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Vol. 13(24), pp. 2385-2393, 11 June, 2014
DOI: 10.5897/AJB2014.13646
Article Number: 18E0C4E45334
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Application of random amplified polymorphic DNA (RAPD) markers to identify *Taxus chinensis* var. *mairei* cultivars associated with parthenogenesis

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Received 20 January, 2014; Accepted 26 May, 2014

The random amplified polymorphic DNA (RAPD) technique has been widely applied to identify different varieties of plants for molecular breeding. However, application of RAPD markers to identify parthenogenesis in plants has not been reported. In this investigation, we used pedigree and RAPD markers to differentiate different *Taxus chinensis* var. *mairei* cultivars that were associated with parthenogenesis. Among 180 and eighty RAPD primers used, 108 primers generated RAPD bands from genomic DNA of *T. chinensis* var. *mairei* cultivars ("Jingzhou" and "Baokang"). Six hundred and thirty-three RAPD loci bands were produced and used to identify eight cultivars with unique banding patterns. Pedigree and RAPD data demonstrated that *T. chinensis* var. *mairei* cultivars with parthenogenesis were separated from others in both "Jingzhou" and "Baokang" cultivars. These results provide evidence for identification of parthenogenesis and confirm that the RAPD technique is especially suitable for identification of *T. chinensis* var. *mairei* cultivars for agricultural purposes.

Key words: Cultivar identification, DNA extraction, parthenogenesis, pedigree, random amplified polymorphic DNA (RAPD), *Taxus chinensis* var. *mairei*.

INTRODUCTION

Molecular markers are of great interest to plant breeders as a source of genetic information on crops and for use in selecting traits to which the markers are linked (Benoit et al., 1997; Mir et al., 2011; Xavier et al., 2011). In classic breeding approach, the breeders expended considerable effort and time in refining the crosses as the tight linkage or association of the desired characters with the obvious phenotypic characters was never unequivocally established (Bublyk et al., 2013; Degani et al., 1998; Doyle and Doyle, 1990). Compared to classic breeding approach, the advancement in using of molecular markers in plant breeding has become very Common

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place (Cires et al., 2013; Gidoni et al., 1994). Molecular markers provide advantage to score multiple morphological mutant traits in a single segregating population in which a trait using morphological markers was not practical due to the undesirable pleiotropic effects of many morphological markers on plant phenotype (Graham et al., 1996; Graham and McNicol, 1995). In addition, molecular markers can function as a tag or label for the gene of interest (Graham et al., 1994; Mir et al., 2011; Xavier et al., 2011), important prerequisites exist. In this study, we attempted to use molecular markers to differentiate different cultivars that are associated with parthenogenesis in *Taxus chinensis* var*. mairei*.

The identification of different varieties of plants is a relevant issue especially when it concerns commercially valuable species such as fruits (Levi et al., 1993; MacPherson et al., 1993). Frequently, the varieties obtained through genetic selection for commercial purposes involving high economical interests.

The molecular approach has proved itself an increasingly valuable tool in the identification of plant varieties (Bublyk et al., 2013; Cires et al., 2013; Morell et al., 1995). Some of the commercially desirable ones, such as strawberries, are reproduced by micropropagation; therefore all individuals belonging to a given variety share an identical genome. In this field one of the most successful techniques is random amplified polymorphic DNA (RAPD) (Welsh and McClelland 1990; Williams et al., 1990) which has two main advantages: it allows random sampling of markers over whole genomic DNA and does not require any previous information on the genome of the organism under investigation (Mir et al. 2011; Xavier et al. 2011). RAPD technique has not been used to identify different cultivars that are associated with parthenogenesis in *T. chinensis* var*. mairei*.

T. chinensis var*. mairei* is an important medical plant species in Southern China. Here we present the results of an application of RAPD markers to identify cultivars that are associated with parthenogenesis. The RAPD technique was chosen because it had been successfully applied in crop to estimate genetic distances among varieties (Graham et al., 1996; Benoit et al., 1997) and to genetically characterize different cultivars (Gidoni et al., 1994; Degani et al., 1998). RAPD also proved itself highly effective in this case.

MATERIALS AND METHODS

Plant materials

Eight cultivars of *T. chinensis* var*. mairei* were collected from Jingzhou and Baokang in Southern China (Jingzhou-F, Jingzhou-M, Jingzhou-FM, Jingzhou-P; Baokang-F, Baokang-M, Baokang-FM, Baokang-P). Stems, leaves, and flower cones of *T. chinensis* var*. mairei* were progressively numbered and were used to identify relationship that is associated with parthenogenesis in *Taxus chinensis* var*. mairei*. All plants were analyzed by RAPD.

