

CHAPTER IV

RESULTS

Sample collections

Thirty-seven giant galanga accessions, 30 cultivated and 7 wild landraces from different areas in Thailand, were collected and shown many morphological characters such as color and size of rhizome in Figure 4.1.



Figure 4.1 Variation of giant galanga in Thailand: (1) KhaLing, (2) KhaSaku, (3) KhaDang, (4) KhaPar, (5) KhaYuek, (6) KhaLeang and (7) KhaYai.

DNA extraction strategies

The quantity of extracted genomic DNA of giant galanga could be measured by spectrophotometer (Table 4.1). This amount of DNA which was extracted by SDS extraction procedure was enough to PCR amplification. Electrophoretic analysis revealed that the quality of DNA was good (Figure 4.2).

Table 4.1 DNA concentration of giant galanga (37 accessions) using SDS extraction procedure.

	Name (color-size type)	DNA concentration (ng/ μ l)
1	KhaDang (red-medium cultivated)	939
2	KhaYuek (white-large cultivated)	1168
3	KhaLeang (yellow-medium wild)	1230
4	KhaSaku (red-small wild)	1553
5	KhaYuek (white-large cultivated)	1394
6	KhaDang (red-medium cultivated)	1610
7	KhaPar (red-medium wild)	1465
8	KhaDang (red-medium cultivated)	2152
9	KhaDang (red-medium cultivated)	1449
10	KhaYuek (white-medium cultivated)	1458
11	KhaYuek (white-large cultivated)	1461
12	KhaYuek (white-large cultivated)	1411
13	KhaYai (white-large cultivated)	1431
14	KhaLing (red-medium wild)	1490
15	KhaTadang (red-large cultivated)	1764
16	KhaDang (red-medium cultivated)	1422
17	KhaLing (red-medium wild)	1294
18	KhaYuek (white-medium cultivated)	1458
19	KhaTadang (red-medium cultivated)	1627
20	Kha (red-medium cultivated)	1266
21	KhaKaw (red-medium cultivated)	1162
22	KhaLeang (yellow-medium wild)	1213

Table 4.1 (continued)

Name (color-size type)	DNA concentration (ng/ μ l)
23 Kha (red-medium cultivated)	1171
24 Kha (red-large cultivated)	1240
25 KhaDang (red-medium cultivated)	1256
26 KhaNoldang (red-large cultivated)	1171
27 Kha (red-medium cultivated)	1188
28 KhaDang (red-medium cultivated)	1144
29 KhaKut (white-large wild)	1149
30 KhaLuang (white-small cultivated)	1219
31 KhaTadang (red-medium cultivated)	1089
32 KhaNol (red-medium cultivated)	1133
33 KhaYai (white-large cultivated)	1157
34 KhaTadang (red-medium cultivated)	1123
35 KhaTadang (red-medium cultivated)	1272
36 KhaDang (red-medium cultivated)	1206
37 KhaDang (red-medium cultivated)	1082



Figure 4.2 Agarose gel electrophoresis of giant galanga DNA (6 samples) by SDS extraction procedure.

Determination of genetic variation of *Alpinia* spp. using RAPD analysis

Randomly amplified polymorphic DNA (RAPD) technique was used to analyse the genetic variation of *Alpinia* spp. for DNA fingerprint. A total of 22 arbitrary primers (10 nucleotides) were screened with 4 giant galanga samples for the suitable RAPD primers (Data not shown). Eight primers (OPA20, OPB18, OPC09, OPD02, OPD11, OPG13, OPK12 and OPAX17) were selected for further studies on genetic variation of giant galanga on the basis of consistent, repeatable and easily scorable results of these primers.

Thirty-seven giant galanga accessions, 30 cultivated and 7 wild landraces from different areas in Thailand, were used in this study for RAPD analysis. Eight primers produced a total of 73 polymorphic bands (Table 4.2). Band sizes ranged from 0.75 to 2.5 kb.

Table 4.2 The total bands and polymorphic bands of giant galanga (37 accessions) based on RAPD analysis.

Primer	Sequence	No. of total bands	No. of polymorphic bands
	5' → 3'		
OPA20	GTTGCGATCC	13	12
OPB18	CCACAGCAGT	13	12
OPC09	CTCACCGTCC	9	9
OPD02	GGACCCAACC	7	7
OPD11	AGCGCCATTG	9	7
OPG13	CTCTCCGCCA	8	8
OPK12	TGGCCCTCAC	10	9
OPAX17	TGGGCTCTGG	10	9

The RAPD patterns and data matrices for each primer are shown hereafter. Only reproducible bands were scored for presence (1) or absence (0). Seventy-three polymorphic bands were observed from 8 primers. The combined matrices were used to construct a dendrogram in which 37 galanga accessions were divided into 5 clusters based on genetic similarity.

RAPD analysis using OPA20 primer

RAPD patterns of giant galanga were generated by OPA20 (Figure 4.3). It showed a total of 13 bands which ranged in sizes from 750 to 1900 bp. Twelve bands were polymorphic and only one was monomorphic. Analysis of each band was shown in Table 4.3. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that a total of three fragments (1100 bp, 1000 bp and 900 bp) were specific to the former.

Cluster 2: accession No. 16 and 17 that were shown the similar bands at 1600 bp and 1500 bp.

Cluster 3: accession No. 18, 19 and 20 that a 1100 bp and 750 bp fragments were observed.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that only a 1000 bp fragment was observed in almost all of the accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 1500 bp and 1000 bp fragments were found in almost all of the accessions.



Figure 4.3 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPA20. Numbers of lanes represent giant galanga accessions as listed in Table 3.2

M: 100 bp DNA Step Ladder size marker.

Table 4.3 Data matrices of giant galanga using OPA20. Band was present(1) and absent(0).

