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# In Vitro Multiplication of *Nepenthes mirabilis* Lour (Druce) with Variations Concentration of Sucrose and BAP

**Enni Suwarsi Rahayu* & Nabila Cyntia Banowati**

Department of Biology, FMIPA, Universitas Negeri Semarang, Kampus UNNES, Gedung D, Jl. Kampus Timur Jl. Sekaran Raya No.6, Sekaran, Kec. Gn. Pati, Kota Semarang, Jawa Tengah 50229, Indonesia

*Corresponding Author: enni_sr@mail.unnes.ac.id

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**Abstract.** *Nepenthes mirabilis* (Lour) Druce is a rare ornamental plant and has high economic value so it needs to be preserved. This study aimed to analyze effect of sucrose and BAP concentrations and their optimal interactions in the in vitro multiplication of *Nepenthes mirabilis*. Explants in the form of in vitro plantlets from Laboratory of Plant Tissue Culture, Department of Biology, Semarang State University. The treatment was carried out by adding sucrose and BAP to murashige & skoog (MS) media with various concentrations. The treatments were arranged in two factor completely randomized design with four replications. Plantlets were planted in the treatment medium for 10 weeks, then every week the growth of shoots and leaves of *N. mirabilis* was observed. The data were analyzed by two-way analysis of variance and Duncan's test. The results showed that concentration of sucrose, BAP, and their interactions affected the growth of explants. Plantlets grown on media with sucrose concentration 20 g/l produced the highest number of shoots, media with concentrations BAP 3 and 2 ppm produced the highest number of shoots and number of leaves. Medium ½ MS with varying concentrations of sucrose 20 g/l with 3 ppm BAP and sucrose 20 g/l with 2 ppm BAP resulted in the highest growth in the number of shoots and number of leaves. The composition of this medium can be used for the multiplication of *N. mirabilis*.

**Key words:** Nepenthes mirabilis; sucrose; BAP; and multiplication

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## INTRODUCTION

*Nepenthes mirabilis* (Lour.) Druce is a carnivorous plant that has high economic value and is attractive. *N. mirabilis* is widely used as an ornamental plant, in ropes, medicine, and others (Handayani, 2021). The liquid in *Nepenthes* contains antibiotic compounds so that it can inhibit the development of pathogenic bacteria (Hidayat, 2015). In general, *N. mirabilis* is used as an ornamental plant. The longer demand for this ornamental plant is increasing so it is experiencing scarcity. Based on the Regulation of the Minister of Environment and Forestry of Republic Indonesia Number P.106/MENLHK/SETJEN/KUM.1/6/2018 about protected plant and animal species, *Nepenthes* is one of the endangered and protected plants.

Propagation has been done through seed germination and cuttings. Propagation through seeds is not effective because it takes a long time while cuttings require more individuals but availability in nature is very limited. An effective alternative for the Multiplication is one of the applications that is applied in plant propagation. The success of in vitro multiplication is influenced by several factors such as sucrose and growth regulators (PGR). Sucrose is a carbon and energy source that is often used by in vitro cultures. Zheng *et al*. (2010) used 30 g/l of sucrose concentration and 2 ppm of BAP concentration as the optimal concentration to optimize the axillary shoot proliferation of *N. mirabilis*. One of the PGRs that are often used for shoot formation is *6-Benzylaminopurine* (BAP).

Research that has been done is just a combination of the hormone cytokinin and auxin. Research on the interaction between sucrose and BAP has never been done. Therefore, it is necessary to study and further research the interaction between the optimal concentration of sucrose and BAP concentration in the shoot propagation of *N. mirabilis*. This study aimed to analyze the effect of sucrose concentration, BAP, and optimal interactions in the in vitro multiplication of *Nepenthes mirabilis*. The optimal concentrations of sucrose and BAP were useful as the basis for the concentration used for the propagation of *N. mirabilis*.

