

VEGETATIVE PROPAGATION OF SELECTED NEPENTHES SPECIES

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ABSTRACT. *The potential of pitcher plant as ornamental plant as well as medicinal values has attracted many studies conducted on the species. However, only a few studies have been carried out to propagate the plant in vitro and none has been reported on rooting of stem cuttings.*

The present study focus on two different methods of vegetative propagation on Nepenthes. Firstly, the in vitro seed germination on N. ampullaria shows that a half-strength Murashige and Skoog media ($\frac{1}{2}$ MS) formulation with high sucrose level enhanced seed germination compared to other media formulation (Knudson-C and Vacin and Went). The seedlings grown on the $\frac{1}{2}$ MS media supplemented with 1.0 mg/L IBA enhanced shoot and root growth as well as in vitro pitcher production. The same media formulation supplemented with 2.0 mg/l 2,4-D, however, reduced shoot but promoted callus growth. Numerous shoot proliferation was enhanced with the addition of 2.5 mg/l BAP.

Secondly, the experiment on rooting of stem cuttings of three Nepenthes species (N. ampullaria Jack, N. mirabilis Lour. And N. rafflesiana Jack) using commercially prepared rooting hormone produced an over all rooting success of 42.5% in sand media and 16.7% in liquid media (using Thiamine and Pyridoxine). The use of commercial rooting hormone significantly ($p=0.01$) enhanced adventitious root formation for all species. However, N. mirabilis treated with Seradix-1 and Seradix-2 planted in sand media has the most cuttings rooted (60% and 53.3% respectively). The results show that Nepenthes could be propagated vegetatively using stem cuttings and in vitro propagation. With the use of commercially prepared rooting hormone large number of planting material could be produced in a short time.

KEYWORDS. Pitcher Plants, Rooting of Stem Cutting, Vegetation Propagation

INTRODUCTION

Pitcher plant is a unique tropical species that has many potential uses, which are not fully explored (Phillips and Lamb, 1989). These include the species' potential for ornamental plants (both indoor and outdoor) since the different shape and colour of the pitchers can be very attractive. At the same time various parts of the plant have been used by natives of

Sarawak, Brunei and Kalimantan to treat some ailments such as stomachache, dysentery and diarrhea. *Nepenthes* is a protected species in Sarawak and to-date most of the studies deal with the ecology and taxonomy aspect of the plant. Only a few studies have been carried out to propagate the plant *in vitro* and none has been reported on rooting of stem cuttings. In Sarawak 18 species has been recorded to thrive in the peat swamp, the kerangas and mountain forests. Among the lowland species, *Nepenthes mirabilis* Merr. and *N. ampullaria* Jack Korth. are the most common pitcher plants found growing on the peat soil, while *N. rafflesiana* Jack. is found in dry and rocky marginal areas. Although all species of *Nepenthes* are totally protected in Sarawak, these three common species are constantly cut and destroyed by farmers as well the road maintenance workers.

Pitcher plant has small seeds and is known to have poor germination rate, thus, natural regeneration through seeds will slow effort to conserve the plant *ex situ*. One way to ensure the species existence is to look for an alternative propagation technique through vegetative propagation such as tissue culture and rooting of stem cuttings. There had been no known good reports on the micro propagation of *Nepenthes* species using seeds and shoots, although Latha and Seenii (1994) has indicated a low success in *N. khasiana* Hook.f.. While Normah and coworkers (1994) had tried nodal cuttings of *N. gracilis* *in vitro* but they were troubled by the heavy fungal and bacterial contamination.

Similarly, to-date, the possibility of propagating *Nepenthes* through vegetative methods has not been well explored by plant researchers. The ability to propagate the plants by vegetative means, will help to minimize disease and pest during transportation of the plants as well as enhance the use of the plants as an ornamentals.

MATERIAL AND METHODS

1. Micro-propagation by Tissue Culture

Seed Germination

The fruits of *N. ampullaria* and *N. mirabilis* were collected in middle of July. The seeds were separated by shaking the dried fruit stalks. The tiny winged seeds were stored in an air-tight container and was placed in the refrigerator at 4°C until used.

