

## CHAPTER 4

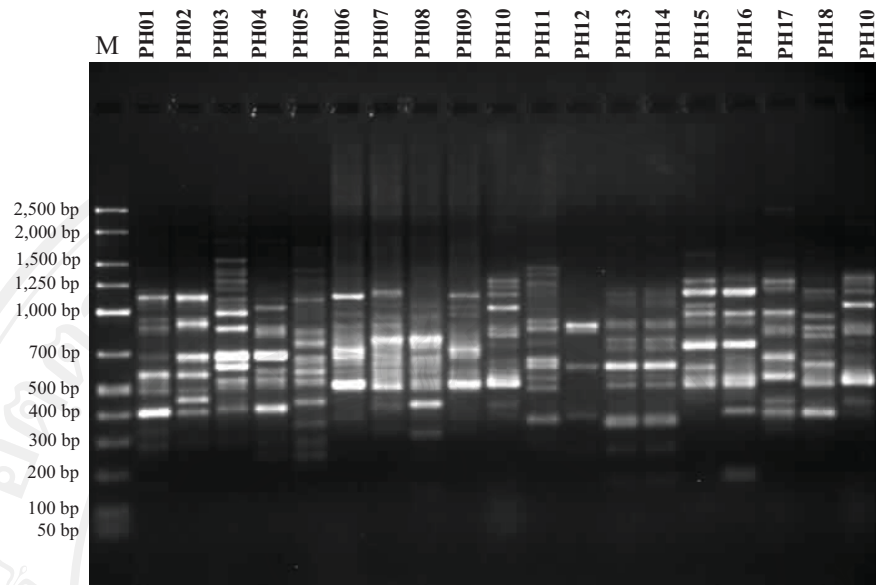
### RESULTS

#### 4.1 Analysis of genetic relationship of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, by RAPD technique

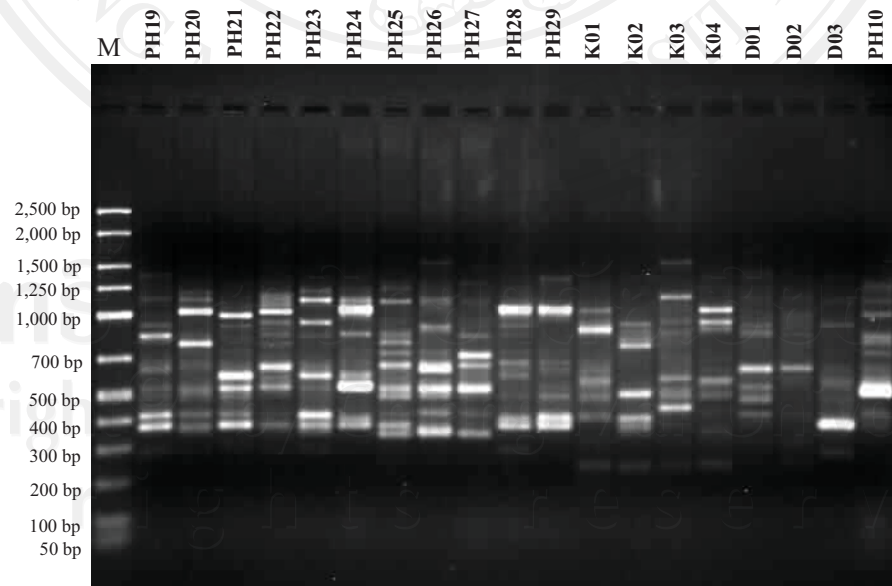
##### RAPD analysis

Thirty-six samples of 30 representative species were collected from 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*. Twenty decamer primers, OPAK01, OPAK10, OPAK11, OPD03, OPD10, and OPF01 - OPF15, were screened. It was found that 6 primers: OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14 generated a total of 84 DNA bands, 2 of which were monomorphic while 82 were polymorphic DNA bands, in the ranges of 223 - 1,686, 378 - 1,644, 300 - 2,300, 375 - 1,500, 327 - 1,550 and 262 - 2,000 bp, respectively (Figure 12 - 23), the number of DNA bands ranged from 12 bands by OPF02 and OPF09 to 17 bands by OPF14 primer, and the average number was 14 bands per primer (Table 9).

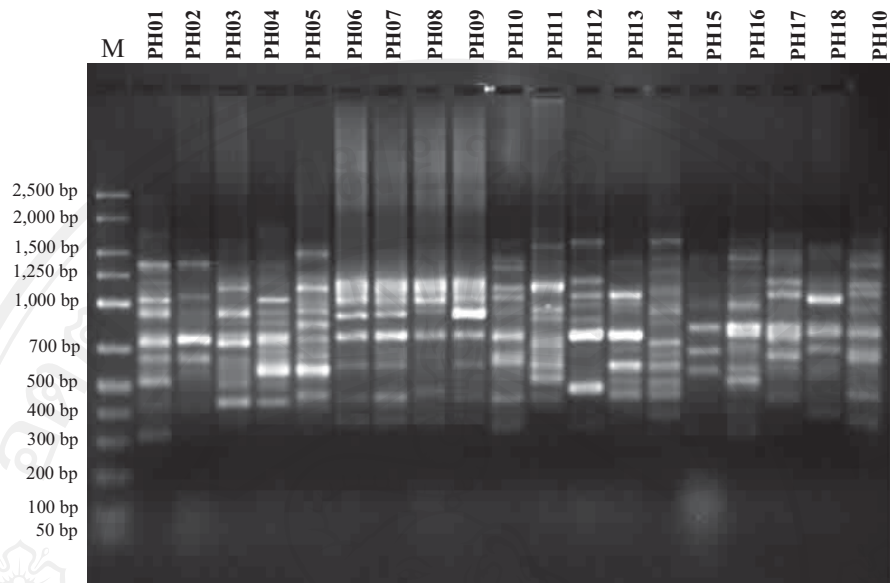
For each section or genus, a total number of DNA bands generated by 6 primers ranged from 27 bands in *P. sumatrana* to 44 bands in *P. corningiana* with 35.7 bands in average (Table 9). The polymorphic DNA bands for each section/genus varied from 0 - 70.24 % with the total of 97.62 % (Table 10). Gene diversity ( $h$ ) of all section/genus was 0.3175: section *Zebrinae* showed the highest gene diversity at 0.2286 whereas the lowest, 0, was found in section *Proboscidioides* (Table 10).



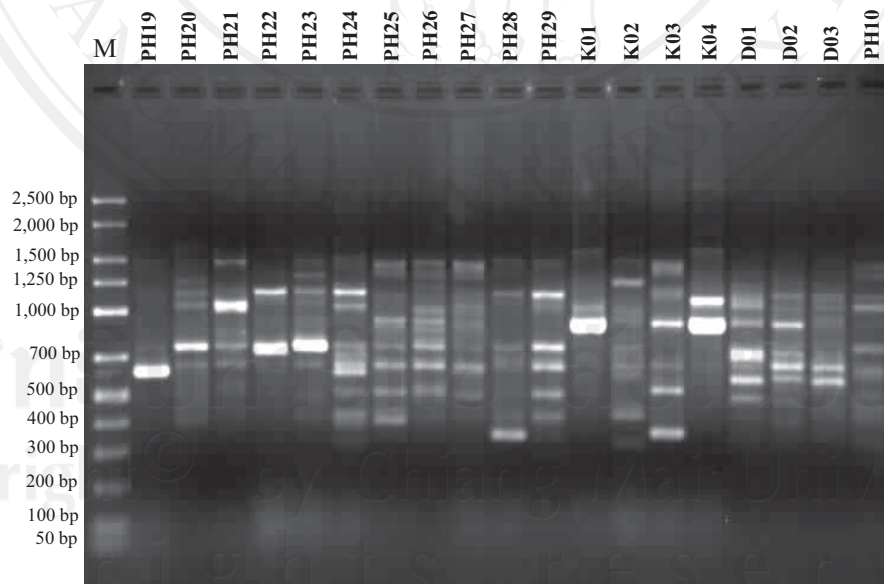
**Figure 12** RAPD profiles of PH01 - PH18 generated by OPAK10 primer.



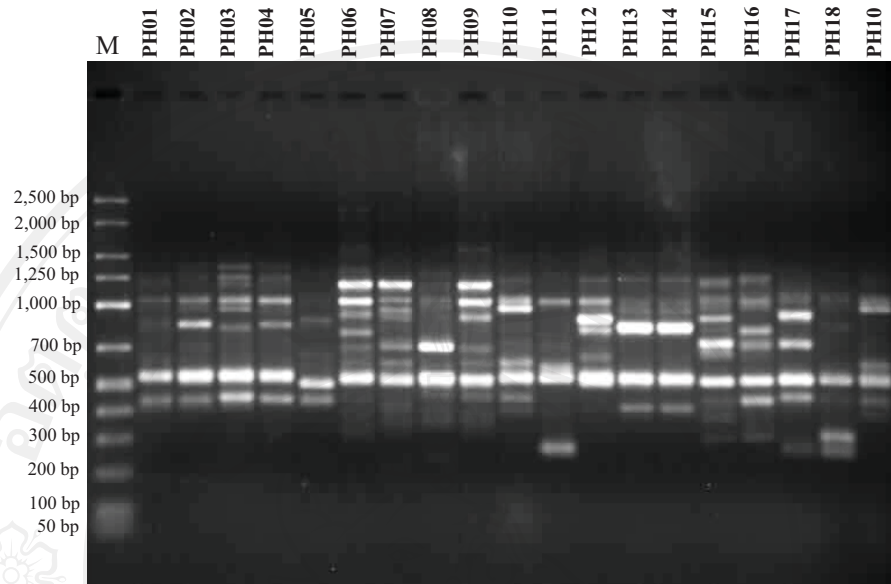
**Figure 13** RAPD profiles of PH19 - D03 generated by OPAK10 primer.



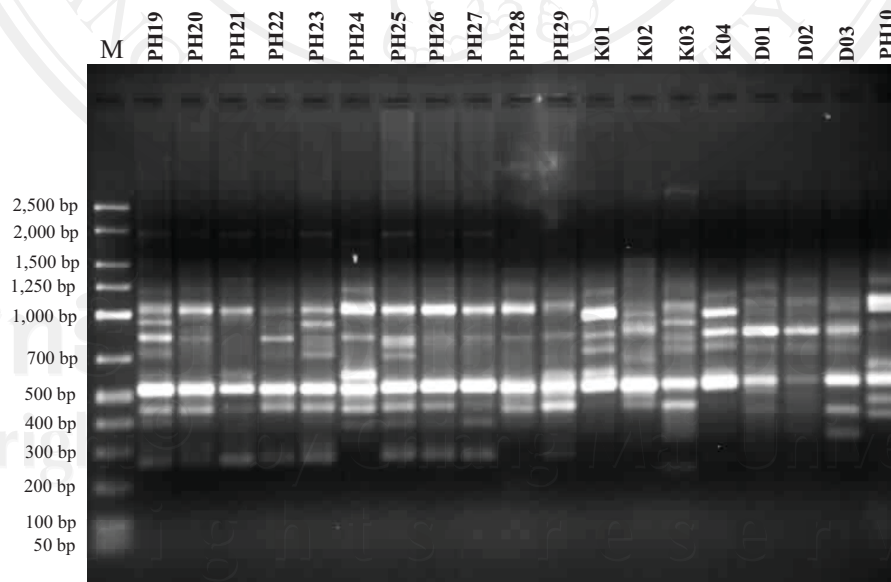
**Figure 14** RAPD profiles of PH01 - PH18 generated by OPD03 primer.



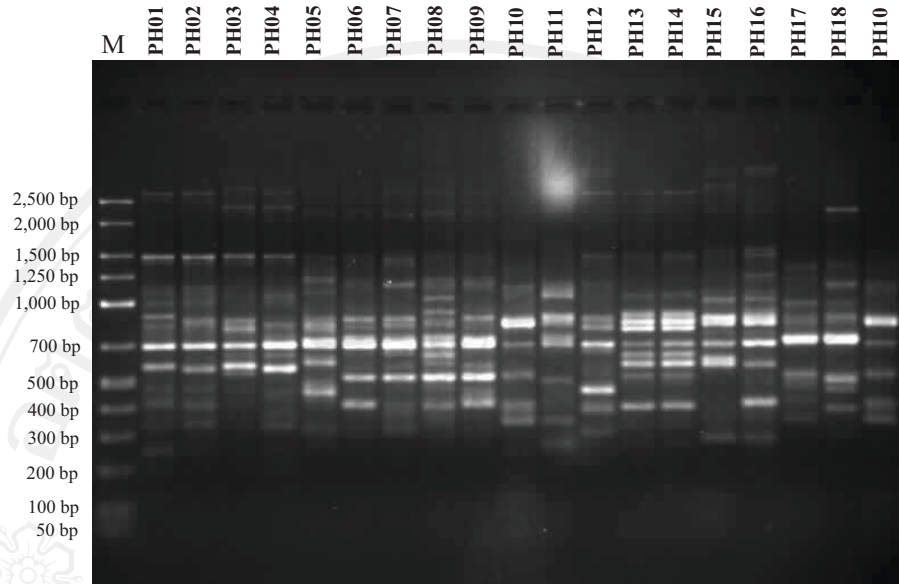
**Figure 15** RAPD profiles of PH19 - D03 generated by OPD03 primer.



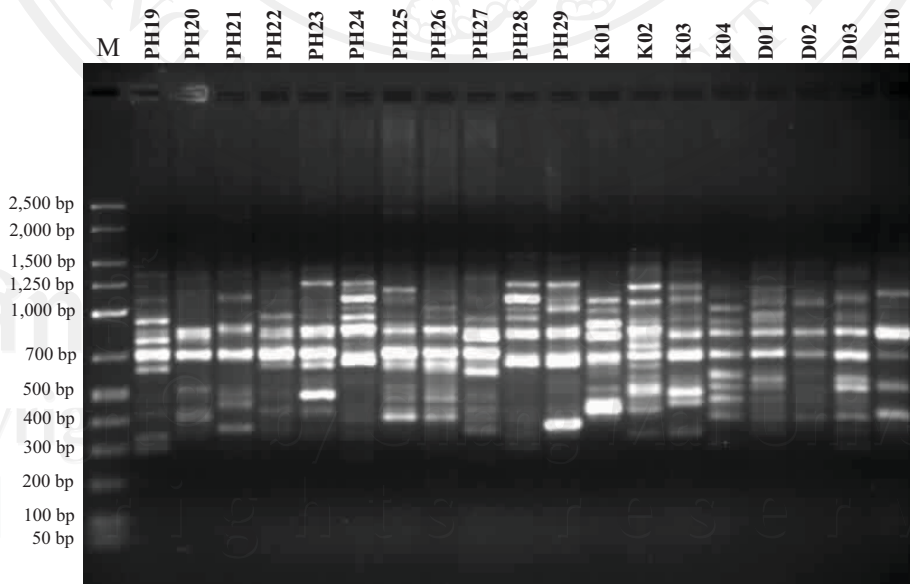
**Figure 16** RAPD profiles of PH01 - PH18 generated by OPF01 primer.



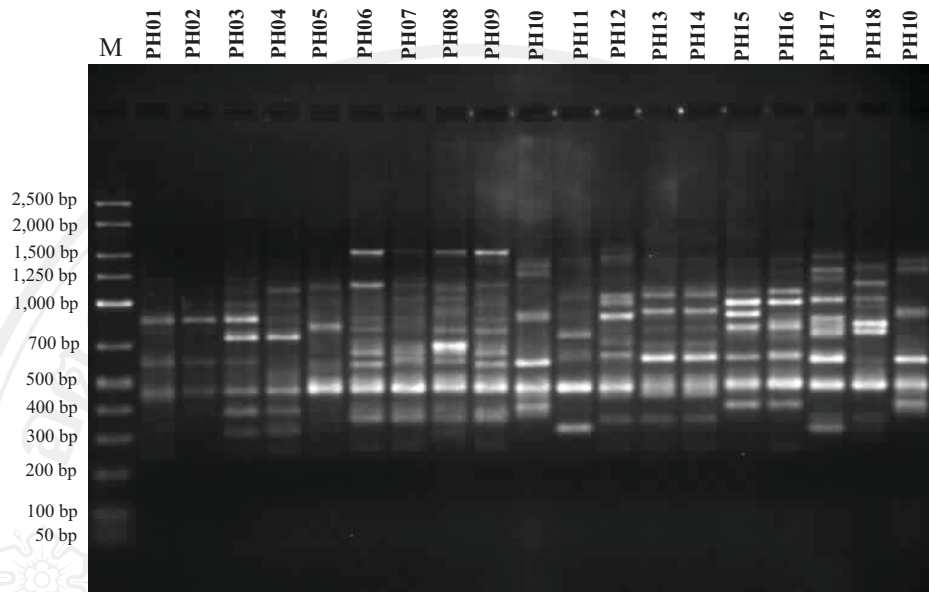
**Figure 17** RAPD profiles of PH19 - D03 generated by OPF01 primer.



