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Phylogenetic Analysis of Thai *Amomum* (Alpinioideae: Zingiberaceae) Using AFLP Markers

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ABSTRACT

The AFLP technique was used to assess the genetic relationships among 45 zingiberaceous plants including 40 collections of *Amomum* and 5 outgroup taxa: *Alpinia*, *Etlingera* 1, *Etlingera* 2, *Elettaria* and *Geostachys*. Cluster analysis using unweighted pair group method with arithmetic mean (UPGMA), based on AFLP data from 122 polymorphic bands generated with five primer combinations, was performed. The grouping of accessions of most species corresponded with their fruit morphological characteristics and were found to be consistent with previous studies. The species of Thai *Amomum* were classified into 3 groups based on AFLP markers: *A. aculeatum* group, *A. biflorum* group, and *A. dealbatum* group. The genetic relationships among genus *Amomum* and other genera in the tribe Alpinioideae are still incompletely understood.

Key words: phylogenetic, *Amomum*, AFLP, Thailand

INTRODUCTION

Amomum Roxb. is one of the largest genera in the ginger family (Zingiberaceae) with about 150-180 species. As currently recognized, *Amomum* occurs from the Himalayas through Southeast Asia, Northern Australia and extends into the central Pacific and is widely distributed in Southeast Asia (Kiew, 1982; Smith, 1985). Sirirugsa (2001) estimated about 15-20 species to be found in Thailand. Plants of *Amomum* are generally evergreen herbs inhabiting wet forests in light gaps and at forest margins (Sakai and Nagamasu, 1998). Many species are used as medicine, spice, condiment and vegetable. Even though the plants from this genus have been long utilized, the identification is still confusing because of the absence of a comprehensive work on the genus and the much confused taxonomic problems. These bring about many changes in their taxonomic status.

Four species of *Amomum* were first recognized by Linnaeus (1753) including: *A. cardamomum*, *A. zingiber*, *A. zerumbet and A. grana-paradisi*. These species have since been transferred to *Elettaria* Maton, *Zingiber* Boehm and *Aframomum* K. Schum. by Burtt and Smith (1972). Baker (1892), classified *Amomum* into 5 sections; *Geanthus*, *Achasma*, *Hornstedtia*, *Euamomum* and *Cenolophon* based on

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morphological characteristics of spike, labellum and anther crest. Schumann (1904) used the characteristics of anther crest and classified *Amomum* into 2 sections and 4 series. Section *Geanthus* was divided into 2 series, series *Oliganthae* and *Polyanthae*, distinguished by the absence of an anther crest. Section *Euamomum* was comprised of series *Lobulatae* and *Integrae*, characterized by an anther crest. Gagnepain (1906) separated *Amomum* into 3 groups based on the characteristics of floral morphology such as anther crest and lateral staminode. Loesener (1930) classified *Amomum* into 2 main groups using anther crest, *Lobulatae* and *Integrae*.

Xia *et al*. (2004) investigated the phylogenetic status of *Amomum* using ITS and *mat*K DNA sequence data. They indicated that *Amomum* as currently defined is polyphyletic with three major groups of species (*A. villosum* Group, *A. tsao-ko* Group and *A. maximum* Group) that do not correspond with any previously recognized sectional classification of the genus. They also mentioned that some morphological characters such as anther crest and fruit type could be useful for classification.

The AFLP technique has been used to study genetic diversity and phylogenetic relationships in a wide range of plant species; Lubberstedt *et al*. (2000) studied relationships among early European maize inbreds, Garcia-Mass *et al*. (2000) used AFLP marker for measuring genetic diversity in melon, Abdalla *et al*. (2001) used AFLP marker for estimating genetic relationships across a wide range of taxonomic levels and for analyzing the evolutionary and historical development of cotton cultivars at the genomic level, Larson *et al*. (2001) studied AFLP variation in agamospermous and dioecious bluegrasses of western North America, Mizumoto *et al.* (2003) used AFLP for studying the diversity of nuclear and chloroplast genome in wild einkorn wheat (*Triticum urartu*).

Because the relationships within genus *Amomum* and other genera in tribe Alpinioideae are still incompletely understood, a more detailed analysis using other molecular techniques is necessary. Knowledge of the genetic relationships among them is essential to the classification of the genus. This study was intended to determine genetic relationships among species of the *Amomum* genus occurring in Thailand using AFLP markers.

MATERIALS AND METHODS

Plant materials

Forty accessions of *Amomum* and 5 accessions of outgroup taxa: *Alpinia*, *Elettaria*, *Etlingera* 1, *Etlingera* 2 and *Geostachys* were used in this study (Table 1). All plant materials were grown and kept at Department of Horticulture, Kasetsart University, Bangkok, Thailand.

DNA isolation and AFLP analysis

Total genomic DNAs were extracted from 100 mg fresh young leaves using Qiagen DNeasy® Plant Mini kit (Qiagen GmbH, Hilden, Germany).

