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Nephentes In Vitro Seedling on Low Ms Media Concentration

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Abstrak

Penerapan bioteknologi kultur jaringan (*in vitro*) merupakan salah satu cara untuk melestarikan dan mengembangkan tanaman kantong semar agar bahan tanaman dapat diproduksi dalam jumlah banyak dan dalam waktu singkat. Konsentrasi tinggi komponen nutrisi anorganik dan komprehensif dalam media MS mendorong pertumbuhan tanaman. Media MS dapat dimodifikasi hanya dengan mengaplikasikannya pada konsentrasi rendah pada beberapa tanaman untuk menghasilkan efek positif. Tujuan penelitian ini adalah untuk mengidentifikasi media semai N. mirabilis terbaik dengan konsentrasi MS rendah. Media semai yang digunakan adalah K1 (MS ½), K2 (1/4), K3 (1/6), K4 (1/8), dan K5 (1/5). Hasil penelitian menunjukkan bahwa K3 (media MS 1/6) merupakan media semai terbaik dan memberikan pengaruh nyata terhadap seluruh parameter penelitian antara lain jumlah benih yang berkecambah, jumlah eksplan hidup, dan jumlah eksplan yang dilengkapi kantong semar.

Kata Kunci: In vitro, Konsentrasi rendah, MS media, Nephentes, Semai

Abstract

The application of tissue culture biotechnology (in vitro) is one way to preserve and develop pitcher plants so that plant material can be produced in large quantities and in a short time. High concentrations of inorganic and comprehensive nutritional components in MS media promote plant growth. MS media can be modified by simply applying it at low concentrations in some plants to yield positive effects. The objective of this study was to identify the best N. mirabilis seedling media using a low MS concentration. The seedling media were K1 (MS ½), K2 (1/4), K3 (1/6), K4 (1/8), and K5 (1/5). The results showed that K3 (MS 1/6 media) was the best seedling media and had a significant impact on all research parameters, including the number of germinated seeds, the number of live explants, and the number of explants with pitcher.

Keywords: In vitro, Low concentration, MS media, Nephentes, Seedling,

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INTRODUCTION

Nephentes has substantial commercial value because it is planted as an exotic decorative plant in Asia, Europe, America, and Australia. The bulk of the habitat for the 85 species that call Indonesia home is found in Kalimantan and Sumatra. Four of these species are considered severely endangered (critical), and the remaining four are considered endangered (threatened). At least 27 of these species are at risk of becoming extinct. Conventional propagation techniques that are most frequently used include sucker splitting, cuttings, and seeds. Nephentes propagation by seed has restrictions on the number of individuals and the time it takes for them to germinate because of segregation. Due to the scarcity of planting material, it is challenging to acquire plants and cultivate them in large quantities. Biotechnology-based seed planting techniques, including tissue culture cultivation, must be taken into consideration in order to generate planting material rapidly and in large quantities (Siregar, 2017). One stage of shoot multiplication or propagation involves in vitro techniques. By inducing a callus or directly stimulating shoot growth, micropropagation, also known as shoot growth stimulation or multiplication, increases the potential of plants in vitro (Arti & Mukarlina, 2017).

This method uses aseptic and controlled conditions to grow and reproduce plant cells, tissues, and organs in solid or liquid media. Plant tissue culture, or micropropagation, is the term for this. In vitro propagation techniques offer numerous benefits over traditional approaches. Among these are the fact that this method uses less planting material or explants than traditional methods, that it can be completed quickly in a limited space (a tissue culture room), that the plant results are disease and pathogen free, that the results are uniform and similar to its parent (a clone), that it is simpler and requires less transportation because only culture bottles need to be sent, and that production speed can be changed to meet market demand. (Purita et al., 2017).

Culture media is one type of media that can be used in tissue culture procedures; factors that affect. One of them, Murahige and Skoog 1962 (MS), is good for plant growth because it contains a lot of complete nutrients and inorganic salts. (Pratama & Nilahayati, 2018). Aseptic plant propagation is performed by tissue culture, which allows the sterilization step to eliminate contamination (Almeida et al., 2020).