DNA extraction

Genomic DNA was extracted as previously described (Tang et al., 2007), using a genomic DNA Isolation kit following the manufacturer's protocol. Small stem, leave, or flower cone pieces were minced by micropestles in the presence of 50 µL of extraction buffer containing 4% hexadecyl trimethylammonium bromide (CTAB), 1.4 mM NaCl, 0.2% 2-mercaptoethanol, 20 mM EDTA and 100 mM Tris-HCl, pH 8. To each minced sample, 350 µL of the same extraction buffer was added and the samples were incubated for 2 h at 50°C. After incubation three purification steps were performed with equal volumes of phenol, phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1), respectively. DNA was precipitated with 2 vols of absolute ethanol and 1/10 vol. 3 mM Na acetate pH 5.2, washed in 70% ethanol, dried and resuspended in TE buffer. The purification procedure was then repeated from the beginning on the previously extracted DNA. This twice-purified DNA finally yielded good amplification products. The amount of DNA was estimated by the minigel method (Maniatis et al., 1982) and the spectrophotometric readings.

RAPD amplification

PCR analysis was performed with a PTC-100TM Programmable Thermal Controller (MJ Research, San Francisco, CA). RAPD amplification reactions were performed in a total volume of 20 µL with the following final concentrations: A total of 100 ng of genomic DNA was used as a template in a 20 µL PCR reaction mix. The PCR mixture consisted of 200 µM each of dATP, dCTP, dGTP, dTTP, 35 pmol of each primer, 2.5 U Taq DNA polymerase (Promega), 1.5 mM $MgCl_2$, and 5 µL 10 x buffer (500 mM KCl, 100 mM Tris-HCl pH 9.0 at 25°C, 1% Triton X-100, and 15 mM $MgCl_2$). Amplifications were carried out in a PTC-100TM Programmable Thermal Controller (MJ Research, San Francisco, CA). The PCR conditions were 95°C for 5 min followed by 29 cycles at 95°C for 60 s, 57°C for 40 s, and 72°C for 90 s. Cycling was followed by a final incubation of 72°C for 10 min. In each thermal cycling a negative control (water instead of template) was included to rule out amplification products due to external contamination. All amplifications were repeated twice for each sample and the PCR products were separated by electrophoresis on 1.0% agarose gels in 1·TAE buffer and were detected by fluorescence under UV light (302 nm) after staining with 0.1% ethidium bromide. A molecular marker of HyperLadder I (Bioline) was used. The results obtained with eight cultivars were confirmed by 180 standard Opern primers (Operon Technologies Inc., Alameda, CA, USA), which yielded satisfying results in the same conditions.

Data analysis

Each gel was run twice and the repeatable bands of each primer were scored as present or absent. The similarity matrix between cultivars was computed using Jaccard's coefficient of similarity (Jaccard, 1908). NTSYS-PC2.1 software was employed for cluster analysis using the data from the similarity matrix and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to construct a dendrogram that represents the genetic relationships among the studied cultivars.

Figure 1. *Taxus chinensis* var. *mairei* cultivars and the development of novel DNA markers for cultivars identification of parthenogenesis. Samples showed in this figure were as follows: **(A)** 'Jingzhou-M'; **(B)** 'Jingzhou-P'; **(C)** 'Jingzhou-F'; **(D)** 'Jingzhou-FM'. Each of *Taxus chinensis* var. *mairei* illustration shows the positions of stem, flower cone, and leave in each cultivar.

RESULTS

The *T. chinensis* var. *mairei* cultivars Jingzhou-M, Jingzhou-P, Jingzhou-F, Jingzhou-FM (Figure 1) and Baokang-M, Baokang-P, Baokang-F, and Baokang-FM used in this study were a diverse group of cultivars. *T. chinensis* var. *mairei* Jingzhou-P and Baokang-P are cultivars that are associated with parthenogenesis. Among 180 RAPD primers (OP A1-20, B1-20, C1-20, D1-20, E1-20, F1-20, G1-20, H1-20, and I1-20) used, 108 primers generated RAPD bands (Table 1) and a total of 633 unique bands were obtained. Examples of RAPD gel bands from *T. chinensis* var. *mairei* cultivars Jingzhou-M, Jingzhou-P, Jingzhou-F, and Jingzhou-FM are showed in Figure 2, in which genomic DNA was amplified using primers OP-A09 (Figure 2A) and OP-D10 (Figure 2B). Examples of RAPD gel bands from *T. chinensis* var. *mairei* cultivars Baokang-M, Baokang-P, Baokang-F, and Baokang-FM are shown in Figure 3, in which genomic DNA was amplified using primers OP-A09 (Figure 3A) and OP-D10 (Figure 3B).