The seeds were wrapped in muslin cloth, run under tap water for 20 minutes; dipped in 30% @Clorox (containing 2.5% sodium hypochlorite) for 3 minutes and rinsed three times in distilled water for 5, 15 and 30 minutes respectively. The seeds were placed in petri-plate containing half-strength MS (Murashige and Skoog), Knudson-C and Vacin and Went media with two levels of sucrose (3 and 6%). Each petri plate contained 15 seeds and each treatment consisted of ten petri-plates. The petri-plates containing *Nepenthes* seeds were placed under aseptic culture room supplemented with cool white fluorescent bulbs and the temperature was kept at 25°C by an air conditioner day and night.

Growth of Seedlings

Three weeks after germination, the two-leaf seedlings were transferred onto $\frac{1}{2}$ MS media containing different BAP (0, 1.0, 2.5 and 5.0 mg/L) and IBA (0, 0.5, 1.0, 2.0 mg/L) combinations and 2,4-D. The length of leaves, roots and number of pitchers formed were measured and the development of callus was observed.

2. Vegetative Propagation By Stem Cuttings

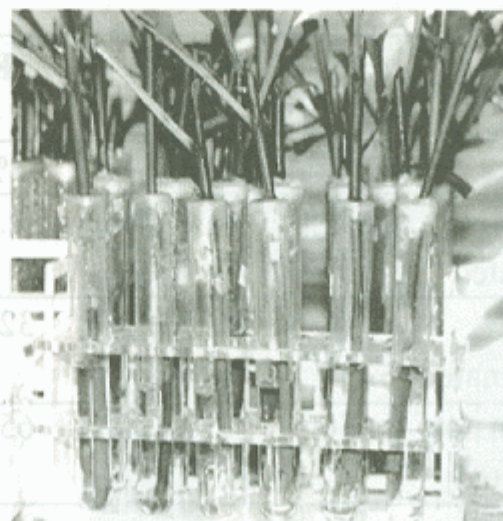
Two different propagation media were used in the study. The first batch was using sand as rooting media while the second batch was using liquid media.

A total of 150 cuttings (10-15 cm long) were collected for each species (*N. ampullaria*, *N. mirabilis* and *N. rafflesiana*). The cuttings were assigned to four commercial hormone treatments (Seradix-1, Seradix-2, MAPA and Ferti-P) where each treatment had 30 cuttings. Seradix-1 and 2, as well as MAPA contained Indole-butyric Acid (IBA) as an active compound, while Ferti-P contained Naphthelene Acetic Acid (NAA). Another 30 cuttings were used as controls. Only one leaf was left on each cutting. Longer leaf was cut into half to further reduce the loss of water through transpiration. The lower end of the cuttings were dipped into hormone talc and properly placed into the rooting bed of sand media.

The bed was equipped with misting system to provide high humidity through intermittent mist for 30 seconds at an interval of 15 minutes. The cuttings were lifted at an interval of 5 days to observe the emergence of adventitious roots. A cutting was considered as rooted if the root protruded about 0.5 cm from the stem. The cuttings were inserted back with extra care into the rooting media to avoid any damage to the roots, marked and left undisturbed until the end of the study (Figure 1).



1a. Cuttings on sand media



1b. Cuttings in liquid media

Figure 1. Rooted cuttings placed on sand media (1a) and in liquid media (1b)

In the second batch, a total of 120 cuttings for each species were used. The liquid media was prepared by dissolving 5m/L of Indolebutyric Acid) (IBA) Naphthelene-Acetic-Acid (NAA), a vitamin (Thiamine-HCL) and a Control. About 20 ml of the solution was poured into a 25mm test tube. The cuttings were placed into the test tube and its mouth was wrapped using aluminum foil to provide firm grip and support. Observation was made on the emergence of the adventitious roots through the transparent glass without lifting the cuttings.

RESULTS AND DISCUSSION

1. In Vitro Propagation

Seed Germination

An overall germination success only recorded an average of 17.5%. However, $\frac{1}{2}$ MS has the most seed germinated with 66% in $\frac{1}{2}$ MS + 6% sucrose and 25% in $\frac{1}{2}$ MS + 3% sucrose (Table 1). The seeds sown on other media did not produced good germination, except Kundson-C with 6% sucrose where 20.3% of the seeds germinated with two tiny leaves emerged from the seed coat after 4 weeks (Fig. 2). Over 25% of the seeds were contaminated by fungi which appeared in the first 3 to 5 days of the experiment. The remaining 55% of the seeds remained clean but they failed to germinate after a period of two months.