AFLP analysis was performed following the method of Vos *et al*. (1995) with minor modifications. From each sample, 2 templates were prepared by digesting 20-50 ng DNA with the restriction enzyme combination *Eco*RI-*Mse*I and by ligating the corresponding oligonucleotide adaptors in a total volume of 10 µl. Preselective PCR amplification with primers corresponding to adaptor core sequences (E+A and M+C) was performed in a 10 µl reaction containing 3 µl of AFLP template. PCR contained 10X PCR buffer, 0.5 µmol/L of each primer, 1 µmol/L of each dNTP, and 1 U *Taq* DNA polymerase (Fermentas, Lithuania) and was performed using a Biosystems Mod. Gene Amp® PCR system 9700 (Biosystems, Montgomeryville, PA). PCR conditions consisted of 1 cycle of 5 min at 50°C, 1 cycle of 3 min at 94°C, 24 cycles of 30 s at 94°C, 24 cycles of 1

Table 1 List of Thai *Amomum* accessions and outgroup taxa used in AFLP study.

Accessions	Species	Collected number	Collected places(provinces)
1	*A. aculeatum* Roxb.	Kaewsri-02	Kanchanaburi
2	*A. biflorum* Jack	Kaewsri-52	Chanthaburi
3	*A. dealbatum* Roxb.	Kaewsri-110	Chiang Mai
4	*A. koenigii* 1	Kaewsri-03	Kanchanaburi
5	*A. koenigii* 2	Kaewsri-29	Nakhon Nayok
6	*A. micranthum* Ridl.	Kaewsri-63	Chanthaburi
7	*A. repoense* Gagnep.	Kaewsri-64	Chanthaburi
8	*A. rivale*1*	Kaewsri-04	Kanchanaburi
9	*A. rivale*2*	Kaewsri-23	Kanchanaburi
10	*A.* cf. *rivale*	Kaewsri-33	Kanchanaburi
11	*A. siamense* Craib	Kaewsri-14	Tak
12	*A. testaceum* 1	Kaewsri-15	Tak (Cultivated)
13	*A. testaceum* 2	Kaewsri-16	Tak (Cultivated)
14	*A. testaceum* 3	Kaewsri-17	Tak (Cultivated)
15	*A. testaceum* 4	Kaewsri-96	Tak (Cultivated)
16	*Amomum* cf. *testaceum*	Kaewsri-86	Chumphon
17	*A. uliginosum*1	Kaewsri-30	Nakhon Nayok
18	*A. uliginosum*2	Kaewsri-92	Tak
19	*A. uliginosum*3	Kaewsri-32	Trat
20	*A.* cf. *villosum*1	Kaewsri-12	Tak (Cultivated)
21	*A.* cf. *villosum*2	Kaewsri-13	Tak
22	*Amomum* sp.1	Kaewsri-01	Kanchanaburi
23	*Amomum* sp.2	Kaewsri-10	Kanchanaburi
24	*Amomum* sp.3	Kaewsri-19	Prachuap Khiri Khan
25	*Amomum* sp.4	Kaewsri-22	Kanchanaburi
26	*Amomum* sp.5	Kaewsri-24	Kanchanaburi
27	*Amomum* sp.6a	Kaewsri-113	Chiang Mai
28	*Amomum* sp.6b	Kaewsri-88	Tak
29	*Amomum* sp.7	Kaewsri-27	Uthai Thani
30	*Amomum* sp.8	Kaewsri-35	Ranong
31	*Amomum* sp.9	Kaewsri-38	Ranong
32	*Amomum* sp.10	Kaewsri-50	Sakon Nakhon
33	*Amomum* sp.11	Kaewsri-68	Chumphon
34	*Amomum* sp.12	Kaewsri-70	Chumphon
35	*Amomum* sp.13	Kaewsri-81	Ranong
36	*Amomum* sp.14	Kaewsri-94	Tak
37	*Amomum* sp.15	Kaewsri-108	Chiang Mai
38	*Amomum* sp.16	Kaewsri-111	Chiang Mai
39	*Amomum* sp.17a	Kaewsri-134	Nan
40	*Amomum* sp.17b	Kaewsri-138	Nan
41	*Alpinia nigra*	-	Cultivated at KU
42	*Elettaria cardamomum*	-	Tak
43	*Etlingera littoralis*	-	Kanchanaburi
44	*Etlingera pavieana*	-	Chanthaburi
45	*Geostachys* sp.	-	Nakhon Nayok

The number 1, 2, 3 or 4 of each species = *Amomum*'s specimens that were collected from different places.

min at 56°C, and 24 cycles of 1 min at 72°C, followed by an extension of 5 min at 72°C. Amplification products were diluted in 100 µl deionized H_2O and 2 µl were used for selective amplification in a total volume of 10 µl containing 1 µmol/L of 10X PCR Buffer, 5 µmol/L of E-specific primer extended by 3 selective neucleotides (Table 2), 5 µmol/L of M-specific primer extended by 3 selective nucleotides (Table 2), 1 U of *Taq* DNA polymerase (Fermentas, Lithuania) and 1 µmol/L of each dNTPs. PCR was performed using a touchdown protocol with initial denaturation of a cycle of 30 s at 94°C, 30 s at 65° C (decreasing the temperature by 1°C after each cycle until 57°C) and 1 min at 72°C, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C with a final extension of 4 min at 72°C. Following amplification, 10 µl of formamide loading dye was added to the PCR products. The products were electrophoresed on 8% non-denaturing polyacrylamide gel. The bands were visualized using silver stain.

Data analysis

Each accession was scored (1) for presence and (0) for absence of each polymorphic band. AFLP bands within accessions were scored as missing if they were poorly resolved on the gel or if the template DNA did not amplify well. Similarity coefficient was calculated on the basis of Dice similarity coefficients (Dice, 1945) and is written as

$$C_{jk} = 2a/(2a+b+c)$$

In which Cjk is similarity coefficient, a is number of AFLP markers present in both j and k accessions, b is number of AFLP markers present only in j accessions and c is number of AFLP markers present only in k accessions. The similarity matrix was subjected to cluster analysis by the unweighted pair-group method with arithmetic mean (UPGMA) and a dendrogram was created using the NTSYS-pc version 2.01d program (Rohlf, 1997).

