



Original Article

## Genetic relationships between 4 *Parkia* spp. and variation in *Parkia speciosa* Hassk. based on random amplified polymorphic DNA (RAPD) markers

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### Abstract

A study was undertaken by RAPD to investigate genetic relationships among the following 4 *Parkia* species, Sator (*Parkia speciosa* Hassk.), Riang (*P. timoriana* Merr.), Khonkhong (*P. leiophylla* Kurz) and Lukding (*P. sumatrana* Miq.) and two plants of *Parkia* spp., namely Tong and Tien were also included. DNA from the leaf samples was isolated using CTAB. Of total 180 primers first screened, 8 primers were chosen to analyse genetic variation of 103 individual plants. A total of 125 amplified fragments was obtained from 8 primers with an average of 15.63 fragments per primer, of which 101 fragments (80.80%) were polymorphic. Some species-specific fragments were also obtained. With *P. speciosa* Hassk., 77 polymorphic fragments (68.75%) were obtained with an average of 14 fragments per primer, but no specific fragment could differentiate Sator khao from Sator dan. Results from the dendrogram revealed 5 groups of *Parkia* with similarity coefficients ranging from 0.437-1.000. Tien was found to be in the same group as Riang while Tong could be separated from other *Parkia* with a close relationship to Khonkhong at a similarity value 0.725.

**Keywords:** *Parkia* spp., RAPD, genetic relationship, similarity coefficient

### 1. Introduction

The genus *Parkia* belongs to the family Leguminosae, sub-family Mimosaceae, and is distributed through both the New and Old World tropical zones. The genus is taxonomically most diverse in the rainforests of the Amazon Basin, but ten species are found in the Indo-Pacific region (Luckow and Hopkins, 1995). In Thailand only four species have been reported: Sator (*P. speciosa* Hassk.), Riang (*P. timoriana* Merr.), Khonkhong (*P. leiophylla* Kurz) and Lukding (*P. sumatrana* Miq.) (Smitinand, 1980). *Parkia* seeds have been eaten raw, fried or pickled in Thailand. Among *Parkia*, Sator (*P. speciosa* Hassk.) is preferred for eating compared to the others species, and is considered to be of high nutritional

value. The protein content is approximately 8-9% fresh weight (Suvachittanont and Pothivakit, 1988). Sator seeds also have a distinctive aroma suggesting the presence of sulphur-containing compounds. The sulphur-containing compounds in the seeds could be cysteine and its derivatives such as glutathione, djenkolic acid and thiazolidine-4-carboxylic acid (Suvachittanont *et al.*, 1996). Based on their pod and eating quality, Sator is divided into 2 types: Sator khao and Sator dan. However, cross pollination among these is widespread and has caused considerable genetic variation (Luckow and Hopkins, 1995), resulting in the current situation where variations in pod, seed size and flavor are found in Sator. Besides 4 species of *Parkia*, 2 other, *Parkia* namely Tong and Tien, were found in Southern Thailand, but there is no information about which of the 2 groups they belonged to. Information on the genetic diversity and relationships among the various *Parkia* spp. is valuable for an improve-

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ment and breeding program.

Morphology is frequently used to characterize different types of crop diversity; however, environmental factors may influence in morphological expression and identical species may have different morphologies. At present, DNA markers have proven to be valuable tools and are widely used for assessment of genetic diversity at the level of species and intra-specific categories. The molecular analysis technique of RAPD has been used for cultivar identification, and to probe genetic relationships and genetic variation in various tree species such as *Lansium* (Nualsri *et al.*, 2001) *Citrus reticulata* Blanco (Toolapong *et al.*, 2003), olives (Belaj *et al.*, 2003a), *Elaeis guineensis* Jacq. (Junmag, 2003), coconuts (Upadhyay *et al.*, 2004), *Calliandra* Benth (Mattagajasingh *et al.*, 2006), cycads (Viljioen and Staden, 2006) and palmyra palm (Promkaew and Nualsri, 2007). The objectives of the present study were i) to evaluate the genetic and relationship among 4 species of *Parkia* include Tong and Tien ii) to analyze the genetic diversity of the single species *P. speciosa* Hassk.

