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Dispersal Molecular Ecology of Some Framework Species for Forest Restoration

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A preliminary molecular ecological study of seed dispersal in fragmented forest restoration plots in northern Thailand

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1. INTRODUCTION

Forest degradation is one of the main environmental problems in many tropical countries, including Thailand. The Thai government has been reforesting many degraded areas, using various reforestation schemes. The Forest Restoration Research Unit of Chiang Mai University (FORRU-CMU, established in 1994, http://www.forru.org) has also been involved in attempts to improve such reforestation schemes for biodiversity conservation, by developing ways to re-establish original forest ecosystems, including former compositional and functional aspects. The framework species method, developed by FORRU was adapted from the method original devised in North Queensland, Australia. The method plants native tree species that grow fast, suppress weeds, are resilient to fire, and attract seed-dispersing wildlife.

Seed dispersal is one of the two, pollen transport being another, methods of passing genetic information through generations of plants (Godoy and Jordano 2001). The distance of seed dispersal depends of modes of dispersal, e.g. animal-, water-, and wind-dispersed seeds. Approximately 46-80 per cent of trees in the Paleotropics (e.g. Southeast Asia) are vertebrated (birds, and mammals, especially bats and primates)-dispersed (Howe and Smallwood 1982). Clark (1998) reported that about 10 percent of trees have long-ranged dispersing seeds. Predicting gene-flow pattern of tropical trees is difficult because behaviors of animal seed dispersers are unpredictable (Hamrick and Loveless 1986, Nason and Hamrick 1997 in Hardesty *et al.* 2005). However, it is crucial to know this information, which can be applied in forest restoration. For example, one can know how far seeds of certain species can disperse from intact forests into forest-restored plots (e.g. Muller-Landau and Hardesty 2005). This process can help increase richness of species in the planted areas (FORRU 2005). Cordeiro and Howe (2003) showed that forest fragmentation severely affect reproductive cycle of *Leptonychia usambarensis* (Sterculiaceae) due to the rareness or disappearance of seed-dispersing birds in small forest fragments. Muller-Landau and Hardesty (2005) stressed that more studies on changes of seed dispersal in fragmented forests are needed because changing dispersal events can affect populations and communities of plants. Howe and Miriti (2004) suggested that knowledge on seed dispersal distance is important for vegetation recovery when disturbance is decreased.

The Chiang Mai Agenda for the restoration of degraded forest lands for wildlife conservation in Southeast Asia stemmed from the proceedings of a workshop on forest restoration for wildlife conservation held in Chiang Mai between January 30th and February 4th 2000. It identified five main areas of needed research in forest restoration. The areas were 1) plantation design, 2) seed dispersal, 3) fire ecology and management, 4) species selection, nursery, and plantation techniques, and 5) social and community issues (Elliott, 2000; Elliott *et al.*, 2000). The proposed research here contributes to one of the priority areas identified, which are #2 seed dispersal (Elliott, 2000).

Patterns of gene flow of tropical tree species can be obtained by either (1) directly observing movements of pollinators and agents of seed dispersal (Webb and Bawa 1983, Howe 1986, Schupp 1993, Boshier *et al.*1995), (2) indirectly estimating using F_{st}-based statistics or spatial autocorrelation (Hamrick *et al.* 1993, Hamilton and Miller 2002, Dick *et al.* 2003, Vekemans and Hardy 2004 in Hardesty *et al.* (2005), or (3) using molecular markers for reconstructing movements of seed and pollen by performing parentage analysis (Aldrich and Hamrick 1998, Ouborg *et al.* 1999, Jordano and Godoy 2002, Sezen *et al.* 2005)." in Hardesty *et al.* (2005).

Molecular tools are increasingly being utilized in ecological studies. Molecular markers enable ecologists to "quantify genetic diversity, track the movements of the individuals, measure inbreeding, identify the remains of individuals, characterize new species, and retrace historical patterns of dispersal" (Freeland 2005). Jordano and Godoy (2002) reported earlier that molecular markers were still under-utilized in studies of seed dispersal. Later, however, Jones and Ardren (2003) reviewed that microsatellite markers are highly powerful for parentage analysis and have been used widely for various genetic studies, e.g. genetic mapping, mating-system analysis, and parentage analysis (Pakkad *et al.* 2001). Hardesty *et al.* (2005) used microsatellite markers to perform parental analysis of

Simarouba amara (Simaroubaceae) in Panama. Molecular ecology studies in Paleotropics (e.g. Southeast Asia) have been few in comparison to Neotropics (e.g. South America) (e.g. Muller-Landau and Hardesty 2005). However, developing of microsatellite markers can be costly. This present, therefore, attempted to use RAPD (Random Amplified Polymorphic DNA), which is less expensive.

2. MATERIALS AND METHODS

2.1 Study site

Forest restoration experimental plots were established in the north of Doi Suthep-Pui National Park (Elliott *et al.* 2003). After discussion with the national park authorities and villagers of Ban Mae Sa Mai (a Hmong hill tribe community in the north of the park), trial plots were positioned along or immediately below the ridges of a degraded watershed area, 2-3 km from the village (18° 52'N, 98° 51'E), at 1,207-1,310 m above sea level. The villagers collaborated closely in all aspects of the experiments, including growing saplings in their own community nursery, as well as planting, maintaining and monitoring plots.

Originally, the study site had been covered with evergreen forest (EGF), cleared approximately 20 years previously, to provide land for cultivation of cabbages, corn, potatoes and other cash crops. The abandoned fields were dominated by herbaceous weeds such as *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae), *Bidens pilosa* L. var. *minor* (Bl.) Sherf, *Ageratum conyzoides* L., *Eupatorium odoratum* L. and *E. adenophorum* Spreng. (all Compositae), *Commelina diffusa* Burm. F. (Commelinaceae) and grasses e.g. *Phragmites vallatoria* (Pluk. ex L.) Veldk., *Imperata cylindrica* (L.) P. Beauv. var. *major* (Nees) C.E. Hubb. ex Hubb. & Vaugh. and *Thysanolaena latifolia* (Roxb. ex Horn.) Honda (both Gramineae). Most of the slopes below the plots were still cultivated (corn, cabbages, carrots etc.), with extensive litchi orchards beyond, providing the villagers with their main source of income.

A few remnant forest trees, sparsely scattered across the plots, provided a potential seed source for natural forest regeneration. The nearest extensive patch of forest lay some 2-3 km from the plots. Compared with soil in undisturbed EGF at a similar elevation, soil in the study site before planting was significantly more acidic and contained significantly less organic matter and nitrogen, more sand and less silt and clay, which may be a result of forest degradation (Table 1, $p<0.05$) (Elliott *et al.*, 2000) (Elliott *et al.* 2003).

The area has two main seasons: the wet season (May - October) and the dry season (mean monthly rainfall below 100 mm, November - April). The dry season is subdivided into the cool-dry season (November to January) and the hot- dry season (February to April). Average annual rainfall, recorded at the nearest weather station to the study site at similar elevation (Kog-Ma Watershed Research Station), was 2,094.9 mm. Extreme temperatures ranged from a minimum of 4.5°C in December to a maximum of 35.5°C in March.

Fire is a major constraint to reforestation in this landscape. Villagers use fire to clear land for cultivation and, despite rules to prevent accidents, fires often "escape" and burn out of control over extensive areas. Frequent anthropogenic fires are a recent occurrence in the evolutionary history of upland EGF and most species have low resistance or resilience.

