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Research article

Assessment of genetic diversity among *Plumeria* spp. using random amplified polymorphic DNA technique

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Abstract

Plumeria (*Plumeria* spp.) is considered as one of the most popular ornamental plants, with many varieties. Therefore, the purpose of this study was to evaluate the genetic diversity of plumeria using random amplified polymorphic DNA (RAPD) markers. Fifty plumeria samples were randomly collected from Dhonburi Rajabhat University, Bangkok, Thailand and the surrounding area. The DNA of these samples was analyzed using 20 RAPD markers. The results showed that only 47 samples could amplify and produce 351 DNA bands. The average percentage polymorphism was 99.58%, the average polymorphic information content was 0.33, the average resolving power was 8.52, the average effective multiplex ratio was 17.45 and the average marker index was 5.80. All these values were highly effective in discriminating among the 47 plumeria samples. Cluster analysis using the unweighted pair-group method with arithmetic average divided the 47 samples into four groups (A–D). Moreover, principle component analysis showed that group A could be separated into three subgroups. From cluster analysis, plumeria could not be divided based on the flower color, petal and leaf shape, while the samples could be separated using the leaf apex. The results showed a high level of genetic variation among these plumeria samples. RAPD markers could be powerful tools for the detection of genetic diversity among *Plumeria* species. The information obtained from this study can be applied to genetic conservation, taxonomic investigation and breeding programs for plumeria in the future.

Introduction

Plumeria (*Plumeria* spp.) commonly known as frangipani and temple tree belongs to the family Apocynaceae and is found in tropical and subtropical areas throughout the world (Koeser et al., 2013). Plumeria is recognized as an excellent flowering ornamental plant in yards and other planned landscapes and is easy to grow in hot and dry areas, with the flowers of most cultivars being highly fragrant and are colorful, with white, red, yellow, pink or multiple colors (Criley, 2005).

Genetic diversity has been evaluated using molecular markers which are considered as useful tools for the identification of plant cultivars; however, not much molecular information used to identify and determine relationships regarding plumeria (Meerow et al., 2006; Zhao et al., 2018). Where there is a lack of genetic information, random markers are suitable to overcome this problem, especially random amplified polymorphic DNA (RAPD) markers since they do not require any specific knowledge of the target DNA sequence as they use only the single primers of the arbitrary nucleotide sequence (Williams et al., 1990). This dominant marker is able to anneal and prime at multiple locations throughout the genome (Kumar and Gurusubramanian, 2011). RAPD markers have been efficiently

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used for genetic diversity study in various plants, including fennel (Choudhary et al., 2018), palm (Santos et al., 2015), soybean (Sharma et al., 2018) and sugarcane (Singh et al., 2017).

There are many different varieties of plumeria in Thailand (Malimart et al., 2013). However, genetic diversity of plumeria has not yet been reported. Therefore, the purpose of this study was to evaluate the genetic diversity of 50 plumeria samples using 20 RAPD markers. The phylogenetic tree, principal component analysis (PCA), polymorphic information content (PIC) value, revolving power (RP), effective multiplex ratio (EMR) and the marker index (MI) were analyzed and discussed. The results obtained from this study can be applied to genetic conservation, taxonomic investigation and breeding programs for plumeria in the future.

Materials and Methods

Plant materials and DNA extraction

Fifty plumeria samples were randomly collected from Dhonburi Rajabhat University, Bangkok, Thailand and the surrounding area. Fresh young leaves from the apex were collected and stored at -20°C. The morphological appearances of each plant were recorded based on the flower color, petals, leaf shape and leaf apex (Table 2). Total genomic DNA from all samples was extracted from leaves using the CTAB isolation protocol from Doyle and Doyle (1987). The quality and quantity of DNA samples were measured using agarose gel electrophoresis and NanoDrop, respectively. DNA samples were diluted to 50 ng/µL for RAPD analysis.

