The Effect of Ms Media Concentration and Coconut Water Addition on Pitcher Plant *(Nepenthes mirabilis)* In Vitro Germination

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**Abstract:** Nepenthes propagation by tissue culture is influenced by several factors, one of which is the growth media. This study aims to determine the appropriate media for Nepenthes in vitro germination. The explant source was Nepenthes seed pod which was obtained from the forest of Uwe River Village, Paser Regency. The seed pod washed then dipped in 96% alcohol and burned in LAF. Then the Nepenthes seeds were planted into MS media (1/2, 1/4, 1/6, 1/8) combined with additional coconut water (100 ml, 200 ml). The results showed that the use of single 1/6 MS media resulted in the highest seed germination rate and the fastest germination time, which was 4 weeks after culture (WAC).

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**I. Introduction**

Nepenthes has a high enough economic value as it is developed as an exotic ornamental plant in Asia, Europe, America, and Australia. Of the 85 species that live in Indonesia, the largest habitat is found in Kalimantan and Sumatra, at least 27 species are threatened with extinction, 4 of which are species with critically endangered (critical) status and 4 others are endangered (threatened)\(^1\).

Conventional propagation that is mostly done is by using seeds, cuttings, and suckers splitting. Propagation of Nepenthes by seed has constraints on the length of time to germinate and the diversity of individuals due to segregation. The method of propagation through cuttings is limited from the number of books and a relatively long time to prepare mother plants that are ready to produce cuttings. Propagation by suckers splitting is limited by the small number of tillers formed as in *Nepenthes mirabilis*.

The application of tissue culture biotechnology or in vitro culture is the right solution to preserve and develop this plant. In vitro culture can be used for shoot propagation and plantlet formation quickly and in large quantities. Tissue culture is a plant propagation technique by isolating plant parts such as leaves, buds, and growing these parts in aseptic media so that they can become complete plants / planlets. The advantage of propagation by tissue culture is that it is able to produce the same plants as its parent and the resulting seeds are healthier or free of pathogens \(^3,4\). In vitro propagation of Nepenthes is able to produce large numbers of plants in a relatively short time to reduce the pressure of uptake in nature \(^5,6\).

The explant is the part of the plant to be cultured. Explant material can be in the form of organs, tissues, or cells. In principle, sterilization of explants is to sterilize from contamination by microorganisms, without killing the explants \(^7,8,9\). Explant sterilization plays a very important role in the success of plant tissue culture activities. In general, plants originating from their natural habitat in the field contain many contaminants in the form of dust, dirt, pests, diseases and various contaminants both on the surface and in the plant tissue. Explant sterilization activities aim to eliminate microorganisms that may be carried during the preparation of explants, which can cause contamination so that it will inhibit the growth of explants into intact plants. Especially in Indonesia, which has a tropical climate that allows contaminants such as bacteria and fungi to grow throughout the year \(^10,11,12\).

Various plant tissue culture studies that have been carried out on the optimization of explant sterilization have shown that chemical compounds such as fungicides, bactericides, 70% and 96% alcohol, spiritus, chlorox, sodium hypochlorite, HgCl₂, H₂O₂, detergents, antiseptic solutions such as betadine and tween can be used as a sterilant \(^13,14,15,16,17\).

The explant parts also plays an important role in the success of the culture. Explants in the form of young tissue are generally better differentiated. Apart from shoot meristems, other parts such as seeds, embryo, cotyledons, young petioles and young leaves are also selected for various reasons. Explant sources such as fruit...
parts and young tissues such as leaves and shoots are easier to sterilize considering that these parts are located far from the main contaminant source, namely soil. The interaction between the sterilization method and the explant source used in this study was based on the type of Nephentes seed pod. The types of seed pod and orchids are similar so they can use the same method as the orchid sterilization method as well as the explants used. Orchid’s pod is washed and then dipped in 70% alcohol, 96% alcohol or spirits and then burned in LAF.

