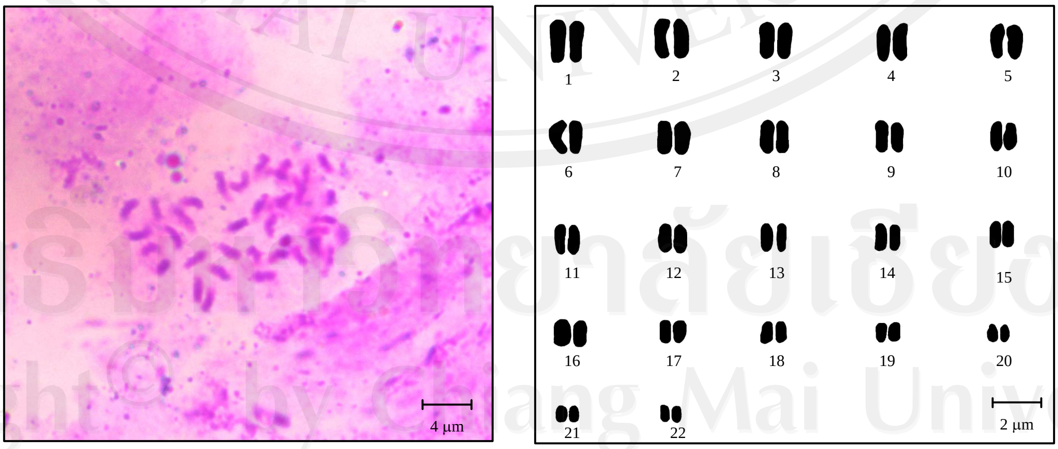
Table 64 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of PH Phak Plang Daeng chromosomes

Number	Ls (µm)	Ll (µm)	LT (µm)	RL	CI
1	0.923	1.075	1.998	0.111	0.538
2	0.730	1.233	1.963	0.109	0.628
3	0.825	0.875	1.700	0.094	0.515
4	0.675	1.013	1.688	0.094	0.600
5	0.778	0.900	1.678	0.093	0.537
6	0.800	0.858	1.658	0.092	0.517
7	0.775	0.833	1.608	0.089	0.518
8	0.545	0.988	1.533	0.085	0.644
9	0.555	0.928	1.483	0.082	0.626
10	0.675	0.790	1.465	0.081	0.539
11	0.480	0.758	1.238	0.069	0.612
12	0.400	0.703	1.103	0.061	0.637
13	0.388	0.660	1.048	0.058	0.630
14	0.378	0.633	1.010	0.056	0.626
15	0.368	0.553	0.920	0.051	0.601
16	0.328	0.580	0.908	0.050	0.639
17	0.313	0.550	0.863	0.048	0.638
18	0.283	0.545	0.828	0.046	0.659
19	0.260	0.478	0.738	0.041	0.647
20	0.225	0.328	0.553	0.031	0.593
21	0.203	0.285	0.488	0.027	0.585
22	0.190	0.273	0.463	0.026	0.589



A

B

Figure 140 Somatic chromosome (2n = 44) (A) and karyogram (B) of PH Phak Plang Daeng

The range of chromosome size and karyotypic formula of 2 varieties of *Basella alba* L. of all accessions collected from 8 provinces were summarized in Tables 65 to 68. The chromosome size of Phak Plang Khao ranged from 3.938 to 0.883 µm in length. The largest chromosome was that of LN sample and the smallest was of LP sample. The chromosome size of Phak Plang Daeng ranged from 3.940 to 0.463 µm in length with the largest chromosome being that of PY sample and the smallest of PH sample.

Table 65 Range of chromosome size concluded from samples of *Basella alba* L. variety Phak Plang Khao from different locations

Accession code	Size (µm)			LT ± sd (µm)	CI ± sd
	Large	Medium	Small		
CM	3.295-2.373	2.313-1.870	1.618-1.438	2.322 ± 0.517	0.629 ± 0.031
CR	2.885-2.195	1.880-1.473	1.138-1.110	1.976 ± 0.518	0.625 ± 0.035
LP	2.628-1.928	1.740-1.363	1.308-0.883	1.611 ± 0.494	0.605 ± 0.025
LN	3.938-2.610	2.425-2.150	1.875-1.225	2.326 ± 0.907	0.628 ± 0.033
MH	3.163-2.183	2.075-1.618	1.573-1.185	2.119 ± 0.540	0.658 ± 0.018
NA	3.785-2.738	2.545-1.933	1.803-1.648	2.446 ± 0.567	0.662 ± 0.035
PY	2.380-1.730	1.703-1.273	1.073	1.748 ± 0.359	0.570 ± 0.028
PH	2.675-2.048	1.950-1.343	1.293	1.892 ± 0.405	0.597 ± 0.020

Table 66 Range of chromosome size concluded from samples of *Basella alba* L. variety Phak Plang Daeng from different locations

Accession code	Size (µm)			LT ± sd (µm)	CI ± sd
	Large	Medium	Small		
CM	2.965-2.180	2.068-1.503	1.465-1.233	1.866 ± 0.471	0.580 ± 0.019
CR	2.158-1.488	1.428-1.090	1.055-0.798	1.413 ± 0.381	0.599 ± 0.024
LP	2.323-1.610	1.550-1.190	1.160-0.855	1.533 ± 0.406	0.645 ± 0.034
LN	2.353-1.505	1.485-1.218	1.135-0.643	1.399 ± 0.512	0.553 ± 0.040
NA	2.610-1.790	1.690-1.370	1.300-0.883	1.667 ± 0.503	0.630 ± 0.048
PY	3.940-2.695	2.558-2.103	1.955-1.250	2.338 ± 0.705	0.610 ± 0.059
PH	1.998-1.238	1.103-1.010	0.920-0.463	1.224 ± 0.474	0.596 ± 0.048

Table 67 Karyotypic formula of *Basella alba* L. variety Phak Plang Khao collected from different sites

Accession code	Karyotypic formula
CM	$L_4^{m} + L_{14}^{sm} + M_8^{sm} + M_6^{m} + S_6^{sm}$
CR	$L_6^{m} + L_{12}^{sm} + M_{14}^{sm} + M_2^{m} + S_2^{sm} + S_2^{m}$
LP	$L_{12}^{m} + L_2^{sm} + M_{10}^{sm} + S_{12}^{sm} + S_2^{m}$
LN	$L_2^{a} + L_{14}^{sm} + M_4^{sm} + S_{12}^{sm} + S_6^{m}$
MH	$L_{20}^{sm} + M_{10}^{sm} + S_8^{sm}$
NA	$L_{12}^{sm} + M_{18}^{sm} + M_2^{m} + S_2^{sm} + S_4^{a}$
PY	$L_{12}^{m} + L_8^{sm} + M_{16}^{m} + S_2^{m}$
PH	$L_{10}^{sm} + L_6^{m} + M_4^{sm} + M_{16}^{m} + S_2^{m}$

Table 68 Karyotypic formula of *Basella alba* L. variety Phak Plang Daeng collected from different sites

Accession code	Karyotypic formula
CM	$L_{12}^{m} + M_8^{sm} + M_{14}^{m} + S_{10}^{m}$
CR	$L_{18}^{m} + L_2^{sm} + M_{12}^{sm} + M_2^{m} + S_6^{m} + S_4^{m}$
LP	$L_6^{m} + L_{14}^{sm} + M_{14}^{sm} + S_8^{sm} + S_2^{a}$
LN	$L_{10}^{m} + L_6^{sm} + M_{12}^{m} + S_{16}^{m}$
NA	$L_6^{a} + L_{12}^{sm} + M_{10}^{m} + M_2^{sm} + S_{12}^{sm} + S_2^{m}$
PY	$L_8^{m} + L_6^{sm} + M_{10}^{sm} + M_4^{m} + S_8^{sm} + S_8^{m}$
PH	$L_{12}^{m} + L_{10}^{sm} + M_6^{sm} + S_{10}^{sm} + S_6^{m}$

From Tables 65-68, difference in size and shape of the chromosomes appeared in different plant accessions within variety of *Basella alba* L. can be observed for qualitative comparison.

6.3 *Gymnema inodorum* Decne.

6.3.1 Root tip preparation

Experiments on root-tip preparation were also done using the same procedure as with the other two species. The results are as follows:

6.3.1.1 Sampling

More dividing cells at metaphase stage were found in the root-tip tissue sampled at 10.00 a.m. than in other treatments. The sampling treatments of 7.00 a.m. and 8.00 including 9.00 a.m. yielded a large number of dividing cells at prophase and pro-metaphase stages, respectively. The anaphase cells occurred more in the treatments of 11.00 and 12.00 a.m. (Figure 141).

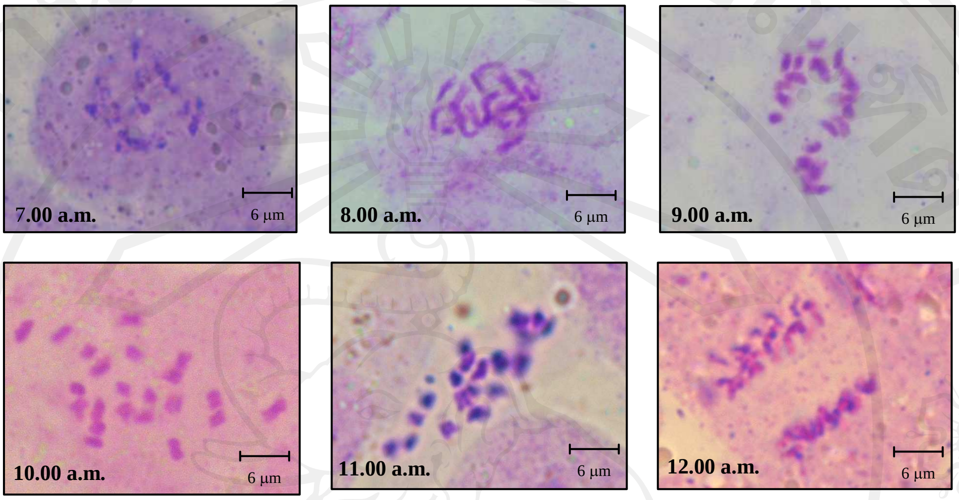


Figure 141 Root-tip chromosome of *Gymnema inodorum* Decne. sampled at different time

6.3.1.2 Pre-treatment

The best PDB treatment was that of 2 hours in the solution since it produced well contracted chromosomes, convenient for configuration evaluation (Figure 142).

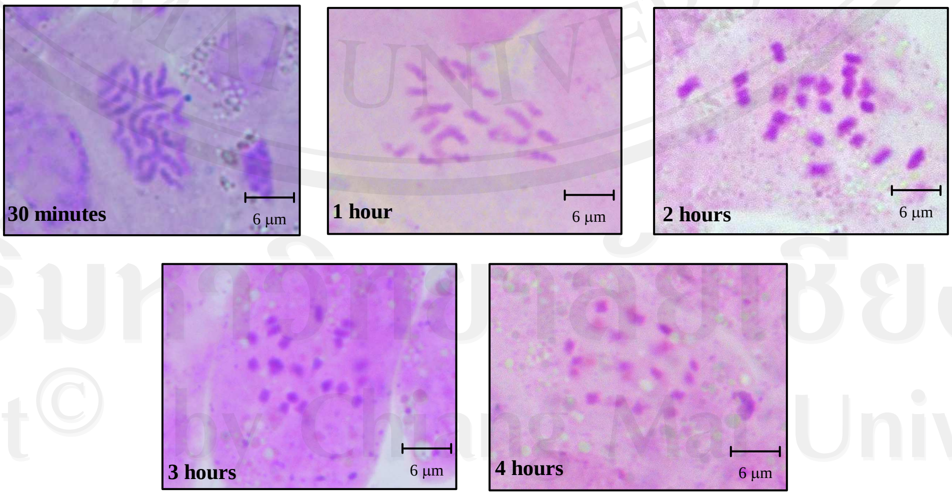


Figure 142 Root-tip chromosome of *Gymnema inodorum* Decne. pre-treated at different duration

6.3.1.3 Staining

Well stained cells with clear image were obtained from staining duration of 4 hours of which considered the best staining treatment as seen in Figure 143.

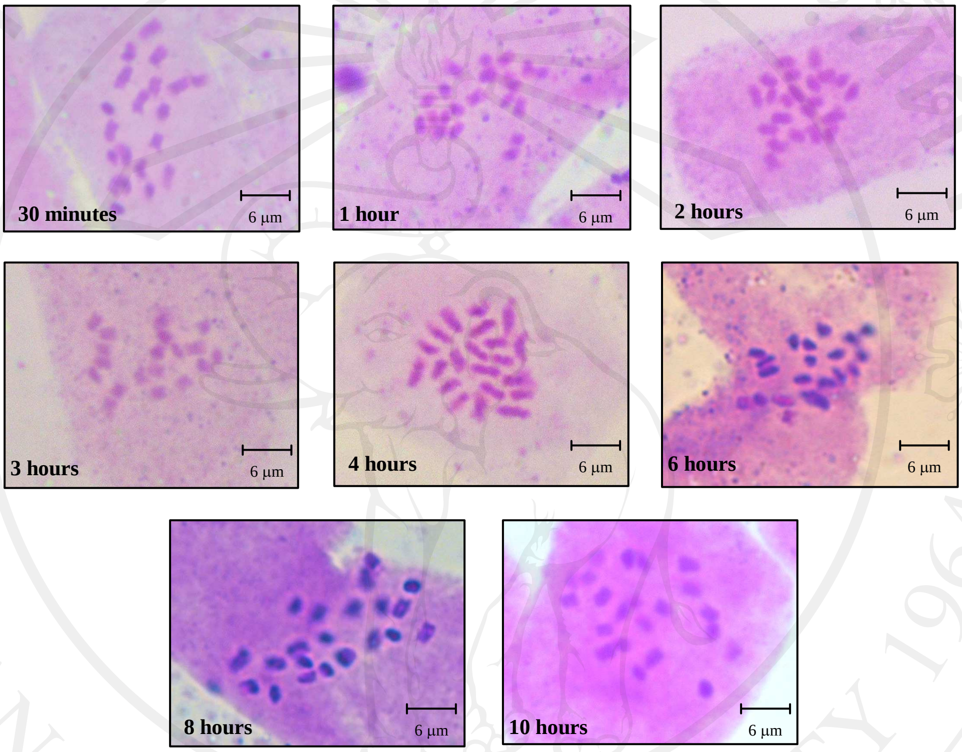


Figure 143 Root-tip chromosome of *Gymnema inodorum* Decne. stained for different duration

Appropriate technique of root-tip tissue preparation for chromosome investigation of *Gymnema inodorum* Decne. as concluded from the experimental results above, comprised sampling at 10.00 a.m., pre-treating for 2 hours in PDB and staining in carbol fuchsin for 4 hours. Chromosome counts from more than 10 cells per specimens revealed chromosome number of 2n = 22.

6.3.2 Karyotypic characteristics

Chromosome configuration and karyogram of *Gymnema inodorum* Decne. were studied following the same methods as carried out in the other two species. Results are as follows:

6.3.2.1 Chiang Mai (CM) samples

Chromosomes were classified into 3 groups. **Large** chromosomes were 1.263-0.946 µm in length, found in the 1st-6th pairs of metacentric chromosomes. **Medium** metacentric chromosomes were 0.945-0.632 µm long, appeared in the 7th-10th pairs. **Small** chromosomes of the 11th pair were 0.631-0.628 µm long, and metacentric (Tables 69 and 70; Figure 144). Karyotypic formula was L_{12}^{m} + M_{8}^{m} + S_{2}^{m}.

Table 69 Size (length) and type of CM *Gymnema inodorum* Decne. chromosomes

Large (1.263-0.946 µm)	**Medium (0.945-0.632 µm)**	**Small (0.631-0.628 µm)**
# 1 metacentric	# 7 metacentric	# 11 metacentric
# 2 metacentric	# 8 metacentric	
# 3 metacentric	# 9 metacentric	
# 4 metacentric	# 10 metacentric	
# 5 metacentric		
# 6 metacentric		

Table 70 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of CM *Gymnema inodorum* Decne. chromosomes

Number	**Ls (µm)**	**Ll (µm)**	**LT (µm)**	**RL**	**CI**
1	0.573	0.690	1.263	0.121	0.547
2	0.513	0.608	1.120	0.107	0.542
3	0.508	0.590	1.098	0.105	0.538
4	0.500	0.585	1.085	0.104	0.539
5	0.498	0.563	1.060	0.101	0.531
6	0.415	0.545	0.960	0.092	0.568
7	0.398	0.505	0.903	0.086	0.560
8	0.433	0.440	0.873	0.083	0.504
9	0.370	0.398	0.768	0.073	0.518
10	0.348	0.355	0.703	0.067	0.505
11	0.313	0.315	0.628	0.060	0.502

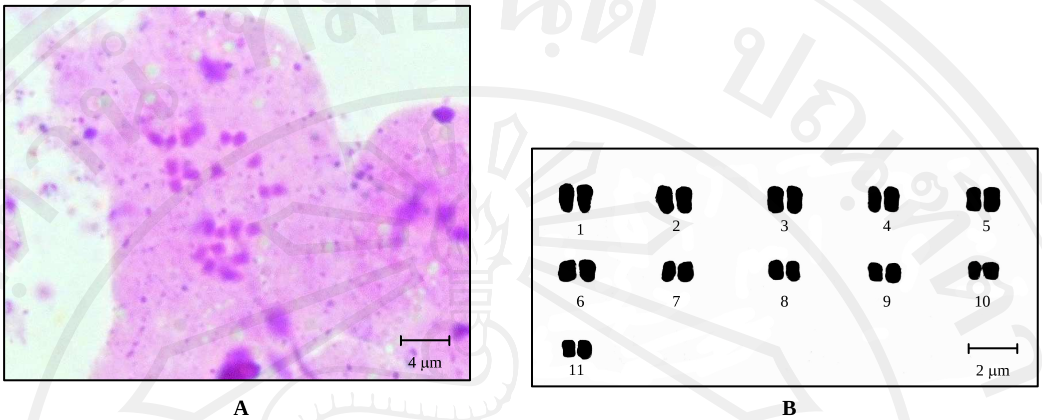


Figure 144 **Somatic chromosome (2n = 22) (A) and karyogram (B) of CM *Gymnema inodorum* Decne.**

6.3.2.2 Chiang Rai (CR) samples

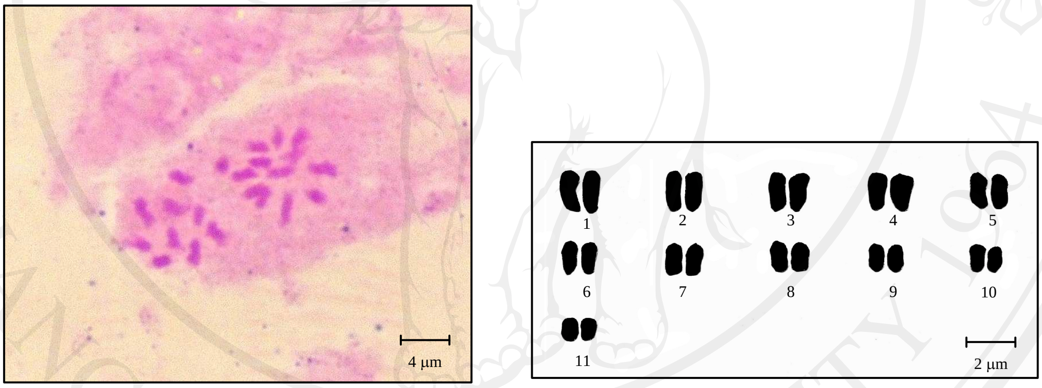
Chromosomes were divided into 3 groups. **Large** chromosomes were 1.910-1.430 µm in length, comprised 5 pairs. The 1st-3th and 5th were metacentric while the 4th was submetacentric. **Medium** chromosomes were 1.429-0.955 µm in length, found in the 6th-10th pairs, being metacentric. **Small** chromosomes of metacentric type of the 11th pair were 0.954-0.950 µm long (Tables 71 and 72; Figure 145). Karyotypic formula was $L_8^m + L_2^{sm} + M_{10}^m + S_2^m$.

Table 71 **Size (length) and type of CR *Gymnema inodorum* Decne. chromosomes**

Large **(1.910-1.430 µm)**	**Medium** **(1.429-0.955 µm)**	**Small** **(0.954-0.950 µm)**
# 1 metacentric	# 6 metacentric	# 11 metacentric
# 2 metacentric	# 7 metacentric	
# 3 metacentric	# 8 metacentric	
# 4 submetacentric	# 9 metacentric	
# 5 metacentric	# 10 metacentric	

Table 72 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of CR *Gymnema inodorum* Decne. chromosomes

Number	Ls (µm)	Ll (µm)	LT (µm)	RL	CI
1	0.793	1.118	1.910	0.120	0.585
2	0.763	0.958	1.720	0.108	0.557
3	0.688	1.005	1.693	0.106	0.594
4	0.668	1.003	1.670	0.105	0.600
5	0.683	0.833	1.515	0.095	0.550
6	0.635	0.793	1.428	0.090	0.555
7	0.553	0.798	1.350	0.085	0.591
8	0.640	0.673	1.313	0.082	0.512
9	0.585	0.655	1.240	0.078	0.528
10	0.560	0.600	1.160	0.073	0.517
11	0.468	0.483	0.950	0.060	0.508



A

B

Figure 145 Somatic chromosome (2n = 22) (A) and karyogram (B) of CR *Gymnema inodorum* Decne.

6.3.2.3 Lampang (LP) samples

Chromosomes were sorted out into 3 groups. **Large** chromosomes, having the length of 1.610-1.206 µm, were found in 7 pairs, the 1st-7th. They were all of metacentric type. **Medium** chromosomes were also metacentric and 1.205-0.805 µm long. They were in the 8th-10th pairs. **Small** chromosomes of the 11th pair, as well in metacentric pattern, were 0.804-0.803 µm long (Tables 73 and 74; Figure 146). Karyotypic formula was $L_{14}^{m} + M_{6}^{m} + S_{2}^{m}$.