Random amplified polymorphic DNA fingerprinting

Twenty RAPD primers (Table 1) were used to amplify the DNA taken from the 50 plumeria samples using two replications. The PCR reaction was performed in a 10 µL volume (50 ng template DNA, 0.5 µM RAPD primer, 1× PCR buffer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase enzyme) using a Bioer Gene Pro Thermal Cycler (USA). The PCR reaction was carried out to perform PCR amplification under the following conditions: 3 min of pre-denaturation at 94°C, followed by 40 cycles consisting of 45 s at 94°C, 45 s at 35.4–43.6°C (varying with the primers) and 90 s at 72°C and then a final extension at 72°C for 5 min. The PCR products were separated using electrophoresis on 1.5% (w/v) agarose gel in 0.5 Tris/borate/ethylenediaminetetraacetic acid buffer and then visualized using ethidium bromide staining and photographed under ultraviolet light.

Table 1 Twenty RAPD primers, the sequences and information of polymorphism

Primer name	Sequence (5'–3')	T_a (°C)	Total bands	Polymorphic bands	Polymorphism (%)	DNA size (bp)	PIC	RP	EMR	MI
OPA-01	CAGGCCCTTC	35.4	15	15	100.00	380–1,350	0.37	8.56	15.00	5.55
OPA-09	GGGTAACGCC	35.4	19	19	100.00	200–1,350	0.38	11.24	19.00	7.22
OPAC-03	CACTGGCCCA	39.5	17	17	100.00	280–1,500	0.30	7.62	17.00	5.10
OPAC-04	ACGGGACCTG	43.6	12	12	100.00	300–1,700	0.30	5.10	12.00	3.60
OPAC-05	GTTAGTGCGG	39.5	20	20	100.00	200–1,600	0.35	10.00	20.00	7.00
OPAH-01	TCCGCAACCA	35.4	14	14	100.00	250–1,350	0.39	8.19	14.00	5.46
OPAH-02	CACTTCCGCT	35.5	18	18	100.00	100–2,000	0.28	7.79	18.00	5.04
OPAH-03	CTCCCCAGAC	39.5	19	19	100.00	200–1,750	0.37	10.29	19.00	7.03
OPAH-05	TTGCAGGCAG	39.5	12	11	91.67	400–1,800	0.25	4.23	10.08	2.52
OPB-01	GTTTCGCTCC	39.5	21	21	100.00	300–2,500	0.30	9.22	21.00	6.30
OPB-04	GGACTGGAGT	35.4	14	14	100.00	250–1,350	0.30	5.87	14.00	4.20
OPB-05	TGCGCCCTTC	35.5	20	20	100.00	220–1,350	0.39	11.31	20.00	7.80
OPE-03	CCAGATGCAC	39.5	18	18	100.00	220–2,200	0.36	9.54	18.00	6.48
OPE-04	GTGACATGCC	43.6	22	22	100.00	220–1,200	0.31	9.97	22.00	6.82
OPE-05	TCAGGGAGGT	39.5	18	18	100.00	400–2,250	0.30	7.92	18.00	5.40
UPB-483	GCACTAAGAC	35.4	21	21	100.00	280–2,000	0.32	9.04	21.00	6.72
UPB-485	AGAATAGGGC	35.4	17	17	100.00	200–1,500	0.34	7.78	17.00	5.78
UPB-486	CCAGCATCAG	39.5	21	21	100.00	120–1,600	0.30	8.32	21.00	6.30
UPB-489	CGCACGCACA	43.6	17	17	100.00	200–1,200	0.40	11.04	17.00	6.80
UPB-499	GGCCGATGAT	39.5	16	16	100.00	250–1,900	0.31	7.44	16.00	4.96
	Minimum		12	11	91.67	100	0.25	4.23	10.08	2.52
	Maximum		22	22	100.00	2,500	0.40	11.31	22.00	7.80
	Average		17.55	17.5	99.58	-	0.33	8.52	17.45	5.80

PIC = polymorphic information content; (RP) = revolving power; (EMR) = effective multiplex ratio; (MI) = marker index