No.	bp	1900	1800	1700	1600	1500	1400	1300	1200	1100	1000	900	750
1		0	0	0	0	1	0	1	0	0	1	0	0
2		0	1	1	0	1	0	0	0	1	1	0	0
3		0	1	1	1	1	1	0	1	1	1	1	1
4		1	0	0	1	1	1	0	0	1	1	0	1
5		0	1	1	1	1	1	0	0	1	1	0	0
6		1	1	1	1	1	1	1	1	1	1	0	1
7		1	1	1	1	1	1	0	0	0	1	0	1
8		0	1	1	0	1	1	0	0	0	1	0	1
9		0	1	1	0	1	1	0	0	1	1	0	1
10		0	1	0	0	1	1	0	0	0	1	0	0
11		0	1	0	1	1	1	0	0	0	1	0	0
12		0	1	1	1	1	1	1	1	1	1	0	1
13		1	1	1	1	1	1	0	0	0	1	0	1
14		1	1	1	1	1	1	0	0	0	1	0	1
15		1	1	1	0	1	1	1	1	1	1	0	1
16		0	0	0	1	1	0	0	0	0	0	0	0
17		0	1	0	1	1	0	1	1	1	1	0	0
18		1	1	0	1	0	0	0	1	1	1	0	1
19		0	0	0	1	0	0	0	0	1	0	1	1
20		0	1	0	0	0	0	1	1	1	1	1	1
21		0	0	0	1	1	0	0	0	0	1	0	0
22		0	1	1	1	1	1	1	1	1	1	1	0
23		0	0	0	0	0	0	1	1	1	1	1	0
24		0	1	1	1	1	1	0	0	1	1	1	0
25		0	1	1	1	1	1	0	0	1	1	1	1
26		1	1	1	1	1	1	0	0	1	1	0	1
27		1	1	1	1	1	1	0	0	1	1	1	1
28		0	0	0	0	0	1	0	0	1	1	1	0
29		0	1	1	0	1	0	0	0	1	1	1	1
30		1	1	1	0	1	0	0	0	1	1	1	1
31		0	0	0	0	1	1	0	0	1	1	0	1
32		1	1	1	1	1	1	1	1	1	1	0	0
33		1	1	1	1	1	1	1	1	1	1	0	1
34		0	0	0	0	0	0	1	1	1	1	0	1
35		1	1	0	1	1	0	0	0	1	1	0	1
36		0	0	0	0	0	1	0	0	1	1	0	0
37		1	1	0	1	1	1	1	1	0	1	0	0

RAPD analysis using OPB18 primer

RAPD patterns of giant galanga were generated by OPB18 (Figure 4.4). It showed a total of 13 bands which ranged in sizes from 800 to 2200 bp. Twelve bands were polymorphic and only one was monomorphic. Analysis of each band was shown in Table 4.4. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that were shown the similar bands at 1500 bp and 1400 bp.

Cluster 2: accession No. 16 and 17 that a total of three fragments (1700 bp, 1300 bp and 950 bp) were specific to the former.

Cluster 3: accession No. 18, 19 and 20 that only a 1200 bp fragment was observed in almost all of the accessions.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21,34, 36, 35 and 37 that the 1900 bp and 900 bp fragments were absent in almost all of the accessions.

Cluster 5: accession No. 1, 2, 3, 5 ,6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 900 bp and 800 bp fragments were absent in all of these accessions.

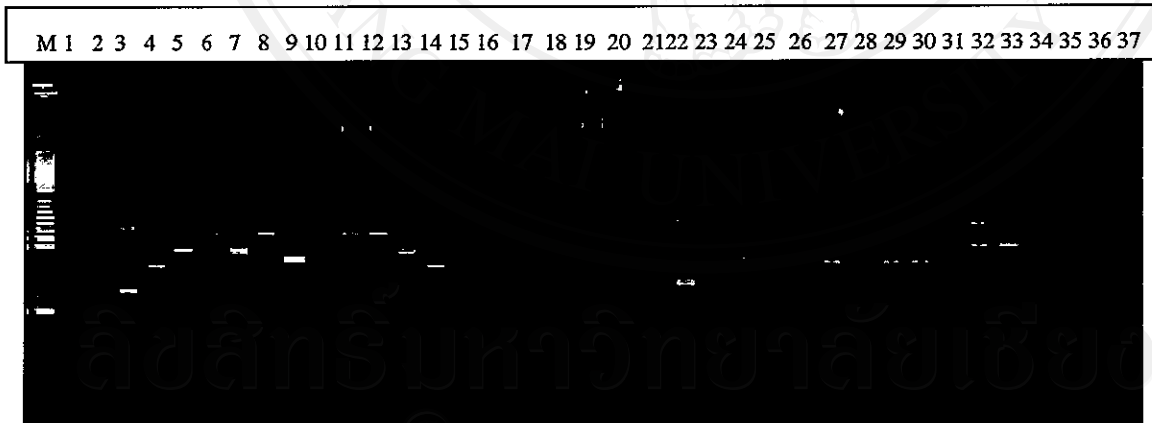


Figure 4.4 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPB18. Numbers of lanes represent giant galanga accessions as listed in Table 3.2
M:100 bp DNA Step Ladder size marker.

Table 4.4 Data matrices of giant galanga using OPB18. Band was present(1) and absent(0).

No.	bp	2200	1900	1800	1700	1600	1500	1400	1300	1200	950	900	800
1		0	0	0	1	0	1	0	0	1	0	0	0
2		0	0	0	1	0	1	1	0	0	0	0	0
3		1	0	1	1	1	1	1	0	1	0	0	0
4		0	0	0	0	0	0	0	1	0	1	0	0
5		1	0	0	0	0	1	0	0	0	0	0	0
6		1	0	0	1	0	0	0	0	0	0	0	0
7		1	0	1	1	0	1	0	0	0	0	0	0
8		1	1	0	1	0	0	1	0	1	0	0	0
9		1	1	0	0	0	0	1	0	0	0	0	0
10		0	0	0	0	0	0	0	0	0	0	0	0
11		1	1	1	1	1	1	1	1	1	1	0	0
12		1	1	1	1	1	1	1	1	1	1	0	0
13		0	0	0	0	0	1	0	0	1	0	0	0
14		1	0	0	0	0	0	0	1	1	1	0	0
15		1	0	0	1	1	1	0	0	1	0	0	0
16		0	0	0	1	0	0	0	1	0	1	0	0
17		0	0	0	1	1	0	0	1	0	1	1	1
18		0	0	1	0	1	0	0	0	1	0	0	0
19		0	0	1	0	1	0	0	0	1	0	0	0
20		0	0	0	0	0	1	1	0	1	0	0	0
21		0	0	0	0	1	0	0	0	1	1	1	0
22		0	0	1	1	1	1	0	1	1	1	0	1
23		0	0	0	0	0	1	1	0	1	1	0	1
24		1	1	1	1	1	0	0	1	1	1	0	1
25		0	0	0	0	1	1	1	1	1	1	0	1
26		0	0	0	0	1	1	1	1	1	1	0	1
27		1	1	1	1	1	1	1	1	1	1	0	1
28		0	0	0	0	0	1	1	0	0	0	0	0
29		0	0	0	0	0	0	0	1	1	1	0	1
30		0	0	0	0	0	0	0	1	1	0	0	1
31		0	0	1	0	0	0	0	0	0	0	0	0
32		0	1	1	0	1	1	1	1	1	0	0	0
33		0	1	0	0	1	1	1	1	1	0	0	0
34		0	0	0	0	0	0	0	0	0	0	1	1
35		0	0	0	0	0	0	0	0	0	1	1	1
36		0	0	0	0	0	0	1	0	0	1	0	0
37		0	0	0	0	0	0	1	0	1	0	0	1

RAPD analysis using OPC09 primer

RAPD patterns of giant galanga were generated by OPC09 (Figure 4.5). It showed a total of 9 polymorphic bands which ranged in sizes from 900 to 2300 bp. Analysis of each band was shown in Table 4.5. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that a total of four fragments (1400 bp, 1300 bp, 1100 and 900 bp) were specific to the former.