To verify whether RAPD markers can be used to identify *T. chinensis* var. *mairei* cultivars, 633 RAPD bands were used to analyze the genetic relationships among the studied cultivars. The repeatable bands of each primer were scored as present or absent. The similarity matrix between cultivars was computed using Jaccard's coefficient of similarity (Jaccard, 1908) and the UPGMA was used to construct a dendrogram that represents the genetic relationships among the studied cultivars. The 8 analyzed cultivars showed different profiles. The RAPD analyses performed using Opern

Table 1. Nucleotide sequences of selected primers with the number of amplified products and fragment size range (kb)

Primer number	Primer code	Primer sequence (5'-3')	Total band	Monomorphic band	Polymorphic band	Polymorphism (%)	Size range (kb)
1	OP A-01	CAGGCCCTTC	4	3	1	25	0.4-1.9
2	OP A-02	TGCCGAGCTG	6	6	0	0	0.8-3.0
3	OP A-03	AGTCAGCCAC	5	5	0	0	0.8-2.5
4	OP A-04	AATCGGGCTG	3	3	0	0	0.4-1.5
5	OP A-05	AGGGGTCTTG	7	5	2	28.6	0.4-1.8
6	OP A-06	GGTCCCTGAC	2	2	0	0	0.4-1.5
7	OP A-07	GAAACGGGTG	8	7	1	12.5	0.4-1.6
8	OP A-08	GTGACGTAGG	7	6	1	14.3	0.4-1.5
9	OP A-09	GGGTAACGCC	6	6	0	0	0.4-4.0
10	OP A-10	GTGATCGCAG	3	3	0	0	0.4-1.9
11	OP A-11	CAATCGCCGT	6	6	0	0	0.8-3.0
12	OP A-12	TCGGCGATAG	5	5	0	0	0.8-2.5
13	OP A-13	CAGCACCCAC	4	4	0	0	0.4-1.5
14	OP A-14	TCTGTGCTGG	7	7	0	0	0.4-1.8
15	OP A-15	TTCCGAACCC	8	8	0	0	0.4-1.5
16	OP A-16	AGCCAGCGAA	9	9	0	0	0.4-1.6
17	OP A-17	GACCGCTTGT	3	3	0	0	0.4-1.5
18	OP B-01	GTTTCGCTCC	4	3	1	25	0.4-1.5
19	OP B-02	TGATCCCTGG	5	3	2	40	0.4-1.9
20	OP B-03	CATCCCCCTG	2	2	0	0	0.8-3.0
21	OP B-04	GGACTGGAGT	6	5	1	16.7	0.8-2.5
22	OP B-05	TGCGCCCTTC	5	4	1	20	0.4-1.5
23	OP B-06	TGCTCTGCCC	4	3	1	25	0.4-1.8
24	OP B-07	GGTGACGCAG	3	3	0	0	0.4-1.9
25	OP B-08	GTCCACACGG	4	3	1	25	0.8-3.0
26	OP B-09	TGGGGGACTC	5	4	1	20	0.8-2.5
27	OP B-10	CTGCTGGGAC	6	4	2	33.3	0.4-1.5
28	OP B-11	GTAGACCCGT	5	4	1	20	0.4-1.8
29	OP B-12	CCTTGACGCA	4	4	0	0	0.4-1.5
30	OP B-13	TTCCCCCGCT	3	3	0	0	0.4-1.6
31	OP B-14	TCCGCTCTGG	6	5	1	16.7	0.4-1.5
32	OP B-15	GGAGGGTGTT	5	5	0	0	0.4-1.5
33	OP B-16	TTTGCCCGGA	7	5	2	28.6	0.4-1.9
34	OP B-17	AGGGAACGAG	3	3	0	0	0.8-3.0
35	OP B-18	CCACAGCAGT	4	3	1	26	0.8-2.5
36	OP B-19	ACCCCCGAAG	5	3	2	40	0.4-1.5
37	OP B-20	GGACCCTTAC	7	6	1	14.3	0.4-1.8
38	OP B-01	GTTTCGCTCC	8	6	2	25	0.4-1.5
39	OP C-11	AAAGCTGCGG	9	6	3	33.3	0.4-1.6
40	OP C-12	TGTCATCCCC	6	6	0	0	0.4-1.5
41	OP C-13	AAGCCTCGTC	7	5	2	28.6	0.4-1.9
42	OP C-14	TGCGTGCTTG	5	5	0	0	0.8-3.0
43	OP C-15	GACGGATCAG	4	4	0	0	0.8-2.5
44	OP C-16	CACACTCCAG	3	3	0	0	0.4-1.5
45	OP C-17	TTCCCCCCAG	5	4	1	20	0.4-1.8
46	OP C-18	TGAGTGGGTG	6	4	2	33.3	0.4-1.5
47	OP C-19	GTTGCCAGCC	7	5	2	28.6	0.4-1.6
48	OP C-20	ACTTCGCCAC	8	5	3	37.5	0.4-1.5

Table 1. Contd.