Table 2 List of 50 plumeria samples, source, flower color, petal and leaf shape

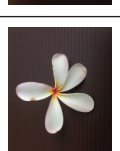
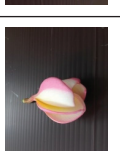
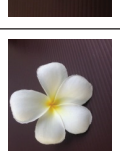
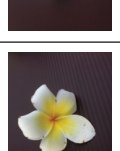
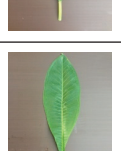
No.	Source	Flower color and petal	Leaf shape and leaf apex	No.	Source	Flower color and petal	Leaf shape and leaf apex
1	Dhonburi Rajabhat University	N/A	Oblanceolate with acute tips 	26	Naval Welfare Department	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 
2	Dhonburi Rajabhat University	N/A	Oblanceolate with acute tips 	27	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 
3	Dhonburi Rajabhat University	**Flower:** white with large yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acuminate tips 	28	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 
4	Wat Mai Phiren	**Flower:** white with large yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Oblanceolate with acuminate tips 	29	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 
5	Wat Mai Phiren	**Flower:** small, red with large yellow **Petal:** narrow petals, pointed tip, no overlap 	Elliptic with acute tips 	30	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 
6	Wat Mai Phiren	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	31	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acute tips 
7	Wat Chinorasaram Worawiharn	**Flower:** small, white with moderate pink and yellow center **Petal:** narrow petals, rounded tip, no overlap 	Oblanceolate with rounded tips, small leaves 	32	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acute tips 
8	Wat Chinorasaram Worawiharn	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	33	Wat Ratchasittharam Ratchaworawihan	**Flower:** brilliant yellow with broad white margin around petal **Petal:** elliptical petals, rounded tip, moderately overlapped 	Oblanceolate with acute tips 
9	Wat Chinorasaram Worawiharn	**Flower:** small, white with large yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Oblanceolate with acuminate tips 	34	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center, pink bands **Petal:** obovate petals, rounded tip, slightly overlapped 	Oblanceolate with acuminate tips 

Table 2 Continued

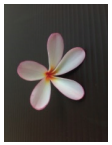
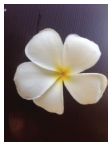
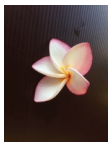
No.	Source	Flower color and petal	Leaf shape and leaf apex	No.	Source	Flower color and petal	Leaf shape and leaf apex
10	Wat Chinorasaram Worawiharn	**Flower:** small, white with moderate pink and yellow center **Petal:** narrow petals, rounded tip, no overlap 	Oblanceolate with rounded tips, small leaves 	35	Wat Ratchasittharam Ratchaworawihan	**Flower:** small, white with large yellow center, pink bands **Petal:** elliptical petals, rounded tip, moderately overlapped 	Oblanceolate with acuminate tips 
11	Wat Khrut	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	36	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with large yellow center **Petal:** elliptical petals, rounded tip, moderately overlapped 	Elliptic with acuminate tips 
12	Wat Khrut	**Flower:** small, white with large yellow center **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 	37	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with large yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 
13	Wat Dong Munlek	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	38	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with large yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 
14	Wat Dong Munlek	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	39	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with reddish-yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 
15	Wat Dong Munlek	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	40	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with reddish-yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 
16	Wat Dong Munlek	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	41	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with large yellow center **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acute tips 
17	Wat Dong Munlek	**Flower:** white with yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 	42	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with large yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 

