+	+	−	+	+	+	+	−	−	+
P7	+	+	−	+	−	−	+	−	−	−	−	+	−	−	+	−	−	+	+	+	−	−	−	+	+
P8	−	+	+	+	+	−	−	+	+	−	+	−	−	+	−	−	+	+	+	+	+	+	+	−	−
P9	+	+	+	+	+	−	+	+	−	−	+	+	−	+	−	−	−	−	+	+	−	+	+	−	−
P10	+	+	+	+	+	−	−	+	−	+	+	+	−	+	−	−	−	−	−	+	+	−	−	−	−
P11	−	+	+	+	+	−	+	+	+	−	+	+	−	+	−	−	−	+	−	+	+	+	−	−	−
P12	+	+	+	+	+	−	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+
P13	+	+	+	+	+	+	+	−	+	−	+	+	−	+	−	−	−	−	+	+	+	−	−	−	+
P14	−	+	+	+	+	+	+	−	+	−	+	−	−	+	−	−	−	−	−	+	+	+	−	−	+
P15	+	+	+	+	+	−	−	−	−	−	+	−	−	+	−	−	−	−	+	+	−	−	−	−	−
P16	−	+	+	+	+	−	+	−	−	−	+	−	−	+	−	+	+	−	+	+	+	+	−	−	+
P17	+	+	+	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	+	+	−	+	−	−	−
P18	−	+	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	+	+	+	+	+	−	−
P19	+	+	+	+	+	−	+	+	−	−	−	−	−	+	−	+	−	+	+	+	+	−	−	−	+
P20	+	+	+	−	−	−	+	−	−	−	+	−	−	+	−	+	+	+	+	+	−	+	+	+	+
P21	−	+	+	+	+	−	+	−	−	−	+	−	−	+	−	+	−	−	+	+	+	+	−	−	+
P22	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	+	−	−	+	+
P23	+	+	+	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	+	−	+	−	−	−
P24	+	−	+	+	−	−	−	−	+	−	−	+	+	+	−	−	+	+	−	−	−	+	+	+	−
P25	+	−	+	+	−	−	+	−	−	−	+	−	−	+	−	−	−	−	+	+	−	−	−	−	−

4 Discussion

Determination of genetic purity of pepper and tomato varieties is an important quality requirement in plant breeding and seed production. Cultivar identification is mostly based on morphological and disease resistance criteria. New DNA-marker techniques represent powerful tools for varieties identification. In the present study, we found two AFLP primer combinations, which generated polymorphic fragments and were able to discriminate one accession from each other among Chinese C. annuum varieties of various types. In comparison, the RAPD markers were not so effective in distinguishing the cultivated peppers. Only a set of five decamer primers did allow the distinguishing of 24 from 25 accessions. Examining thirty-four pepper varieties of different types by Paran et al[17]., a similar result, namely that AFLP markers were more efficient than RAPD markers in detecting polymorphism in peppers were reported.

The result was reverse in the tested tomato varieties. The revealed DNA polymorphism was higher by using RAPD than by AFLP. There were two RAPD reactions enough for discrimination among 21 varieties. This result supports the conclusion by Rus-Kortekaas et al.[10] describing a high percentage of bands sharing in RAPD markers of *Lycopersicon*. Using four reselected RAPD primers they could identify 11 of 15 varieties by unique combination of polymorphic bands.

But there was none out of 20 AFLP primer combinations to distinguish the screened 22 tomato varieties. From this it follows that the selection of molecular marker technique suitable for discriminating of plant varieties depends on materials. Moreover, the result could be influenced strongly by screened material. We found that 61% of RAPD fragments were polymorphic for 22 Chinese tomato varieties. Williams and St. Clair[18] checked 48 accessions of *Lycopersicon* ssp. by RAPD revealing 37% informative fragments. Noli et al.[19] characterized 67 cultivated tomato accessions and eight accessions of wild Lycopersicon species. For all together they found 56% polymorphic RAPD fragments (from 6 primers). If they took into account only the cultivated accessions the percentage of polymorphic bands was reduced to 32%. RAPD and AFLP are PCR based techniques. It is useful that both do not require specific sequence information. Moreover, an advantage of the proposed protocol is the use of total plant DNA isolated using simple fast method. This is important since the DNA-extraction step could be cost and labor consumpted. Hazhizume et al.[20] have shown that DNA prepared with a simple method gave the same RAPD pattern like high-purified DNA. We have utilized DNA from a fast method[21] for RAPD analyses as well as for AFLP's. AFLP is an able technique in which we get robust and reliable DNA marker from the genome[22]. But compared with RAPD technique, it is expensive for reagents and instruments, the method includes more steps than that of RAPD. Controlling the reproducibility of the generation of RAPD marker depends on the standardization of many factors like suitable DNA concentration, the thermal cycler for amplification and Taq-polymerase purchased from one company[23]. If kept in the stable amplification condition, the RAPD reaction also can generate intensely reproducible informative fragments, moreover, it is a rapid, simple, economic technique. Our present study suggests that different methods could be employed to different cultivars. In a great amount for these Chinese pepper and tomato varieties, it is sufficient to get variation revealed by RAPD marker. Only in few cases it is necessarily to utilize additional molecular marker technique.

RAPD analysis with two primers gives completely new DNA profiles in comparison to the reactions with only one corresponding primer. This is a simple modification enabling more information about the genetic variation in the plant materials. The result is in accordance to Klein-Lankhorst et al.[24] as well as Kochieva and Suprunova[25].

The RAPD and AFLP data represent information for traits of pepper and tomato varieties, which could be of practical use for variety identification and registration as well as for actual breeding. There is a possibility to find commercial varieties purposely or unintentionally mixed with other varieties. So the polymorphic RAPD and AFLP markers are effective to control the commercial varieties qualities and to discriminate the self-pollinated pepper and tomato varieties whose genetic bases are narrow. It is hopeful that molecular markers will be an important additional technique, moreover could be a substitute of

morphological markers.

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