2.2 Sampling method

Leaf samples of non-planting seedlings species were collected from 3 plots: non-planted control, 1999, and 2002 plots. The numbers of the plots represent the planting years. Leaf samples of adult trees, 3 individuals per species, of the same non-planted species found in the forest restoration plots were collected from two nearby protected forests. The leaf samples were analysed using the HAT-RAPD technique (Anuntalabhochai *et al.* 2000).

3. RESULTS AND DISCUSSION

A list of non-planted seedlings is in Table 1. Table 2 shows the locations of each of the non-planted species. Table 3 shows the amounts of the extracted DNA. Figures 1 to 17 depict the results of the HAT-RAPD.

4. CONCLUSION

The primers, OPN 02 and OPN 03, used in the HAT-RAPD technique could differentiate some species from the other species, but could not distinguish among the individuals within the same species.

5. RECOMMENDATION

This is a need for further research on screening more primers for molecular study of seed dispersal in forest restoration areas.

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Table 1 Non-planted species, and their dispersal mechanisms, found in the forest restoration plots

No.	Species	Family	Dispersal mechanism	References
1	*Alangium kurzii* Craib	Alangiaceae	Animal	1, 4
2	*Albizia chinensis* (Osb.) Merr.	Mimosaceae	Wind	4
3	*Antidesma bunius* (L.) Spreng. var. *bunius*	Euphorbiaceae	Animal	4
4	*Antidesma ghaesembilla* Gaerthn.	Euphorbiaceae	Animal	-
5	*Artocarpus lakoocha* Roxb.	Moraceae	Animal	4
6	*Bombax anceps* Pierre var. *anceps*	Bombaceae	Wind	NA
7	*Dalbergia stipulacea* Roxb.	Papilionoideae	Wind	NA
8	*Dalbergia cultrate* Graham ex Benth.	Papilionoideae	Wind	4
9	*Eugelhardtia spicata* var. *spicata*	Juglandaceae	Wind	4
10	*Eugenia albiflora* Duth. ex Kurz.	Myrtaceae	Animal	NA
11	*Ficus hirta* Vahl. var. *hirta*	Moraceae	Animal	1
12	*Ficus hispida* L.f.	Moraceae	Animal	4
13	*Ficus subulata* var. *subulata*	Moraceae	Animal	4
14	*Glochidion acuminatum* var. *siamense*	Euphorbiaceae	Animal	4
15	*Harrisonia perforate* (Blanco) Merr.	Simaroubaceae	NA	-
16	*Lithocarpus lindleyanus* (Wall.)	Fagaceae	NA	-
17	*Litsea cubeba* (Lour.)	Lauraceae	Animal	2, 4
18	*Litsea monopetala* (Roxb.)	Lauraceae	Animal	4
19	*Machilus bombycina* King. ex Hk.f.	Lauraceae	Animal	4
20	*Maesa ramentacea* Roxb.A.DC.	Myrsinaceae	Animal	4
21	*Marhamia stipulata* var. *kerrii*	Bignoniaceae	Wind	4
22	*Micromelum minutum* (G.Forst.) Wight & Arn.	Rutaceae	Animal	-
23	*Micromelum minutum* (G.Forst.) Wight & Arn.	Rutaceae	NA	-

Table 1 Cont.

No.	*Species*	Family	Dispersal mechanism	References
24	*Phoebe lanceolata* Nees.	Lauraceae	Animal	1, 4
25	*Pterocarpus macrocarpus* Kurz	Papilionoideae	Wind	-
26	*Rhus chinensis* Mill.	Anacardiaceae	NA	4
27	*Rhus rhetsoides* Craib	Anacardiaceae	Animal	5
28	*Schima wallichii* (DC.) Korth	Theaceae	Wind	3, 4
29	*Spondias axillaries*	Anacardiaceae	Animal	5
30	*Sterculia villosa* Roxb.	Sterculiaceae	Animal	4
31	*Stereospermum colais* (Buch.-Ham. ex Dillwtn) Mabb.	Bignoniaceae	Wind	4
32	*Symplocos sumuntia*	Symplocaceae	NA	-

Note

1. Kitamura *et al.* (2002)

2. Sri-ngernyuang *et al.* (2003)

3. Elliott *et al.* (2003)

4. Navakitbumrung (2003)

5. FORRU (2005)

NA = Not available

Table 2 Non-planted species found in the forest restoration plots

No.	Species	Plot		
		1999	2002	Control
1	*Alangium kurzii* Craib	-	-	✓
2	*Albizia chinensis* (Osb.) Merr.	-	-	✓
3	*Antidesma bunius* (L.) Spreng. var. *bunius*	-	-	✓
4	*Antidesma ghaesembilla* Gaerthn.	-	-	✓
5	*Artocarpus lakoocha* Roxb.	-	✓	-
6	*Bombax anceps* Pierre var. *anceps*	-	✓	-
7	*Dalbergia stipulacea* Roxb.	-	✓	-
8	*Dalbergia cultrate* Graham ex Benth.	✓	-	-
9	*Eugelhardtia spicata* var. *spicata*	-	-	✓
10	*Eugenia albiflora* Duth. ex Kurz.	-	✓	-
11	*Ficus hirta* Vahl. var. *hirta*	-	-	✓
12	*Ficus hispida* L.f.	-	-	✓
13	*Ficus subulata* var. *subulata*	✓	-	-
14	*Glochidion acuminatum* var. *siamense*	✓	-	-
15	*Harrisonia pere*	-	✓	-
16	*Lithocarpus lindleyanus* (Wall.)	✓	-	-
17	*Litsea cubeba* (Lour.)	-	✓	✓
18	*Litsea monopetala* (Roxb.)	✓	-	✓
19	*Machilus bombycina* King. ex Hk.f.	✓	-	-
20	*Maesa ramentacea* Roxb. A. DC.	-	-	✓
21	*Marhamia stipulata* var. *kerrii*	-	-	✓
22	*Micromelum minutum* (G.Forst.) Wight & Arn.	-	-	✓
23	*Micromelum minutum* (G.Forst.) Wight & Arn.	✓	-	-
24	*Phoebe lanceolata* Nees.	✓	-	✓

Table 2 Cont.

No.	Species	Plot		
		1999	2002	Control
25	*Pterocarpus macrocarpus* Kurz	-	-	✓
26	*Rhus chinensis* Mill.	-	✓	-
27	*Rhus rhetsoides* Craib	✓	-	✓
28	*Schima wallichii* (DC.) Korth	✓	-	-
29	*Spondias axillaries*	-	✓	✓
30	*Sterculia villosa* Roxb.	-	-	✓
31	*Stereospermum colais* (Buch.-Ham. ex Dillwtn) Mabb.	-	✓	-
32	*Symplocos sumuntia*	✓	-	-

Table 3 Amount of extracted DNA (mg)