RESULTS

Five informative AFLP primer combinations generated a total of 364 reproducible amplification fragments across all species of *Amomum*, among which 122 bands were polymorphic (Table 2). The number of amplified AFLP bands per primer pair varied from 66 to 81 with an average of 72.8 bands. The average number of polymorphic bands detected was 24.4 per primer combination. The fragment sizes were determined by comparing each one with the standard DNA ladder, ranging from about 140 to 726 base pairs (bp). Two primer combinations (E-AGG, M-CAA (Figure 2) and E-ACC, M-CAA) produced 30 polymorphic bands, a relatively higher numbers of polymorphisms compared to the other primers used in this study.

Table 2 AFLP primer pairs and their number of amplified and polymorphic bands for phylogenetic study of Thai *Amomum*.

Primer combinations (*Eco*RI+3/*Mse*I+3)	Amplified bands	No. of polymorphic bands
E-AGG, M-CAA	81	30
E-ACC, M-CTA	73	17
E-ACC, M-CAA	66	30
E-AGC, M-CTC	74	25
E-AGG, M-CTC	70	20
Total	364	122
Mean	72.8	24.4

Cluster analysis

Cluster analysis using UPGMA (unweighted pair group method with arithmetic mean) was performed to examine genetic relationships among Thai *Amomum* species. A dendrogram was produced from the UPGMA cluster analysis of genetic similarity (GS) matrix for 45 accessions, 40 accessions of *Amomum* species and 5 accessions of out taxa, based on AFLP markers varied from 43% to 88% with a total average genetic similarity of 74.5% (Table 3). Two main clusters (A and B) were separated at 57% GS. The A cluster was separated into 2 groups: C and D, at 58% genetic similarity. The D group is subdivided into 2 subgroups (I and II) at 59% GS while the B Cluster generated 2 groups (E and F) at 59% GS (Figure 3).

The A cluster is characterized by spiny fruit (rarely smooth fruit). The C group contains *Amomum koenigii* 1, *A. koenigii* 2, *A.uliginosum* 1, *Amomum* sp.9, *Etlingera littoralis*, *A. aculeatum*, *Amomum* sp.12 and *Geostachys* sp. while D group is divided into two subgroups (I and II). Subgroup (I) consists of *A. testaceum* 1, *A. testaceum* 2, *A. testaceum* 3 and *Amomum* cf. *testaceum*. Subgroup (II) consists of *A. testaceum* 4, *A.* cf. *villosum*2, *Amomum* sp.5, *Amomum* sp.7, *A. uliginosum*2, *A.* cf. *villosum*1, *A. rivale*1, *A. micranthum*, *A. rivale*2, *A.* cf.*rivale*, *Amomum* sp. 8, *Amomum* sp.4, *A. biflorum* and *Amomum* sp.13. The B cluster is characterized by smooth, ridged or wing fruit (rarely spiny fruit). This cluster contains E and F groups. The E group consists *Amomum* sp.16, *Amomum* sp.3, *Amomum* sp.2, *Amomum* sp.17a, *A. siamense*, *Amomum* sp.6b, *Amomum* sp.6a, *Elettaria cardamomum*, *Amomum* sp.17b, *A.dealbatum*, *Amomum* sp.15, *Amomum* sp.10 and *Alpinia nigra*. The F group contains *Amomum* sp.1, *Amomum* sp.14, A. *repoense*, and *Etlingera pavieana*. Regarding the out group taxa; *Alpinia nigra*, *Elettaria cardamomum* and *Etlingera pavieana* were inserted in B group while *Etlingera littoralis* and *Geostachys* sp. were placed in A group.

DISCUSSION

In this study, 40 accessions of Thai *Amomum* species were fingerprinted including 5 outgroup taxa. One hundred twenty two polymorphic AFLP markers were produced from five primer combinations. UPGMA cluster analysis (Rohlf, 1997) with genetic similarity of 57% separated *Amomum* into 2 main clusters: A consists of C and D groups and B consists of E and F groups (Figure 1).

Regarding the C group, *A. koenigii* 1 and *A.koenigii* 2 were collected from Kanchanaburi and Nakhon Nayok provinces, respectively. It is clear that both collections are closely related (74%), even though the peduncular lengths vary greatly. The plants from Nakhon Nayok have a much shorter peduncle than those found in Kanchanaburi. The variation in phenotype could be caused by differences in their respective habitats. The placement of this species is similar to morphological analysis that placed it in spiny fruit group. This result is confirmation of the paraphyletic relationship between *A.koenigii* and the spiny fruit species (*A. uliginosum* and *A. aculeatum*). *A.aculeatum* and *Amomum* sp.12 are placed together at 90% GS. These closely related species are similar in leafy stem but differ in peduncular length, colour and size of labellum. From the results, the species *Amomum* sp.12 should be established as a new variety. However, this is difficult to decide from only a single plant. More collections are needed to solve this problem.