49	OP D-01	ACCGCGAAGG	9	6	3	33.3	0.4-1.5
50	OP D-02	GGACCCAACC	6	5	1	16.7	0.4-1.9
51	OP D-03	GTCGCCGTCA	5	5	0	0	0.8-3.0
52	OP D-04	TCTGGTGAGG	4	4	0	0	0.8-2.5
53	OP D-05	TGAGCGGACA	5	4	1	20	0.4-1.5
54	OP D-06	ACCTGAACGG	6	4	2	33.3	0.4-1.8
55	OP D-07	TTGGCACGGG	7	4	3	42.5	0.4-1.5
56	OP D-08	GTGTGCCCCA	8	6	2	25	0.4-1.6
57	OP D-09	CTCTGGAGAC	5	5	0	0	0.4-1.9
58	OP D-10	GGTCTACACC	3	3	0	0	0.8-3.0
59	OP E-16	GGTGACTGTG	6	5	1	16.7	0.8-2.5
60	OP E-17	CTACTGCCGT	7	5	2	28.6	0.4-1.5
61	OP E-18	GGACTGCAGA	8	6	2	25	0.4-1.8
62	OP E-19	ACGGCGTATG	5	4	1	20	0.4-1.5
63	OP E-20	AACGGTGACC	6	5	1	16.7	0.4-1.6
64	OP F-01	ACGGATCCTG	7	5	2	28.6	0.4-1.5
65	OP F-02	GAGGATCCCT	9	7	2	22.2	0.4-1.5
66	OP F-03	CCTGATCACC	7	5	2	28.6	0.4-1.9
67	OP F-04	GGTGATCAGG	4	4	0	0	0.4-1.5
68	OP F-05	CCGAATTCCC	5	4	1	20	0.4-1.6
69	OP G-11	TGCCCGTCGT	3	3	0	0	0.4-1.5
70	OP G-12	CAGCTCACGA	6	5	1	16.7	0.8-2.5
71	OP G-13	CTCTCCGCCA	7	5	2	28.6	0.4-1.5
72	OP G-14	GGATGAGACC	8	6	2	25	0.4-1.5
73	OP G-15	ACTGGGACTC	9	7	2	22.2	0.4-1.8
74	OP G-16	AGCGTCCTCC	7	6	1	14.3	0.4-1.5
75	OP G-17	ACGACCGACA	6	5	1	16.7	0.4-1.6
76	OP G-18	GGCTCATGTG	4	4	0	0	0.4-1.5
77	OP G-19	GTCAGGGCAA	5	5	0	0	0.4-1.5
78	OP G-20	TCTCCCTCAG	7	5	2	28.6	0.4-1.9
79	OP H-01	GGTCGGAGAA	8	6	2	25	0.4-1.5
80	OP H-02	TCGGACGTGA	9	7	2	22.2	0.4-1.6
81	OP H-03	AGACGTCCAC	7	6	1	14.3	0.4-1.5
82	OP H-04	GGAAGTCGCC	6	5	1	16.7	0.8-2.5
83	OP H-05	AGTCGTCCCC	4	3	1	25	0.4-1.9
84	OP H-06	ACGCATCGCA	7	7	0	0	0.8-2.5
85	OP H-07	CTGCATCGTG	6	5	1	16.7	0.4-1.5
86	OP H-08	GAAACACCCC	4	4	0	0	0.4-1.8
87	OP H-09	TGTAGCTGGG	3	3	0	0	0.4-1.5
88	OP H-10	CCTACGTCAG	7	7	0	0	0.4-1.6
89	OP I-01	ACCTGGACAC	8	7	1	12.5	0.4-1.5
90	OP I-02	GGAGGAGAGG	9	8	1	11.1	0.4-1.5
91	OP I-03	CAGAAGCCCA	7	7	0	0	0.4-1.9
92	OP I-04	CCGCCTAGTC	5	5	0	0	0.4-1.5
93	OP I-05	TGTTCCACGG	4	3	1	25	0.4-1.6
94	OP I-06	AAGGCGGCAG	6	6	0	0	0.4-1.5
95	OP I-07	CAGCGACAAG	8	7	1	12.5	0.4-1.5
96	OP I-08	TTTGCCCGGT	9	7	2	22.2	0.4-1.8
97	OP I-09	TGGAGAGCAG	7	6	1	14.3	0.4-1.5
98	OP I-10	ACAACGCGAG	6	6	0	0	0.4-1.7

Table 1. Contd.