Table 73 Size (length) and type of LP *Gymnema inodorum* Decne. chromosomes

Large (1.610-1.206 μm)	Medium (1.205-0.805 μm)	Small (0.804-0.803 μm)
# 1 metacentric	# 8 metacentric	# 11 metacentric
# 2 metacentric	# 9 metacentric	
# 3 metacentric	# 10 metacentric	
# 4 metacentric		
# 5 metacentric		
# 6 metacentric		
# 7 metacentric		

Table 74 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of LP *Gymnema inodorum* Decne. chromosomes

Number	Ls (μm)	Ll (μm)	LT (μm)	RL	CI
1	0.698	0.913	1.610	0.113	0.567
2	0.668	0.870	1.538	0.108	0.566
3	0.610	0.865	1.475	0.103	0.586
4	0.625	0.838	1.463	0.102	0.573
5	0.645	0.805	1.450	0.102	0.555
6	0.638	0.800	1.438	0.101	0.557
7	0.613	0.625	1.238	0.087	0.505
8	0.568	0.580	1.148	0.080	0.505
9	0.525	0.563	1.088	0.076	0.517
10	0.490	0.538	1.028	0.072	0.523
11	0.365	0.438	0.803	0.056	0.545

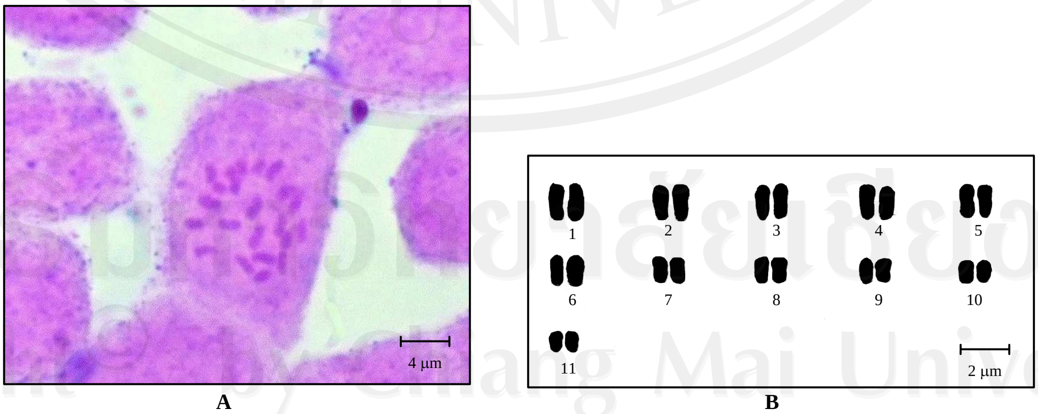


Figure 146 Somatic chromosome (2n = 22) (A) and karyogram (B) of LP *Gymnema inodorum* Decne.

6.3.2.4 Lamphun (LN) samples

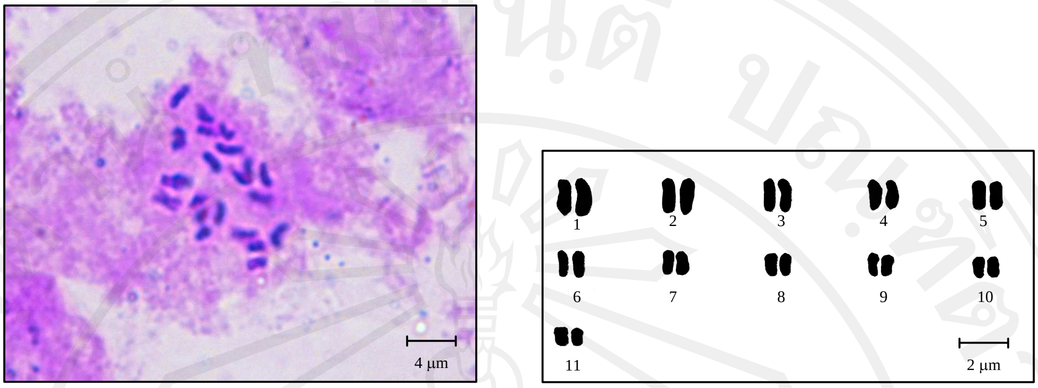
Chromosomes were grouped in three. **Large** chromosomes were 1.550-1.154 µm in length. They were those of submetacentric, belonging to the 1st and 2nd pairs, and metacentric of the 3rd-6th. **Medium** chromosomes were 1.153-0.775 µm long, found in the 7th-10th pairs, being metacentric. **Small** chromosomes were metacentric and 0.774-0.758 µm long in the 11th pair (Tables 75 and 76; Figure 147). Karyotypic formula was $L_4^{sm} + L_8^{m} + M_8^{m} + S_2^{m}$.

Table 75 Size (length) and type of LN *Gymnema inodorum* Decne. chromosomes

Large **(1.550-1.154 µm)**	**Medium** **(1.153-0.775 µm)**	**Small** **(0.774-0.758 µm)**
# 1 submetacentric	# 7 metacentric	# 11 metacentric
# 2 submetacentric	# 8 metacentric	
# 3 metacentric	# 9 metacentric	
# 4 metacentric	# 10 metacentric	
# 5 metacentric		
# 6 metacentric		

Table 76 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of LN *Gymnema inodorum* Decne. chromosomes

Number	Ls (µm)	Ll (µm)	LT (µm)	RL	CI
1	0.590	0.960	1.550	0.120	0.619
2	0.568	0.943	1.510	0.117	0.624
3	0.605	0.870	1.475	0.114	0.590
4	0.575	0.750	1.325	0.103	0.566
5	0.558	0.628	1.185	0.092	0.530
6	0.545	0.628	1.173	0.091	0.535
7	0.503	0.565	1.068	0.083	0.529
8	0.470	0.528	0.998	0.077	0.529
9	0.488	0.500	0.988	0.077	0.506
10	0.398	0.463	0.860	0.067	0.538
11	0.373	0.385	0.758	0.059	0.508



A B

Figure 147 **Somatic chromosome (2n = 22) (A) and karyogram (B) of LN *Gymnema inodorum* Decne.**

6.3.2.5 Mae Hong Son (MH) samples

Chromosomes were allocated into 3 groups. **Large** chromosomes were 1.545-1.158 μm long and metacentric, in the 1st-6th pairs. **Medium** chromosomes, being metacentric, were 1.157-0.772 μm in length, found in the 7th-10th pairs. **Small** chromosomes were metacentric and 0.771-0.770 μm long. They were in the 11th pair (Tables 77 and 78; Figure 148). Karyotypic formula was $L_{10}^{m} + M_{10}^{m} + S_{2}^{m}$.

Table 77 **Size (length) and type of MH *Gymnema inodorum* Decne. chromosomes**

Large **(1.545-1.158 μm)**	**Medium** **(1.157-0.772 μm)**	**Small** **(0.771-0.770 μm)**
# 1 metacentric	# 6 metacentric	# 11 metacentric
# 2 metacentric	# 7 metacentric	
# 3 metacentric	# 8 metacentric	
# 4 metacentric	# 9 metacentric	
# 5 metacentric	# 10 metacentric	

Table 78 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of MH *Gymnema inodorum* Decne. chromosomes

Number	Ls (μm)	Ll (μm)	LT (μm)	RL	CI
1	0.698	0.848	1.545	0.124	0.549
2	0.648	0.763	1.410	0.113	0.541
3	0.518	0.730	1.248	0.100	0.585
4	0.538	0.700	1.238	0.100	0.566
5	0.525	0.650	1.175	0.095	0.553
6	0.525	0.608	1.133	0.091	0.536
7	0.515	0.525	1.040	0.084	0.505
8	0.475	0.515	0.990	0.080	0.520
9	0.488	0.490	0.978	0.079	0.501
10	0.450	0.455	0.905	0.073	0.503
11	0.383	0.388	0.770	0.062	0.503

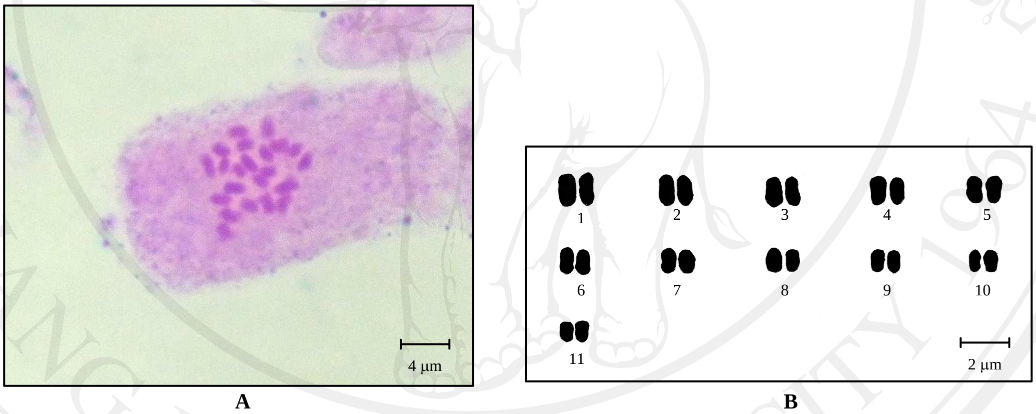


Figure 148 Somatic chromosome (2n = 22) (A) and karyogram (B) of MH *Gymnema inodorum* Decne.

6.3.2.6 Nan (NA) samples

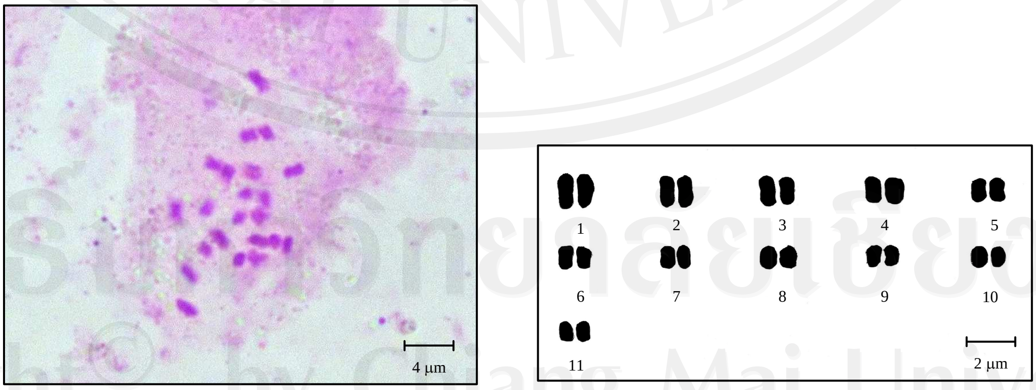
Chromosomes were grouped in three. **Large** chromosomes were 1.613-1.206 μm in length in the 1st-3rd pairs. They were metacentric in type. **Medium** chromosomes found in the 4th-10th pairs were metacentric and 1.205-0.806 μm long. **Small** chromosomes of the 11th pair were of metacentric type and 0.805-0.800 μm long (Tables 79 and 80; Figure 149). Karyotypic formula was $L_6^m + M_{14}^m + S_2^m$.

Table 79 Size (length) and type of NA *Gymnema inodorum* Decne. chromosomes

Large (1.613-1.206 µm)	Medium (1.205-0.806 µm)	Small (0.805-0.800 µm)
# 1 metacentric	# 4 metacentric	# 11 metacentric
# 2 metacentric	# 5 metacentric	
# 3 metacentric	# 6 metacentric	
	# 7 metacentric	
	# 8 metacentric	
	# 9 metacentric	
	# 10 metacentric	

Table 80 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of NA *Gymnema inodorum* Decne. chromosomes

Number	Ls (µm)	Ll (µm)	LT (µm)	RL	CI
1	0.800	0.813	1.613	0.133	0.504
2	0.665	0.765	1.430	0.118	0.535
3	0.650	0.710	1.360	0.113	0.522
4	0.585	0.618	1.203	0.100	0.514
5	0.473	0.568	1.040	0.086	0.546
6	0.460	0.550	1.010	0.084	0.545
7	0.453	0.545	0.998	0.083	0.546
8	0.460	0.483	0.943	0.078	0.512
9	0.418	0.430	0.848	0.070	0.507
10	0.410	0.428	0.838	0.069	0.510
11	0.385	0.415	0.800	0.066	0.519



A B

Figure 149 Somatic chromosome (2n = 22) (A) and karyogram (B) of NA *Gymnema inodorum* Decne.

6.3.2.7 Phayao (PY) samples

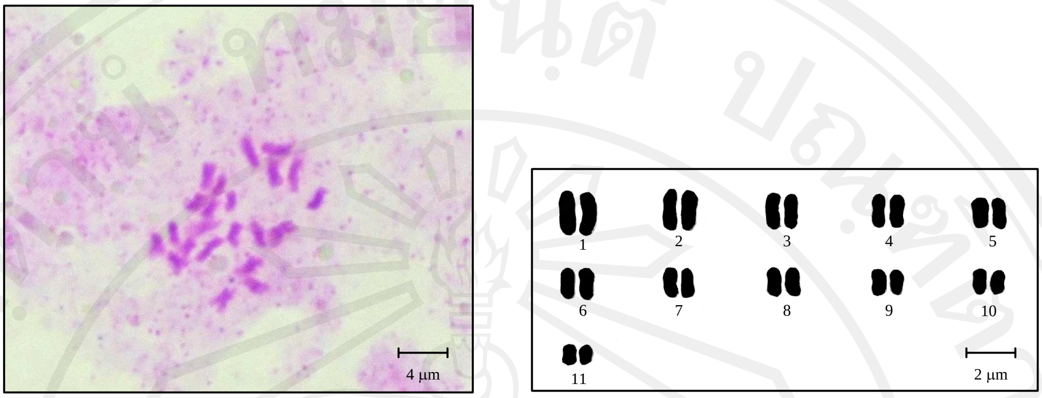
Chromosomes were classified into 3 groups. **Large** chromosomes were 2.008-1.463 µm long, and metacentric, appeared in the 1st-3rd pairs. **Medium** chromosomes were 1.462-1.004 µm in length, composed of 7 pairs, the 4th, 5th, 7th-10th being metacentric while the 6th submetacentric. **Small** chromosomes of the 11th pair were 1.003-0.918 µm long and of metacentric type (Tables 81 and 82; Figure 150). Karyotypic formula was $L_6^m + M_{12}^m + M_2^{sm} + S_2^m$.

Table 81 Size (length) and type of PY *Gymnema inodorum* Decne. chromosomes

Large (2.008-1.463 µm)	Medium (1.462-1.004 µm)	Small (1.003-0.918 µm)
# 1 metacentric	# 4 metacentric	# 11 metacentric
# 2 metacentric	# 5 metacentric	
# 3 metacentric	# 6 submetacentric	
	# 7 metacentric	
	# 8 metacentric	
	# 9 metacentric	
	# 10 metacentric	

Table 82 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of PY *Gymnema inodorum* Decne. chromosomes

Number	Ls (µm)	Ll (µm)	LT (µm)	RL	CI
1	0.805	1.203	2.008	0.131	0.599
2	0.838	1.023	1.860	0.122	0.550
3	0.720	0.868	1.588	0.104	0.546
4	0.675	0.783	1.458	0.095	0.537
5	0.583	0.798	1.380	0.090	0.578
6	0.520	0.790	1.310	0.086	0.603
7	0.585	0.690	1.275	0.083	0.541
8	0.578	0.673	1.250	0.082	0.538
9	0.533	0.625	1.158	0.076	0.540
10	0.513	0.560	1.073	0.070	0.522
11	0.438	0.480	0.918	0.060	0.523



A B

Figure 150 Somatic chromosome (2n = 22) (A) and karyogram (B) of PY *Gymnema inodorum* Decne.

6.3.2.8 Phrae (PH) samples

Chromosomes were sorted out into 3 groups. **Large** chromosomes were 1.598-1.196 µm long, included metacentric chromosomes of the 1st-5th pairs. **Medium** chromosomes were 1.195-0.799 µm long, occurred in the 6th -10th pairs. They were metacentric chromosomes. **Small** chromosomes of metacentric type, having the length of 0.798-0.793 µm, were found in the 11th pair (Tables 83 and 84; Figure 151). Karyotypic formula was $L_{10}^{m} + M_{10}^{m} + S_{2}^{m}$.

Table 83 Size (length) and type of PH *Gymnema inodorum* Decne. chromosomes

Large (1.598-1.196 µm)	Medium (1.195-0.799 µm)	Small (0.798-0.793 µm)
# 1 metacentric	# 6 metacentric	# 11 metacentric
# 2 metacentric	# 7 metacentric	
# 3 metacentric	# 8 metacentric	
# 4 metacentric	# 9 metacentric	
# 5 metacentric	# 10 metacentric	

Table 84 Average length of short arm (Ls) and long arm (Ll), chromosome length (LT), relative length (RL) and centromeric index (CI) of PH *Gymnema inodorum* Decne. chromosomes

Number	Ls (μm)	Ll (μm)	LT (μm)	RL	CI
1	0.660	0.938	1.598	0.125	0.587
2	0.615	0.788	1.403	0.110	0.561
3	0.658	0.733	1.390	0.109	0.527
4	0.610	0.723	1.333	0.104	0.542
5	0.615	0.643	1.258	0.098	0.511
6	0.560	0.590	1.150	0.090	0.513
7	0.490	0.520	1.010	0.079	0.515
8	0.488	0.518	1.005	0.079	0.515
9	0.448	0.483	0.930	0.073	0.519
10	0.415	0.493	0.908	0.071	0.543
11	0.388	0.405	0.793	0.062	0.511

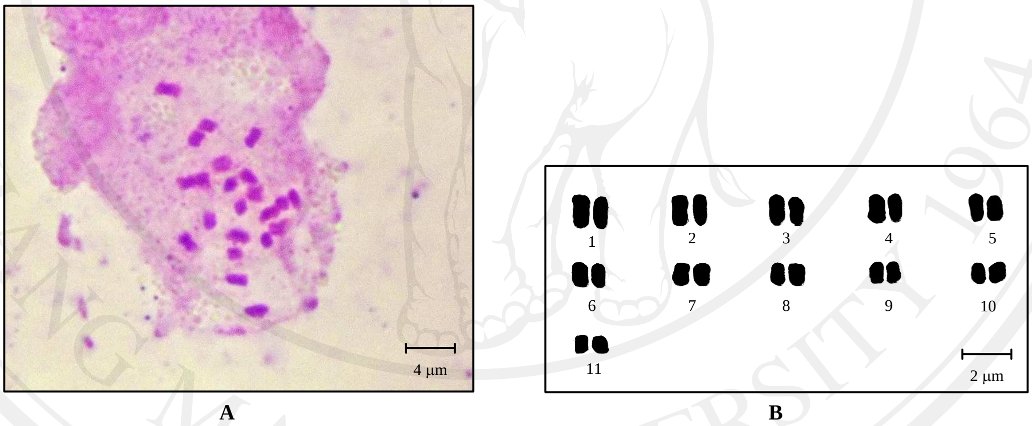


Figure 151 Somatic chromosome (2n = 22) (A) and karyogram (B) of PH *Gymnema inodorum* Decne.

The range of chromosome size and the karyotypic formula obtained from *Gymnema inodorum* Decne. accessions collected from 8 provinces were summarized in Tables 85 and 86, respectively. Chromosome size ranged from 2.008 to 0.628 μm with the largest belonging to the PY sample and the smallest in CM sample.

Table 85 Range of chromosome size concluded from samples of *Gymnema inodorum* Decne. from different locations

Accession code	Size (μm)			LT ± sd (μm)	CI ± sd
	Large	Medium	Small		
CM	1.263-0.960	0.903-0.703	0.628	0.951 ± 0.196	0.532 ± 0.023
CR	1.910-1.515	1.428-1.160	0.950	1.450 ± 0.283	0.554 ± 0.035
LP	1.610-1.238	1.148-1.028	0.803	1.298 ± 0.254	0.545 ± 0.028
LN	1.550-1.173	1.068-0.860	0.758	1.172 ± 0.268	0.552 ± 0.042
MH	1.545-1.175	1.133-0.905	0.770	1.130 ± 0.226	0.533 ± 0.029
NA	1.613-1.360	1.203-0.838	0.800	1.098 ± 0.268	0.524 ± 0.016
PY	2.008-1.588	1.458-1.073	0.918	1.389 ± 0.326	0.552 ± 0.028
PH	1.598-1.258	1.150-0.908	0.793	1.162 ± 0.253	0.531 ± 0.025

Table 86 Karyotypic formula of *Gymnema inodorum* Decne. collected from different sites

Accession code	Karyotypic formula
CM	$L_{12}^{m} + M_{8}^{m} + S_{2}^{m}$
CR	$L_{8}^{m} + L_{2}^{sm} + M_{10}^{m} + S_{2}^{m}$
LP	$L_{14}^{m} + M_{6}^{m} + S_{2}^{m}$
LN	$L_{4}^{sm} + L_{8}^{m} + M_{8}^{m} + S_{2}^{m}$
MH	$L_{10}^{m} + M_{10}^{m} + S_{2}^{m}$
NA	$L_{6}^{m} + M_{14}^{m} + S_{2}^{m}$
PY	$L_{6}^{m} + M_{12}^{m} + M_{2}^{sm} + S_{2}^{m}$
PH	$L_{10}^{m} + M_{10}^{m} + S_{2}^{m}$

Tabulated data shown in Tables 85 and 86 provided useful information for relatedness comparison of *Gymnema inodorum* Decne. accessions located in different sites of the 8 surveyed provinces.

7. Isozyme pattern

Isozyme patterns of individual plant species were investigated via polyacrylamide gel electrophoresis technique proposed by Schmidt (1980), Shields *et al*. (1986) and Chokthaweepanich (2002) involving 8 enzyme systems, i.e. acid phosphatase, esterase, glucose dehydrogenase, glutamate oxaloacetate transminase, leucine aminopeptidase, malate dehydrogenase, peroxidase and shikimate dehydrogenase. The plant proteins used for determination were extracted from the leaves of mature plants grown in cultivation plots.

Banding patterns and zymograms resulting from individual enzyme system recorded in separate palnt species were gathered, analysed and evaluated to produce dendrograms of which exhibited genetical relationship of the accessions within species. Results are as follows:

7.1 *Peliosanthes teta* Andr.

A total of 72 samples of *Peliosanthes teta* Andr. accessions were taken and designated with numerical sample codes for isozyme pattern investigations. They were allocated according to the provinces of collection being 1-6 for Chiang Mai samples, 7-17 for Chiang Rai samples, 18-33 for Lampang samples, 34-46 for Lamphun samples, 47-48 for Mae Hong Son samples, 49-54 for Nan samples, 55-62 for Phayao samples and 63-72 for Phrae samples. Banding patterns and zymograms obtained from separate enzyme system are shown below.

7.1.1 Acid phosphatase (ACP)

Six band types were found at the Rf of 0.164, 0.200, 0.264, 0.336, 0.445 and 0.527 with the band thickness of 2-4 mm from ACP enzyme system. Each sample was seen with 1-4 bands (Figures 152 and 153). The whole samples were allocated into 16 groups, as shown in Table 87.

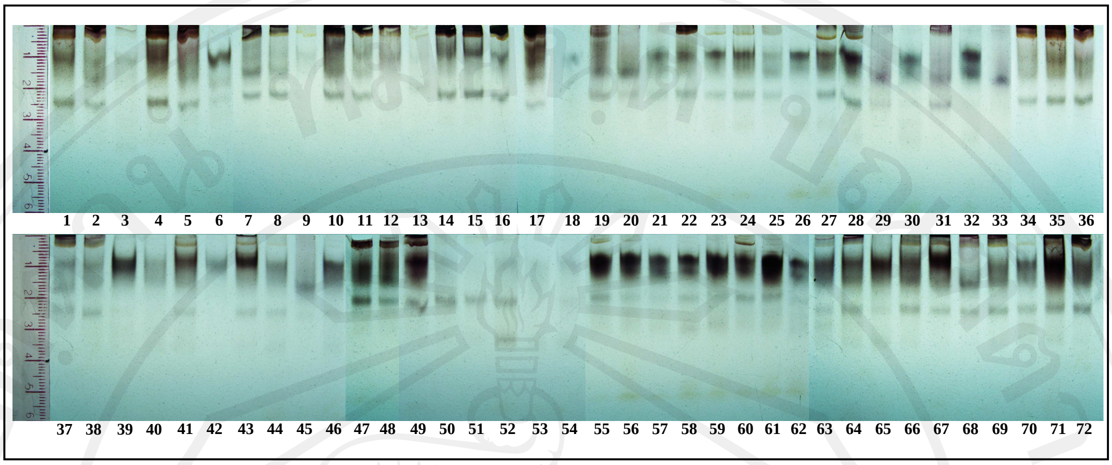


Figure 152 ACP banding pattern of *Peliosanthes teta* Andr.