Table 2 Continued

No.	Source	Flower color and petal	Leaf shape and leaf apex	No.	Source	Flower color and petal	Leaf shape and leaf apex
18	Wat Dong Munlek	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	43	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center, pink bands **Petal:** elliptical petals, pointed tip, slightly overlapped 	Oblanceolate with acuminate tips 
19	Wat Dong Munlek	**Flower: pink** with yellow center **Petal:** elliptical petals, pointed tip, moderately overlapped 	Obovate with acuminate tips 	44	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 
20	Wat Dong Munlek	**Flower:** white with yellow center, deep pink bands **Petal:** elliptical petals, pointed tip, moderately overlapped 	Oblanceolate with acuminate tips 	45	Wat Ratchasittharam Ratchaworawihan	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 
21	Wat Dong Munlek	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 	46	Prasat Neurological Institute	**Flower:** white with yellow center **Petal:** obovate petals, rounded tip, no overlap 	Oblanceolate with rounded tips 
22	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	47	Prasat Neurological Institute	**Flower:** white with large yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Oblanceolate with acuminate tips 
23	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	48	Prasat Neurological Institute	**Flower:** white with yellow center, pink bands **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acuminate tips 
24	Naval Welfare Department	**Flower:** white with large yellow center **Petal:** elliptical and twisted petals, pointed tip, moderately overlapped 	Elliptic with acuminate tips 	49	Prasat Neurological Institute	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Oblanceolate with acuminate tips 
25	Naval Welfare Department	**Flower:** red with yellow center **Petal:** elliptical petals, pointed tip, slightly overlapped 	Elliptic with acute tips 	50	Prasat Neurological Institute	**Flower:** white with large yellow center **Petal:** elliptical petals, rounded tip, slightly overlapped 	Oblanceolate with acuminate tips 

Data analysis

The DNA banding patterns were scored and converted in terms of a binary code as present (1) or absent (0). The data were investigated for genetic similarity among plumeria samples using the Jaccard genetic similarity coefficient and the dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA). The reliability of the nodes was investigated using bootstrap analysis of 10,000 pseudo-samples. All these analyses were completed using FreeTree (Hampl et al., 2001). Principal component analysis (PCA) was undertaken using the Numerical Taxonomy System software, Version 2.1 (Rohlf, 2000). The discriminatory power, efficiency and utilization of RAPD markers were quantified using the following values. The polymorphic information content (PIC) value of each RAPD marker was calculated using the formula: $PIC_i = 2f_i(1 - f_i)$ described by Roláng-Ruiz et al. (2000), where PIC_i is the polymorphism information content of primer i calculated using the average PIC value from all loci of primer i and PIC values range from 0 to 0.5; f_i is the frequency of the present marker bands; and $1 - f_i$ is the frequency of the absent marker bands. The resolving power (RP) of each RAPD marker was calculated using the formula: $RP = \sum I_b$ described by Prevost and Wilkinson (1999), where I_b is the fragment informativeness which was calculated using the formula: $I_b = 1 - [2 \times |0.5 - p|]$, where p is the proportion of accession containing the fragment. The effective multiplex ratio (EMR) was calculated using the formula: EMR = np(np/n) described by Chesnokov and Artemyeva (2015), where np is the number of polymorphic loci and n is the total loci number. The marker index (MI) was calculated using the formula: MI = PIC × EMR described by Chesnokov and Artemyeva (2015).

Results

DNA polymorphism

The 20 RAPD primers were screened to evaluate the genetic diversity of the 50 plumeria samples. Of the 50 samples, only 47 could be amplified. In total, 351 bands were produced and varied from 12 bands per primer (OPAC-04 and OPAH-05) to 22 bands per primer (OPE-04) with an average of 17.55 bands per primer. The polymorphic bands varied from 11 (OPAH-05) to 22 (OPE-04) with an average of 17.5 bands per primer. The percentage polymorphism of each primer ranged from 91.67% to 100% with all primers having 100% polymorphism except for OPAH-05 (91.67%). The fragment size ranged from 100 bp to 2,500 bp (Table 1).