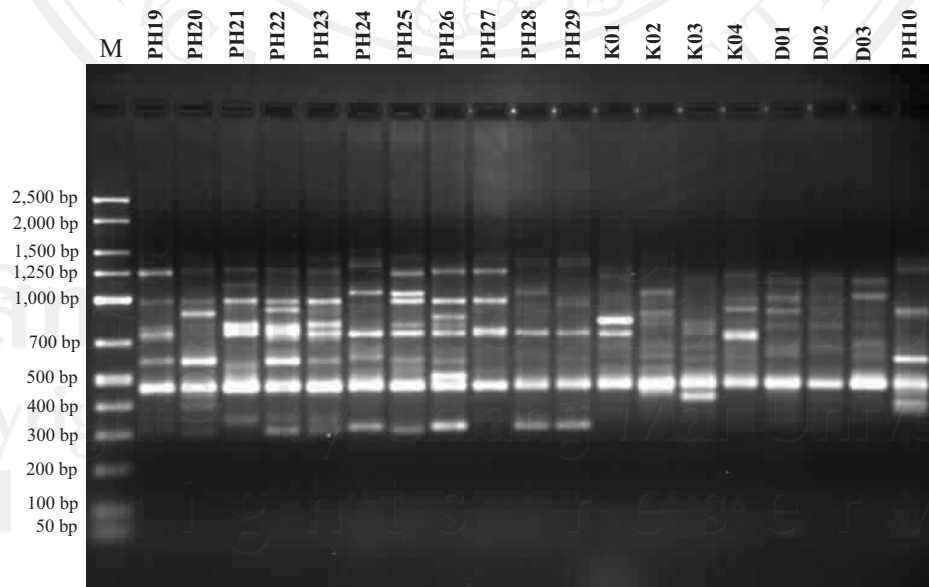
**Figure 18** RAPD profiles of PH01 - PH18 generated by OPF02 primer.



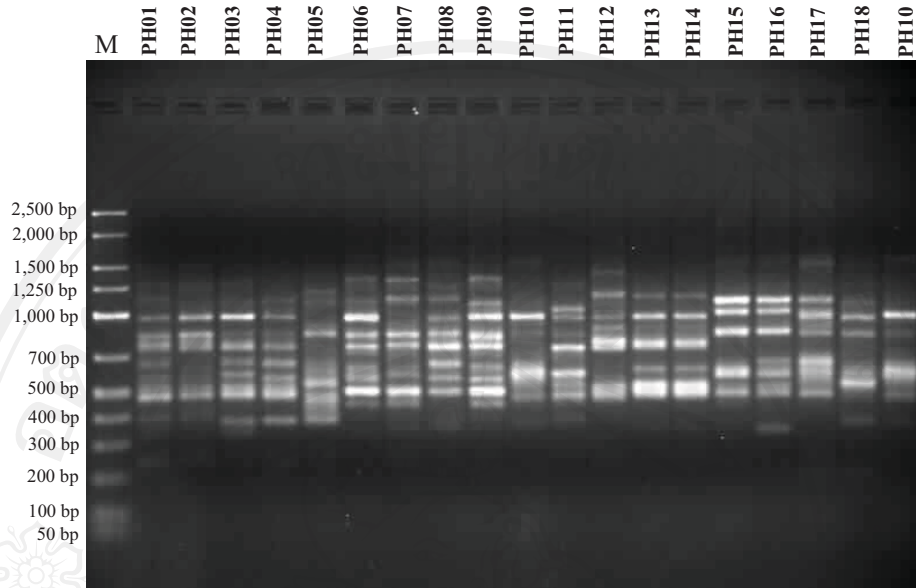
**Figure 19** RAPD profiles of PH19 - D03 generated by OPF02 primer.



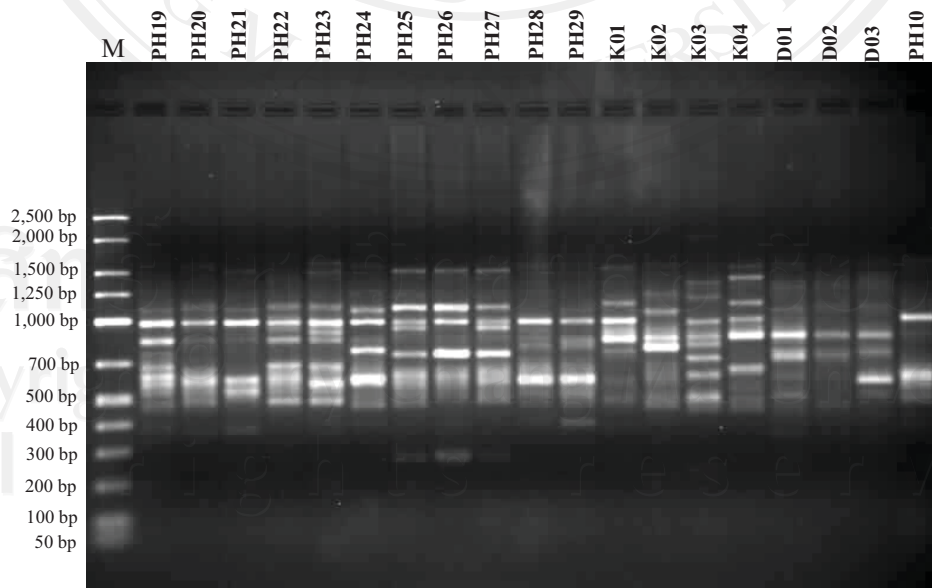
**Figure 20** RAPD profiles of PH01 - PH18 generated by OPF09 primer.



**Figure 21** RAPD profiles of PH19 - D03 generated by OPF09 primer.



**Figure 22** RAPD profiles of PH01 - PH18 generated by OPF14 primer.



**Figure 23** RAPD profiles of PH19 - D03 generated by OPF14 primer.

**Table 9** Number of DNA bands of 36 samples from 30 species of *Phalaenopsis* and two related genera, *Doritis* and *Kingidium*, generated by OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14 primers.

Code	Section/genus	Species	Number of DNA band						Total no. of DNA band	Average no. of DNA band
			OPAK	OPD	OPF	OPF	OPF	OPF		
PH01	<i>Phalaenopsis</i>	<i>P. amabilis</i>	8	8	6	8	5	4	39	6.5
PH02	<i>Phalaenopsis</i>	<i>P. aphrodite</i>	7	5	6	6	7	5	36	6.0
PH03	<i>Phalaenopsis</i>	<i>P. schilleriana</i>	8	6	5	3	7	7	36	6.0
PH04	<i>Phalaenopsis</i>	<i>P. philippinensis</i>	6	7	6	6	6	7	38	6.3
		Total	13	9	6	9	11	7	55	9.2
PH05	<i>Proboscidioides</i>	<i>P. lowii</i>	8	8	5	5	3	7	36	6.0
		Total	8	8	5	5	3	7	36	6.0
PH06	<i>Parishianae</i>	<i>P. gibbosa</i> 1	5	8	7	4	7	6	37	6.2
PH07	<i>Parishianae</i>	<i>P. gibbosa</i> 2	8	8	8	4	3	7	38	6.3
PH08	<i>Parishianae</i>	<i>P. lobbii</i>	8	7	5	6	6	8	40	6.7
PH09	<i>Parishianae</i>	<i>P. parishii</i>	8	7	6	5	5	8	39	6.5
		Total	12	8	9	7	9	10	55	9.2
PH10	<i>Polychilos</i>	<i>P. cornu-cervi</i>	7	9	5	4	5	6	36	6.0
PH11	<i>Polychilos</i>	<i>P. mannii</i>	9	9	3	5	3	9	38	6.3
		Total	10	12	6	7	7	9	51	8.5
PH12	<i>Stauroglottis</i>	<i>P. equestris</i>	10	7	4	6	5	8	40	6.7
PH13	<i>Stauroglottis</i>	<i>P. lindenii</i> 1	8	6	4	4	4	6	32	5.3
PH14	<i>Stauroglottis</i>	<i>P. lindenii</i> 2	10	10	6	6	5	5	42	7.0
		Total	12	11	6	8	6	9	52	8.7
PH15	<i>Fuscatae</i>	<i>P. viridis</i>	9	5	2	3	5	5	29	4.8
PH16	<i>Fuscatae</i>	<i>P. fuscata</i>	9	7	5	9	4	9	43	7.2
		Total	10	7	5	9	5	9	45	7.5
PH17	<i>Amboinenses</i>	<i>P. amboinensis</i> 1	9	5	7	5	7	8	41	6.8
PH18	<i>Amboinenses</i>	<i>P. amboinensis</i> 2	9	4	4	6	6	7	36	6.0
PH19	<i>Amboinenses</i>	<i>P. javanica</i>	9	4	2	3	5	6	29	4.8
PH20	<i>Amboinenses</i>	<i>P. micholitzii</i>	9	5	5	2	6	4	31	5.2
PH21	<i>Amboinenses</i>	<i>P. venosa</i>	10	5	4	4	5	5	33	5.5
		Total	14	8	8	9	9	11	59	9.8
PH22	<i>Zebrinae</i>	<i>P. sumatrana</i>	8	2	3	4	6	4	27	4.5
PH23	<i>Zebrinae</i>	<i>P. corningiana</i>	9	6	6	8	7	8	44	7.3
PH24	<i>Zebrinae</i>	<i>P. hieroglyphica</i>	8	7	6	4	6	5	36	6.0
PH25	<i>Zebrinae</i>	<i>P. violacea</i>	8	5	6	3	5	7	34	5.7
PH26	<i>Zebrinae</i>	<i>P. violacea</i> var. <i>sumatra</i>	8	6	5	5	5	6	35	5.8
PH27	<i>Zebrinae</i>	<i>P. bellina</i>	7	6	4	3	7	6	33	5.5
PH28	<i>Zebrinae</i>	<i>P. pulchra</i>	11	6	4	5	5	4	35	5.8
PH29	<i>Zebrinae</i>	<i>P. mariae</i>	9	6	7	6	5	4	37	6.2
		Total	12	12	12	10	10	13	69	11.5



Table 9 Continued.

Code	Section/genus	Species	Number of DNA band						Total no. of DNA band	Average no. of DNA band
			OPAK	OPD	OPF	OPF	OPF	OPF		
			10	03	01	02	09	14		
K01	<i>Kingidium</i>	<i>K. braceana</i>	8	3	6	5	4	5	31	5.2
K02	<i>Kingidium</i>	<i>K. deliciosa</i>	9	6	4	6	2	6	33	5.5
K03	<i>Kingidium</i>	<i>K. minus</i>	10	5	2	5	4	6	32	5.3
K04	<i>Kingidium</i>	<i>K. philippinensis</i>	12	3	3	6	6	6	36	6.0
		Total	14	10	9	9	7	11	60	10.0
D01	<i>Doritis</i>	<i>D. pulcherrima</i>	8	4	7	5	5	5	34	5.7
D02	<i>Doritis</i>	<i>D. pulcherrima</i> 'dwarf'	11	5	7	2	3	6	34	5.7
D03	<i>Doritis</i>	<i>D. pulcherrima</i> var. <i>buyssoniana</i>	10	5	6	5	4	4	34	5.7
		Total	14	5	7	7	6	6	45	7.5
Average			8.6	6.0	5.0	4.9	5.1	6.1	35.7	6.0
Range			5-12	2-10	2-8	2-9	2-7	4-9	27-44	4.5-7.3
Total			16	13	14	12	12	17	84	14

**Table 10** Gene diversity and number of polymorphic bands among 8 sections of *Phalaenopsis* and two related genera, *Doritis* and *Kingidium*.

Section/genus	n	Gene diversity (h)	No. of polymorphic band	% Polymorphic band
<i>Phalaenopsis</i>	4	0.1555	35	41.67
<i>Proboscidioides</i>	1	0	0	0
<i>Parishianae</i>	4	0.1513	33	39.29
<i>Polychilos</i>	2	0.1331	27	32.14
<i>Stauroglottis</i>	3	0.1635	32	38.10
<i>Fuscatae</i>	2	0.0986	20	23.81
<i>Amboinenses</i>	5	0.1992	47	55.95
<i>Zebrinae</i>	8	0.2286	59	70.24
<i>Kingidium</i>	4	0.2073	49	58.33
<i>Doritis</i>	3	0.0951	21	25.00
Total	36	0.3175	82	97.62

### Analysis of genetic relationship

Genetic relationships among 36 samples of 30 species from 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, were evaluated using POPGENE version 1.32 program (Yeh *et al.*, 1999). The results of 6 primer combinations analysis among 25 *Phalaenopsis* species showed the genetic distance values in the ranges of 0.14 between *P. violacea* and *P. violacea* var. *sumatra*, and *P. violacea* and *P. bellina*, to 0.79 between *P. corningiana* and *P. lowii*. When compared the 25 *Phalaenopsis* species to the related genera, *Doritis* and *Kingidium*, displayed the genetic distance values in the ranges of 0.29 between *D. pulcherrima* ‘dwarf’ and *P. javanica*, to 0.67 between *K. philippinensis* and *P. amboinensis* 1 (Table 11). The dendrogram from UPGMA cluster analysis of 6 primer combinations could distinguish and divide the genus *Phalaenopsis* and related genera into 9 major groups at genetic distance of 0.20 (Figure 24). The bootstrap confidence values for clusters were in the ranges of 35.7 - 92.5 % (Figure 24).

**Group 1:** Consisted of 2 sections from 3 subgroups, which were supported by 37.5 % bootstrap value;

subgroup 1: consisted of 2 sections, 1) section *Amboinenses*, i.e. *P. javanica* and *P. micholitzii* and 2) section *Zebrinae*, i.e. *P. sumatrana*, which were supported by 39.5 % bootstrap value.

Subgroup 2: consisted of 1 section, section *Zebrinae*, i.e. *P. hieroglyphica*, *P. mariae* and *P. pulchra*, which were supported by 52.9 % bootstrap value.

Subgroup 3: consisted of 1 section, section *Zebrinae*, i.e. *P. bellina*, *P. violacea* and *P. violacea* var. *sumatra*, which were supported by 70.2 % bootstrap value.

**Group 2:** Consisted of 2 sections from 2 subgroups, which were supported by 49.7 % bootstrap value;

Subgroup 1: consisted of 1 section, section *Fuscatae*, i.e. *P. fuscata* and *P. viridis*. which were supported by 52.5 % bootstrap value.

Subgroup 2: consisted of 2 sections, 1) section *Amboinenses*, i.e. *P. amboinensis* 1, *P. amboinensis* 2 and *P. venosa* and 2) section *Zebrinae*, i.e. *P. corningiana*, which were supported by 50.2 % bootstrap value.

**Group 3:** Consisted of 1 section, section *Stauroglottis*, i.e. *P. equestris*, *P. lindenii* 1 and *P. lindenii* 2, which were supported by 42.5 % bootstrap value.

**Group 4:** Consisted of 2 sections, 1) section *Phalaenopsis*, i.e. *P. amabilis*, *P. aphrodite*, *P. philippinensis* and *P. schilleriana*, and 2) section *Polychilos*, i.e. *P. cornu-cervi*, which were supported by 47.5 % bootstrap value.

**Group 5:** Consisted of genus *Kingidium*, *K. delisiosa* and *K. philippinensis*, which were supported by 49.5 % bootstrap value.

**Group 6:** Consisted of genus *Kingidium*, *K. braceana* and *K. minus*, which were supported by 39.2 % bootstrap value.

**Group 7:** Consisted of genus *Doritis*, *D. pulcherrima*, *D. pulcherrima* ‘dwarf’ and *D. pulcherrima* var. *buyssoniana*, which were supported by 87.1 % bootstrap value.

**Group 8:** Consisted of 2 sections, 1) section *Proboscidioides*, i.e. *P. lowii*, and 2) section *Polychilos*, i.e. *P. mannii*, which were supported by 47.9 % bootstrap value.

**Group 9:** Consisted of 1 section, section *Parishianae*, i.e. *P. gibbosa* 1, *P. gibbosa* 2, *P. lobbii* and *P. parishii*, which were supported by 57.9 % bootstrap value.

Genetic distance values among 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, ranged from 0.07 between section *Zebrinae* and section *Amboinenses*, to 0.46 between section *Zebrinae* and section *Proboscidioides* (Table 12). The dendrogram from UPGMA cluster analysis of 6 primer combinations could distinguish and divide the genus *Phalaenopsis* and related genera into 2 major groups at genetic distance of 0.15 (Figure 25).

**Group 1:** Consisted of 7 sections from genus *Phalaenopsis*, i.e. section *Amboinenses*, *Zebrinae*, *Phalaenopsis*, *Parishianae*, *Polychilos*, *Fuscatae* and *stauroglottis*, and genus *Kingidium*.