## 2. Materials and Methods

Leaf samples of 103 plants of *Parkia* species, including Tong and Tien were collected from the Trang Horticultural Research Center in Trang province, private plantations in Songkhla, Surat Thani, Patthalung and Loei provinces, and the Sakaerat Environmental Research Center, Nakhon Ratchasima province (Table 1). Some morphological characters of each species were also recorded.

The total genomic DNA of each plant was extracted from young leaves using the cetyltrimethylammonium bromide (CTAB) method introduced by Doyle and Doyle (1990). Random amplified polymorphic DNA (RAPD) analysis was performed according to the methodology of Williams *et al.*, (1990). Each amplification mixture of 25 µl

contained 25 mM MgCl<sub>2</sub>, 10x *Taq* buffer, 100 µM of each dNTP, 0.3 mM of primer, 1.5 units of *Taq* polymerase and 60 ng of template DNA. The thermal profile for RAPD-PCR followed the pattern of 41 cycles of 94°C for 2 min 37°C for 1 min and 72°C for 2 min., followed by 1 cycle of 94°C for 30 sec 35°C for 1 min and finally 72°C for 7 min. The PCR products were then electrophoresed in 1.5% (w/v) agarose gels in 0.5X TBE buffer at 100 V. The gels were stained with ethidium bromide for 15 min and viewed under ultraviolet light with gel documentation. One hundred and eighty primers were first screened with individual of 4 samples of Sator and 2 samples of Rieng. Primers with reproducible patterns and clear cut polymorphisms were chosen to use for analysis of 103 DNA samples. RAPD profiles were manually scored as 1 for presence or 0 for absence of a band. The scores were entered into a database program (NTedit) and compiled in a binary matrix for statistical analysis. Jaccard's (1908) similarity coefficient values for each pairwise comparison between plants were calculated and a similarity coefficient matrix was constructed. The unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis was performed on genetic similarity matrixes, and relationships among species were visualized as a dendrogram using the NTSYS-pc Exeter Software version 2.1 (Rohlf, 2002).

## 3. Results

### 3.1 Morphological characters

Morphological characters of the collected *Parkia* spp. such as leaf and leaflet shape and size, inflorescences, pods and seeds were recorded. On the basis of leaf apex, 2 different forms were observed. The leaf apex of Sator, Lukding, Tong and Tien was rounded while Rieng and Khonkhong had an acutely forward-bent leaf apex. (Figure 1 ai and aii). The leaflet size of Lukding was the largest when compared

Table 1. List of samples with abbreviation and place of collection

Name	Abbreviation	Place of collection	No. of samples
Sator	St	Trang province	11
		Surat Thani province	13
		Songkhla province	14
Riang	Ri	Trang province	2
		Surat Thani province	5
		Songkhla province	5
Khonkhong	Kg	Trang province	4
		Loei province	1
Lukding	Ld	Trang province	5
		Nakhon Ratchasima province	7
Tong	Tg	Trang province	3
		Patthalung province	1
Tien	Ti	Surat Thani province	1
Total			103