The discriminatory power, efficiency and utilization of RAPD markers were quantified using the PIC, RP, EMR and MI. From Table 1, PIC values ranged from 0.25 (OPAH-05) to 0.40 (UPB-489) with a mean of 0.33. The RP is a parameter characterizing the ability to distinguish genotypes and in the current study it ranged between 4.23 (OPAH-05) and 11.31 (OPB-05) with an average of 8.52. Furthermore, the EMR which is the polymorphic markers generated per assay showed the efficiency of the marker and this varied from 10.08 (OPAH-05) to 22.00 (OPE-04) with an average of 17.45. Lastly, MI, a parameter used to estimate the utility of the marker system, was lowest with OPAH-05 (2.52) and highest with OPB-05 (7.80) with a mean of 5.80.

Genetic diversity and clustering

The genetic diversity of the 47 plumeria samples was described using the UPGMA method and a phylogenetic tree showed the best discrimination among the 47 samples which could be separated into four groups (Fig. 1 and Fig. 2): Group A contained 16 samples, group B contained eight samples, group C contained five samples and group D contained 18 samples. Most plumeria samples in group A were collected from the Naval Welfare Department and Wat Ratchasittharam Ratchaworawihan. Moreover, almost all the plumeria (eight from nine samples) with a white flower with a yellow center and being an oblanceolate shape with a rounded apex were grouped into group D. Considering the leaf apex, almost all the plumeria from groups A, B and C had an acuminate tip while almost all the plumeria from group D had a rounded tip. However, it was not possible to differentiate between any of the plumeria based on flower color, petals and leaf shape. In addition, the results from PCA indicated that group A could be also divided into three subgroups (A-I, A-II and A-III) corresponding to the phylogenetic tree (Fig. 1–3) while PCA could not separate groups B, C and D from each other (Fig. 3).

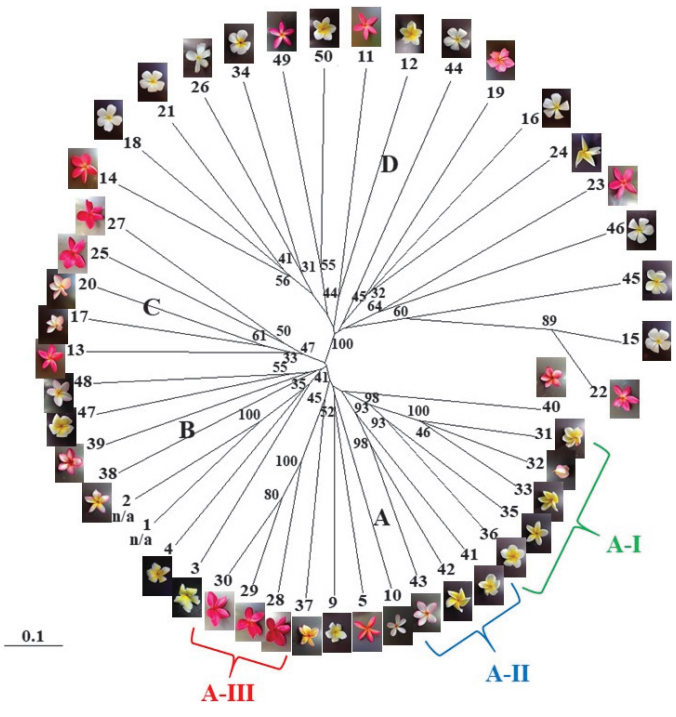


Fig. 1 Phylogenetic tree of 47 plumeria samples, where numbers at nodes are bootstrap values (30% and higher are shown) obtained from 10,000 replications and flower images show flower color and petals of each sample.