99	OP I-11	ACATGCCGTG	5	5	0	0	0.4-1.5
100	OP I-12	AGAGGGCACA	4	4	0	0	0.4-1.6
101	OP I-13	CTGGGGCTGA	8	8	0	0	0.4-1.5
102	OP I-14	TGACGGCGGT	7	6	1	14.3	0.4-1.9
103	OP I-15	TCATCCGAGG	6	6	0	0	0.4-1.5
104	OP I-16	TCTCCGCCCT	7	7	0	0	0.4-1.6
105	OP I-17	GGTGGTGATG	9	9	0	0	0.4-1.8
106	OP I-18	TGCCCAGCCT	5	3	2	40	0.4-1.5
107	OP I-19	AATGCGGGAG	6	6	0	0	0.4-1.9
108	OP I-20	AAAGTGCGGG	8	6	2	25	0.4-1.5

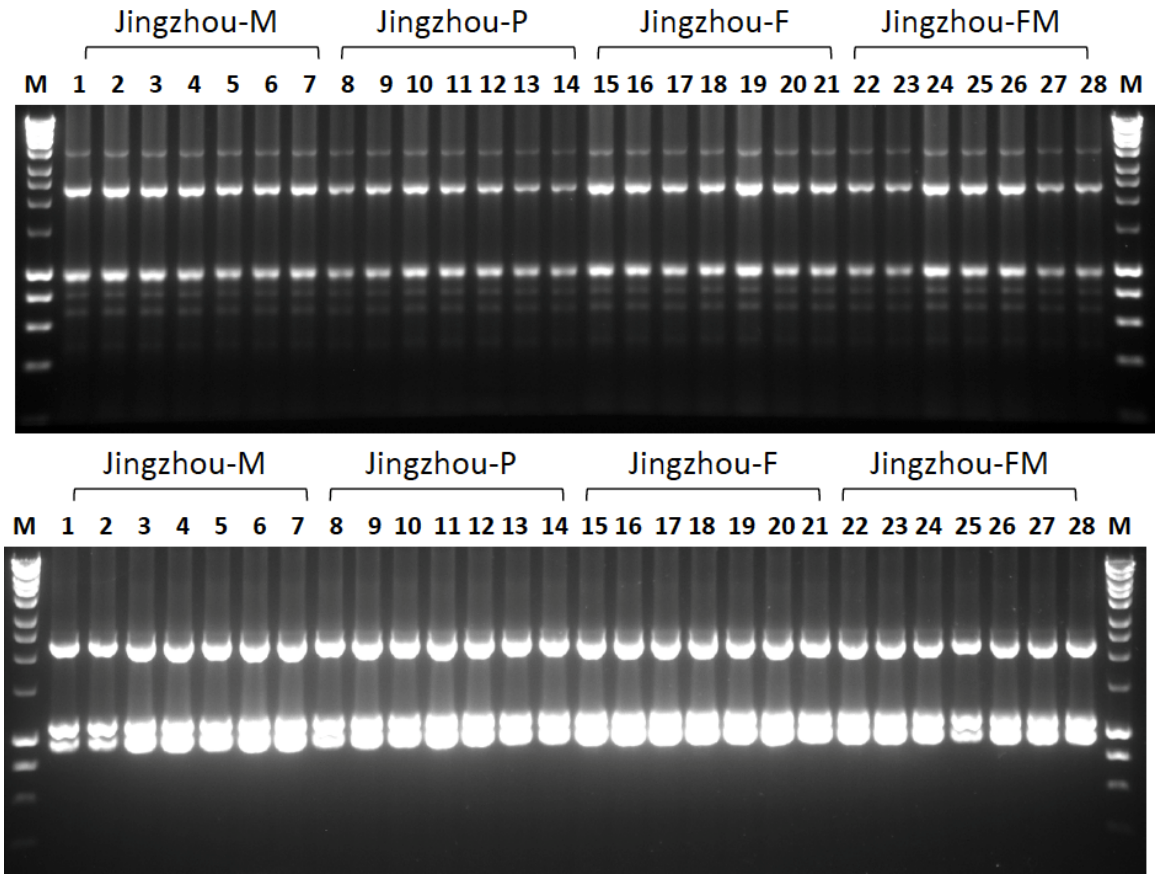

Figure 2. Cultivars-specific patterns detected by primers using the sequence data of each polymorphic band. Representative banding pattern as revealed by primers OP-A09 (A) and OP-D10 (B) (Lanes M: DNA Markers; 1, 8, 15, and 22: PCR bands amplified from DNA of flower cone; 2, 9, 16, and 23: PCR bands amplified from DNA of stem; 3-7, 10-14, 17-21, and 24-28: PCR bands amplified from DNA of leave in *Taxus chinensis* var. *mairei* cultivars Jingzhou-M, Jingzhou-P, Jingzhou-F, Jingzhou-FM, respectively).

primers suggested that 4 out of 8 cultivars did not belong to the same group, because they showed unambiguous, reproducible and consistent bands not shared by the 'Baohkang' samples (Figures 2 and 3). The remaining 4 cultivars had profiles whose bands were all shared by the 'Jingzhou' pattern. These differences in profiles could be ascribed to genetic information.

