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Original Article

Analysis of genetic diversity evaluation of *Lysiphyllum strychnifolium* (Craib) A. Schmitz in Thailand using amplified fragment length polymorphism markers

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ABSTRACT

Lysiphyllum strychnifolium (Craib) A. Schmitz, a Leguminosae-Caesalpiniodeae species, is colloquially known in Thai as Ya Nang Daeng. In Thai traditional medicine, the leaves, stems and roots have been used to relieve fever and alcohol toxication. The stem has also been used for anti-HIV-1 integrase, anti-allergy and anti-cancer treatment. Confusion over herbal drugs has arisen from the homonymity and morphological characteristics, which present many similarities within its family. Thus, germplasm collection and genetic diversity analysis are prerequisites for a breeding program. In this study, the amplified fragment length polymorphism (AFLP) marker was used to identify and elucidate the phylogenetic relationships among 98 accessions of Ya Nang Daeng throughout Thailand. Thirteen AFLP primer combinations generated 379 bands, of which 359 were polymorphic. The value of the similarity coefficient varied from 0.52 to 0.93, with a mean of 0.73. A phylogenetic tree was constructed using the unweighted major clusters at a cut-off genetic mean (UPGMA) clustering method. The phylogenetic tree derived from the AFLP data showed that the Ya Nang Daeng accessions could be divided into two clusters. Cluster 1 could be further classified into five subgroups, while a similarity coefficient of 0.23 could be used to separate an outgroup from Ya Nang Daeng. The result of principal coordinate analysis and morphological grouping corresponded to the UPGMA clustering. This study showed that the AFLP technique is a useful tool for estimating DNA polymorphism of the genetic diversity and differentiation of related plants. This genetic information will provide useful information for a breeding program in the future.

Introduction

Lysiphyllum strychnifolium (Craib) A. Schmitz (synonym *Bauhinia strychnifolia* Craib. *L. strychnifolium*) is a woody climbing plant which has the vernacular names Ya Nang Daeng or Kha Yan. It is belongs to the Caesalpinaceae family, an endemic species to Thailand and belongs to the Caesalpinaceae family. Furthermore, it is distributed especially in the dry deciduous dipterocarp forest in northern Thailand. In addition to the species, there is variation in the foliage morphological characteristics of an intraspecific population (Larsen et al., 1984). It has been used as a traditional medicinal plant that serves to relieve fever, act as an anti-allergic agent and to mitigate the effects of alcohol intoxication (Wutthithammavet, 1997). It has a rather high percentage of phenol which has an anti-oxidant effect in regard to internal organs (Sayompark et al., 2012). Flavanonol could suppress growth of cancer cells (Yuenyongsawad et al., 2013). Isolated quercetin from the stem also could be applied as an anti HIV-1 integrase and has anti-allergy properties (Bunluepuech and Tewtrakul, 2011; Bunluepuech et al., 2013). Because this species has several vernacular names and natural variation in phytochemical portions and substances, there is some risk that some extracts may have no or even adverse medical benefit, presenting confusion in its utilization (Inthusaitrakul, 2009).

Morphological study on *L. strychnifolium* diversity is quite complicated, because of its narrow distribution, the similarity of characters, the long vegetative growth phase and the difficulty in flowering (Larsen et al., 1984). Nowadays, molecular markers have been broadly used and applied for plant classification. This methodology is accurate and precise for genetic diversity evaluation. The amplified fragment length polymorphism (AFLP) marker is regarded as high quality and was developed by Vos et al. (1995). AFLP can

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identify and evaluate a multi-locus without DNA sequencing information. For this reason, it has been ideal for use in the genetic evaluation of several plants including: velvet bean (*Mucana* sp.) by Capo-chichi et al. (2001), *Oxytopis compestris* (Schönswetter et al., 2004), *Gardenia jasminodes* (Han et al., 2007), *Punica granatum* (Moslemi et al., 2010) and *Ocimum* spp. (Moghaddam et al., 2011). However, it has been used only to study plants in the same family with no study focusing on the same genus.

