



Types of Media for Seeds Germination and Effect of BA on Mass Propagation of *Nepenthes mirabilis* Druce

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ABSTRACT

Nepenthes mirabilis Druce seeds need light for their germination. The best medium for seeds germinated was half strength MS medium supplemented with 0.1 % activated charcoal. The organic substances such as coconut water, potato, and banana were not enhanced seed germinated.

Shoot tips of *N. mirabilis* about 1 cm. long were cultured on half strength MS medium supplemented with 2.0 mg/l BA gave the best result in the highest average height of plant (1.3 cm). Half strength of MS medium supplemented with 1.0, 2.0, and 3.0 mg/l BA gave the same result in the highest average number of leaf per plant, leaf width, leaf length. But numbers of root and root length were not significant different in each concentration of BA.

After cultured shoot tip in half strength MS medium supplemented with 2.0 mg/l BA for 20 weeks. It was found that the bases of some shoots tip were formed the highest percentage of callus. The average number of new shoot tip (4.2 shoots) proliferated at the base of shoot tip which cultured in half strength MS medium contained with 2.0 mg/l BA also.

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1. Introduction

The plant tissue culture technique plays an important role in the preservation and micropropagation of *Nepenthes* sp. that is endangered or on the brink of extinction (Iankova *et al.* 2001; Bhatia *et al.* 2002). There are a number of reports on the *in vitro* propagation of other carnivorous plants as an effort for their preservation. Adams *et al.* (1979a) described a method for *Cephalotus follicularis* (Australian pitcher plant) by shoot tip culture, and Adams *et al.* (1979b) also described a method for *Pinguicula moranensis* (Butterwort) through leaf culture. Crouch *et al.* (1990) and Van Wares (1985) reported *in vitro* propagation of *Drosera rotundifolia* L. by leaf culture. Jang and Park (1999) reported a method for mass propagation of *Drosera rotundifolia* L. through shoot culture. Beebe (1980) and Parliman *et al.* (1982b) reported a method for producing adventitious bud from leaves. Minocha (1985) also reported an *in vitro* propagation method from mature leaf segments. This work studied a suitable and rapid *in vitro* micropropagation method for *Nepenthes mirabilis* Druce through optimization of medium and for shoot proliferation.

2. Mathematical Model

Nepenthes mirabilis Druce. seeds were sterilized with 15% Clorox (bleaching solution) and 0.01% tween 80 (v/v) for 20 min. and again with 5% Clorox for 10 min. and then washed three times with sterilized distilled water for 3 min. each. Fifty sterilized seeds were placed on different media (as Table 1) with aseptic technique. All cultures were placed at 25 ± 2 °C under cool white florescent light ($37 \mu\text{molm}^{-2} \text{S}^{-1}$) for 16/8 h. photoperiod and dark condition were treated also.

2.1 Media for seed germination

Fifty sterilized seed were cultured on MS, $\frac{1}{2}$ MS VW, $\frac{1}{2}$ VW, VW or MS supplemented with 100gm. ripen banana, 50 gm. potato and 50 gm cc. coconut water, 0.01% activated charcoal, (as Table 1). Each medium were contained with 0.25% gelrite, 2% sucrose, adjusted pH 5.8. Each media were put in bottles with 75 cc.

2.2 Media for shoot and callus induction

Young shoots tip about 1 cm. long were used as explants. Half strength MS medium were used as basal medium supplemented with 0,0.5,1.0, 2.0, and 3.0 mg/l BA, 2% sucrose, 0.25 % gelrite and adjusted pH 5.8 with 75 ml per bottle. All cultures were sterilized with autoclave at 121 °C for

20 min. The explants were subcultured into the same medium for 4 times every 4 weeks.

2.3 Statistical analysis

The data were subjected to one way analysis of variance (ANOVA) to assess treatment differences and interaction using the SPSS version 11.0 significance between means was tested by Turkey's Test ($p \leq 0.05$). This experiment with 25 replications per treatments. The percentage of seed germination was recorded after 16 weeks of cultured, and callus induction was recorded after cultured 12 weeks and shoot proliferation were recorded after 20 weeks of culture.