Cluster 2: accession No. 16 and 17 that a 1900 bp RAPD fragment was fixed in these accessions.

Cluster 3: accession No. 18, 19 and 20 that the 2300 bp, 1400 bp, 1300 bp, 950 bp and 900 bp fragments were absent in all of the accessions.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that only a 900 bp fragment was observed in almost of these accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 1300 bp and 1200 bp fragments were absent in almost of the accessions.



Figure 4.5 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPC09. Numbers of lanes represent giant galanga accessions as listed in Table 3.2

M: 100 bp DNA Step Ladder size marker.

Table 4.5 Data matrices of giant galanga using OPC09. Band was present(1) and absent(0).

No.	hp	2300	2200	1900	1400	1300	1200	1100	950	900
1	0	0	0	1	0	0	0	1	1	
2	0	0	0	1	0	0	0	1	1	
3	1	0	0	1	0	0	1	1	1	
4	1	1	1	1	1	1	0	1	1	
5	1	0	0	1	0	0	1	0	0	
6	1	1	1	0	0	0	0	1	1	
7	0	0	0	0	0	0	1	1	1	
8	1	0	1	0	0	0	1	1	1	
9	1	1	1	0	0	1	1	1	1	
10	1	0	1	0	1	0	0	1	1	
11	1	1	1	0	0	0	0	1	1	
12	1	1	1	0	0	0	0	1	1	
13	0	0	1	0	0	0	0	1	1	
14	0	1	1	1	0	0	0	1	0	
15	0	0	1	0	0	0	0	1	0	
16	0	0	1	0	0	0	0	1	0	
17	0	1	1	0	0	0	0	0	0	
18	0	1	1	0	0	0	0	0	0	
19	0	0	0	0	0	0	1	0	0	
20	0	0	0	0	0	0	1	1	0	
21	1	0	1	1	1	1	1	1	0	
22	1	0	1	1	1	1	0	1	1	
23	0	0	0	1	1	0	1	0	1	
24	1	1	1	1	1	0	1	1	1	
25	0	1	1	1	1	0	1	1	1	
26	0	1	0	1	1	0	0	1	1	
27	1	1	1	1	1	0	1	1	1	
28	1	1	1	1	1	0	1	1	1	
29	1	1	1	1	1	0	1	1	1	
30	1	1	1	1	1	1	1	1	1	
31	0	0	0	1	1	0	1	0	1	
32	1	1	1	1	1	1	1	0	1	
33	1	1	1	1	1	1	1	0	1	
34	0	0	0	0	0	0	1	0	1	
35	0	0	0	1	1	1	1	1	1	
36	1	1	0	0	0	0	1	1	1	
37	0	1	0	1	0	1	1	1	1	

RAPD analysis using OPD02 primer

RAPD patterns of giant galanga were generated by OPD02 (Figure 4.6). It showed a total of 7 polymorphic bands which ranged in sizes from 1050 to 2300 bp. Analysis of each band was shown in Table 4.6. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that a 1050 bp RAPD fragment was fixed in these accessions.

Cluster 2: accession No. 16 and 17 that the 1500 bp and 1050 bp fragments were present in all of the accessions.

Cluster 3: accession No. 18, 19 and 20 that a total of four fragments (2300 bp, 2100 bp, 1700 and 1300 bp) were specific to the former.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that only a 1500 bp fragment was observed in all of these accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 1700 bp and 1300 bp fragments were fixed in all of the accessions.

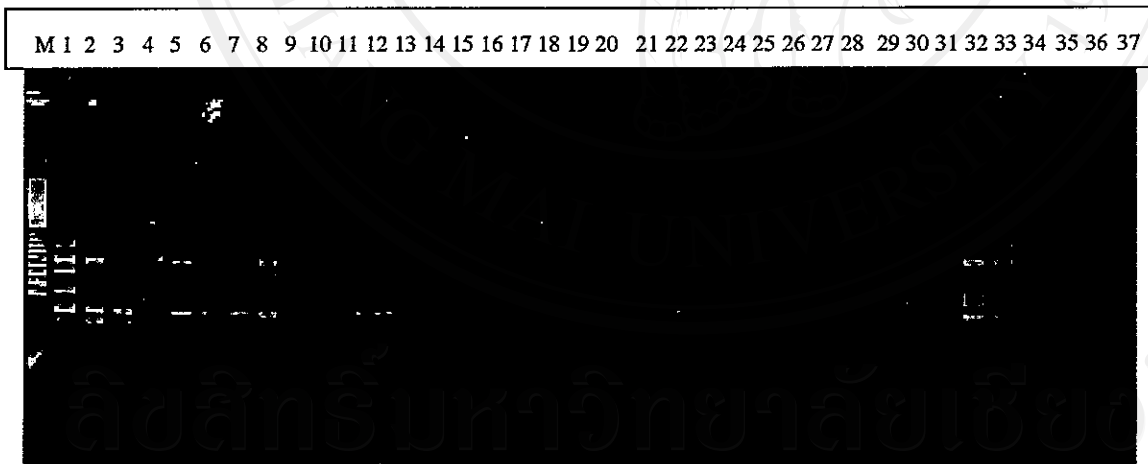


Figure 4.6 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPD02. Numbers of lanes represent giant galanga accessions as listed in Table 3.2

M: 100 bp DNA Step Ladder size marker.

Table 4.6 Data matrices of giant galanga using OPD02. Band was present(1) and absent(0).

No.	bp	2300	2100	1700	1500	1300	1150	1050
1		0	1	1	1	1	0	1
2		0	1	1	1	1	0	0
3		0	0	1	1	1	0	0
4		0	0	1	1	0	1	0
5		1	1	1	1	1	0	0
6		0	1	1	0	1	0	0
7		0	1	1	0	1	1	0
8		1	1	1	1	1	1	1
9		0	1	1	0	1	1	0
10		0	0	1	0	1	0	0
11		0	1	1	0	1	0	0
12		0	1	1	0	1	0	0
13		0	0	1	1	1	1	0
14		0	1	1	1	0	1	0
15		0	1	1	0	1	1	0
16		0	0	0	1	0	0	1
17		0	0	1	1	0	0	1
18		1	1	1	0	1	0	0
19		1	1	1	0	1	0	0
20		1	1	1	0	1	0	0
21		1	0	1	1	1	0	0
22		1	1	1	1	1	0	0
23		1	0	0	1	0	0	1
24		1	1	0	1	0	1	1
25		0	0	0	1	0	1	1
26		1	0	0	1	1	1	1
27		1	1	0	1	1	1	1
28		0	0	0	0	0	1	1
29		0	0	1	1	1	1	1
30		0	0	1	1	1	1	1
31		0	0	0	1	0	1	1
32		1	1	1	1	1	1	1
33		1	1	1	1	1	1	1
34		0	0	1	1	0	0	1
35		0	0	0	1	0	1	1
36		0	0	0	1	0	0	0
37		0	0	0	1	0	0	0

RAPD analysis using OPD11 primer

RAPD patterns of giant galanga were generated by OPD11 (Figure 4.7). It showed a total of 9 bands which ranged in sizes from 1300 to 2500 bp. Seven bands were polymorphic and two bands were monomorphic. Analysis of each band was shown in Table 4.7. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that a 1300 bp fragment was fixed in these accessions.