Apart from above, the database of genetic diversity of *L. strychnifolium* has not been concluded with certainty. Consequently, this research studied genetic diversity evaluation and intraspecific classification of the species based on a comparison of morphological characteristics and AFLP markers for improvement and conservation of its germplasm.

Materials and methods

Plant material and genomic DNA extraction

In total, 98 accessions (Table 1) were collected from both natural habitats and cultivated sites in 47 provinces of Thailand. All accessions were grown in the field in the Department of Agronomy, Kasetsart University, Bangkok, Thailand. Genomic DNA was extracted from leaf tissue according to the FTA® Plantsaver cards. A disc from the dried FTA tissue print was removed using a clean Haris® micro punch. The disc was washed twice in 80 μL of FTA purification reagent, incubated for five minutes for each wash, followed by repeated twice washing with 180 μL of $T_1E_{0.1}$ buffer incubating for two minutes for each wash. The disc to dry for one hour at room temperature. After drying the disc was transferred to a polymerase chain reaction (PCR) tube for AFLP analysis.

AFLP analysis

AFLP analysis was performed based on the method described by Vos et al. (1995) and Kladmook et al. (2010). Genomic DNA on an FTA disc was digested and ligated overnight at 37 °C with the *Eco*RI and *Mse*I in a final volume of 25 μL containing digestion and ligation solution (1 unit of T4 DNA ligase, 0.25 unit of *Eco*RI and *Mse*I, 10 pmol of *Eco*RI and *Mse*I adapter, 2.5 μL 10× fast reaction buffer, 2.5 μL 10× T4 DNA ligase buffer). For pre-selective amplification, 5 μL of digestion/ligation mixture amplified in 25 μL of PCR reaction containing 2.5 μL 10× buffer for PCR, 5pM of *Eco*RI and *Mse*I adapter-directed primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 1 unit of *Taq* DNA polymerase. PCR reactions were performed with the following profile: 30 cycles of 94 °C for 30 s, 56 °C for 60 s and 72 °C for 60 s. It was then checked for the presence of a smear of fragment (100–1000 bp in length) using agarose electrophoresis. Selective amplification (second PCR) of the diluted pre-selective amplification products was carried out using 13 primer combinations (Table 2). Selective PCR reactions were performed with the following profile: 1 cycle of 94 °C for 30 s, 65 °C for 30 s and 72 for 60 s, then 12 cycles of 94 °C of 30 s, 65 °C for 30 s (with decreasing ramp of 0.7 °C each cycle) and 72 °C for 60 s followed by 23 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 60 s. The second PCR products were run on a 6% denaturing polyacrylamide gels in 1× TBE buffer. Gels were run at 300 V for about 2.5 h. The AFLP fragments were detected using silver staining.

Data analysis

Band profiles generated using AFLP were scored and converted into binary matrices on the basis of the presence (1) or absence (0) of selected band. NTSYS-pc version 2.20k (Rohlf, 2005) was used to analyze the binary matrix. The polymorphism information content (PICs) was calculated as: $PICs = 1 - p^2 - q^2$, where p = band frequency and q = no-band frequency (Ghislain et al., 1999; Alem et al., 2011). A calculation of similarity was performed using Jaccard's similarity index (JSI) (Jaccard, 1908). These similarity coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) (Sneath and Sokal, 1973). Then the principal coordinate analysis (PCoA) was used to analyze the partitioning of genetic variance among different populations. The morphological characteristic of all accessions was observed in terms of planar shapes, apex shapes, base shape (Simpson, 2006), petiole color and branch color (RHS color chart).

Results

A collection of accessions was harvested through every region of Thailand. Ya Nang Daeng could be found only in 13 of the 77 provinces of Thailand, with 98 accessions. These were divided into two main groups, with the main group 1 was a natural habitat distributed only in four provinces and contained 77 accessions. The other main group 2 was cultivated, consisting of 20 accessions. In addition, *Cayratia* sp. was found which has the same vernacular name as *L. strychnifolium*, so it was grouped as an outgroup. All accessions were cultured and grown under the same conditions and then their morphology was studied.