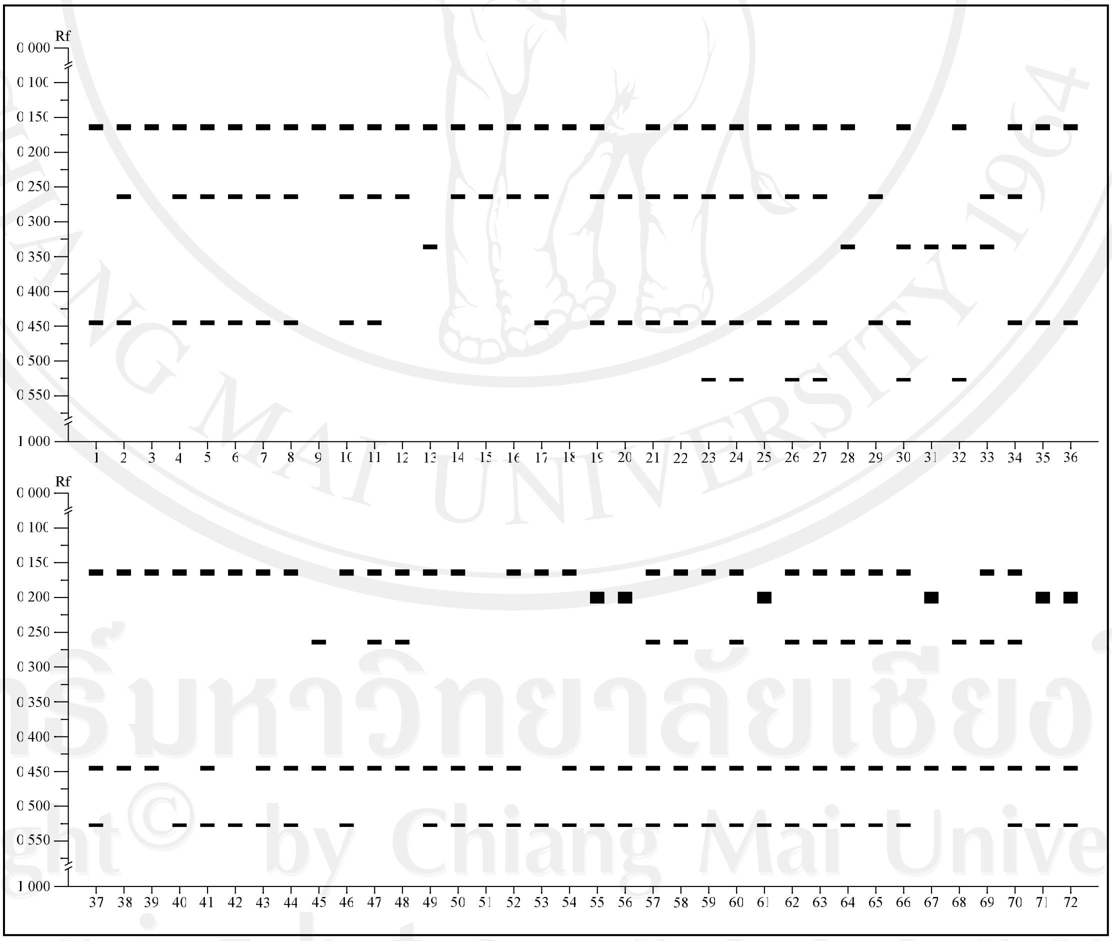


Figure 153 ACP zymogram of *Peliosanthes teta* Andr.

Table 87 ACP banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)]						Sample code
		0.164 [3]	0.200 [4]	0.264 [2]	0.336 [2]	0.445 [2]	0.527 [2]	
A	2	✓				✓		1, 35, 36, 38, 39
B	3	✓		✓		✓		2, 4, 5, 6, 7, 8, 10, 11, 17, 19, 21, 22, 25, 34, 47, 48, 69
C	1	✓						3, 9, 18
D	2	✓		✓				12, 14, 15, 16
E	2	✓			✓			13, 28
F	2			✓		✓		20, 29, 45, 68
G	4	✓		✓		✓	✓	23, 24, 26, 27, 57, 58, 60, 62, 63, 64, 65, 66, 70
H	4	✓			✓	✓	✓	30
I	1					✓		31
J	3	✓			✓		✓	32
K	2			✓	✓			33
L	3	✓				✓	✓	37, 41, 43, 44, 46, 49, 50, 52, 54, 59
M	2	✓					✓	40, 42, 53
N	2					✓	✓	51
O	3		✓			✓	✓	55, 56, 61, 71, 72
P	2		✓			✓		67

7.1.2 Esterase (EST)

Eight Rf bands were produced from EST enzyme system with various distance of 0.109, 0.136, 0.164, 0.236, 0.255, 0.273, 0.364 and 0.764, having the band thickness of 2-5 mm (Figures 154 and 155). One to three band types appeared in each sample. The samples were classified into 9 groups, accordingly, as shown in Table 88.

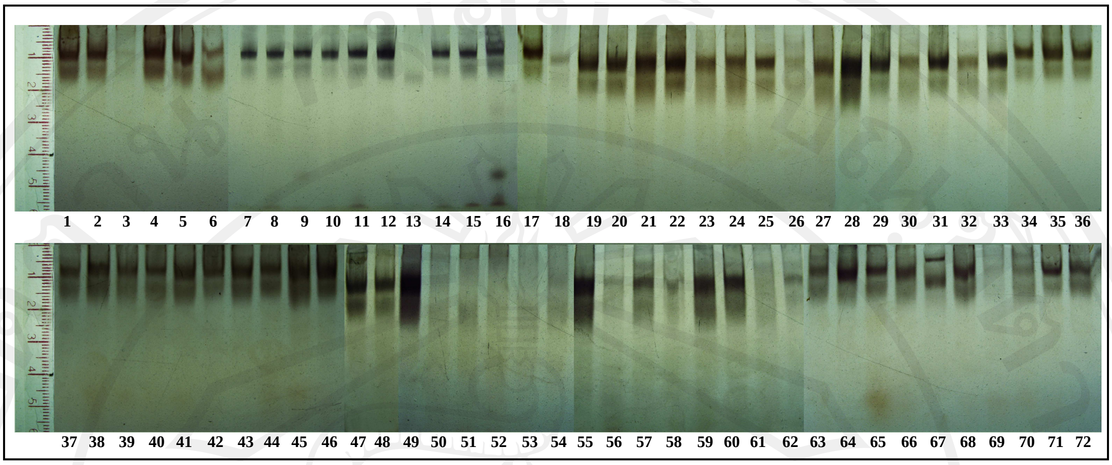


Figure 154 **EST banding pattern of *Peliosanthes teta* Andr.**

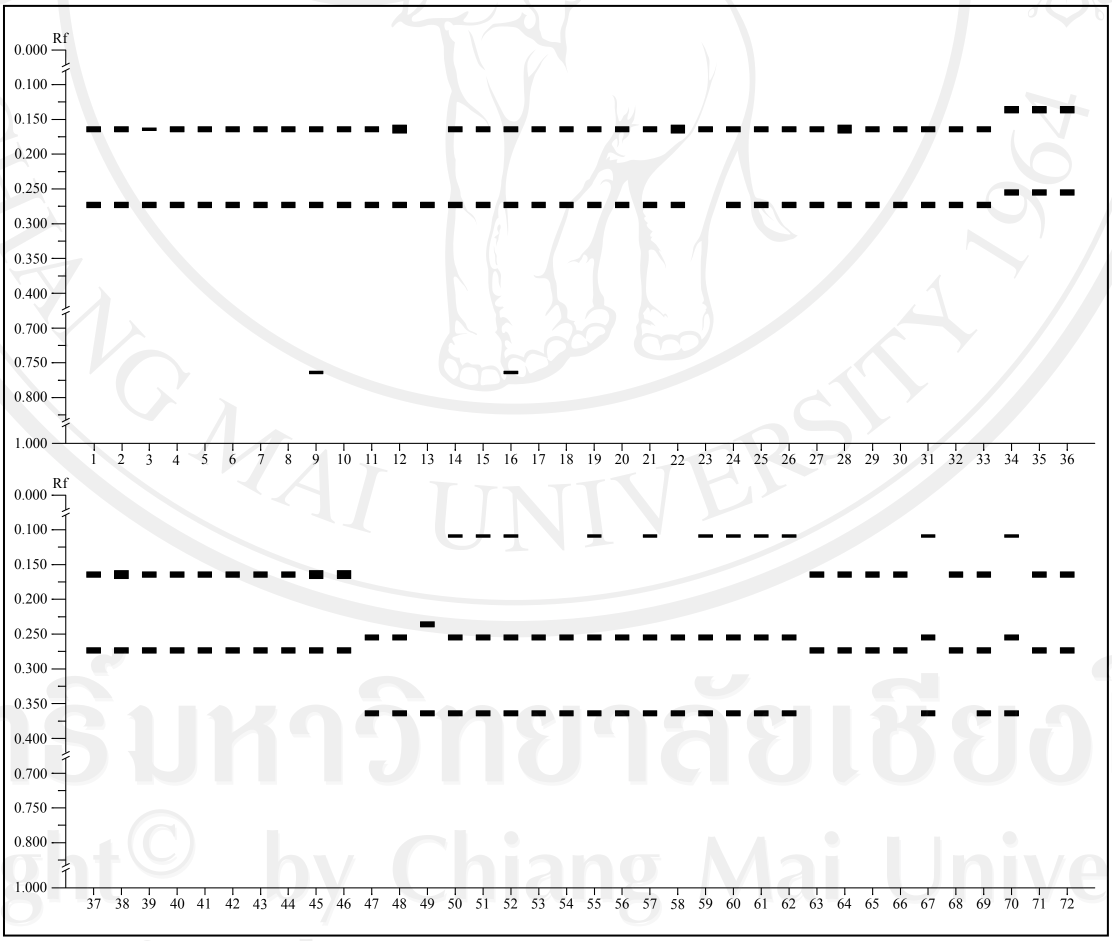


Figure 155 **EST zymogram of *Peliosanthes teta* Andr.**

Table 88 EST banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)]								Sample code
		0.109 [2]	0.136 [5]	0.164 [2,4,5]	0.236 [4]	0.255 [4]	0.273 [4]	0.364 [4]	0.764 [2]	
A	2			✓			✓			1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 14, 15, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31,32, 33, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 63, 64, 65, 66, 68, 71, 72
B	3			✓			✓		✓	9, 16
C	1						✓			13
D	1			✓						23
E	2		✓			✓				34, 35, 36
F	2					✓		✓		47, 48, 53, 54, 56, 58
G	2				✓			✓		49
H	3	✓				✓		✓		50, 51, 52, 55, 57, 59, 60, 61, 62, 67, 70
I	3			✓			✓	✓		69

7.1.3 Glucose dehydrogenase (GDH)

GDH enzyme system failed to produce banding from extracted samples.

7.1.4 Glutamate oxaloacetate transminase (GOT)

There were 6 band types shown in GOT gel, at the Rf of 0.264, 0.300, 0.436, 0.445, 0.600 and 0.618 with the thickness of 2-5 mm (Figures 156 and 157). Each sample showed 2-3 band types. The samples were sorted out into 5 groups, as tabulated in Table 89, according to the banding patterns.

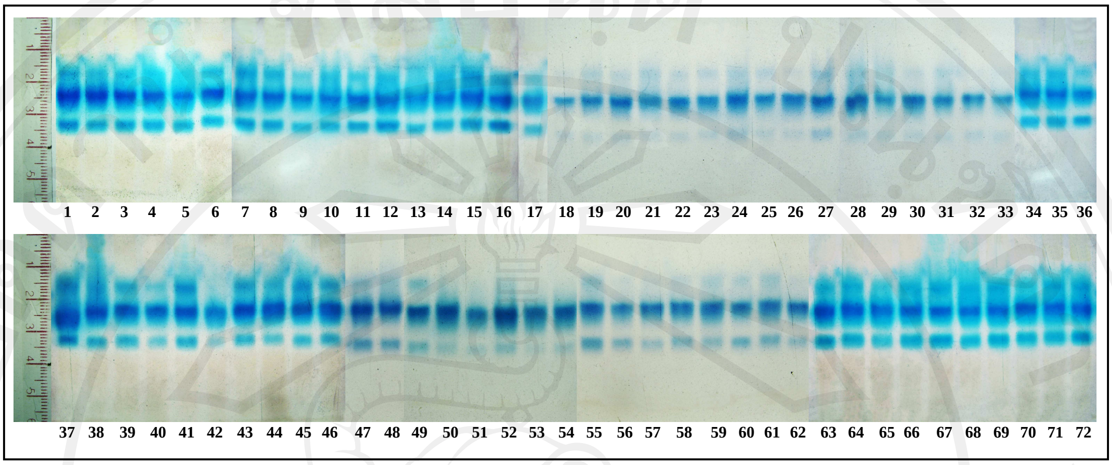


Figure 156 GOT banding pattern of *Peliosanthes teta* Andr.

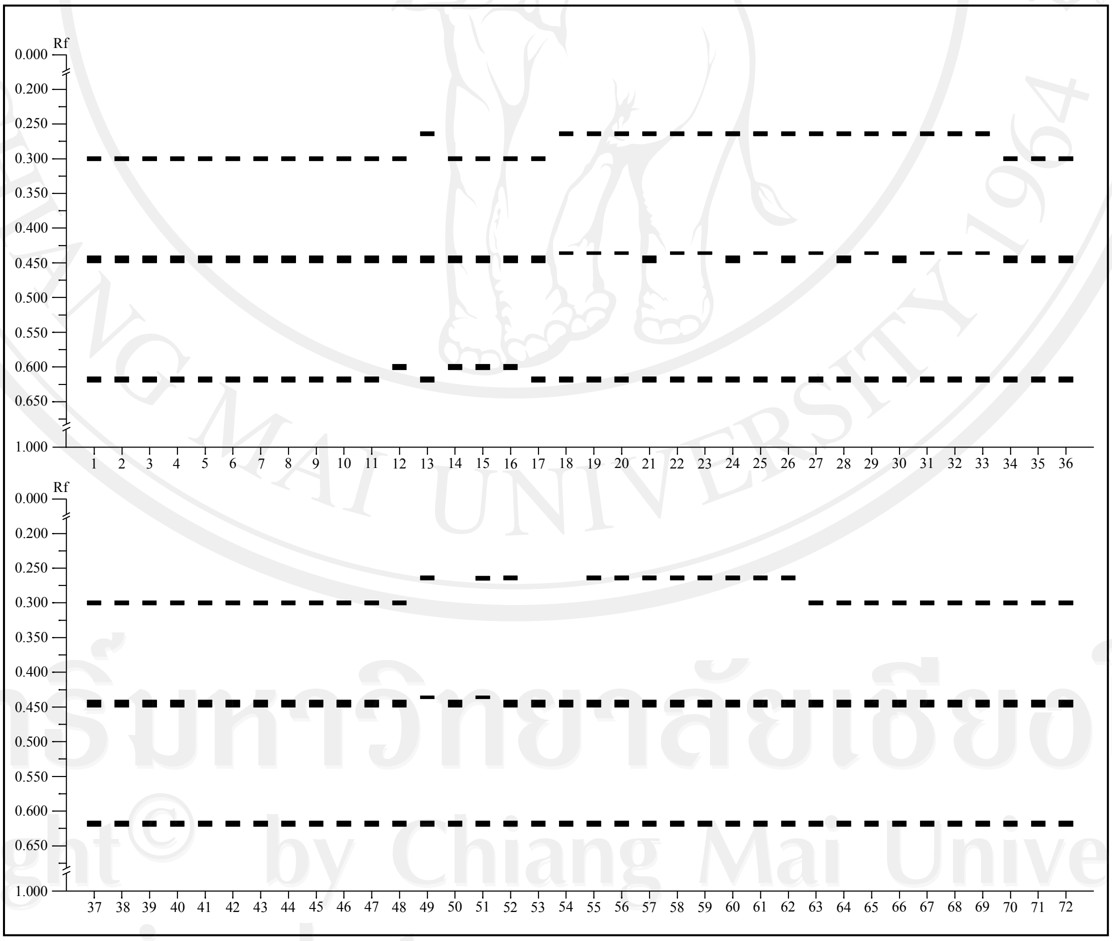


Figure 157 GOT zymogram of *Peliosanthes teta* Andr.

Table 89 GOT banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)]						Sample code
		0.264 [3]	0.300 [3]	0.436 [2]	0.445 [5]	0.600 [4]	0.618 [4]	
A	3		✓		✓		✓	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72
B	3		✓		✓	✓		12, 14, 15, 16
C	3	✓			✓		✓	13, 21, 24, 26, 28, 30, 52, 55, 56, 57, 58, 59, 60, 61, 62
D	3	✓		✓			✓	18, 19, 20, 22, 23, 25, 27, 29, 31, 32, 33, 49, 51
E	2				✓		✓	50, 53, 54

7.1.5 Leucine aminopeptidase (LAP)

Different 3 bands were formed in LAP gel at the Rf of 0.545, 0.564 and 0.655, with the band thickness of 2-6 mm (Figures 158 and 159). Each sample expressed 1-2 band types. The whole set of samples was then classified into 3 groups, as presented in Table 90.

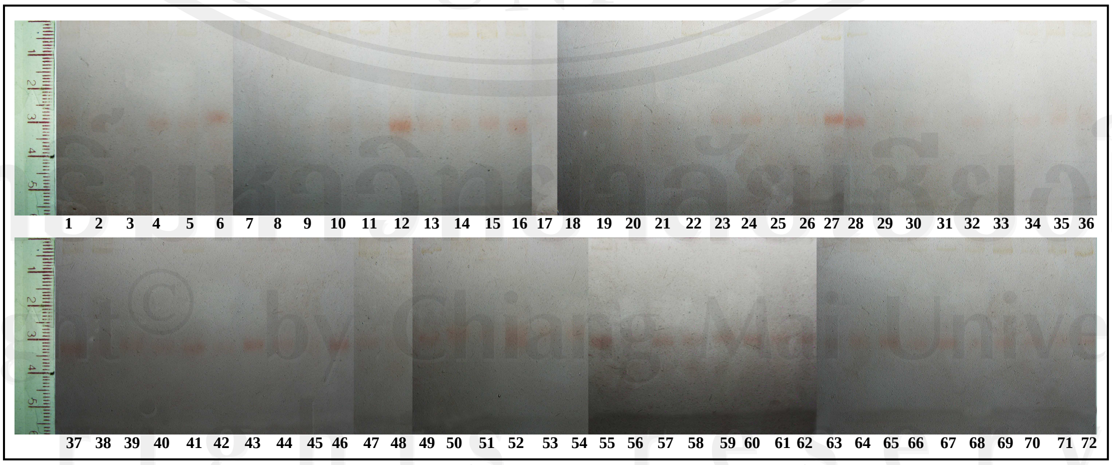


Figure 158 LAP banding pattern of *Peliosanthes teta* Andr.

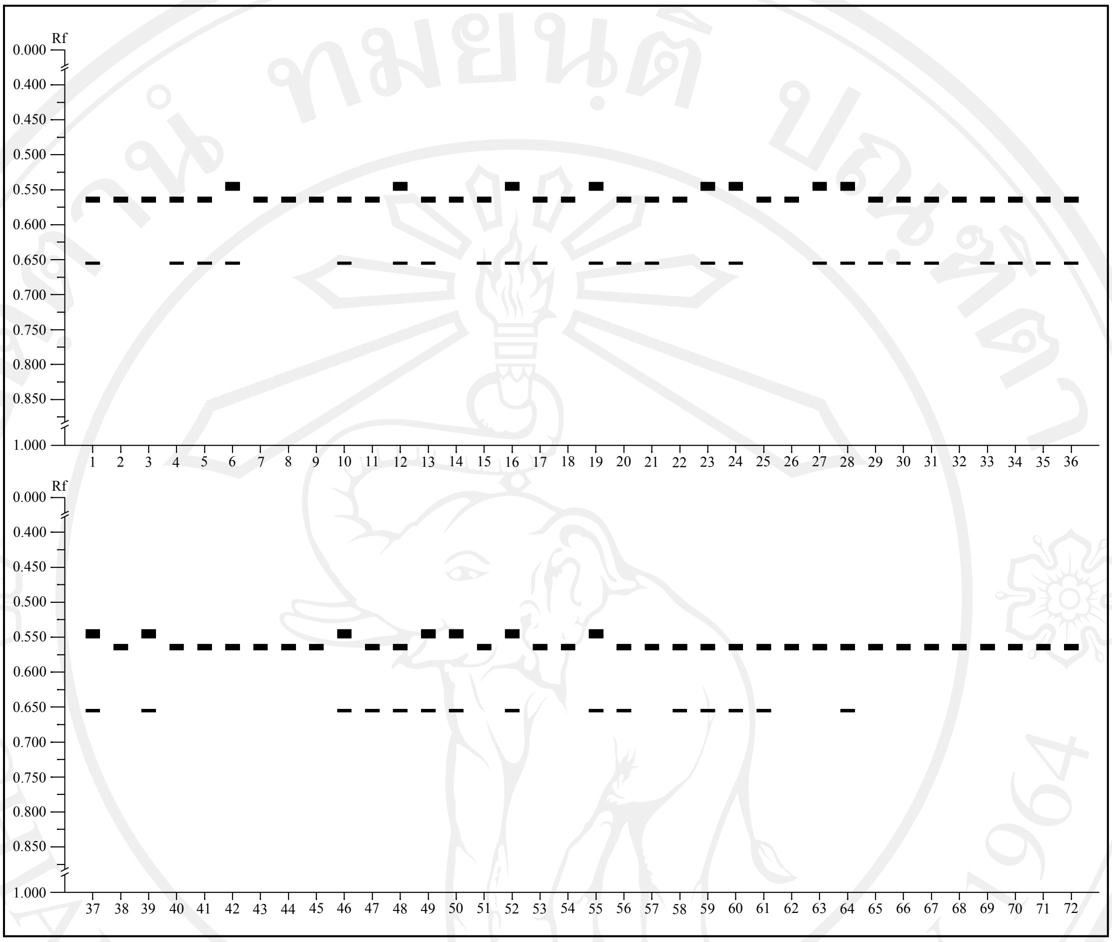


Figure 159 LAP zymogram of *Peliosanthes teta* Andr.

Table 90 LAP banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)]			Sample code
		0.545 [6]	0.564 [4]	0.655 [2]	
A	2		✓	✓	1, 4, 5, 10, 13, 15, 17, 20, 21, 29, 30, 31, 33, 34, 35, 36, 47, 48, 56, 58, 59, 60, 61, 64
B	1		✓		2, 3, 7, 8, 9, 11, 14, 18, 22, 25, 26, 32, 38, 40, 41, 42, 43, 44, 45, 51, 53, 54, 57, 62, 63, 65, 66, 67, 68, 69, 70, 71, 72
C	2	✓		✓	6, 12, 16, 19, 23, 24, 27, 28, 37, 39, 46, 49, 50, 52, 55

7.1.6 Malate dehydrogenase (MDH)

MDH gel performed 8 band types at the Rf of 0.073, 0.255, 0.309, 0.455, 0.618, 0.636, 0.664 and 0.673, 2-5 band types in each sample. The thickness of the bands were between 2-8 mm (Figures 160 and 161). The tested samples were accordingly divided into 15 groups, as shown in Table 91.

