In vitro culture of *Melastoma malabathricum* (L.) from internodes on Murashige & Skoog (1962) modified medium supplemented with thidiazuron and 2,4-dichlorophenoxyacetic acid

N Zakiyah, R Yuniati and W Handayani
Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

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

Abstract. *Melastoma malabathricum* (L.) is a shrub that has potential uses in the fields of medicine and phytoremediation although further research on this species is warranted, including on its propagation, particularly in vitro culture. Here, research regarding the in vitro culture of *M. malabathricum* from internodes was conducted to determine its response on Murashige and Skoog (MS) modified medium supplemented with thidiazuron (TDZ) (1, 2, and 3 mg/L), 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5 and 1 mg/L), or a combination of both. Our results showed that internodes responded by forming calluses on all treatment media except the medium supplemented with 0.5 mg/l 2,4-D and 3 mg/L TDZ. Compact calluses were formed upon supplementation with a single dose of TDZ (1, 2, and 3 mg/L) and a combination of growth regulators. Meanwhile, semi-compact calluses were formed upon supplementation with a single dose of 2,4-D (0.5 and 1 mg/L) and a combination of growth regulators. Generally, callus formation was observed on 5–19 days of culture. Internode explants cultured on medium supplemented with a single dose of 2,4-D showed optimal response, whereas those cultured on medium supplemented with the combination of TDZ and 2,4-D showed weak response.

Keywords: Melastoma malabathricum, internode, thidiazuron, 2,4-D

1. Introduction

*Melastoma malabathricum* L. (*Melastoma*) is an aluminium (Al) accumulator plant which grows in tropical acid soils [1]. This plant is able to grow in polluted soil and is tolerant to acidic conditions [2]. Another benefit of *M. malabathricum* is to have efficacy as a traditional medicine, such as wound healer [3] and toothache [4]. Therefore, a good propagation protocol for *M. malabathricum* is needed which could be utilized for transformation and secondary metabolites production experiments.

Generally, the in vitro propagation of *Melastomataceae* plants involves the use of leaves as an explant [5, 6]. To the best of our knowledge, internode has never been used as explants for in vitro culture of *M. malabathricum*. The use of plant growth regulators (PGRs), such as cytokinin and auxins may affect the results of in vitro cultures [7, 8]. The use of a combination of both types of PGRs has been reported can produce callus [9, 10] and shoot [11]. The aim of this research was to determine the response of *M. malabathricum* internode to various concentrations of Thidiazuron (TDZ) and 2,4-Dichlorophenoxyacetic acid (2,4-D).
2. Materials and method
MS medium was used as the basal medium. This medium was supplemented with 2,4-D and TDZ at concentrations between 0 and 3 mg/L according to the experimental scheme (table 1).
Explant used in this study is a segment of internode from 16-months-old M. malabathricum seedlings. Internodes as explants (length 0.5 ± 1 cm) were obtained from the second to the fifth shoot buds. Explants were placed horizontally on MS medium. All cultures were maintained in a culture room at 25 ± 1 °C, with a 16 hour light/8 hour dark photoperiod cycle.

3. Results and discussion
3.1. Percentage of explants forming calluses
The results showed that the medium with the highest percentage of callus formation was observed on two treatment media, M1 and M2. Both treatments have the highest percentage, i.e. 90 %. The lowest percentage of callus formation (5 %) was observed on medium supplemented with 1 mg/L 2,4-D and 3 mg/L TDZ (M12) . Meanwhile, from 12 types of treatment medium used, explant culture on medium supplemented with 0.5 mg/L 2,4-D and 3 mg/L TDZ (M11) did not form callus. The percentage of explants forming callus on each treatment is presented in figure 1.

Explants culture on a medium supplemented with only TDZ at concentrations of 1, 2, or 3 mg/L showed 85 %, 80 % and 50 % callus formation, respectively. The high percentage of callus formation was considered to be the effect of TDZ, which can modify the concentration of endogenous plant hormones, including auxin which trigger callus formation [10, 11].

Table 1. Combination of plant growth regulator.

| Thidiazuron (mg/L) | 2,4-Dichlorophenoxyacetic acid (mg/L) |
|-------------------|---------------------------------------|
| 0                 | M1                                    |
| 1                 | M4                                    |
| 2                 | M7                                    |
| 3                 | M10                                   |
| 0                 | M2                                    |
| 0.5               | M5                                    |
| 1                 | M8                                    |
|                  | M11                                   |
|                  | M12                                   |

Figure 1. The mean percentage of callus induction from internode explant of Melastoma malabathricum 8 weeks after the culture incubation.
Medium supplemented with 2,4-D alone, such as M2 and M3 also showed high percentages of callus formation: 90 % and 60 %, respectively. This is in accordance with the statement of Taji et al. [12]. That callus can be formed because of the role of 2,4-D as auxin in elongation and cell multiplication. The combination of 2,4-D and TDZ can also trigger callus formation in M5, M6, M8, M9, and M12 treatment. We consider that callus formation is triggered by the action of PGRs added to the treatment medium.

