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Optimization of Surface Sterilization and Organogenesis Protocol for Monstera spp.

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Abstrak

In vitro micropropagation provides faster and more economical solutions to increase world ornamental plant demands. The present study focuses on surface sterilization and multiple shoot induction of Monstera adansonii and M. deliciosa. Combination of sterilant agent such as 70% ethanol (1 min) + 10% NaClO (5 min) + 5% NaClO (5 min) + 2% NaClO (5 min) has highest M. deliciosa (80.53%) and M. adansonii (65.77%) culture viability. Meanwhile, the highest callus formation on M. deliciosa was achieved in Murashige and Skoog medium supplemented with 0.5 ppm BAP + 0.1 ppm TDZ. Taken all together, this study achieved a sterilization protocol and callus induction of M. deliciosa and could be considered for large-scale multiplication. In addition, serve as a base for further detailed analysis of organogenesis induction for both Monstera species

Keywords: micropropagation, Monstera, organogenesissurface sterilization

1. Introduction

Ornamental plants are an important part of agriculture and horticulture and have great significance for beautifying and improving humans' living environment, cultivating human sentiment, and promoting structural adjustments in the industry (Zheng et al. 2021). Ornamental plants such as Monstera adansonii and M. deliciosa, are the most beloved and popular products in the fast-changing horticulture industry. International trade, urbanization, climate change, sustainability-oriented consumption patterns, and the COVID-19 pandemic have increased the supply and demand of plants (Benderradji et al. 2012; Gabellini et al. 2022)

Monstera spp. conventional propagation methods such as seed planting, nodes, and shoot cutting, could not keep up with all the demands as they produced a low number of products in a short time and large losses due to pests, pathogen which could cause disease (Bettaieb 2018; Carvalho et al. 2019). As the solution, in vitro micropropagation can increase plants' mass production on a large scale in a short time (Kumari et al. 2017). The advantages of micropropagations are high fecundity, often producing more robust plants, and space efficiency compared to a conventional technique (Roberts and Schum 2003; Cardoso et al. 2018).

In vitro micropropagation of Monstera spp. through seedlings (Fonnesbech and Fonnesbech 1980), lateral buds and nodes (Zanca and Zaffari 2013), leaf and disk stem (Palomeque et al. 2019) have also been investigated. Most of the studies were concerned with surface sterilization protocols, browning on the explants, and the effect of various plant growth hormones (PGRs) on shoot multiplication efficiency. A simple and efficient protocol for ornamental plants is needed to increase the supply of the demands. Furthermore, there are no experiments on, M. adansonii and M. deliciosa successful organogenesis.

This study aimed to determine effective surface sterilization protocols by using different sterilizing agents, the response of different types of Monstera spp. explants to various culture media components including lamina, petiole, and midribs, test the effects of PGRs including cytokinins (6-benzylaminopurine, thidiazuron) on organogenesis of Monstera spp. explants,

and to develop a procedure for mass production for ornamental plants needed to increase the supply of the demands.

2. Methods

This research was done at the Plant Tissue Culture Laboratorium, Faculty of Biotechnology, the Atma Jaya Catholic University of Indonesia from 2021 to 2022. The materials needed were Monstera adansonii and M. deliciosa plants, sterilant agents, and PGR. There were several stages of methods, including plant preparation, surface sterilization, organogenesis induction, explant growth measurement, and data analysis.

2.1. Plant Preparation

Monstera adansonii, and M. deliciosa were collected Larch Studio Nursery (Kemang, Jakarta). Stock plants were then placed in Green House (Atma Jaya University, BSD, Tangerang) and sprayed with Bactocyn (2%) every two days for three weeks. Monstera spp. explants (1 cm midribs) were used as experimental material.

2.2. Surface Sterilization

Monstera spp. explants were sterilized according to (Palomeque et al. 2019) with modifications (Table 1). Explants were washed in commercial liquid soap and rinsed in running water for 20-30 minutes then sterilized with sterilizing agents such as 70% ethanol and commercial sodium hypochlorite (NaClO) Explant was then rinsed in sterilized distilled water four times for 5 minutes each, placed on sterilized blotting paper, and finally placed on an organogenesis medium. To evaluate optimum surface sterilization, explants were observed every day. The number of sterile explants, i.e., those plants which were without bacterial, yeast, or fungal contamination and still viable, was recorded after 8 weeks (Daud et al. 2012). The data was then compared in each method by using statistical analysis.