D group is divided into two subgroups (I and II). Subgroup D (I) consists of *A. testaceum* 1, *A. testaceum* 2, *A. testaceum* 3 and *Amomum* cf. *testaceum*. Regarding *A. testaceum* species complex, the dendrogram suggests that this species can be separated into at least three varieties; especially *A. testaceum* 4 which was isolated from the group. The placement of *A. testaceum* is rather close to the spiny fruit species (*Amomum* sp.1 and *Amomum* sp.14). This result does agree with Xia *et al*. (2004) whose work was based on ITS and

*Mat*K genes. They placed *A.testaceum* among the spiny fruit species of *A.villosum* group. A possible explanation for this was a paraphyletic origin of *A. testaceum* complex. Although its morphological characteristics are different, its genotype is close to spiny fruit species. The *Amomum* cf. *testaceum* that was collected from Chumphon is also placed in this group. Its leafy stem is similar to *A. testaceum* but dif fers in its hairiness on the lower surface of leaves.

Figure 1 Some species of *Amomum* used in AFLP study.
A. *A. aculeatum* Roxb. B. *A. biflorum* Jack C. *A. dealbatum* Roxb.
D. *A.koenigii* Gmelin. E. *A. repoense* Pierre ex Gagnep. F. *A. rivale* Ridl.
G. *A. testaceum* Ridl. H. *A. uliginosum* K?nig ex Retz. I. *A. siamense* Craib

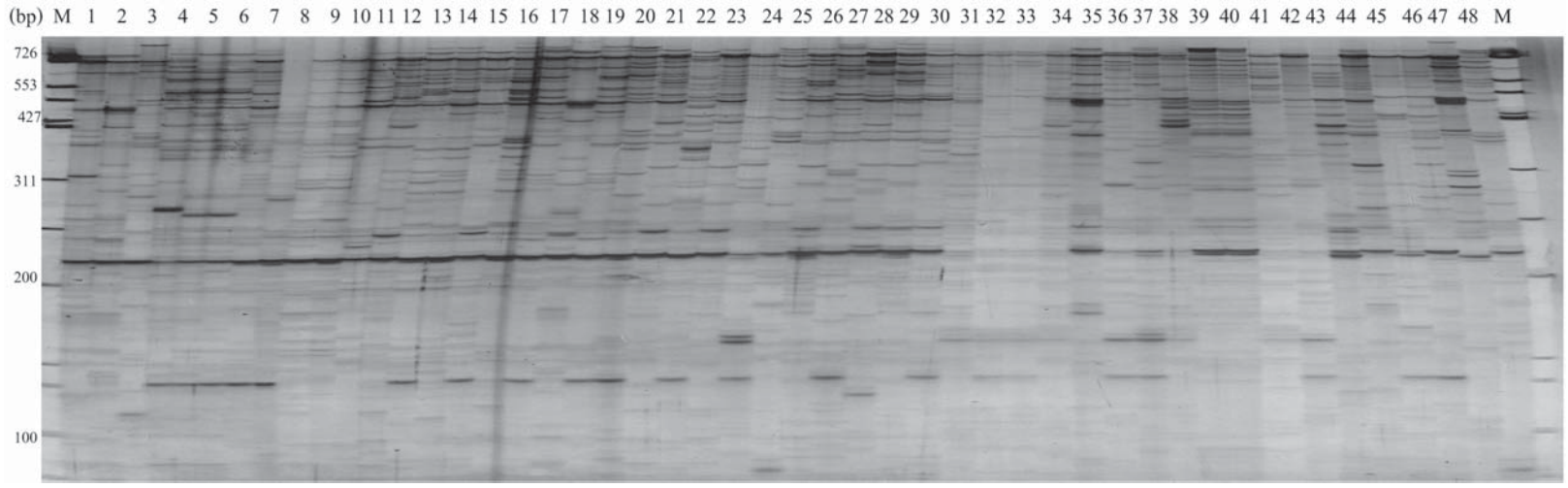

Figure 2 AFLP fingerprint of Thai *Amomum* species and out-groups using E-AGG, M-CAA primer pair. 1. *A. koenigii*1 , 2. *A. koenigii* 2, 3. *Amomum* sp.16, 4. *A. testaceum*1, 5. *A. testaceum* 2, 6. *A. testaceum* 3, 7. *A. testaceum* 4, 8. *Amomum* sp.1, 9. *A. aculeatum* Roxb., 10. *Amomum* sp.12, 11. *A. rivale*1, 12. *A. rivale*2, 13. *A.*cf. *villosum*1, 14. *A.* cf. *villosum*2, 15. *Amomum* sp.4, 16. *Amomum* sp.5, 17. *Amomum* sp.7, 18. *A. uliginosum*1, 19. *A. uliginosum*2, 20. *A. uliginosum*3, 21. *A Amomum* cf. *rivale.*, 22. *Amomum* sp.17a, 23. *Amomum* sp.8, 24. *Amomum* sp.10, 25. *A. biflorum* Jack , 26. *A. micranthum* Ridl., 27. *Amomum* sp.11, 28. *Amomum* sp.13, 29. none use, 30. none use, 31. *A. siamense* Craib,32. *.Amomum* sp.3, 33. *Amomum* sp.2, 34. *Amomum* sp.6b, 35. *A. uliginosum4*, 36. *Amomum* sp.17b, 37. *A. repoense* Gagnep., 38. *Amomum* sp.6a, 39. none use, 40. *Amomum* sp.14, 41. *Amomum* sp.15, 42. *A. dealbatum* Roxb., 43. *Elettaria cardamomum*, 44. *Etlingera littoralis*, 45. *Etlingera pavieana*, 46. *Alpinia nigra*, 47. *Geostachys* sp., 48.*Amomum* cf. *testaceum* and M=ϕX*Hin*fI