The newly germinated seeds were transferred onto $\frac{1}{2}$ MS containing 2.0 mg/l 2,4-D + 2.5 mg/l BAP developed callus and later proliferated numerous new shoots.

Table 1. Percentage (%) of *Nepenthes* seeds germinated on different culture media

Parameter	$\frac{1}{2}$ MS + Sucrose		Knudson-C + Sucrose		Vacin & Went + Sucrose		Control
	3%	6%	3%	6%	3%	6%	
Seeds (%) Germinated	25.0	66.0	2.0	20.3	2.5	0%	6.7
% Contaminated	18.5	12.5	32.5	22.0	28.5	25.8	48.4
Clean but not germinated	56.5	21.5	65.5	57.7	69.0	74.2	44.9

The study indicates that good germination of *Nepenthes* seeds can be obtained and the ½MS formulation seems to be the best germination media for *N. ampullaria*. Surface sterilization using 30% @Clorox can be used to reduce fungal contamination. However, no known studies on *Nepenthes*'s seed germination reported any successful findings. Although studies by Redwood and Bowling (1990), Rathore et al (1991) and Latha and Seeni (1994) on *N. khasiana* claimed to be able to germinate the seeds but did not mentioned the percentage of success.



Figure 2. Germinated seeds (*N. ampullaria*) on $\frac{1}{2}$ MS with 6% sucrose.

Growth of Germinated Seeds

Seedlings grown on $\frac{1}{2}$ MS supplemented with 1.0 mg/L IBA without BAP produced the best growth with an average of 8.8 ± 0.44 leaves and average leaf length of 17.7 ± 0.15 mm; 9.9 ± 1.13 number of roots at average length of 16.4 ± 0.11 mm (Fig. 3).

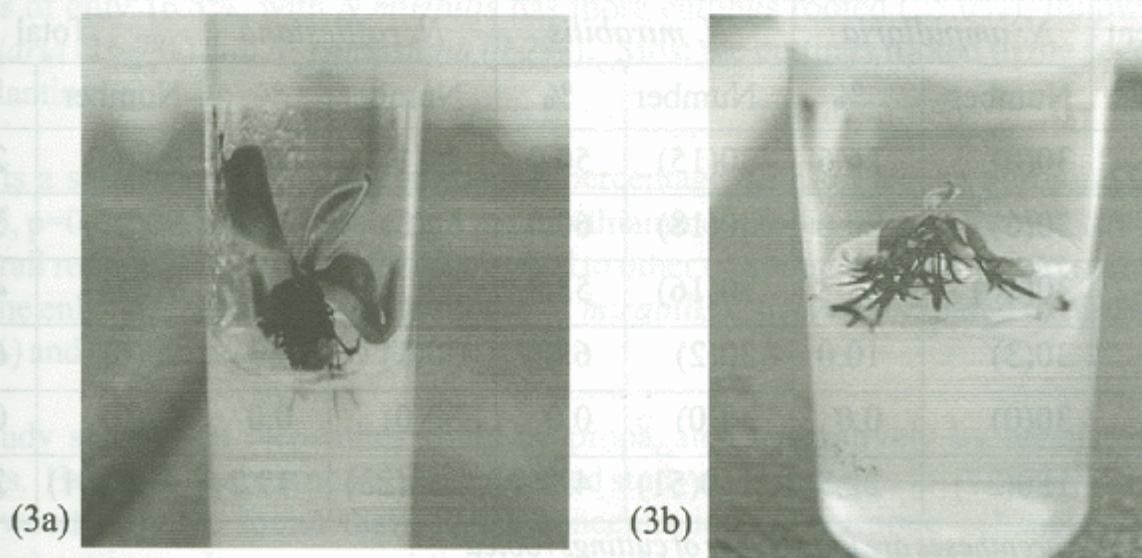


Figure 3. In vitro seedlings growth showing the number and length of leaves (3a) and number and length of roots (3b) after 10 weeks in culture.