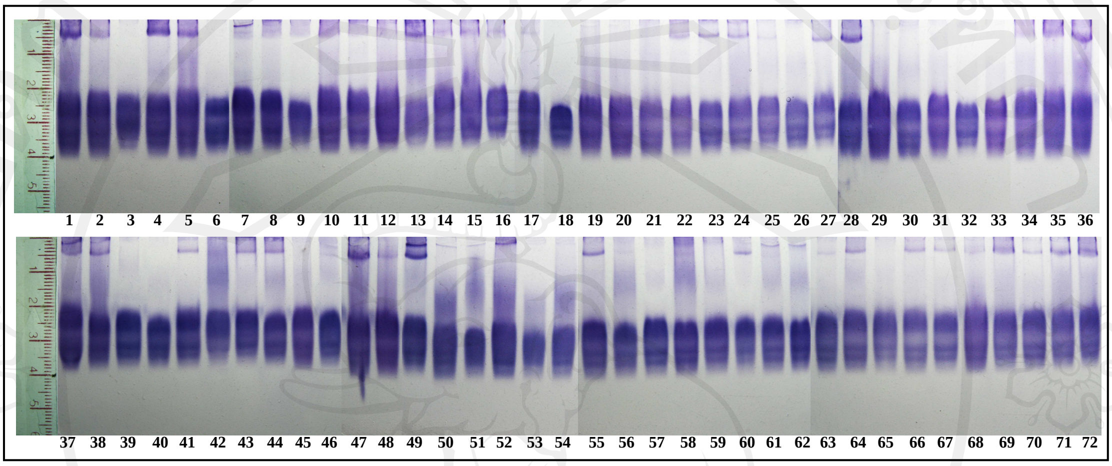


Figure 160 MDH banding pattern of *Peliosanthes teta* Andr.

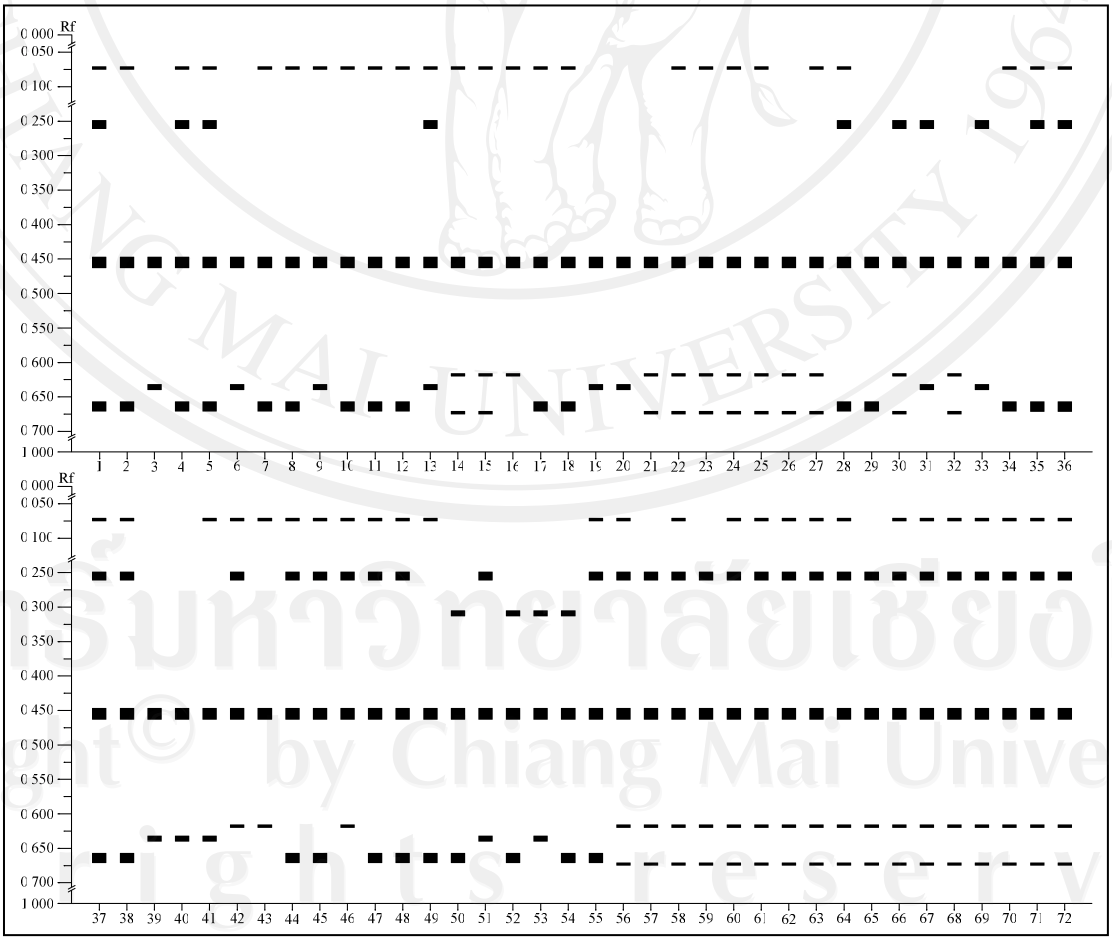


Figure 161 MDH zymogram of *Peliosanthes teta* Andr.

Table 91 MDH banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)] 0.073 [2]	0.255 [6]	0.309 [8]	0.455 [8]	0.618 [2]	0.636 [4]	0.664 [7]	0.673 [2]	Sample code
A	4	✓	✓		✓			✓		1, 4, 5, 28, 35, 36, 37, 38, 44, 45, 47, 48, 55
B	3	✓			✓			✓		2, 7, 8, 10, 11, 12, 17, 18, 34, 49
C	2				✓		✓			3, 6, 19, 20, 39, 40
D	3	✓			✓		✓			9, 41
E	4	✓	✓		✓		✓			13
F	4	✓			✓	✓			✓	14, 15 22, 23, 24, 25, 27
G	3	✓			✓	✓				16, 43
H	3				✓	✓			✓	21, 26, 32
I	2				✓			✓		29
J	4		✓		✓	✓			✓	30, 57, 59, 65
K	3		✓		✓		✓			31, 33, 51
L	4	✓	✓		✓	✓				42, 46
M	3			✓	✓			✓		50, 52, 54
N	3			✓	✓		✓			53
O	5	✓	✓		✓	✓			✓	56, 58, 60, 61, 62, 63, 64, 66, 67, 68, 69, 70, 71, 72

7.1.7 Peroxidase (POX)

The bands at the Rf of 0.127, 0.145, 0.164, 0.255, 0.291, 0.300 and 0.391 were found in POX gel with the band thickness of 2-6 mm. One to three band types appeared in separate samples (Figures 162 and 163). With these bands the set of the whole samples were divided into 9 groups (Table 92).

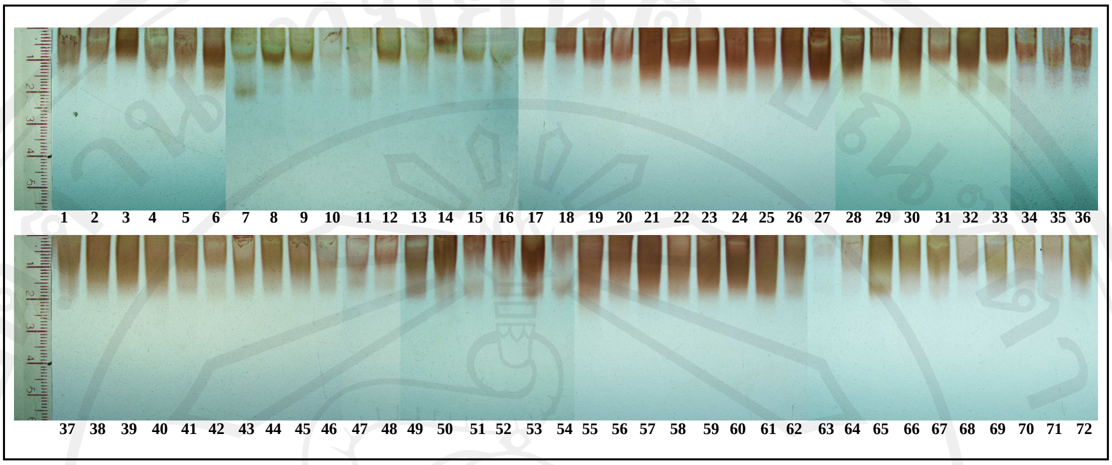


Figure 162 **POX banding pattern of *Peliosanthes teta* Andr.**

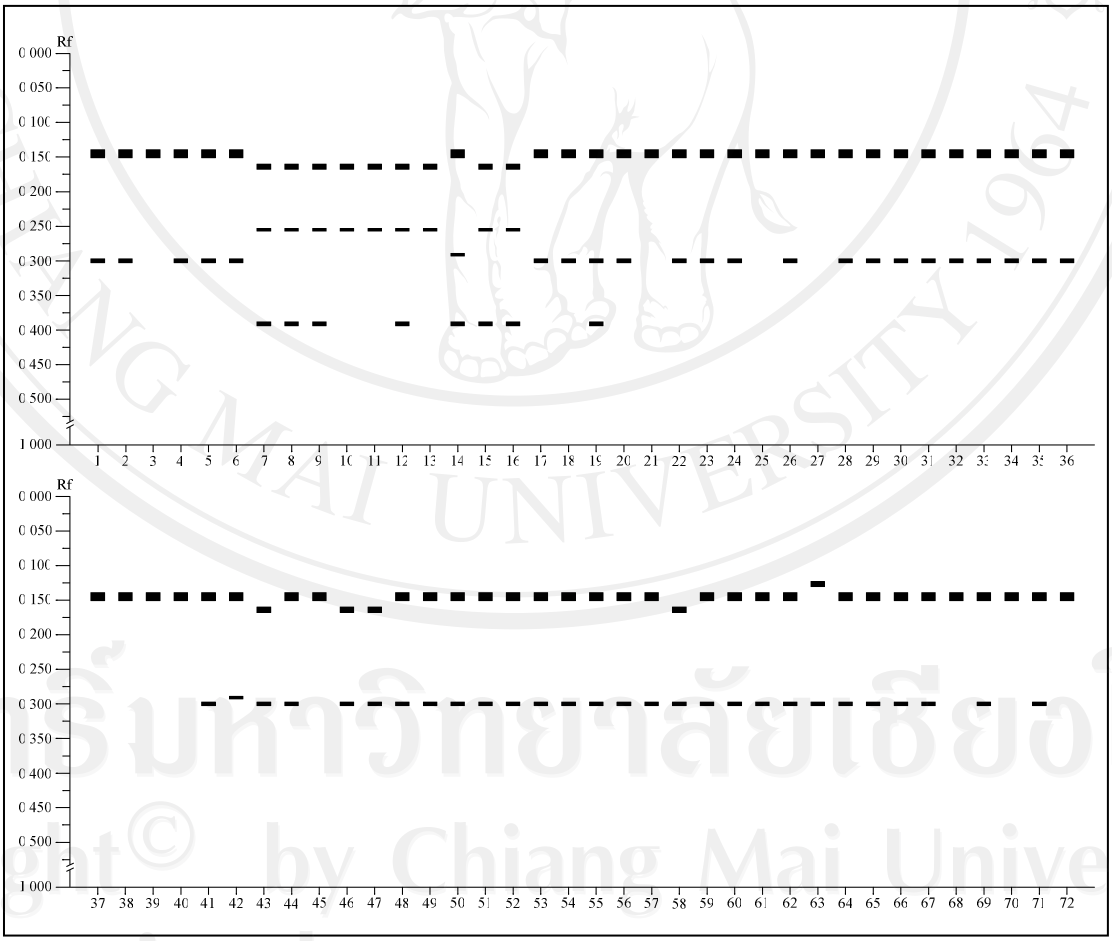


Figure 163 **POX zymogram of *Peliosanthes teta* Andr.**

Table 92 POX banding of *Peliosanthes teta* Andr.

Group	Number of band	Rf [Thickness (mm)]							Sample code
		0.127 [2]	0.145 [6]	0.164 [4]	0.255 [2]	0.291 [2]	0.300 [2]	0.391 [3]	
A	2		✓				✓		1, 2, 4, 5, 6, 17, 18, 20, 22, 23, 24, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 41, 44, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64, 65, 66, 67, 69, 71
B	1		✓						3, 21, 25, 27, 37, 38, 39, 40, 45, 68, 70, 72
C	3			✓	✓			✓	7, 8, 9, 12, 15, 16
D	2			✓	✓				10, 11, 13
E	3		✓			✓		✓	14
F	3		✓				✓	✓	19
G	2		✓			✓			42
H	2			✓			✓		43, 46, 47, 58
I	2	✓					✓		63

7.1.8 Shikimate dehydrogenase (SKD)

SKD enzyme system was unaccomplished in differentiating the samples.

7.1.9 Genetic relationship

Six enzyme systems, i.e. ACP, EST, GOT, LAP, MDH and POX were successful in reproducing the band patterns obtaining from different samples, providing the sample grouping of 16, 5, 6, 3, 15 and 9 sets of representative phenotypes, respectively (Figure 164). Cluster analysis was developed via NTSYSpc programme version 2.21i (Rohlf, 2005), using the bands across all enzyme systems and entries coding as; present = 1 and absent = 0. Results shown in the dendrogram (Figure 165) and Table 7 could indicate that 72 samples of *Peliosanthes teta* Andr. collected from 8 provinces were in 5 separate groups, according to their genetical relatedness, at the coefficient indices of 64%-72%.

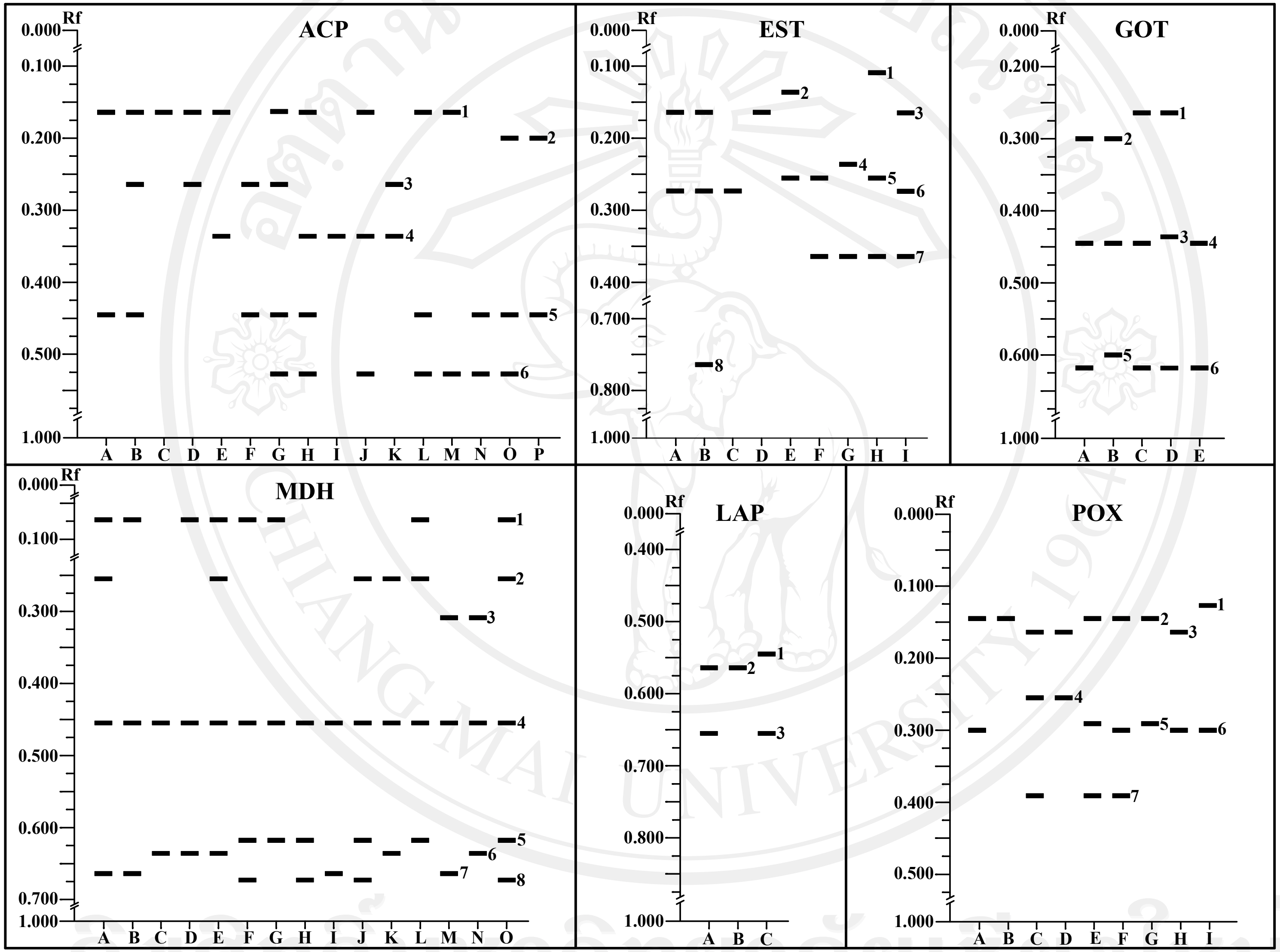


Figure 164 Schematic zymogram of representative phenotypes of *Peliosanthes teta* Andr. reproduced from ACP, EST, GOT, MDH, LAP and POX enzyme systems

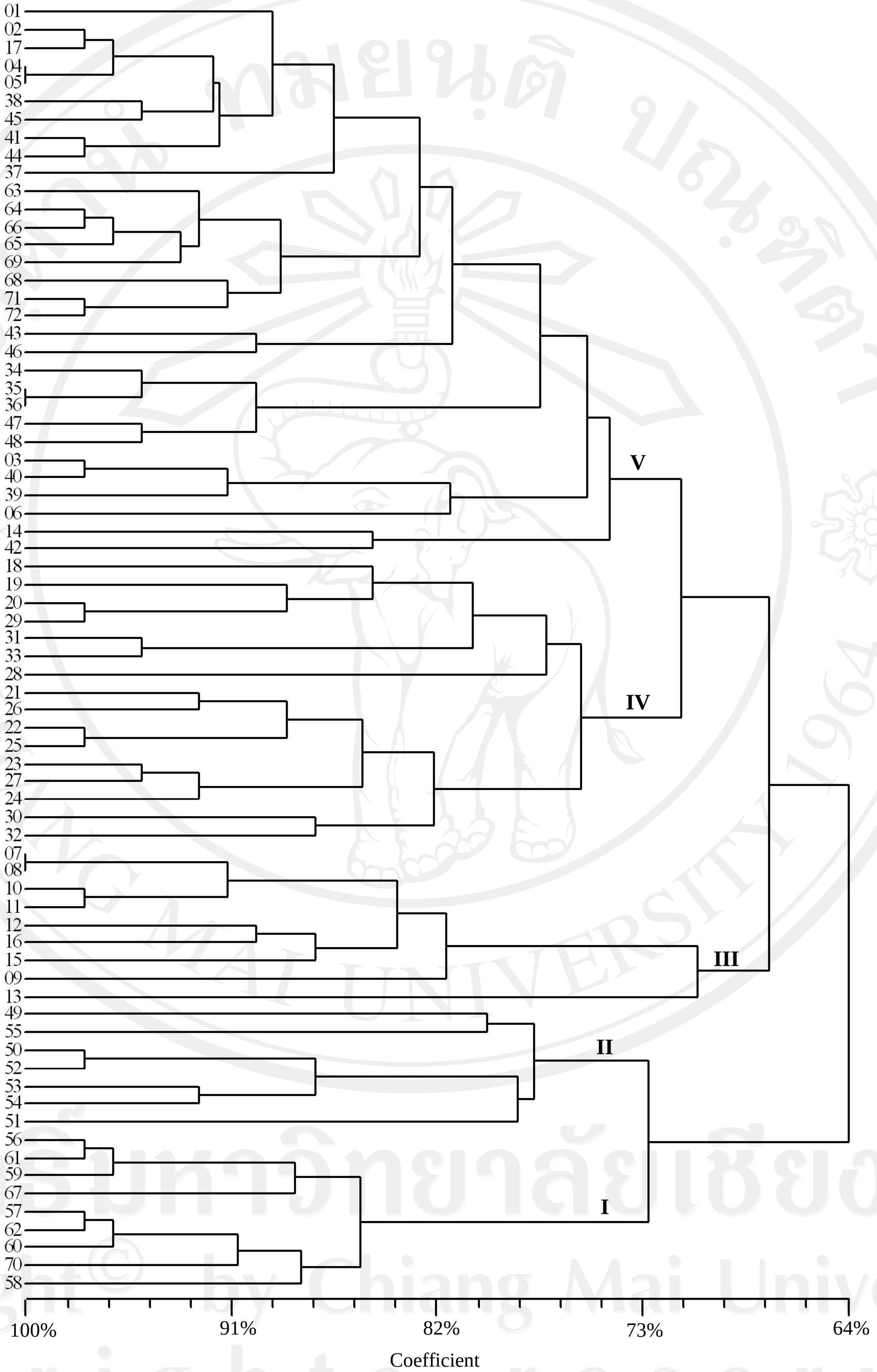


Figure 165 Dendrogram showing genetical relatedness of *Peliosanthes teta* Andr. accessions

Table 93 **Total number of samples with accession codes of classified *Peliosanthes teta* Andr. via polyacrylamide gel electrophoresis**

Group	Total number of samples	Sample code*
I	9	56, 57, 58, 59, 60, 61, 62, 67, 70
II	7	49, 50, 51, 52, 53, 54, 55
III	9	7, 8, 9, 10, 11, 12, 13, 15, 16
IV	16	18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33
V	31	1, 2, 3, 4, 5, 6, 14, 17, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 63, 64, 65, 66, 68, 69, 71, 72

* blue = Chiang Mai; pink = Chiang Rai; black = Lampang; green = Lamphun; yellow = Mae Hong Son; red = Nan; purple = Phayao; orange = Phrae

7.2 *Basella alba* L.

Samples of *Basella alba* L., 80 in accession number, were gathered from the plants in cultivation plots and coded before being prepared for isozyme pattern investigations. They were numbered according to surveyed provinces as 1-10 for Chiang Mai's, 11-20 for Chiang Rai's, 21-30 for Lampang's, 31-40 for Lamphun's, 41-49 for Mae Hong Son's, 50-60 for Nan's, 61-70 for Phayao's and 71-80 for Phrae's. Results are as follows:

7.2.1 Acid phosphatase (ACP)

ACP gel performed 9 band types at the Rf of 0.355, 0.364, 0.427, 0.482, 0.518, 0.536, 0.555, 0.582 and 0.800. The thickness of the bands were from 1 to 7 mm and the samples each produced 1-4 band types as seen in Figures 166 and 167. The band patterns used in separating the samples indicated the group of 15 from the whole set of samples, as tabulated in Table 94.

