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

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Abstract: *Nephentes 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 Nephentes in vitro germination. The explant source was Nephentes seed pod which was obtained from the forest of Uwe River Village, Paser Regency. The pod washed then dipped in 96% alcohol and burned in LAF. Then the Nephentes seeds were planted into MS media (½ , ¼ , 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

Nephentes 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 endengered (critical) status and 4 others are endengered (threatened)[1].

Conventional propagation that is mostly done is by using seeds, cuttings, and suckers splitting. Propagation of Nephentes 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 [2,3]. In vitro propagation of Nephentes is able to produce large numbers of plants in a relatively short time to reduce the pressure of uptake in nature [3,4,5,6].

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, $HgCl_2$, H_2O_2, 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 [18,19,20,21,22,23,24].

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 [25,26].

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[27,28,29]. The use of leaf explants of *Echinaceae purpurea* (L.) on MS media was able to produce the highest flavonoid content [30]. Media with 1/8 nitrogen modified is the best medium for the growth of *N. ampullaria* Jack shoots [31]. MS media which was added with activated charcoal apparently supported the *N. mirabilia* seeds germination and supported the growth of plantlet height, plantlet weight, and growth of orchid shoots [32,33].

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 plantingS. 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 strenght 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*) [34,35]. 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* [3,36]. 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 [37]. 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* [31].

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 ^{0}C. After that, store (incubate) culture bottles on culture shelves 2-3 weeks before use to prevent media contaminants.

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:

K1 = MS ½
K2 = MS ¼
K3 = MS 1/6
K4 = MS 1/8
A1 = MS ½ + CW 100 ml
A2 = MS ½ + CW 200 ml
A3 = MS ¼ + CW 100 ml
A4 = MS ¼ + CW 200 ml
A5 = MS 1/6 + CW 100 ml
A6 = MS 1/6 + CW 200 ml
A7 = MS 1/8 + CW 100 ml
A8 = MS 1/8 + CW 200 ml

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 1/6 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 [37]. Similar results were also found in the study of the effect of low MS media on *Cymbidium* orchid subcultures [38]. 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 [39]. 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 [40,41].

Table 1 also showed there was a slight diffrence 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.

Tabel 1. The effect of media treatment on in vitro culture *N. mirabilis*

Media	time of seed germination (WAC)	percentage seed germinated (%)
K1	6	45
K2	6	50
K3	4	80
K4	5	75
A1	7	35
A2	7	30
A3	6	50
A4	6	45
A5	5	60
A6	5	40
A7	6	25
A8	7	15

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.

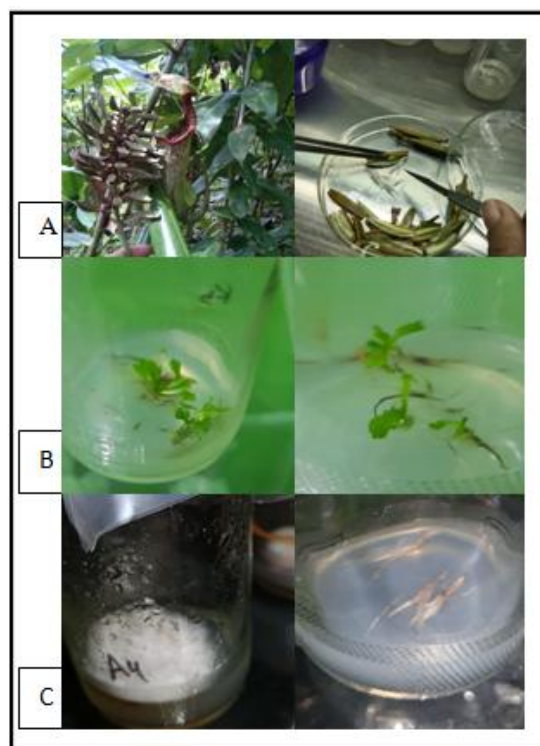

Figure 1 : Seed pod and pitcher of *N. mirabilis* (A); germinated seeds (B); contamination , ungerminated seed (C)

IV. Conclusion

The best media to germinate Nephentes seeds is 1/6 MS media. It showed the highest n 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

[1]. Hidayati, N. 2020. Kantong Semar, Tumbuhan Unik Pemakan Serangga. http://ksdae.menlhk.go.id/info/7502/kantong-semar,-tumbuhan-unik-pemakan-serangga.html. Diakses pada 20 Mei 2021.