The DNA from the above mentioned 8 cultivars was also

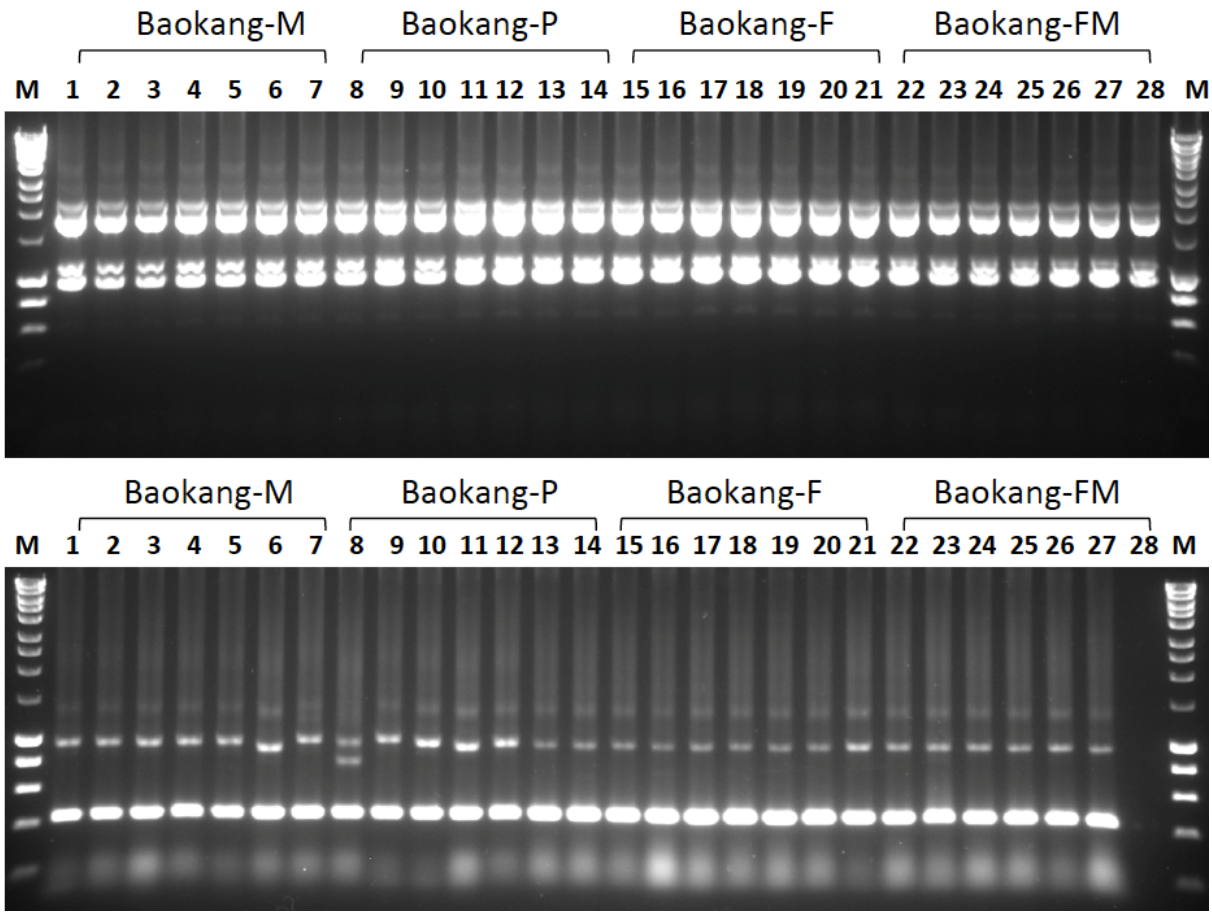

Figure 3. RAPD profiles of *Taxus chinensis* var. *mairei* cultivar (Baokang) with primer. Representative banding pattern as revealed by primers RAPD-OP-H06 (A) and RAPD-OP-I18 (B) (Lanes M: DNA Markers; 1, 8, 15, and 22: PCR bands amplified from DNA of flower cone; 2, 9, 16, and 23: PCR bands amplified from DNA of stem; 3-7, 10-14, 17-21, and 24-28: PCR bands amplified from DNA of leave in *Taxus chinensis* var. *mairei* cultivars Baokang-M, Baokang-P, Baokang-F, Baokang-FM, respectively). Lane 28 in Baokang-FM is the negative control.

re-amplified by Opern primers with different amounts of template and the results are clearly shared the complete profile, except Jingzhou-P, which showed a slightly different profile from that of Jingzhou-M, Jingzhou-F, and Jingzhou-FM.

By varying template amount, subsequent amplifications with the same primer always yielded a perfectly identical profile to the 'Jingzhou' one. The same results were obtained after re-extracting and re-amplifying DNA from Jingzhou-P. This supports the fact that a critical issue in RAPD experiments is the amount of template employed and, consequently, the correct quantification of extracted DNA. The 4 cultivars showing a profile identical to 'Jingzhou' with Opern primers Group A, B, C, D, E, F, G, H, and I also yielded profiles identical to 'Baokang' with the previously listed OPA primers. The other 4 cultivars did not show profiles identical to 'Baokang'. The results of RAPD analyses were double-checked.