Table 3 Dice's coefficient of similarity matrix from AFLP fingerprints of 40 accessions of *Amomom* and 5 outgroup taxa.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	*A. koenigii* 1	1.00																					
2	*A. koenigii* 2	0.71	1.00																				
3	*Amomum* sp. 16	0.58	0.57	1.00																			
4	*A. testaceum* 1	0.56	0.54	0.45	1.00																		
5	*A. testaceum* 2	0.56	0.51	0.47	0.78	1.00																	
6	*A. testaceum* 3	0.57	0.57	0.51	0.78	0.88	1.00																
7	*A. testaceum* 4	0.50	0.55	0.47	0.59	0.62	0.64	1.00															
8	*Amomum* sp. 1	0.64	0.60	0.60	0.50	0.53	0.57	0.60	1.00														
9	*A. aculeatum*	0.59	0.65	0.60	0.66	0.63	0.64	0.56	0.61	1.00													
10	*Amomum* sp. 12	0.58	0.63	0.57	0.64	0.62	0.60	0.60	0.64	0.90	1.00												
11	*A. rivale* 1	0.63	0.63	0.61	0.65	0.67	0.68	0.61	0.68	0.65	0.61	1.00											
12	*A. rivale* 2	0.57	0.59	0.65	0.57	0.55	0.63	0.65	0.67	0.60	0.59	0.76	1.00										
13	*A.* cf. *villosum* 1	0.54	0.56	0.51	0.57	0.52	0.52	0.64	0.52	0.61	0.57	0.65	0.60	1.00									
14	*A.* cf. *villosum* 2	0.50	0.53	0.43	0.53	0.53	0.53	0.73	0.53	0.58	0.59	0.56	0.64	0.75	1.00								
15	*Amomum* sp. 4	0.61	0.63	0.58	0.68	0.68	0.71	0.64	0.60	0.67	0.64	0.80	0.74	0.71	0.62	1.00							
16	*Amomum* sp. 5	0.57	0.60	0.53	0.60	0.58	0.60	0.65	0.57	0.66	0.65	0.64	0.67	0.77	0.83	0.68	1.00						
17	*Amomum* sp. 7	0.53	0.55	0.52	0.62	0.68	0.64	0.69	0.59	0.60	0.66	0.67	0.67	0.65	0.70	0.75	0.71	1.00					
18	*A. uliginosum* 1	0.67	0.83	0.61	0.57	0.56	0.59	0.53	0.60	0.68	0.64	0.63	0.60	0.60	0.51	0.63	0.64	0.55	1.00				
19	*A. uliginosum* 2	0.53	0.57	0.47	0.65	0.68	0.65	0.69	0.57	0.62	0.64	0.64	0.67	0.71	0.74	0.77	0.74	0.94	0.58	1.00			
20	*A. uliginosum* 3	0.53	0.52	0.57	0.57	0.56	0.54	0.63	0.62	0.56	0.58	0.67	0.64	0.67	0.64	0.69	0.65	0.75	0.58	0.74	1.00		
21	*Amomum* cf.*rivale*	0.55	0.57	0.69	0.59	0.57	0.62	0.63	0.64	0.62	0.58	0.72	0.85	0.62	0.64	0.78	0.68	0.66	0.61	0.67	0.67	1.00	
22	*A.momum* sp.17a	0.53	0.52	0.58	0.53	0.51	0.48	0.53	0.56	0.60	0.60	0.63	0.56	0.60	0.48	0.60	0.53	0.60	0.50	0.57	0.58	0.60	1.00