[2]. Dinarty, D., Subekti, U., Alitalia, Y. 2009. Kultur Jaringan Nephentes (Nephentes mirabilis). Kumpulan Makalah seminar Ilmiah Perhorti.

[3]. Nuryadin, E., Sugiyono, Elly, P. 2017. Pengaruh Zat Pengatur Tumbuh Terhadap Multiplikasi Tunas Dan Bahan Penyangga Pada Pembentukan Plantlet Nephentes (Nephentes adrianii) Dengan Kultur In Vitro. Bioeksperimen 3(2): 31-45.

[4]. Sukamto, L.A. 2011. Pengaruh Eksplan Dan ZPT Terhadap Pertumbuhan Nepenthes albomarginata Secara In Vitro. J.Tek. Ling. 12 (1): 103 – 109.

[5]. Nuryadin, E. 2018. Pengaruh BAP dan NAA Terhadap Waktu Pertumbuhan Tanaman Nephentes(Nepenthes adrianii) Secara In-Vitro. BIOSFER, J.Bio. & Pend.Bio. 3 (1).

[6]. Rohayah, S. 2021. Pengaruh berbagai macam Media Murashige and Skoog (MS), Kombinasi Air Kelapa dan Naphthalene Acetic Acid (NAA) Terhadap Pertumbuhan Tunas Nephentes(Nepenthes ventricosa) Secara In Vitro. [Diploma Thesis]. Fakultas Sains dan Teknologi. Program Studi Pertanian. Agroteknologi. UIN Sunan Gunung Djati Bandung.

[7]. Previaningrum, H.A., Qadir, A., Isnaini, Y. 2021. Konservasi In Vitro Nephentes (Nepenthes rafflesiana Jack.) dengan Metode Slow Growth. J. Jejaring Matematika dan Sains 03 (01): 07-10.

[8]. Pancaningtyas, S. dan Ismayadi, C. 2011. Sterilisasi Ulang pada Perbanyakan Somatic Embryogenesis Kakao (Theobroma cacao L.) untuk Penyelamatan Embrio Terkontaminasi. Pelita Perkebunan 27 (1).

[9]. Sandra, E. 2013. Kultur Jaringan Skala Rumah Tangga. IPB Press. Bogor.

[10]. Isnaini, Y. 2015. Kultur Jaringan Nephentes (Nephentes spp) Di Kebun Raya LIPI Dan Pemanfaatannya Oleh Masyarakat. Warta Kebun Raya 13 (2).

[11]. Balitbiogen. 2003. Perbanyakan Bibit Jati Melalui Kultur Jaringan. Bogor: Balai Penelitian Bioteknologi dan Sumberdaya Genetik Pertanian.

[12]. Natasha, K. dan Restiani, R. 2019. Optimasi Sterilisasi Eksplan Pada Kultur In Vitro Ginseng Jawa (Talium paniculatum). Prosiding Symbion (Symposium on Biology Education), Prodi Pendidikan Biologi, FKIP, Universitas Ahmad Dahlan.

[13]. Surya, M.I. dan Ismaini, L. 2021. Perbandingan Metode Sterilisasi Untuk Perbanyakan Rubus rosifolius Secara In Vitro. Al-Kauniyah: J.Biologi: 14(1): 127-137.

[14]. Elfiani dan Jakoni. 2015. Sterilisasi Eksplan Dan Sub Kultur Anggrek, Sirih Merah Dan Krisan Pada Perbanyakan Tanaman Secara In Vitro. J. Dinamika Pertanian XXX (2): 117-124.