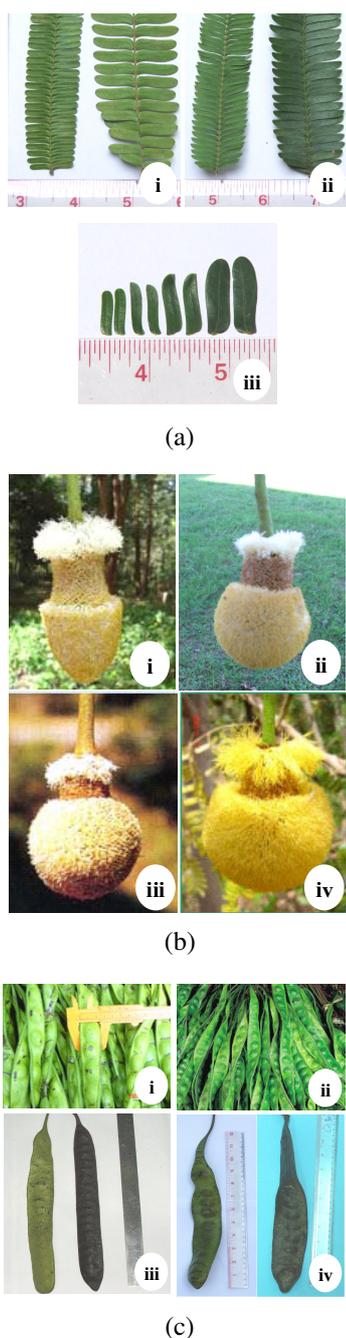


Figure 1. Rounded leaf apex of Sator and Lukding (a.i) and acute-vent forward leaf apex of Rieng and Khonkhong (a.ii). Leaflet size of Sator, Rieng, Khonkhong and Lukding, respectively (a.iii). Inflorescence colors of Sator (b.i), Rieng (b.ii), Khonkhong (b.iii) and Lukding (b.iv). Pod shape and seed arrangement of Lukding (c.i), Sator (c.ii), Rieng (c.iii) and Tong (c.iv).

to Khonkhong, Rieng and Sator (Figure 1 a.iii). The flowers of all *Parkia* species were characterized by a compound inflorescence, consisting of a capitular. It was found that the staminodial flower color and seed arrangement of Lukding were different from there of the other *Parkia* species. The

Lukding staminodial flowers had a dark yellow color while the flower color of the other *Parkia* species was white (Figure 1 b.i-iv). The seed arrangement of Lukding was clearly different from the other species. The seeds of 3 *Parkia* spp., plus Tong and Tien, were transversely arranged while the seed arrangement of Lukding was vertical along the pod (Figure c). The pod shape of Rieng and Tong was straight, strap-shaped and flat, while the other *Parkia* spp. exhibited a slightly twisted strap-shape (Figure 1 c.iii-iv).

### 3.2 RAPD analysis

One hundred and eighty primers were first screened with Sator khao, Sator dan and Rieng, 8 primers were chosen for the study on genetic diversity evaluation in 103 individuals of 4 *Parkia* spp. including Tong and Tien (Table 2). From the 8 primers, a total of 125 fragments were obtained, of which 101 fragments 80.80%, showed polymorphisms. The highest number of polymorphic fragments was found in primer OPT-01 and the lowest in primer OPR-02 and OPB-18. Two DNA fragments, 450 bp amplified by primer OPAB-03 (Figure 2a) and 880 bp of primer OPR-02 (Figure 2b), were found specific to Lukding. A specific fragments was also found in Khonkhong, 425 bp of primer OPR-02 (Figure 2b). Within *P. speciosa* Hassk., 112 fragments were generated by the 8 primers with an average of 14 fragments per primer, of which 77 fragments (68.75%) were polymorphic fragments (Table 2). The results of DNA pattern in *P. speciosa* Hassk. (Sator khao and Sator dan), found that no specific fragment could be used to distinguish between Sator khao and Sator dan. However, 2000 bp and 350 bp fragments generated by primer OPB-17 and OPR-02 were noted to be found in particular samples collected from Songkhla province while 2125 bp and 600 bp fragments generated by primer OPB-17 and OPR-01 were found only in samples from Trang and Surat Thani provinces (data not shown).

### 3.3 Genetic similarity and cluster analysis

From all samples analyzed, the genetic similarity matrix showed an average range from 0.437 to 1.000. Frequency distribution analysis showed that 75% of similarity measures fell between 0.494 and 0.719 (Figure 3). The highest similarity value (1.000) was observed between the Khonkhong samples (85 and 86) and Lukding samples (87-89) and the lowest similarity value (0.437) was obtained between the Sator and Lukding. Within the Sator groups, genetic similarity ranged from 0.533-0.946. Cluster analysis of the genetic similarity values was performed to generate a dendrogram illustrating the overall genetic relationships among the *Parkia* spp. studied. From the dendrogram, all of 103 plants could be separated into five clusters: I Sator, II Rieng and Tien, III Tong, IV Khonkhong and V Lukding (Figure 4). In *P. speciosa* Hassk., the results from a dendrogram generated by 8 primers indicated that Sator khao and Sator dan cannot be clearly separated (Figure 5), as no

Table 2. Primers producing polymorphic DNA bands in RAPD patterns of *Parkia* spp., Tong and Tien.