The 64 selective recombined primers between *Eco*RI primer+3 (E-ANN) and *Mse*I primer+3 (M-CNN) showed only 13 primers having several DNA bands which were clear and discriminative to accession. Overall there were 359 (94.72%) DNA bands that were polymorphic, containing an average of 27.12 polymorphic bands per recombined primer, the values of PICs ranged from 0.154 to 0.377 with a mean of 0.253 and similarity values ranged from is 0.38–0.97 with a mean of 0.69. The dendrogram was designed using NTYSYS version 2.20k with the UPGMA methodology (Fig. 1). The credibility of dendrogram was assayed using cophenetic correlation coefficient (r = 0.8768). The dendrogram of accessions could be classified into two major clusters with 0.52 similarities. Cluster I could be divided into five subgroups with 0.61 similarities, while the Ya Nang Daeng outgroup had a similarity value of 0.23 (Fig. 1). DNA fingerprint clustering was not related to locality. Nevertheless, UPGMA clustering was done according to morphological characteristics (Fig. 3). In the subgroup Ia, the foliage morphology showed these accessions having a cuspidate apex, petioles being green combined with red tones and branches red combined with green tones. Ib displayed the characteristics of a cuspidate apex and green (N144C, 144A, 144B and 143C) petioles and green (N144C, 144A, 144B and 143C) branches. In Ic, the leaves had a cuspidate apex, red (172A and 173A) petioles and dark red (181A, 183A, 183B and 184C) branches. Id had a caudate apex, with petioles being red combined with green tones and dark red (181A, 183A, 183B and 184C) branches. Lastly Ie had traits of leaves with a caudate apex, with the petioles being green combined with red tones and red (180A and 180B) branches. Likewise, in Cluster II, the leaf morphology of all accessions consisted of a caudate apex, with the petioles being red combined with green tones and red (180A and 180B) branches. In addition to the leaf shape and base characteristics, there was a low rate of variation which could not be formed into a subgroup. PCoA classified two major clusters (Fig. 2). The PCoA clustering was in accord with the UPGMA clustering but could not separate cluster I into subgroups.

Discussion

Accession gathering

There are only four provinces in Thailand (Lampang, Tak, Sukhothai, Kampaengpetch) where there is the natural habitat of Ya

Table 1
List of Ya Nang Daeng accessions used in this study.