Cluster 2: accession No. 16 and 17 that a total of three fragments (2500 bp, 1700 bp and 1500 bp) were specific to the former.

Cluster 3: accession No. 18, 19 and 20 that the similar bands were the 2500 bp and 1500 bp bands.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that a 1400 bp fragment was absent in almost all of the accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 2500 bp fragment was present and the 2100 bp fragment was absent in all of these accessions.

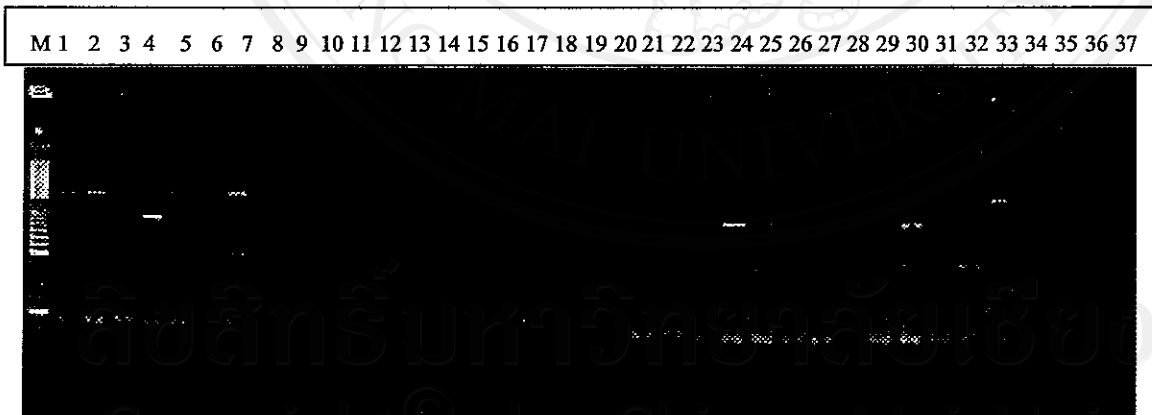


Figure 4.7 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPD11. Numbers of lanes represent giant galanga accessions as listed in Table 3.2
M: 100 bp DNA Step Ladder size marker.

Table 4.7 Data matrices of giant galanga using OPD11. Band was present(1) and absent(0).

No.	bp	2500	2100	1900	1700	1500	1400	1300
1		1	0	0	1	1	0	0
2		1	0	0	1	0	0	0
3		1	0	0	1	1	0	0
4		0	1	0	1	0	0	0
5		1	0	0	1	1	0	0
6		1	0	1	1	1	1	1
7		1	0	1	1	1	1	1
8		1	0	0	0	1	0	0
9		1	0	0	0	1	0	0
10		1	0	0	0	0	1	0
11		1	0	0	0	1	0	0
12		1	0	0	1	1	0	0
13		1	0	0	1	1	0	0
14		0	1	0	1	0	1	0
15		1	0	0	1	0	1	0
16		1	0	0	1	1	0	0
17		1	0	0	1	1	0	0
18		1	0	0	1	1	0	0
19		1	0	0	0	1	0	0
20		1	0	0	0	1	0	0
21		1	0	1	1	1	1	1
22		1	1	1	1	1	1	1
23		0	0	0	0	0	0	1
24		0	0	1	1	0	1	1
25		0	0	1	0	0	1	1
26		0	0	1	0	1	1	1
27		0	0	1	0	1	1	1
28		0	0	1	0	0	1	1
29		0	1	1	0	1	1	1
30		0	1	1	0	1	1	1
31		0	0	1	0	1	1	1
32		0	0	1	0	1	1	1
33		1	0	0	0	1	1	0
34		0	0	0	0	0	1	0
35		0	0	0	0	0	1	0
36		0	0	0	0	0	1	0
37		0	0	0	0	0	1	1

RAPD analysis using OPG13 primer

RAPD patterns of giant galanga were generated by OPG13 (Figure 4.8). It showed a total of 8 polymorphic bands which ranged in sizes from 950 to 2200 bp. Analysis of each band was shown in Table 4.8. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that the 2200 bp, 2100 bp and 950 bp fragments were absent in all of the accessions.

Cluster 2: accession No. 16 and 17 that a total of four fragments (2200 bp, 1700 bp, 1600 and 1300 bp) were specific to the former.

Cluster 3: accession No. 18, 19 and 20 that the 2100 bp, 1600 bp, 1300 bp, 1100 bp, 1050 bp and 950 bp were observed in the accessions.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21,34, 36, 35 and 37 that a 1050 bp fragment was present in all of these accessions.

Cluster 5: accession No. 1, 2, 3, 5 ,6, 7, 8, 9, 11, 12, 13, 15 and 10 that a 2100 bp fragment was fixed in all of the accessions.

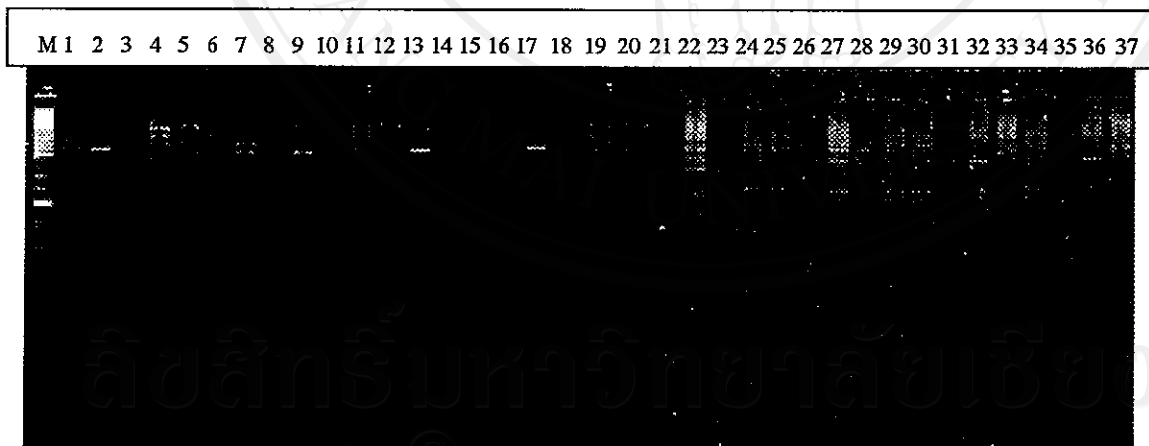


Figure 4.8 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPG13. Numbers of lanes represent giant galanga accessions as listed in Table 3.2
M: 100 bp DNA Step Ladder size marker.