On the other hand seedlings grown on $\frac{1}{2}$ MS media with high BAP 5.0 mg/l and IBA 2.0 mg/l produced many small plants with tiny rosette leaves. This might indicate that cytokinin such as BAP did not help much in vegetative growth of the seedlings, but for tissue culture high BAP helps to produce more small plants required for sub-culture as cytokinin is known to increase cell division.

The seedlings grown on $\frac{1}{2}$ MS without growth hormone remained small and stunted. The results failed to agree with those reported by Latha and Seeni (1994) in which they reported that seedlings could grow into bigger size for five months through subculture onto the same media without any growth hormone and cytokinin.

Callus development from the germinated seeds was induced by 2.0 mg/l 2,4-D + 2.5mg/l BAP in $\frac{1}{2}$ MS formulation after a period of 1-2 months.

2. Vegetative Propagation By Rooting of Cuttings

Rooting Success

In general, only 26.4% of the cuttings planted in sand media rooted (Table 2). Cuttings of *N. mirabilis* produced the best rooting percentage with an overall success of 42.5%. This was followed by *N. ampullaria* with an overall success of 22.5% and *N. rafflesiana* with the least (19.2%). Most of the cuttings rooted in the fifth weeks after planting (Fig 4).

Table 2. The number of cuttings rooted and rooting percentage with respect to hormone treatment in sand media.

Treatment	<i>N. ampullaria</i>		<i>N. mirabilis</i>		<i>N. rafflesiana</i>		Total	
	Number	%	Number	%	Number	%	Number	%
Control	30(8)	26.6	30(15)	50.0	30(1)	3.3	90(24)	26.7
Seradix-1	30(6)	20.0	30(18)	60.0	30(3)	10.0	90(27)	30.0
Seradix-2	30(10)	33.3	30(16)	53.3	30(18)	60.0	90(44)	48.9
MAPA	30(3)	10.0	30(2)	6.6	30(1)	3.3	90(6)	6.6
Ferti-P	30(0)	0.0	30(0)	0.0	30(0)	0.0	90(0)	0.0
Total	150(27)	22.5	150(51)	42.5	150(23)	19.2	450(101)	26.4

The number in parentheses are the number of cuttings rooted

Hormone treatment significantly influenced adventitious root formation ($=79.9$ $p=0.01$). Cuttings treated with Seradix-2 produced the highest rooting percentage with an overall average of 48.9%. At species level, *N. rafflesiana* dipped into Seradix-2 powder has 60% rooting success. This was followed by *N. mirabilis* (53.3%) and *N. ampullaria* (33.3%). *N. ampullaria* showed some degree of sensitivity to hormone treatment where rooting was much better with lower hormone (Seradix-1 with 60%) and those without hormone also showed good rooting (50%). The other two commercial hormones (MAPA and Ferti-P) failed to show any convincing results in all species (Table 3).