The best outcomes can be obtained by using MS media in different plant tissue cultures. It turns out that simply using less composition, MS media can be altered. Numerous studies employing those alteration. Using MS media at 25%–50%, some

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findings have been able to guarantee good growth. The maximum flavonoid concentration was achieved using Echinaceae purpurea (L.) leaf explants grown in MS ½ media. The ideal media for Nepenthes ampullaria shoot growth is 1/8 nitrogen-modified media (0.553 g.l-1). MS 1/2 media demonstrate increased plantlet weight, growth, and number of orchid shoots (Nasution et al., 2021). The highest average growth was observed in the parameters of plant height, number of shoots, length of shoots, number of leaves, number of roots, and length of roots in potato plant tissue culture using MS ½ media (Setiawati et al., 2018; Putri et al., 2021). The media with half strength of Murashige and Skoog (MS ½) had the highest average number of pitchers planted (Isnaini & Novitasari, 2023).

Considering plant height, number of roots, and number of shoots, modified MS media up to MS ¼ remains suitable for growing potato explants. The MS 1/8 media effectively adjusts the nitrogen content throughout the developmental phases of N. mirabilis shoots. Plantlet weight, plantlet height, and growth in orchid shoots were facilitated by half-strength MS media supplemented with activated charcoal. Compared with full-strength MS media, the growth presentation, quantity, and height of Cymbidium orchid shoots were increased on MS ¼ and MS ½ media. According to another study, N. mirabilis plants with a single media MS 1/6 (0.738 g.l-1) had the greatest amount of seed germination and the quickest germination period, occurring 4 weeks after culture (Arsela, 2022; Fatonah et al., 2016; Munarti & Kurniasih, 2014; D. A. Siregar, 2017; Sitorus et al., 2012; Yusnita, 2015) . Changing the nitrogen concentration in MS 1/8 media is suitable for the growth of N. mirabilis shoots (Siregar, 2017).

In tissue culture, sugar plays a very important role in plant tissue culture. In the context of tissue culture, sugar serves as the primary energy source and an important building block for the growth and development of plant explants. Some key benefits of sugar in plant tissue culture: In vitro, explants usually cannot perform photosynthesis effectively because they are in controlled in vitro conditions. Plants need light for photosynthesis, but in tissue culture, they are usually grown in sterile conditions and often in bottles or closed containers that limit access to natural light. Sugar in culture media also acts as an osmotic regulator, helping to regulate osmotic pressure within the cells and in the surrounding environment. Sugar also plays a role in the processes of differentiation and morphogenesis, where plant cells undergo specialization to form new structures and organs.

In addition to the main functions mentioned above, sugar can also aid in cellular protection and adaptation to various stress conditions. The addition of sugar to the culture

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media can also affect the pH of the media, which is important for the growth and development of explants. To put it another way, 20–30 g.l-1 of sugar is added to the culture media as an energy source. To solidify the culture media, a media compactor, also known as agar, is added in an 8 g.l-1 culture media (Apriliani, 2021). This study provides insights into the use of reduced MS media concentrations to optimize the in vitro propagation of Nephentes, which can be applied to other plant species as well. PPM (Plant Preservative Mixture) is an exceptionally broad-spectrum biocide that effectively lowers the amount of microbial contamination in vitro.

Although most studies on Nephentes pitcher plant propagation have been conducted, investigations into in vitro culture are still necessary, particularly in Paser Regency. Thus, more studies are required to produce a modified media with low concentration of MS media.

METHODS

This study was conducted at the Tissue Culture Laboratory, Faculty of Agriculture and Digital Business, Universitas Muhammadiyah Kalimantan Timur, Paser Campus. The study was conducted from June to July 2023.

Culture Media

Base media containing low concentrations of MS media are K1 (MS ½), K2 (1/4), K3 (1/6), K4 (1/8), and K5 (1/5). Added 30 g of sucrose and 7 g of agar and 20 cc.l-1 of PPM to the media. The media was then heated, poured into a 20 ml culture bottle, and sealed with plastic. Next, the media were autoclaved for 10 minutes at 121 psi pressure. To avoid media contamination, store (incubate) culture bottles on culture shelves for 2–3 weeks before use.

LAFC and culture instruments sterilization

Laminar Air Flow (LAF) cleaning involves scrubbing the surface with 70% alcohol and then patting it dry with tissue. Set up sterile culture supplies in the LAF, including tissue, plastic rubber, dissection tools, bunsen burner, 95% alcohol glasses, petridishes, and clean seed pod explants can be sterilized by washing them with dish soap and scrub the surface to eliminate any residual contaminants. After that, rinse the pods under running water until it no longer smells like soap or foams. Apply a 75% alcohol spray to both hands while wearing a lab coat and mask before beginning any LAF activity. Soak the dissection instrument in 95% alcohol to prepare it.