No.	Floristic region	Province	District	Planar shape	Apex shape	Base shape	Petiole color	Branch color
1[b]	SE	Prachin Buri	Kabin Buri	Lance-ovate	Cuspidate	Rounded	G-R	R-G
2[b]	SE	Prachin Buri	Kabin Buri	Lance-ovate	Cuspidate	Rounded	G-R	R-G
3[b]	SE	Prachin Buri	Kabin Buri	Lance-ovate	Cuspidate	Rounded	G-R	R-G
4[b]	SE	Prachin Buri	Kabin Buri	Lanceolate	Cuspidate	Rounded	G-R	R-G
5[b]	C	Sa Kaeo	Wang Nam Yen	Lance-ovate	Cuspidate	Rounded	172A	181A
6	N	Tak	Mueang Tak	Ovate	Cuspidate	Rounded	172A	183A
7	N	Tak	Mueang Tak	Lance-ovate	Acute	Cordate	173A	183B
8	N	Tak	Mueang Tak	Lanceolate	Cuspidate	Rounded	G-R	R-G
9	N	Tak	Mueang Tak	Ovate	Cuspidate	Cordate	G-R	R-G
10	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Rounded	G-R	R-G
11	N	Tak	Mueang Tak	Ovate	Cuspidate	Cordate	G-R	R-G
12	N	Tak	Mueang Tak	Ovate	Cuspidate	Cordate	G-R	R-G
13	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Rounded	G-R	R-G
14	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Rounded	G-R	R-G
15	N	Tak	Mueang Tak	Ovate	Cuspidate	Cordate	G-R	R-G
16	N	Tak	Mueang Tak	Ovate	Cuspidate	Rounded	G-R	R-G
17	N	Tak	Mueang Tak	Lance-ovate	Acute	Rounded	G-R	R-G
18	N	Tak	Mueang Tak	Ovate	Cuspidate	Rounded	G-R	R-G
19	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Cordate	G-R	R-G
20	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Cordate	G-R	R-G
21	N	Tak	Mueang Tak	Ovate	Cuspidate	Cordate	G-R	R-G
22	N	Tak	Mueang Tak	Lance-ovate	Cuspidate	Cordate	G-R	R-G
23	N	Tak	Sam Ngao	Ovate	Cuspidate	Cordate	G-R	R-G
24	N	Tak	Sam Ngao	Lance-ovate	Caudate	Cordate	R-G	180A
25	N	Tak	Sam Ngao	Ovate	Cuspidate	Cordate	G-R	R-G
26	N	Tak	Sam Ngao	Ovate	Cuspidate	Rounded	G-R	R-G
27	N	Lampang		Ovate	Cuspidate	Cordate	G-R	R-G
28	N	Lampang		Lance-ovate	Caudate	Cordate	R-G	180B
29	N	Lampang		Ovate	Cuspidate	Rounded	173A	183B
30	N	Lampang		Lance-ovate	Cuspidate	Rounded	173A	183A
31	N	Lampang		Lance-ovate	Cuspidate	Rounded	172A	183A
32	N	Lampang	Thoen	Lance-ovate	Cuspidate	Rounded	173A	184A
33	N	Lampang	Mae Phrik	Lance-ovate	Cuspidate	Rounded	172A	183B
34	N	Lampang	Mae Phrik	Lance-ovate	Cuspidate	Cordate	180A	181A
35	N	Lampang	Mae Phrik	Lance-ovate	Cuspidate	Rounded	180A	183B
36	N	Lampang	Mae Phrik	Lance-ovate	Cuspidate	Rounded	180B	183B
37	N	Lampang	Mae Phrik	Ovate	Cuspidate	Rounded	180A	183A
38	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Cuspidate	Rounded	180A	183A
39	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Cordate	180B	183A
40	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Cordate	144A	144B
41	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Cuspidate	Rounded	G-R	R-G
42	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Acute	Rounded	172A	184A
43	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Cordate	173A	183A
44	N	Sukhothai	Ban Dan Lan Hoi	Lanceolate	Caudate	Rounded	R-G	180B
45	N	Sukhothai	Ban Dan Lan Hoi	Widely ovate	Obtuse	Cordate	N144C	144B
46	N	Sukhothai	Ban Dan Lan Hoi	Widely ovate	Cuspidate	Cordate	180A	183B
47	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Rounded	172A	184A
48	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Acute	Rounded	173A	181A
49	N	Sukhothai	Ban Dan Lan Hoi	Widely ovate	Acute	Cordate	173A	183A
50	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Cuspidate	Cordate	G-R	R-G
51	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Rounded	172A	183B
52	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Cuspidate	Rounded	172A	183A
53	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Rounded	173A	183A
54	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180A
55	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180B
56	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Caudate	Rounded	G-R	180B
57	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Cuspidate	Cordate	N144C	144A
58	N	Sukhothai	Ban Dan Lan Hoi	Widely ovate	Caudate	Cordate	R-G	180A
59	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Cordate	R-G	180A
60	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180A
61	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180A
62	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180B
63	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180A
64	N	Sukhothai	Ban Dan Lan Hoi	Lance-ovate	Caudate	Rounded	R-G	180B
65	N	Sukhothai	Ban Dan Lan Hoi	Ovate	Caudate	Rounded	R-G	180A
66	N	Kamphaeng Phet	Phran Kratai	Ovate	Caudate	Rounded	R-G	180A
67	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Rounded	R-G	180B
68	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Cordate	R-G	180A
69	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Rounded	R-G	180B
70	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Rounded	R-G	184A
71	N	Kamphaeng Phet	Mueang Kamphaeng Phet	Lance-ovate	Caudate	Rounded	R-G	183B
72	N	Kamphaeng Phet	Mueang Kamphaeng Phet	Lance-ovate	Caudate	Rounded	R-G	183A
73	N	Kamphaeng Phet	Mueang Kamphaeng Phet	Ovate	Caudate	Rounded	G-R	180A