Table 3 (Continued)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
23	*Amomum* sp. 8	0.55	0.57	0.66	0.57	0.57	0.59	0.61	0.62	0.59	0.55	0.77	0.85	0.65	0.62	0.77	0.67	0.63	0.61	0.66	0.69	0.92	0.60	1.00
24	*Amomum* sp. 10	0.55	0.50	0.63	0.54	0.53	0.48	0.57	0.59	0.57	0.58	0.60	0.57	0.60	0.53	0.60	0.51	0.53	0.58	0.58	0.61	0.63	0.61	0.66
25	*A. biflorum* Jack	0.58	0.58	0.61	0.65	0.65	0.68	0.61	0.65	0.60	0.61	0.71	0.71	0.59	0.56	0.69	0.64	0.67	0.64	0.67	0.67	0.71	0.55	0.71
26	*A. micranthum* Ridl.	0.53	0.54	0.62	0.61	0.60	0.63	0.71	0.63	0.63	0.62	0.73	0.75	0.63	0.63	0.64	0.61	0.64	0.57	0.64	0.67	0.76	0.56	0.74
27	*Amomum* sp. 11	0.50	0.54	0.51	0.64	0.69	0.64	0.71	0.60	0.55	0.57	0.62	0.67	0.61	0.67	0.68	0.71	0.81	0.59	0.81	0.73	0.70	0.53	0.65
28	*Amomum* sp. 13	0.54	0.67	0.65	0.67	0.61	0.63	0.60	0.60	0.66	0.64	0.73	0.75	0.60	0.60	0.71	0.61	0.68	0.64	0.68	0.70	0.78	0.56	0.78
29	*A. siamense* Craib	0.54	0.62	0.57	0.58	0.60	0.57	0.54	0.55	0.60	0.64	0.70	0.57	0.58	0.55	0.62	0.58	0.59	0.64	0.59	0.64	0.60	0.60	0.65
30	*Amomum* sp. 3	0.52	0.53	0.61	0.53	0.51	0.53	0.55	0.64	0.56	0.52	0.63	0.51	0.51	0.48	0.60	0.47	0.57	0.55	0.55	0.61	0.53	0.52	0.57
31	*Amomum* sp. 2	0.50	0.53	0.66	0.51	0.51	0.50	0.57	0.67	0.51	0.49	0.60	0.56	0.48	0.47	0.57	0.43	0.53	0.53	0.50	0.58	0.57	0.50	0.55
32	*Amomum* sp. 6b	0.58	0.60	0.58	0.54	0.53	0.51	0.53	0.60	0.57	0.58	0.67	0.60	0.57	0.60	0.63	0.60	0.60	0.57	0.58	0.64	0.63	0.64	0.64
33	*A. uliginosum* 4	0.59	0.67	0.65	0.58	0.58	0.58	0.60	0.57	0.63	0.62	0.65	0.64	0.60	0.58	0.67	0.64	0.62	0.74	0.62	0.62	0.68	0.57	0.67
34	*Amomum* sp.17b	0.57	0.56	0.60	0.57	0.63	0.58	0.62	0.69	0.63	0.60	0.68	0.64	0.63	0.52	0.65	0.60	0.60	0.62	0.62	0.59	0.64	0.64	0.67
35	*A. repoense* Gagnep.	0.53	0.52	0.58	0.50	0.54	0.54	0.55	0.70	0.56	0.52	0.58	0.59	0.57	0.54	0.57	0.51	0.52	0.52	0.55	0.63	0.63	0.64	0.63
36	*Amomum* sp. 6a	0.59	0.54	0.60	0.50	0.47	0.47	0.45	0.55	0.60	0.57	0.59	0.53	0.50	0.43	0.53	0.49	0.56	0.51	0.54	0.51	0.54	0.60	0.54
37	*Amomum* sp. 14	0.53	0.53	0.57	0.55	0.57	0.53	0.64	0.67	0.57	0.60	0.65	0.66	0.57	0.61	0.57	0.61	0.59	0.56	0.57	0.60	0.64	0.48	0.62
38	*Amomum* sp. 15	0.57	0.54	0.56	0.57	0.58	0.55	0.56	0.57	0.61	0.57	0.70	0.66	0.66	0.55	0.65	0.58	0.54	0.54	0.59	0.62	0.67	0.68	0.70
39	*A. dealbatum* Roxb.	0.61	0.64	0.57	0.56	0.57	0.62	0.52	0.70	0.60	0.53	0.63	0.60	0.64	0.54	0.67	0.57	0.55	0.64	0.60	0.60	0.66	0.60	0.67
40	*Elettaria cardamomum*	0.65	0.56	0.60	0.44	0.52	0.53	0.51	0.74	0.57	0.62	0.62	0.58	0.53	0.50	0.51	0.50	0.56	0.57	0.54	0.59	0.53	0.62	0.56
41	*Etlingera littoralis*	0.69	0.63	0.64	0.56	0.56	0.59	0.52	0.60	0.59	0.60	0.60	0.62	0.60	0.57	0.60	0.64	0.58	0.71	0.58	0.60	0.63	0.57	0.64
42	*Etlingera pavieana*	0.58	0.58	0.57	0.54	0.57	0.54	0.57	0.71	0.64	0.63	0.67	0.54	0.51	0.47	0.61	0.50	0.58	0.55	0.58	0.61	0.55	0.61	0.53
43	*Alpinia nigra*	0.52	0.55	0.55	0.53	0.53	0.50	0.60	0.56	0.56	0.50	0.61	0.50	0.62	0.56	0.61	0.56	0.55	0.52	0.60	0.58	0.55	0.49	0.58
44	*Geostachys* sp.	0.57	0.60	0.59	0.55	0.57	0.53	0.60	0.64	0.69	0.65	0.64	0.57	0.61	0.58	0.65	0.61	0.59	0.67	0.62	0.65	0.65	0.60	0.62
45	*Amomum* cf. *testaceum*	0.53	0.49	0.61	0.70	0.70	0.68	0.58	0.57	0.60	0.60	0.63	0.67	0.47	0.53	0.63	0.56	0.53	0.49	0.53	0.55	0.67	0.57	0.66

Table 3 (Continued)