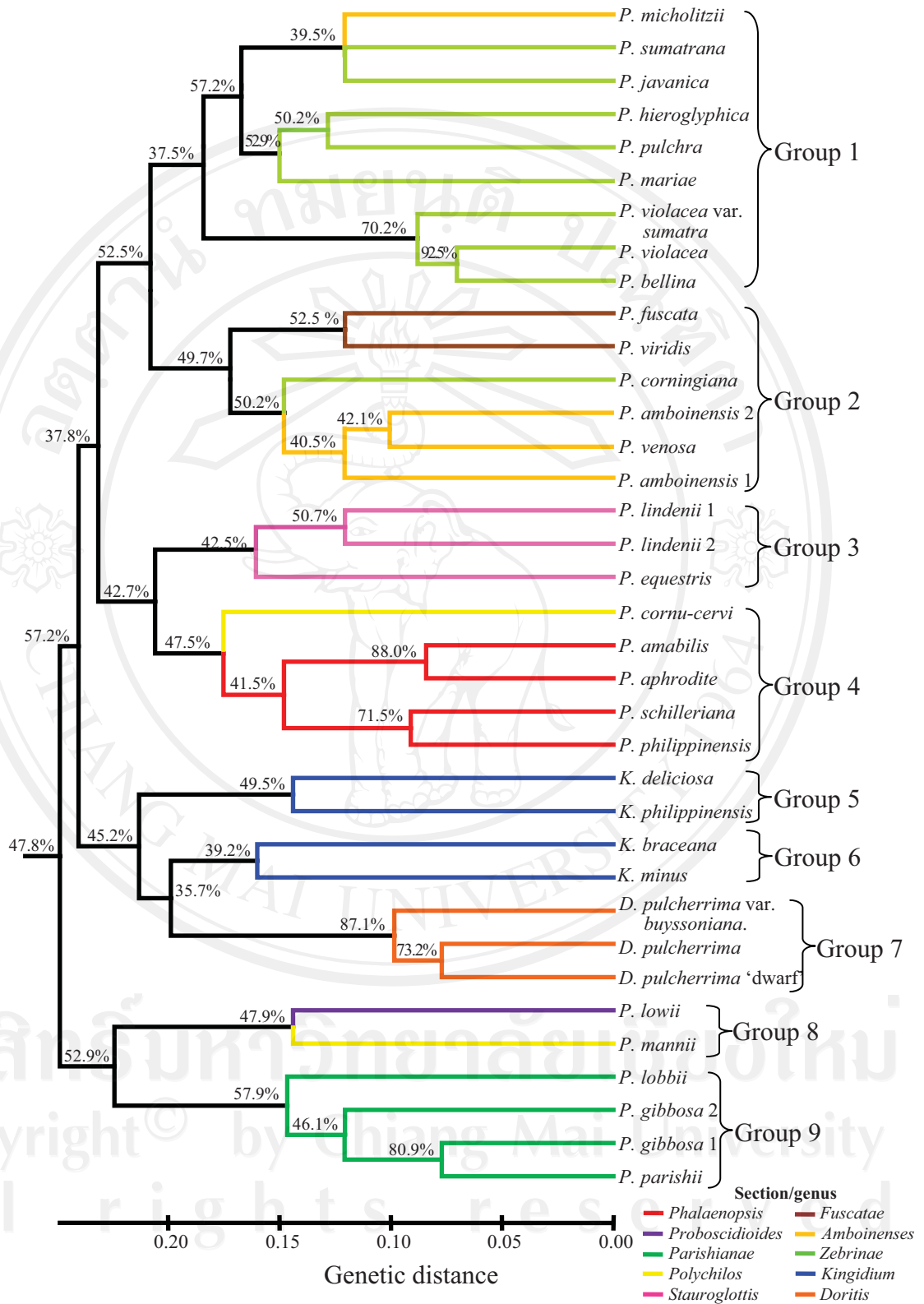
**Group 2:** Consisted of section *Proboscidioides* from genus *Phalaenopsis* and genus *Doritis*.

**Table 11** Genetic distance of 36 samples from 30 species of *Phalaenopsis* (PH01 - PH29) and 2 related genera, *Doritis* (D01 - D03) and *Kingidium* (K01 - K04), based on 6 primer combinations, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14.

Code	PH 01	PH 02	PH 03	PH 04	PH 05	PH 06	PH 07	PH 08	PH 09	PH 10	PH 11	PH 12	PH 13	PH 14	PH 15	PH 16	PH 17	PH 18
PH01	0																	
PH02	0.17	0																
PH03	0.29	0.34	0															
PH04	0.26	0.30	0.20	0														
PH05	0.40	0.41	0.44	0.44	0													
PH06	0.48	0.46	0.39	0.42	0.67	0												
PH07	0.54	0.48	0.52	0.56	0.37	0.23	0											
PH08	0.54	0.52	0.52	0.44	0.48	0.29	0.27	0										
PH09	0.41	0.42	0.35	0.46	0.58	0.15	0.26	0.32	0									
PH10	0.35	0.37	0.27	0.41	0.41	0.29	0.41	0.41	0.39	0								
PH11	0.56	0.50	0.42	0.50	0.29	0.41	0.39	0.39	0.30	0.39	0							
PH12	0.39	0.41	0.52	0.41	0.52	0.50	0.56	0.48	0.50	0.60	0.46	0						
PH13	0.42	0.34	0.44	0.41	0.48	0.46	0.48	0.48	0.50	0.34	0.46	0.37	0					
PH14	0.39	0.37	0.44	0.41	0.41	0.54	0.56	0.60	0.58	0.41	0.50	0.27	0.24	0				
PH15	0.44	0.46	0.32	0.39	0.46	0.44	0.54	0.62	0.44	0.35	0.34	0.46	0.32	0.35	0			
PH16	0.44	0.46	0.42	0.39	0.42	0.52	0.50	0.58	0.41	0.42	0.41	0.62	0.46	0.42	0.27	0		
PH17	0.44	0.35	0.42	0.46	0.50	0.44	0.50	0.54	0.37	0.46	0.44	0.67	0.46	0.54	0.44	0.24	0	
PH18	0.35	0.44	0.41	0.41	0.52	0.50	0.65	0.56	0.39	0.41	0.35	0.48	0.52	0.56	0.35	0.32	0.29	0
PH19	0.37	0.42	0.35	0.39	0.46	0.48	0.58	0.46	0.44	0.42	0.48	0.42	0.50	0.58	0.41	0.56	0.48	0.35
PH20	0.34	0.42	0.39	0.54	0.54	0.48	0.54	0.54	0.41	0.32	0.52	0.58	0.39	0.54	0.41	0.52	0.37	0.29
PH21	0.41	0.42	0.50	0.54	0.54	0.52	0.54	0.54	0.41	0.46	0.37	0.58	0.42	0.58	0.37	0.41	0.27	0.23
PH22	0.37	0.46	0.32	0.39	0.58	0.48	0.58	0.50	0.41	0.46	0.44	0.42	0.42	0.58	0.37	0.56	0.41	0.32
PH23	0.35	0.48	0.44	0.48	0.79	0.46	0.56	0.56	0.39	0.44	0.54	0.65	0.56	0.60	0.54	0.46	0.32	0.30
PH24	0.42	0.60	0.48	0.37	0.44	0.58	0.60	0.41	0.54	0.44	0.50	0.52	0.48	0.56	0.50	0.50	0.39	0.30
PH25	0.46	0.48	0.44	0.48	0.60	0.50	0.52	0.52	0.42	0.48	0.42	0.56	0.41	0.52	0.42	0.46	0.42	0.34
PH26	0.37	0.42	0.39	0.39	0.58	0.48	0.58	0.50	0.41	0.39	0.44	0.50	0.35	0.50	0.44	0.52	0.44	0.32
PH27	0.44	0.50	0.42	0.46	0.67	0.48	0.62	0.54	0.48	0.46	0.48	0.42	0.39	0.50	0.41	0.56	0.44	0.29
PH28	0.44	0.54	0.46	0.42	0.54	0.56	0.58	0.42	0.56	0.46	0.48	0.58	0.54	0.54	0.48	0.52	0.44	0.39
PH29	0.48	0.62	0.54	0.54	0.62	0.60	0.58	0.54	0.65	0.46	0.56	0.67	0.58	0.58	0.52	0.56	0.60	0.50
K01	0.44	0.42	0.50	0.46	0.58	0.52	0.58	0.50	0.44	0.58	0.56	0.42	0.54	0.62	0.44	0.56	0.52	0.50
K02	0.48	0.54	0.58	0.54	0.46	0.56	0.50	0.54	0.52	0.62	0.52	0.50	0.50	0.50	0.41	0.48	0.52	0.50
K03	0.39	0.44	0.48	0.44	0.37	0.62	0.60	0.52	0.54	0.60	0.46	0.41	0.65	0.48	0.39	0.58	0.62	0.52
K04	0.42	0.48	0.44	0.48	0.52	0.58	0.52	0.44	0.54	0.52	0.50	0.37	0.56	0.48	0.39	0.62	0.67	0.44
D01	0.46	0.52	0.48	0.44	0.44	0.50	0.52	0.48	0.50	0.44	0.54	0.48	0.56	0.56	0.46	0.39	0.46	0.37
D02	0.46	0.48	0.48	0.41	0.34	0.62	0.56	0.48	0.58	0.44	0.50	0.44	0.56	0.65	0.54	0.54	0.54	0.44
D03	0.46	0.48	0.60	0.44	0.37	0.54	0.48	0.41	0.58	0.44	0.50	0.48	0.48	0.60	0.46	0.50	0.50	0.41

Table 11 Continued.

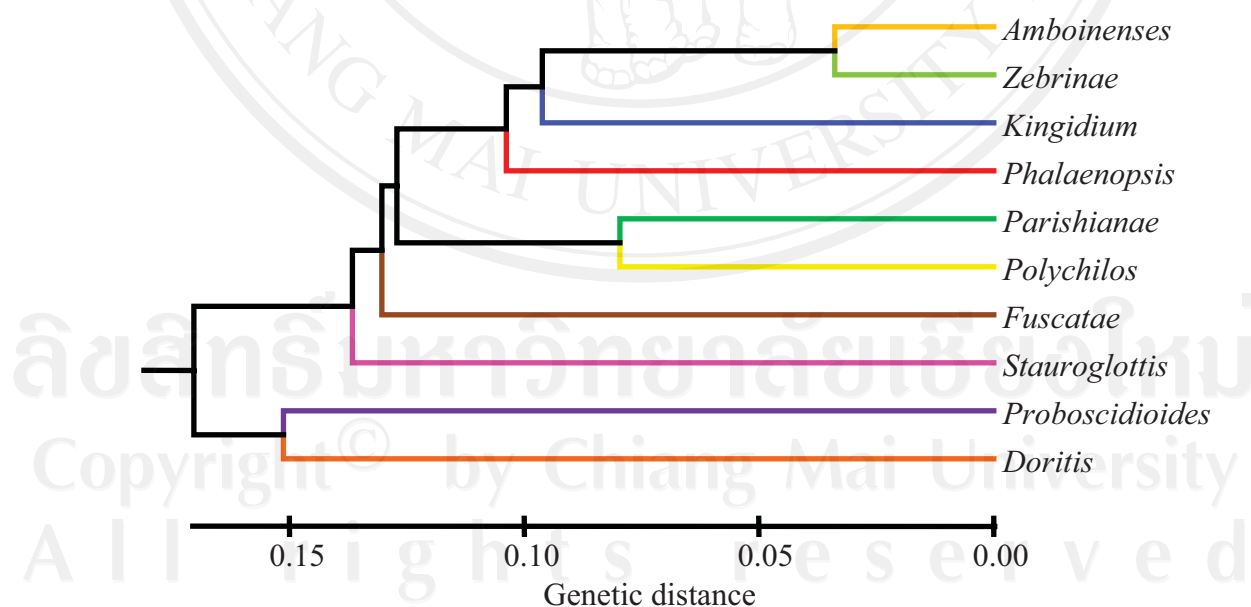
Code	PH 19	PH 20	PH 21	PH 22	PH 23	PH 24	PH 25	PH 26	PH 27	PH 28	PH 29	K 01	K 02	K 03	K 04	D 01	D 02	D 03
PH19	0																	
PH20	0.24	0																
PH21	0.44	0.30	0															
PH22	0.24	0.24	0.27	0														
PH23	0.46	0.32	0.29	0.35	0													
PH24	0.32	0.32	0.29	0.32	0.44	0												
PH25	0.42	0.32	0.35	0.42	0.44	0.37	0											
PH26	0.34	0.30	0.30	0.34	0.35	0.28	0.14	0										
PH27	0.37	0.24	0.30	0.34	0.42	0.35	0.14	0.21	0									
PH28	0.37	0.37	0.34	0.30	0.42	0.26	0.50	0.44	0.48	0								
PH29	0.37	0.41	0.56	0.41	0.46	0.39	0.39	0.34	0.44	0.30	0							
K01	0.41	0.52	0.41	0.48	0.54	0.50	0.46	0.41	0.48	0.48	0.56	0						
K02	0.41	0.56	0.56	0.52	0.50	0.50	0.39	0.41	0.48	0.52	0.44	0.44	0					
K03	0.35	0.62	0.58	0.50	0.74	0.52	0.56	0.54	0.62	0.39	0.46	0.42	0.32	0				
K04	0.39	0.46	0.39	0.39	0.52	0.44	0.52	0.54	0.54	0.35	0.54	0.29	0.50	0.41	0			
D01	0.32	0.50	0.42	0.39	0.48	0.37	0.41	0.39	0.46	0.42	0.42	0.39	0.35	0.44	0.37	0		
D02	0.29	0.56	0.46	0.42	0.60	0.34	0.48	0.42	0.50	0.42	0.42	0.42	0.46	0.41	0.48	0.15	0	
D03	0.39	0.50	0.46	0.46	0.60	0.37	0.44	0.50	0.46	0.42	0.42	0.46	0.35	0.37	0.37	0.18	0.21	0



**Figure 24** Dendrogram of 36 samples from 30 species of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, based on Nei's (1972) genetic distance clustering by the UPGMA method from 6 primer combinations. Bootstrap confidence values for clusters were indicated on the left of each node.

**Table 12** Genetic distance of 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, based on 6 primer combinations, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14.

Section/genus	1	2	3	4	5	6	7	8	9	10
<i>Phalaenopsis</i> (1)	0									
<i>Proboscidioides</i> (2)	0.36	0								
<i>Parishianae</i> (3)	0.27	0.41	0							
<i>Polychilos</i> (4)	0.23	0.27	0.16	0						
<i>Stauroglottis</i> (5)	0.23	0.38	0.34	0.28	0					
<i>Fuscatae</i> (6)	0.25	0.37	0.33	0.21	0.27	0				
<i>Amboinenses</i> (7)	0.19	0.42	0.25	0.21	0.28	0.23	0			
<i>Zebrinae</i> (8)	0.20	0.46	0.25	0.22	0.24	0.27	0.07	0		
<i>Kingidium</i> (9)	0.24	0.34	0.29	0.31	0.28	0.27	0.20	0.19	0	
<i>Doritis</i> (10)	0.30	0.30	0.34	0.32	0.38	0.37	0.27	0.24	0.22	0



**Figure 25** Dendrogram of 8 sections of *Phalaenopsis* and 2 related genera, *Doritis* and *Kingidium*, based on Nei's (1972) genetic distance clustering by the UPGMA method from 6 primer combinations.



## 4.2 Studies on crossability of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*

Twenty-four interspecific, i.e. 21 intersectional and 3 intrasectional, and 20 intergeneric crosses were made. From a total of 264 pollinations, 36 fruits were set in 24 crosses, which was only 13.64 % fruit setting (Table 16). Fruit setting percentage of intersectional, intrasectional and intergeneric hybridizations were 14.07, 5.88 and 14.29 % (Table 16), respectively. Intersectional cross between section *Phalaenopsis*: *P. schilleriana* x section *Polychilos*: *P. cornu-cervi* showed the greatest number of fruit setting, 83.33 % (Table 13), only one intrasectional cross was found, 33.33 % fruit setting in section *Parishianae*: *P. gibbosa* 1 x *P. parishii* (Table 14), while the intergeneric cross between *P. violacea* x *D. pulcherrima* ‘dwarf’ showed the greatest number of fruit setting, 66.67 % (Table 15).

When germination and number of viable seeds were taken into account, number of crossability was reduced. Only 7 crosses could yield viable seeds which showed that low crossability, 2.65 %, was found among the intersectional, intrasectional and intergeneric hybridizations. Seeds of each fruit were sown under aseptic condition. After culturing for 6 months, number of obtained plantlets were counted. The following crosses: four intersectional hybridizations, 1) section *Phalaenopsis*: *P. amabilis* x section *Polychilos*: *P. cornu-cervi*, 2) section *Phalaenopsis*: *P. schilleriana* x section *Polychilos*: *P. cornu-cervi*, 3) section *Zebrinae*: *P. violacea* x section *Polychilos*: *P. cornu-cervi* and 4) section *Zebrinae*: *P. violacea* x section *Amboinenses*: *P. javanica*, one intrasectional hybridization, section *Parishianae*: *P. gibbosa* 1 x *P. parishii* and two intergeneric hybridizations, genus *Doritis*: *D. pulcherrima* ‘dwarf’ x genus *Phalaenopsis*: *P. equestris* and

2) genus *Doritis*: *D. pulcherrima* ‘dwarf’ x genus *Kingidium*: *K. minus* could yield their progenies. Other crosses, even though had fruit set, yielded no viable seed. It was found that cross between *P. schilleriana* x *P. cornu-cervi* gave the greatest number of hybrid seedlings, 1,000 plantlets, while other crosses, *D. pulcherrima* ‘dwarf’ x *K. minus*, *D. pulcherrima* ‘dwarf’ x *P. equestris*, *P. violacea* x *P. cornu-cervi*, *P. amabilis* x *P. cornu-cervi*, *P. gibbosa* 1 x *P. parishii* and *P. violacea* x *P. javanica*, showed various numbers of hybrid seedlings, 650, 500, 200, 100, 50 and 40 plantlets, respectively (Table 17).

Six-month old hybrid seedlings were transplanted from *in vitro* to 70 % shaded house condition. After six months, seedlings of the intersectional, intrasectional and intergeneric hybridizations showed the total of 74.58 % survival rate (Table 18). Seedlings of cross *P. schilleriana* x *P. cornu-cervi* showed the greatest survival rate, 81.25 %, whereas those of crosses *D. pulcherrima* ‘dwarf’ x *P. equestris*, *D. pulcherrima* ‘dwarf’ x *K. minus*, *P. violacea* x *P. javanica*, *P. amabilis* x *P. cornu-cervi*, *P. violacea* x *P. cornu-cervi* and *P. gibbosa* 1 x *P. parishii* yielded only 74.5, 70.8, 70, 64.29, 58 and 43.33 % survival rate, respectively (Table 18).