Table 4.8 Data matrices of giant galanga using OPG13. Band was present(1) and absent(0).

No.	bp	2200	2100	1700	1600	1300	1100	1050	950
1		1	1	1	1	1	0	1	1
2		1	1	0	1	1	0	1	0
3		0	1	0	1	1	0	1	0
4		0	1	1	1	0	1	1	0
5		0	1	1	1	1	0	1	0
6		0	1	1	1	1	0	0	0
7		0	1	1	1	0	0	0	0
8		0	1	0	1	0	0	0	0
9		0	1	1	1	1	0	0	0
10		0	1	0	0	1	0	0	0
11		0	1	1	1	1	1	0	1
12		0	1	1	1	1	0	0	0
13		0	1	0	1	1	0	0	0
14		0	1	1	1	0	0	1	0
15		1	1	1	1	1	0	0	0
16		1	0	1	1	1	0	0	0
17		1	1	1	1	1	0	1	0
18		0	1	0	1	1	1	1	1
19		0	1	1	1	1	1	1	1
20		1	1	1	1	1	1	1	1
21		0	0	1	0	1	0	1	0
22		1	1	1	1	1	1	1	1
23		0	0	1	0	1	1	1	0
24		0	1	1	1	0	1	1	0
25		0	1	1	1	0	1	1	0
26		0	1	1	1	0	0	1	0
27		1	1	1	1	1	1	1	1
28		0	0	0	1	0	0	0	0
29		1	1	1	1	0	1	1	0
30		1	1	1	1	1	1	1	0
31		0	1	1	0	0	1	1	0
32		1	1	1	0	0	1	1	0
33		1	1	1	1	0	0	1	0
34		1	1	1	1	1	1	1	0
35		0	1	0	0	0	0	1	0
36		1	1	1	1	1	1	1	0
37		1	1	1	1	1	0	1	0

RAPD analysis using OPK12 primer

RAPD patterns of giant galanga were generated by OPK12 (Figure 4.9). It showed a total of 10 bands which ranged in sizes from 950 to 2500 bp. Nine bands were polymorphic and only one was monomorphic. Analysis of each band was shown in Table 4.9. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that the 2500 bp, 1600 bp and 1200 bp fragments were observed in all of the accessions.

Cluster 2: accession No. 16 and 17 that a total of two fragments (1400 bp and 1200 bp) were specific to the former.

Cluster 3: accession No. 18, 19 and 20 that the 2500 bp, 2000 bp, 1400 bp and 1100 bp fragments were present in all of these accessions.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that a 1100 bp fragment was absent in almost all of the accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that a 1300 bp fragment was present in almost all of these accessions.

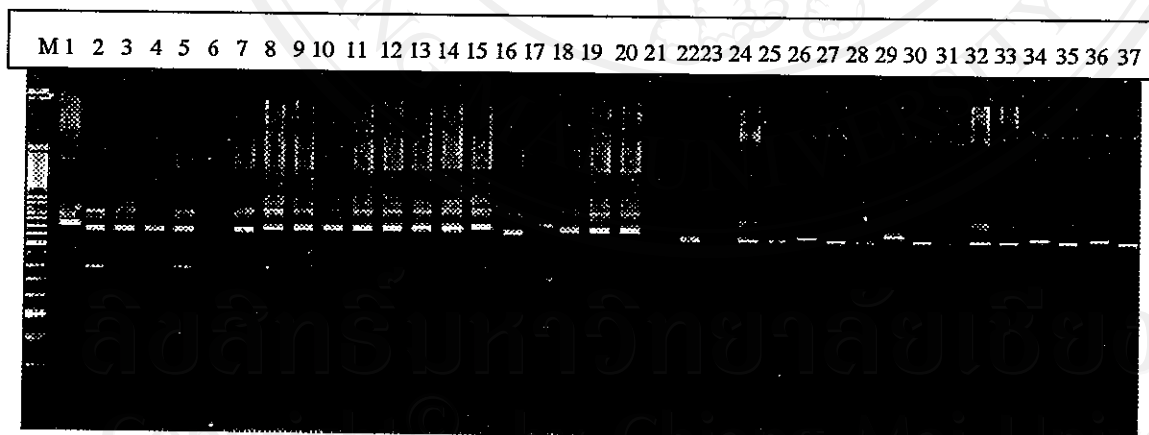


Figure 4.9 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPK12. Numbers of lanes represent giant galanga accessions as listed in Table 3.2
M: 100 bp DNA Step Ladder size marker.

Table 4.9 Data matrices of giant galanga using OPK12. Band was present(1) and absent(0).

No.	bp	2500	2300	2000	1600	1400	1300	1200	1100	950
1		0	1	1	0	1	1	1	1	0
2		1	0	1	1	0	1	0	0	0
3		1	1	1	1	0	1	0	0	0
4		0	0	1	1	1	1	0	0	0
5		1	0	1	1	0	1	0	0	1
6		0	0	0	0	1	1	0	1	0
7		1	1	1	1	1	0	0	0	0
8		1	1	1	1	1	1	1	1	1
9		1	1	1	0	1	1	1	1	1
10		0	0	0	0	1	1	1	1	1
11		1	1	1	1	1	1	1	1	1
12		1	0	1	1	1	1	1	1	1
13		1	0	1	0	1	1	1	1	1
14		1	0	1	1	1	1	1	1	1
15		1	0	1	0	1	1	1	1	1
16		0	0	0	1	1	0	1	0	0
17		0	0	0	0	1	0	1	0	1
18		1	0	1	0	1	0	0	1	0
19		1	0	1	1	1	0	0	1	0
20		1	1	1	1	1	0	0	1	0
21		0	0	0	1	0	0	0	0	0
22		1	0	0	1	0	0	1	0	0
23		1	0	0	1	0	0	1	0	0
24		0	1	1	1	1	0	1	0	1
25		0	0	0	0	0	0	1	0	1
26		0	0	1	0	0	0	0	0	1
27		0	0	0	0	0	0	0	0	1
28		1	0	0	1	1	0	1	0	0
29		0	0	1	1	1	0	1	1	1
30		0	0	1	1	1	0	1	0	1
31		0	0	1	0	0	0	1	1	1
32		1	1	1	0	0	0	1	0	0
33		1	1	1	0	0	0	1	0	0
34		0	0	1	0	0	1	1	0	0
35		1	1	1	0	0	1	1	0	0
36		1	1	1	0	0	1	1	0	1
37		1	1	1	0	0	1	1	0	1

RAPD analysis using OPAX17 primer

RAPD patterns of giant galanga were generated by OPAX17 (Figure 4.10). It showed a total of 10 bands which ranged in sizes from 950 to 2500 bp. Nine bands were polymorphic and only one was monomorphic. Analysis of each band was shown in Table 4.10. Band characteristics of giant galanga clusters were observed as follows:

Cluster 1: accession No. 23 and 28 that the 1000 bp and 950 bp fragments were fixed in these accessions.