(*continued on next page*)

Table 1 (*continued*)

No.	Floristic region	Province	District	Planar shape	Apex shape	Base shape	Petiole color	Branch color
74	N	Kamphaeng Phet	Mueang Kamphaeng Phet	Ovate	Caudate	Rounded	G-R	180B
75	N	Kamphaeng Phet	Kosamphi Nakhon	Lance-ovate	Caudate	Rounded	R-G	183B
76	N	Kamphaeng Phet	Kosamphi Nakhon	Lance-ovate	Caudate	Cordate	R-G	184A
77	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Rounded	R-G	183B
78	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Rounded	G-R	180A
79	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Cordate	R-G	183A
80	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Cordate	G-R	180B
81	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Cordate	G-R	180A
82	N	Kamphaeng Phet	Phran Kratai	Lance-ovate	Caudate	Cordate	G-R	180A
83[b]	N	Sakon Nakhon	Kham Ta Kla	Ovate	Caudate	Rounded	G-R	180A
84[b]		unknown		Lance-ovate	Caudate	Rounded	G-R	180A
85[b]	E	Chaiyaphum	Kaeng Khro	Lance-ovate	Caudate	Cordate	G-R	180A
86[b]	NE	Udon Thani	Chai Wan	Lanceolate	Caudate	Rounded	R-G	180B
87[b]	NE	Sakon Nakhon	Sawang Daen Din	Lance-ovate	Caudate	Cordate	R-G	180B
88[b]	NE	Udon Thani	Ban Dung	Ovate	Caudate	Rounded	R-G	180A
89[b]	NE	Udon Thani	Phen	Ovate	Caudate	Cordate	R-G	180A
90[b]	E	Ubon Ratchathani	Na Chaluai	Lanceolate	Caudate	Cordate	R-G	180B
91[b]	E	Ubon Ratchathani	Khong Chiam	Lance-ovate	Caudate	Rounded	R-G	183B
92[b]	E	Ubon Ratchathani	Trakan Phuet Phon	Lance-ovate	Caudate	Rounded	R-G	184A
93[b]	E	Ubon Ratchathani	Phibun Mangsahan	Lance-ovate	Caudate	Cordate	G-R	180A
94[b]	E	Amnat Charoen	Mueang Amnat Charoen	Lance-ovate	Caudate	Rounded	G-R	180A
95[b]	E	Amnat Charoen	Senangkhanikhom	Ovate	Caudate	Rounded	R-G	180B
96[b]	E	Yasothon	Kut Chum	Widely ovate	Caudate	Cordate	R-G	180A
97[b]	NE	Kalasin	Mueang Kalasin	Lanceolate	Caudate	Cordate	R-G	180B
98[a]	C	Sa Kaeo	Wang Sombun	Ovate	Acute	Rounded	184C	184C

G-R: green combined with red tone; R-G: red combined with green tone.
[a] *Cayratia* sp. (outgroup).
[b] Cultivated habit.

