In vitro culture of leaf explant *Melastoma malabathricum* L. on Murashige & Skoog (1962) modified medium with thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA)

R Nauli, R Yuniati and W Handayani

Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: windri.h@sci.ui.ac.id

Abstract. *Melastoma malabathricum* L. is a plant potentially useful in phytoremediation, and its successful propagation would facilitate further research in this application. The present study examined in vitro culture of leaf explants using MS medium supplemented with thidiazuron (TDZ; 0, 0.1, 1 and 2 mg/L) and 1-naphthaleneacetic acid (NAA; 0, 0.1 and 1 mg/L) alone and in combination. Leaf explants responded to all treatments by forming callus, except on medium with a combination of 2 mg mg/L TDZ and 1 mg/L NAA. The callus formed tended to be friable-compact and compact in texture, and browning tended to occur in callus formed on media containing NAA alone. The medium containing 1 mg mg/L NAA and 0.1 mg/L TDZ gave the best results for callus formation (100 %). Callus formed faster on medium containing 1 mg/L NAA alone. Both callus and adventitious roots formed on media containing NAA alone, with 1 mg/L NAA giving the best results. One explant growing on the medium containing 2 mg/L TDZ and 0.1 mg/L NAA formed both callus and shoots.

Keywords: *Melastoma malabathricum* L., NAA, TDZ, callus, adventitious shoot, adventitious root

1. Introduction

*Melastoma malabathricum* L. is a shrub that is commonly used in traditional medicine to cure certain diseases [1] and can also have ability for phytoremediation application [2]. In order to support further research for their potential, a satisfactory propagation method is needed, and this can be achieved using in vitro culture techniques. In vitro plant propagation (micropropagation) can rapidly provide plants in large quantities [3]. This method has been used in some Melastomataceae via adventitious shoot induction from leaf explants of *M. affine* [4], *Tigridiopalma magnifica* [5] and *M. malabathricum* [6].

The use of a single cytokinin, such as thidiazuron (TDZ), has been proved to induce the formation of adventitious shoots in leaf explants of *M. affine* [4] and *T. magnifica* [5]. Ghimire et al. [6] used cytokinin combined with the 1-Naphthaleneacetic Acid (NAA) as auxin to induce the formation of adventitious shoots on leaf explants of *M. malabathricum*. Based on study by Ghimire et al [6], the use of Murashige & Skoog (1962) (MS) medium containing a combination of TDZ (0.1, 1 and 2 mg/L) and NAA (0.1, 1 and 2 mg/L) can induce the formation of adventitious shoots on leaf explants of *M. malabathricum*. Therefore, this present study aimed to investigate the response from leaf explants of...
2. Materials and method

2.1. Culture media and plant materials
In total, 12 treatment media were used in this study based on Murashige & Skoog (1962) (MS) modified medium [7] containing 30 g/L commercial sugar, 8 g/L agar, and varying concentrations of TDZ (0, 0.1, 1, and 2 mg/L) and NAA (0, 0.1 and 1 mg/L) (table 1). Prior to use, pH was adjusted to 5.6–5.8 and the media were autoclaved for 15 min at 121 °C. The donor plant for explant was 16-month-old *M. malabathricum* plants grown via in vitro culture of seed that were free from contamination.

2.2. Explant culture
The second and the third leaves (length 5 ± 3 cm) from shoot buds of donor plants were used as explants. Leaves were excised aseptically from donor plants and cut into 0.5 × 0.5 cm segments with incision on the adaxial surface. Each explant was inoculated into a culture bottle containing 10 mL of treatment medium. Each treatment consisted of 20 replicates. The explants then cultured with the abaxial surface in contact with the treatment medium. After that, cultures were incubated at 24 ± 2 °C under a 16/8h light/dark photoperiod for 60 days.

3. Results and discussion

Figure 1 showed that the explants responded to treatment media M1–M11 by forming callus to some extent, whereas the combination of 2 mg/L TDZ and 1 mg/L NAA (treatment M12) produced no response. The fact that callus formed in treatment M1 [MS medium without plant growth regulators (PGRs)] indicates that the concentration of endogenous hormones in the explants was enough to stimulate explant cell division and callus formation [8, 9]. Callus formation in treatments M2–M11 were likely related to the role of the exogenous cytokinin and auxin in triggering cell division and proliferation [8, 10].