Cluster 2: accession No. 16 and 17 that a total of seven fragments (2200 bp, 2000 bp, 1700 bp, 1600 bp, 1500 bp, 1000 bp and 950 bp) were specific to the former.

Cluster 3: accession No. 18, 19 and 20 that the 1600 bp, 1500 bp, 1000 bp and 950 bp fragments were present except the 2200 bp fragment which was absent in all of these accessions.

Cluster 4: accession No. 4, 14, 22, 32, 33, 24, 27, 25, 26, 29, 30, 31, 21, 34, 36, 35 and 37 that a 2200 bp fragment was absent in almost all of the accessions.

Cluster 5: accession No. 1, 2, 3, 5, 6, 7, 8, 9, 11, 12, 13, 15 and 10 that the 1700 bp fragment was present and the 1600 bp fragment was absent in all of these accessions.



Figure 4.10 Amplification patterns obtained from 37 giant galanga accessions using RAPD primer OPAX17. Numbers of lanes represent giant galanga accessions as listed in Table 3.2 M: 100 bp DNA Step Ladder size marker.

Table 4.10 Data matrices of giant galanga using OPAX17. Band was present(1) and absent(0).

No. \ bp	2500	2200	2000	1800	1700	1600	1500	1000	950
1	0	0	0	0	1	0	1	0	0
2	0	0	0	0	1	0	1	0	0
3	0	0	0	0	1	0	1	0	0
4	1	0	0	0	1	0	1	1	1
5	1	0	0	0	1	0	0	0	0
6	1	1	1	1	1	0	0	0	1
7	1	1	1	1	1	0	0	1	1
8	1	0	1	1	1	0	0	0	0
9	1	1	1	1	1	0	1	1	1
10	1	0	1	1	1	0	0	0	0
11	1	1	1	1	1	0	0	0	0
12	1	1	1	1	1	0	1	1	0
13	0	0	0	1	1	0	1	0	0
14	0	0	0	0	1	0	1	1	1
15	0	0	0	0	1	0	0	0	0
16	0	1	1	0	1	1	1	1	1
17	0	1	1	0	1	1	1	1	1
18	0	0	0	0	0	1	1	1	1
19	0	0	0	0	0	1	1	1	1
20	1	0	1	1	1	1	1	1	1
21	0	0	0	0	0	1	1	1	1
22	1	0	1	0	0	1	1	0	0
23	0	0	0	0	0	0	0	1	1
24	1	0	1	0	1	1	1	1	1
25	0	0	0	0	0	0	1	1	1
26	0	0	0	0	0	0	1	1	1
27	1	0	1	1	1	1	1	1	1
28	0	0	0	0	0	0	1	1	1
29	0	0	0	0	0	0	1	1	1
30	0	0	1	0	1	1	1	1	1
31	0	0	1	0	0	0	1	1	1
32	0	0	1	1	1	1	1	0	0
33	1	1	1	1	1	1	1	0	0
34	0	0	0	0	0	0	0	1	1
35	0	0	1	1	1	0	0	1	1
36	0	0	0	0	0	1	1	1	1
37	0	0	1	1	1	0	0	1	1

Based on RAPD bands, genetic distances among the 37 accessions were calculated and a dendrogram was constructed by UPGMA method (Figure 4.11). The accessions were divided into five major clusters. The first cluster consisted of two red-medium cultivated rhizome accessions. The second cluster consisted of one red-medium cultivated and one red-medium wild rhizome accessions. The third cluster included two red-medium cultivated and one white-medium cultivated rhizome accessions. The fourth was the largest cluster, including 13 cultivated- and 4 wild accessions, most of which had red-medium rhizome. The fifth cluster included two wild- and eleven cultivated accessions that six white-, six red- and one yellow rhizome accessions.

As for this dendrogram, giant galangas were classified into five clusters without any relation with their morphological characters such as type (Figure 4.11.1), color of rhizome (Figure 4.11.2) and the collection sites which were indicated by the regions of Thailand (Figure 4.11.3).

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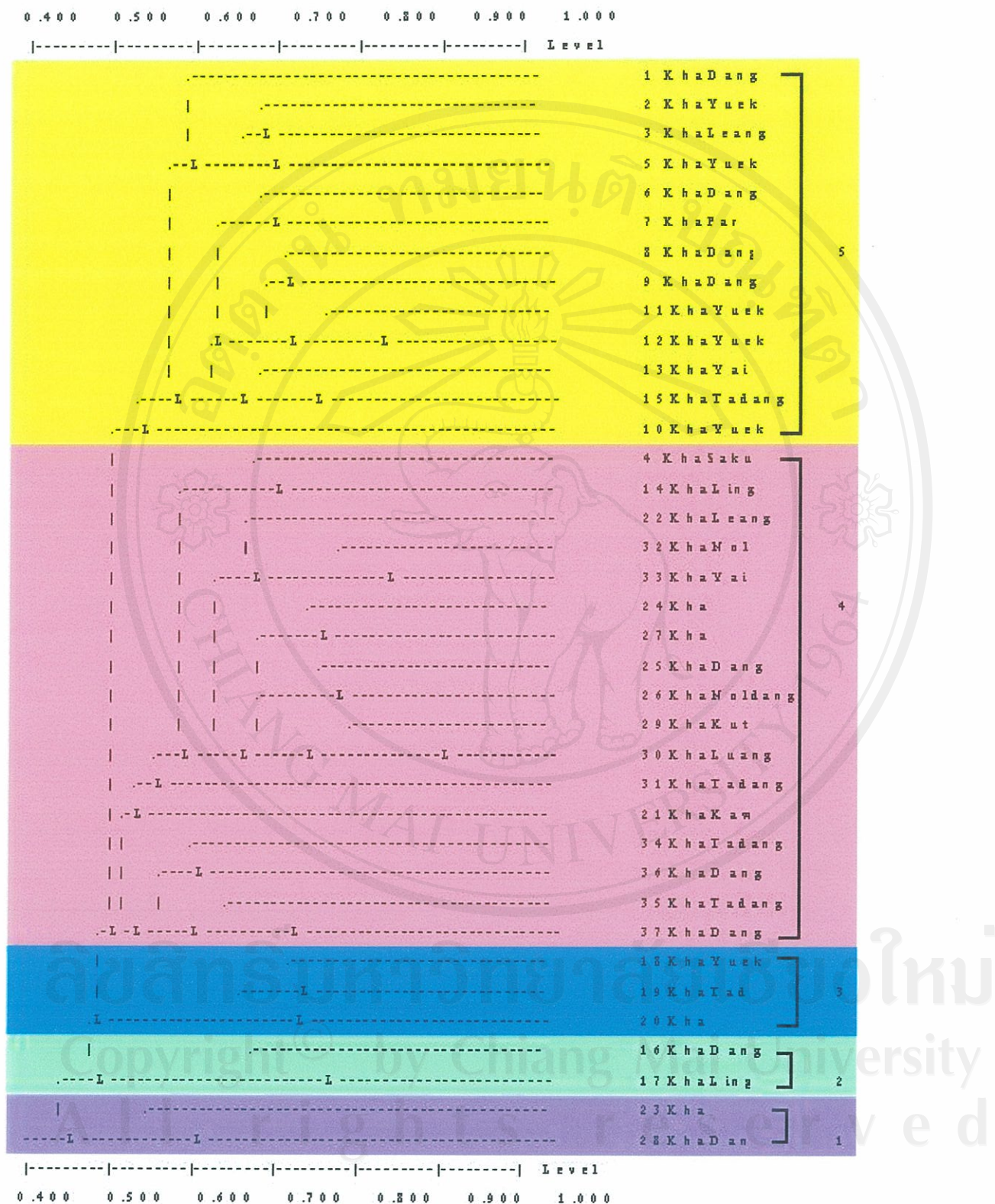