**Table 13** Results of intersectional hybridization of *Phalaenopsis* species.

No.	Parent 1 (P1)	Parent 2 (P2)	No. of pollinated flower		No. of fruit setting		% fruit setting	
			P1xP2	P2xP1	P1xP2	P1xP2	P1xP2	P2xP1
Section <i>Phalaenopsis</i> x Section <i>Polychilos</i>			11	8	7	0	63.64	0
1	<i>P. amabilis</i>	<i>P. cornu-cervi</i>	5	3	2	0	40.00	0
2	<i>P. schilleriana</i>	<i>P. cornu-cervi</i>	6	5	5	0	83.33	0
Section <i>Phalaenopsis</i> x Section <i>Parishianae</i>			6	5	1	0	9.09	0
3	<i>P. aphrodite</i>	<i>P. parishii</i>	3	3	0	0	0	0
4	<i>P. schilleriana</i>	<i>P. gibbosa</i> 1	3	2	1	0	33.33	0
Section <i>Phalaenopsis</i> x Section <i>Proboscidioides</i>			3	3	0	0	0	0
5	<i>P. schilleriana</i>	<i>P. lowii</i>	3	3	0	0	0	0
Section <i>Phalaenopsis</i> x Section <i>Zebrinae</i>			3	2	0	0	0	0
6	<i>P. schilleriana</i>	<i>P. violacea</i>	3	2	0	0	0	0
Section <i>Proboscidioides</i> x Section <i>Parishianae</i>			8	5	0	0	0	0
7	<i>P. lowii</i>	<i>P. gibbosa</i> 1	5	3	0	0	0	0
8	<i>P. lowii</i>	<i>P. parishii</i>	3	2	0	0	0	0
Section <i>Proboscidioides</i> x Section <i>Polychilos</i>			10	5	0	0	0	0
9	<i>P. lowii</i>	<i>P. cornu-cervi</i>	10	5	0	0	0	0
Section <i>Parishianae</i> x Section <i>Polychilos</i>			3	2	0	0	0	0
10	<i>P. parishii</i>	<i>P. cornu-cervi</i>	3	2	0	0	0	0
Section <i>Parishianae</i> x Section <i>Stauroglottis</i>			3	2	1	1	33.33	33.33
11	<i>P. parishii</i>	<i>P. equestris</i>	3	2	1	1	33.33	33.33
Section <i>Parishianae</i> x Section <i>Zebrinae</i>			3	3	0	1	0	33.33
12	<i>P. parishii</i>	<i>P. violacea</i>	3	3	0	1	0	33.33
Section <i>Polychilos</i> x Section <i>Stauroglottis</i>			5	5	1	1	20.00	20.00
13	<i>P. cornu-cervi</i>	<i>P. equestris</i>	5	5	1	1	20.00	20.00
Section <i>Polychilos</i> x Section <i>Amboinenses</i>			6	5	0	0	0	0
14	<i>P. cornu-cervi</i>	<i>P. amboinensis</i> 2	3	3	0	0	0	0
15	<i>P. cornu-cervi</i>	<i>P. javanica</i>	3	2	0	0	0	0
Section <i>Polychilos</i> x Section <i>Zebrinae</i>			5	5	0	4	0	80.00
16	<i>P. cornu-cervi</i>	<i>P. violacea</i>	5	5	0	4	0	80.00
Section <i>Stauroglottis</i> x Section <i>Amboinenses</i>			4	2	0	0	0	0
17	<i>P. equestris</i>	<i>P. amboinensis</i> 2	2	1	0	0	0	0
18	<i>P. equestris</i>	<i>P. javanica</i>	2	1	0	0	0	0
Section <i>Stauroglottis</i> x Section <i>Zebrinae</i>			3	2	0	0	0	0
19	<i>P. equestris</i>	<i>P. violacea</i>	3	2	0	0	0	0
Section <i>Amboinenses</i> x Section <i>Zebrinae</i>			2	2	0	1	0	50.00
20	<i>P. amboinensis</i> 2	<i>P. violacea</i>	2	2	0	1	0	50.00
Section <i>Amboinenses</i> x Section <i>Zebrinae</i>			2	2	1	0	50.00	50.00
21	<i>P. javanica</i>	<i>P. violacea</i>	2	2	1	0	50.00	50.00
Total			77	58	11	8	14.29*	13.79*

\* Calculated with total value

**Table 14** Results of intrasectional hybridization of *Phalaenopsis* species.

No.	Parent 1 (P1)	Parent 2 (P2)	No. of pollinated flower		No. of fruit setting		% fruit setting	
			P1xP2	P2xP1	P1xP2	P1xP2	P1xP2	P2xP1
Section <i>Phalaenopsis</i>								
1	<i>P. aphrodite</i>	<i>P. schilleriana</i>	4	3	0	0	0	0
Section <i>Parishianae</i>								
2	<i>P. gibbosa</i> 1	<i>P. parishii</i>	3	3	1	0	33.33	0
Section <i>Amboinenses</i>								
3	<i>P. amboinensis</i> 2	<i>P. javanica</i>	2	2	0	0	0	0
Total			9	8	1	0	11.11*	0

\*Calculated with total value

**Table 15** Results of intergeneric hybridization of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*.

No.	Parent 1 (P1)	Parent 2 (P2)	No. of pollinated flower		No. of fruit setting		% fruit setting	
			P1xP2	P2xP1	P1xP2	P1xP2	P1xP2	P2xP1
Genus <i>Phalaenopsis</i> x Genus <i>Doritis</i>			22	19	3	3	13.64	15.79
1	<i>P. amabilis</i>	<i>D. pulcherrima</i> 'dwarf'	5	5	1	1	20.00	20.00
2	<i>P. cornu-cervi</i>	<i>D. pulcherrima</i>	2	3	0	0	0	0
3	<i>P. cornu-cervi</i>	<i>D. pulcherrima</i> 'dwarf'	5	3	0	1	0	33.33
4	<i>P. equestris</i>	<i>D. pulcherrima</i> 'dwarf'	2	2	0	1	0	50.00
5	<i>P. violacea</i>	<i>D. pulcherrima</i>	3	2	0	0	0	0
6	<i>P. violacea</i>	<i>D. pulcherrima</i> 'dwarf'	2	2	0	0	66.67	0
7	<i>P. violacea</i>	<i>D. pulcherrima</i> var. <i>buyssoniana</i>	3	2	2	0	0	0
Genus <i>Phalaenopsis</i> x Genus <i>Kingidium</i>			24	19	5	3	20.83	15.79
8	<i>P. lowii</i>	<i>K. deliciosa</i>	3	3	1	1	33.33	33.33
9	<i>P. cornu-cervi</i>	<i>K. deliciosa</i>	3	2	0	0	0	0
10	<i>P. cornu-cervi</i>	<i>K. minus</i>	3	2	0	0	0	0
11	<i>P. equestris</i>	<i>K. deliciosa</i>	5	2	0	0	0	0
12	<i>P. equestris</i>	<i>K. minus</i>	5	4	2	2	40.00	50.00
13	<i>P. violacea</i>	<i>K. deliciosa</i>	2	3	1	0	50	0
14	<i>P. violacea</i>	<i>K. minus</i>	3	3	1	0	33.33	0
Genus <i>Doritis</i> x Genus <i>Kingidium</i>			14	14	2	0	14.29	0
15	<i>D. pulcherrima</i>	<i>K. deliciosa</i>	2	2	0	0	0	0
16	<i>D. pulcherrima</i>	<i>K. minus</i>	2	2	0	0	0	0
17	<i>D. pulcherrima</i> 'dwarf'	<i>K. deliciosa</i>	2	2	0	0	0	0
18	<i>D. pulcherrima</i> 'dwarf'	<i>K. minus</i>	4	3	2	0	50.00	0
19	<i>D. pulcherrima</i> var. <i>buyssoniana</i>	<i>K. deliciosa</i>	2	3	0	0	0	0
20	<i>D. pulcherrima</i> var. <i>buyssoniana</i>	<i>K. minus</i>	2	2	0	0	0	0
Total			60	52	10	6	16.67*	11.54*

\*Calculated with total value

**Table 16** Number of pollinated flowers and number of fruit setting from intersectional, intrasectional and intergeneric hybridization of genus *Phalaenopsis* and related genera, *Doritis* and *Kingidium*.

No.	Female parent (section/genus)	Male parent (section/genus)	No. of pollinated flower *	No. of fruit setting *	% fruit setting *
1	<i>Phalaenopsis</i>	<i>Polychilos</i>	19	7	36.84
2	<i>Phalaenopsis</i>	<i>Parishianae</i>	11	1	9.09
3	<i>Phalaenopsis</i>	<i>Proboscidioides</i>	6	0	0
4	<i>Phalaenopsis</i>	<i>Zebrinae</i>	5	0	0
5	<i>Proboscidioides</i>	<i>Parishianae</i>	13	0	0
6	<i>Proboscidioides</i>	<i>Polychilos</i>	15	0	0
7	<i>Parishianae</i>	<i>Polychilos</i>	5	0	0
8	<i>Parishianae</i>	<i>Stauroglottis</i>	5	2	40.00
9	<i>Parishianae</i>	<i>Zebrinae</i>	6	1	16.67
10	<i>Polychilos</i>	<i>Stauroglottis</i>	10	2	20.00
11	<i>Polychilos</i>	<i>Amboinenses</i>	11	0	0
12	<i>Polychilos</i>	<i>Zebrinae</i>	10	4	40.00
13	<i>Stauroglottis</i>	<i>Amboinenses</i>	6	0	0
14	<i>Stauroglottis</i>	<i>Zebrinae</i>	5	0	0
15	<i>Amboinenses</i>	<i>Zebrinae</i>	8	2	25.00
Total of intersectional hybridization			135	19	14.07**
16	<i>Phalaenopsis</i>	<i>Phalaenopsis</i>	7	0	0
17	<i>Parishianae</i>	<i>Parishianae</i>	6	1	16.67
18	<i>Amboinenses</i>	<i>Amboinenses</i>	4	0	0
Total of intrasectional hybridization			17	1	5.88**
19	<i>Phalaenopsis</i>	<i>Doritis</i>	41	6	14.63
20	<i>Phalaenopsis</i>	<i>Kingidium</i>	43	8	18.60
21	<i>Doritis</i>	<i>Kingidium</i>	28	2	7.14
Total of intergeneric hybridization			112	16	14.29**
Total			264	36	13.64**

\* Included reciprocal cross

\*\* Calculated with total value

**Table 17** Number of seedlings per fruit obtained *in vitro* from 24 crosses.

No.	Crosses	No. of seedling per fruit obtained <i>in vitro</i>
1	<i>P. amabilis</i> x <i>P. cornu-cervi</i>	100
2	<i>P. amabilis</i> x <i>D. pulcherrima</i> 'dwarf'	No viable seedling
3	<i>P. schilleriana</i> x <i>P. gibbosa</i> 1	No viable seedling
4	<i>P. schilleriana</i> x <i>P. cornu-cervi</i>	1,000
5	<i>P. lowii</i> x <i>K. deliciosa</i>	No viable seedling
6	<i>P. gibbosa</i> 1 x <i>P. parishii</i>	50
7	<i>P. parishii</i> x <i>P. cornu-cervi</i>	No viable seedling
8	<i>P. parishii</i> x <i>P. equestris</i>	No viable seedling
9	<i>P. cornu-cervi</i> x <i>P. equestris</i>	No viable seedling
10	<i>P. equestris</i> x <i>P. equestris</i>	
11	<i>P. equestris</i> x <i>P. cornu-cervi</i>	No viable seedling
12	<i>P. equestris</i> x <i>K. minus</i>	No viable seedling
13	<i>P. javanica</i> x <i>P. violacea</i>	No viable seedling
14	<i>P. violacea</i> x <i>P. parishii</i>	No viable seedling
15	<i>P. violacea</i> x <i>P. cornu-cervi</i>	200
16	<i>P. violacea</i> x <i>P. javanica</i>	40
17	<i>P. violacea</i> x <i>D. pulcherrima</i> 'dwarf'	No viable seedling
18	<i>P. violacea</i> x <i>K. minus</i>	No viable seedling
19	<i>D. pulcherrima</i> 'dwarf' x <i>P. amabilis</i>	No viable seedling
20	<i>D. pulcherrima</i> 'dwarf' x <i>P. cornu-cervi</i>	No viable seedling
21	<i>D. pulcherrima</i> 'dwarf' x <i>P. equestris</i>	500
22	<i>D. pulcherrima</i> 'dwarf' x <i>K. minus</i>	600
23	<i>K. deliciosa</i> x <i>P. lowii</i>	No viable seedling
24	<i>K. minus</i> x <i>P. equestris</i>	No viable seedling

**Table 18** Number of transplanted seedlings and number of survival plantlets after 6 months transplanting of 7 crosses.

No.	Crosses	No. of transplanted seedling	No. of survival plantlet after 6 months transplanting	% survival plantlet
1	<i>P. amabilis</i> x <i>P. cornu-cervi</i>	70	45	64.29
2	<i>P. schilleriana</i> x <i>P. cornu-cervi</i>	800	650	81.25
3	<i>P. gibbosa</i> 1 x <i>P. parishii</i>	30	13	43.33
4	<i>P. violacea</i> x <i>P. cornu-cervi</i>	20	14	70.00
5	<i>P. violacea</i> x <i>P. javanica</i>	100	58	58.00
6	<i>D. pulcherrima</i> 'dwarf' x <i>P. equestris</i>	400	298	74.50
7	<i>D. pulcherrima</i> 'dwarf' x <i>K. minus</i>	500	354	70.80
Total		1,920	1,432	74.58*

\*Calculated with total value

### 4.3 Characterizations of F<sub>1</sub> progenies derived from intersectional and intergeneric hybrids of *Phalaenopsis* and related genera, *Doritis* and *Kingidium*, by RAPD technique

#### Descriptions of F<sub>1</sub> progenies

F<sub>1</sub> progenies derived from the crosses, *P. schilleriana* x *P. cornu-cervi*, *D. pulcherrima* ‘dwarf’ x *P. equestris*, and *D. pulcherrima* ‘dwarf’ x *K. minus* could yield flowers within a year. Phenotypic characteristics, i.e. leaf length and width, leaf color, flower width, flower color and number of pollinia were recorded. The flower and leaf descriptions of parental lines and their F<sub>1</sub> progenies were described as follows:

#### *P. schilleriana* x *P. cornu-cervi*

Leaf of *P. schilleriana* was dark green gray bar and spot with 8.0 x 20.0 cm in size while that of *P. cornu-cervi* was green with 4.0 x 15.0 cm in size. Leaf color of H1 and H9 were green while the others were dark green. Leaf width and length were 5.5 - 7.5 and 15.5 - 20.0 cm, respectively (Table 19). Flower of *P. schilleriana* was pink with 6.0 cm wide while that of *P. cornu-cervi* was yellow with reddish brown bar and spot with 4.0 cm wide. Ten random progenies showed segregation of flower characters. Flower colors of H1 and H10 were light pink, H5, H6 and H7 were pink, H8 and H9 were dark pink, and H2, H3 and H4 were brown. Flower widths of 10 progenies were 4.0 - 5.5 cm. All parents and their 10 progenies had two pollinia (Table 20 and Figure 26).

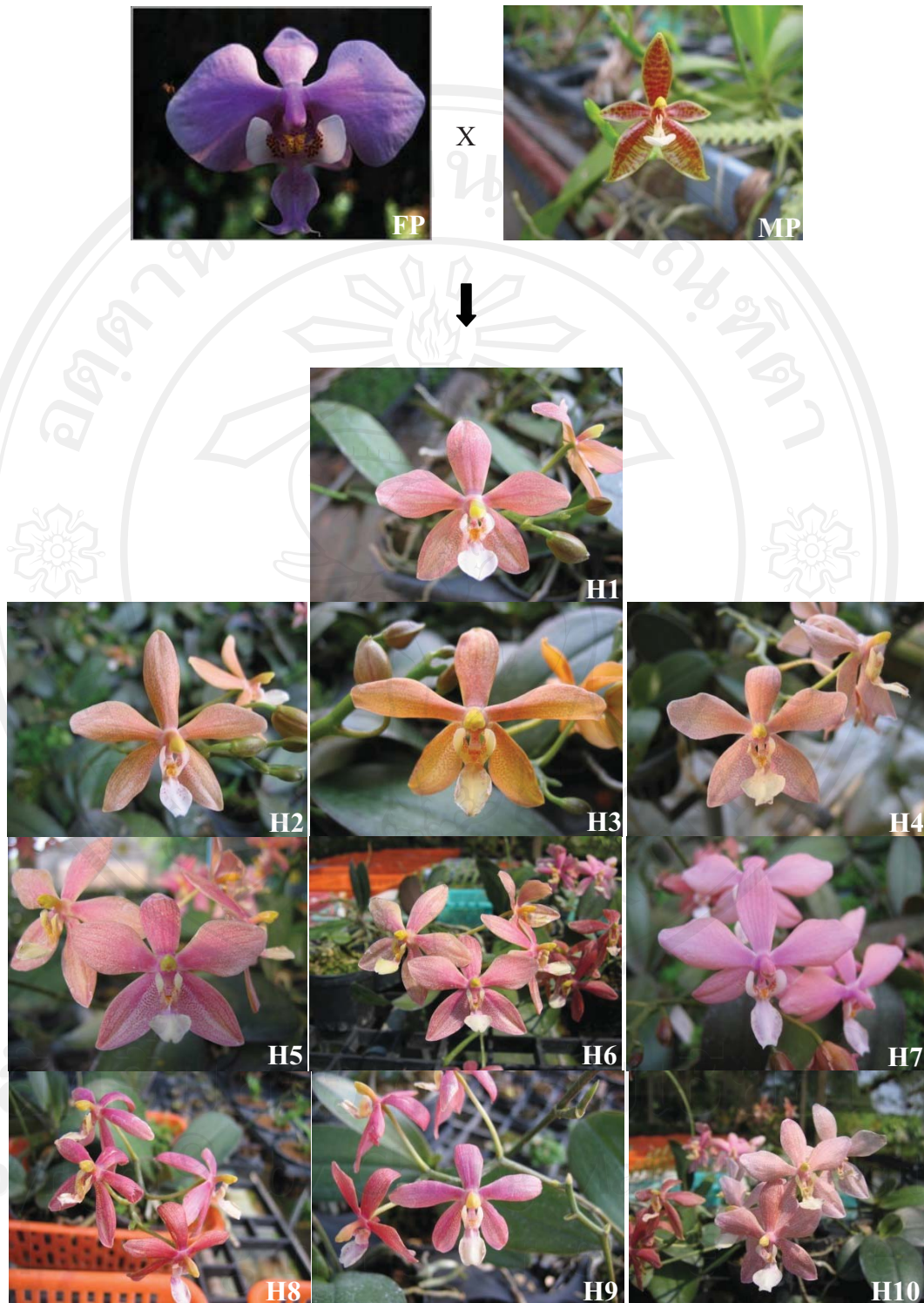
**Table 19** Leaf descriptions of *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10).