It was observed that callus formed at the wounded part of the explants. The presence of wounds on the explants may increase the absorption of nutrient and PGRs contained in the culture medium [11], causing an increase in cell division activity and triggering callus formation [8]. Callus that formed at the wounded part of explants may be due to the accumulation of auxins at the point of the wound, which may then trigger cell proliferation and callus formation in that part [12]. In the present study, the percentage of explants forming callus on media containing TDZ only or NAA only was greater than (M2, M3, M5, and M9) or no less than (M4) that on media containing combinations of TDZ and NAA (M6, M7, M8, M10, M11, and M12). There were two media in which 100 % of explants formed callus: M2 (0.1 mg/L TDZ) and M9 (1 mg/L NAA) (figure 1).

In the treatments with TDZ alone, as TDZ concentration increased, the percentage of explants forming callus decreased (M2–M4). The same trend was repeated in media in which TDZ was combined with NAA (M6–M8 and M10–M12), where increasing the concentration of TDZ and NAA caused a decrease in the percentage of explants forming callus. It is possible that as the exogenous PGRs (TDZ+NAA) concentration increased in these media, to the point at which the physiological response of callus formation by the explant was inhibited at higher concentration of TDZ.

Callus grew faster on leaf explants cultured on media containing NAA alone (M5 and M9) than on media with TDZ alone (M2, M3, M4) (figure 2), with the fastest growth rate achieved for M9 (1 mg/L NAA); callus growth rate tended to be slower on media with combined TDZ and NAA. In addition, in the TDZ + NAA combined media, as the concentration of either PGR increased, the time taken to form callus increased (figure 2).
The callus growth rate on explants, in relation to the speed and ability of the cells to divide, depends on the type and concentration of PGR used as well as the physiological condition of the explants. The time required by explant cells for the completion of the cell cycle and then cell division varies [10]. In addition, the type of cell and the presence of explant cells in distinct phases of the cell cycle can also affect cell division rate [10, 13].

Callus obtained in this study tended to have a friable-compact to compact texture and various color. At the 4th week of observation, the color of callus tended to be light green, yellowish green, green, or brownish green (figure 3). Meanwhile after 8 weeks, the callus tended to be yellowish green, brown, dark brown or black (figure 4). Callus at week 8 also showed that browning tends to happen on media containing single NAA (as opposed to media containing TDZ either alone or in combination with NAA). It is known that the use of auxin at certain concentrations can increase the production of ethylene, thereby accelerating senescence [10], which could explain this result.

| Table 1. Treatment media used in this study combining NAA and TDZ. |
|-----------------------------------------------|
| NAA (mg/L) | 0 | 0.1 | 1 | 2 |
| TDZ (mg/L) | M1 | M2 | M3 | M4 |
| 0.1 | M5 | M6 | M7 | M8 |
| 1 | M9 | M10 | M11 | M12 |

Figure 1. Percentage of leaf explants of *Melastoma malabathricum* forming callus on different media. See table 1 for the treatment medium combination.

Figure 2. Average days of callus growth on leaf explants of *Melastoma malabathricum*. 
Figure 3. Colors of callus at week 4: (a) light green, (b) yellowish green, or (c) green.

Figure 4. Colors of callus at week 8: (a) brown, (b) dark brown, or (c) black.

The results showed that explants responded to the media containing NAA alone (M5 and M9) by forming callus and adventitious roots (figure 5), likely induced by the NAA in the culture medium. According to George et al. [8], the addition of exogenous auxin is generally required for the formation of roots on explants. The formation of roots on leaf explants cultured on MS medium containing NAA has also been reported in *T. magnifica* [6], *M. affine* [5], and *Nicotiana tabacum* [9].

The medium producing the highest percentage (100%) of explants forming adventitious roots was M9 (1 mg/L NAA), and explants on medium M9 also produced the fastest growing adventitious roots (table 2). Therefore, in this study, 1 mg/L NAA proved to be the best concentration for root induction on leaf explants of *M. malabathricum*.