3. Results

Seeds were permitted to swell after sowed 3 weeks and continue to germinate after 5 weeks. Their cotyledons came out after 7 weeks. After 16 weeks, the best medium for seeds germination was half strength MS medium supplemented with 0.1 % activated charcoal where as 87.57% (Table 1). When compared percentage of seeds germination. It was significant difference ($p \leq 0.05$) between each media. Percentage of seeds germinated in MS, half strength MS and VW, half strength VW supplemented with activated charcoal was the second. *N. mirabilis* seeds do not germinated in VW, half strength VW, MS, half strength MS which supplemented with banana, coconut water and potato as showed in table 1. *N. mirabilis* seeds in these media turned brown after cultured for 4 weeks and all seeds could not germinated.

3.1 Shoot Induction

After cultured *N. mirabilis* explants on half strength MS medium supplemented with 0, 0.5, 1.0, 2.0, and 3.0 mg/l BA for 20 weeks *in vitro*, plantlets were formed. The parameters, plants height, number of leaf per plantlet, leaf length, number of root and root length were recorded as in Table 2. The result showed that half strength MS medium supplemented with 0 and 2.0 mg/l BA gave the highest average height where as 1.86 and 1.3 cm, respectively. When counted number of leaf per plantlet, half strength MS medium supplemented with 0.5 mg/l BA gave the lowest number of leaf (7.8 leaves). But number of leaf per plant among each media which supplemented with 1.0, 2.0 and 3.0 mg/l BA were not significant difference. In half strength MS supplemented with 1.0, 2.0 and 3.0 mg/l BA gave the highest average leaf width (0.78, 0.82, and 1.0 cm per leaf,

respectively). The highest average leaf length was from plantlet which cultured in half strength MS medium without BA (2.92 cm.). Root formation was most effective and their average root length was longest in half strength MS medium with free BA. However, the numbers of root and root length were not significant different in each treatments.

Table 1: Average percentage of *N. mirabilis* seeds germination in each media (after cultured 16 weeks).

Media	Activated charcoal(0.1%)	Banana (100gm)	Potato (50gm)	Coconut water (150cc.)	% germination*
MS	-	-	-	-	25.67d
½MS	-	-	-	-	29.16cd
VW	-	-	-	-	38.62c
½VW	-	-	-	-	35.02c
MS	+	-	-	-	46.67b
½MS	+	-	-	-	87.57a
VW	+	-	-	-	34.67c
½VW	+	-	-	-	44.33b
½VW	+	+	+	-	28.94d
½VW	+	+	+	-	24.65d
½MS	+	+	+	-	17.5e
½VW	+	+	+	+	15.05e
½VW	+	+	-	-	8.2ef
½VW	+	+	+	-	10.0ef
VW	-	+	+	+	4.0ef
VW	-	+	-	-	0 f
VW	-	+	+	-	0f
½VW	-	+	+	+	0f
½MS	-	+	-	-	0f
½MS	-	+	+	-	0f
½MS	-	+	+	+	0f

* mean followed by the same letters are not significant difference ($p \leq 0.05$) using Turkey Test. Evaluation was made after 16 weeks

+ supplemented with : activated charcoal, banana, coconut water or potato
 - do not supplemented with : activated charcoal, banana, coconut water or potato

Callus Induction and Shoot Proliferation

After subcultured young shoot tip of *N. mirabilis* every 4 weeks into the same medium for 3 times, the result showed that some explants formed new shoots at the base of explants and at the edge of leaf (fig 1a) (Table 3). The highest average new shoots was proliferated in half strength MS contained with 2 mg/l BA (4.2 shoots), followed by half strength MS contained with 3 mg/l

(3.66 shoots) (Figure 1b). Callus were induced in half strength MS medium supplemented with BA and formed at the base of some explants. In half strength MS medium supplemented with 3 % BA gave the highest average percentage callus induction (50%).