## METHODS

Explants were obtained from the Laboratory of Plant Tissue Culture, Department of Biology, Semarang State University. This research was in September – December 2021 at the Laboratory of Plant Tissue Culture, Department of Biology, Semarang State University. The plant material in this study was *an N. mirabilis* plantlet which had an overall length of 3 cm and had 6 leaves. The research was carried out experimentally by using a completely randomized design with two factors that is sucrose and BAP concentration. Sucrose concentration consisted of four levels, i.e. concentration of 0 g/l, 10 g/l, 20 g/l, and 30 g/l, while BAP concentration consisted of four levels, they were 0 ppm, 1 ppm, 2 ppm, and 3 ppm. The growth medium was ½ MS with the addition of BAP hormone and sucrose according to the treatment level, Myo-inositol, and vitamins. After the pH of the media solution is appropriate, the solution is added with 1,6 g of agarose as a compactor. Sterile plantlets were planted in Laminar Air Flow (LAF). Plantlets that met the criteria were selected and planted into the treatment culture media. The research unit was one bottle containing one plantlet and repeated four times. Plantlets that have been planted are incubated at a temperature of 20-24$^{o}$C, air humidity 63%, irradiated with a 1600 lux TL lamp for 16 hours of light and 8 hours of darkness for 10 weeks with observations once a week.

## RESULTS AND DISCUSSION

The results of the in vitro multiplication study of *N. mirabilis* with varying concentrations of sucrose and BAP are presented in Table 1. The data were analyzed using two-way ANOVA. Parameters that had a significant effect on in vitro multiplication of *N. mirabilis* were the time of emergence of shoots, several shoots, and the number of leaves. This significant effect comes from the concentration of sucrose, the concentration of BAP, and their interactions. The data with significant influence were further tested using Duncan Multiple Range Test (DMRT). DMRT test results are presented in Table 1.

The concentration of BAP affects the growth of shoots and leaves. The concentration of sucrose affects increasing the number of shots. Without the addition of BAP, the plant was able to stimulate the early shoots to appear faster. This is probably because the endogenous cytokinin hormone is sufficient for plants to affect the speed of cell

**Table 1.** Summary of duncan test on number of shoots, number of leaves, Percentage of live shoot, and morphology of shoots due to sucrose concentration and BAP concentration

| No | Treatment | Parameters | | | |
|---|---|---|---|---|---|
| | | Number of shoots | Number of leaves | Percentage of live shoot | Morphology of shoots |
| 1 | $S_0B_0$ | 0.50 d | 4.00 cde | 50.00 | Yellowish green |
| 2 | $S_1B_0$ | 0.50 d | 4.75 bc | 50.00 | Yellowish green |
| 3 | $S_2B_0$ | 0.50 d | 2.25 e | 50.00 | Yellowish green |
| 4 | $S_3B_0$ | 0.75d | 5.75 ab | 75.00 | Yellowish green |
| 5 | $S_0B_1$ | 1.00 cd | 3.50 cde | 75.00 | Yellowish green |
| 6 | $S_1B_1$ | 0.75 d | 4.50 bcd | 75.00 | Yellowish green |
| 7 | $S_2B_1$ | 2.25 abc | 2.75 de | 100.00 | Yellowish green |
| 8 | $S_3B_1$ | 2.50 ab | 2.50 e | 100.00 | Whitish green |
| 9 | $S_0B_2$ | 0.50 d | 5.75 ab | 50.00 | Yellowish green |
| 10 | $S_1B_2$ | 1.25 bcd | 2.25e | 75.00 | Yellowish green |
| 11 | **$S_2B_2$** | 3.00 a | **6.50 a** | 100.00 | Light green |
| 12 | **$S_3B_2$** | **3.50 a** | 4.00 cde | 100.00 | Light green |
| 13 | $S_0B_3$ | 1.00 cd | 3.25 cde | 50.00 | Brownish |
| 14 | $S_1B_3$ | 2.75 a | 3.25 cde | 100.00 | Light green |
| 15 | $S_2B_3$ | 3.25 a | 2.75 de | 100.00 | Whitish green |
| 16 | $S_3B_3$ | 2.25 abc | 3.25 cde | 100.00 | Yellowish green |