Media in tissue culture propagation generally use MS media (Murashige Skoog). This medium contains macro and micro nutrients needed for plant growth. The addition of organic materials such as potato extract, corn extract, onion extract, coconut water is expected to save on the use of chemicals for MS media, replace substances for tissue culture and enrich the nutrient content in tissue culture media so as to encourage the growth of explants. The use of leaf explants of Echinacea purpurea (L.) on MS media was able to produce the highest flavonoid content. Media with 1/8 nitrogen modified is the best medium for the growth of N. ampullaria Jack shoots. MS media which was added with activated charcoal apparently supported the N. mirabilis seeds germination and supported the growth of plantlet height, plantlet weight, and growth of orchid shoots.

Additional growth regulators that are often added in tissue culture are cytokinins and auxin groups. The cytokinin group used in this study was coconut water. Coconut water is a natural ingredient that has cytokinin activity for cell division and encourages organ formation. Coconut water is good for growth regulators, namely young coconut water. The content of growth regulators in young coconut water is higher than in old coconut water because growth regulators tend to be produced in young tissues that are still actively dividing (meristems). The role of cytokinins usually works together with auxin to stimulate cell division and affect differentiation pathways. In addition, coconut water also contains sugar, lipids, amino acids, nitrogen compounds, organic acids and enzymes. These components encourage the growth of cultures so that the function of synthetic cytokinins can be replaced by coconut water. The concentration of coconut water commonly used in tissue culture is 2 - 20%. Application of coconut water as much as 10% was effective in in vitro shoot multiplication of Patchouli with a percentage of 100% live shoots. In vitro shoot propagation containing 15% coconut water produced an average of 4.6 shoots within 8 weeks after culture (WAC).

With the addition of 100 ml/l young coconut water, shoots appear faster in Raja Bulu banana cultures. Cattleya Orchid tissue culture media containing the addition of a combination of 50 ml/l carrot extract and 200 ml/l coconut water, showed an average increase in the number of shoots and plantlet height at 16 weeks after planting. Sorghum tissue culture using additional 200 ml/l coconut water was able to increase shoot height. The combination of MS media added with 10% coconut water was able to accelerate the emergence of leaves, shoots and pouches and was able to increase the highest number of leaves and roots in N. ventricosa tissue culture and the best results in Cymbidium orchid subculture activities.

The use of a half strength MS media with the addition of NAA and BAP was able to stimulate the growth of N. ampullaria, N. gracilis and N. mirabilis micro cuttings as well as stimulate the Lili germination (Lilium longiflorum). Another study using a half strenght MS media with the addition of auxin in tissue culture of N. mirabilis was able to increase the number of shoots in 10 weeks of culture and could induce shoots of N. adrianii. An increase in the number of leaves/explants, the number of pitcher/explant and the number of roots/explant of N. ventricosa was obtained in a half strenght MS media treatment.

Another study on tissue culture of N. rafflesiana using 1/8 MS media was able to increase the number of pitcher, number of roots and the percentage of live explants, and roots. The treatment of reducing nitrogen in 1/8 MS media turned out to be able to stimulate shoot growth, pitcher formation as well as the best morphological appearance and bag color in tissue culture of N. ampullaria. Hopefully this research can be used as a basis for propagation of Nephentes in East Kalimantan, especially in Paser Regency, as a way of conserving local plants. In addition, this research is also expected to be used by students who want to do their final research on plant propagation in vitro, especially Nephentes.

II. Material And Method

This research was conducted in February – June 2022 at the Plant Tissue Culture Laboratory, STIPER Muhammadiyah Tanah Grogot. The research is divided into 4 stages including:

1. Media preparation

   The treatment medium used was MS (Murashige Skoog) media (½, ¼, 1/6, 1/8) combined with additional coconut water (100 ml, 200 ml) added sucrose 30 g and agar 7 g. The media is cooked and put into a glass bottle as much as 20 ml and then covered with plastic. The media then put into an autoclave to be sterilized for 15 minutes at a temperature of 121 °C. After that, store (incubate) culture bottles on culture shelves 2-3 weeks before use to prevent media contaminants.
The Effect of Ms Media Concentration and Coconut Water Addition on Pitcher Plant...