[15]. Yuliana, N. 2018. Optimasi Sterilisasi Eksplan Dan Pengaruhnya Terhadap Kestabilan In Vitro Tunas Vegetatif Tanaman Sirsak (Annona muricata L. Var Ratu). [Masters thesis]. Fakultas Biologi. Institut Teknologi Sepuluh Nopember.

[16]. Rahmawati, L. dan Lukmana, M. 2019. Pengaruh Lama Perendaman Sterilisasi Eksplan Daun Karet (Hevea brasiliensis) Secara In Vitro. Ziraa'ah 44 (3): 301-308.
[17]. Hamdani, S., Nugraha, D., Berliani, T., Baroroh, U. 2020. Teknik Sterilisasi Eksplan Tunas Kentang Granola Kembang (Solanum tuberosum L.) untuk Kultur In Vitro. J. Kartika Kimia 3 (2): 60-69.
[18]. Handayani, E., Isyadi, M.B., Aris, I., Alawiyah, R.L.M.N., Ayuningtias, N., Permatasari, F., Rineksane, I.A. 2021. Optimasi Sterilisasi Endosperma Kepel (Stelecthocarpus burahol [BI] Hook F. & Th). JBE 6 (2): 113-121.
[19]. Irawati. 2005. Pembentukan Kalus Dan Embriogenesis Kultur Pelepah Daun Dan Daun Caladium Hibrida. Berita Biologi 7 (5).
[20]. Sari, D.Y.I. 2009. Pengaruh Sumber Eksplan Dan Konsentrasi Zat Pengatur Tumbuh Terhadap Pembentukan Kalus Tanaman Gambir (Uncaria gambir Roxb.). Sainmatika 6 (1).
[21]. Anggraeni, T.D.A., Sulistyowati, E., Puewati, R.D. 2012. Pengaruh Komposisi Media Dan Sumber Eksplan Terhadap Induksi Kalus, Perkecambahan, Dan Pertumbuhan Tunas Embrio Somatik Jarak Pagar. Buletin Tanaman Tembakau, Serat, dan Minyak Industri 4 (2).
[22]. Sitinjak , M.A., Isda, M.N., Fatonah, S. 2015. Induksi Kalus Dari Eksplan Daun In Vitro Keladi Tikus (Typhonium sp.) Dengan Perlakuan 2,4-D Dan Kinetin. Al-Kauniyah J. Biologi 8 (1): 32-39.
[23]. Triana F. 2015. Induksi Kalus Pada Eksplan Daun Tanaman Binahong (Anredera cordifolia) Secara In Vitro Dengan Konsentrasi 2,4-D Dan BAP Yang Berbeda. [Skripsi]. Program Studi Pendidikan Biologi Fakultas Keguruan Dan Ilmu Pendidikan Universitas Muhammadiyah Surakarta.
[24]. Damanik, I.T.S., Rosmayati, Siregar, L.A.M. 2017. Pengaruh Jenis Eksplan dan Komposisi Zat Pengatur Tumbuh Terhadap Induksi Kalus Pada Tanaman Binahong (Anredera cordifolia (Ten.) Steenis). J. Agroteknologi FP USU 5 (3): 532-536.
[25]. Purba, R.V., Yuswanti, H., Astawa, I.N.G. 2017. Induksi Kalus Eksplan daun Tanaman Anggur (Vitis vinivera L.) dengan Aplikasi 2,4-D Secara In Vitro. E-Jurnal Agroekoteknologi Tropika 6 (2).
[26]. Widoretno, W dan Mastuti, R. 2019. Petunjuk Praktikum Kultur Jaringan Tumbuhan. Laboratorium Kultur Jaringan Tumbuhan Jurusan Biologi Fakultas MIPA Universitas Brawijaya Malang.
[27]. Dewanti, P., Wafa, A., Handoko, F., Sasmita, H.D. 2020. Buku Modul Pelatihan Anggrek Secara In Vitro. LP3DI Press. UPT Agrotechnopark Universitas Jember.
[28]. Karjadi, A.K. dan Buchory, A. 2008. Pengaruh Komposisi Media Dasar, Penambahan BAP, dan Pikloram terhadap Induksi Tunas Bawang Merah. J. Hort. 18 (1):1-9.