Cluster analysis of the pedigree data yielded results that were more or less expected (Figure 4). The *T. chinensis* cultivars `Baokang' and `Jingzhou' did not join, but 'Jingzhou-M, Jingzhou-P, and Jingzhou-FM' did join 'Jingzhou-F', which also has *T. chinensis* var. *mairei* germplasm in its background. 'Baokang-M', 'Baokang-P', 'Baokang-FM', 'Baokang-F' were all joined together. The Mantel matrix correlation test generated a value of r = 0.95, suggesting a very good fit of the data to the resulting dendrogram (Table 2). Overall, 633 RAPD loci were used to calculate the similarity estimates, a number that Fu et al. (2002) deemed to be within an acceptable range. Of this number, most were polymorphic due to the inclusion of the *T. chinensis* var. *mairei*. Eight cultivars were identified specifically within these sets of primers. When compared amongst themselves, the *T. chinensis* var. *mairei* displayed from 74.9% ('Jingzhou-F') to 82.1% (Jingzhou-M) similarity, with the average at 75% for

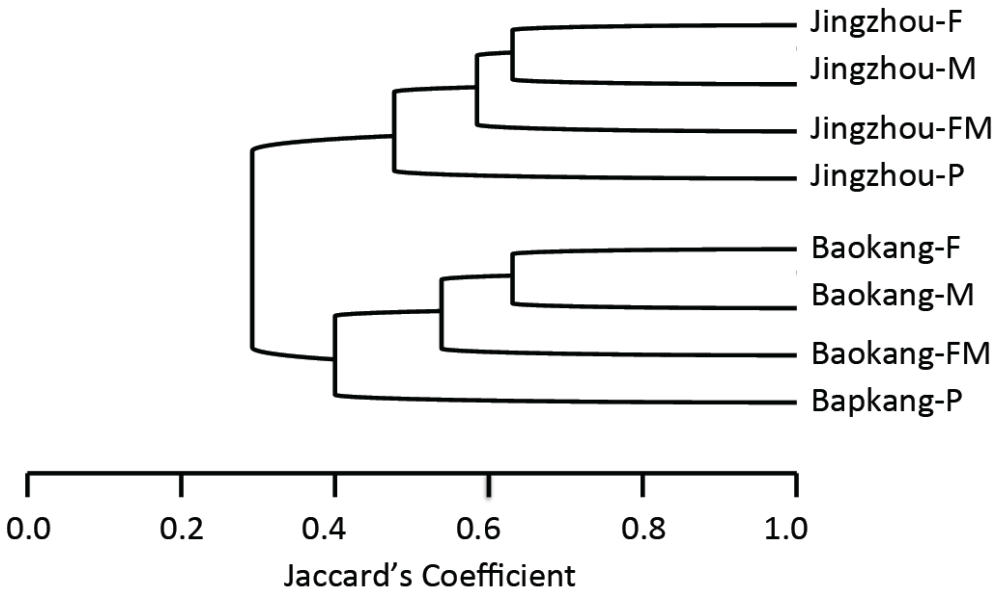

Figure 4. Dendogram illustrating genetic similarity (Jaccard's coefficient) among eight *Taxus chinensis* var*. mairei* cultivar generated by UPGMA cluster analysis calculated from 633 RAPD bands produced by 108 primers.