Code	Leaf		
	Width (cm)	Length (cm)	Color
FP	8.0	20.0	Dark green with gray bar and spot
MP	4.0	15.0	Green
H1	5.5	17.5	Dark green
H2	6.8	18.0	Green
H3	7.0	15.7	Dark green
H4	6.5	17.5	Dark green
H5	7.5	18.5	Dark green
H6	5.8	15.5	Dark green
H7	8.0	16.2	Dark green
H8	6.5	17.5	Dark green
H9	5.7	20.0	Green
H10	6.5	19.5	Dark green

**Table 20** Flower descriptions of *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10).

Code	Flower		
	Width (cm)	Color	No. of pollinia
FP	6.0	Pink	2
MP	4.0	Yellow with reddish brown bar and spot	2
H1	4.5	Light pink	2
H2	4.8	Brown	2
H3	5.0	Brown	2
H4	4.0	Brown	2
H5	4.5	Pink	2
H6	5.2	Pink	2
H7	4.7	Pink	2
H8	4.0	Dark pink	2
H9	5.5	Dark pink	2
H10	5.2	Light pink	2





**Figure 26** Flowers of *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10).

***D. pulcherrima* ‘dwarf’ x *P. equestris***

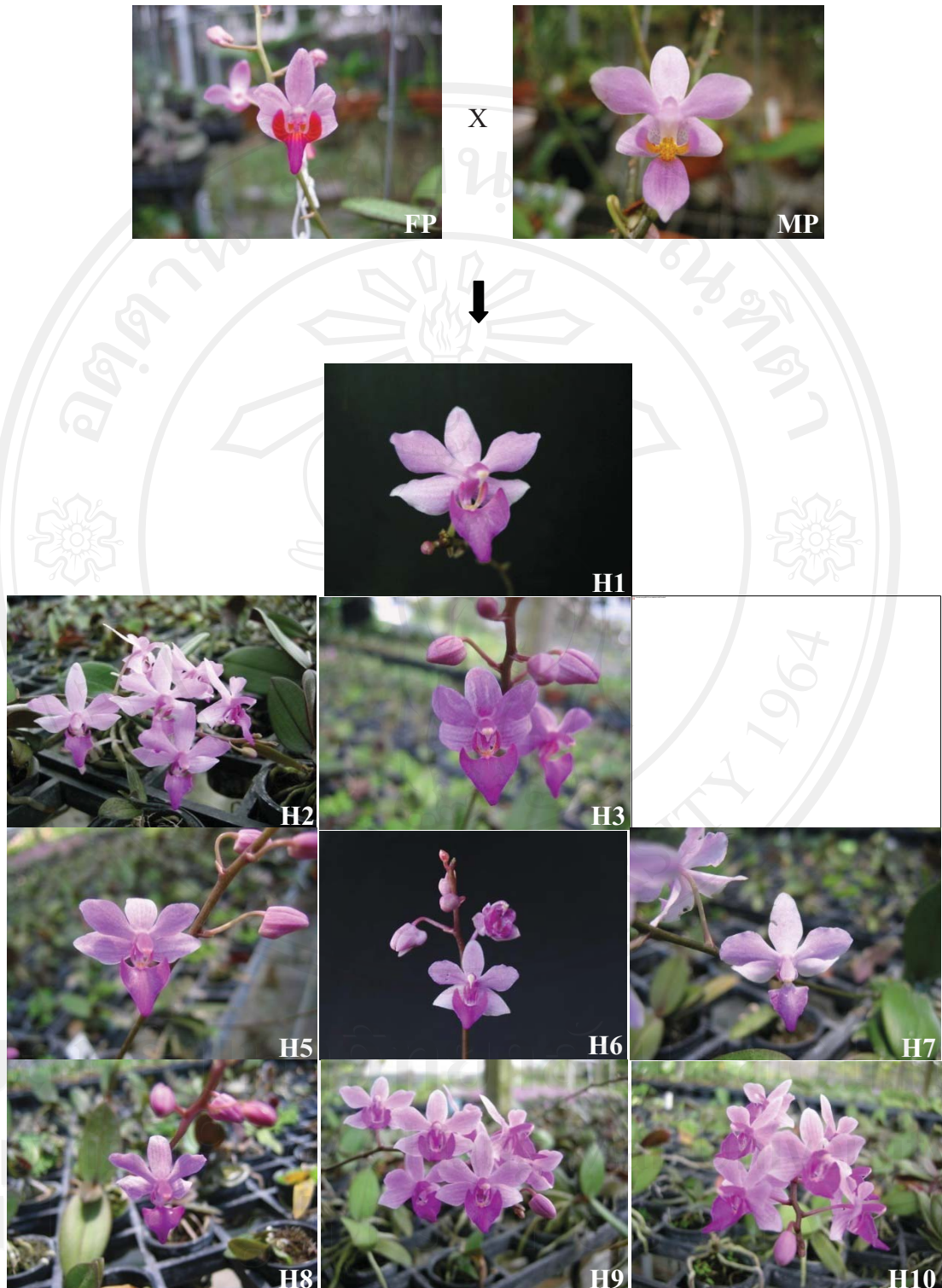
Leaf of *D. pulcherrima* ‘dwarf’ was dark green with 3.0 x 5.0 cm wide while that of *P. equestris* was green with 3.5 x 12.0 cm wide. Leaf color of H4 and H8 were green while the others were dark green. Leaf width and length were 2.5 - 3.5 and 5.0 - 7.2 cm, respectively (Table 21). Flower of *D. pulcherrima* ‘dwarf’ was dark pink with 2.0 cm wide while that of *P. equestris* was pink with 3.0 cm wide. Ten random progenies showed segregation of flower characters. Flower color of H1, H2 and H7 were light pink, H5, H6, H8, H9 and H10 were pink, H3 and H4 were dark pink. Flower widths of 10 progenies were 2.0 - 3.2 cm. All 10 progenies had four pollinia which were similar to female parent while the male parent had two pollinia (Table 22 and Figure 27).

**Table 21** Leaf descriptions of *D. pulcherrima* ‘dwarf’ (FP), *P. equestris* (MP) and their ten progenies (H1 - H10).

Code	Leaf		
	Width (cm)	Length (cm)	Color
FP	3.0	5.0	Dark green
MP	3.5	12.0	Green
H1	3.0	6.5	Dark green
H2	2.7	5.5	Dark green
H3	3.0	5.7	Dark green
H4	3.2	6.0	Green
H5	2.5	5.0	Dark green
H6	3.0	5.5	Dark green
H7	2.5	5.0	Dark green
H8	2.7	6.5	Green
H9	3.0	6.7	Dark green
H10	3.5	7.2	Dark green

**Table 22** Flower descriptions of *D. pulcherrima* ‘dwarf’ (FP), *P. equestris* (MP) and their ten progenies (H1 - H10).

Code	Flower		
	Width (cm)	Color	No. of pollinia
FP	2.0	Dark pink	4
MP	3.0	Light pink	2
H1	2.7	Light pink	4
H2	3.0	Light pink	4
H3	2.2	Dark pink	4
H4	2.5	Dark pink	4
H5	3.2	Pink	4
H6	2.0	Pink	4
H7	2.5	Light pink	4
H8	3.0	Pink	4
H9	2.7	Pink	4
H10	2.5	Pink	4



**Figure 27** Flowers of *D. pulcherrima* ‘dwarf’ (FP), *P. equestris* (MP) and their ten progenies (H1 - H10).

***D. pulcherrima* ‘dwarf’ x *K. minus***

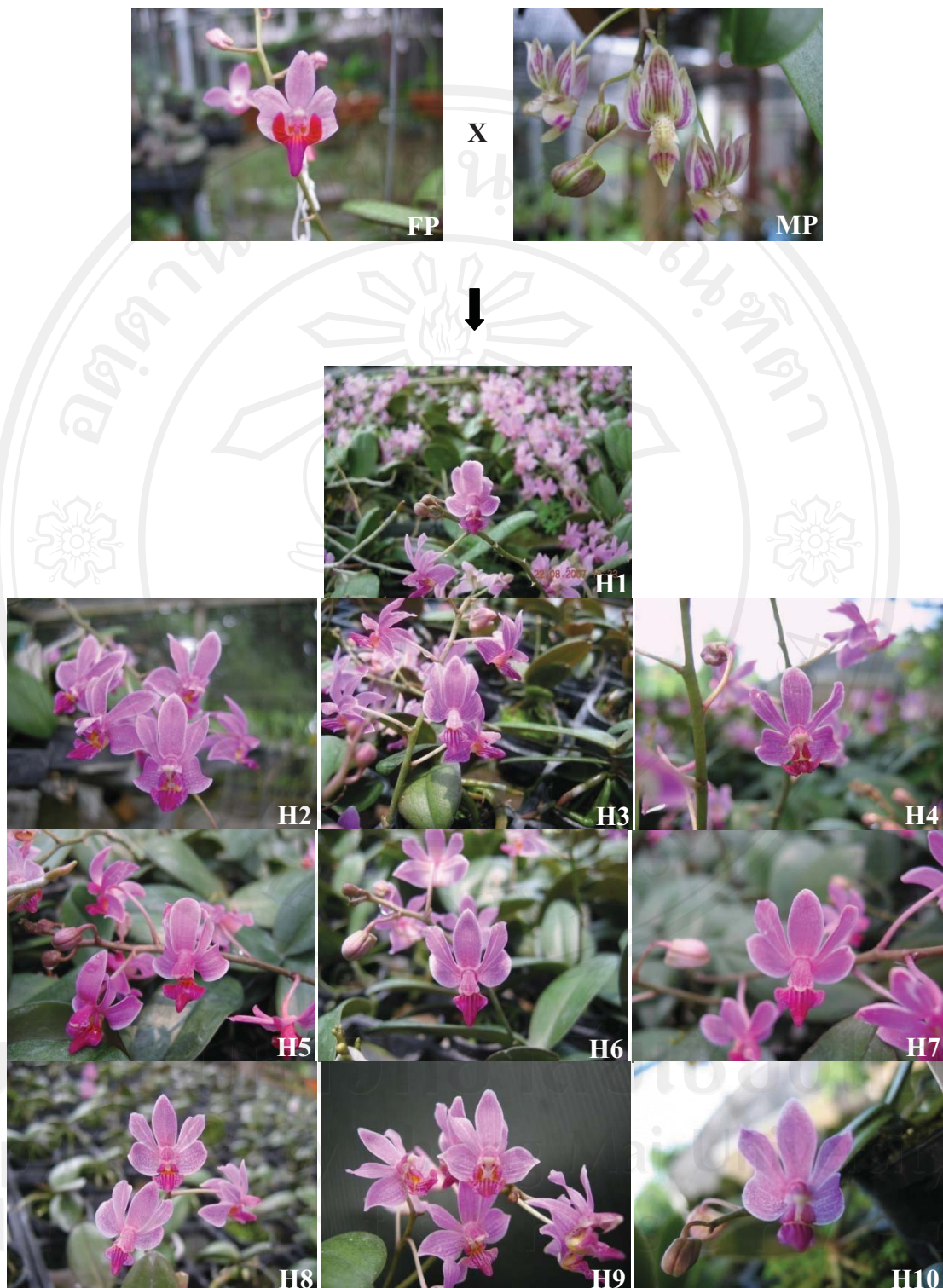
Leaf of *D. pulcherrima* ‘dwarf’ was dark green with 3.0 x 5.0 cm wide while that of *K. minus* was green with 3.5 x 8.0 cm wide. Leaf color of all 10 progenies were dark green. Leaf width and length were 2.7 - 3.5 and 6.0 - 8.5 cm, respectively (Table 23). Flower of *D. pulcherrima* ‘dwarf’ was dark pink with 2.0 cm wide while that of *K. minus* was white with purple bar and spot with 2.5 cm wide. Ten random progenies showed segregation of flower characters. Flower color of H1, H6, H8, H9 and H10 were pink, and H2, H3, H4, H5 and H7 were dark pink. Flower widths of 10 progenies were 2.0 - 3.2 cm. All parents and their 10 progenies had two pollinia (Table 24 and Figure 28).

**Table 23** Leaf descriptions of *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10).

Code	Leaf		
	Width (cm)	Length (cm)	Color
FP	3.0	5.0	Dark green
MP	3.5	8.0	Green
H1	3.0	5.7	Dark green
H2	3.5	6.0	Dark green
H3	3.2	6.5	Dark green
H4	2.7	7.2	Dark green
H5	3.0	7.0	Dark green
H6	3.5	6.5	Dark green
H7	2.8	8.5	Dark green
H8	3.0	8.0	Dark green
H9	3.5	7.0	Dark green
H10	3.2	6.5	Dark green

**Table 24** Flower descriptions of *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10).

Code	Flower		No. of pollinia
	Width (cm)	Color	
FP	2.0	Dark pink	4
MP	2.5	White with purple bar and spot	4
H1	2.0	Pink	4
H2	1.8	Dark pink	4
H3	2.0	Dark pink	4
H4	2.5	Dark pink	4
H5	2.0	Dark pink	4
H6	2.2	Pink	4
H7	2.0	Dark pink	4
H8	1.8	Pink	4
H9	2.2	Pink	4
H10	2.0	Pink	4



**Figure 28** Flowers of *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10).

## Molecular characterization of F<sub>1</sub> progenies

### Primer screening

The twenty decamer primers were evaluated for amplification of 3 compatible crosses and their 10 progenies. The number of primers giving polymorphic DNA bands varied among crosses. Suitable primers for each cross could be described as follows: 6 primers, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14 for cross *P. schilleriana* x *P. cornu-cervi*; 4 primers, OPAK10, OPF01, OPF02 and OPF09 for cross *D. pulcherrima* 'dwarf' x *P. equestris*; and 5 primers, OPAK10, OPD03, OPF02, OPF09 and OPF14 for cross *D. pulcherrima* 'dwarf' x *K. minus*. The DNA fingerprints were presented showing polymorphic RAPD markers from either parent that appeared in hybrid banding.

### RAPD analysis

#### *P. schilleriana* x *P. cornu-cervi*

Parental lines, *P. schilleriana* and *P. cornu-cervi* and their 10 progenies were analyzed with 6 primers, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14.

Banding patterns showed polymorphic DNA bands in the ranges of 422 - 2,050, 275 - 1,602, 394 - 1,500, 358 - 2,364, 310 - 1,411 and 373 - 1,582 bp, respectively (Figure 29 - 34).

OPAK10 primer yielded 14 DNA bands in the ranges of 422 - 2,050 bp, 2 DNA bands, 520 and 1,020 bp, were monomorphic and 12 DNA bands were polymorphic. Seven DNA bands specific to female parent, 422, 678, 879, 1,301, 1,411, 1,546 and 1,842 bp, and 3 DNA bands specific to male parent, 844, 1,152 and 2,050 bp,



were found in their progenies. The 1,288-bp DNA band was found in both parents and H1 progeny whereas the 1,440-bp DNA band was found only in H1 and H9 progenies (Figure 29).

OPD03 primer yielded 15 DNA bands in the ranges of 275 - 1,602 bp, 2 DNA bands, 435 and 745 bp, were monomorphic and 13 DNA bands were polymorphic. The 1,129-bp DNA band specific to female parent, and 7 DNA bands specific to male parent, 275, 319, 600, 1,030, 1,106, 1,308 and 1,400 bp, were found in their progenies whereas the 669-bp DNA band was found only in H9 progeny (Figure 30).

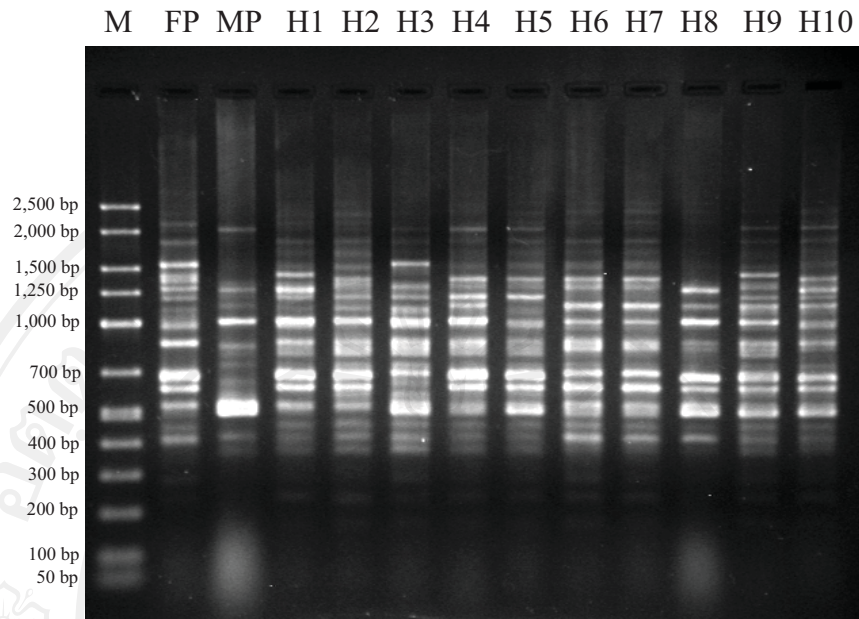
OPF01 primer yielded 11 DNA bands in the ranges of 394 - 1,500 bp, 3 DNA bands, 451, 545 and 966 bp, were monomorphic and 8 DNA bands were polymorphic. Four DNA bands specific to female parent, 1,034, 1,236, 1,361 and 1,500 bp, and 3 DNA bands specific to male parent, 394, 1,222 and 1,463 bp, were found in their progenies (Figure 31).

