APPENDICES

APPENDIX A

DNA Fingerprint Method.

- Total genomic DNA was extracted from young leaves, using the hexadecyltrimethylammonium bromide (CTAB) method following the procedure of Doyle and Doyle (1990) with some modifications.
- Preheat CTAB buffer in centrifuge tube to 65°C in a water bath
- Grind 0.8 of fresh tissue in 10 ml CTAB buffer using a preheated
- Transfer mixture to centrifuge tube and incubate at 65°C for 20 minutes
- Transfer the aqueous phase to a new tube, add 2/3 volume of cold isopropanal, mix gently
- Spin at 500 g for 5 minutes at room temp., discard solution
- Wash pellet in wash buffer at room temperature for 5 minutes
- Spin briefly, discard solution
- Air-dry the pellet and resuspend in 200 ml autoclaved deionized water
- Add half volume of 7.5 M NH_4OAc and 2.5 volume of cold EtOH
- Spin at 6,000-7,000 g for 10 minutes at 4°C, discard solution
- Air-dry the pellet and resuspend in appropriate amount of TE (or keep at the freeze)
- Initial RAPD profiles were generated using ten random decamer primers (AB01-AB10). All polymerase chain reactions were carried out in 25 µl total volume, containing 1X PCR buffer (Promega®), 0.15 mM MgCl2, 0.32 mM dNTPs, 1.28 µM of primer, 0.08 unit of *Tag* DNA polymerase (Promega®), and 8 ng of genomic DNA template.
- Negative controls with distilled water instead of DNA were included in each run in order to verify the absence of contamination.
- DNA amplification were performed in a thermal cycles (Hybaid, USA) programmed for 3 min pre-denaturation at 95° C followed by 40 cycles of 1 min denaturation at 94° C, 1 min annealing at 40° C, and 2 min extension at 72° C, with a final extension of 7 min at 72° C.
- Amplification products were electrophoresed in 1.5% (w/v) agarose gel containing ethidium bromide (0.5 gm/ml).

- Data were scored for the presence or absence of amplification fragments and entered into a binary data matrix. Similarity values from the matrices were used for cluster analyses via the unweighted pair group method.

The screening of primers resulted in 8 decamer primers which showed maximum and minimum number of bands were produced by the primers AB 03 to AB 10 respectively.