One explant responded to the medium containing 2 mg/L TDZ and 0.1 mg/L NAA (M8) by forming callus and adventitious shoots (figure 6) on day 58. The formation of shoots was likely caused by the use a specific combination and concentration of PGRs. The use of higher concentrations of cytokinin than auxins in culture media can induce the formation of adventitious shoots on explants [8, 10], and this result is consistent with that of Ghimire et al. [7], who showed that adventitious shoots could be formed on leaf explants of *M. malabathricum* cultured on medium containing 2 mg/L TDZ and 0.1 mg/L NAA. The results showed that explants took a long time to form adventitious shoots, possibly related to the age of the explants (already 16 months old) and a reduced responsiveness. Therefore, young explants tend to be more responsive and easier to grow than older explants [8, 14].
Figure 5. Leaf explants of *Melastoma malabathricum* showing callus and root formation on (a) medium M5 (0.1 mg/L NAA) and (b) medium M9 (1 mg/L NAA), showed by arrow.

Table 2. The effect of treatment with NAA on adventitious root formation from leaf explants of *Melastoma malabathricum*.

| Medium | PGRs (mg/L) | Percentage of explants forming roots (%) | Average days to root induction |
|--------|-------------|------------------------------------------|------------------------------|
|        | TDZ  | NAA |                                      |                              |
| M5     | -    | 0.1 | 85                                     | 20.88 ± 6.45                 |
| M9     | -    | 1   | 100                                    | 9.5 ± 4.52                   |

Figure 6. Adventitious shoot formed on leaf explant of *Melastoma malabathricum*.

4. Conclusion
Leaf explants of *M. malabathricum* responded to all treatment media, except for the medium containing a combination of 2 mg/L TDZ and 1 mg/L NAA (M12). Explants responded well to media containing NAA (0.1 mg/L, 1 mg/L) and TDZ (0.1 mg/L, 1 mg/L) by forming callus, and somewhat less well to media containing a combination of TDZ and NAA at varying concentrations. Browning tended to occur in callus on media containing NAA alone. Callus obtained in the study tended to be friable-compact to compact in texture. The media in which callus production was the highest (100 %) were M2 (0.1 mg/L TDZ alone) and M9 (1 mg/L NAA alone). The fastest growing callus formation was achieved in leaf explants cultured on MS medium containing 1 mg/L NAA (M9). Explants also responded to the media
containing NAA alone (M5 and M9) by forming both callus and adventitious roots. Therefore, the use of 1 mg/L NAA proved to be best for root induction on leaf explants of *M. malabathricum*.

**Acknowledgments**

This research supported by PITTA 2017 Grant from Universitas Indonesia.

**References**

[1] Grosvenor P W, Gothard P K, McWilliam N C, Supriono A and Gray D O 1995 *J. Ethnopharmacol.* **45** 75-95
[2] Abdullah J O and Yong W T L 2007 *Transgenic Plant Journal* **1** 237-43
[3] Jha TB and Ghosh B 2005 *Plant and Tissue Culture Basic and Applied* (India: Universities Press)
[4] Ma G, Li Y, Jiao G, Fu X and Lin Y 2007 *Floriculture. Ornamental. Biotech.* **1** 27-9
[5] Zeng S J, Li L N, Wu K L, Chen Z L and Duan J 2008 *Pak. J. Bot.* **40** 1179-84
[6] Ghimire B K et al. 2016 *Plant. Cell. Tissue. Organ. Cult.* **124** 517-29
[7] Murashige T and Skoog F 1962 *Physiol. Plant.* **15** 473-97
[8] George E F, Hall M A and De Klerk G-J 2008 *Plant Propagation by Tissue Culture* 3rd edition (Netherlands: Springer)
[9] Nisak K, Nurhidayati T and Purwani K I 2012 *Jurnal Sains dan Seni Pomits* **1** 1-6
[10] Hopkins W G and Huner N P A 2009 *Introduction to Plant Physiology* (Danvers: John Wiley & Sons, Inc.)
[11] Pierik R L M 1987 *In Vitro Culture of Higher Plants* (Dordrecht: Martinus Nijhoff Publisher)
[12] Ahmad N, Faisal M, Anis M and Aref I M 2010 *S. Afr. J. Bot.* **76** 597-600
[13] Pulianmackal A J, Kareem A V K, Durgaprasad K, Trivedi Z B and Prasad K 2014 *Front. Plant. Sci.* **5** 1-16
[14] Smith R 2013 *Plant Tissue Culture Techniques and Experiments* 3rd edition (USA: Academic Press)