OPF02 primer yielded 11 DNA bands in the ranges of 358 - 2,364 bp, the 874-bp DNA band was monomorphic and 10 DNA bands were polymorphic. Six DNA bands specific to female parent, 589, 731, 818, 1,500, 1,787 and 2,364 bp, and 2 DNA bands specific to male parent, 527 and 1,111 bp, were found in their progenies whereas the 358- and 424-bp DNA bands were found only in progenies (Figure 32).

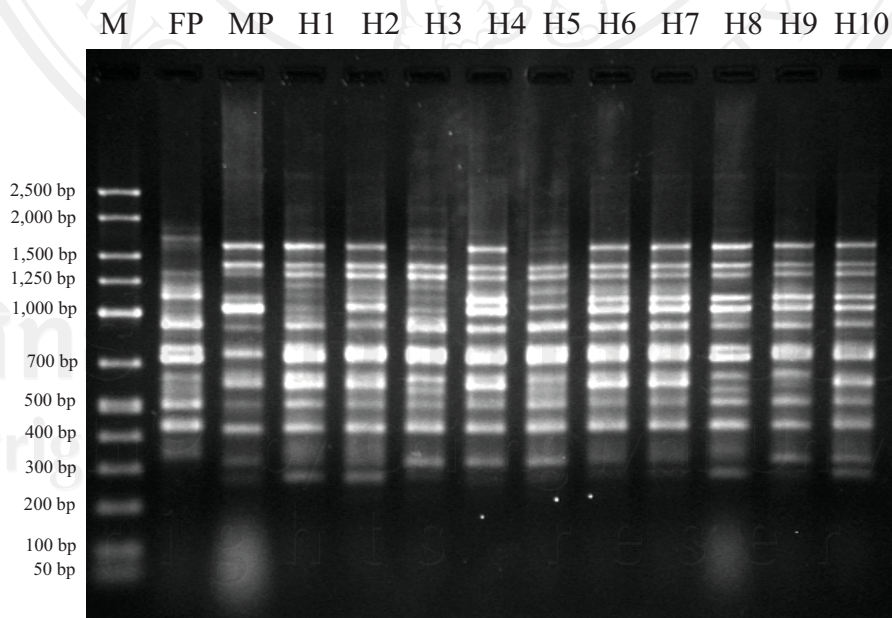
OPF09 primer yielded 13 DNA bands in the ranges of 310 - 1,411 bp, the 464-bp DNA band was monomorphic and 12 DNA bands were polymorphic. Six DNA bands specific to female parent, 310, 404, 740, 852, 981 and 1,411 bp, and 4 DNA bands specific to male parent, 395, 589, 667 and 1,262 bp, were found in their progenies (Figure 33).

OPF14 primer yielded 8 DNA bands in the ranges of 373 - 1,582 bp, the 990-bp DNA band was monomorphic and 7 DNA bands were polymorphic. Four DNA bands specific to female parent, 373, 429, 679 and 748 bp, and 3 DNA bands specific to male parent, 591, 848 and 1,582 bp, were found in their progenies (Figure 34).

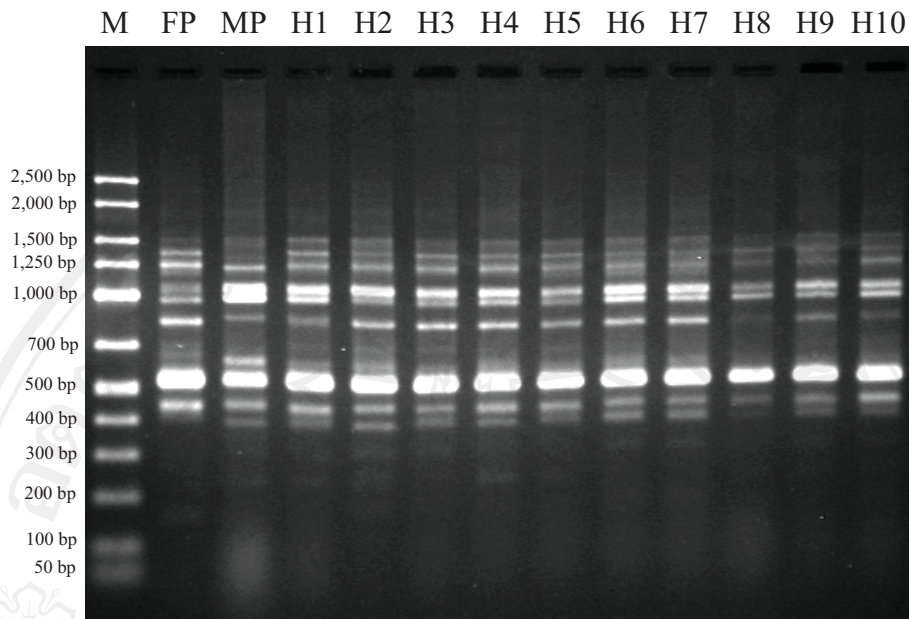
Banding patterns from 6 primer combinations were analyzed for genetic similarity between parental lines and their 10 progenies using principle component analysis (PCA). The PCA was performed with the NTSYS-pc version 2.01 program (Rohlf, 2000). It was found that PCA diagram distributed all ten progenies widely between female parent (FP), *P. schilleriana*, and male parent (MP), *P. cornu-cervi* (Figure 35).



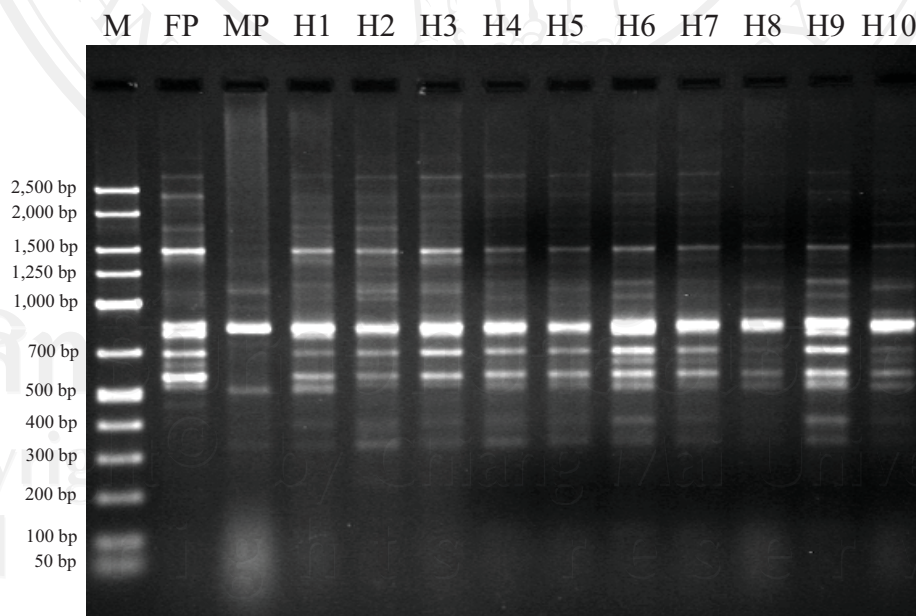
**Figure 29** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) after amplification with OPAK10 primer.



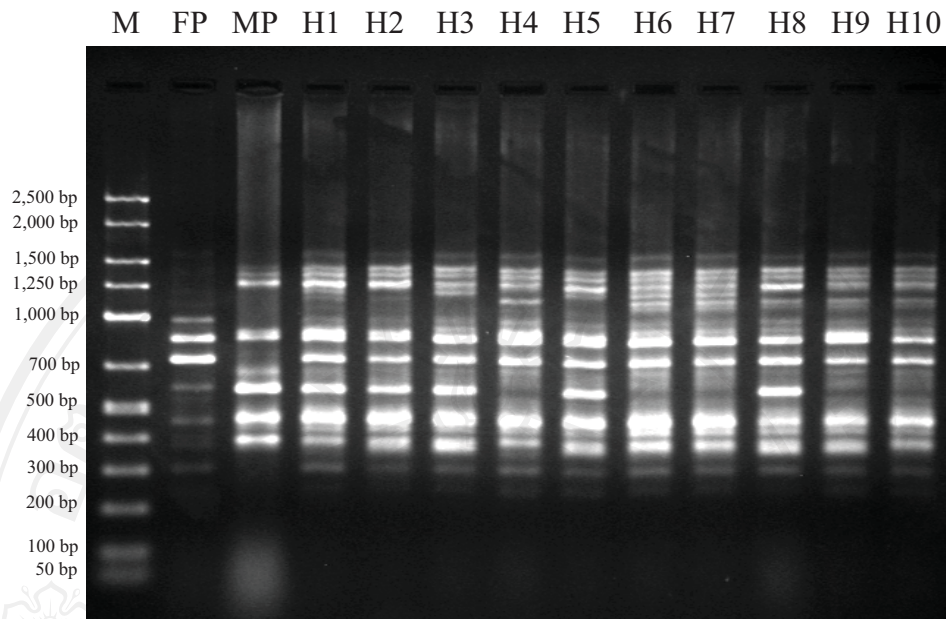
**Figure 30** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) after amplification with OPD03 primer.



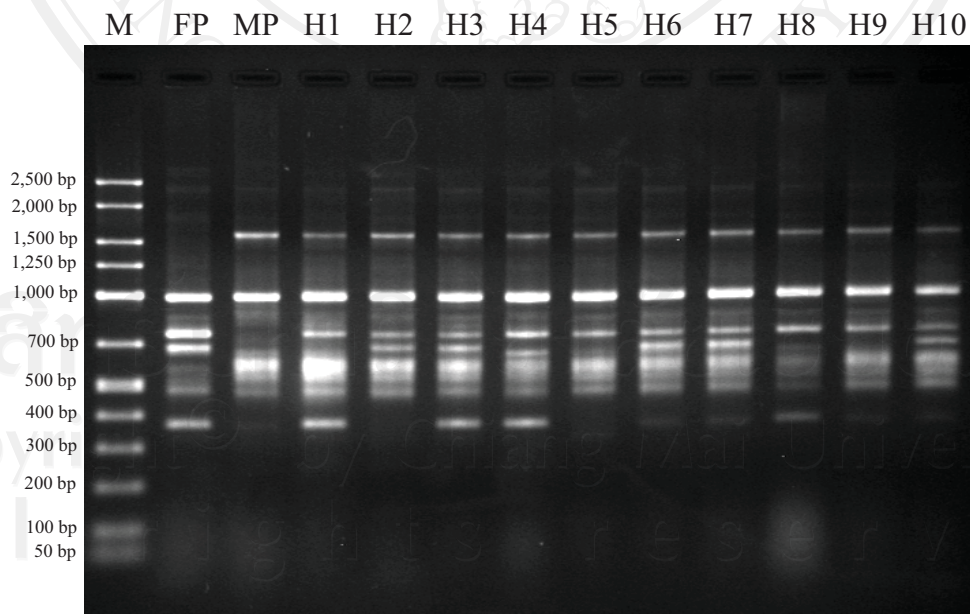
**Figure 31** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - 10) after amplification with OPF01 primer.



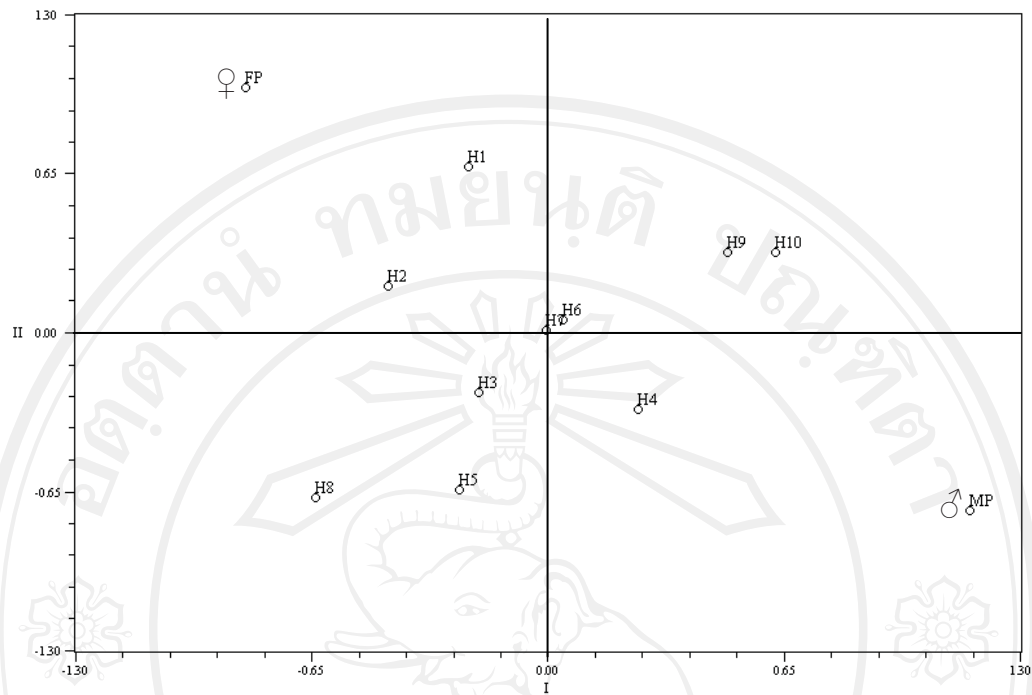
**Figure 32** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) after amplification with OPF02 primer.



**Figure 33** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) after amplification with OPF09 primer.



**Figure 34** RAPD profiles obtained from *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) after amplification with OPF14 primer.



**Figure 35** Principle component analysis (PCA) diagram illustrated the genetic similarity of *P. schilleriana* (FP), *P. cornu-cervi* (MP) and their ten progenies (H1 - H10) evaluated with 6 primer combinations, OPAK10, OPD03, OPF01, OPF02, OPF09 and OPF14.

***D. pulcherrima* ‘dwarf’ x *P. equestris***

Parental lines, *D. pulcherrima* ‘dwarf’ and *P. equestris* and their 10 progenies were analyzed with 4 primers, OPAK10, OPF01, OPF02 and OPF09. Banding patterns showed polymorphic DNA bands in the ranges of 262 - 425, 100 - 574, 127 - 405 and 150 - 519 bp, respectively (Figure 36 - 39).

OPAK10 primer yielded 3 polymorphic DNA bands in the ranges of 262 - 425 bp. Two DNA bands specific to female parent, 350 and 425 bp, and 262-bp DNA band specific to male parent, were found in their progenies (Figure 36).

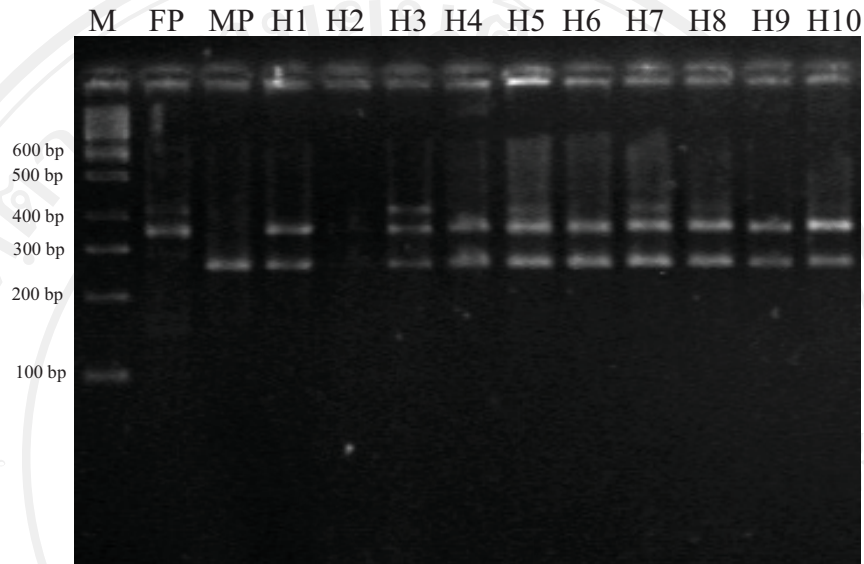
OPF01 primer yielded 11 DNA bands in the ranges of 100 - 574 bp, 4 DNA bands, 176, 235, 279 and 348 bp, were monomorphic and 7 DNA bands were polymorphic. Two DNA bands specific to male parent, 100 and 149 bp, were found in their progenies whereas 5 DNA bands, 372, 420, 475, 500 and 574 bp, were found only in their progenies (Figure 37).

OPF02 primer yielded 7 polymorphic DNA bands in the ranges of 127 - 405 bp. The 127-bp DNA band specific to female parent and 4 DNA bands specific to male parent, 195, 220, 254 and 350 bp, were found in their progenies whereas 2 DNA bands, 370 and 405 bp, were found only in their progenies (Figure 38).