Figure 4.11 A dendrogram of 37 giant galanga accessions from Thailand based on RAPDs using UPGMA method.



Figure 4.11.1 A dendrogram of 37 giant galanga accessions from Thailand based on RAPDs

Using UPGMA method (Between clusters and type of galanga).

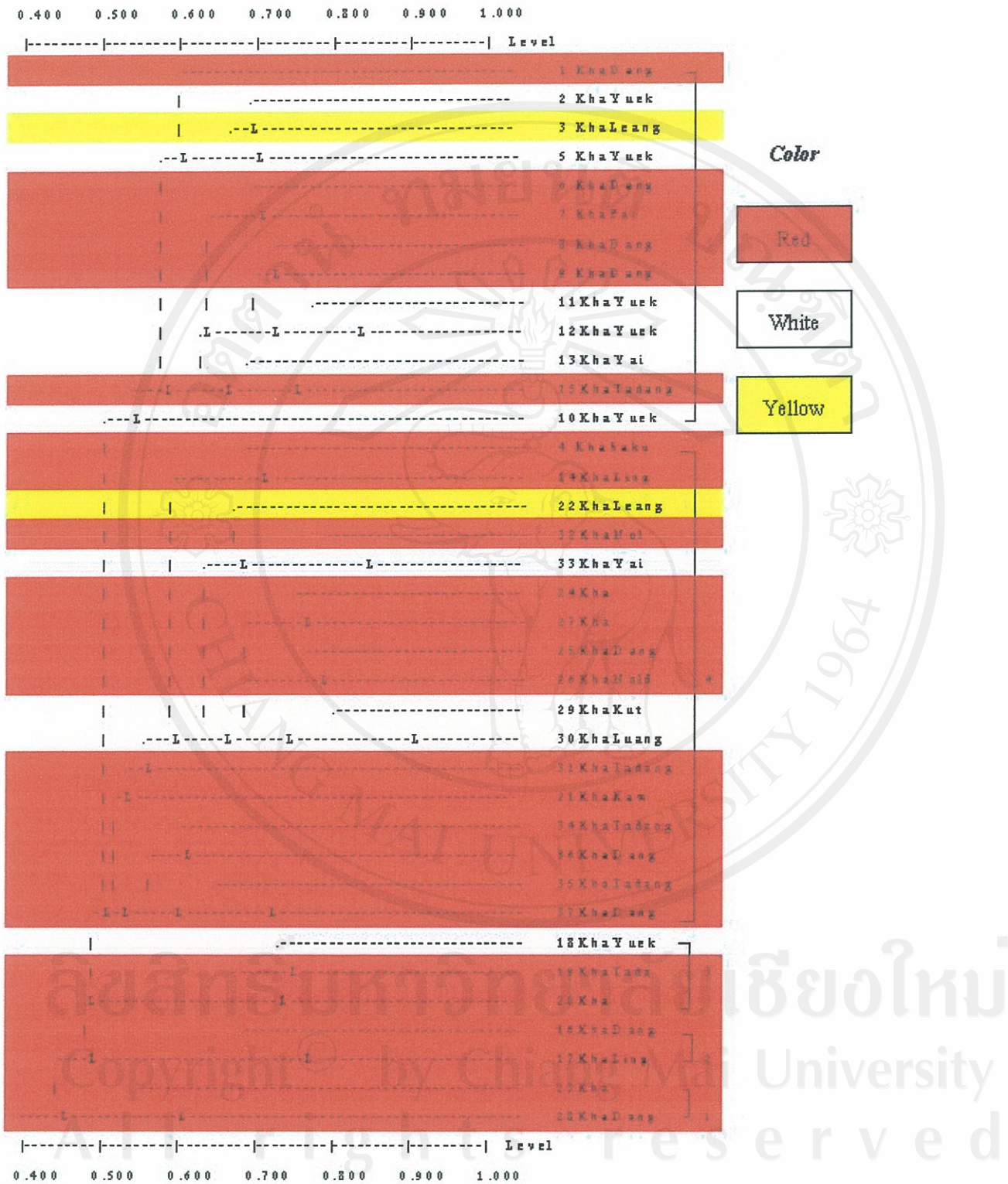


Figure 4.11.2 A dendrogram of 37 giant galanga accessions from Thailand based on RAPDs using UPGMA method (Between clusters and color of rhizomes).