[29]. Nursetiadi, E. 2008. Kajian Macam Media Dan Konsentrasi BAP Terhadap Multiplikasi Tanaman Manggis (Garcinia mangostana L.) Secara In Vitro. [Skripsi]. Fakultas pertanian. Universitas Sebelas Maret. Surakarta.
[30]. Herawati, D., Mukarlina, Zakiah, Z. 2021. Multiplikasi Anggrek Dendrobium sp Dengan Penambahan Ekstrak Jagung Dan NAA Secara In Vitro. Bioma 6 (1): 38-47.
[31]. Pratomo, G.S. 2016. Pengaruh Jenis Media Dengan Hormon Tumbuh NAA-BAP Terhadap Pertumbuhan Dan Kandungan Flavonoid Kalus Daun Echinaceae purpurea (L.) Moench. J. Surya Medika Volume 1(2): 50-53.
[32]. Siregar, D.A. 2018. Modifikasi Konsentrasi Nitrogen Pada Medium MS (Murashige Skoog) Terhadap Pembentukan Kantong Nepenthes ampullaria Jack Secara In Vitro. J. Education and Development 6 (1): 137-140.
[33]. Jala, A. 2012. Types of Media for Seeds Germination and Effect of BA on Mass Propagation of Nepenthes mirabilis Druce. American Transaction on Engineering & Applied Sciences 1 (2).
[34]. Nasution, L.Z., Manurung, E.D., Hasibuan, M., Hardayani, M.A. 2021. Pengaruh Arang Aktif (Charcoal) pada Media MS untuk Meningkatkan Pertumbuhan Anggrek pada Kultur In Vitro. Prosiding Seminar Nasional. Fakultas Pertanian UNS 5 (1).
[35]. Deswiniyanti, N.W., Lestari, N.K.D., Astarini, I.A., Hardini, Y. 2020. Perbanyakan Lili (Lilium longiflora Thunb.) Secara In Vitro Dengan Zat Pengatur Tumbuh BAP dan NAA. J. Hort. Indonesia 11 (2): 131-139.
[36]. Novitasari, Y dan Isnaini Y. 2021. Propagation of Pitcher Plant (Nephentes gracilis KORTH and Nephentes reinwardtiana MIQ.) Through Callus Induction. AGRIC 33 (3): 81-92.
[37]. Pratama, J dan Nilahayati. 2018. Modifikasi Media MS Dengan Penambahan Air Kelapa Untuk Subkultur I Anggrek Cymbidium. J. Agrium 15 (2): 96-109.
[38]. Kunita, L.Y., Susiyanti, Isminingsih, S., Isnaini, Y. 2011. Pertumbuhan Tanaman Kantong Semar (Nephentes rafflesiana Jack.) Dengan Modifikasi Konsentrasi Media Dan pH Secara In Vitro. Jur. Agroekotek 3 (1): 24-33.
[39]. Asmono, S.L. dan Lestari, K.A. 2020. Respon Pertumbuhan Planlet Stevia (Stevia rebaudiana B.) Terhadap Penambahan Bahan Organik Pada Beberapa Konsentrasi Media MS. J. Penelitian Pertanian Terapan 20 (3): 177-182.
[40]. Khompat, K., Takhao, W., Jantasilp, A. 2007. Factors affecting in vitro seed germination and shoot multiplication of a pitcher plant (Nepenthes mirabilis (Lour.) Druce). Songklanaklarin J. of Science and Technology 29 (2).
[41]. Nongrum, I., Kumaria, S., Tandon, P. 2009. Multiplication Through In Vitro Seed Germination And Pitcher Development in Nepenthes khasiana Hook. f., a Unique Insectivorous Plant of India. J. of Horticultural Science and Biotechnology 84 (3): 329-332.

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