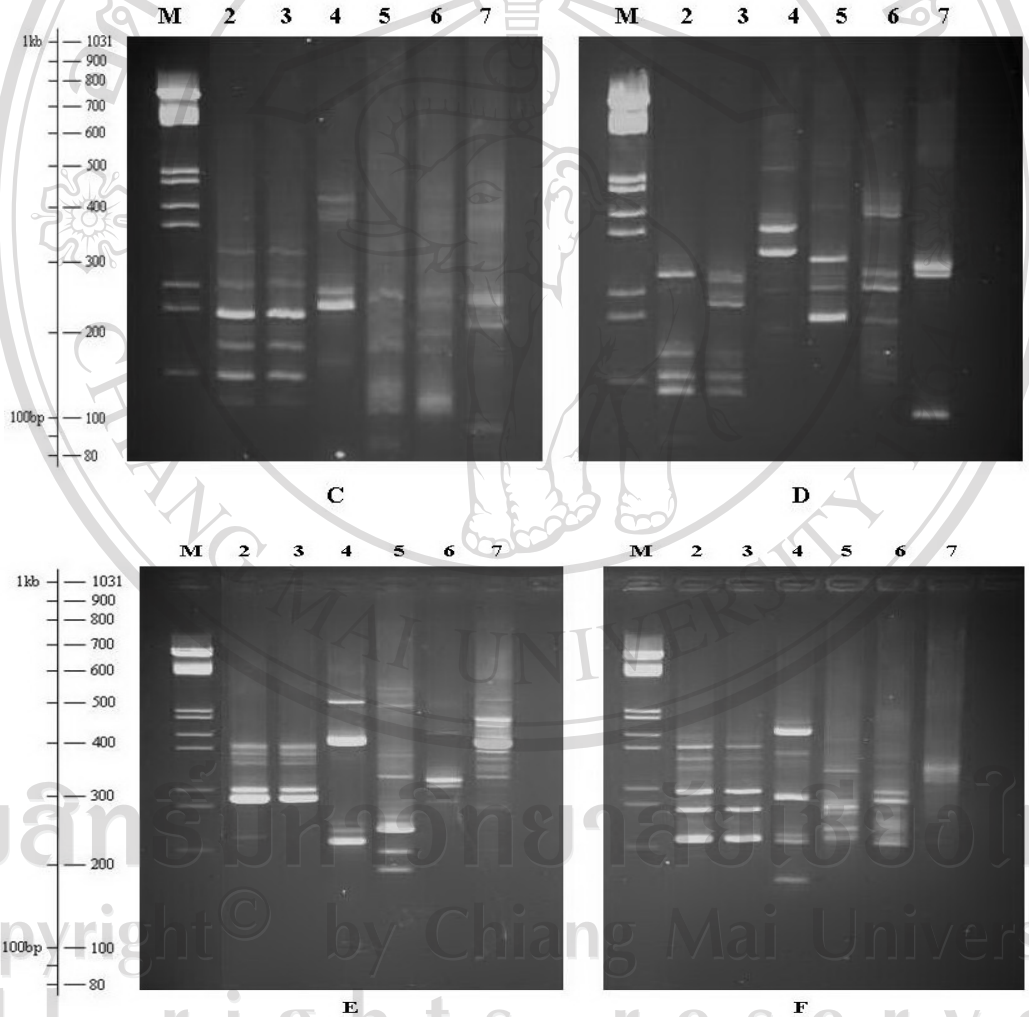

Figure 49 RAPD patterns of six species of plant by the primer AB 03(C), AB 04 (D), AB 05(E) and AB 06 (F): Lane M=marker, 2= *Parabaena sagittata*, 3= *P. sagittata*, 4= *Rhaphidophora glauca*, 5= *Rhaphidophora peepla*, 6=*Philodendron monstera* and 7=*Epipremnum aureus*.