Number	ID and Plant species	DNA (mg)	Plot
1	02-5-28 *Micromelum minutum* (G.Forst.) Wight & Arn.	2	2002
2	02-1-2 *Litsea monopetala* (Roxb.) Pers.	4	2002
3	807 (827) *Bombax* sp.	10	1999
4	02-1-6 *Litsea monopetala* (Roxb.) Pers	4	2002
5	02-5-19 *Antidesma ghaesembilla* Gaertn.	0.5	2002
6	826-control *Eugenia albiflora* Duth. ex Kurz	5	Control
7	929-control *Rhus chinensis* Mill.	2	Control
8	02-1-14 *Litsea monopetala* (Roxb.) Pers	1	2002
9	02-5-50 *Micromelum minutum* (G.Forst.) Wight & Arn.	4	2002
10	02-5-32 *Antidesma ghaesembilla* Gaertn.	6	2002
11	863-Control *Sterculia villosa* Roxb.	10	Control
12	02-1-7 *Litsea monopetala* (Roxb.) Pers	3	2002
13	02-1-27 *Litsea cubeba* (lour.) Pers. var. *cubeba*	4	2002
14	02-1-4 *Litsea cubeba* (lour.) Pers. var. *cubeba*	4	2002
15	984- control *Ficus hirta* Vahl var. *hirta*	1	Control
16	02-5-18 *Micromelum minutum* (G.Forst.) Wight & Arn.	6	2002
17	02-1-24 *Markhamia stipulata* (Wall.) Seem. ex K. Sch.	4	2002
18	02-5-4 *Maesa ramentacea* (Roxb.) A. DC.	6	2002
19	874-control *Dalbergia stipulacea* Roxb.	6	Control
20	02-1-12 *Litsea monopetala* (Roxb.) Pers	4	2002
21	929 *Rhus chinensis* Mill.	4	1999
22	02-1-15 *Litsea cubeba* (lour.) Pers. var. *cubeba*	12	2002
23	02-1-20 *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	2002
24	02-1-23 *Litsea monopetala* (Roxb.) Pers	1	2002
25	02-1-17 *Litsea cubeba* (lour.) Pers. var. *cubeba*	4	2002

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
26	829 *Stereospermum colais* (B.-H. ex Dillw.) Mabb.	1	1999
27	932-Control *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	Control
28	833-Control *Dalbergia stipulacea* Roxb.	13	Control
29	02-5-51 *Micromelum minutum* (G.Forst.) Wight & Arn.	10	2002
30	02-8-15 *Micromelum minutum* (G.Forst.) Wight & Arn.	10	2002
31	806 *Spondias xillaries* Roxb.	2	1999
32	02-1-5 *Litsea monopetala* (Roxb.) Pers	2	2002
33	02-2-1 *Litsea monopetala* (Roxb.) Pers	2	2002
34	02-8-1 *Micromelum minutum* (G.Forst.) Wight & Arn.	6	2002
35	02-4-12 *Engelhardia spicata* Lechen. ex Bl. var. *integra* (Kurz) Mann.	2	2002
36	02-8-9 *Litsea monopetala* (Roxb.) Pers	4	2002
37	02-5-7 *Phoebe lanceolata* (Wall. ex Nees) Nees.	2	2002
38	02-2-8 *Litsea monopetala* (Roxb.) Pers	2	2002
39	02-5-1 *Maesa ramentacea* (Roxb.) A. DC.	6	2002
40	02-8-4 *Rhus rhetsoides* Craib.	4	2002
41	02-2-16 *Antidesma ghaesembilla* Gaertn.	2	2002
42	02-2-11 *Antidesma bunius* (L.) Spreng.	1	2002
43	02-6-3 *Litsea monopetala* (Roxb.) Pers	1	2002
44	02-11-21 *Litsea monopetala* (Roxb.) Pers	3	2002
45	02-5-20 *Antidesma ghaesembilla* Gaertn.	2	2002
46	02-4-13 *Litsea monopetala* (Roxb.) Pers	2	2002
47	02-4-11 *Litsea monopetala* (Roxb.) Pers	2	2002
48	02-4-4 *Litsea monopetala* (Roxb.) Pers	2	2002
49	02-10-2 *Bombax anceps* Pierre var. *anceps*	4	2002

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
50	02-4-7 *Litsea cubeba* (lour.) Pers. var. *cubeba*	2	2002
51	02-2-9 *Litsea monopetala* (Roxb.) Pers	3	2002
52	02-5-8 *Litsea monopetala* (Roxb.) Pers	2	2002
53	02-8-19 *Artocarpus lakoocha* Roxb.	3	2002
54	02-2-10 *Antidesma ghaesembilla* Gaertn.	1	2002
55	02-2-1 *Litsea monopetala* (Roxb.) Pers	4	2002
56	02-8-18 *Litsea monopetala* (Roxb.) Pers	1	2002
57	02-3-9 *Antidesma ghaesembilla* Gaertn.	1	2002
58	02-5-29 *Sterculia villosa* Roxb.	2	2002
59	02-5-33 *Litsea monopetala* (Roxb.) Pers	2	2002
60	02-5-34 *Antidesma ghaesembilla* Gaertn.	4	2002
61	02-6-12 *Markhamia stipulata* (Wall.) Seem. ex K. Sch.	4	2002
62	02-3-11 *Litsea monopetala* (Roxb.) Pers	2	2002
63	02-3-5 *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	2002
64	02-3-1*Litsea monopetala* (Roxb.) Pers	2	2002
65	02-4-1 *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	2002
66	02-6-1 *Markhamia stipulata* (Wall.) Seem. ex K. Sch	4	2002
67	02-6-2 *Litsea monopetala* (Roxb.) Pers	1	2002
68	02-6-9 *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	2002
69	02-6-13 *Artocarpus lakoocha* Roxb.	6	2002
70	02-6-14 *Litsea cubeba* (lour.) Pers. var. *cubeba*	1	2002
71	02-6-15 *Ficus hispida* L.f. var. *hispida*	1	2002
72	02-6-16 *Pterocarpus macrocarpus* Kurz.	6	2002
73	02-6-18 *Ficus hirta* Vahl var. *hirta*	3	2002
74	02-10-1*Litsea monopetala* (Roxb.) Pers	2	2002

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
75	02-10-5 *Spondias axillaris* Roxb.	1	2002
76	02-10-05 *Spondias axillaris* Roxb.	1	2002
77	02-10-6 *Albizia chinensis* (Osb.) Merr.	6	2002
78	02-10-6 *Albizia chinensis* (Osb.) Merr.	1	2002
79	02-11-1 *Litsea monopetala* (Roxb.) Pers	1	2002
80	02-11-03 *Litsea monopetala* (Roxb.) Pers	1	2002
81	02-11-4 *Litsea monopetala* (Roxb.) Pers	1	2002
82	02-11-6 *Litsea monopetala* (Roxb.) Pers	2	2002
83	02-11-09 *Litsea monopetala* (Roxb.) Pers	1	2002
84	02-11-13 *Litsea monopetala* (Roxb.) Pers	1	2002
85	02-11-21 *Litsea monopetala* (Roxb.) Pers	1	2002
86	312 *Litsea monopetala* (Roxb.) Pers	4	Forest
87	313 *Litsea monopetala* (Roxb.) Pers	10	Forest
88	314 *Artocarpus lakoocha* Roxb.	2	Forest
89	317 *Dalbergia cultrata* Grah. ex Bth.	2	Forest
90	318 *Machilus bombycina* King ex Hk.f.	1	Forest
91	323 *Litsea monopetala* (Roxb.) Pers	10	Forest
92	349 *Litsea monopetala* (Roxb.) Pers	6	Forest
93	F316 *Schima wallichii* (DC.) Korth.	2	Forest
94	F322 *Schima wallichii* (DC.) Korth.	3	Forest
95	F341*Rhus rhetsoides* Craib	4	Forest
96	3211*Litsea monopetala* (Roxb.) Pers	0.5	Forest
97	3251*Litsea monopetala* (Roxb.) Pers	1	Forest
98	1 *Archidendron clyperia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	0.5	1999