Primer	Sequence (5' → 3')	Amplified fragments	Monomorphic fragments	Polymorphic fragments
OPB-04	GACTGGAGT	17	1	16
OPB-17	AGGGAACGAG	16	4	12
OPB-18	CCACAGCAGT	13	5	8
OPC-02	GTGAGGCGTC	13	4	9
OPR-01	TGCGGGTCCT	17	2	15
OPR-02	CACAGCTGCC	11	3	8
OPT-01	GGGCCACTCA	21	4	17
OPAB-03	TGGCGCACAC	17	1	16
Total		125	24	101
Polymorphic (%)		-	-	80.80

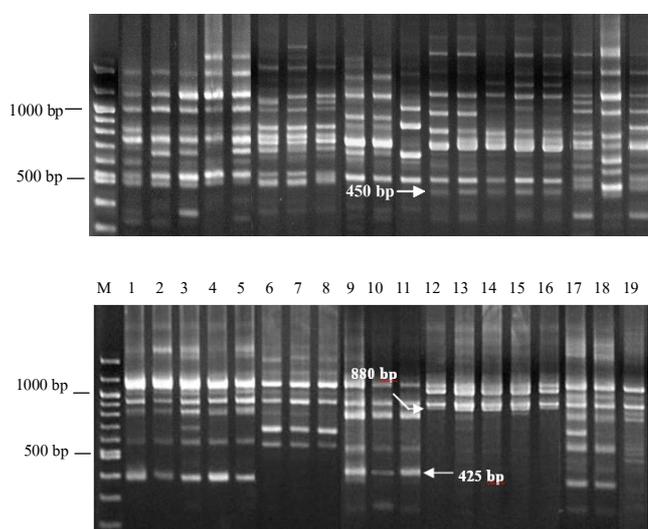
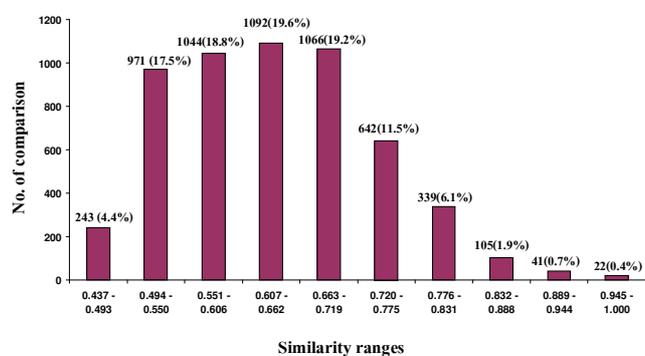
Figure 2. RAPD patterns of four species of *Parkia*, Tong and Tien amplified by primer OPAB-03 (a) and OPR-02 (b). Lane M = 100 bp ladder; 1-5 = Sator; 6-8 = Riang; 9-11 = Khonkhong; 12-16 = Lukding; 17-18 = Tong; and 19 = Tien.Figure 3. Distribution of genetic similarity values obtained from pairwise comparison between 103 plants, 4 species of *Parkia*, Tong and Tien.

Table 3. Primers producing polymorphic DNA bands in RAPD patterns of Sator groups (Sator khao and Sator dan).

Primer	Amplified fragments	Monomorphic fragments	Polymorphic fragments
OPB-04	15	4	11
OPB-17	16	5	11
OPB-18	12	6	6
OPC-02	13	5	8
OPR-01	16	4	12
OPR-02	9	4	5
OPT-01	19	5	14
OPAB-03	12	2	10
Total	112	35	77
Polymorphic (%)	-	-	68.75

specific fragment was found between those two types of Sator.