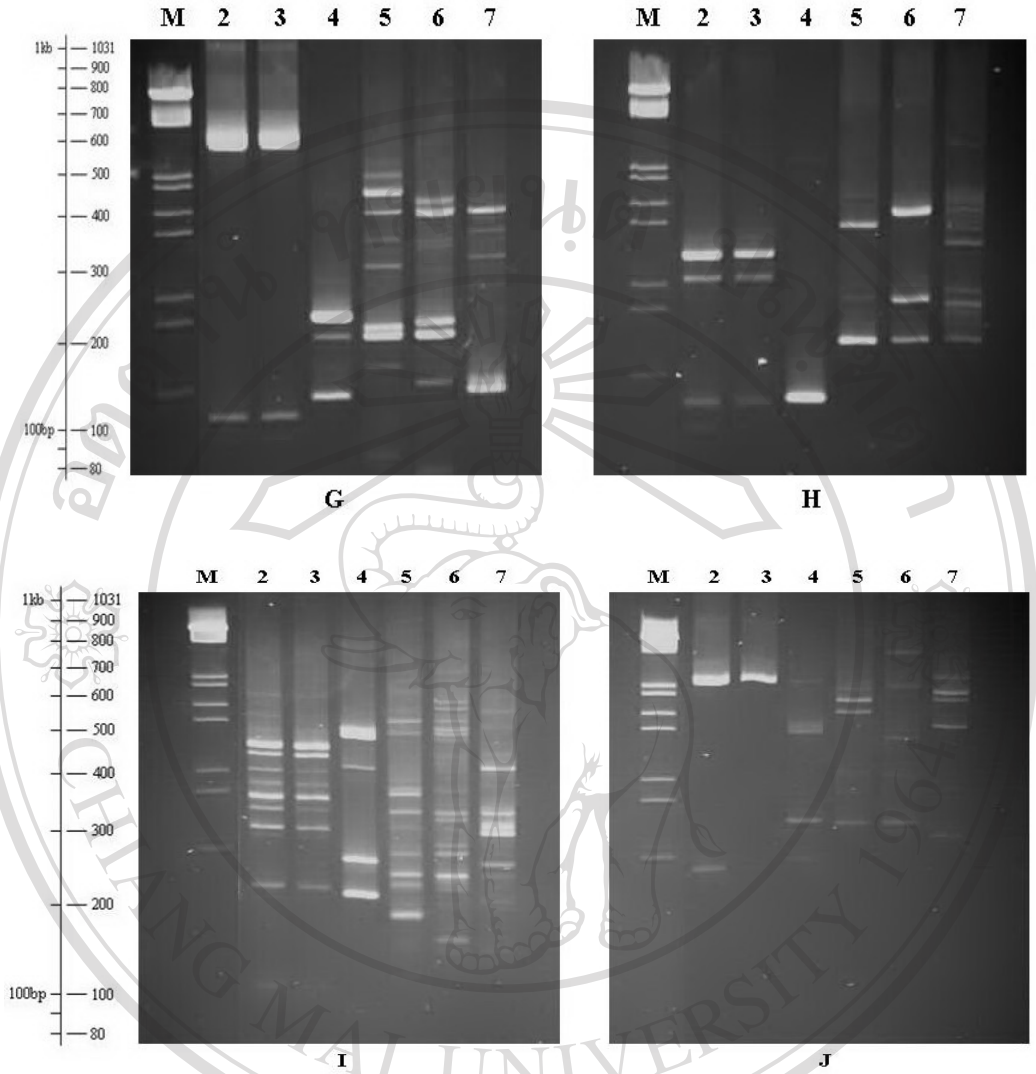

Figure 49 RAPD patterns of six species of plant by the primer AB 07(G), AB 08 (H), AB 09(I) and AB 10 (J) Lane M=marker, 2= *Parabaena sagittata*, 3= *P. sagittata*, 4= *Rhaphidophora glauca*, 5= *Rhaphidophora peepla*, 6=*Philodendron monstera* and 7=*Epipremnum aureus*.

APPENDIX B

Effects of different concentrations of IBA on rooting of creepers.

RPF-KW12 rooted in 8,000 mg/l IBA gave the highest root number in week 4 (3.93) but was not significant from other concentrations. However, *Selaginella siamensis* rooted without IBA gave significantly different results in root length in week 2 (1.05 cm) and week 4 (5.45 cm) shown in Table 14.

Table 14 Effects of IBA concentrations on root numbers and root length of *S. siamensis*.

Treatment	Root number				Root length (cm)			
	Week				Week			
	1	2	3	4	1	2	3	4
Control	-	0.02c	2.68	3.65	-	1.05ab	2.68	5.45a
Seradix 0.8 %	-	1.4a	2.65	2.24	-	1.70a	1.65	4.0b
IBA 4000 mg/l	-	0.5b	2.83	3.80	-	0.8b	1.83	2.80c
IBA 8000 mg/l	-	1.43a	1.85	3.93	-	1.72a	2.85	3.75b
IBA 12000 mg/l	-	1.05a	2.53	2.85	-	1.35a	2.03	2.63c
F-Test	-	*	ns	ns	-	*	ns	*
C.V. (%)	-	13.49	12.15	18.92	-	10.84	12.33	14.08

Means values in the same column with different letters were significantly different at $P < 0.05$ by DMRT

Gynostemma pentaphyllum rooted without IBA gave significantly different results in root numbers (4.0, 6.0 and 6.58) and root length (4.30, 7.0 and 8.38 cm) in week 2, 3 and 4 (Table 15).

Table 15 Effects of IBA concentrations on root numbers and root length of *G. pentaphyllum*.