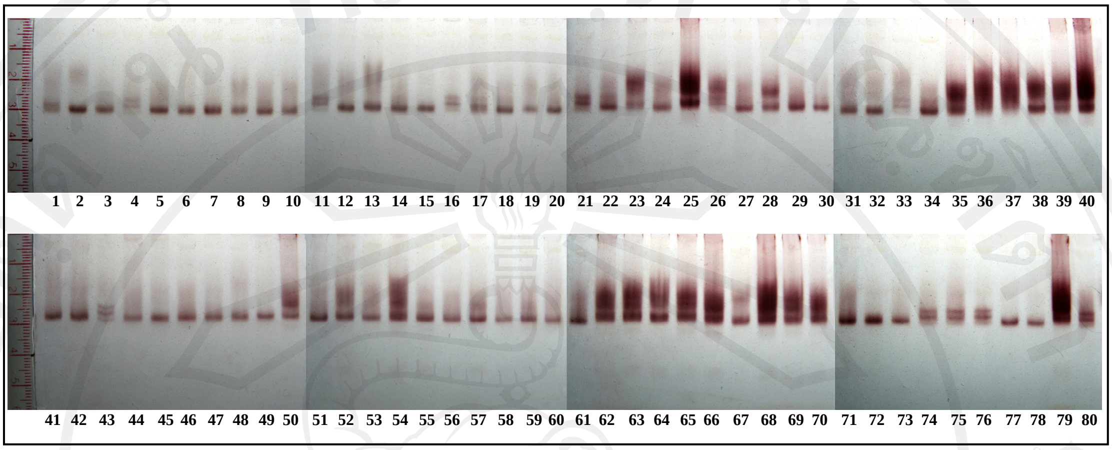


Figure 166 ACP banding pattern of *Basella alba* L.

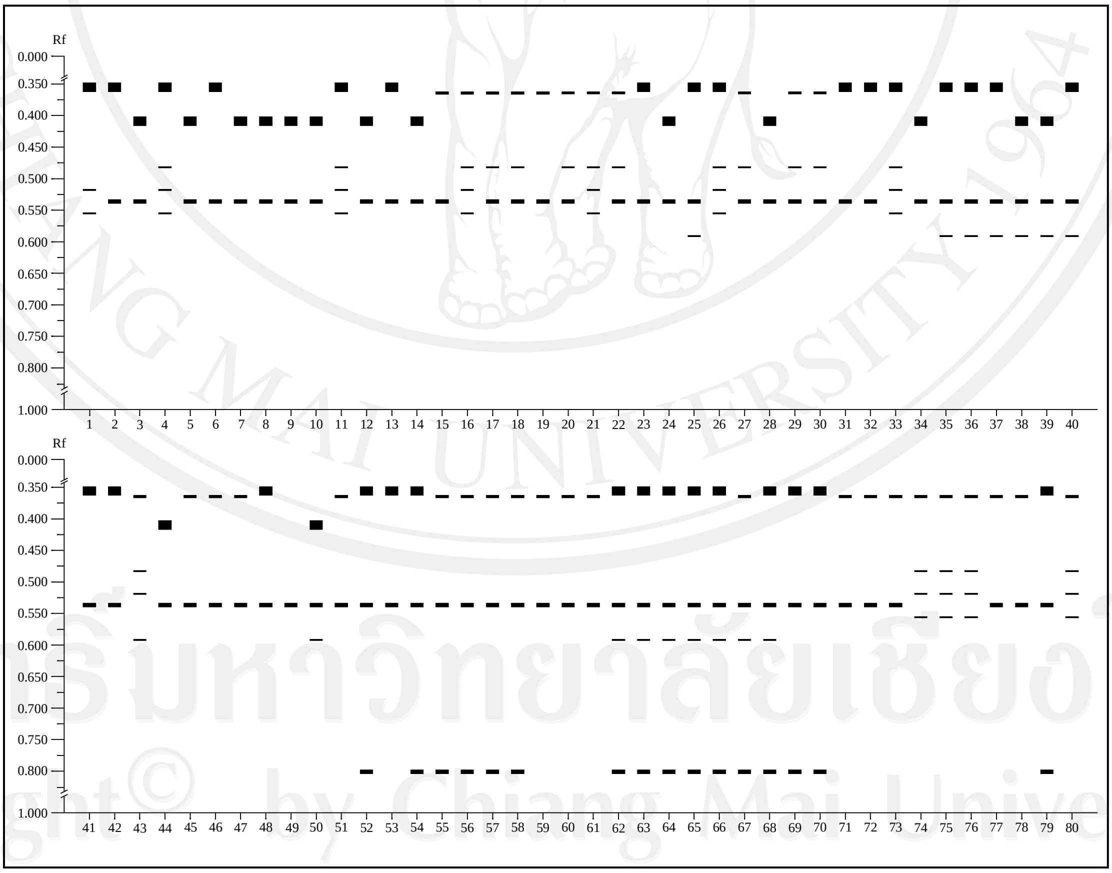


Figure 167 ACP zymogram of *Basella alba* L.

Table 94 ACP banding of *Basella alba* L.

Group	Number of band	Rf [Thickness (mm)] 0.355 [7]	0.364 [2]	0.427 [7]	0.482 [1]	0.518 [1]	0.536 [3]	0.555 [1]	0.582 [1]	0.800 [3]	Sample code
A	3	✓				✓		✓			1
B	2	✓					✓				2, 6, 13, 23, 31, 32, 41, 42, 48, 53
C	2			✓			✓				3, 5, 7, 8, 9, 10, 12, 14, 24, 28, 34, 44
D	4	✓			✓	✓		✓			4, 11, 26, 33
E	2		✓				✓				15, 19, 45, 46, 47, 51, 59, 60, 61, 71, 72, 73, 77, 78
F	4		✓		✓	✓		✓			16, 21, 74, 75, 76, 80
G	3		✓		✓		✓				17, 18, 20, 22, 27, 29, 30
H	3	✓					✓		✓		25, 35, 36, 37, 40
I	3			✓			✓		✓		38, 39, 50
J	4		✓		✓	✓			✓		43
K	1						✓				49
L	3	✓					✓			✓	52, 54, 69, 70, 79
M	3		✓				✓			✓	55, 56, 57, 58
N	4	✓					✓		✓	✓	62, 63, 64, 65, 66, 68
O	4		✓				✓		✓	✓	67

7.2.2 Esterase (EST)

EST enzyme system yielded 6 band types with the Rf of 0.236, 0.291, 0.345, 0.436, 0.545 and 0.664 and the thickness of the bands 2 to 4 mm, as shown in Figures 168 and 169. Separate sample revealed the band types of 3-6 in number. Results of the band evaluation classified the total samples into 6 groups, as seen in Table 95.

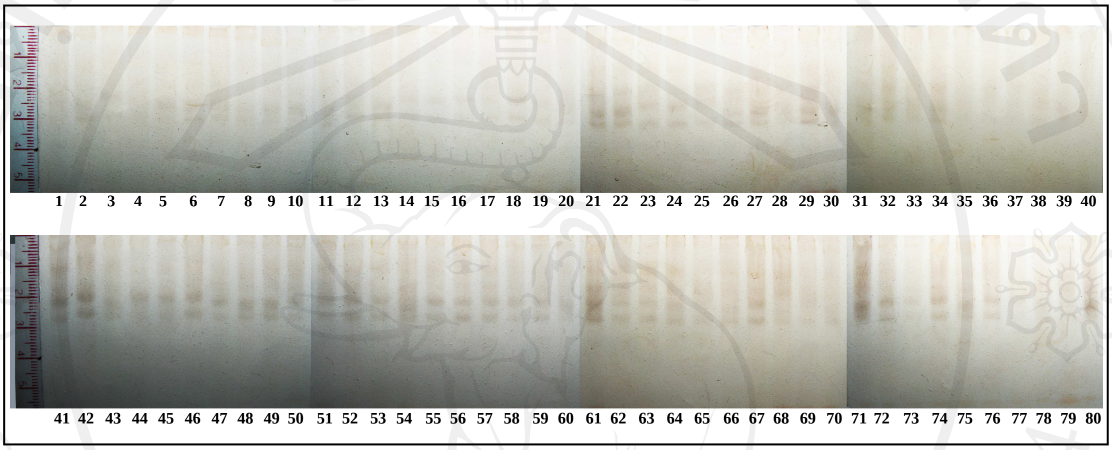


Figure 168 EST banding pattern of *Basella alba* L.

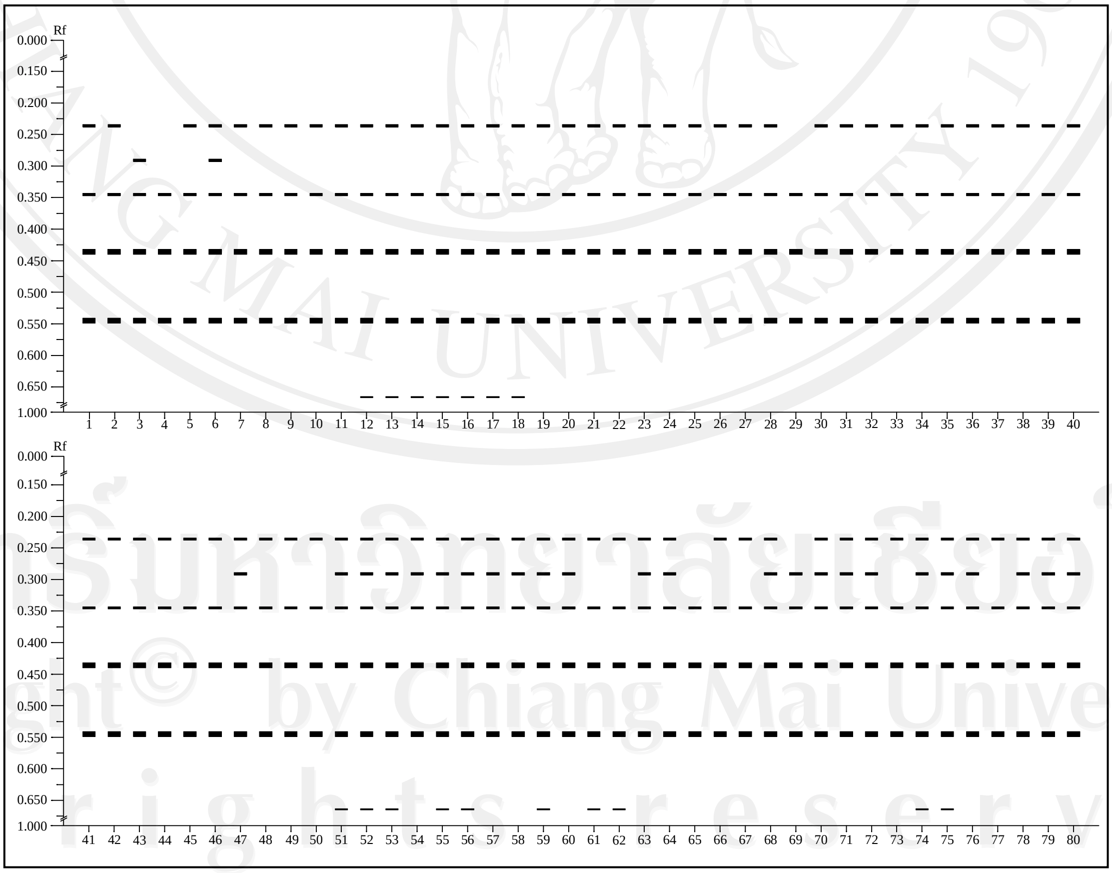


Figure 169 EST zymogram of *Basella alba* L.

Table 95 EST banding of *Basella alba* L.

Group	Number of band	Rf [Thickness (mm)]						Sample code
		0.236 [2]	0.291 [2]	0.345 [2]	0.436 [4]	0.545 [4]	0.664 [2]	
A	4	✓		✓	✓	✓		1, 2, 5, 7, 8, 9, 10, 11, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 48, 49, 50, 66, 67, 73, 77
B	4		✓	✓	✓	✓		3, 69
C	3			✓	✓	✓		4, 29, 65
D	5	✓	✓	✓	✓	✓		6, 47, 54, 57, 58, 60, 63, 64, 68, 70, 71, 72, 76 78, 79, 80
E	5	✓		✓	✓	✓	✓	12, 13, 14, 15, 16, 17, 18, 61, 62
F	6	✓	✓	✓	✓	✓	✓	51, 52, 53, 55, 56, 59, 74, 75

7.2.3 Glucose dehydrogenase (GDH)

GDH enzyme system yielded no band in the gel.

7.2.4 Glutamate oxaloacetate transminase (GOT)

Six band types were found existing in the gel of GOT enzyme system. The Rf measured from the gel were at 0.309, 0.345, 0.364, 0.436, 0.464 and 0.482. These bands ranged from 1-6 mm in thickness (Figures 170 and 171). Individual sample expressed 2-4 band types. The band complement could indicate the samples composing of 7 groups, as presented in Table 96.

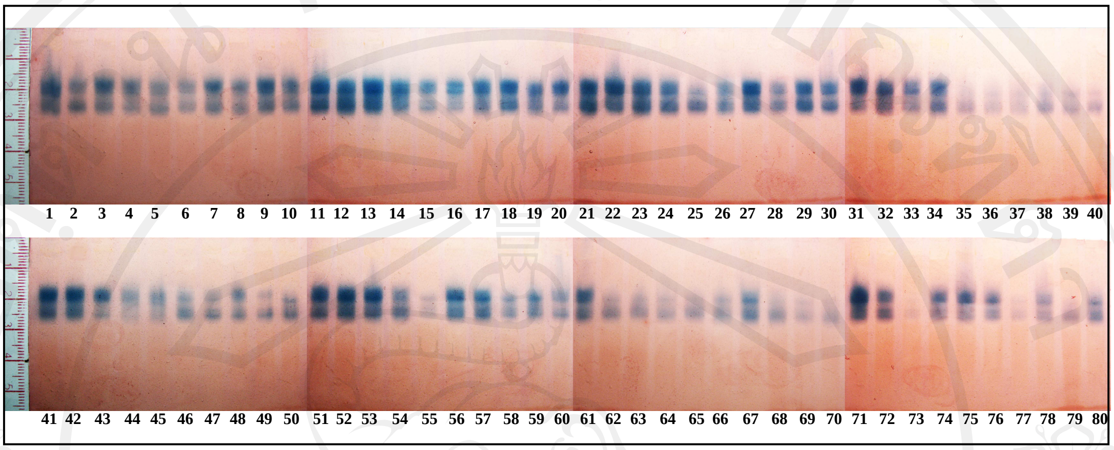


Figure 170 GOT banding pattern of ***Basella alba*** **L.**

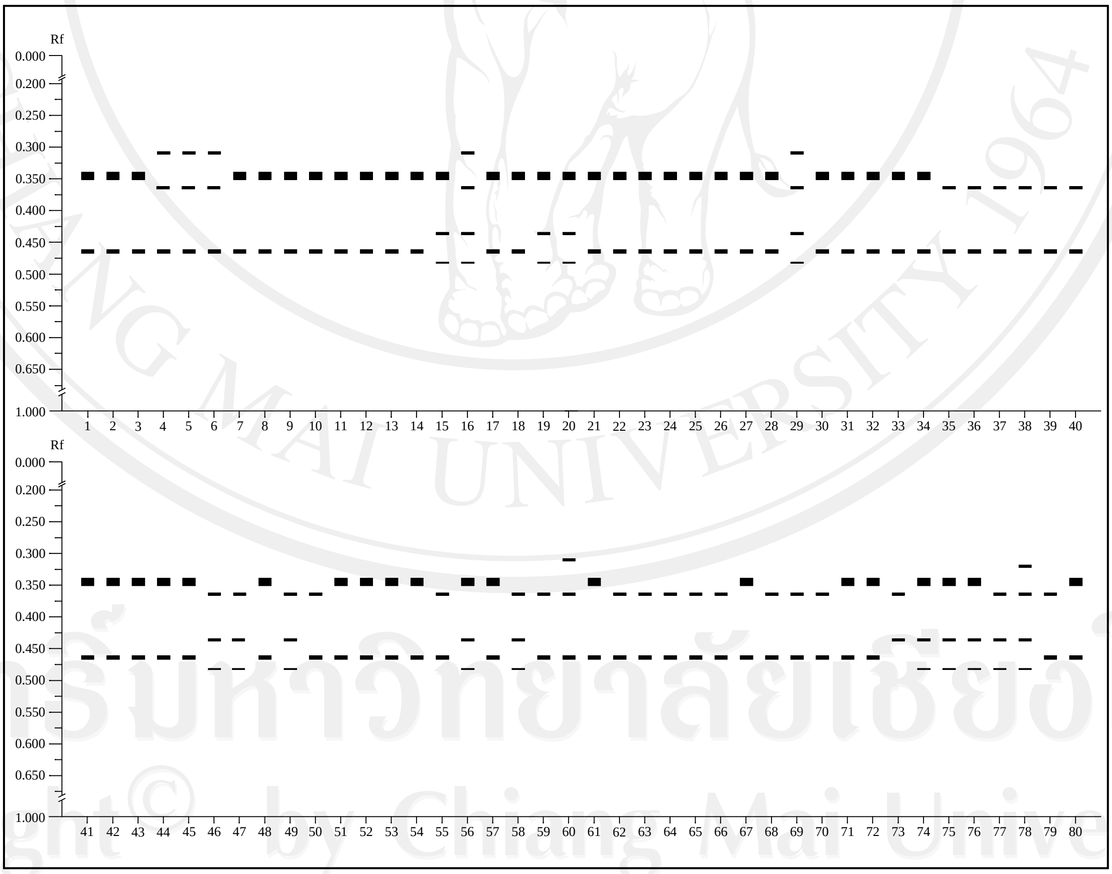


Figure 171 GOT zymogram of ***Basella alba*** **L.**

Table 96 GOT banding of *Basella alba* L.

Group	Number of band	Rf [Thickness (mm)] 0.309 [2]	0.345 [6]	0.364 [2]	0.436 [2]	0.464 [3]	0.482 [1]	Sample code
A	2		✓			✓		1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 41, 42, 43, 44, 45, 48, 51, 52, 53, 54, 57, 61, 67, 71, 72, 80
B	3	✓		✓		✓		4, 5, 6, 60
C	3		✓		✓		✓	15, 19, 20, 56, 74, 75, 76
D	4	✓		✓	✓		✓	16, 29, 78
E	2			✓		✓		35, 36, 37, 38, 39, 40, 50, 55, 59, 62, 63, 64, 65, 66, 68, 69, 70, 79
F	3			✓	✓		✓	46, 47, 49, 58, 77
G	2			✓	✓			73

7.2.5 Leucine aminopeptidase (LAP)

LAP enzyme system were not able to reproduce any band in the gel.

7.2.6 Malate dehydrogenase (MDH)

The number of band type developed by MDH enzyme system was 7, existed at the Rf of 0.064, 0.082, 0.100, 0.182, 0.391, 0.573 and 0.645. The thickness of the bands ranged from 1-9 mm (Figures 172 and 173). Each of the samples consisted of 2-5 bands. The complement of these bands divided the samples into 20 groups, as seen in Table 97.

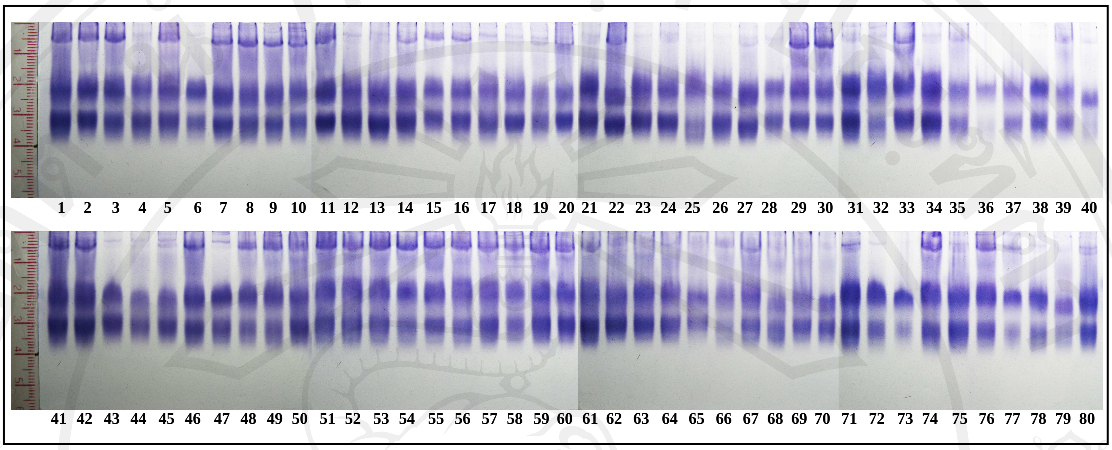


Figure 172 MDH banding pattern of *Basella alba* L.

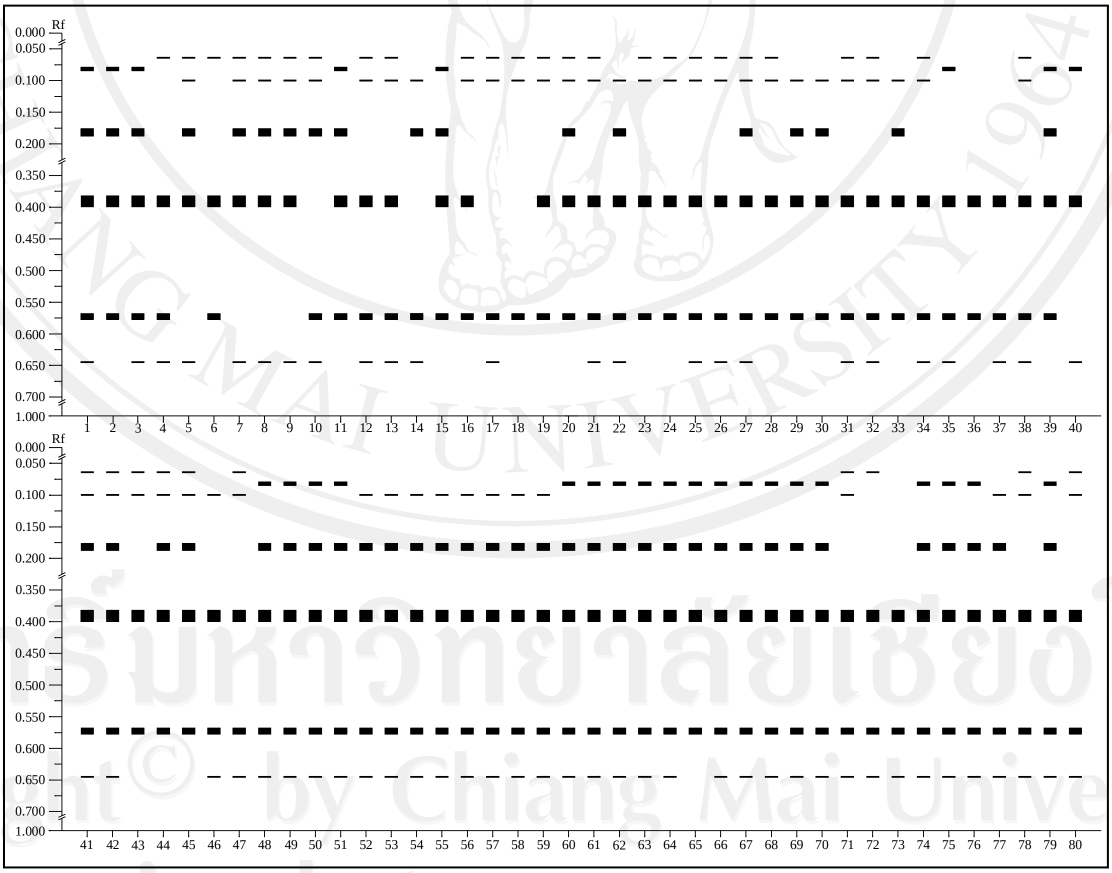


Figure 173 MDH zymogram of *Basella alba* L.