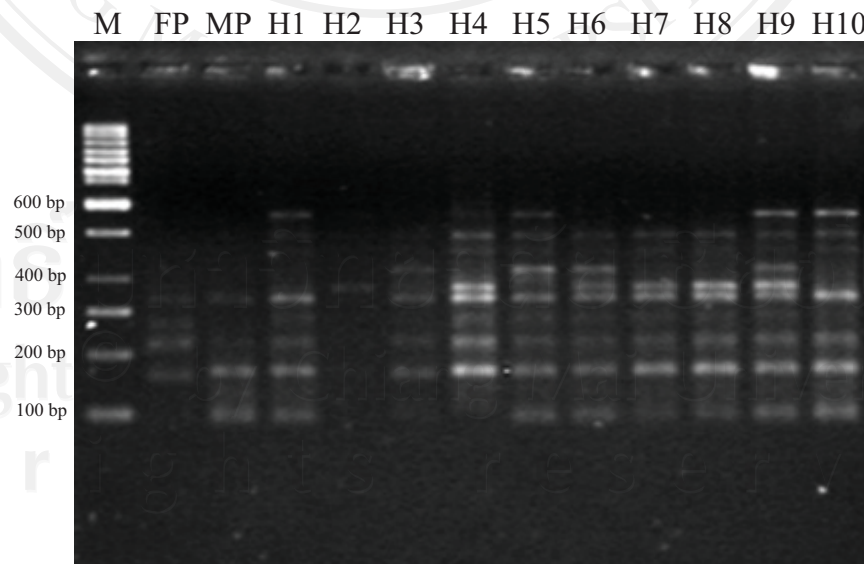
OPF09 primer yielded 8 DNA bands in the ranges of 150 - 519 bp, the 417-bp DNA band was monomorphic and 7 DNA bands were polymorphic. Four DNA bands specific to female parent, 150, 198, 359 and 500 bp, were found in their progenies whereas 3 DNA bands, 450, 474 and 510 bp, were found only in their progenies (Figure 39).

Genetic similarity between parental lines and their 10 progenies using principle component analysis (PCA) showed that 8 progenies, H1, H2, H3, H5, H6,

H7, H8, and H9 were clustered along with male parent, *P. equestris*, while H4 and H10 were distributed between female and male parents (Figure 40).

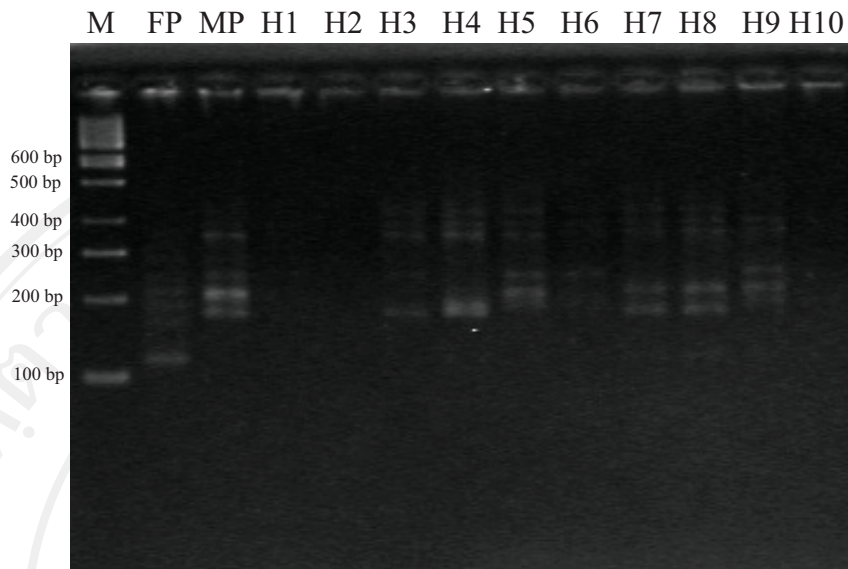


**Figure 36** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *P. equestris* (MP) and their ten progenies (H1 - H10) after amplification with OPAK10 primer.

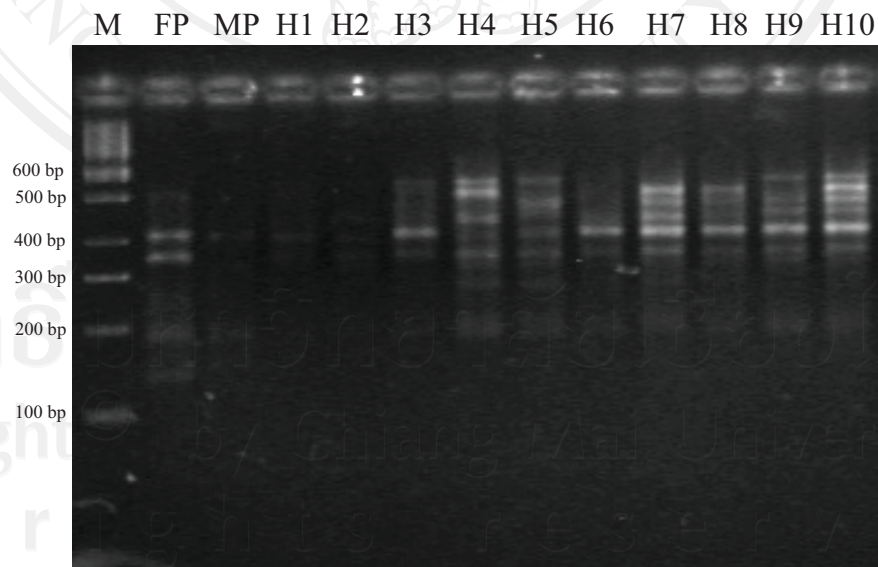


**Figure 37** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *P. equestris* (MP) and their ten progenies (H1 - H10) after amplification with OPF01 primer.

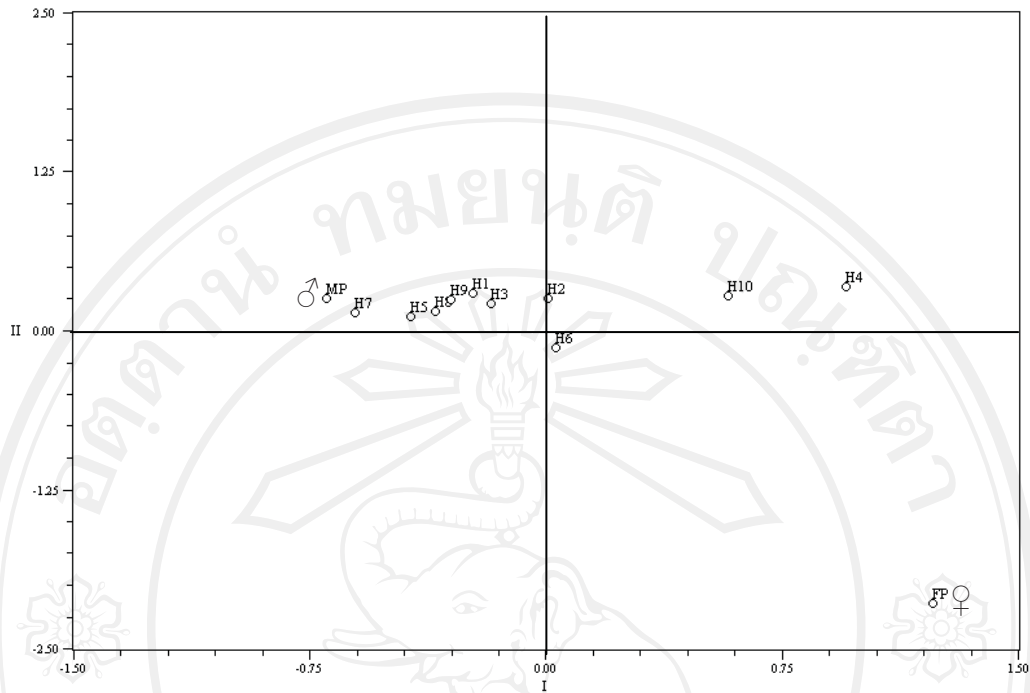




**Figure 38** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *P. equestris* (MP) and their ten progenies (H1 - H10) after amplification with OPF02 primer.



**Figure 39** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *P. equestris* (MP) and their ten progenies (H1 - H10) after amplification with OPF09 primer.



**Figure 40** Principle component analysis (PCA) diagram illustrated the genetic similarity of *D. pulcherrima* ‘dwarf’ (FP), *P. equestris* (MP) and their ten progenies (H1 - H10) evaluated with 4 primer combinations, OPAK10, OPF01, OPF02 and OPF09.

***D. pulcherrima* ‘dwarf’ x *K. minus***

Parental lines, *D. pulcherrima* ‘dwarf’ and *K. minus* and their 10 progenies were analyzed with 5 primers, OPAK10, OPD03, OPF02, OPF09 and OPF14. Banding patterns showed polymorphic DNA bands in the ranges of 210 - 1,200, 310 - 1,362, 118 - 1,000, 382 - 1,250 and 623 - 1,579 bp, respectively (Figure 41 - 45).

OPAK10 primer yielded 8 DNA bands in the ranges of 210 - 1,200 bp, 2 DNA bands, 530 and 727 bp, were monomorphic and 6 DNA bands were polymorphic. Two DNA bands specific to female parent, 210 and 1,200 bp, and 4 DNA bands specific to male parent, 295, 428, 1,000 and 1,123 bp, were found in their progenies (Figure 41).

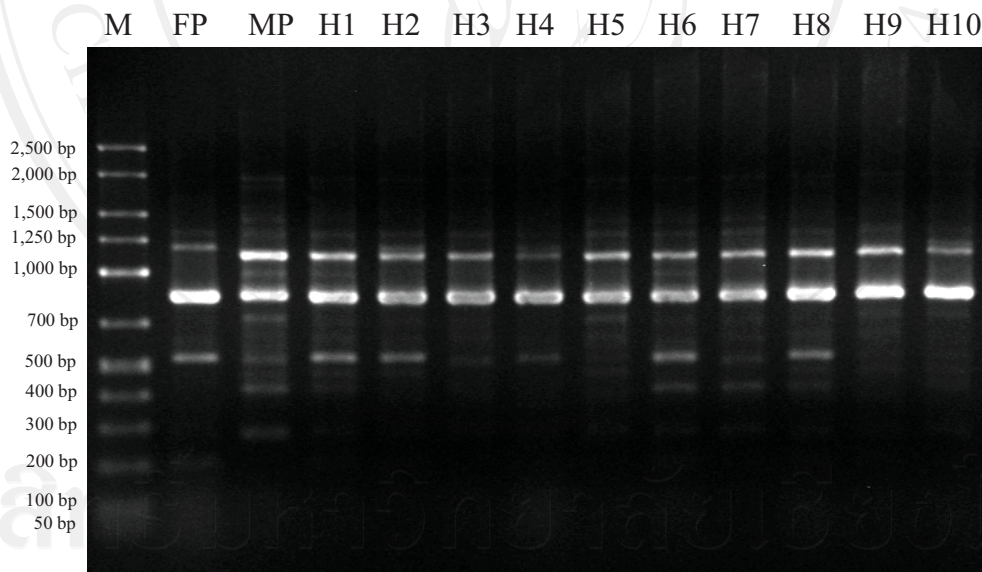
OPD03 primer yielded 8 DNA bands in the ranges of 310 - 1,362 bp, 4 DNA bands, 394, 693, 915 and 1,362 bp, were monomorphic and 4 DNA bands were polymorphic. The 310-bp DNA band specific to female parent, and 3 DNA bands specific to male parent, 421, 591 and 1,123 bp, were found in their progenies (Figure 42).

OPF02 primer yielded 6 DNA bands in the ranges of 118 - 1,000 bp, the 840-bp DNA band was monomorphic and 5 DNA bands were polymorphic. Two DNA bands specific to female parent, 570 and 1,000 bp, and 3 DNA bands specific to male parent, 118, 347 and 700 bp, were found in their progenies (Figure 43).

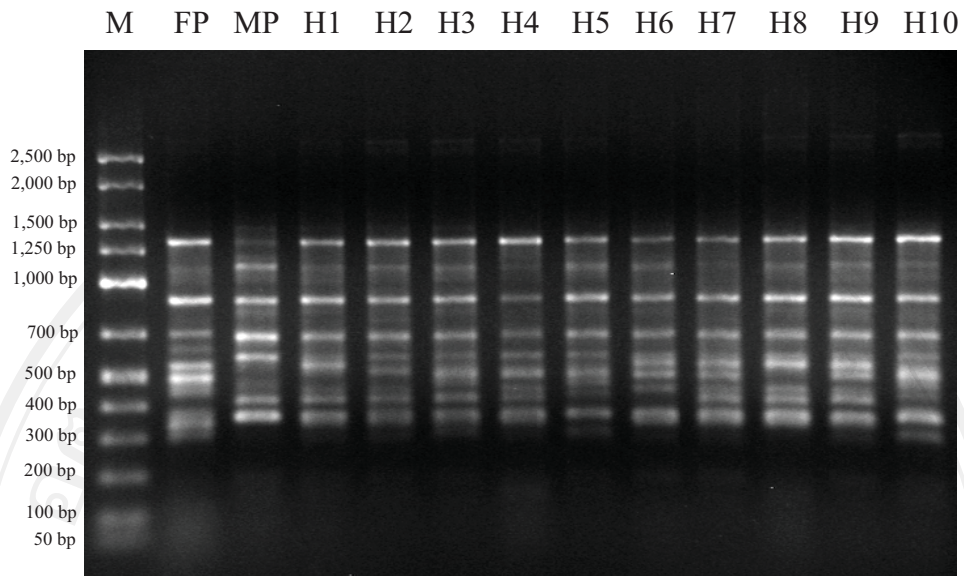
OPF09 primer yielded 9 DNA bands in the ranges of 382 - 1,250 bp, the 459-bp DNA band was monomorphic and 8 DNA bands were polymorphic. Three DNA bands specific to female parent, 400, 754 and 1,000 bp, and 4 DNA bands specific to male parent, 382, 562, 648 and 1,250 bp, were found in their progenies. The 875-bp DNA band was found in both parents, and H6 and H10 progenies (Figure 44).

OPF14 primer yielded 6 polymorphic DNA bands in the ranges of 623 - 1,579 bp. Two DNA band specific to female parent, 700 and 1,210 bp, and 4 DNA bands specific to male parent, 623, 850, 1,005 and 1,579 bp, were found in their progenies (Figure 45).

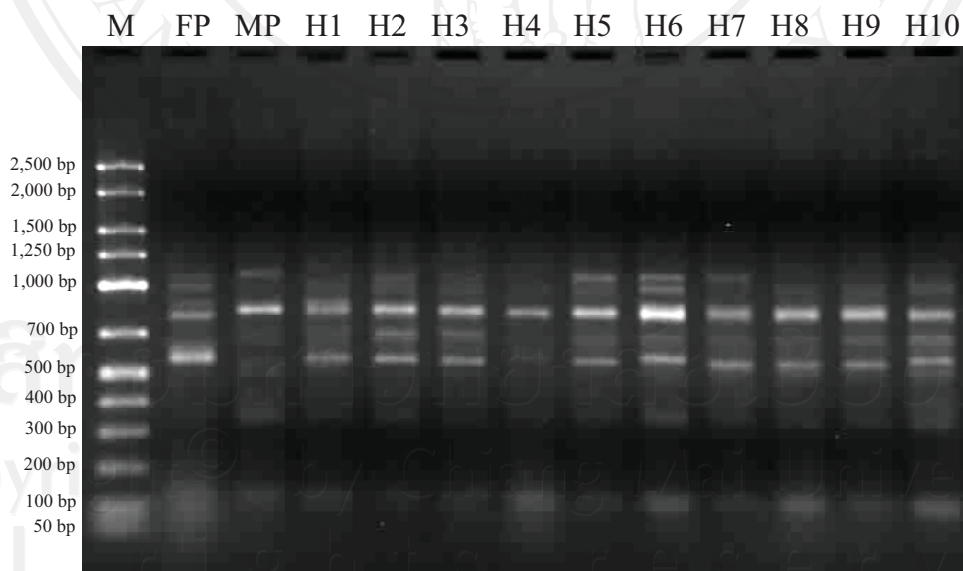
Genetic similarity between parental lines and their 10 progenies using principle component analysis (PCA) showed that the 2 progenies, H3 and H4 were clustered along with female parent, *D. pulcherrima* 'dwarf', while H2 was clustered along with male parent, *K. minus*. The other 7 progenies, H1, H5, H6, H7, H8, H9 and H10, were widely distributed between female and male parents (Figure 46).



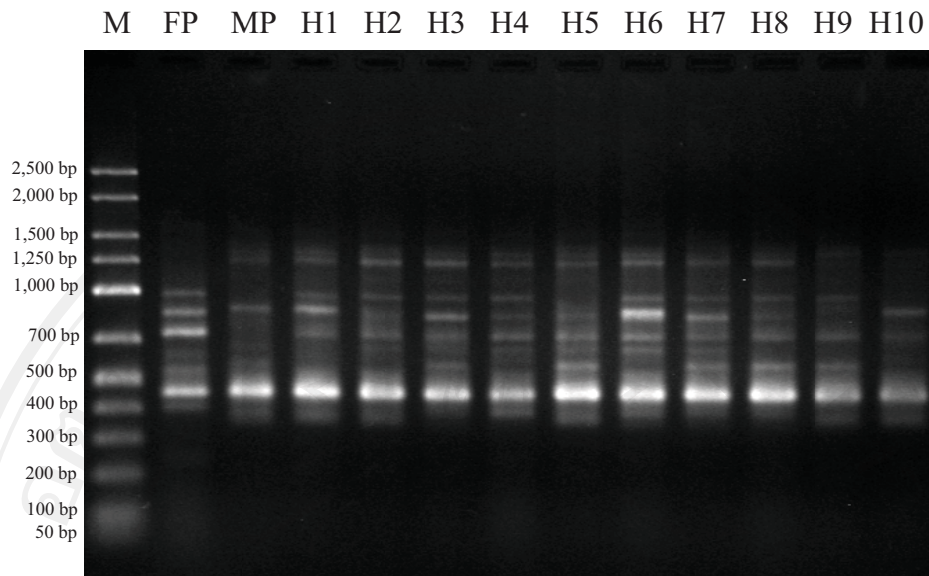
**Figure 41** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *K. minus* (MP) and their ten progenies (H1 - H10) after amplification with OPF14 primer.