Nang Daeng and these provinces are in the northern region of the floristic map and all accessions were found in dry deciduous dipterocarp forest. In these regions, the plant has a rather long vegetative growth phase of 4–5 yr. There is a low rate of reproductive growth, with a low rate of fruiting set which was not correlated with the number of florets and inflorescence (Larsen et al., 1984). When the ripened legume was dehisced, the seed was quite weighty because of the endosperm, so that the ripe fruit would fall down onto the forest floor. However, the seed has a low rate of germination and living because it was eaten by insects and worms and this factor has a direct effect on its narrow distribution (Campbell et al., 1996). Moreover, vegetative parts of the immature plant, root and stem are removed for utilization. The soil condition in the natural habitat of Ya Nang Daeng consisted of three layers (upper loamy topsoil, lower gravelly sandy clay loam, stone layer 50 cm below the topsoil) making the soil difficult to dig in order to remove the Ya Nang Daeng, so it still exists in the wild (Pongamphi and Reungpradisth, 1973).

DNA fingerprint analysis

The DNA fingerprints from 13 primers had a total of 379 DNA bands, with a mean of 29.15 bands per primer, where 359 (94.72%) bands were polymorphic. The percentage of polymorphic bands in the current study was higher than reported by Capo-chichi et al. (2001) but comparable to Schönswetter et al. (2004) who studied Leguminosae species. The polymorphism information content (PIC) provides an indication of AFLP marker efficiency for the sample classification (Rolden-Ruiz et al., 2000). In the current study, the mean PIC of each primer was between 0.154 (M-CTT/E-ACA) and 0.377 (M-CAC/E-ACA). The mean PIC of all bands was 0.253 which is somewhat high and this value could indicate an the efficiency of the sample classification. The PICs in this study were in harmony with a study of diversity analysis in Indian genotypes of linseed using AFLP markers which reported PIC values between 0.19 and 0.31 with a mean of 0.23 (Chandrawati et al., 2014). The M-CAC/E-ACA

Table 2
Average number of bands, number of alleles and proportion of polymorphic bands obtained for the 98 accessions from 13 selective primer combinations.

Primer combination	Total band No.	Polymorphic band No.	% polymorphism band	PICs
M-CTA/E-AAC	30	28	93.33	0.197
M-CTT/E-AAC	31	29	93.55	0.245
M-CAA/E-AAG	28	27	96.43	0.252
M-CAC/E-AAG	25	23	92.00	0.203
M-CTG/E-AAG	27	24	88.89	0.266
M-CAC/E-ACA	31	29	93.55	0.377
M-CAG/E-ACA	32	31	96.88	0.334
M-CAT/E-ACA	21	21	100	0.206
M-CTT/E-ACA	28	27	96.43	0.154
M-CAA/E-ACG	31	30	96.77	0.273
M-CAG/E-ACG	29	26	89.66	0.215
M-CTT/E-ACG	35	34	97.14	0.288
M-CAC/E-AGG	31	30	96.77	0.280
Total	379	359	–	–
Average	29.15	27.12	94.72	0.253

E = pre-amplification primer (GACTGCGTACCAATTC) of *Eco*RI; M = pre-amplification primer (GATGAGTCCTGAGTAA) of *MseI*; PIC = polymorphism information content.