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
99	212 *Castanopsis tribuloides* (Sm.) A. DC.	2	1999
100	27 *Markhamia stipulata* (Wall.) Seem. ex K. Sch	1	1999
101	200 *Aporosa villosa* (Lindl.) Baill.	0.2	1999
102	65 *Schima wallichii* (DC.) Korth.	1	1999
103	186 *Phoebe lanceolata* (Wall. ex Nees) Nees	0 *	1999
104	12 *Litsea monopetala* (Roxb.) Pers	1	1999
105	6 *Litsea monopetala* (Roxb.) Pers	1	1999
106	4 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	0 *	1999
107	16 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	0.5	1999
108	188 *Ficus hirta* Vahl var. *hirta*	2	1999
110	185 *Sterculia lanceolata* Car. var. *lanceolata*	1	1999
111	184 *Litsea monopetala* (Roxb.) Pers	1	1999
112	44 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	1	1999
113	214 *Markhamia stipulata* (Wall.) Seem. ex K. Sch	0	1999
114	195 *Phoebe lanceolata* (Wall. ex Nees) Nees	1	1999
115	3 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	Milky sap	1999
116	182 *Schima wallichii* (DC.) Korth.	0.2	1999
117	32 *Phoebe lanceolata* (Wall. ex Nees) Nees	1	1999
118	64 *Phoebe lanceolata* (Wall. ex Nees) Nees	1	1999
119	198 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	0.5	1999

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
120	213 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.2	1999
121	20 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
122	50 *Litsea monopetala* (Roxb.) Pers	1	1999
123	49 *Litsea monopetala* (Roxb.) Pers	0.1	1999
124	48 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.2	1999
125	42 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.5	1999
126	47 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
127	46 *Phoebe lanceolata (*Wall. ex Nees) Nees	2	1999
128	45 *Litsea monopetala* (Roxb.) Pers	1	1999
129	181 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	1	1999
130	33 *Litsea monopetala* (Roxb.) Pers	2	1999
131	9 *Schima wallichii* (DC.) Korth.	1	1999
132	34 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
133	37 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
134	208 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	0.5	1999
135	209 *Markhamia stipulate* (Wall.) Seem. ex K. Sch	1	1999
136	55 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.1	1999
137	206 *Ficus hirta* Vahl var. *hirta*	10	1999
138	60 *Litsea monopetala* (Roxb.) Pers	2	1999
139	207 *Dalbergia cultrata* Grah. ex Bth.	1	1999
140	61 *Castanopsis acuminatissima* (Bl.) A. DC.	0.1	1999
141	199 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	0.1	1999
142	Unknown	0.1	1999

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
143	202 *Turpinia pomifera* (Roxb.) Wall. ex DC	12	1999
144	201 *Litsea monopetala* (Roxb.) Pers	0 *	1999
145	210 *Markhamia stipulata* (Wall.) Seem. ex K. Sch	1	1999
146	192 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
147	203 *Eugenia fruiticosa* (DC.) Roxb.	0.2	1999
148	194 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.2	1999
149	Unknown	0.2	1999
150	190 *Litsea monopetala* (Roxb.) Pers	0.1	1999
151	193 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	20	1999
153	196 *Phoebe lanceolata (*Wall. ex Nees) Nees	10	1999
154	189 *Schima wallichii* (DC.) Korth.	1	1999
155	205 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.2	1999
156	187 *Aporosa villosa* (Lindl.) Baill.	0.1	1999
157	7 *Litsea monopetala* (Roxb.) Pers	0 *	1999
158	13 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
159	15 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	1.5	1999
160	11 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.1*	1999
161	67 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.1	1999
162	19 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
163	2 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	0.1	1999
164	5 *Archidendron clypearia* (Jack) Niels. ssp. *clypearia* var. *clypearia*	1	1999
165	68 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
166	56 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.1	1999
167	59 *Litsea monopetala* (Roxb.) Pers	0.2	1999

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
168	63 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.3	1999
169	204 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
170	17 *Schima wallichii* (DC.) Korth.	2	1999
171	66 *Phoebe lanceolata (*Wall. ex Nees) Nees	3	1999
172	8 *Litsea monopetala* (Roxb.) Pers	1	1999
173	54 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
174	10 *Litsea monopetala* (Roxb.) Pers	1	1999
175	14 *Litsea monopetala* (Roxb.) Pers	5	1999
176	62 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.2	1999
177	58 *Litsea monopetala* (Roxb.) Pers	0.3	1999
178	57 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.4	1999
179	51 *Phoebe lanceolata (*Wall. ex Nees) Nees	0.5	1999
180	53 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
181	52 *Phoebe lanceolata (*Wall. ex Nees) Nees	2	1999
182	99-322 *Schima wallichii* (DC.) Korth.	1	1999
183	99-323 *Litsea monopetala* (Roxb.) Pers	1	1999
184	99-324 *Micromelum minutum* (G.Forst.) Wight & Arn.	1	1999
185	99-325 *Litsea monopetala* (Roxb.) Pers	1	1999
186	99-326 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
187	99-327*Litsea monopetala* (Roxb.) Pers	2	1999
188	99-328 *Litsea monopetala* (Roxb.) Pers	2	1999
189	99-329 *Litsea monopetala* (Roxb.) Pers	1	1999
190	99-322 *Schima wallichii* (DC.) Korth.	0.5	1999
191	99-308 *Litsea monopetala* (Roxb.) Pers	0.2	1999
192	99-307 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
193	99-306 *Ficus subulata* Bl. var. *subulata*	1	1999
194	99-305 *Micromelum minutum* (G.Forst.) Wight & Arn.	2	1999
195	99-305 unknown	5	1999
196	99-303 *Litsea monopetala* (Roxb.) Pers	7	1999
197	99-302 *Symplocos sumunita* B.-H. ex D.Don	8	1999
198	99-301 *Rhus rhetsoides* Craib	1	1999
199	99-300 *Rhus rhetsoides* Craib	0.2	1999
200	99-321 *Litsea monopetala* (Roxb.) Pers	0.3	1999
201	99-320 *Glochidion acuminatum* var. *siamense*	0.4	1999
203	99-319 *Machilus bombycina* King ex Hk.f.	1	1999
204	163 *Phoebe lanceolata (*Wall. ex Nees) Nees	1	1999
205	99-317 *Dalbergia cultrata* Grah. ex Bth.	1	1999
206	99-316 *Schima wallichii* (DC.) Korth.	2	1999
207	99-315 *Phoebe lanceolata (*Wall. ex Nees) Nees	2	1999
208	99-314 *Artocarpus lakoocha* Roxb.	2	1999
209	99-313 *Litsea monopetala* (Roxb.) Pers	3	1999
210	99-312 *Litsea monopetala* (Roxb.) Pers	4	1999
211	99-311 *Lithocarpus lindleyanus* (Wall.) A. Camus	1	1999
212	99-310 *Litsea monopetala* (Roxb.) Pers	1	1999
213	F301 *Litsea monopetala* (Roxb.) Pers	2	Forest
214	F334 *Litsea monopetala* (Roxb.) Pers	5	Forest
215	F340 *Rhus rhetsoides* Craib	0.2	Forest
216	F342 *Litsea monopetala* (Roxb.) Pers	0.5	Forest
217	F328 *Litsea monopetala* (Roxb.) Pers	0.3	Forest
219	F327 *Aporusa villosa* (lindl.) Baill.	1	Forest