Table 97 MDH banding of *Basella alba* L.

Group	Number of band	Rf [Thickness (mm)]							Sample code
		0.064 [1]	0.082 [3]	0.100 [1]	0.182 [6]	0.391 [9]	0.573 [5]	0.645 [1]	
A	5		✓		✓	✓	✓	✓	1, 3, 48, 49, 50, 51, 60, 61, 62, 63, 64, 66, 67, 68, 69, 70, 74, 75, 76, 79
B	4		✓		✓	✓	✓		2, 11, 15, 39, 65
C	4	✓				✓	✓	✓	4, 72
D	5	✓		✓	✓	✓		✓	5, 7, 8, 9
E	3	✓				✓	✓		6
F	5	✓		✓	✓		✓	✓	10
G	5	✓		✓		✓	✓	✓	12, 13, 21, 25, 26, 31, 32, 34, 38, 47, 71 78, 80
H	4			✓	✓		✓	✓	14
I	4	✓		✓		✓	✓		16, 19, 23, 24, 28, 43
J	4	✓		✓			✓	✓	17
K	3	✓		✓			✓		18
L	5	✓		✓	✓	✓	✓		20, 44, 45
M	5			✓	✓	✓	✓	✓	22, 52, 53, 54, 55, 56, 57, 58, 59, 77
N	6	✓		✓	✓	✓	✓	✓	27, 41, 42
O	4			✓	✓	✓	✓		29, 30, 33
P	4		✓			✓	✓	✓	35
Q	2					✓	✓		36
R	3					✓	✓	✓	37, 73
S	3		✓			✓		✓	40
T	4			✓		✓	✓	✓	46

7.2.7 Peroxidase (POX)

POX enzyme system was able to differentiate the sample extracts into 7 band types at the Rf of 0.064, 0.125, 0.218, 0.309 0.345, 0.382 and 0.582 with the band thickness of 2-6 mm (Figures 174 and 175). Individual sample showed 2-5 band types. The samples were then separated into 16 groups, according to the band patterns, as tabulated in Table 98.

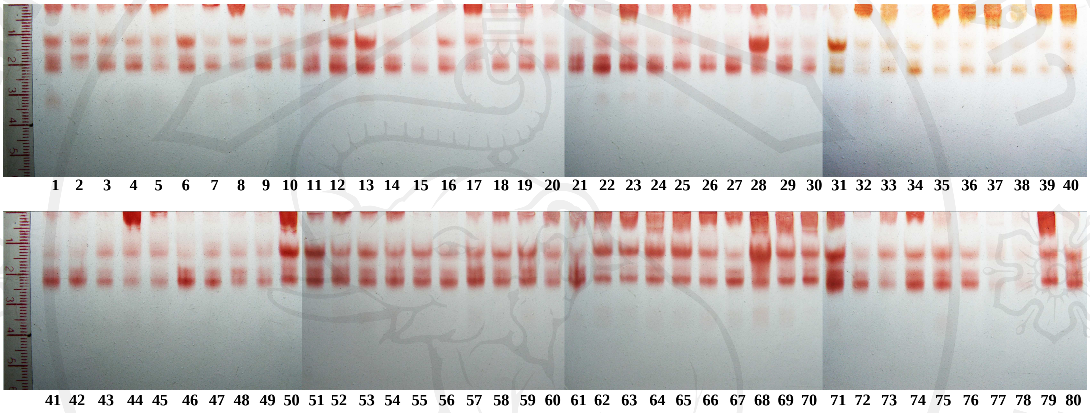


Figure 174 POX banding pattern of *Basella alba* L.

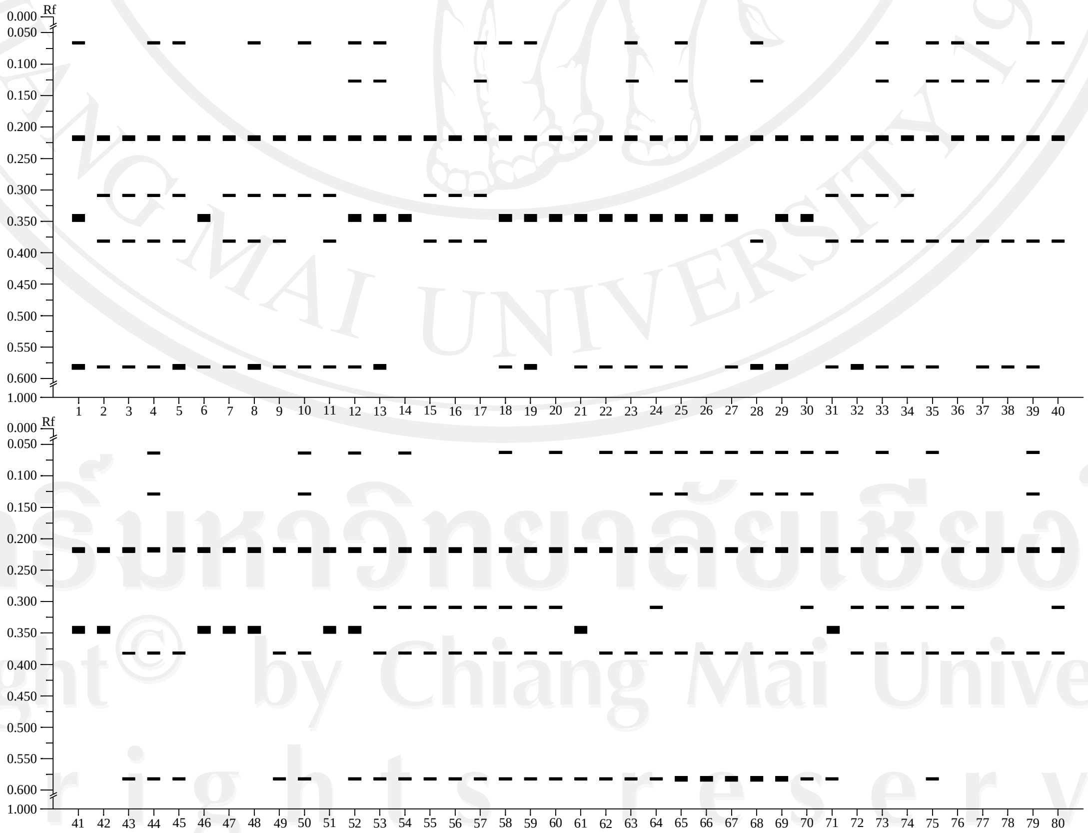


Figure 175 POX zymogram of *Basella alba* L.

Table 98 POX banding of *Basella alba* L.

Group	Number of band	Rf [Thickness (mm)] 0.064 [2]	0.125 [2]	0.218 [4]	0.309 [2]	0.345 [6]	0.382 [2]	0.582 [2,4,5]	Sample code
A	4	✓		✓		✓		✓	1, 18, 19, 52, 71
B	4			✓	✓		✓	✓	2, 3, 7, 9, 11, 31, 32, 34, 53, 55, 56, 57, 59
C	5	✓		✓	✓		✓	✓	4, 5, 8, 54, 58, 60, 75
D	3			✓		✓		✓	6, 21, 22, 24, 27, 29, 61
E	4	✓		✓	✓			✓	10
F	5	✓	✓	✓		✓		✓	12, 13, 23, 25
G	2			✓		✓			14, 20, 26, 30, 41, 42, 46, 47, 48, 51
H	3			✓	✓		✓		15, 16, 72, 74, 76, 80
I	5	✓	✓	✓	✓		✓		17
J	5	✓	✓	✓			✓	✓	28, 35, 37, 39, 44, 50, 65, 68, 69
K	6	✓	✓	✓	✓		✓	✓	33, 64, 70
L	4	✓	✓	✓			✓		36, 40, 79
M	3			✓			✓	✓	38, 43, 45, 49
N	4	✓		✓			✓	✓	62, 63, 66, 67
O	4	✓		✓	✓		✓		73
P	2			✓			✓		77, 78

7.2.8 Shikimate dehydrogenase (SKD)

SKD enzyme system failed to develop protein bands in the gel.

7.2.9 Genetic relationship

Five enzyme systems capable of reproducing the band patterns from extracted samples of *Basella alba* L. comprised ACP, EST, GOT, MDH and POX. Banding patterns from these enzyme systems could allocate the whole samples into 15, 6, 7, 20 and 16 groups, respectively (Figure 176). Bands across all enzyme systems and entries were analysed as mentioned in 7.1.9 and the dendrogram was then produced as shown in Figure 177. Classification analysed from the coefficient indices of 45%-59% are seen in Table 99, dividing accessions of *Basella alba* L. into 9 groups, according to their relatedness.

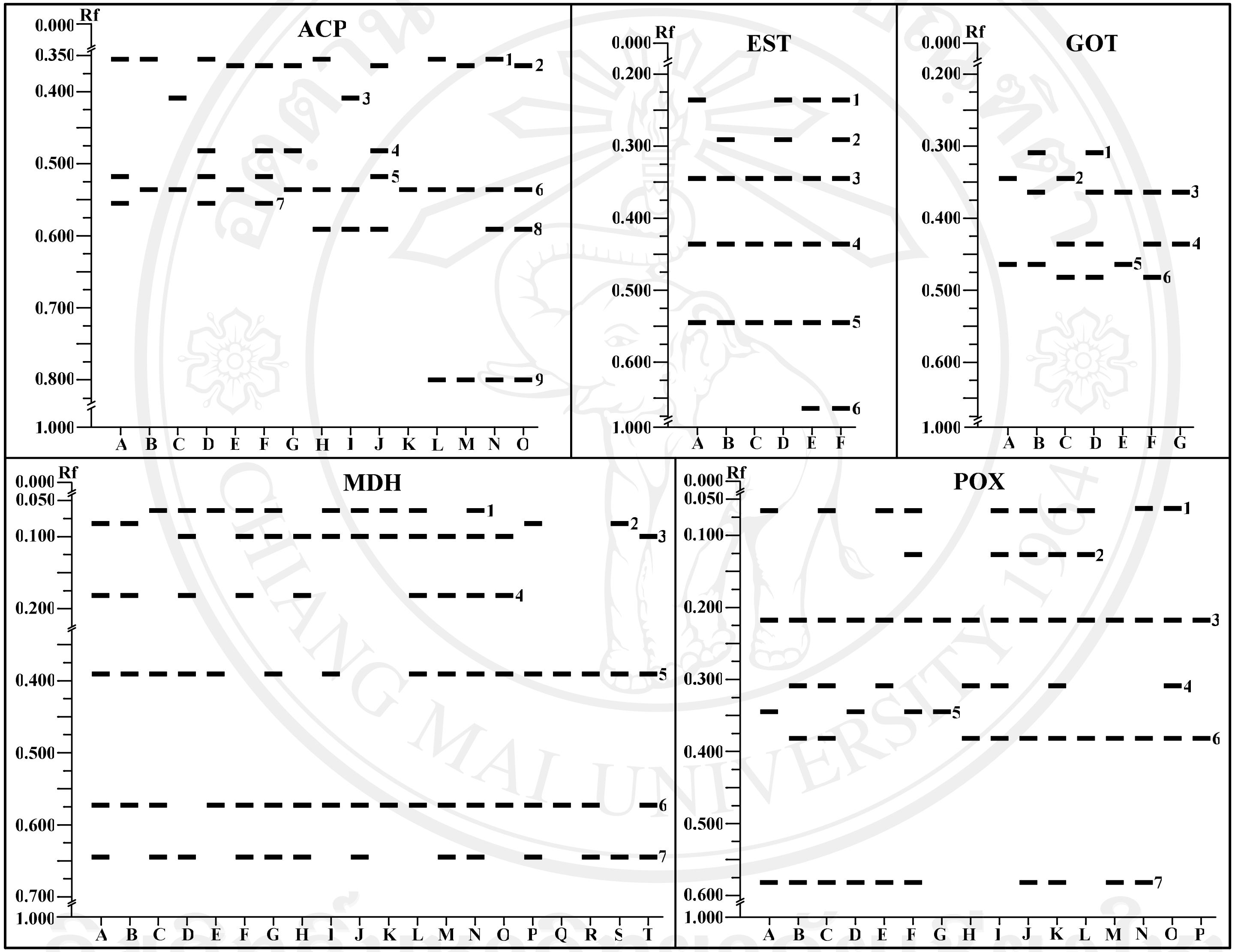


Figure 176 Schematic zymogram of representative phenotypes of *Basella alba* L. reproduced from ACP, EST, GOT, MDH and POX enzyme systems

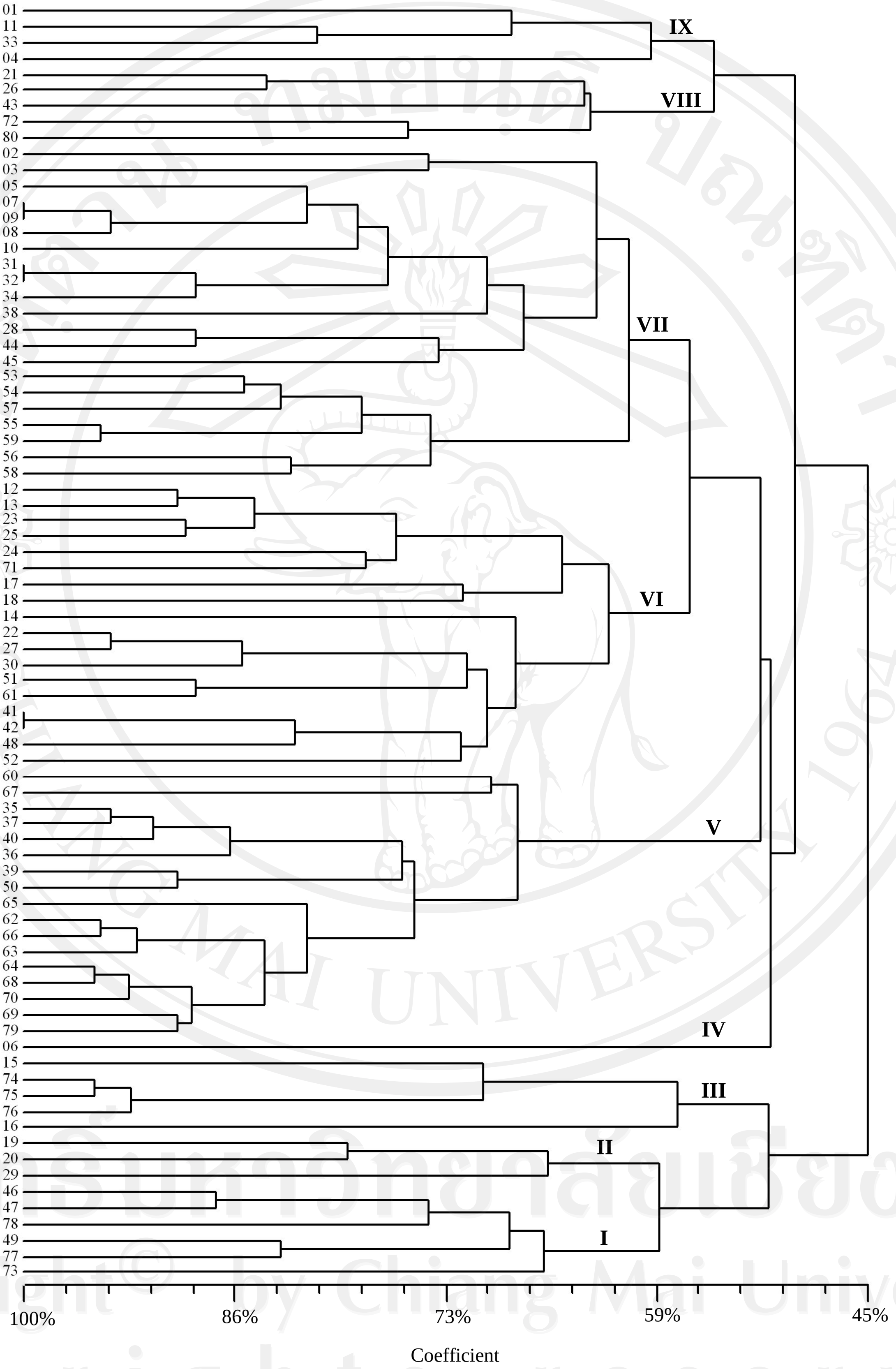


Figure 177 Dendrogram showing genetical relatedness of *Basella alba* L. accessions

Table 99 Total number of samples with accession codes of classified *Basella alba* L. via polyacrylamide gel electrophoresis

Group	Total number of samples	Sample code*
I	6	46, 47, 49, 73, 77, 78
II	3	19, 20, 29
III	5	15, 16, 74, 75, 76
IV	1	6
V	17	35, 36, 37, 39, 40, 50, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 79
VI	18	12, 13, 14, 17, 18, 22, 23, 24, 25, 27, 30, 41, 42, 48, 51, 52, 61, 71
VII	21	2, 3, 5, 7, 8, 9, 10, 28, 31, 32, 34, 38, 44, 45, 53, 54, 55, 56, 57, 58, 59
VIII	5	21, 26, 43, 72, 80
IX	4	1, 4, 11, 33

* blue = Chiang Mai; pink = Chiang Rai; black = Lampang; green = Lamphun; yellow = Mae Hong Son; red = Nan; purple = Phayao; orange = Phrae

7.3 *Gymnema inodorum* Decne.

Eighty samples of *Gymnema inodorum* Decne. accessions were studied for their relatedness in the same manner as done with *Peliosanthes teta* Andr. (7.1) and *Basella alba* L. (7.2). Sample codes for different accessions from targeted provinces were 1-11 for Chiang Mai, 12-14 for Chiang Rai, 15-17 for Lampang, 18-34 for Lamphun, 35-43 for Mae Hong Son, 44-60 for Nan, 61-70 for Phayao and 71-80 for Phrae. Results are as follows:

7.3.1 Acid phosphatase (ACP)

The gel obtained from ACP enzyme system showed 5 band types at the Rf of 0.273, 0.291, 0.345, 0.355 and 0.391 with the band thickness ranged from 3 to 10 mm. Single sample bore 1-2 band types (Figures 178 and 179). The banding pattern classified the tested samples into 6 groups, as shown in Table 100.

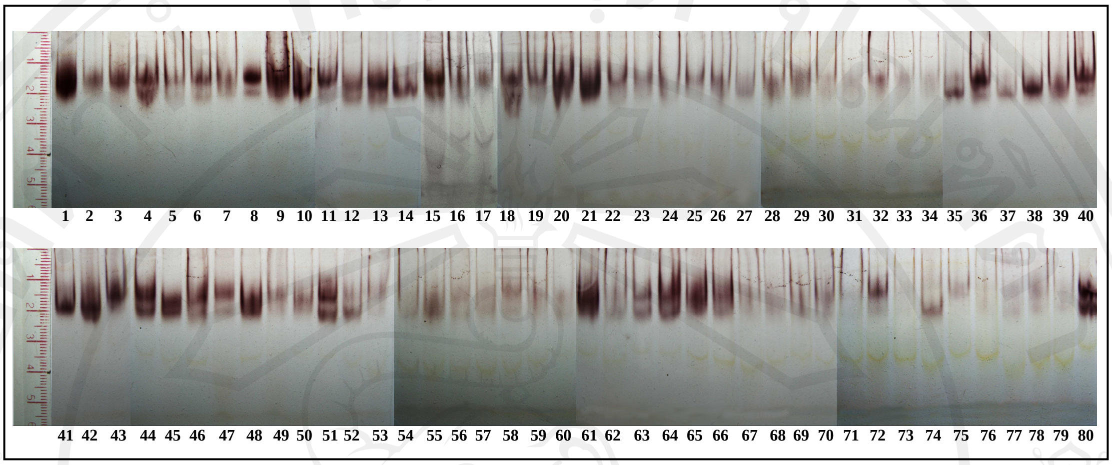


Figure 178 ACP banding pattern of *Gymnema inodorum* Decne.

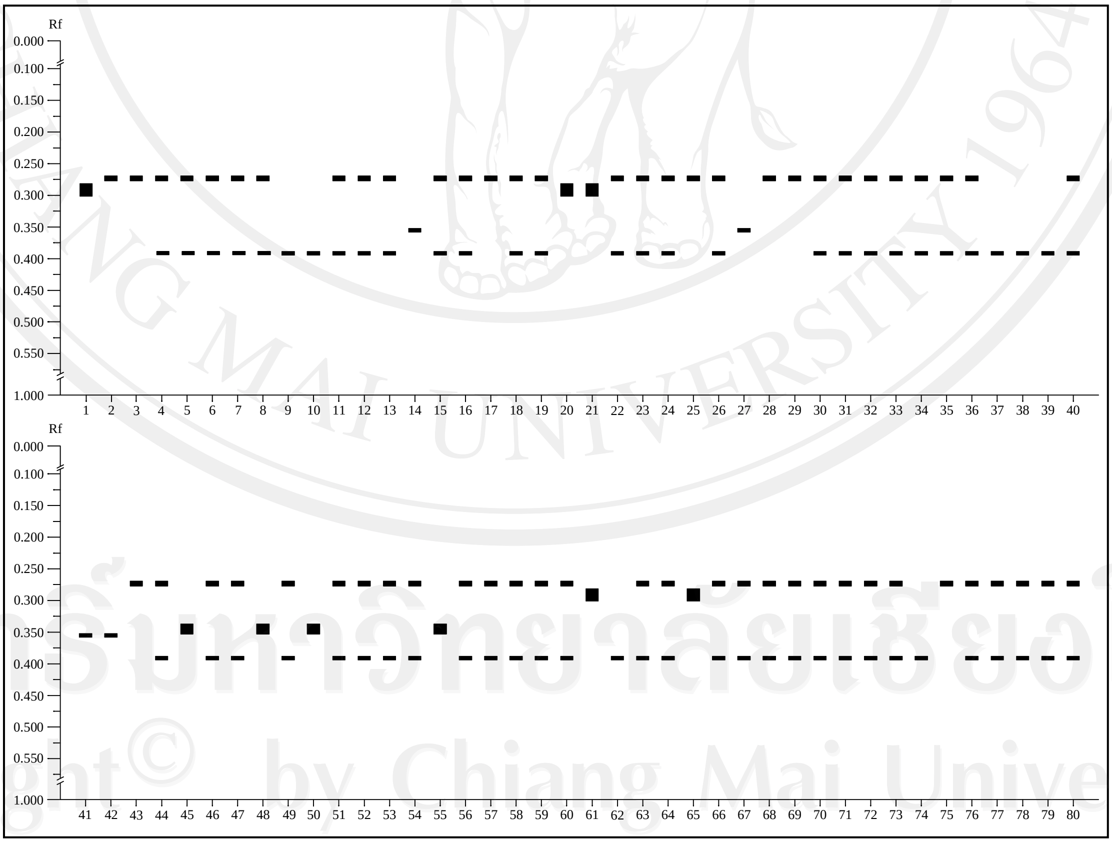


Figure 179 ACP zymogram of *Gymnema inodorum* Decne.