Information:
S = Sucrose concentration consisted 0 g/l, 10 g/l, 20 g/l, and 30 g/l.
B = BAP concentration consisted 0 ppm, 1 ppm, 2 ppm, and 3 ppm

**Figure 1.** Number of shoots $S_3B_2$ treatment after 10 weeks

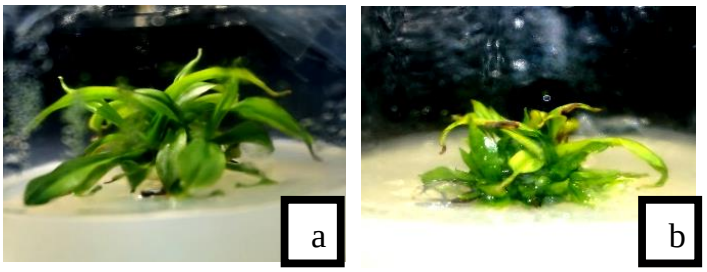


**Figure 2.** Leaves of treatment a) $S_3B_0$ and b) $S_2B_2$ for 10 weeks

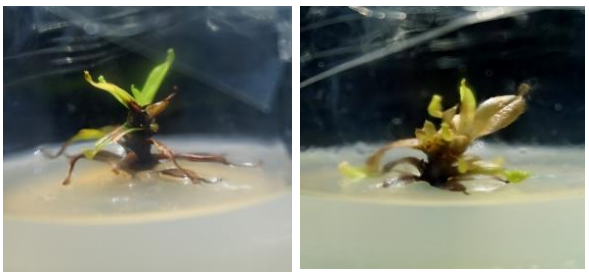

**Figure 3.** Shoots from $S_0B_3$ treatments are brownish

division which leads to the formation of shoot primordia so that the initial shoot time appears faster. The optimal concentration of sucrose is the concentration of 20 g/l. BAP concentrations of 2 – 3 ppm increased the yield of shoots and leaves.

Table 1 shows that the S3B2 treatment was not significantly different from the S2B3 and S2B2 treatments in the number of shots. S3B2 treatment produced a large number of shoots Figure. 1. Table 1 shows that the S2B2 treatment was not significantly different from the S3B0 treatment and the S0B2 treatment in the number of leaves. S2B2 treatment produced a large number of leaves. The percentage of the live shoot can be seen in Table 1. The morphology of shoots was seen from the color produced by the new shoots. The data are presented in Table 1.

The time of emergence of the shoot is influenced by the speed of cell division and the ability of cell differentiation to form new shoots. Cytokinin hormones accelerate the transition of the G1-synthesis phase and the G2-mitotic phase. Cytokinins activate the synthesis of RNA, an enzyme that plays a role in cell division, and increase protein synthesis. The process of cell division is influenced by the activity of Cyclin-Dependent Kinase (CDK). The cell division cycle requires cooperation between CDK and several types of cyclins. The G1-Synthesis phase transition is regulated by cyclin-D. The active complex of cyclin-D with CDK type A will activate the E2F promoter which activates transcriptional genes involved in the synthesis phase (Dewitte & Murray, 2003).

Shoots appear when the ratio between cytokinin hormone is higher than auxin hormone so that the cell does cell division continuously and differentiates towards the formation of shoots

(Urry et al., 2017). Nuryadin (2018) reported that the time of shoot initiation was not influenced by the interaction of the BAP hormone with NAA but from the BAP hormone independently.