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
220	F331 *Glochidon kerrii* Craib.	2	Forest
221	F319 *Litsea monopetala* (Roxb.) Pers	1	Forest
222	F330 *Litsea monopetala* (Roxb.) Pers	1	Forest
223	F310 *Litsea monopetala* (Roxb.) Pers	1	Forest
224	F348 *Litsea monopetala* (Roxb.) Pers	2	Forest
225	F303 *Litsea monopetala* (Roxb.) Pers	1	Forest
226	F304 *Albizia garrettii* Niels.	1	Forest
227	F305 *Litsea monopetala* (Roxb.) Pers	3	Forest
228	F306 *Litsea monopetala* (Roxb.) Pers	4	Forest
229	F307 *Litsea monopetala* (Roxb.) Pers	1	Forest
230	F335 *Litsea monopetala* (Roxb.) Pers	1	Forest
231	F345 *Litsea monopetala* (Roxb.) Pers	2	Forest
232	F338 *Prunus cerasoides* D.Don	3	Forest
233	F302 *Litsea monopetala* (Roxb.) Pers	1	Forest
234	F309 *Litsea monopetala* (Roxb.) Pers	5	Forest
235	F336 *Litsea cubeba (*lour.) Pers. var. *cubeba*	0.2	Forest
236	F331*Glochidon kerii*i Craib.	0.5	Forest
237	F332 *Engelhardia spicata* Lechen. Ex Bl. var. *integra* (Kurz) Mann.	1	Forest
238	F337 *Rhus rhetsoides* Craib	1	Forest
239	F333 *Litsea monopetala* (Roxb.) Pers	1	Forest
240	F308 *Litsea monopetala* (Roxb.) Pers	2	Forest
241	F326 *Maesa ramentacea* (Roxb.) A.DC.	2	Forest
242	F324 *Litsea monopetala* (Roxb.) Pers	1	Forest
243	321 *Artocarpus lakoocha* Roxb.	1	Forest

Table 3 Cont.

Number	ID and Plant species	DNA (mg)	Plot
244	346 *Wendlandia tinctoria* (Roxb.) DC. ssp. *floribunda* (Craib) Cowan	1	Forest
245	347 *Litsea monopetala* (Roxb.) Pers.	2	Forest
246	344 *Litsea monopetala* (Roxb.) Pers.	3	Forest
247	350 *Litsea monopetala* (Roxb.) Pers.	10	Forest
248	02-5-34 *Antidesma ghaesembilla* Gaertn.	2	2002
249	02-5-33 *Litsea monopetala* (Roxb.) Pers.	5	2002
250	02-5-29 *Sterculia villosa* Roxb.	1	2002

Note Different numbers of the plant species represent the different individuals.

Figure 1 HAT-RAPD DNA fingerprints using OPN 03 primer

M = 100 basepair ladder

Band 1 = *Markhamia stipulata* var. *kerrii*

Band 2 = *Archidendron clypearia*

Band 3 = Band 4 = *Albizia chinensis*

Band 5 = Band 6 = Band 8 = *Dalbergia cultrata*

Band 7 = Band 9 = Band 10 = *Lithocarpus polystachyus*

Band 11 = *Litsea cubeba*

Band 12 = *Litsea monopetala*

Band 13 = 365 *Antidesma bunius*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 1 that the primer OPN 03 can distinguish *Albizia chinensis* from the other tree species (see Bands 3 and 4), but there was no distinction among the individuals of *Albizia chinensis*.

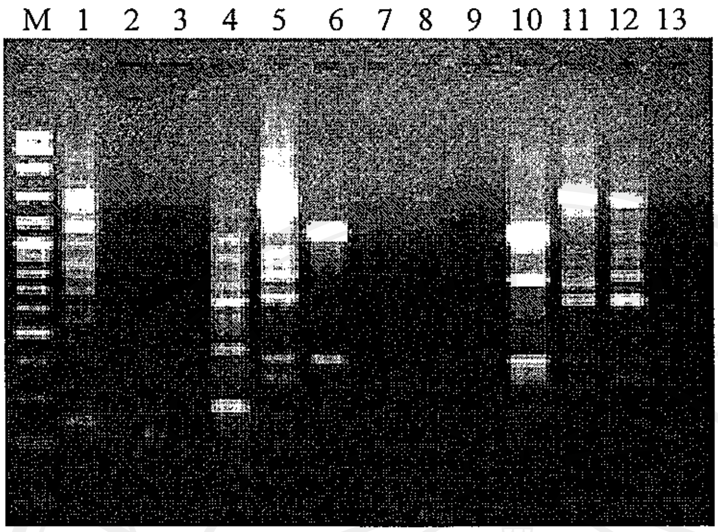

Figure 2 HAT-RAPD DNA fingerprints using OPN 03 primer

M = 100 basepair ladder

Band 1 = *Litsea monopetala*

Band 2 = Band 3 = *Antidesma bunius*

Band 4 = *Alangium kurzii*

Band 5 = Band 11 = Band 12 = *Glocidion acuminatum*

Band 6 = Band 10 = *Engelhardia spicata*

Band 7 = Band 8 = Band 9 = *Sterculia villosa*

Band 13 = *Antidesma ghaesembilla*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 2 that the primer OPN 03 can distinguish *Glocidion acuminatum* from the other tree species (see Bands 5, 11, and 12), but there was no distinction among the individuals of *Glocidion acuminatum*.

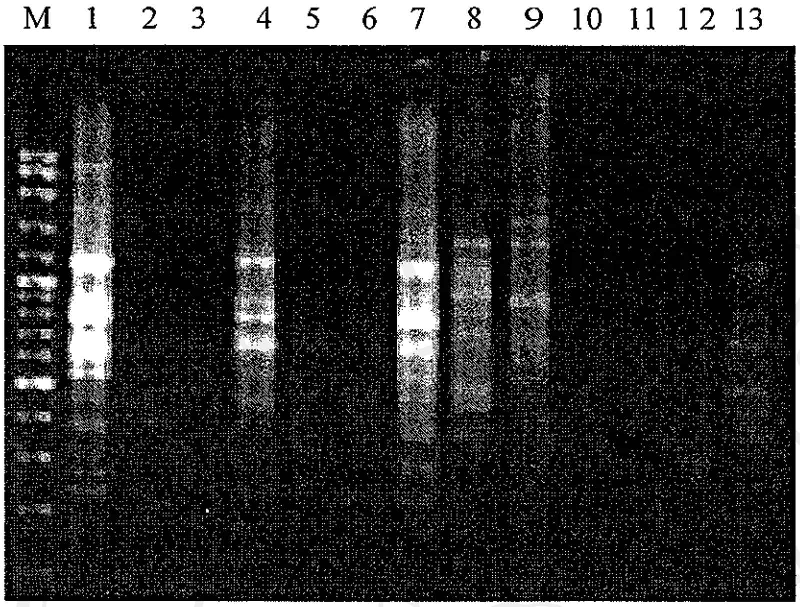

Figure 3 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 basepair ladder

Band 1 = Band 4 = Band 7 = *Phoebe lanceolata*

Band 2 = Band 3 = Band 5 = *Turpinia pomifera*

Band 6 = Band 8 = *Castasnopsis diversifolia*

Band 9 = *Dalbergia stipulata*

Band 10 = *Ficus hirta*

Band 11 = *Wendlandia tinctoria*

Band 12 = *Pterocarpus macrocarpus*

Band 13 = *Markamia stipulata* var. *kerrii*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 3 that the primer OPN 02 can distinguish *Phoebe lanceolata* from the other tree species (see Bands 1, 4, and 7), but there was no distinction among the individuals of *Phoebe lanceolata*.