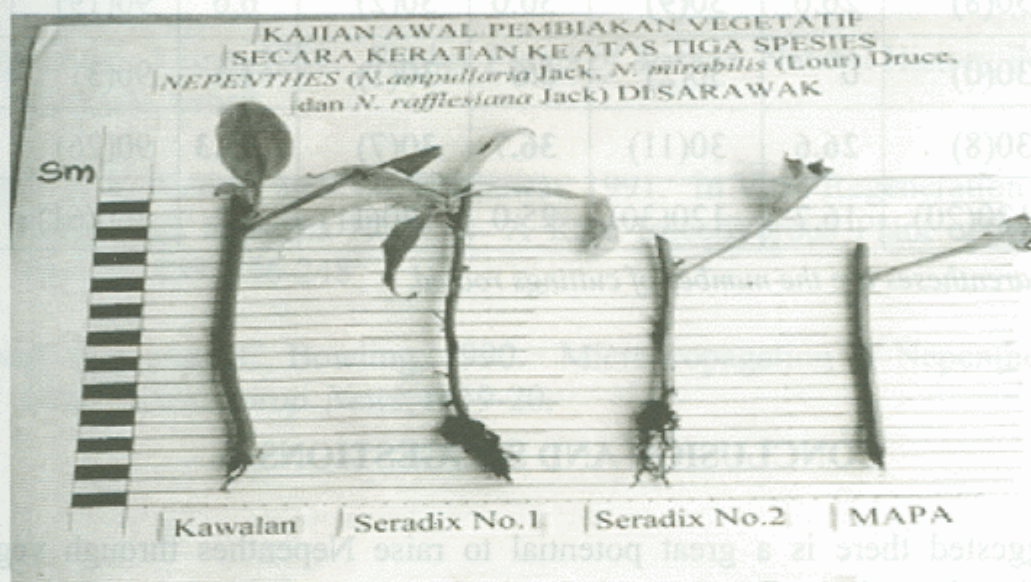


Figure 3. Rooted stem cuttings treated different rooting hormone

For cuttings placed in liquid media containing different hormone produced an overall rooting success of only 16.5%, with *N. mirabilis* has more cuttings rooted (25.0%), followed by *N. ampullaria* (16.7%) and *N. rafflesiana* (9.2%). Most the cuttings rooted in the seven weeks after planting.

There is a significant difference in rooting percentage with respect to hormone treatment ($\chi^2=22.5$, $p=0.001$). Liquid containing vitamin (thiamine) produced more rooted cuttings with an overall rooting success of 28.7% compared to other treatments (Table 4). At species level, thiamine enhanced more cuttings to root in *N. mirabilis* (36.7%), compared to *N. ampullaria* (26.6%) and *N. rafflesiana* (23.3%).

The study shows that *Nepenthes* could be propagated vegetatively by means of rooting cuttings. However, there is no known published study on *Nepenthes* for comparison. Studies using woody species found that lower concentration of hormone enhanced rooting in softwood cuttings. It is presumed that non-woody cuttings behaves similarly as softwood cuttings as shown by the results that cuttings treated with Seradix-1 and Seradix-2 rooted much better than the other hormone that contained higher concentration of IBA and NAA.

Table 4. Number of cutting rooted and rooting percentage with respect to hormone treatment in liquid media.

Treatment	<i>N. ampullaria</i>		<i>N. mirabilis</i>		<i>N. rafflesiana</i>		Total	
	Number	%	Number	5	Number	%	Number	%
Control	30(4)	13.3	30(8)	26.6	30(1)	3.3	90(13)	14.4
IBA	30(8)	26.6	30(9)	30.0	30(2)	6.6	90(19)	21.1
NAA	30(0)	0	30(2)	6.6	30(1)	3.3	90(3)	3.3
Thiamine	30(8)	26.6	30(11)	36.7	30(7)	23.3	90(26)	28.7
Total	120(20)	16.7	120(30)	25.0	120(11)	9.2	360(61)	16.5

The number in parentheses are the number of cuttings rooted.

CONCLUSION AND SUGGESTIONS

The study suggested there is a great potential to raise *Nepenthes* through vegetative propagation either through tissue culture via in vitro seed germination as well through the conventional macro propagation by means of rooting of stem cuttings. Propagation by means of in vitro culture requires more basic experiments to be tried to find out the appropriate media and sterilization techniques for pitcher plants. Although ½ MS media produced a promising results a more thorough study should be done to find the optimum auxin and cytokinin combinations. The problem of browning could be reduced if the explants is obtained from a rejuvenated mother plant. This could be achieved by cutting back the mother plant at a regular interval and the new shoots should have the juvenile characteristics.

For rooting of cuttings, improvement in the misting system to ensure high humidity will enhance better results. Rooting in liquid media should be given priority in future study since it needs less space and no misting system is required.

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Currently, no species in the Borneo region is included in Appendix I and only one species is included in Appendix II. This is *Aquilaria malaccensis* of Unakam, which was listed in 1994. No species of the region is included in Appendix III. However, many activities of some NGOs are aiming at including a large number of timber tree species worldwide in Appendix II. These activities are based on the fact that these trees are regarded as critically endangered, endangered or vulnerable World List of Threatened Trees of the World Conservation Monitoring Centre (WCMC) and in the Red List by the World Conservation Union (IUCN). A variety of species from Borneo are on this list, e.g. *Agathis borneensis*, *Calophyllum rotundifolium*, *Cynometra inaequalifolia*, *Durio* species, *Eusideroxylon zwageri*, *Koompassia* species and *Lophopetalum* species.