Treatment	Root number				Root length (cm)			
	Week				Week			
	1	2	3	4	1	2	3	4
Control	-	4.0a	6.0a	6.58a	-	4.30a	7.00a	8.38a
Seradix 0.8 %	-	2.3ab	1.95c	2.68cd	-	2.60ab	2.95bc	4.48bc
IBA 4000 mg/l	-	0.8b	0.73d	1.43d	-	2.10b	1.73c	3.23c
IBA 8000 mg/l	-	3.38a	4.8b	5.38b	-	3.68a	5.80b	7.18a
IBA 12000 mg/l	-	2.70ab	3.05b	3.78c	-	3.00a	4.05b	5.58b
F-Test	-	*	*	*	-	*	*	*
C.V. (%)	-	10.05	12.15	8.9	-	9.30	13.10	12.08

Means values in the same column with different letters were significantly different at $P < 0.05$ by DMRT

Trachelospermum asiaticum started rooting on week 3 and 4 in all rooting treatments. The plant rooted in Seradix 0.8% gave the best rooting result which was significantly different in root numbers (0.23 and 0.20) and root length (0.53 and 1.20 cm) as shown in Table 16.

Table 16 Effects of IBA concentrations on root numbers and root length of *T. asiaticum*.

Treatment	Root number				Root length (cm)			
	Week				Week			
	1	2	3	4	1	2	3	4
Control	-	-	0.08c	0.05b	-	-	0.08c	0.05c
Seradix 0.8 %	-	-	0.23a	0.20a	-	-	0.53a	1.20a
IBA 4000 mg/l	-	-	0.06c	0.05b	-	-	0.05c	0.05c
IBA 8000 mg/l	-	-	0.13b	0.1b	-	-	0.43b	0.85a
IBA 12000 mg/l	-	-	0.13b	0.75ab	-	-	0.43b	0.58b
F-Test	-	-	*	*	-	-	*	*
C.V. (%)	-	-	12.73	19.28	-	-	14.28	12.88

Means values in the same column with different letters were significantly different at $P < 0.05$ by DMRT

Hoya thomsoni gave significantly different results in both root numbers and root lengths every week when rooted in 8,000 mg/l IBA. The root numbers received were 1.48, 1.98 and 2.9, and root lengths were 17.8, 2.18 and 4.7 cm (Table 17).

Table 17 Effects of IBA concentrations on root numbers and root length of *H. thomsoni*.

Treatment	Root number week				Root length (cm) Week			
	1	2	3	4	1	2	3	4
Control	-	1.40a	1.08ab	1.35c	-	1.70a	2.08ab	3.15c
Seradix 0.8 %	-	1.0b	1.18ab	2.38a	-	1.10bc	2.18ab	4.18ab
IBA 4000 mg/l	-	1.03b	1.18ab	1.8b	-	1.33b	2.33ab	3.5bc
IBA 8000 mg/l	-	1.48a	1.98a	2.9a	-	1.78a	2.68a	4.7a
IBA 12000 mg/l	-	0.5c	0.88b	1.38c	-	0.80c	1.88b	3.18c
F-Test	-	*	*	*	-	*	*	*
C.V. (%)	-	14.80	12.20	8.90	-	18.40	10.25	10.03

Means values in the same column with different letters were significantly different at $P < 0.05$ by DMRT

The results shown in Table 18 below showed that *Solena amplexicaulis* rooted in 4,000 mg/l IBA gave significantly different root numbers (2.33, 9.58 and 10.75) and root lengths (2.63, 10.58 and 10.15 cm).

Table 18 Effects of IBA concentrations on root numbers and root length of *S. amplexicaulis*

Treatment	Root number week				Root length (cm) week			
	1	2	3	4	1	2	3	4
Control	-	1.28c	3.15b	3.7b	-	1.18bc	4.15b	5.50b
Seradix 0.8 %	-	0.5c	5.33b	8.75a	-	0.80c	6.33b	10.55a
IBA 4000 mg/l	-	2.33a	9.58a	10.75a	-	2.63a	10.58a	10.15a
IBA 8000 mg/l	-	1.43b	5.25b	5.78b	-	1.73b	6.25b	7.57b
IBA 12000 mg/l	-	0.85bc	4.95b	5.58b	-	1.15bc	5.95b	5.50b
F-Test	-	*	*	*	-	*	*	*
C.V. (%)	-	12.2	17.3	16.05	-	13.09	9.05	16.05

The Means in the same column followed by different letters were significant difference at $p = 0.05$ by DMRT

Pothos sp. rooted in Seradix 0.8 % and IBA 4,000 mg/l gave significantly different results in both root number and root length (Table 19).