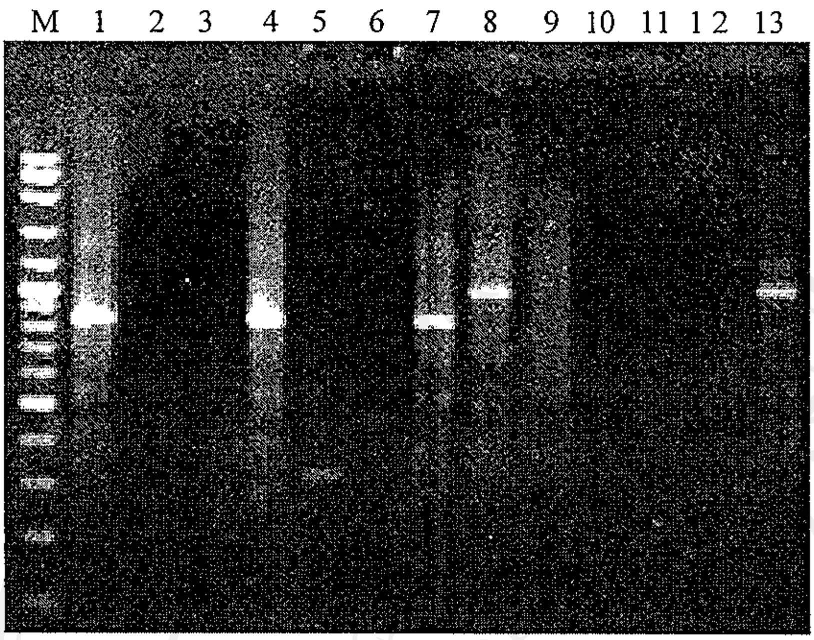

Figure 4 HAT-RAPD DNA fingerprints using OPN 03 primer

M = 100 basepair ladder

Band 1 = Band 4 = Band 7 = *Phoebe lanceolata*

Band 2 = Band 3 = Band 5 = *Turpinia pomifera*

Band 6 = Band 8 = *Castasnopsis diversifolia*

Band 9 = *Dalbergia stipulata*

Band 10 = *Ficus hirta*

Band 11 = *Wendlandia tinctoria*

Band 12 = *Pterocarpus macrocarpus*

Band 13 = *Markamia stipulate* var. *kerrii*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 4 that the primer OPN 03 can distinguish *Phoebe lanceolata* from the other tree species (see Bands 1, 4, and 7), but there was no distinction among the individuals of *Phoebe lanceolata*.

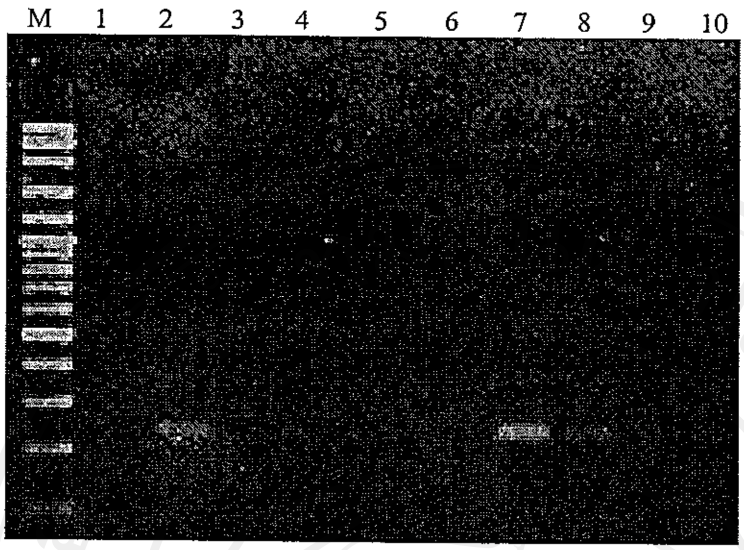

Figure 5 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 basepair ladder

Band 1 = *Wendlendia scabra*

Band 2 = Band 8 = *Castanopsis tribuloides*

Band 3 = *Wendlandia tinctoria*

Band 4 = Band 6 = *Aporusa* sp.1

Band 5 = Band 9 = *Pterocarpus macrocarpus*

Band 7 = *Archidendron clypearia*

Band 10 = *Castanopsis diversifolia*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 5 that the primer OPN 02 can not distinguish the differences among the species.

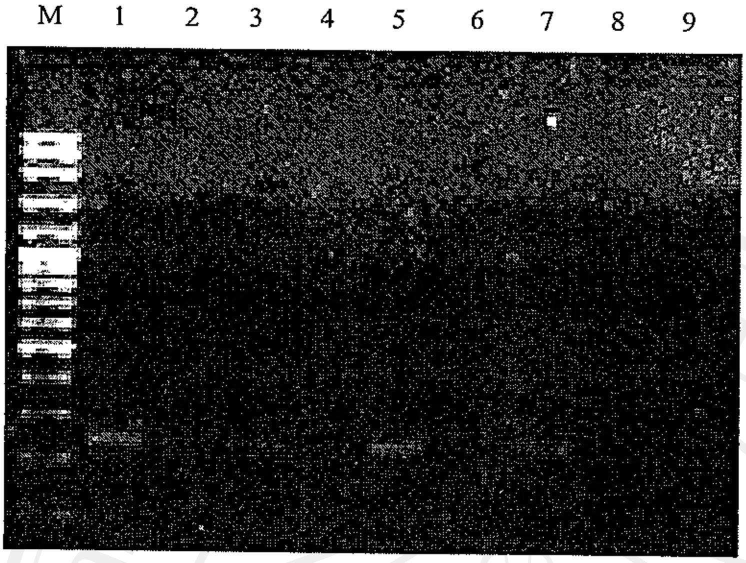

Figure 6 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = *Archidendron clypearia*

Band 2 = *Schima wallichii*

Band 3 = *Sterculia lanceolata*

Band 4 = Band 5 = *Maesa ramentosa*

Band 6 = *Albizia chinensis*

Band 7 = *Litsea monopetala*

Band 8 = *Ficus subincisa*

Band 9 = *Wendlendia scabra*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 6 that the primer OPN 02 can not distinguish the differences among the species.

M 1 2 3 4 5 6 7 8 9 10 11 12

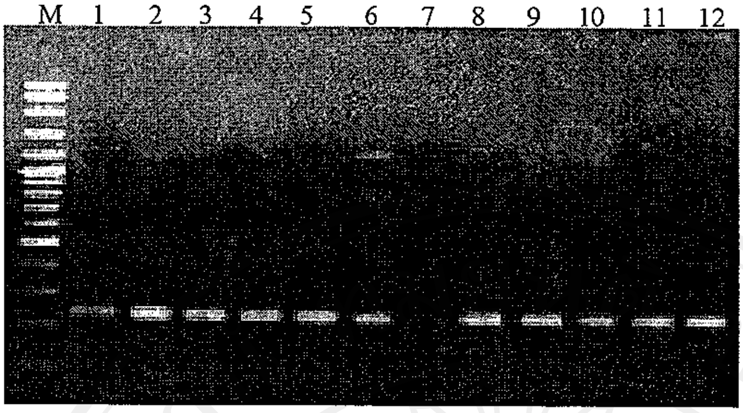

Figure 7 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 3 = Band 4 = Band 5 = Band 6 = Band 7 = Band 10 = *Litsea glutinosa*

Band 2 = Band 8 = Band 9 = Band 12 = *Litsea cubeba*

Band 11 = *Marhamia stipulata* var. *kerrii*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 7 that the primer OPN 02 can not distinguish the differences among the species.