3.2. Time required for callus induction
The induction of callus in explants occurred within 6 to 20 days after inoculation. The fastest formation of callus occurs on M2 medium. Meanwhile, the medium with 1 mg/L 2,4-D with 3 mg/L TDZ (M12) was the slowest in inducing callus on explant. However, there is a medium which is unable to induce callus, ie, medium with administration of 0.5 mg/L 2,4-D with 3 mg/L TDZ (M11). The results can be seen in figure 2.

The treatment medium with the addition of TDZ (M4; M7; and M10) showed the average callus formed at day 11.5; 12.8; and 13.4. The use of TDZ in the medium, one of which can trigger callus growth. TDZ plays a role in cell membrane modification, nutrient uptake and assimilation [13]. The role of TDZ is supposed to increase cell activity in splitting and producing callus effectively.

Meanwhile, the treatment medium with the addition of 2,4-D (M2 and M3) resulted in average callus growth rate on day 6.8 and 7.7. The average value indicates the callus growing in the first week after planting. The growth rate of callus on the medium with the addition of 2,4-D is due to 2,4-D as PGR which is capable of triggering the growth of callus [12, 14].

In previous studies, 2,4-D which a type of synthetic auxin, has been considered to be optimal for callus induction in both monocots and dicots [15]. TDZ is the most active cytokinin for the induction of shoots in plant cultures [16]. Callus induction, in general can occur in the presence of a single auxin at high concentrations or in combination with low concentrations of cytokinin.

3.3. Number of calluses formed
Callus formed from the internode explant of Melastoma malabathricum is categorized using growth score based on the number of calluses formed. The scores for the number of calluses formed at 8th week are shown in table 2.

A significant trend was found on M2 (0.5 mg/L 2,4-D) and M3 (1 mg/L 2,4-D) which formed callus with score 2. The use of 2,4-D as PGRs on culture medium was known to triggered formation of callus [15]. Soltanipol et al. (2011) report that internode explant and petiole of Monarda didyma (bee balm plant) supplemented with 2,4-D and BAP are able to formed callus with high percentage [17].

![Figure 2](image-url)  
**Figure 2.** Average time required (days) for callus growth from *Melastoma malabathricum* internode explant.
Table 2. Scores of the number of calluses formed by *M. malabathricum* internode explants cultured on treatment media.

| Medium | Number of explants forming calluses | Score 1 | Number of calluses (%) | Score 2 | Score 3 | Score 4 |
|--------|-------------------------------------|---------|------------------------|---------|---------|---------|
| M1     | 18                                  | 50      | 50                     | 0       | 0       |
| M2     | 18                                  | 17      | 72                     | 6       | 0       |
| M3     | 13                                  | 62      | 23                     | 8       | 0       |
| M4     | 17                                  | 0       | 29                     | 53      | 18      |
| M5     | 6                                   | 67      | 33                     | 0       | 0       |
| M6     | 3                                   | 100     | 0                      | 0       | 0       |
| M7     | 16                                  | 19      | 31                     | 31      | 19      |
| M8     | 7                                   | 100     | 0                      | 0       | 0       |
| M9     | 4                                   | 100     | 0                      | 0       | 0       |
| M10    | 11                                  | 18      | 64                     | 18      | 0       |
| M11    | 0                                   | 0       | 0                      | 0       | 0       |
| M12    | 1                                   | 100     | 0                      | 0       | 0       |

Score 1: Less callus formed  
Score 2: Moderate callus formed  
Score 3: Many callus formed  
Score 4: Excessive callus formed

Medium supplemented with both TDZ and 2,4-D produced calluses with an average score of 1 (less) for callus formation on medium supplemented with a combination of cytokinin and auxin, low auxin concentrations and high cytokinin concentrations are used in general [4]. According to Trimulyono et al. [18], the difference in callus size may be due to the difference in callus sensitivity in absorbing and utilizing nutrients and growth regulators from the growth medium.

4. Conclusion
*Melastoma malabathricum* (L.) internode as an explant can respond to treatment medium by forming calluses. The explants forms callus throughout the treatment medium, except M11 treatment (0.5 mg/L 2,4-D with 3 mg/L TDZ. The 2nd and 5th order of *M. malabathricum* (L.) internodes is likely to form more calluses.

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