		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
24	*Amomum* sp. 10	1.00																					
25	*A. biflorum* Jack	0.60	1.00																				
26	*A. micranthum* Ridl.	0.62	0.67	1.00																			
27	*Amomum* sp. 11	0.57	0.73	0.63	1.00																		
28	*Amomum* sp. 13	0.64	0.74	0.75	0.64	1.00																	
29	*A. siamense* Craib	0.65	0.57	0.58	0.57	0.64	1.00																
30	*Amomum* sp. 3	0.69	0.58	0.57	0.51	0.64	0.60	1.00															
31	*Amomum* sp. 2	0.66	0.58	0.57	0.53	0.60	0.57	0.88	1.00														
32	*Amomum* sp. 6b	0.69	0.55	0.60	0.53	0.65	0.82	0.63	0.58	1.00													
33	*A. uliginosum* 4	0.59	0.65	0.66	0.57	0.74	0.64	0.60	0.57	0.62	1.00												
34	*Amomum* sp. 17b	0.70	0.70	0.58	0.67	0.63	0.63	0.62	0.64	0.65	0.52	1.00											
35	*A. repoense* Gagnep.	0.71	0.61	0.68	0.57	0.60	0.54	0.61	0.58	0.60	0.54	0.62	1.00										
36	*Amomum* sp. 6a	0.62	0.56	0.57	0.52	0.60	0.67	0.57	0.57	0.71	0.52	0.66	0.56	1.00									
37	*Amomum* sp. 14	0.54	0.70	0.66	0.61	0.64	0.52	0.60	0.64	0.47	0.61	0.60	0.59	0.43	1.00								
38	*Amomum* sp. 15	0.67	0.65	0.67	0.57	0.64	0.60	0.54	0.53	0.65	0.58	0.72	0.64	0.69	0.55	1.00							
39	*A. dealbatum* Roxb.	0.64	0.63	0.54	0.56	0.59	0.59	0.61	0.63	0.64	0.59	0.74	0.66	0.60	0.57	0.70	1.00						
40	*Elettaria cardamomum*	0.60	0.57	0.63	0.49	0.53	0.66	0.59	0.59	0.68	0.55	0.61	0.67	0.69	0.53	0.61	0.59	1.00					
41	*Etlingera littoralis*	0.55	0.67	0.54	0.57	0.59	0.62	0.55	0.57	0.60	0.71	0.62	0.58	0.53	0.60	0.53	0.69	0.60	1.00				
42	*Etlingera pavieana*	0.64	0.63	0.59	0.56	0.59	0.60	0.63	0.63	0.57	0.54	0.67	0.69	0.59	0.64	0.60	0.63	0.57	0.52	1.00			
43	*Alpinia nigra*	0.67	0.55	0.56	0.54	0.59	0.56	0.61	0.57	0.58	0.54	0.54	0.61	0.54	0.54	0.53	0.60	0.51	0.49	0.64	1.00		
44	*Geostachys* sp.	0.70	0.64	0.64	0.57	0.64	0.67	0.65	0.62	0.70	0.63	0.71	0.62	0.57	0.60	0.64	0.62	0.64	0.54	0.64	0.57	1.00	
45	*Amomum* cf. *testaceum*	0.60	0.64	0.65	0.62	0.65	0.51	0.53	0.60	0.55	0.54	0.59	0.63	0.53	0.54	0.65	0.57	0.53	0.55	0.57	0.44	0.54	1.00