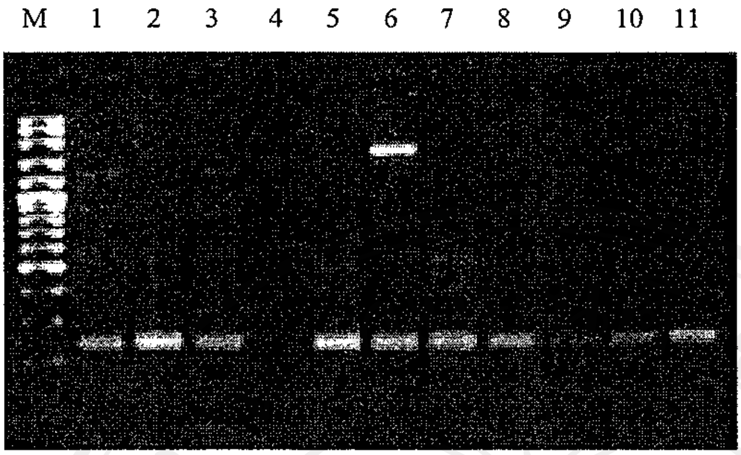

Figure 8 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = Band 3 = Band 8 = Band 9 = *Litsea glutinosa*

Band 4 = Band 7 = Band 11 = *Antidesma ghaesembilla*

Band 5 = *Antidesma bunius*

Band 6 = *Alangium kurzii*

Band 10 = *Litsea cubeba*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 8 that the primer OPN 02 can distinguish *Alangium kurzii* from the other tree species (see Band 6).

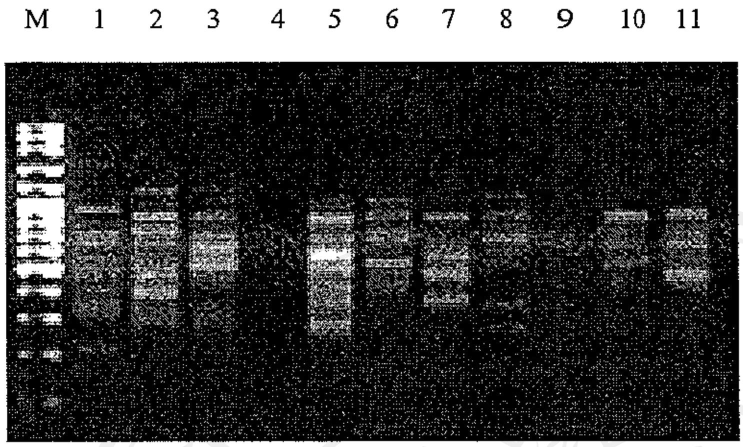

Figure 9 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = Band 3 = Band 8 = Band 9 = *Litsea glutinosa*

Band 4 = Band 7 = Band 11 = *Antidesma ghaesembilla*

Band 5 = *Antidesma bunius*

Band 6 = *Alangium kurzii*

Band 10 = *Litsea cubeba*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 9 that the primer OPN 02 can not distinguish the differences among the species.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

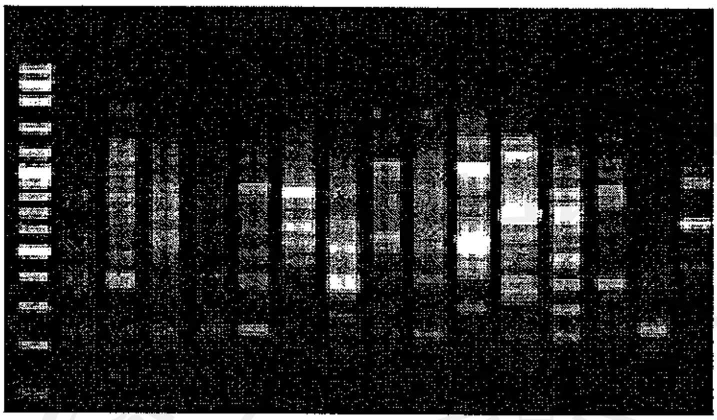

Figure 10 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 4 = *Litsea cubeba*

Band 2 = Band 3 = Band 5 = Band 7 = Band 9 = *Litsea glutinosa*

Band 6 = *Eugelhardia spicata* var. *spicata*

Band 8 = Band 10 = *Marhamia stipulata* var. *kerrii*

Band 11 = *Artocarpus lakoocha*

Band 12 = *Litsea cubeba*

Band 13 = *Ficus hispida* var. *hispida*

Band 14 = *Pterocarpus macrocarpus*

Band 15 = *Ficus hertalayrock*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 10 that the primer OPN 02 can not distinguish the differences among the species.

M 1 2 3 4 5 6 7 8 9 10 11 12

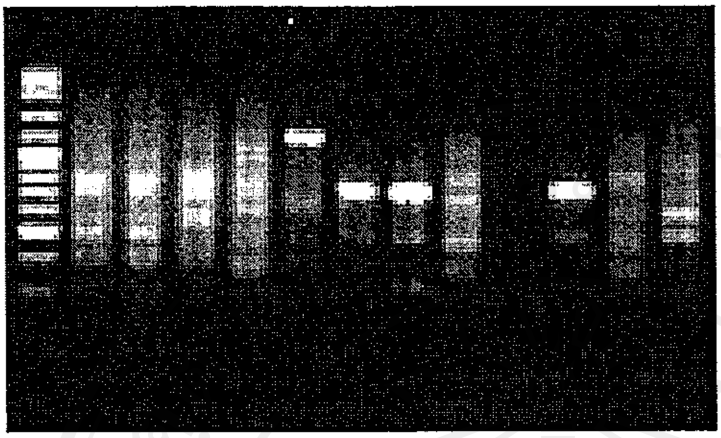

Figure 11 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = *Maesa ramentosa*

Band 3 = *Phoebe lanceolata*

Band 4 = Band 8 = Band 11 = *Litsea glutinosa*

Band 5 = Band 13 = Band 14 = *Micromelum minutum*

Band 6 = Band 7 = Band 10 = Band 12 = *Antidesma ghaesembilla*

Band 9 = *Sterculia villosa*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 11 that the primer OPN 02 can distinguish *Maesa ramentosa* from the other tree species (see Bands 1 and 2), but there was no distinction among the individuals of *Maesa ramentosa*.

Figure 12 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 4 = *Micromelum minutum*

Band 2 = *Rhus rhetsoides*

Band 3 = *Litsea glutinosa*

Band 5 = *Litsea monopetala*

Band 6 = *Artocarpus lakoocha*

Band 7 = งิ้วป่า

Band 8 = *Spondias axillaris*

Band 9 = *Albizia chinensis*

Band 10 = Band 11= Band 12 = Band 13 = Band 14 = Band 15 = Band 16 = *Litsea glutinosa*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 12 that the primer OPN 02 can not distinguish the differences among the species.