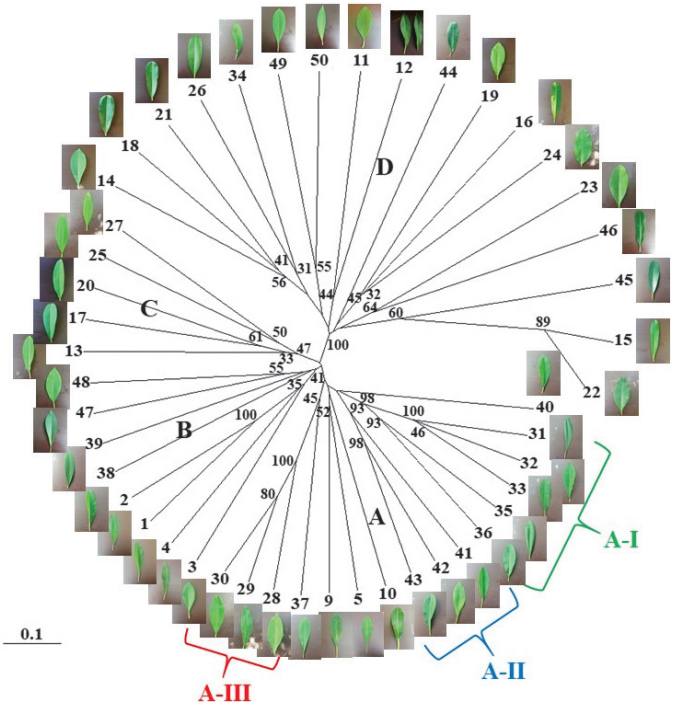


Fig. 2 Phylogenetic tree of 47 plumeria samples, where numbers at nodes are bootstrap values (30% and higher are shown) obtained from 10,000 replications and leaf images show leaf shape of each sample.

Discussion

Genetic diversity data of plumeria is a necessary component for breeders to help in the selection of the parental line in a breeding program and also can be applied to genetic conservation and taxonomic investigation in this plant in the future. In Thailand in particular, there has not been any genetic diversity study of plumeria using molecular markers. Thus, the results from the current study provided important data for genetic diversity assessment of *Plumeria* spp. in Thailand. The present results demonstrated that the selected markers were effective, discriminating and useful for analyzing these plumeria samples, as shown by the PIC, RP, EMR and MI values. The average PIC was 0.33, whereas the highest value in dominant markers is 0.5. Thus, the average PIC obtained could be considered as providing high discriminating power. This was consistent with Innark et al. (2014) who evaluated the genetic diversity of cucumber germplasm using inter simple sequence repeat markers. Their results had a mean PIC value of 0.25 which indicated a high capacity to characterize the cucumber germplasm. Similar results were also reported by Basyuni et al. (2018) who observed the highest PIC score of 0.49 with a mean of 0.33, which confirmed the utility of RAPD markers to assess the genetic diversity in oil palm. In addition to the PIC, the RP has the ability based on primers to discriminate among large numbers of genotypes, as reported by Prevost and Wilkinson (1999). In the current study, OPB-05 had the highest RP value (11.31) and a high PIC value (0.39), suggesting that this marker was the best marker for distinguishing