Table 19 Effects of IBA concentrations on root numbers and root length of *Pothos* sp.

Treatment	Root number Week				Root length (cm) week			
	1	2	3	4	1	2	3	4
control	-	0.80b	0.80b	0.68b	-	0.68b	1.40b	2.08b
Seradix 0.8 %	-	1.98a	3.95a	5.58a	-	1.95a	3.35a	4.68a
IBA 4000 mg/l	-	1.93a	3.58a	4.83a	-	1.83a	3.20a	4.88a
IBA 8000 mg/l	-	0.60b	1.30b	0.38b	-	0.72b	1.03b	1.98b
IBA 12000 mg/l	-	0.87b	1.41b	0.38b	-	0.56b	1.50b	1.82b
F-Test	-	*	*	*	-	*	*	*
C.V. (%)	-	20.01	18.1	12.8	-	17.03	14.80	11.20

Means values in the same column with different letters were significantly different at $P < 0.05$ by DMRT

APPENDIX C

Botanical Microtechnique (Johanson's 1940, Plant Microtechnique.)

1. Collection & Fixation: The first step in preparing materials for wax embedding is to 'fix' the materials. This both kills and preserves the cells so that no further changes take place.

- Fixation of plant samples in **FAA or Formalin-Acetic Acid alcohol**

50% or 70% ethyl alcohol	90	ml
glacial acetic acid	5	ml
formalin	5	ml

or

95% ethyl alcohol	50	ml
glacial acetic acid	5	ml
formalin	10	ml
distilled water	35 ml	

- Time to fix materials is 18-24 hours or 1-2 weeks depending on plants.

2. Dehydration: Once it has been fixed, the next stage is to slowly dehydrate the material to slowly remove all the water. In order not to destroy the delicate tissues, this is done gradually in a series of alcohol/water solutions of increasing strengths.

Solution for dehydration	50%	70%	85%	95%	100%
distilled water	50	30	15	-	-
95% ethyl alcohol	40	50	50	45	-
TBA	10	20	35	55	75
Absolute alc.	-	-	-	-	25

Table 20 The following TBA / Isopropyl alcohol for dehydrating.

dehydrating	step 1	step 2	step 3	step 4	step 5	step 6	step 7
approx total % alcohol	50	70	85	95	100+erythrosin	Pure TBA (3 time)	TBA+liq. Paraffine 1:1
Times	1 night	1 night	1 night	1 night	1 night	1 night	> night

Incubate tissues with paraffin at 58-60˚C for 1 week. These are average times and may be extended for harder tissues.

3. Embedding: Place materials on top of cooled paraffin wax (just sufficiently solidified-not cooled completely) and just cover with the TBA. Finally the specimens are ready for making blocks and sectioning. The cuts blocked in paraffin sections were taken with a manual microtome-rotational. These sections went through stages of pasting in slides, distensions in a heated slide and coloring by hematoxylin and they were finally fixed in Canada balsam and studied under a microscope.

APPENDIX D

Study on vase life.

The colour of the leaves was recorded by using Minolta Chromameter Model CR-300. The leaves were randomly sampled for measurement. Recorded system called CIELAB were used to receive L* value which were lightness and darkness. L* scale varies from 0-100.

L* near zero means less light or dark.

L* near 100 means light.

The others values recorded were a* and b*; the positive a* value means the sample had red colour while the negative a* value means green; the positive b* value means yellow while the negative b* value means blue; a* and b* values were used in the following equations.

Chroma = $(a^* + b^*)^{1/2}$

Hue angle = arctangent (a*/b*)

Leaf freshness.

There were 3 criterias used to measure leaf freshness:

Decurve and recurve of the petiole were rated by using score rating from erected to decurve (0-5).

Leaf wiltness used score rating from fresh to wilt (0-5).

Laminar freshness: score rating from green to yellow (0-5).

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