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Seed preparation

Nephentes seed pods harvested from Desa Sungai Uwe were used as the explant source. The ripe pod that is used is crimson or dark green in color, approximately 3–4 months old, and has not cracked. Each pods contain more than 30 seeds. This research used a stalked panicle with 20 seed pods.

Seed sterilization

In order to eliminate dust contamination, the seed pod is gently cleaned by scrubbing the surface with soap, rinsed and dry it. Subsequently, the pod is submerged in 96% alcohol and ignited with a Bunsen flame in Laminar Air Flow Cabinet (LAFC). After the flame goes out, started to disect the pod by using blade and tweezer. The tweezer helps to extract the seeds. Place all the seeds in a petridish. Plant the seeds explant section into the media bottle that has been prepared, seal the bottle's mouth with bunsen burner, and then cover it with the plastic cover again. The explants that have been planted in the culture media are arranged in the culture room with controlled temperature and lighting. Typically, the temperature in the culture room is maintained between 21°C and 25°C, with lighting adjusted to simulate optimal natural conditions for the growth of Nephentes.

Data analysis

This study was conducted 10 times using a non-factorial completely randomized design (CRD) with five treatments and 10 replications. The data obtained were analyzed using analysis of variance (Anova) to determine the statistical significance of the observed results. The Duncan Multiple Range Test (DMRT) is used to compare the means among treatments when Anova indicates a significant difference. Statistical analysis helps determine which treatment has the most significant effect on the growth of Nephentes seedling. Weekly observations were conducted until 4 weeks after inoculation (WAI). The following parameters were noted: the number of germinated seeds, the number of live explants, and the number of explants with pitcher.

RESULTS AND DISCUSSION

Observations conducted up to 4 WAI showed that all cultures could grow well and form pitchers in any media. The number of germinated seeds, the number of live explants, and the number of explants with pitchers were positively impacted by all media treatments. There were differences in all experimental parameters according to the statistical test results (Table 1 and Table 2).

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Tabel 1. Analysis of Variance (ANOVA) per Treatment on Observed Parameters

Treatment	Germinated seeds	Explants with pitcher	Live explants
	*	*	*
K1 (MS ½)	c	c	b
K2 (MS ¼)	cd	cd	a
K3 (MS 1/6)	d	cd	c
K4 (MS 1/8)	a	a	a
K5 (MS 1/5)	b	b	ab
CV	0,14	0,14	0,14

Note: Using the DMRT test, the numbers that follow the same letter in each column are not substantially different at the 5% level; ns = no significant differences; * = significantly different at the 5% level; CV= coefficient of variation

Tabel 2. The Effect of Modified *In Vitro* Media on Observed Parameters

Treatment	Germinated seeds	Explants with pitcher	Live explants
	*	*	*
K1 (MS ½)	8,05c	7,9c	7,9b
K2 (MS ¼)	8,15cd	8,1cd	8,15a
K3 (MS 1/6)	**8,3d**	**8,65cd**	**8,35c**
K4 (MS 1/8)	7,5a	7,75a	8,05a
K5 (MS 1/5)	7,85b	7,8b	7,8ab
CV	0,14	0,14	0,14

Note: The numbers with the same letter in each column are not significantly different at the 5% level using the DMRT test

Numerous investigations were carried out to generate pitchers in Nepenthes. According to the addition, an initial investigation revealed that the number of pitchers, number of roots, percentage of live plantlets, percentage of plantlet pitchers, and percentage of rooted plantlets in N. rafflesiana were all best influenced by the media concentration of MS 1/8 (Isnaini & Novitasari, 2023). A further study on the development of pitchers in N. ampullaria and N. mirabilis, which do better on MS ½ media (Yelli, 2013). According to a different study in N. ampullaria cultivated on MS media devoid of nitrogen produced the greatest number of pitchers (Siregar, 2018).

A higher concentration of salt in the MS 1/2 media can create less than ideal osmotic conditions for the explants, especially for specific plants. Excessive mineral salts can cause osmotic stress, reduce water uptake by explant cells, and ultimately inhibit growth. In some cases, excessively high concentrations of minerals can lead to toxicity that directly damages plant tissues.

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Although the salt concentration is lower than MS 1/2, MS 1/4 may still have sufficient mineral content to induce some level of stress on the explants. This condition may be better than M1, but it is not optimal enough to support maximum growth. This indicates that pitcher plant may be more tolerant of media with lower nutrients. The MS 1/6 media provides a more balanced nutritional condition for the explants. The lower salt concentration may reduce the risk of toxicity and osmotic stress, as well as allow for more efficient absorption of water and nutrients. In the context of pitcher plants, which may naturally prefer media conditions with more moderate or lower nutrients, MS 1/6 media provides an optimal environment for in vitro growth.