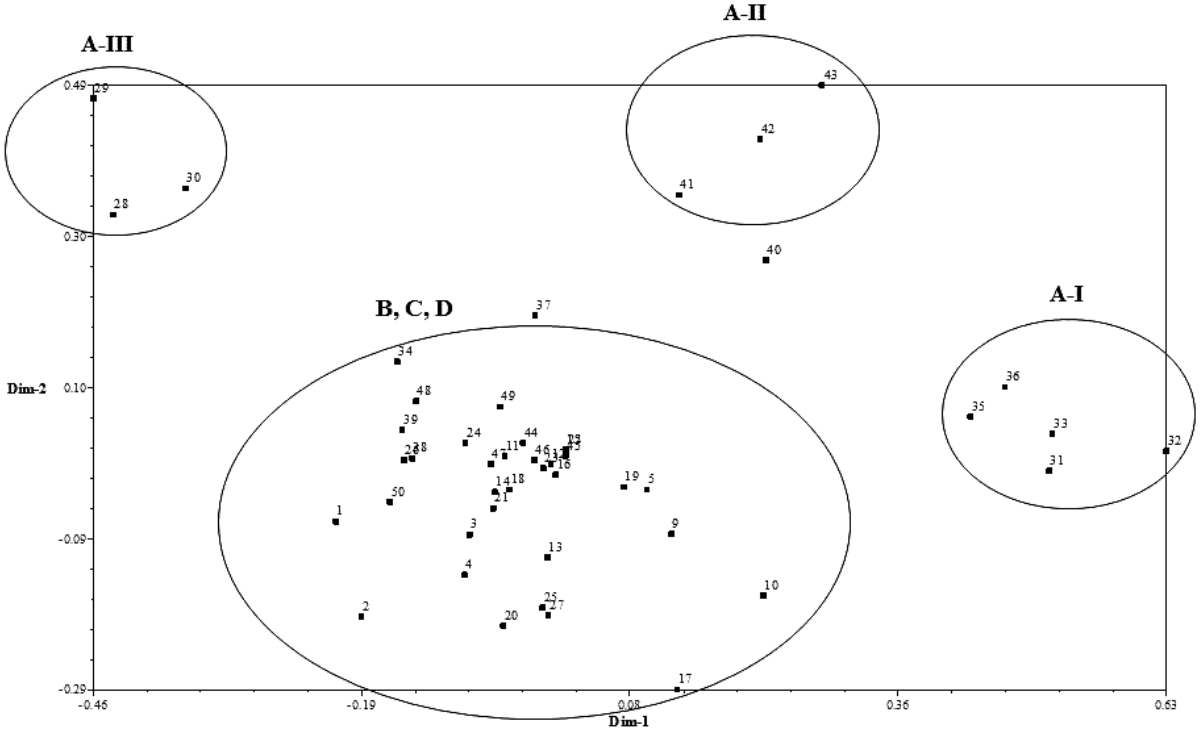


Fig. 3 Principal component analysis of 47 plumeria samples

among the different plumeria samples in this study. Moreover, EMR and MI values were used to estimate the efficiency and utility of the marker system, respectively. The higher the EMR value, the more efficient the marker system is and in the current study the EMR and MI were correlated. A higher EMR provided a higher MI because the MI is the product of the PIC and EMR which corresponded with the report by Choudhary et al. (2018). Most of primers (19 from 20) in the current study had 100% polymorphism which indicated that the selected RAPD primers were suitable to access plumeria genetic diversity. With the dominant marker, the RAPD primer can randomly bind to many different loci in the plumeria genome, though three plumeria samples (numbers 6, 7 and 8) could not amplify with any of the 20 RAPD primers. It is suggested that these three DNA samples should be analyzed with other dominant markers for confirmation of the DNA quality.

The genetic diversity and cluster analysis resulted in the dendrogram dividing the samples into four groups which did not correspond to the flower color, petals and leaf shape.It is common that information from neutral markers which mostly locate in the non-coding regions of genomes does not correlate with phenotypes. For example, Tongsom et al. (2015) reported that the genetic analysis of *Vanda* section Ascocentrum based on nucleotide sequences of the *rbc*L gene and the *trn*H-*psb*A intergenic spacer region did not conform to the morphology and taxonomy. On the contrary, the leaf apex differed with most of the samples in groups A, B and C having an acuminate tip while most of samples in group D had a rounded tip. Moreover, the bootstrap value which separated groups A, B and C from group D was 100 confirmed the separation based on leaf apex.

The current investigation showed that although samples were collected from neighboring locations, there was nonetheless high genetic diversity. One explanation for this is that young plants were brought from many sources to plant in the same location. Interestingly, almost all the plumeria (eight from nine samples) that had a white flower with a yellow center and oblanceolate leaves with a rounded apex were clustered only into group D. Thus, the amplified fragments may relate with the genes that are involved in differentiation between varieties. It is suggested that the specific markers be investigated for separating samples or for the further division of samples based on morphological appearances.

In the current study, the genetic diversity of plumeria samples was assessed using RAPD markers. The DNA polymorphism and cluster analyses indicated high genetic diversity in these samples. The results confirmed that RAPD markers could be powerful tools for the detection of genetic diversity among *Plumeria* species. The information obtained from this study can be applied to genetic conservation, taxonomic investigation and breeding programs for plumeria in the future.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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