The number of shoots was influenced by BAP concentration, sucrose concentration, and their interactions. The sucrose concentration of 20 g/l, 3 ppm of BAP concentration, and the interaction of 30 g/l sucrose concentration with 2 ppm of BAP concentration gave optimal results in increasing the number of shoots because at that concentration plantlets used energy optimally for cell division. Cell division that occurs will affect the number of daughter cells formed. Each daughter cell will differentiate into shoot primordia. The presence of hormonal balance in plants can increase the effect on the number of shots. Dinarti et al. (2010) reported that a concentration of 2 ppm *N. mirabilis* culture produced lower shoot numbers than the other treatments. This is different from research by Devi et al. (2013) where concentration 2 ppm of BAP is the best concentration in increasing the number of shoots of *N. Khasiana.* Shoot life is supported by the presence of sucrose and cytokinin hormones. BAP is included in one type of synthetic cytokinin hormone. Cytokinin hormones play a role in cell division in cells that are still active in cell division. Low concentrations of cytokinin hormones will encourage cells to do cell division in tissues while high concentrations will inhibit cell growth so that over time the plant will experience death (Sutriana et al., 2012). ucrose plays an important role in supporting shoot life. Where sucrose is a component needed in the life of *N. mirabilis*. Sucrose as a carbon source plays a role in cell division, cell enlargement, and cell differentiation (Baskara et al., 2018). Environmental conditions such as temperature, humidity, light and oxygen are other supporting factors (Cryssanti et al., 2019). Purba (2021) stated that without the addition of BAP in the medium, explants can live well. This may be due to explant conditions, endogenous hormones, and lighting.Mahadi (2017) reported the same thing that the high percentage of live shoots was influenced by the explant conditions, the type and composition of the media, as well as the ZPT content given.

Normal shoots are light green. The green color of the shoots is possible due to absorption of light by the pigment chlorophyll a in large quantities, causing the color of the shoots to turn green. Another possibility is the formation of a light green color from the formation of chlorophyll from elemental nitrogen from the culture medium. The absorption of light by chlorophyll b in large quantities produces a yellowish green color in plants (Duca, 2015).

Shoots in the treatment without sucrose with a concentration of 3 ppm BAP showed a brownish color. The brown color is thought to be due to occurrence of senescence in plants. Senescence or aging is a process of decreasing the main pigment, namely chlorophyll in plants (Taiz et al., 2015). Waryastuti et al. (2017stated that at low concentrations of BAP it produces a yellowish-green color in plants.

Leaf initiation is characterized by the appearance of periclinal divisions in the outer surface layer and lower layer of the apical meristem. This division occurs continuously so that a bulge is formed. These protrusions undergo cell division and develop into leaf primordia (Srivastava, 2002 The number of leaves was affected by the concentration of BAP and the interaction of sucrose concentration with BAP concentration. Duncan's test results showed that the concentration of BAP 2 ppm gave a large number of leaves of 4.62. This is because the concentration is optimal in stimulating many cell division processes. A lot of cell division will affect the number of leaf primordia produced. The results of the Duncan test with a sucrose concentration of 20 g/l with a BAP concentration of 2 ppm resulted in a large number of leaves of 6.50. This was possible because the variation of sucrose concentration with BAP was optimal in stimulating leaf growth.

Triyastuti et al. (2018) stated that the concentration of 20 g/l was the optimal concentration for producing the highest number of leaves. Cryssanti et al. (2019) reported that low concentrations of BAP were able to produce a large number of leaves. Previaningrum et al. (2021) found that the use of PGR in low concentrations showed an increase in the number of leaves.

## CONCLUSION

Based on the research, it can be concluded that media with a sucrose concentration of 20 g/l produced the highest number of shoots, and media with concentrations of 3 and 2 ppm bap produced the highest number of shoots and leaves. Media with varying concentrations of sucrose 20 gl with 3 ppm bap and sucrose 20 gl with 2 ppm bap resulted in the highest growth in the number of shoots and number of leaves in the multiplication of *N. mirabilis.*

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