According to the Anova results, the relationship between germinated seed with leaf and pitcher production. In N. gracilis, the protein profile of three distinct phases of plant development: young leaves, leaves with developing leaves, and developing leaves. It was discovered that the protein profiles of the pitcher development phases are comparable. However, compared to other processes, the developed stages have a low protein content. This study suggests a potential connection between pitcher production and the gene expressed in the leaves.

Explant tissue contains a phenolic substance that inhibits plant growth because it regulates the transfer of auxin and other phytohormones. Through its inhibition of IAA oxidase and prevention of polar transport, the phenolic molecule regulates the auxin concentration gradient. Owing to this control, the phenolic molecule has the ability to interfere with plant growth and lower plant growth rates.

The media composition (K3) yields the best response across all observed parameters, according to 5% level DMRT test results (Table 1). The highest seed germination and fastest germination time, which is 4 WAI on N. mirabilis, are produced by a single media MS 1/6, according to other studies. The results demonstrated that media MS 1/6 was the best media for N. mirabilis in terms of plantlet height growth, chlorophyll content, and stomata index. After 16–17 weeks of culture on N. mirabilis and N. khasiana seeds, the media containing MS 1/6 produced the greatest number of shoots and pitchers. The variables of number of pitchers and number of roots were significant for concentration media 1/8 MS in N. rafflesiana Jack culture. The ability of the Nephentes plant to adapt to a nutrient-poor environment is suggested to be connected to this. The pitcher forms in an attempt to survive and obtain extra nutrients that the growing environment is unable to supply (Arfa, 2018; Arsela, 2022; Kunita et al., 2011).

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Table 2 displays the highest number of pitcher formations on media MS 1/6. Nepenthes can be found in exceptionally nutrient-poor environments, such as Desa Sungai Uwe. The species forms a pitcher with a jug-like shape to attract, trap, store, and digest its prey in order to survive (Figure 1). As a result, the findings prove that the results are consistent with what occurred in the natural environment and are similar to in vitro cultures. Findings from other investigation on how low MS media affected Cymbidium orchid subcultures were also discovered. By using lower MS media was able to yield the greatest number of roots and shoots on the growth of Stevia plantlets (Asmono & Lestari, 2020). The use of MS media with lower concentrations, such as MS 1/6, can increase cost efficiency and reduce nutrient toxicity. This is important because excessively high concentrations of mineral salts in the culture media can cause stress to the plants, inhibit growth, or even lead to cell death. The MS 1/6 media, with its lower concentration, provides a more conducive environment for the growth of Nephentes pithcer plant, especially under in vitro conditions..

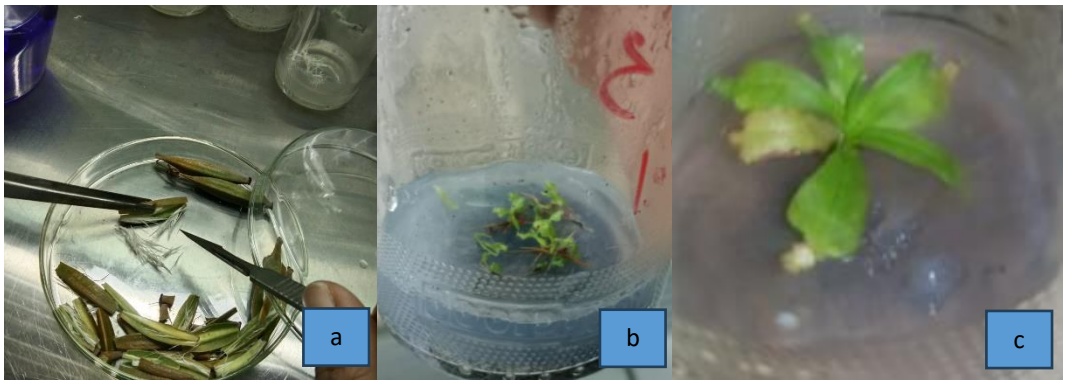

Figure 1. Seed initiation (a), explant in media MS 1/6 (K3) (b), explant with pitcher (c).

CONCLUSION

The use of a lower MS media concentration (MS 1/6) was able to provide the best growth response, to support plant growth consistent with what occurred in the natural environment and similar to in vitro cultures. The study successfully identified an optimal combination of MS media concentration that led to the best growth response of Nephentes seed explants in vitro, which can contribute to the development of efficient micropropagation protocols for this crop

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