2. **Explant preparation**
The explant source was Nephentes seed pod from the forest of Uwe River village. The pod used is ripe pod about 3-4 months old, dark green (brown) / red in coloured and has not been cracked.

3. **Explant sterilization**
Nephentes seed pod is washed gently by brushing the surface of the seed pod with soap to remove dust contamination. Then the pod is dipped in 96% alcohol and burned using a Bunsen fire in the LAF.

4. **Inoculation**
Before using the LAF chamber is sprayed with 70% alcohol and then dried using a tissue. Then turn on the UV lamp in LAF for 30 minutes. Culture tools like tweezers, scalpel, petri disc and culture bottles were sterilized with 70% alcohol. Each culture bottle contained 8-10 Nephentes seeds and carried out 5 replications. Observations were made for 4 months (16 weeks after culture). Observational variables included seed germination time and percentage of seed germination. This study used a completely randomized design consisting of 12 treatment levels with 5 replications to produce 60 experimental units (bottles). The treatments in the study were:

<table>
<thead>
<tr>
<th></th>
<th>Media</th>
<th>Time of Seed Germination (WAC)</th>
<th>Percentage Seed Germinated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>MS ½</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>K2</td>
<td>MS ¼</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>K3</td>
<td>MS ⅓</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>K4</td>
<td>MS ⅓ + CW 100 ml</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td>A1</td>
<td>MS ⅓ + CW 200 ml</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>A2</td>
<td>MS ⅓ + CW 100 ml</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>A3</td>
<td>MS ⅓ + CW 200 ml</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>A4</td>
<td>MS ⅓ + CW 100 ml</td>
<td>6</td>
<td>45</td>
</tr>
<tr>
<td>A5</td>
<td>MS ⅓ + CW 200 ml</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>A6</td>
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</tr>
<tr>
<td>A7</td>
<td>MS ⅓ + CW 200 ml</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>A8</td>
<td>MS ⅓ + CW 200 ml</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>

III. **Result**

Nephentes seed pod used as a source of explants by dipping it in 96% alcohol and burning it was able to reduce the amount of contamination in the culture. Of all the experimental units there was only 10% fungal contamination during the culture period.

The media treatment used with the addition of coconut water was not able to accelerate the time of seed germination and increase the percentage of the number of germinated seeds. Table 1 showed that MS ⅓ media without the addition of coconut water was able to speed up seed germination time and increase the percentage of the number of seeds that germinated. The percentage of plant development tends to be higher in media with a fairly low concentration. Similar results were also found in the study of the effect of low MS media on *Cymbidium* orchid subcultures. On the growth of Stevia plantlets, it was also found that the use of lower MS media was able to produce the most shoots and roots. The highest number of shoots and number of pitcher was obtained on media with 1/6 MS observed for 16-17 weeks of culture on *N. mirabilis* and *N. Khasiana* seeds.

Table 1 also showed there was a slight difference between media K3 and K4 in percentage seed germinated. This is thought to be related to the characteristics of the Nephentes plant which is able to adapt to a nutrient-poor environment. The formation of the pitcher as a way of survival to get additional nutrients that are not provided by the growing environment.

The culture observations were continued in period of 4 months (16 weeks after culture). This was done because in each culture media treatment there were still seeds that capable of germinating and grows well (not germinating at the same time) (Fig. 1B). This may be influenced by the ability of each seed to absorb nutrients from the media. There were several seeds in each treatment bottle that did not germinate (Fig. 1C) until the observation period end.
The Effect of Ms Media Concentration and Coconut Water Addition on Pitcher Plant...

IV. Conclusion
The best media to germinate Nephentes seeds is 1/6 MS media. It showed the highest fastest response to germinate, almost 80% seeds germinated in 4 weeks after culture. Adding coconut water did not able to boost the end results. There were few seeds that unable to germinated until the observation end.

Reference

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Figure 1 : Seed pod and pitcher of N. mirabilis (A); germinated seeds (B); contamination, ungerminated seed (C)