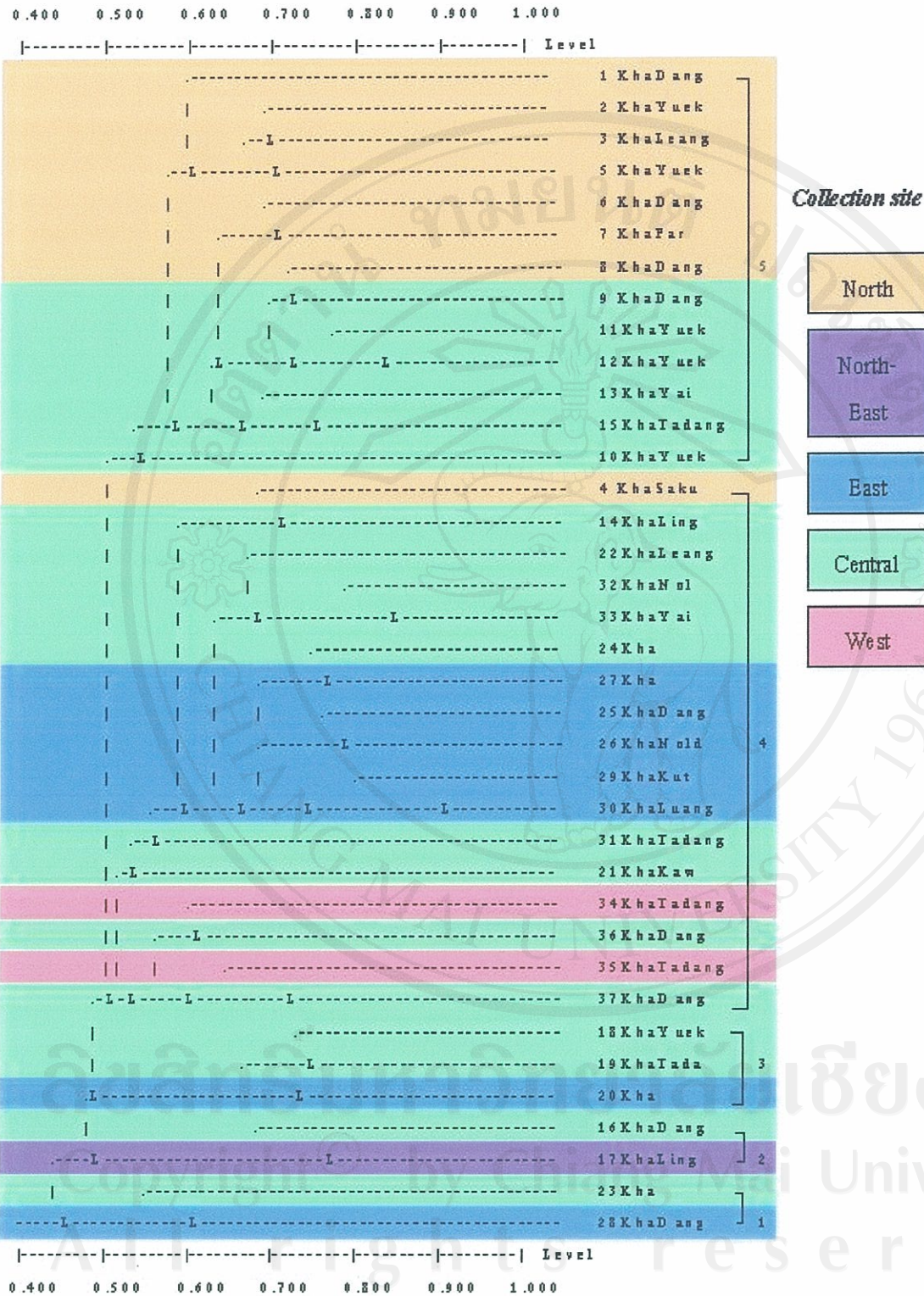


Figure 4.11.3 A dendrogram of 37 giant galanga accessions from Thailand based on RAPDs using UPGMA method (Between clusters and collection sites).

Analysis of anti-fungal agent

It was shown that 1'-acetoxychavicol acetate was the active ingredient in galanga crude extract, and had efficiency to control *Colletotrichum gloeosporioides* (Penz.) Sacc. (Jariyanusorn, 2002). By using GC-MS and antifungal efficiency studies, similar results were reported by Srisornkapol (1996) and Lertvirasawat (1997). Therefore, the quantity of anti-fungal agent (1'-acetoxychavicol acetate) in this study was referred to by the quantity of crude extract in each sample (Table 4.11).

Table 4.11 The clusters of giant galanga accessions and the quantity of the crude extract.

Cluster	Accession	Quantity of crude extract (%w/dw)
1	23 Kha (Bankrokanya, Samut Prakan)	4.66
	28 KhaDang (Nongbon, Srakaew)	2.62
2	16 KhaDang (Bansalaloi, Lop Buri)	4.18
	17 KhaLing (Khon Kaen)	6.17*
3	18 KhaYuek (Bansakaew, Kamphaengphet)	2.52
	19 KhaTadang (Banaoyklongthakhuy, Lop Buri)	5.39
	20 Kha (Takai, Chachoengsao)	4.98
4	KhaSaku (Khugtaphao, Uttaradit)	-
	14 KhaLing (Banyantar, Nakhon Sawan)	1.53#
	22 KhaLeang (Bankrokanya, Samut Prakan)	-
	32 KhaNol (Banna, Nakhon Nayok)	4.58
	33 KhaYai (Banna, Nakhon Nayok)	2.88
	24 Kha (Barpar, Samut Prakan)	3.18
	27 Kha (Lapsing, Chanthaburi)	6.37*
	25 KhaDang (Panumsarakam, Chachoengsao)	5.44*
	26 KhaNoldang (Nayararm, Rayong)	3.98
	29 KhaKut (Bantayag, Srakaew)	5.10
30 KhaLuang (Taham, Prachin Buri)	6.14*	

Table 4.11 (continued)

Cluster	Accession	Quantity of crude extract (%w/dw)
	31 KhaTadang (Banna, Nakhon Nayok)	4.03
	21 KhaKaw (Bankrokanya, Samut Prakan)	7.45*
4	34 KhaTadang (Jombuo, Rachaburi)	3.04
	36 KhaDang (Uthong, Suphan Buri)	4.13
	35 KhaTadang (Danmakhathia, Kanchanaburi)	5.16
	37 KhaDang (Pothong, Angthong)	4.37
	1 KhaDang (Khugtaphao, Uttaradit)	1.66#
	2 KhaYuek (Khugtaphao, Uttaradit)	1.68#
	3 KhaLeang (Khugtaphao, Uttaradit)	-
	5 KhaYuek (Banhuahad, Uttaradit)	1.50#
	6 KhaDang (Banhuahad, Uttaradit)	-
	7 KhaPar (Banhiha, Uttaradit)	-
5	8 KhaDang (Tron, Uttaradit)	1.85#
	9 KhaDang (Kosumpee, Kamphaengphet)	3.78
	11 KhaYuek (Bankang9, Nakhon Sawan)	2.10
	12 KhaYuek (Bankang3, Nakhon Sawan)	2.10
	13 KhaYai (Banyantar, Nakhon Sawan)	-
	15 KhaTadang (Bankardan, Nakhon Sawan)	-
	10 KhaYuek (Bansakaew3, Kamphaengphet)	-

Note: *: Top 5 of galanga accessions which had high crude extract quantity.

: Bottom 5 of galanga accessions which had low crude extract quantity.

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