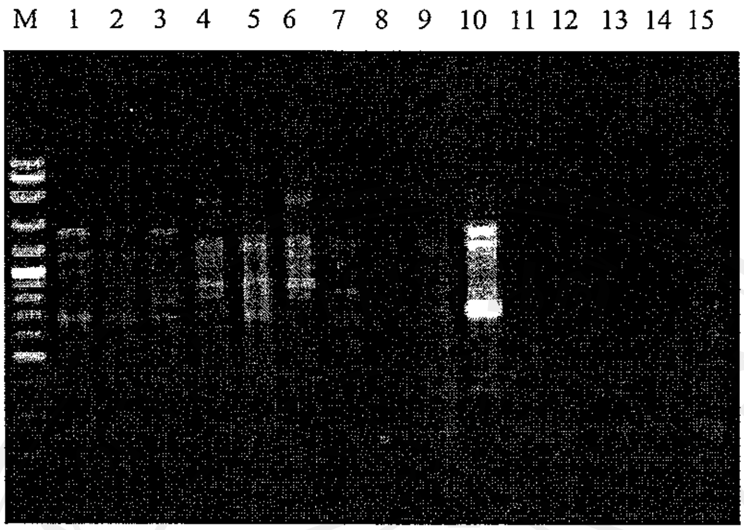

Figure 13 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = Band 3 = Band 8 = Band 9 = *Archidendron clypearia*

Band 4 = Band 6 = *Litsea monopetala*

Band 5 = *Schima wallichii*

Band 7 = Band 10 = Band 11 = Band 12 *Phoebe lanceolata*

Band 13 = Band 14 = Band 15 = *Markhamia stipulata* var. *kerrii*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 13 that the primer OPN 02 can distinguish *Litsea monopetala* from the other tree species (see Bands 4 and 6), but there was no distinction among the individuals of *Litsea monopetala.*

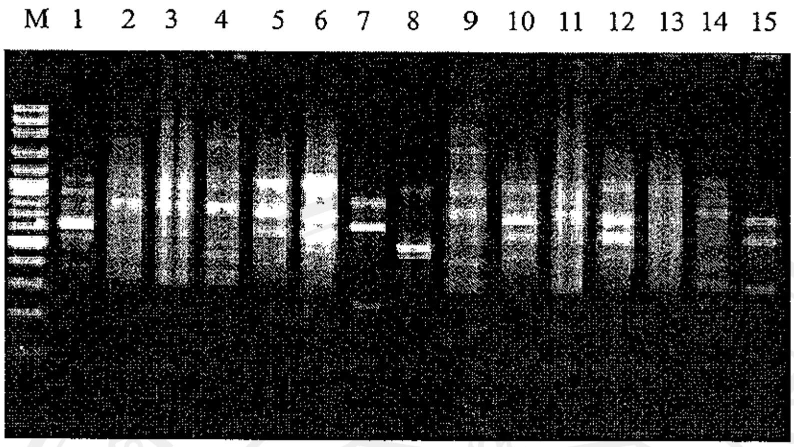

Figure 14 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = *Archidendron clypearia*

Band 2 = Band 3 = Band 4 = Band 9 = Band 11 = Band 14 = *Litsea monopetala*

Band 5 = Band 6 = Band 10 = Band 12 = Band 13 = Band 15 = *Phoebe lanceolata*

Band 7 = *Schima wallichii*

Band 8 = *Markhamia stipulata* var. *kerrii*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 14 that the primer OPN 02 can not distinguish the differences among the species.

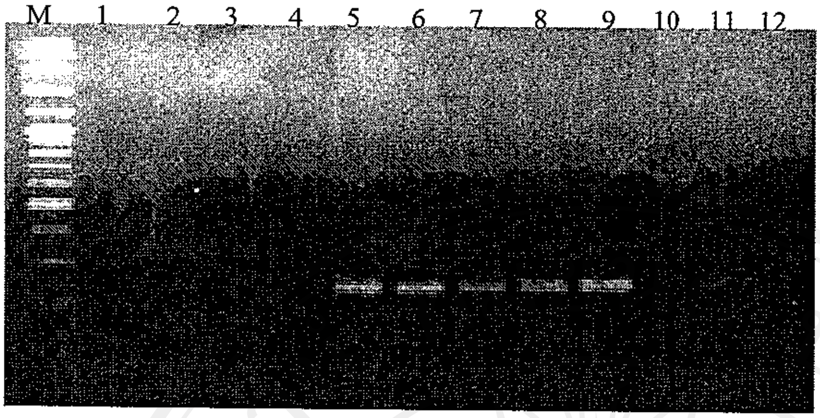

Figure 15 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = Band 5 = Band 6 = Band 7 = Band 8 = Band 9 = Band 10 = Band 11 = *Phoebe lanceolata*

Band 3 = Band 4 = Band 12 *Litsea monopetala*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 15 that the primer OPN 02 can not distinguish the differences among the species.

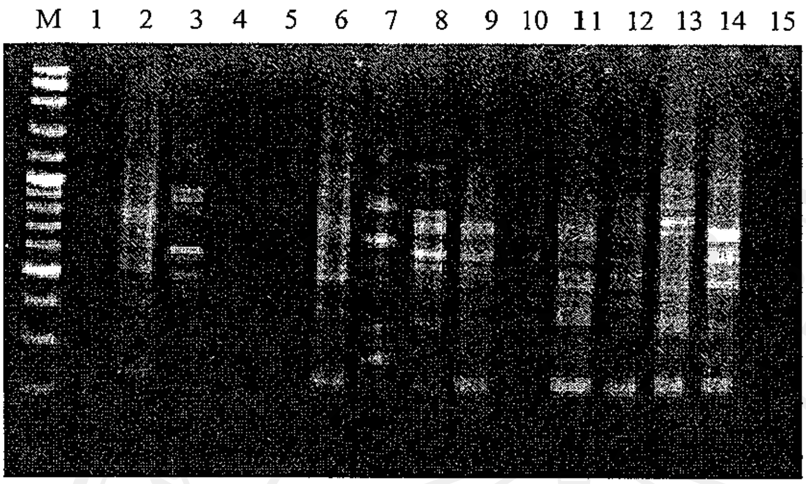

Figure 16 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = Band 2 = Band 11 = Band 14 = *Litsea monopetala*

Band 3 = *Castanopsis acuminatissima*

Band 4 = Band 5 = Band 6 =Band 8 = Band 9 = Band 10 = Band 12 = Band 13 = *Phoebe lanceolata*

Band 7 = *Schima wallichii*

Band 15 = *Antidesma acidum*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 16 that the primer OPN 02 can not distinguish the differences among the species.

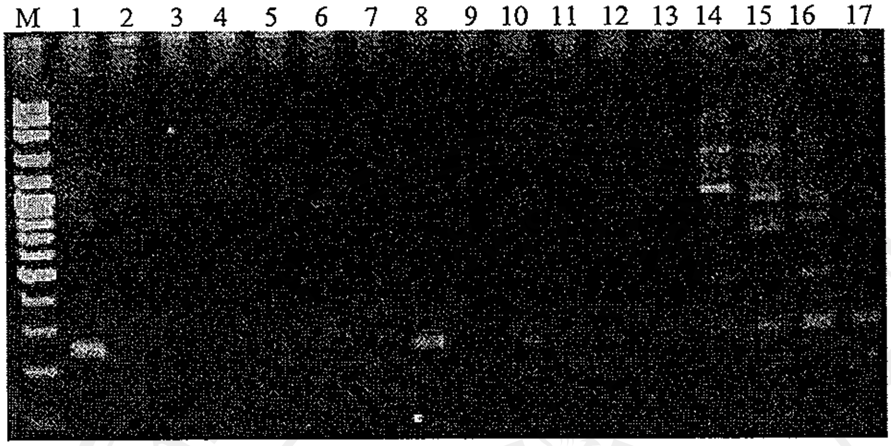

Figure 17 HAT-RAPD DNA fingerprints using OPN 02 primer

M = 100 base pair ladder

Band 1 = *Albizia chinensis*

Band 2 = Band 10 = *Ficus hirta* var. *roxburghii*

Band 3 = *Wendlandia tinctoria*

Band 4 = Band 5 = Band 11 *Schima wallichii*

Band 6 = Band 12 = *Litsea monopetala*

Band 7 = *Sterculia lanceolata*

Band 8 = Band 13 = Band 15 = Band 16 = Band 17 = *Phoebe lanceolata*

Band 9 = *Aporusa octenda*

Band 14 = *Wendlandia tinctoria*

Note Different bands of the same plant species represent the different individuals.

It is shown in Figure 17 that the primer OPN 02 can not distinguish the differences among the species.