Table 2: Effect of BA concentration in half strength MS medium with activated charcoal on shoot induction in *Nepenthes mirabilis* Druce (after cultured 20 weeks).

BA conc. Mg/l	Plant height (cm)*	No. of leaf/ Plant	Leaf width(cm)*	Leaf length (cm)*	No. of root (root) ns	Root length (cm) ns
0	1.86±0.4 ^c	9.8±1.30 ^{ab}	0.82±0.20 ^{ab}	2.92±0.2 ^a	4.2±0.44	2.56±0.43
0.5	0.42±0.08 ^a	7.8±1.30 ^a	0.56±0.15 ^a	0.86±0.4 ^d	3.8±1.4	2.24±0.49
1.0	0.70±0.12 ^{ab}	9.8±2.16 ^{ab}	0.78±0.27 ^{ab}	1.0±0.38 ^{cd}	3.6±0.54	2.26±0.45
2.0	1.300.67 ^b ^c	8.8±0.83 ^{ab}	0.82±0.17 ^{ab}	1.44±0.55 ^{bc}	3.6±0.65	2.74±0.50
3.0	0.92±0.35 ^{ab}	10.6±1.14 ^{ab}	1.00±0.15 ^b	2.34±0.82 ^b	4.6±0.41	3.04±1.2

* a b c d- Average compared mean within column by Turkey's test at (p≤ 0.05)
ns: non significant difference

Table 3: Effects of various BA concentration in half strength MS medium on callus induction and new shoot proliferation in *N. mirabilis* Druce (after cultured 20 weeks).

BA conc. mg/l	% callus induction	No. of new shoots*
0	0	0 ^d
0.5	30	1.88 ^c
1.0	70	3.22 ^{ab}
2.0	85	4.2 ^a
3.0	50	3.66 ^a

* a b c d- Average compared mean within column by Turkey's test at (p≤ 0.05)

4. Discussion

It was revealed in this study showed that *Nepenthes mirabilis* seeds were germinated only in light condition, as reported from Jala (2010a). It was showed that red light and white light gave the highest numbers of seeds germination index and gave healthy seedlings. Research works from Hangarter (1997), and Winslow (1999) reported that many plants species responded to the environment with optimal growth and development according to the light that they received. Colbach (2002) reported that some seeds germinated under different light. *N. mirabilis* took 5

*Corresponding author (A.Jala). Tel/Fax: +66-87028-3073. E-mail address: anchaleejala@yahoo.com. ©2012. American Transactions on Engineering & Applied Sciences. Volume 1 No.2 ISSN 2229-1652 eISSN 2229-1660. Online Available at <http://TUENGR.COM/ATEAS/V01/163-171.pdf>

weeks for germination. Research works from Rasmussen (1995) reported that some plants seeds need long time for germination due to their dormancy. Stoutamire (1983) reported that orchid seeds could not germinate due to their testa which cover with lipid layer. Fast (1983) reported that there are some abscissic acid (ABA) at their testa and Van der Kinderen (1987) found that orchid seeds have inhibitor substance in the seeds. The percentage germination may be due to difference in the response of seeds to light treatment and seeds dormancy.

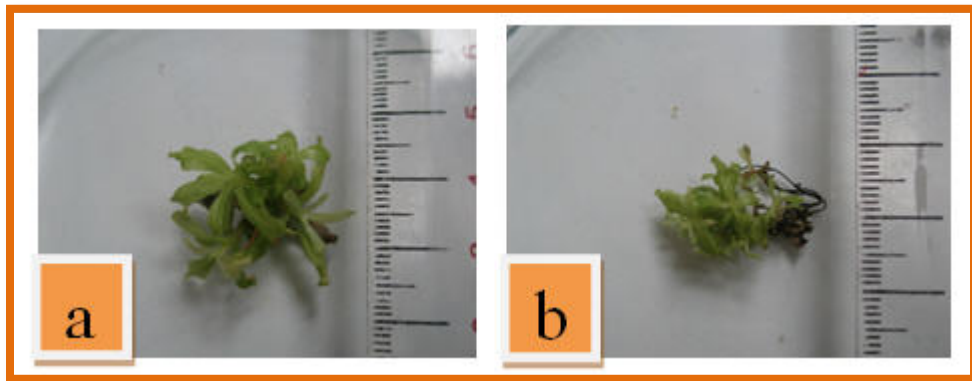


Figure 1: The highest average new shoots proliferated in half strength MS contained with a) 2 mg/l BA b) 3mg/l BA