#### 4. Discussion

Accurate characterization of plants requires an understanding of the degree of polymorphism as revealed by different techniques. On the basis of morphological characters and agronomic traits such as leaf, leaf apex, inflorescence, pod and seed arrangement etc., *Parkia* spp. are taxonomically diverse (Hopkins, 1986). Smitinand (1980) and Suree and Anan (1997) reported that 4 species of *Parkia* were found in Thailand: Sator (*P. speciosa* Hassk.), Riang (*P. timoriana* Merr.), Khonkhong (*P. leiophylla* Kurz) and Lukding (*P. sumatrana* Miq.). There are a few reports of genetic diversity of this genus at DNA level. One such is on the genetic diversity of *P. timoriana* Merr. based on RAPD markers studied by Thangjam *et al.*, (2003) in India.

The objective of the present study was to investigate

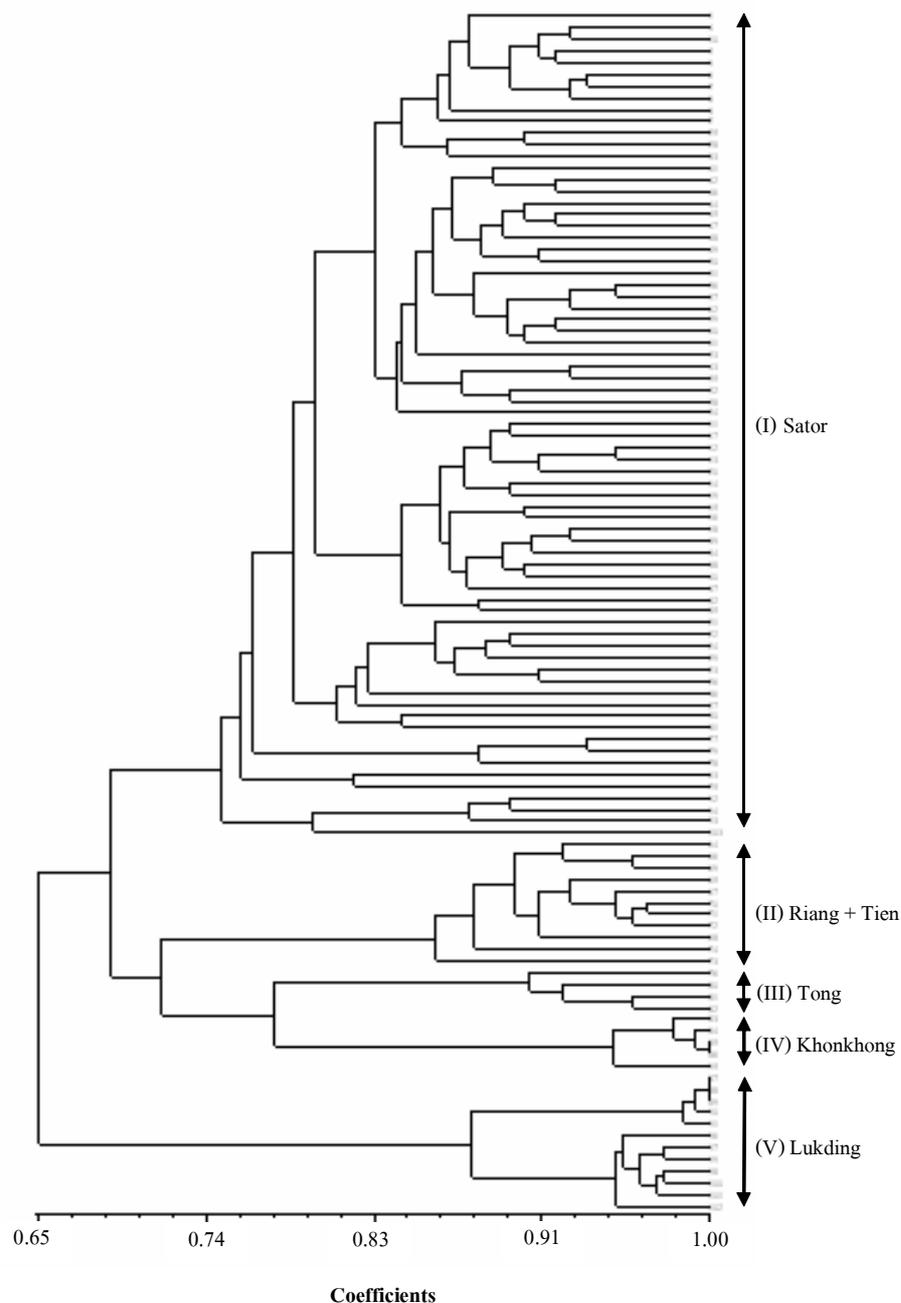


Figure 4. Dendrogram generated by using 101 RAPD markers analysis showing relationship between 103 plants of *Parkia*, Tong and Tien. The scale portrays a similarity index based on Jaccard's coefficient, and the dendrogram was developed using UPGMA clustering procedure.