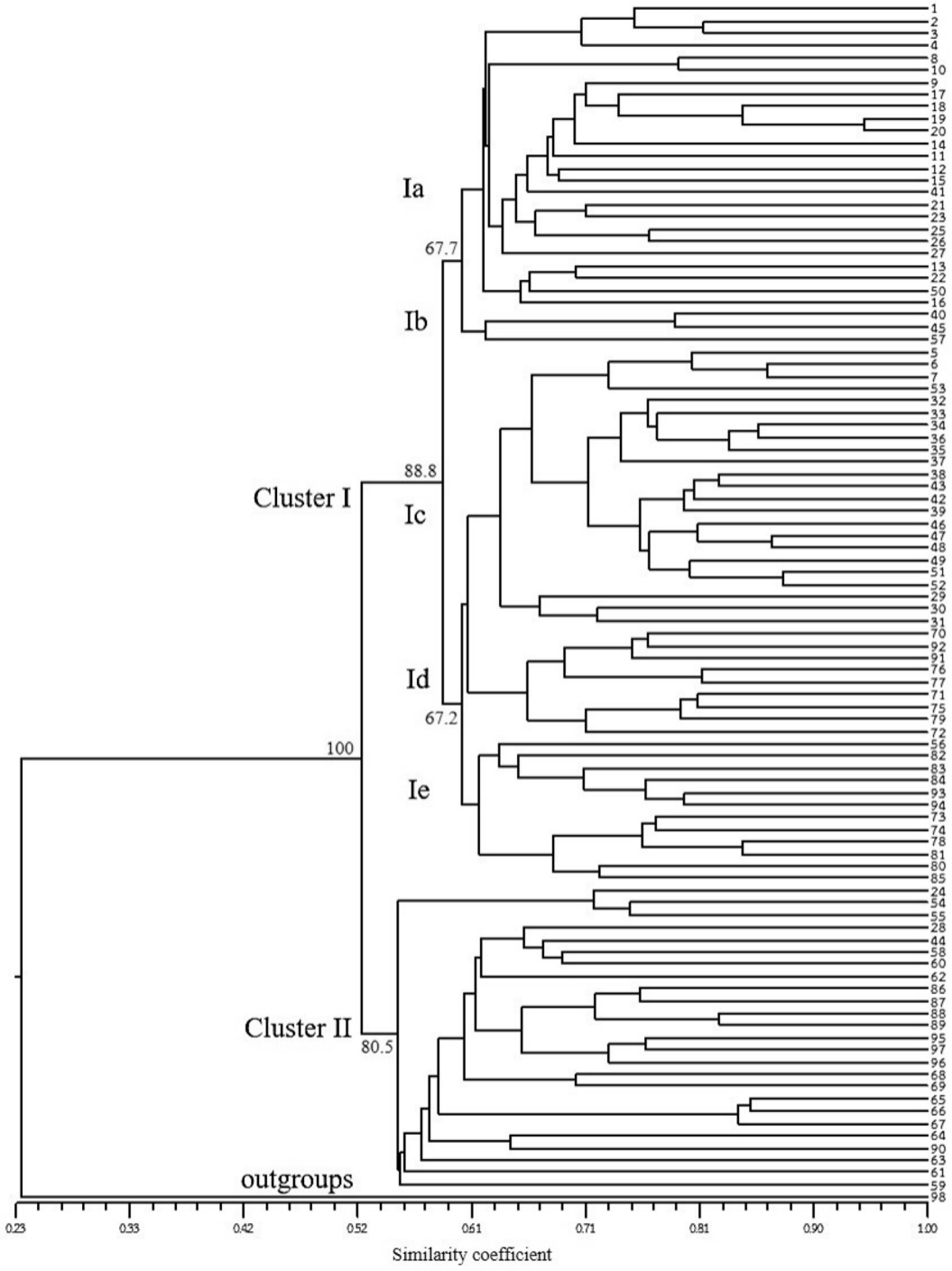

Fig. 1. UPGMA phylogenetic tree of 98 accessions using AFLP markers based on Jaccard's similarity coefficient. Support for branching pattern was determined using 1000 bootstrap replication.

primer had the highest PIC value (0.377), indicating this primer could have high efficiency for sample classification.

Genetic relation analysis

The similarity coefficient values varied from 0.52 to 0.93, with a mean of 0.73. These results revealed that the accessions had genetic differences. The phylogenic tree explained 0.23 similarity and could clearly separate *Cyratia* sp. as an outgroup. The dendrogram divided Ya Nang Daeng into two major clusters. Cluster I had a similarity value of 0.61 (Fig. 1), consisting of five subgroups. The morphological characteristics of all subgroups were different which was related to the UPGMA clustering. The cophenetic correlation coefficient (r) was 0.8617, indicating a high level of correlation (Moslemi et al., 2010; Kladmook et al., 2010). Analysis of correlation using PCoA presented Ya Nang Daeng in three clusters (Fig. 2). A comparison between the UPGMA and PCoA results indicated 0.52 similarity. However, PCoA could not separate Cluster I into subgroups. Both the morphological information and the AFLP data were related. Based on morphological characteristics, Ya Nang Daeng could be separated intraspecifically, although the leaf shape and leaf base were not helpful in classification because expression of morphological traits is