Table 2. Genetic similarity matrix of eight *Taxus chinensis* var*. mairei* cultivars generated by 108 RAPD primers

Parameter	Jingzhou-F	Jingzhou-M	Jingzhou-FM	Jingzhou-P	Baokang-F	Baokang-M	Baokang-FM	Baokang-P
Jingzhou-F	1.000							
Jingzhou-M	0.749	1.000						
Jingzhou-FM	0.735	0.796	1.000					
Jingzhou-P	0.821	0.864	0.824	1.000				
Baokang-F	0.769	0.736	0.729	0.754	1.000			
Baokang-M	0.892	0.825	0.736	0.812	0.817	1.000		
Baokang-FM	0.786	0.768	0.815	0.726	0.763	0.789	1.000	
Baokang-P	0.843	0.689	0.739	0.796	0.812	0.816	0.786	1.000

RAPD marker data.

DISCUSSION

RAPD results are reliable for the remaining pairwise comparisons (Welsh and McClelland, 1990; Parent et al., 1993). Pedigree and RAPD similarity matrices produced results that were comparable (Bublyk et al., 2013; Cires et al., 2013; Parent and Pagé, 1992; Trople and Moore, 1999).

In the present investigation, the RAPD results were completely reliable because the percentage of 'Jingzhou' and 'Baokang' identification was 100% (8 out of 8). The results were accepted as strong evidence. This study confirms that the RAPD technique, which is easy, fast and inexpensive, is especially suitable for identification of *T. chinensis* var*. mairei* cultivars for agricultural purposes. Each primer yields a typical electrophoretic profile and in this way the number of marker bands suitable for variety fingerprinting can be improved simply by performing further amplifications with different primers.

On the other hand, the sensitivity of the RAPD technique requires repeated amplifications with different amounts of template to avoid artifacts and obtain reliable results. The Mantel test revealed a correlation of r = 0.95, a very good fit of the similarity matrix data to the cluster analysis. In the RAPD dendrogram, 'Baokang' and 'Jingzhou' was a distinct cluster from the *T. chinensis* var*. mairei* cultivars. This result is likely more accurate than that of the pedigree dendrogram because of the taxonomic designation of 'Jingzhou' as a *T. chinensis* var.

mairei.

However, some discrepancies became evident. ‘Jingzhou-P’ and ‘Baokang-P’ are cultivars that are associated with parthenogenesis. Surprisingly, ‘Jingzhou-P’ and ‘Baokang-P’ did not join together, and were not in the same cluster. Yet, ‘Baokang-F’ and ‘Baokang-M’ did join together, and had the highest similarity percentage of any pair of cultivars (75%). This is comparable to the result in the pedigree similarity matrix (71%). Baokang-M shows more similarity to ‘Baokang-F’ than to ‘Baokang-P’. Thus, the contribution of ‘Baokang-M’ to both ‘Baokang-FM’ and ‘Baokang-F’ could account for their degree of similarity detected in this study. When the matrices of the pedigree data and the RAPD data were tested with the Mantel matrix correlation the result was correlation (r = 0.95), therefore the pedigree and RAPD matrices may be considered as relating to one another in a meaningful way.

In conclusion, from the results obtained in this investigation, there is a correlate relationship between the pedigree and RAPD data. The pedigree and RAPD data did correlate for cultivars that shared many of the same founding markers, as the results tended to overestimate relatedness. If a more accurate assessment of pedigree relatedness among cultivars was used, then the results may have been more precise. In general, RAPD marker data proved to be a good method of assessing genetic relatedness among different *T. chinensis* var. *mairei* cultivars that are associated with parthenogenesis. Therefore, RAPD markers can effectively differentiate closely related *T. chinensis* var. *mairei* cultivars involved in parthenogenesis, as well as more distantly related *T. chinensis* cultivars.

ACKNOWLEDGEMENTS

We wish to thank Dr. Martine for kind technical assistance. This work was supported by a grant from the Education Committee of Hubei Providence of China (grant no. D20101306), and a grant from the National Natural Science Foundation of China (grant no. 31270740).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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