the relationships between 4 species of *Parkia*, and genetic variation in *P. speciosa* Hassk. specifically using RAPD markers. Genetic discrimination among 103 genotypes of *Parkia* was assessed with 8 RAPD primers to estimate genetic diversity. A high level of polymorphisms was observed in this study. About 80.80% of the polymorphic fragments found in *Parkia* spp. and 68.75% of the polymorphic fragments in *P. speciosa* Hassk. were obtained using the 8 primers. The results indicated a high degree of diversity in *Parkia* spp. Three specific RAPD markers were identified.

The fragments of 880 bp of primer OPR-02 and 450 bp generated by primer OPAB-03 were found only in Lukding. There was one fragment which specific to Khonkhong: 425 bp of primer OPR-02. The knowledge of a specific marker is very useful for certification of plant material as true-to-type (Chowdhury *et al.*, 2002) and can be used for Marker-Assisted Selection (MAS) in a plant breeding program. Among *P. speciosa* Hassk., no specific fragment was found which could differentiate Sator khao from Sator dan. This may be because crossing between these two types is very

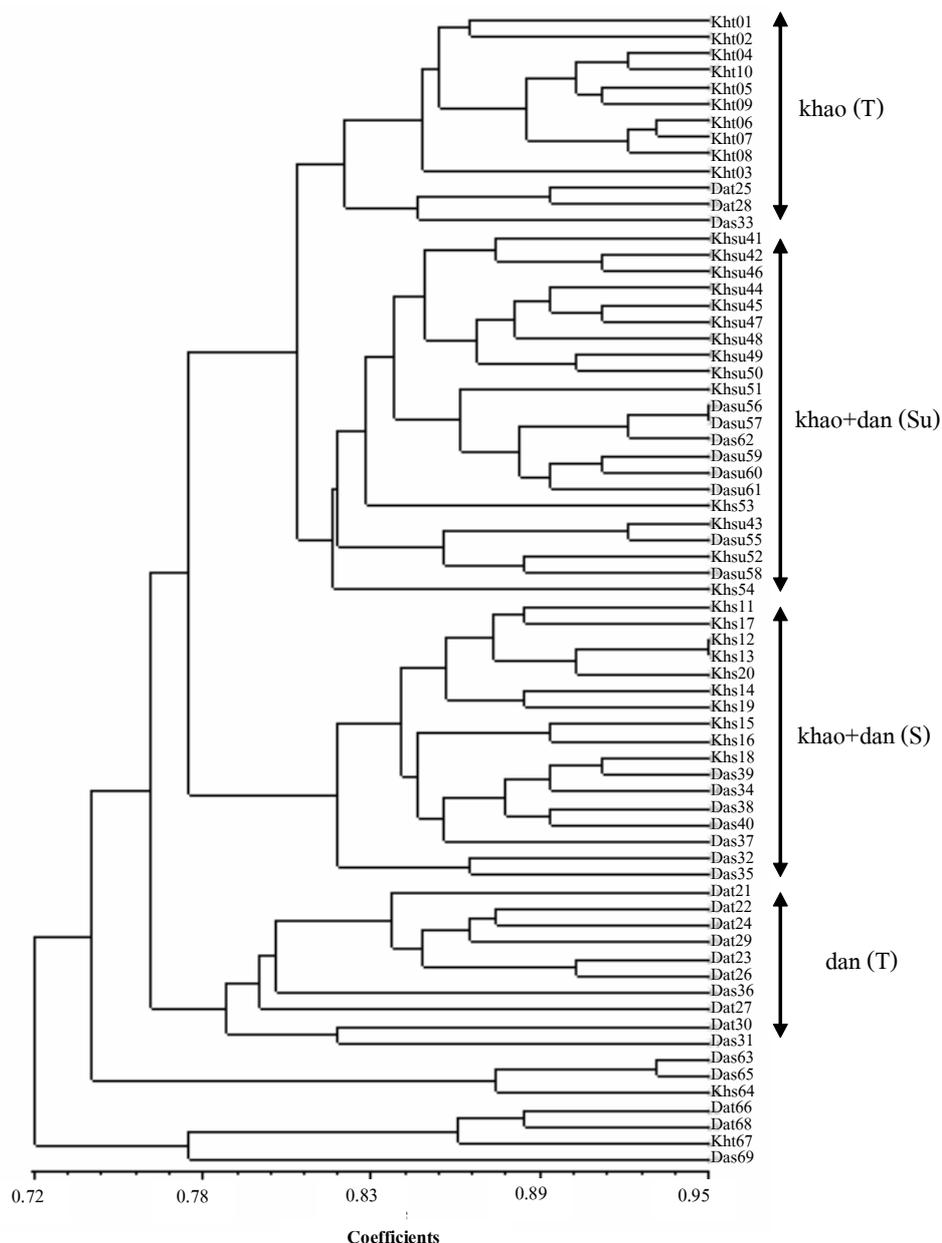


Figure 5. UPGMA dendrogram obtained in different 69 genotypes of *P. speciosa* Hassk. from Trang (T), Songkhla (S) and Surat Thani (Su) provinces based on similarity values.

common, and probably causes heterozygosity in the genetic background, resulting in no specific fragment to be observed. However, some fragments were observed to be associated with the location of sample collection, for example 2000 bp and 325 bp fragments of primer OPB-17 and OPR-02 were only found in *P. speciosa* Hassk. collected from Songkhla province. This finding of location-specific fragments supports the hypothesis of an autochthonal original as well as the limited diffusion of *P. speciosa* Hassk. from its original zone. A similar finding was reported by Belaj *et al.* (2003b), who studied olives. In this study, the similarity coefficients between species varied from 0.437-1.000, indicating a high level of genetic diversity, and approximately 75% of the