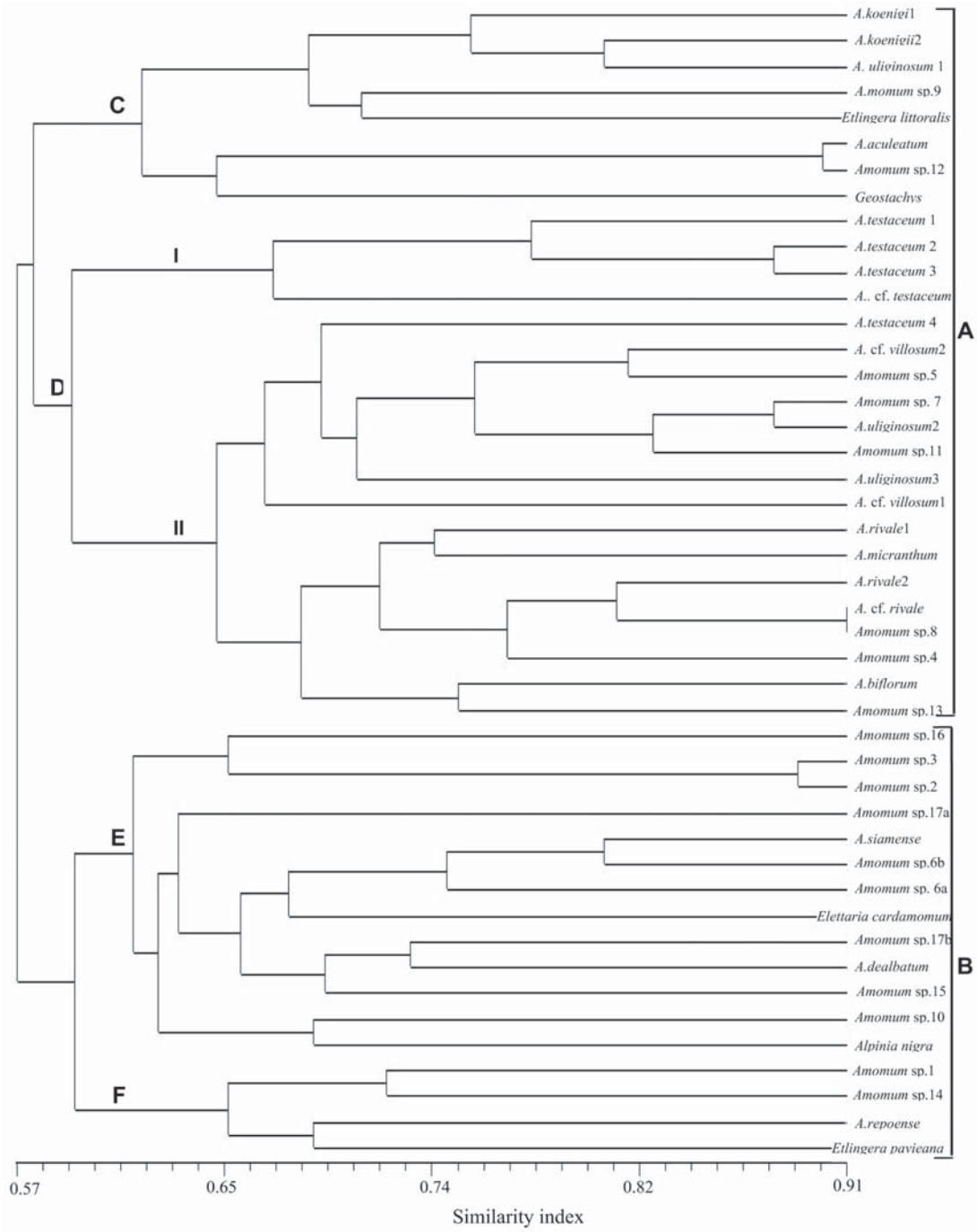

Figure 3 Dendrogram depicting the genetic relationship of 45 accessions of *Amomum* based on AFLP fingerprint, using similarity coefficient by DICE, clustering with UPGMA.

Subgroup D (II) consists of *A. testaceum* 4, *A.* cf. *villosum*, *Amomum* sp.5, *Amomum* sp.7, *A. uliginosum* 2, *Amomum* sp.11, *A. uliginosum* 3, *A. villosum* 1, *A. rivale* 1, *A. micranthum*, *A. rivale* 2, *A.* cf. *rivale*, *Amomum* sp.8, *Amomum* sp.4 and *Amomum* sp.13. All members have spiny fruit and leafy stem less than 1.50 m tall. Regarding *uliginosum* 2 and 3 which were collected from Tak province, they were separated from *A. uliginosum* 1 and 4 (from Nakhon Nayok and Ranong provinces, respectively). Their morphological characteristics differ from the ones in C group in its shorter leafy stem and smaller inflorescence. A possible explanation for this is that their morphological characteristics were the result of long time adaptation in the surrounding habitats which resulted in two ecotypes of *A. uliginosum.*

B cluster consists of E and F groups. It is characterized by smooth, ridged or winged fruit (rarely spiny fruit).

E and F groups include *Amomum* sp.16, *Amomum* sp.3, *Amomum* sp.2, *Amomum* sp.17a, *A. siamense*, *Amomum* sp.6b, *Amomum* sp.6a, *Amomum* sp. 17b, *A. dealbatum*, *Amomum* sp. 15, *Amomum* sp. 10, *Elettaria cardamomum*, *Alpinia nigra*, *Amomum* sp. 1, *Amomum* sp.14, *A. repoense* and *Etlingera pavieana*.

The dendrogram suggests the placement of smooth fruit (*Amomum* sp.16) between spiny and winged fruit. Similar to the result of *Amomum* sp.1 and 10 both of which are spiny fruit but were placed among winged fruit species. *Amomum* sp.17a and 17b from Nan province are similar in their morphology but were placed in different clusters. More study is needed to properly identify the position of these species. *A.siamense* with fruit longitudinally ridged is also placed in this group. This species should be closely related to winged fruit species. Although the cluster is not completely separated from the others, all winged fruit species are clearly placed. Therefore, the results have the tendency to be consistent with the *A.maximum* group of Xia *et al.* (2004).

The outgroup taxa (*Alpinia, Elettaria, Etlingera* and *Geostachys*) are placed among *Amomum* species. The result indicates a closer relationship among them and the spiny fruit species of *Amomum*. This result is similar to Xia *et al.* (2004) who found that *Etlingera littoralis* was placed in the clade of *A.villosum* group. The results then confirmed that *Etlingera* is related to the genus *Amomum*. Furthermore, some species of *Alpinia, Elettaria* and *Geostachys* are also closely related to the genus *Amomum*.

Twenty-six representives of Thai *Amomum* can be classified into 3 groups by using AFLP evidence: *A. aculeatum, A. biflorum* and *A. dealbatum* groups.

The *A. aculeatum* group consists of 4 species: *A. koenigii, A. uliginosum, A. aculeatum* and *Amomum* sp. 12. Species in this group have smooth and spotted or spiny fruit, anther crest 3 lobes, leafy stem stout and usually more than 1.5 m tall.

The *A. biflorum* group contains 10 species: *A. testaceum*, *Amomum* cf. *villosum*, *Amomum* sp.4, *Amomum* sp.5, *Amomum* sp.7, *Amomum* sp.8, *A.rivale*, *A. micranthum*, *Amomum* sp.11 and *Amomum* sp.13. All members of this group are defined by smooth or spiny fruit. Most species of this group are spiny fruit. In the case of smooth fruit, its fruit shape is usually globular and fruit colour is white or pale brown. The leafy stem is usually slender and shorter than 1.5 m.

The *A. dealbatum* group contains 12 species of *Amomum*: *A. dealbatum*, *A. repoense*, *A. siamense*, *Amomum* sp.1, *Amomum* sp.2, *Amomum* sp.3, *Amomum* sp.6, *Amomum* sp.10, *Amomum* sp.14, *Amomum* sp.15, *Amomum* sp.16 and *Amomum* sp.17. The species in this group are characterized by winged, ridged or smooth fruit (rarely spiny fruit and 3 lobes) and entire, round or truncate anther crest.

CONCLUSION

AFLP markers classified Thai *Amomum* species into three groups (*A. aculeatum* group, *A. biflorum* group, and *A. dealbatum* group) which correspond to the fruit and leafy stem characteristics.

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