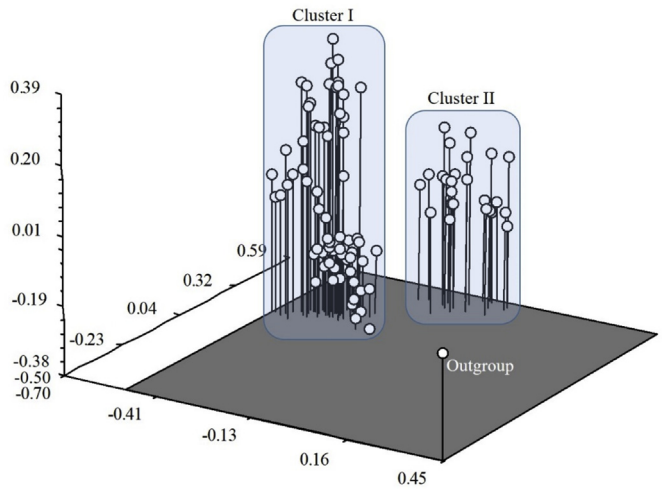

Fig. 2. Distribution of pomegranate accessions revealed by principal coordinate analysis based on amplified fragment length polymorphism data.

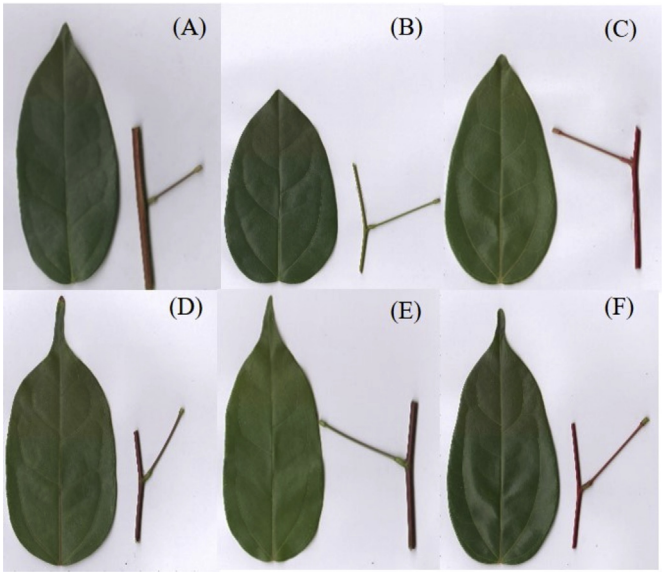

Fig. 3. Morphology of *Lysiphyllum strychnifolium*: (A) subgroup Ia; (B) subgroup Ib; (C) subgroup Ic; (D) subgroup Id; (E) subgroup Ie; (F) cluster II.

controlled by multiple genes which are posited on multi loci. The traits have the same expression (Palmer et al., 1988). Intraspecific morphological variation is caused by acclimation to native habitation (Pratt and Clark, 2004) and phenotypic plasticity which is an ability of the same genotypic organisms to express as different phenotypes (Duminil and Michele, 2009).

AFLP markers could be collaborated usefully with morphological information. These markers are efficient for genetic diversity evaluation and separation which is beneficial for germplasm collection and the breeding program of Ya Nang Daeng. The genetic similarity of Ya Nang Daeng ranged from 0.52 to 0.93, indicating the close correlation of germplasm. The dendrogram of 98 accessions showed a clear outgroup split off from Ya Nang Daeng. Furthermore, Ya Nang Daeng was divided into two major clusters and five subgroups based on morphology and molecular markers.

Conflict of interest

The authors declare that there are no conflicts of interest.

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