Table 100 ACP banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]					Sample code
		0.273 [4]	0.291 [10]	0.345 [8]	0.355 [3]	0.391 [3]	
A	1		✓				1, 20, 21, 61, 65
B	1	✓					2, 3, 17, 25, 28, 29, 43, 75
C	2	✓				✓	4, 5, 6, 7, 8, 11, 12, 13, 15, 16, 18, 19, 22, 23, 24, 26, 30, 31, 32, 33, 34, 35, 36, 40, 44, 46, 47, 49, 51, 52, 53, 54, 56, 57, 58, 59, 60, 63, 64, 66, 67, 68, 69, 70, 71, 72, 73, 76, 77, 78, 79, 80
D	1					✓	9, 10, 37, 38, 39, 62, 74
E	1				✓		14, 27, 41, 42
F	1			✓			45, 48, 50, 55

7.3.2 Esterase (EST)

Seven band types were figured from EST enzyme system. The bands were present at the Rf of 0.109, 0.373, 0.400, 0.509, 0.600, 0.855 and 0.927 with 2-7 mm band thickness (Figures 180 and 181). Each column appeared on the gel held 3-6 band types. The band patterns could divide the samples into 14 groups (Table 101).

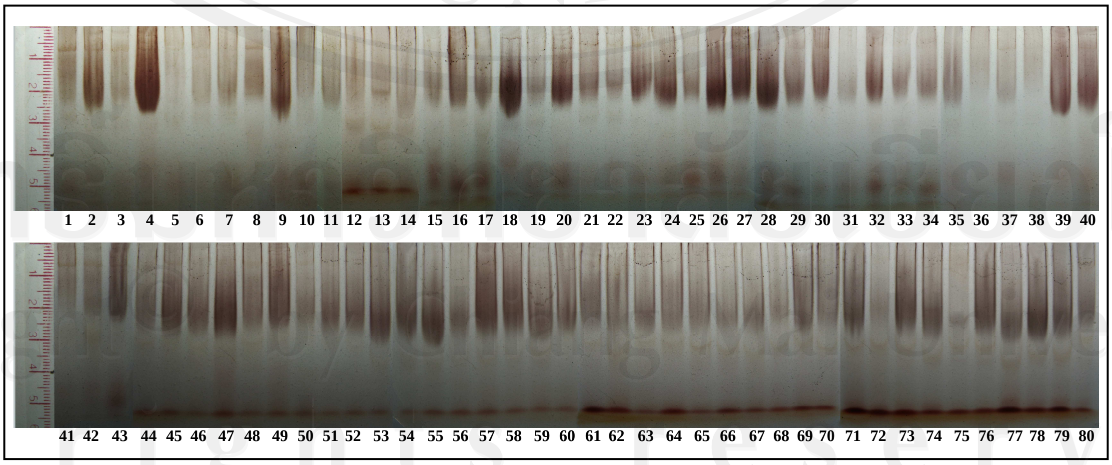


Figure 180 EST banding pattern of *Gymnema inodorum* Decne.

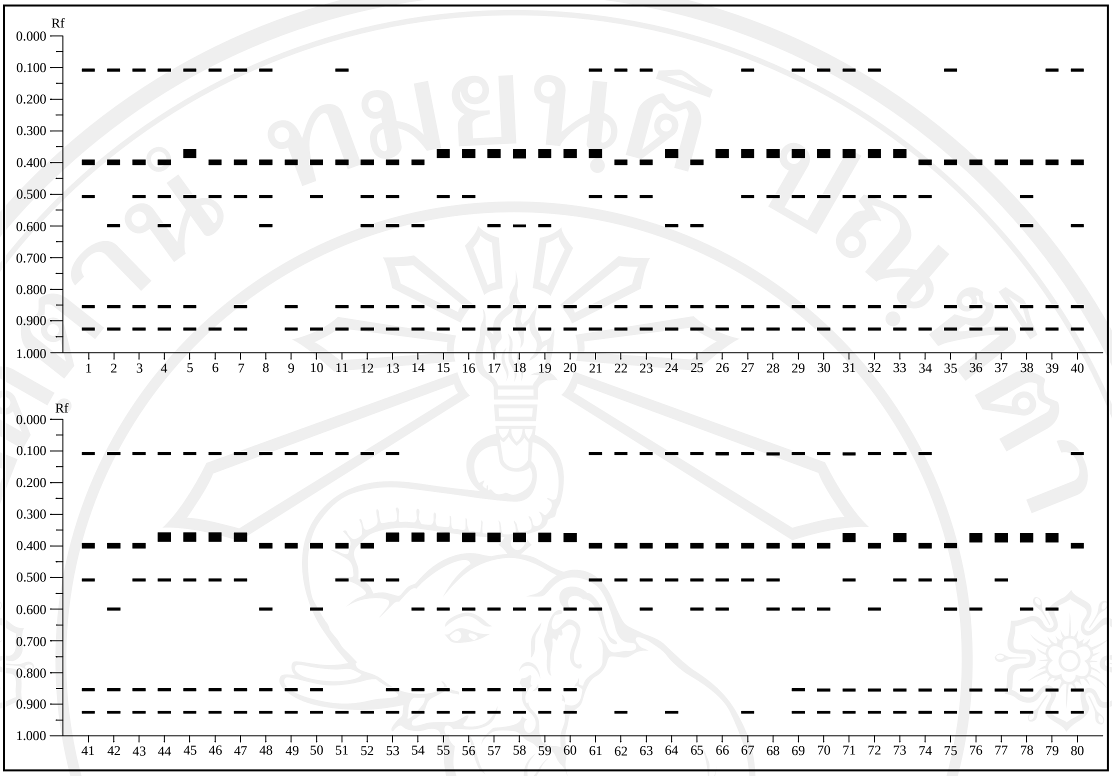


Figure 181 EST zymogram of *Gymnema inodorum* Decne.

Table 101 EST banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]							Sample code
		0.109 [2]	0.373 [7]	0.400 [4]	0.509 [2]	0.600 [2]	0.855 [2]	0.927 [2]	
A	5	✓		✓	✓		✓	✓	1, 3, 7, 22, 23, 41, 43, 74
B	5	✓		✓		✓	✓	✓	2, 40, 42, 48, 50, 69, 70, 72
C	6	✓		✓	✓	✓	✓	✓	4
D	5	✓	✓		✓		✓	✓	5, 21, 27, 29, 30, 31, 32, 44, 45, 46, 47, 53, 71, 73
E	4	✓		✓	✓			✓	6, 51, 52, 62, 64, 67
F	4	✓		✓	✓	✓			8, 61, 63, 65, 66, 68
G	3			✓			✓	✓	9, 36, 37
H	3			✓	✓			✓	10, 34
I	4	✓		✓			✓	✓	11, 35, 39, 49, 80
J	5			✓	✓	✓	✓	✓	12, 13, 38, 75
K	4			✓		✓	✓	✓	14, 25
L	4		✓		✓		✓	✓	15, 16, 28, 33, 77
M	4		✓			✓	✓	✓	17, 18, 19, 24, 54, 55, 56, 57, 58, 59, 60, 76, 78, 79
N	3		✓				✓	✓	20, 26

7.3.3 Glucose dehydrogenase (GDH)

Different band types, 6 altogether, were conspicuous on the gel of GDH enzyme system. They appeared at the Rf of 0.164, 0.655, 0.691, 0.709, 0.782 and 0.855 with the band thickness of 2 mm (Figures 182 and 183). Individual column produced 1 to 2 band types. The tested samples were allocated into 12 groups in accordance with their relatedness, as shown in Table 102.

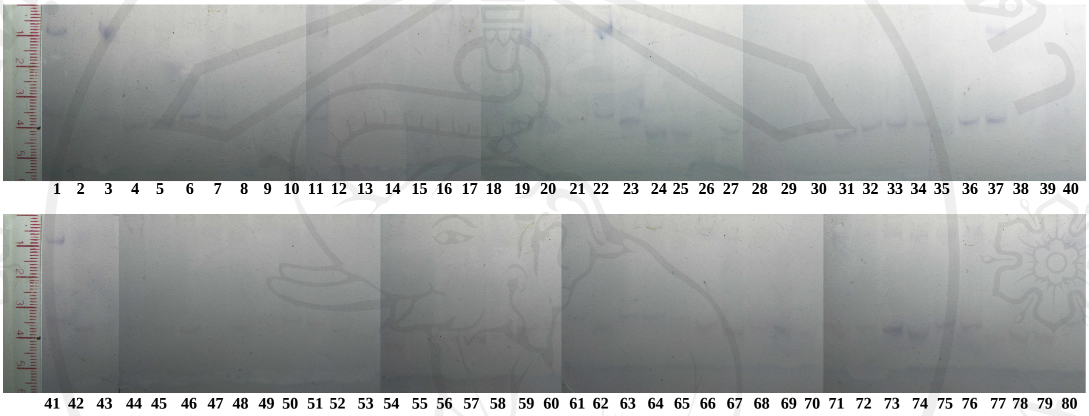


Figure 182 GDH banding pattern of *Gymnema inodorum* Decne.

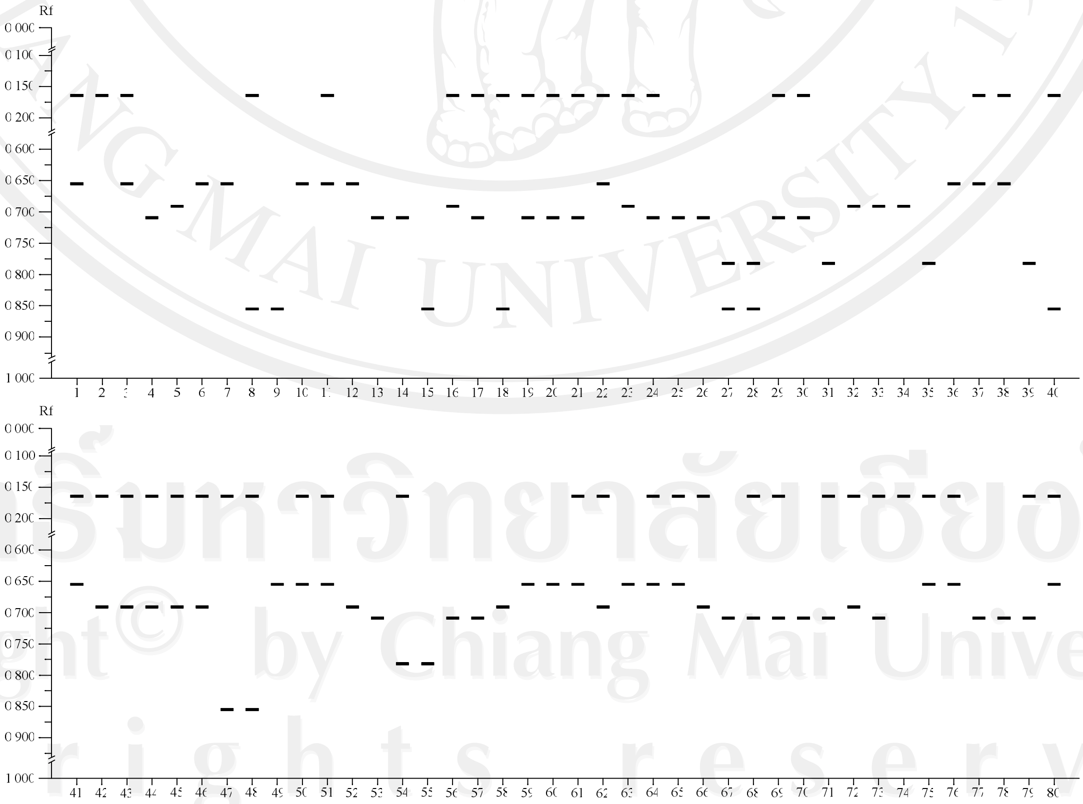


Figure 183 GDH zymogram of *Gymnema inodorum* Decne.

Table 102 GDH banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]						Sample code
		0.164 [2]	0.655 [2]	0.691 [2]	0.709 [2]	0.782 [2]	0.855 [2]	
A	2	✓	✓					1, 3, 11, 22, 37, 38, 41, 50, 51, 61, 64, 65, 75, 76, 80
B	1	✓						2, 74
C	1				✓			4, 13, 14, 25, 26, 53, 56, 57, 67, 70, 77, 78
D	1			✓				5, 32, 33, 34, 52, 58
E	1		✓					6, 7, 10, 12, 36, 49, 59, 60, 63
F	2	✓					✓	8, 18, 40, 47, 48
G	1						✓	9, 15
H	2	✓		✓				16, 23, 42, 43, 44, 45, 46, 62, 66, 72
I	2	✓			✓			17, 19, 20, 21, 24, 29, 30, 68, 69, 71, 73, 79
J	2					✓	✓	27, 28
K	1					✓		31, 35, 39, 55
L	2	✓				✓		54

7.3.4 Glutamate oxaloacetate transminase (GOT)

The band types occurred in GOT enzyme system gel were 5 in number, at the Rf of 0.191, 0.318, 0.473, 0.482 and 0.581 with the band thickness of 3-9 mm. One to three band types were seen in each sample column (Figures 184 and 185). Nine groups of samples were indicated from the banding patterns of these columns (Table 103).

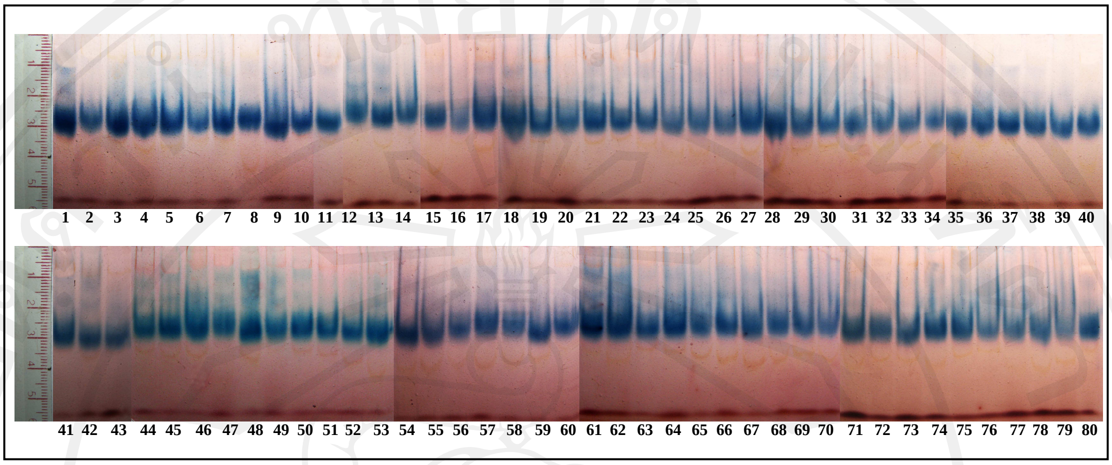


Figure 184 GOT banding pattern of *Gymnema inodorum* Decne.

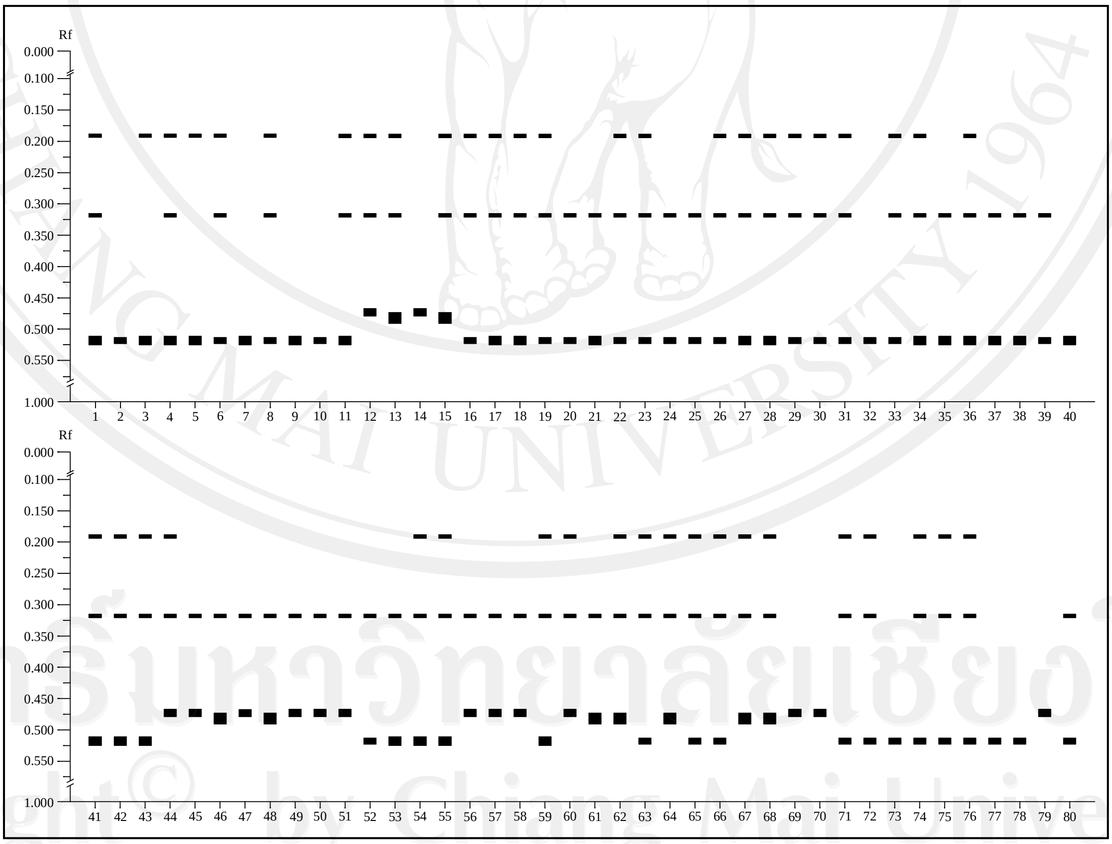


Figure 185 GOT zymogram of *Gymnema inodorum* Decne.

Table 103 GOT banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]					Sample code
		0.191 [3]	**0.318 [3]**	**0.473 [6,4]**	**0.482 [9]**	**0.581 [5,7]**	
A	3	✓	✓			✓	1, 4, 6, 8, 11, 16, 17, 18, 19, 22, 23, 26, 27, 28, 29, 30, 31, 33, 34, 36, 41, 42, 43, 54, 55, 59, 63, 65, 66, 71, 72, 74, 75, 76
B	1					✓	2, 7, 9, 10, 32, 40, 73, 77, 78
C	2	✓				✓	3, 5
D	3	✓	✓	✓			12, 44, 60
E	3	✓	✓		✓		13, 15, 62, 64, 67, 68
F	1			✓			14, 69, 70, 79
G	2		✓			✓	20, 21, 24, 25, 35, 37, 38, 39, 52, 53, 80
H	2		✓	✓			45, 47, 49, 50, 51, 56, 57, 58
I	2		✓		✓		46, 48, 61

7.3.5 Leucine aminopeptidase (LAP)

Only 3 band types were developed from LAP enzyme system. They showed at the Rf of 0.200, 0.709 and 0.800 with the band thickness of 2-6 mm. Single column of the samples yielded 1-3 band types (Figures 186 and 187). The whole samples were allocated into 6 groups, according to the banding patterns (Table 104).

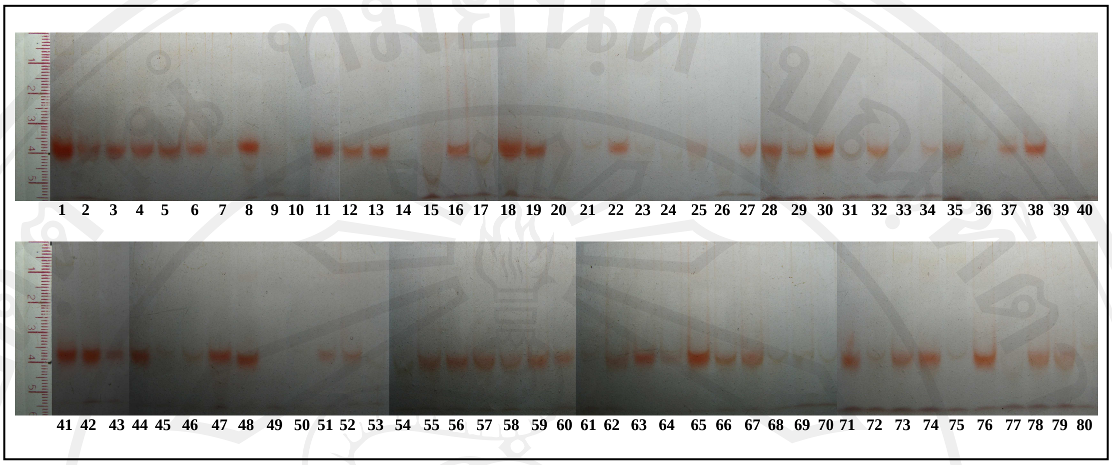


Figure 186 LAP banding pattern of *Gymnema inodorum* Decne.

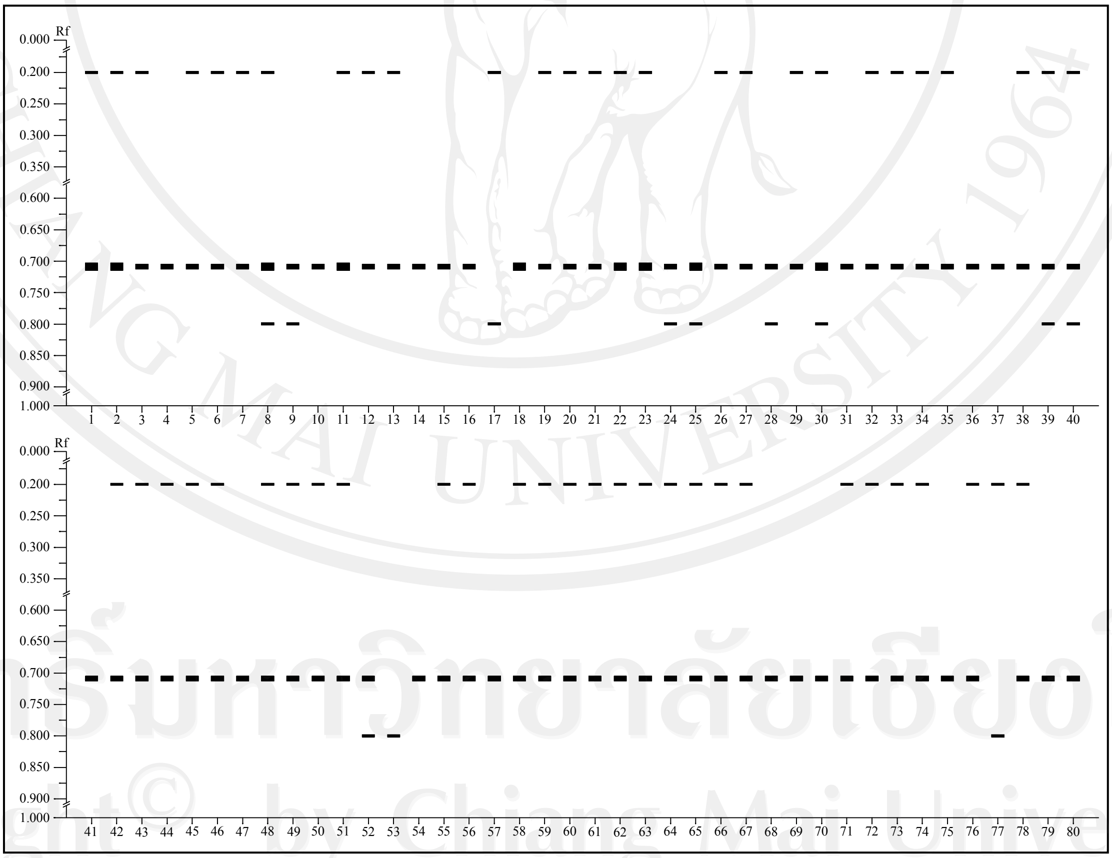


Figure 187 LAP zymogram of *Gymnema inodorum* Decne.