When cultured *N. mirabilis* on MS and VW media with different concentration and supplemented with different concentration of organic substance which were potato, coconut water, banana and activated charcoal. The suitable medium for *N. mirabilis* germination and gave the highest percentage germination (87.57%) was half strength MS medium supplemented with 0.1% activated charcoal. This result was the same as other carnivorous plant. *Dionaea muscipula* Ellis cultured in $\frac{1}{2}$ MS medium. It was the most effective medium for shoot induction from shoot tip and young leaves (Jang et al. 2003). It might be the inorganic elements in MS and VW full strength were effected to seeds germination. In the other medium it gave low percentage of germination. Research works from Jala (2010b), Pierik and et al (1988) reported that germination of orchid seeds could not occurred due to organic element in the medium. In this experiment had many kinds of organic substance including potato, coconut water and banana. Ratsek (1932), Raghavan (1966) and Woodroof (1979) reported that in coconut water have fructose, glucose and sucrose about 5.25,7.25,and 9.18 mg/l respectively. Pierik et al (1988) cultured *Papiopedillum ciliolare* seeds in VW medium supplemented with coconut water and potato. It was showed that germination of *Papiopedillum ciliolare* seeds were decreased when increased concentration of

sugar. Types of sugar which came from potato, banana and coconut water were effected on seed germination also, due to the sugar when autoclave for sterilization, the heat have hydrolyzed sugar to fructose and glucose (Arditi and Ernst, 1993). The difference in the response of seeds to germination may be due to the organic substance.

When cultured shoot tip of *N. mirabilis* in difference concentration of BA, it was showed that ½ MS supplemented with 2 mg/l BA gave the highest average new shoots (4.2 shoots) and percentage of callus induction (85%). These results are consistent to other carnivorous plant. In *Dionaea muscipula* Ellis, zeatin was found the most effective cytokinin in inducing adventitious shoot from segment of leaves (Minocha, 1985). Jang *et al* (2003) reported that micropropagation of *Dionaea muscipula* Ellis by using seeds and shoots multiplication was highest in ½ MS supplemented with 0.5 mg/l kinetin. *Nepenthes macfarlanei*, tropical carnivorous plants, were used BA for induction new shoots from cotyledon seedling (Chua and Henshaw, 1999).

5. Conclusion

Nepenthes mirabilis Druce seeds were germinated within 4 weeks after sowing in MS, half strength MS, VW, half strength VW media and supplemented with and without combination of 100gm banana, 0.1% charcoal, 15% coconut water and 50gm potato. The best medium for *N. mirabilis* seeds germinated was half strength MS medium contained with 0.1% activated charcoal where as 87.57%. During seeds germinated, they needed light condition for incubation and germination also.

Shoot tips about 1 cm. long were cultured on half strength MS medium supplemented with 2.0 mg/l BA and without BA gave the highest average height of plants where as 1.3 – 1.86 cm., respectively. Half strength MS medium supplemented with 1.0, 2.0, and 3.0 mg/l BA gave the same result on the highest average number of leaf per plant, leaf width, and leaf length. But numbers of root and root length were not significant difference in each concentration of BA.

Some shoot tips which cultured on half strength MS medium supplemented with 2.0 mg/l BA were formed callus at the base of explants which gave the highest percentage of callus induction

and the highest average number of new shoots (4.2 shoots) also.

This study described a protocol for seed germination, induction callus and new shoots of *Nepenthes mirabilis* Druce through tissue culture methods. However, acclimatization of carnivorous plants need further studies in order to increase acclimatization rates.

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