2.3. Organogenesis Induction

Monstera spp. were planted in organogenesis induction media according to Imelda et al. (2007) with modification. A control medium (MS0) consisted of 4.43 g/L Murashige and Skoog (1962) (MS) instant medium, 30 g/L sucrose, and 10 g/L agar. To induct organogenesis on Monstera spp., the explant was placed in MS0 supplemented with various concentrations of PGR (Table 2). Growth medium then pH adjusted to 5.7 before autoclaving at 121°C and 15 psi for 15 minutes. After sterilization, explants were then cultured organogenesis medium. Monstera spp. were set in dark conditions for indirect organogenesis for 4-8 weeks. Explants were then subcultured every 3 months into the same medium.

2.4. Monstera sp. Callus Growth Measurement

All data were expressed as mean ± standard error. After 6 weeks, Monstera spp. callus growth area was quantified with the ImageJ application (Schneider et al. 2012) from photos taken on the sterile Petri dishes, including a ruler for measurement and black colored paper. The percentage of Monstera sp. callus area was then calculated using the formula below

$$\%\ Callus\ area = \frac{callus\ area}{leaf\ area} \times 100\%$$

2.5. Data Analysis

The data were analyzed using IBM SPSS Statistics (SPSS Inc. 2008). Normality and homogeneity of the data were then analyzed with Kolmogorov-Smirnov and Levene's Test respectively. Then if the data did not meet the assumptions of parametric tests (i.e., ANOVA, Independent T-Test), non-parametric tests were used (i.e., Kruskal–Wallis). If the data met the assumptions of the ANOVA test, then they were used followed post-hoc by Tukey's test at the 5% probability level.

3. Result and Discussion

3.1. Gambar dan Tabel

Table 1 Standardization of surface sterilization treatments for *Monstera* spp.

Explants' species	Method	Sterilization agent	Duration	Explant	Oxidation	Contamination	Culture viability	p value of culture viability
Monstera deliciosa	3	70% ethanol + 20% NaClO + 10% NaClO + 2% NaClO	1 min +5 min + 5 min + 5 min	Midrib	3.33 ± 0.88	1.67 ± 0.33	9.00 ± 0.58a	0.00
	4	70% ethanol + 10% NaClO + 5% NaClO + 2% NaClO	1 min +10 min + 10 min + 10 min	Midrib	0.67 ± 0.33	6.67 ± 0.88	37.67 ± 0.67b	
Monstera adansonii	3	70% ethanol + 20% NaClO + 10% NaClO + 2% NaClO	1 min +5 min + 5 min + 5 min	Midrib	1.67 ± 0.88	7 ± 1.15	3.00 ± 1.15a	0.00
	4	70% ethanol + 10% NaClO + 5% NaClO + 2% NaClO	1 min +10 min + 10 min + 10 min	Midrib	15.67 ± 2.33	8 ± 0.58	24.33 ± 0.88b	

All data were expressed as mean ± standard error. Different lowercase letters in each column indicate significant differences among different treatments

Table 2 Effects of TDZ and BAP on callus development of *Monstera deliciosa*

Media	Callus Area %
T0.1B0.25	11.39 ± 2.18a
T0.1B0.5	17.96 ± 3.07a
T0.1B1.0	13.99 ± 0.70a
T0.2B0.25	10.56 ± 1.36a
T0.2B0.5	8.55 ± 0.00a
T0.2B1.0	16.88 ± 1.98a
T0.3B02.5	14.33 ± 3.46a
T0.3B0.5	17.16 ± 0.06a
T0.3B1.0	15.59 ± 2.51a

All data were expressed as mean ± standard error. Different lowercase letters in each column indicate significant differences among different treatments