Table 104 LAP banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]			Sample code
		0.200 [2]	0.709 [4,6]	0.800 [2]	
A	2	✓	✓		1, 2, 3, 5, 6, 7, 11, 12, 13, 19, 20, 21, 22, 23, 26, 27, 29, 32, 33, 34, 35, 38, 42, 43, 44, 45, 46, 48, 49, 50, 51, 55, 56, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 71, 72, 73, 74, 76, 78
B	1		✓		4, 10, 14, 15, 16, 18, 31, 36, 37, 41, 47, 54, 57, 68, 69, 70, 75, 79, 80
C	3	✓	✓	✓	8, 30, 39, 40
D	2		✓	✓	9, 24, 25, 28, 52
E	2	✓		✓	17, 77
F	1			✓	53

7.3.6 Malate dehydrogenase (MDH)

The band types detected from MDH gel were 8 altogether at the Rf of 0.318, 0.445, 0.473, 0.573, 0.609, 0.645, 0.682 and 0.782 with the band thickness of 2-9 mm. Separate column on the gel comprised 2-4 band types (Figures 188 and 189). Fourteen groups of the samples were declared from banding pattern analyses, as tabulated in Table 105.

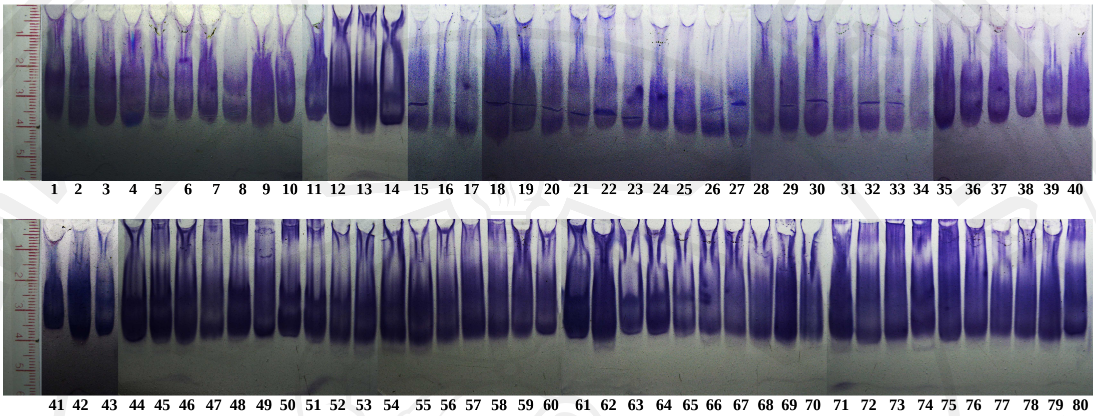


Figure 188 MDH banding pattern of *Gymnema inodorum* Decne.

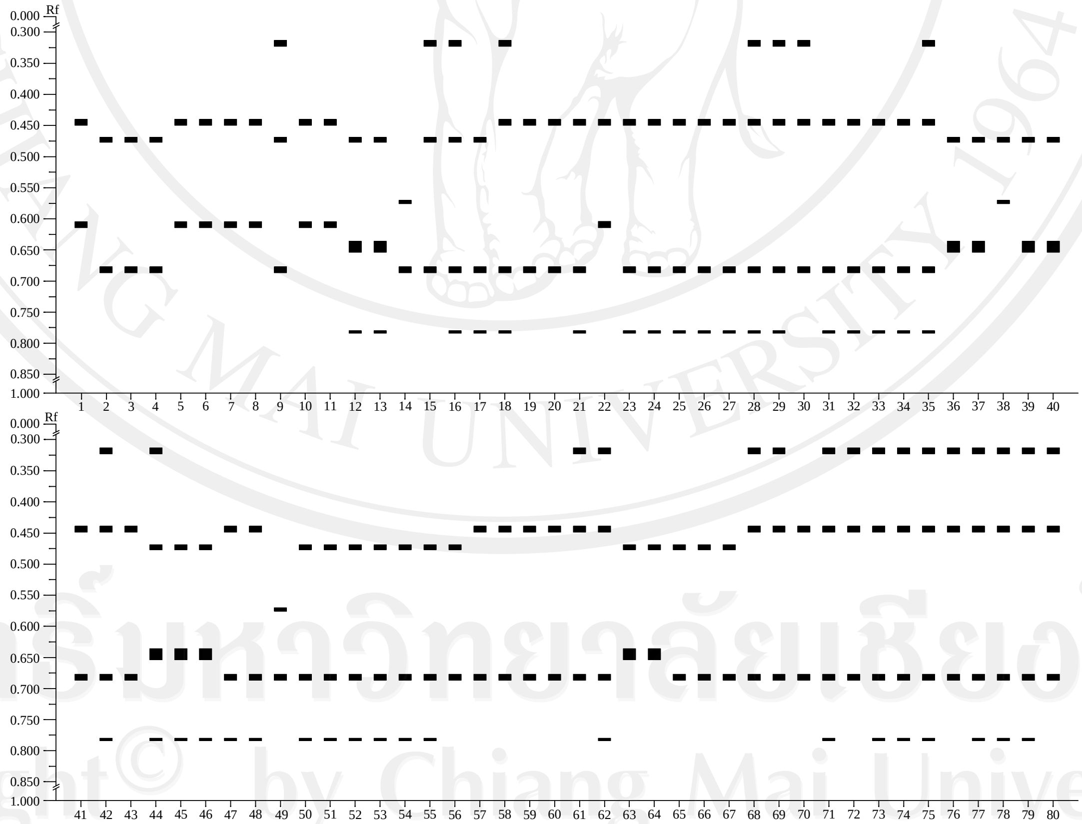


Figure 189 MDH zymogram of *Gymnema inodorum* Decne.

Table 105 MDH banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]								Sample code
		0.318 [5]	0.445 [5]	0.473 [4]	0.573 [3]	0.609 [5]	0.645 [9]	0.682 [5]	0.782 [2]	
A	2		✓			✓				1, 5, 6, 7, 8, 10, 11, 22
B	2			✓				✓		2, 3, 4, 56, 65, 66, 67
C	3	✓		✓				✓		9, 15
D	3			✓			✓		✓	12, 13, 45, 46
E	2				✓			✓		14, 49
F	4	✓		✓				✓	✓	16
G	3			✓				✓	✓	17, 50, 51, 52, 53, 54, 55
H	4	✓	✓					✓	✓	18, 28, 29, 35, 42, 62, 71, 73, 74, 75, 77, 78, 79
I	2		✓					✓		19, 20, 41, 43, 57, 58, 59, 60, 70
J	3		✓					✓	✓	21, 23, 24, 25, 26, 27, 31, 32, 33, 34, 47, 48
K	3	✓	✓					✓		30, 61, 68, 69, 72, 76, 80
L	2			✓			✓			36, 37, 39, 40, 63, 64
M	2			✓	✓					38
N	4	✓		✓			✓		✓	44

7.3.7 Peroxidase (POX)

Six band types appeared in POX gel. They were located at the Rf of 0.445, 0.455, 0.464, 0.473, 0.482 and 0.564 with the band thickness of 2-12 mm. The columns separately showed 1 to 2 band types (Figures 190 and 191). Banding patterns were analysed and the result was used to classify the total tested samples into 8 groups (Table 106).

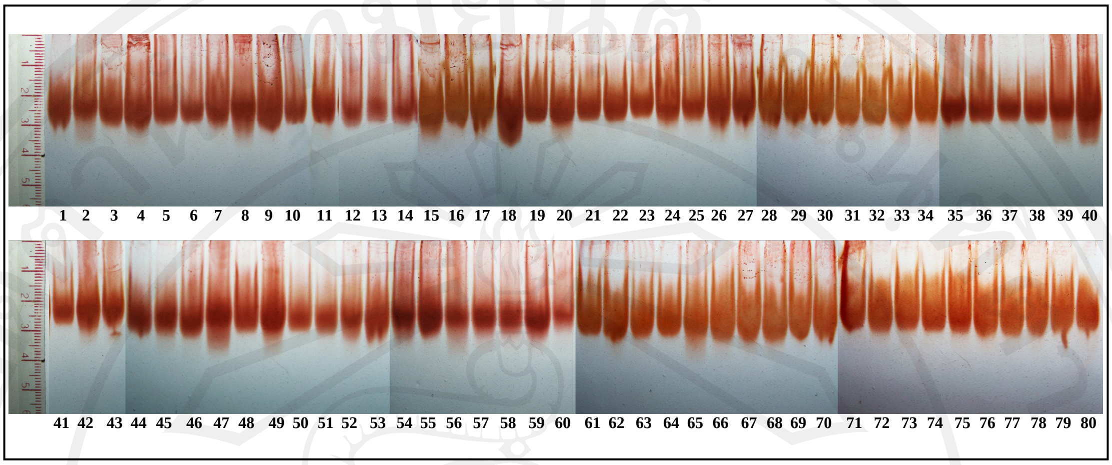


Figure 190 POX banding pattern of *Gymnema inodorum* Decne.

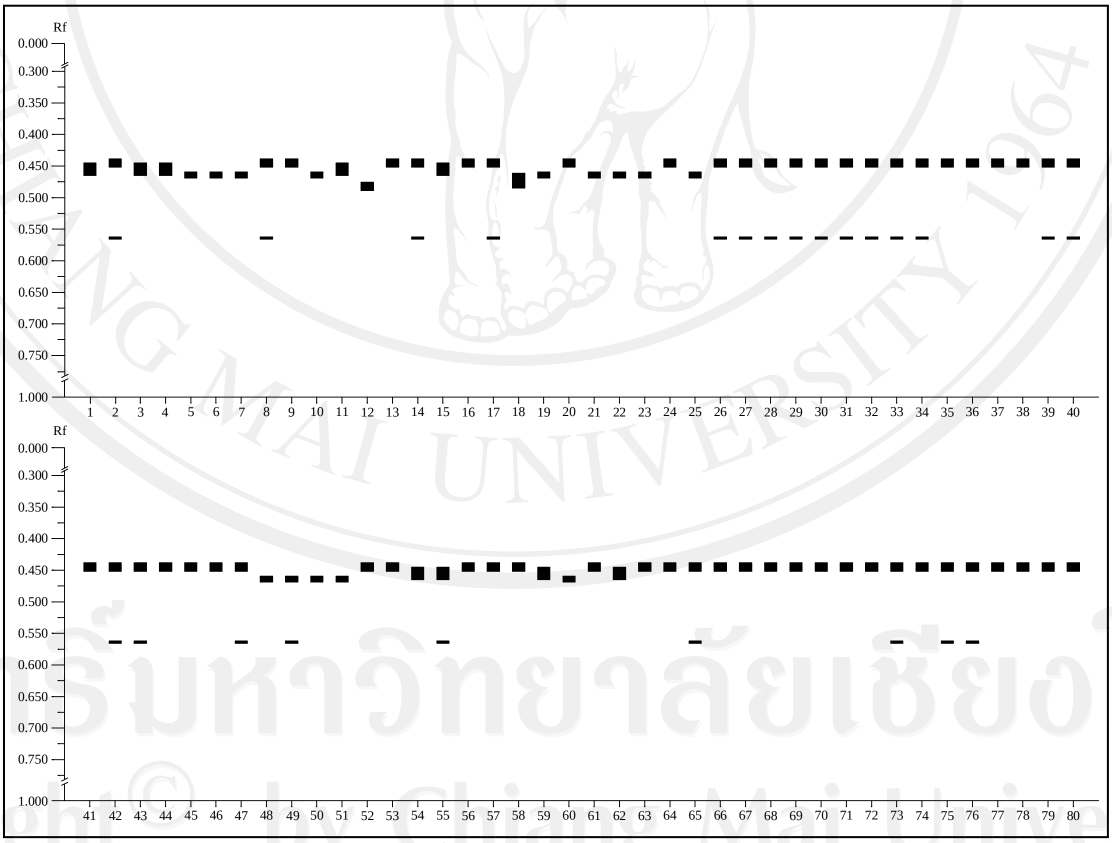


Figure 191 POX zymogram of *Gymnema inodorum* Decne.

Table 106 POX banding of *Gymnema inodorum* Decne.

Group	Number of band	Rf [Thickness (mm)]						Sample code
		0.445 [7]	0.455 [10]	0.464 [5]	0.473 [12]	0.482 [7]	0.564 [2]	
A	1		✓					1, 3, 4, 11, 15, 54, 59, 62
B	2	✓					✓	2, 8, 14, 17, 26, 27, 28, 29, 30, 31, 32, 33, 34, 39, 40, 42, 43, 47, 65, 73, 75, 76
C	1			✓				5, 6, 7, 10, 19, 21, 22, 23, 25, 48, 50, 51, 60
D	1	✓						9, 13, 16, 20, 24, 35, 36, 37, 38, 41, 44, 45, 46, 52, 53, 56, 57, 58, 61, 63, 64, 66, 67, 68, 69, 70, 71, 72, 74, 77, 78, 79, 80
E	1					✓		12
F	1				✓			18
G	2			✓			✓	49
H	2		✓				✓	55

7.3.8 Shikimate dehydrogenase (SKD)

SKD was the only enzyme system unable to produce banding pattern for *Gymnema inodorum* Decne.

7.3.9 Genetic relationship

Results indicated in 7.3.1 to 7.3.8 as described above could be concluded that the enzyme systems of ACP, EST, GDH, GOT, LAP, MDH and POX separated *Gymnema inodorum* Decne. samples into 6, 14, 12, 9, 6, 14 and 8 groups, respectively (Figure 192). Bands across all enzyme systems and entries were analysed and relatedness of the accessions gathered from targeted locations appeared, as shown in Figure 193 and Table 107, at the coefficient indices of 70%-79%, in nine separate groups.

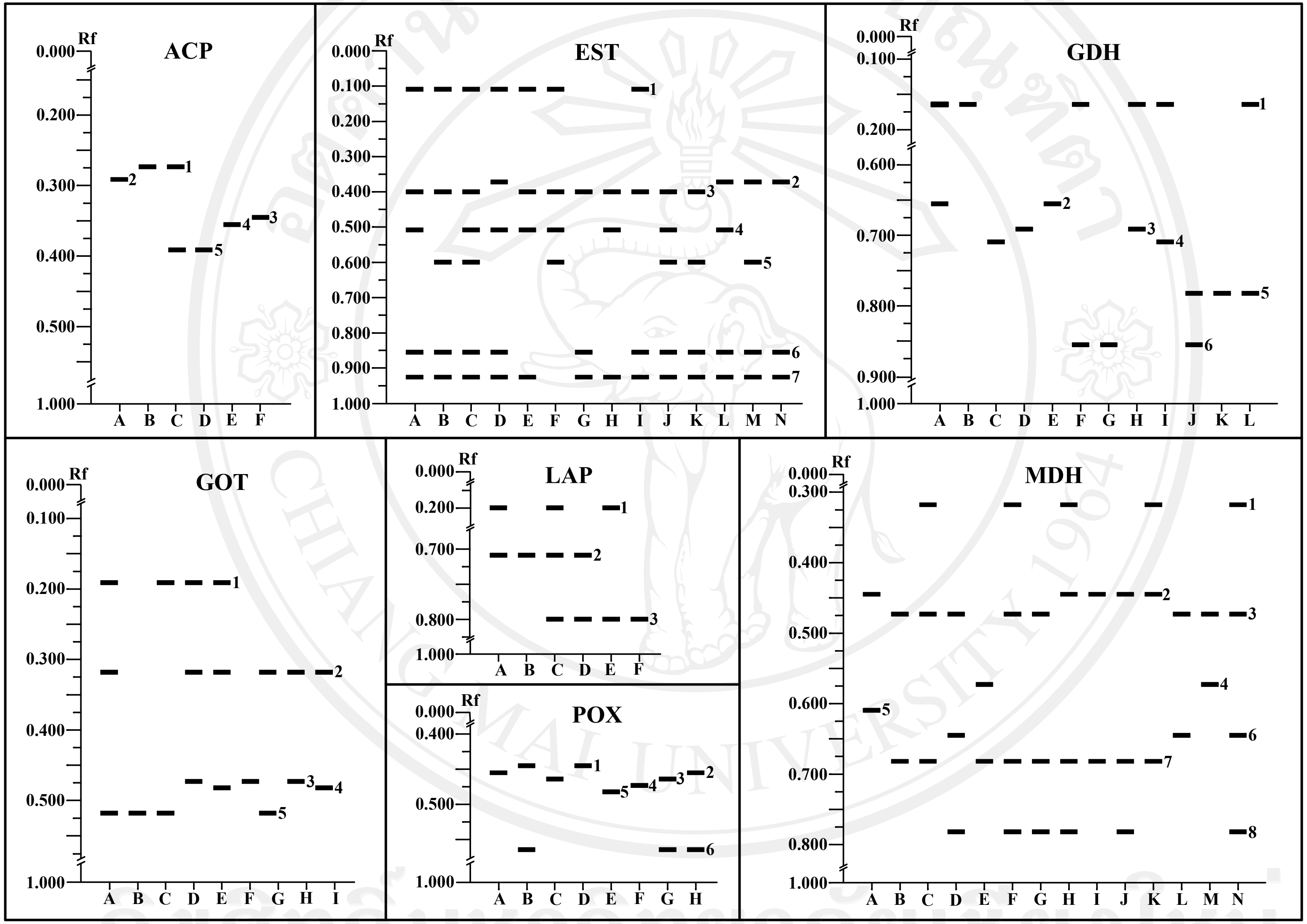


Figure 192 Schematic zymogram of representative phenotypes of *Gymnema inodorum* Decne. reproduced from ACP, EST, GDH, GOT, LAP, POX and MDH enzyme systems

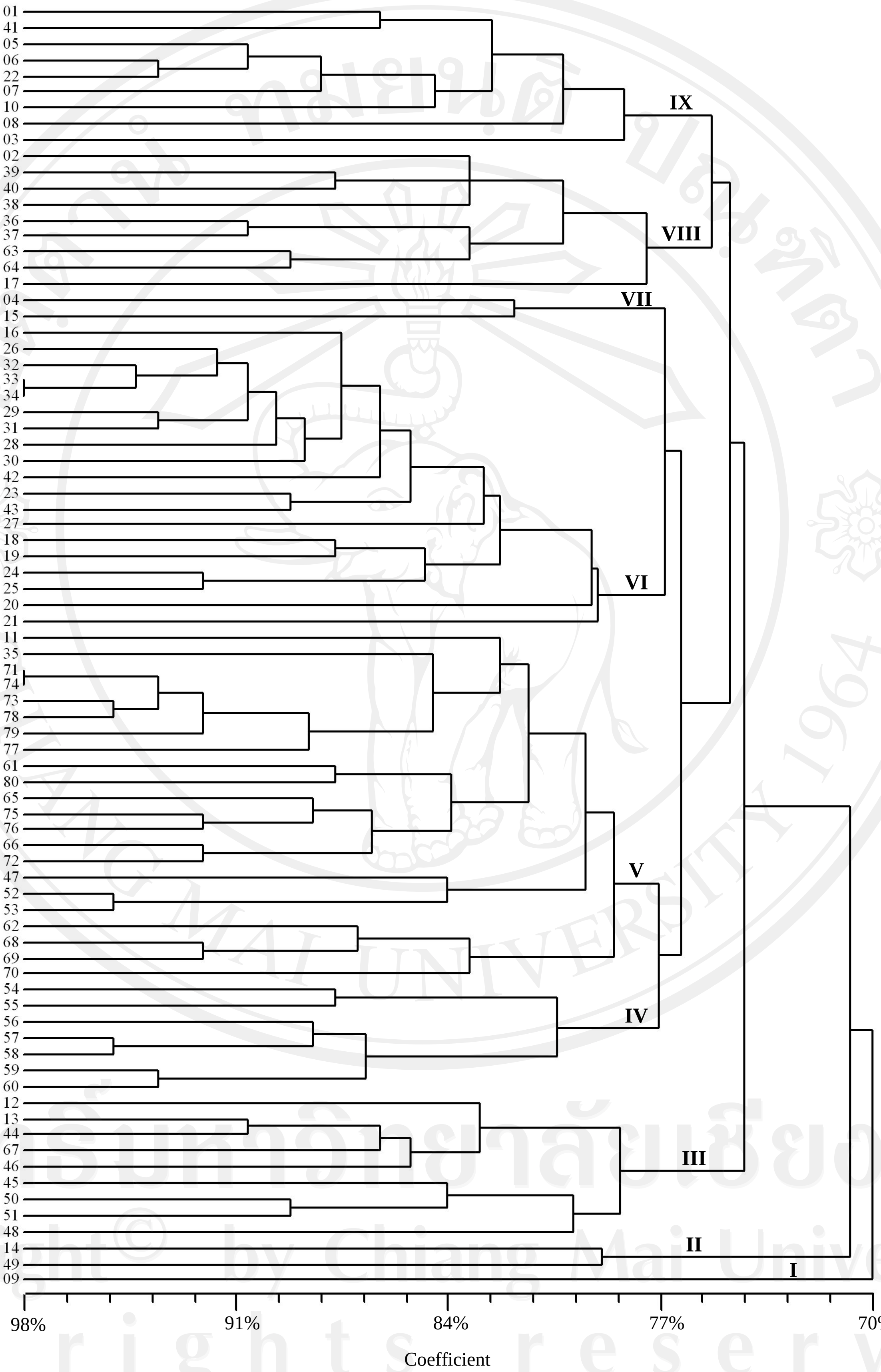


Figure 193 Dendrogram showing genetical relatedness of *Gymnema inodorum* Decne. accessions

Table 107 **Total number of samples with accession codes of classified *Gymnema inodorum* Decne. via polyacrylamide gel electrophoresis**

Group	Total number of samples	Sample code*
I	1	9
II	2	14, 49
III	9	12, 13, 44, 45, 46, 48, 50, 51, 67
IV	7	54, 55, 56, 57, 58, 59, 60
V	22	11, 35, 47, 52, 53, 61, 62, 65, 66, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80
VI	19	16, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 42, 43
VII	2	4, 15
VIII	9	2, 17, 36, 37, 38, 39, 40, 63, 64
IX	9	1, 3, 5, 6, 7, 8, 10, 22, 41

* blue = Chiang Mai; pink = Chiang Rai; black = Lampang; green = Lamphun; yellow = Mae Hong Son; red = Nan; purple = Phayao; orange = Phrae