populations studied revealed similarity value 0.494-0.719 (Figure 3). Among *P. speciosa* Hassk., the similarity values ranged from 0.533 to 0.946. Identical DNA patterns were found in 1 pair of Lukding and 3 samples of Khonkhong, indicating that each pair derived vegetatively from one original clone (data not shown). Propagation in *Parkia* spp. can be done by seed and top grafting (Bumrungruk, 1994), but currently grafting is preferred because it produces true-to-type.

As shown by the dendrogram in Figure 5, the 103 genotypes found in this study of 4 *Parkia* spp., including Tong and Tien can be clustered into 5 groups, which corresponds well with the morphological identification of the

same species by Smitinand (1980). In the natural environment, the flowers of each *Parkia* spp. bloom at a different period so crossing between species is unusual, and there are no reports of crossing ability among these 4 *Parkia* spp. Tien was found in the same group as Rieng, but unfortunately we had only 1 sample of this plant with no flowers or pods. Leaf morphology shows that Tien has a rounded apex which differentiates it from Rieng, but leaving open the possibility that Tien mutated from Rieng. Tong can be separated into another group somewhere between Rieng and Khonkhong, but closer to Khonkhong with a similarity value 0.775. In *P. speciosa* Hassk., clusters from the dendrogram revealed some mixing between Sator khao and Sator dan, but it was noted that the clusters were related to the area of collection, a finding further supported by the location-specific fragments found in this study. Thangjam *et al.* (2003) also reported that discrimination in 8 genotypes of *P. timoriana* Merr. could be related to collection in different areas.

The results of this study indicate that RAPD can be efficiently used for genetic diversity analysis in *Parkia*. However, several factors may affect the estimates of genetic relationships among plant species such as the number of markers used, distribution of the markers in the genome, the working samples and the nature of the evolutionary mechanisms underlying the variation measured (Chowdhury *et al.*, 2005).

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