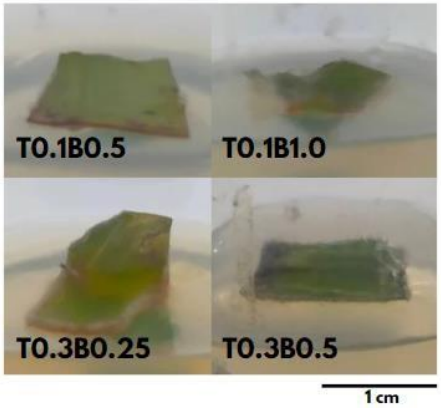

Figure 1 *Monstera adansonii* callus growth after 6 weeks

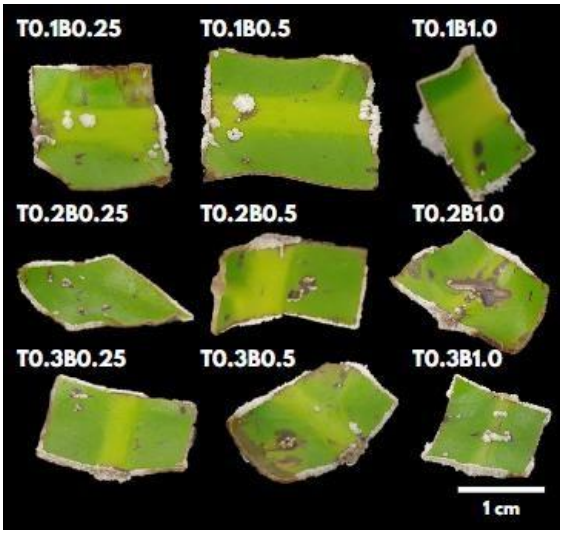

Figure 2 *Monstera deliciosa* callus growth after 6 weeks

3.2 Surface Sterilization of Monstera spp.

Monstera deliciosa surface culture on midrib explant sterilization in method 4 (70% ethanol 1 min + 10% NaClO 10 min + 5% NaClO 10 min + 2% NaClO 10 min) achieved high viability percentages (80.53%) witha low percentage of oxidation (1.77%) even when the explant was washed for a longer duration. Method 3 has higher oxidation rate (23.81%) and produced low amounts of viability (64.29%) (Table 1).

Similar to M. deliciosa, M. adansonii midrib explant surface culture sterilization using method 4 achieved highest viability percentages (65.77%). Although in lower NaClO percentages, the explant still oxidized in higher percentages (23.81%) compared to Method 3. Method 3 has a higher contamination rate (70%) and produced low amounts of viability (13.33%).

Both Monstera surface sterilization, a lower concentration of NaClO and longer duration of exposure resulted in higher explant viability. High contamination on the explant may be avoided by using different types, higher concentrations, and longer exposure to sterilization agents (Singh 2018). This method also resulted low oxidation compared to previous method by Palomeque et al. (2019) without using antioxidant. NaClO sterilization agent is effective against contamination but in higher concentrations could induce browning or blackening in explants. Due to their strong oxidizing properties, NaClO is extremely reactive with nucleic

acids, amines, and amino acids. (Mihaljević et al. 2013). Oxidation of phenolic compounds present in the tissues when excised and exposed to sterilant may induced browning on the explant (Prosanta et al. 2015; Permata and Susandarini 2022).

3.3 Organogenesis Induction of Monstera spp.

After 6 weeks, M. adansonii did not produced callus as the explant did not response to PGRs in the media (Figure 1). Monstera deliciosa callus growth was observed through white callus proliferation on the surface and/or from within the explant (Figure 2). The callus growth was friable and did not produce shoot nor roots. Better callus growth responses (17.51% and 17.18%) were observed in cultures initiated from T0.2B0.25 and T0.2B1.0 media respectively (Table 5).

Organogenesis media of M. deliciosa contained BAP and TDZ. M. deliciosa callus growth is at its lowest in media with a low concentration of BAP and TDZ. At high BAP and TDZ concentrations, callus growth may be inhibited. On M. adansonii callus growth, explants became yellow after callus induction which indicated death on plant tissue. Low callus production was caused by less endogenous auxin existing inside of the explant. The induction of callus is stimulated by the intermediate auxin/cytokinin ratio. Low callus production was caused by less endogenous auxin existing inside of the explant. The intermediate ratio of auxin and cytokinin promotes callus induction (Ikeuchi et al. 2013).

In this research, combination of BAP and TDZ is not suitable for inducing callus for Monstera spp. micropropagation. Even more, shoot produced during the organogenesis induction using midrib explant. Therefore, the combination of PGRs to induce organogenesis on M. deliciosa still need to be explored.

Organogenesis induction been successfully done by Palomeque et al. (2019) but with different species of Monstera that has been used in this study. The M. acuminata plant buds' explant was cut into 1 mm thick disk. The explants were then planted in MS medium supplemented with BAP 1 mg/L + IAA 0.5 mg/ L + NAA 0.1 mg/L. After 35 days of organogenesis induction, there were white callus friable callus growth in explant and shoot growth was observed after 45 days of induction.

4. Conclusions

In the present study, highlighted M. deliciosa and M. adansonii sterilization by using 70% ethanol 1 min + 10% NaClO 10 min + 5% NaClO 10 min + 2% NaClO 10 min was produced explants with a high percentage of viability (80.53% and 65.77% respectively) with a low percentage of oxidation. Organogenesis micropropagation on 0.5 ppm BAP + 0.1 ppm TDZ could induce callus in M. deliciosa midrib explant, however this combination of PGR was not suitable to inducing organogenesis. This work could serve as a base for further detailed analysis of M. adansonii and M. deliciosa surface sterilization and optimum organogenesis.

Citation

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