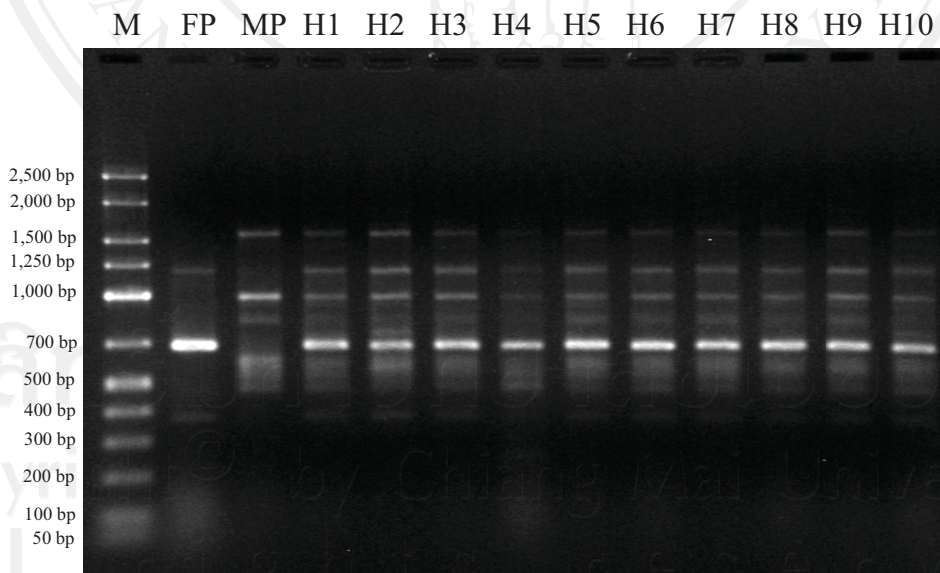
**Figure 42** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *K. minus* (MP) and their ten progenies (H1 - H10) after amplification with OPD03 primer.



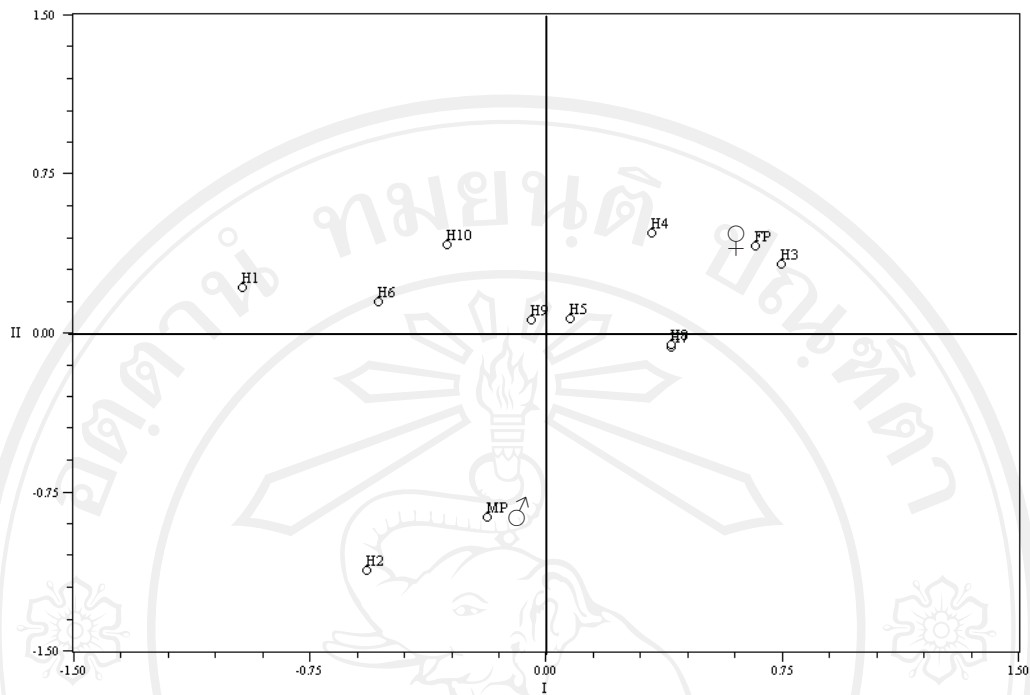
**Figure 43** RAPD profiles obtained from *D. pulcherrima* 'dwarf' (FP), *K. minus* (MP) and their ten progenies (H1 - H10) after amplification with OPF02 primer.



**Figure 44** RAPD profiles obtained from *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10) after amplification with OPF09 primer.



**Figure 45** RAPD profiles obtained from *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10) after amplification with OPF14 primer.



**Figure 46** Principle component analysis (PCA) diagram illustrated the genetic similarity of *D. pulcherrima* ‘dwarf’ (FP), *K. minus* (MP) and their ten progenies (H1 - H10) valuated with 5 primer combinations, OPAK10, OPD03, OPF02, OPF09 and OPF14.

#### 4.4 Specific marker for flower color pattern of *Phalaenopsis cornu-cervi* by AFLP technique

##### Screening of suitable primer combinations

Sixty-four primer combinations of 8 *EcoRI* and 8 *MseI* primers: *EcoRI*+AC, *EcoRI*+AG, *EcoRI*+AAC, *EcoRI*+AAG, *EcoRI*+AGA, *EcoRI*+ATC, *EcoRI*+ATG, *EcoRI*+ATT, *MseI*+CAA, *MseI*+CAG, *MseI*+CAT, *MseI*+CCA, *MseI*+CTA, *MseI*+CTC, *MseI*+CTG and *MseI*+CTT, with 2 - 3 selective bases at 3' end were tested on three types of twelve *P. cornu-cervi*: 1) three plants of reddish brown flower, 2) six plants of yellow flower with reddish brown bars and spots, and 3) three plants of pure yellow flower (no spot or bar). After selective amplification step, agarose gel electrophoresis of plant No. 1 of *P. cornu-cervi* in reddish brown flower group was used to screen for suitable primer combinations. The results showed that the thirty primer combinations (red number in Figure 47) with polymorphic DNA bands and high resolution could be used to find the specific marker, which was found only in reddish brown flower and yellow flower with reddish brown bar and spot groups.

##### Analysis of AFLP profiles

Twelve *P. cornu-cervi* were reproducibly tested with thirty selected primer combinations. After selective amplification step, the denatured PCR products were separated on 6 % denaturing polyacrylamide gel electrophoresis. Four primer combinations, *EcoRI*+AC/*MseI*+CAT, *EcoRI*+AG/*MseI*+CAG, *EcoRI*+AGA/*MseI*+CAG and *EcoRI*+ATT/*MseI*+CCA showed polymorphic DNA bands with high



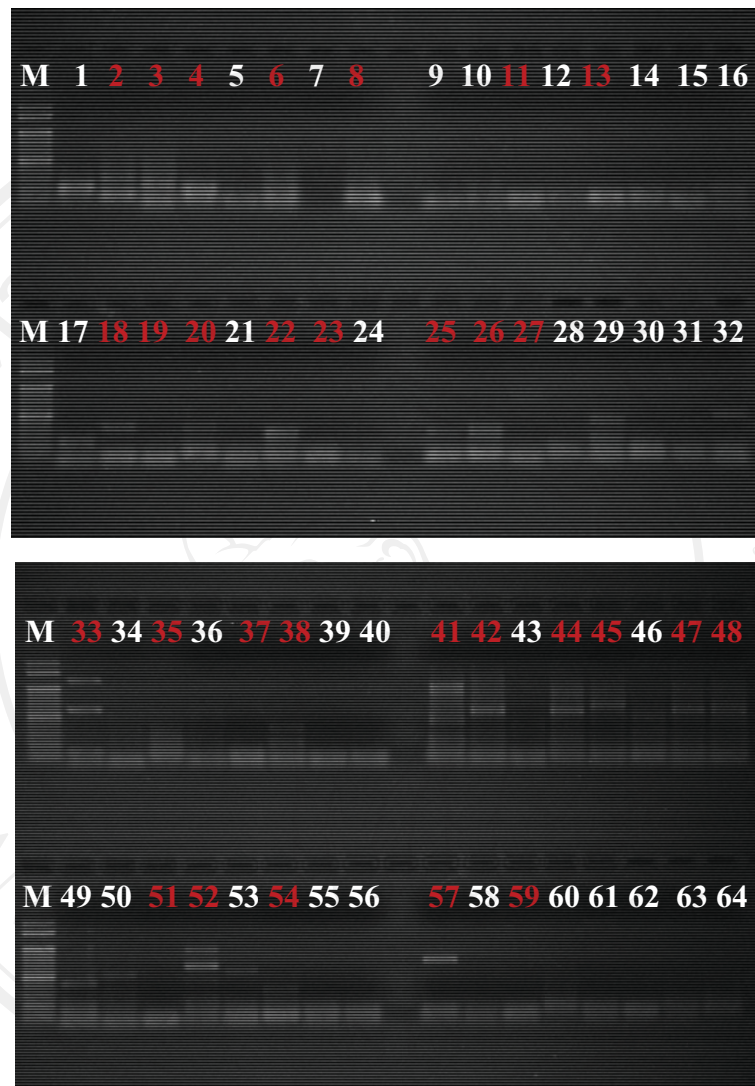
resolution and could be used to find the specific marker, which was found only in reddish brown flower and yellow flower with reddish brown bar and spot groups. The results showed 77 monomorphic DNA bands and 48 polymorphic DNA bands in the ranges of 180 - 2,800 bases (Figure 48).

*EcoRI*+AC/*MseI*+CAT primer combination yielded 57 DNA bands, 39 monomorphic DNA bands and 18 polymorphic DNA bands, in the ranges of 220 - 2,800 bases.

*EcoRI*+AG/*MseI*+CAG primer combination yielded 48 DNA bands, 32 monomorphic DNA bands and 16 polymorphic DNA bands, in the ranges of 250 - 2,700 bases. This primer combination showed two specific DNA bands at 270 and 275 bases, which were found in all plants of reddish brown flower and yellow flower with reddish brown bar and spot groups.

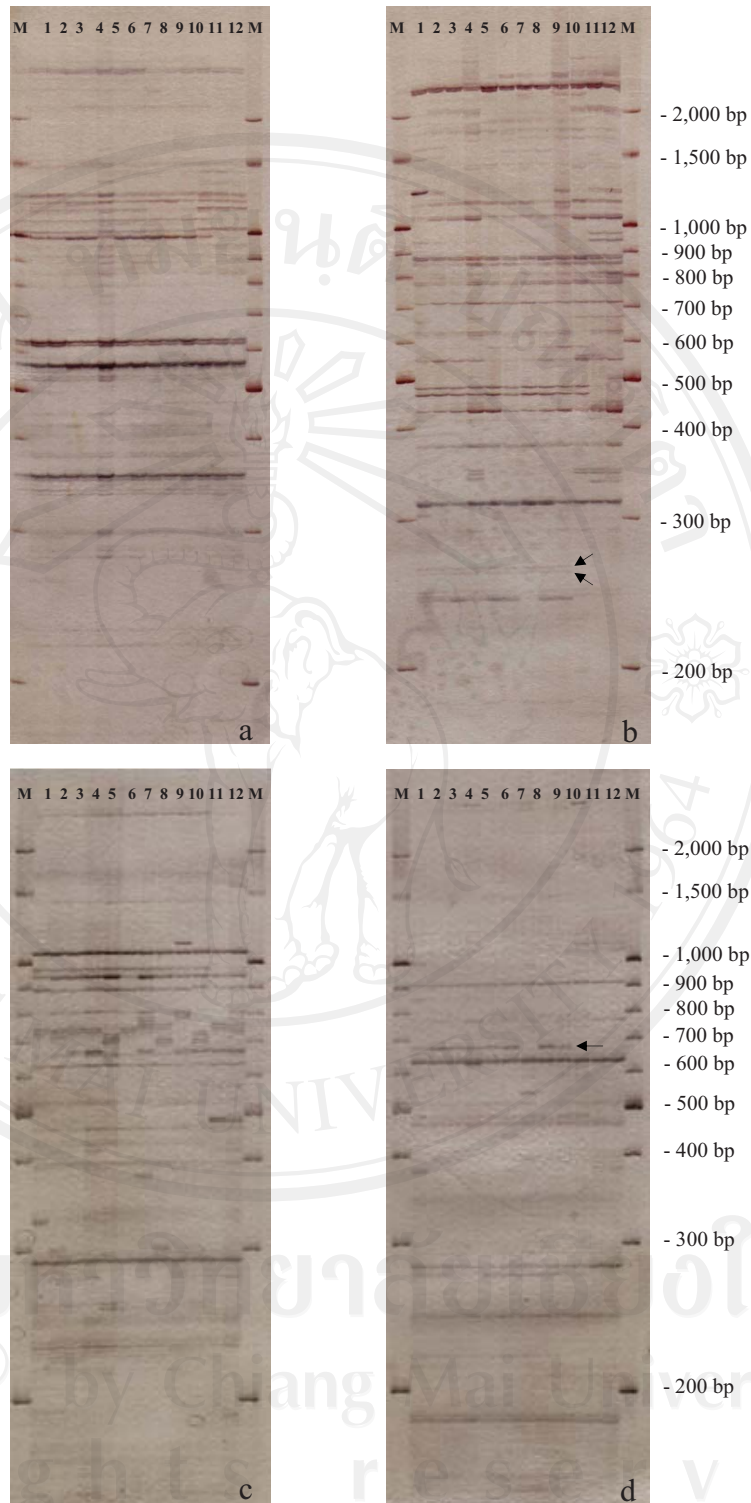
*EcoRI*+AGA/*MseI*+CAG primer combination yielded 37 DNA bands, 23 monomorphic DNA bands and 14 polymorphic DNA bands, in the ranges of 240 - 2,500 bases.

*EcoRI*+ATT/*MseI*+CCA primer combination yielded 32 DNA bands, 20 monomorphic DNA bands and 12 polymorphic DNA bands, in the ranges of 180 - 2,500 bases. This primer combination showed one specific DNA band at 670 bases, which was found in all 3 plants of reddish brown flower and in 5 out of 6 plants of yellow flower with reddish brown bar and spot groups.



**Figure 47** AFLP profiles of *P. cornu-cervi* generated by 64 primer combinations.

(M): 100 - 2,000 bp marker and (1 - 64): 64 primer combinations previous stated in Table 8. The label in red numbers indicated the suitable primer combinations for AFLP analysis.



**Figure 48** AFLP profiles of 12 *P. cornu-cervi* generated by 4 *EcoRI* and *MseI* primer combinations. *EcoRI*+AC/*MseI*+CAT (a), *EcoRI*+AG/*MseI*+CAG (b), *EcoRI*+ATT/*MseI*+CCA (c) and *EcoRI*+AGA/*MseI*+CAG (d) primer combinations. (M): 100 - 2,000 bp marker; (1 - 3): reddish brown flower; (4 - 9): yellow flower with reddish brown bars and spots; (10 - 12): pure yellow flower. The arrows indicated the 3 specific DNA bands.

### Analysis of DNA sequences

The two specific DNA bands generated by *EcoRI*+AG/*MseI*+CAG primer combination which were found in all plants of specified groups were directly excised from dried polyacrylamide gel. The gel pieces were incubated in TE buffer for 15 min at 90 °C and 1µl was used to reamplify the fragment with the *EcoRI*+AG/*MseI*+CAG primer combination using selective amplification condition. The fragments were then cloned with Clone JET™ PCR Cloning Kit and automated sequencing (Ward Medic, Ltd.). The sequencing of two DNA fragments revealed 229 and 278 bases (Figure 49). DNA sequences were compared with DNA sequences database at National Center for Biotechnology Information (NCBI) GenBank. After BLAST searches of the sequences, the 229- and 278-base sequences showed the same 78.7 % homology to the *Citrus reticulata* AFLP marker AFLP-4 genomic sequence which linked to the seedless trait (Xiao *et al.*, 2009), and also the 229- and 278-base sequences showed 65.8 and 67.6 % homology, respectively, to the ATP synthase gamma chain mRNA of *Pyrus communis*.

#### 229 bases

5'- GACTGCGTACCAATTCAGGAAGAGAGAGACTTAGCACTGCCAGAAAT  
AAAGAGGATTTGGATTGGATCTGAGGTTGAAGGATTTTGGCAGGAAATAG  
TTGCTGAACAAAAGCAATTAAGTGTACTCAGGACTCATCATGACTGCG  
TACCAATTCAGCAAAAAGTGAAGTAAAGGGTCTGTTTGGGGCAGCTGTGGAT  
TATTTATCATCTGCTGTTACTCAGGACTCATC -3'

#### 278 bases

5'- GACTGCGTACCAATTCAGGAAGAGAGAGACTTAGCACTGCCAGAAAT  
AAAGAGGATTTGGATTGGATCTGAGGTTGAAGGATTTTGGCAGGAAATAG  
TTGCTGAACAAAAGCAATTTACTGTTACTCAGGACTCATCATGACTGCGT  
ACCAATTCAGGAAGAGAGAGACTTAGCACTGCCAGAAATAAAGAGGATT  
TGGATTGGATCTGAGGTTGAAGGATTTTGGCAGGAAATAGTTGCTGAACA  
AAAAGCAATTTACTGTTACTCAGGACTCATC -3'

**Figure 49** DNA sequences of two specific DNA bands of *P. cornu-cervi*.