The effects of thidiazuron and 2,4-D on the regeneration of Melastoma malabathricum L. cultured leaves

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Abstract. Melastoma malabathricum L. has potential for exploitation of its pharmacological and phytoremediation properties. We cultivated M. malabathricum leaves using in vitro cultures in Murashige and Skoog (MS) media supplemented with thidiazuron (TDZ) (0, 0.1 and 0.2 mg.L⁻¹), 2,4-dichlorophenoxyacetic acid (2,4-D) (0, 1, 2 and 3 mg.L⁻¹) or both. We studied the effect of TDZ alone or combined with 2,4-D on the growth and development of cultured leaves. Explants were cultured on solid MS medium supplemented with TDZ (0, 1, 2 and 3 mg.L⁻¹) or 2,4-D (0, 0.1 and 0.2 mg.L⁻¹) or both. All treatments induced callus with different colours and textures. TDZ, 2,4-D or both induced callus formation in approximately 75–95 %, 95–100 % or 45–90 % of explants. Adventitious root was produced in the presence of 0.1 mg L⁻¹ (70 %) and 0.2 mg.L⁻¹ (60 %) 2,4-D. Adventitious shoot formation was initiated in the presence of 1 mg.L⁻¹ (15 %), 2 mg.L⁻¹ (5 %) and 3 mg.L⁻¹ (5 %) TDZ. Callus formation was induced by 0.1 mg.L⁻¹ 2,4-D (63 %), 0.2 mg.L⁻¹ 2,4-D (50 %), 2 mg.L⁻¹ TDZ (42 %) and 3 mg.L⁻¹ TDZ (50 %), which were higher than other treatments. Callus, adventitious root or adventitious shoot was induced from leaves using 12 different media.

Keywords: Thidiazuron, 2,4-dichlorofenoxyacetic acid, Melastoma malabathricum, callus

1. Introduction

Melastoma malabathricum is a member of the family Melostomataceae. This plant has potential as a source of pharmacologicals that relieve toothaches [1] and overcome indigestion [2]. Further studies know that M. malabathricum can accumulate > 10,000 mg of aluminium (Al) per kilogram of leaves without a sign of toxicity. Therefore, M. malabathricum can be designated for Al accumulator plant [3]. The ability of M. malabathricum to accumulate [4] and detoxify Al [5] indicates its potential as a phytoremediation agent. Therefore, in vitro cultivation should be useful to study this mechanism.

The most frequently used combinations of plant growth regulators (PGRs) to propagate Melastoma are thidiazuron (TDZ) and 1-naphtalene acetic acid (NAA) [6], NAA and 6-benzylaminopurine (BAP) [7] and TDZ and BAP [8]. Ma et al. [7] showed that callus and adventitious shoots are induced by treating Melastoma affine with 1 mg L⁻¹ TDZ. However, explants do not respond to 2,4-D 1 mg.L⁻¹ [7]. Moreover, Zhang et al. [8] showed that single concentrations of TDZ (0.22 mg.L⁻¹, 0.55 mg.L⁻¹ or 1.1 mg.L⁻¹), induce Tibouchina aspera to form callus and adventitious shoots. Further, 0.221–1.1 mg.L⁻¹ 2,4-D able to induce callus, although necrosis follows [8].
The effects of TDZ combined with 2,4-D are known for the leaves of *Aeschynanthus radicans*, a member of *Gesneriaceae* [9], and *Momordica charantia*, a member of *Curcubitaceae* [10]. Further, we are the studies on the use of TDZ and 2,4-D for treating the leaves of *M. malabathricum* still unknow. Based on that, this work aims to identify the effects of TDZ and 2,4-D on the growth and development of the leave culture of *M. malabathricum*. Specifically, we treated the leaves of *M. malabathricum* cultured in Murashige and Skoog (MS) media containing TDZ (0 mg.L⁻¹, 1 mg.L⁻¹, 2 mg.L⁻¹ and 3 mg.L⁻¹) or 2,4-D (0 mg.L⁻¹, 0.1 mg.L⁻¹ and 0.2 mg.L⁻¹) or both. The results reported here will likely provide a foundation for future advanced research.

2. Methodology

The explant was excised from 16-month-old parental plants leaf growth from seed in sterile condition and cut into segmented with size 0.5 cm × 0.5 cm. Explants were cultivated on MS [11] modification media. Table 1 shows the concentration of the PGRs. 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. The features observed included, the percentage of explants that formed callus, roots and shoots and the numbers of callus.

3. Results and discussion

3.1. Callus response

The effects of the PGRs are shown in Figure 1. Eight PGRs treatment showed callus formation above 60 %, with 2,4-D 0.2 mg.L⁻¹, TDZ from 1-2 mg.L⁻¹ and the combination within 2,4-D and TDZ at those concentration. The differences response from each explant to form callus may be related to the physiology of explant and their interaction with the PGRs added to the media. The calluses with various colors and textures were induced using 12 different treatments. Generally, the initial response of explant with the PGRs were indicated by the elongation and swelling. Besides that, treatment with TDZ or TDZ combined with 2,4-D, influenced the various green color of callus and treatment with 2,4-D influenced the light-brown-to-brown color of callus. The results indicate that PGRs influenced the color of callus. TDZ is a cytokine that stimulates the transcription of the chloroplast genome and slows the ageing of plant organs by preventing the degradation of chlorophyll [12, 13]. In contrast, some reduction of chlorophyll formation in the presence of 2,4-D occurs in callus cultures of peas, tomatoes and potatoes, at some point auxin can inhibit chlorophyll biosynthesis [14]. Furthermore, auxin can increase ethylene production, which in turn, stimulates senescence [15].

Table 2 showed trends in the number of samples and the quantity of callus formed, which showed tendency the callus formed after 4 weeks tend to range in the ‘very few’ category (score 1) to ‘medium’ (score 3). Basically, callus can be formed in all parts of explant and medium treatment. We can differentiate the abundance from the callus in each explant and make a qualitatively scoring.

| TDZ (mg.L⁻¹) | 2,4-D (mg.L⁻¹) |
|-------------|---------------|
| 0           | 0             | 0.1 | 0.2 |
| 0           | M1            | M2  | M3  |
| 1           | M4            | M5  | M6  |
| 2           | M7            | M8  | M9  |
| 3           | M10           | M11 | M12 |
Figure 1. Percentage of leaf explants of *M. malabathricum* that formed callus after 60 days.

Table 2. Percentages of callus formed and the quality after four weeks.

| Medium | Number of samples forming callus | Score 1 | Score 2 | Score 3 | Score 4 |
|--------|----------------------------------|---------|---------|---------|---------|
|        |                                  | a       | b       | c       | d       |
| M1     | 5                                | 80      | 20      | 0       | 0       |
| M2     | 18                               | 6       | 50      | 0       | 0       |
| M3     | 19                               | 21      | 47      | 0       | 0       |
| M4     | 16                               | 38      | 13      | 44      | 6       |
| M5     | 7                                | 57      | 43      | 0       | 0       |
| M6     | 6                                | 67      | 33      | 0       | 0       |
| M7     | 14                               | 36      | 21      | 43      | 0       |
| M8     | 1                                | 100     | 0       | 0       | 0       |
| M9     | 3                                | 100     | 0       | 0       | 0       |
| M10    | 7                                | 57      | 29      | 14      | 0       |
| M11    | 1                                | 0       | 100     | 0       | 0       |
| M12    | 1                                | 100     | 0       | 0       | 0       |

Note: a<sup>very few;</sup> b<sup>infrequent;</sup> c<sup>medium;</sup> d<sup>abundance</sup>

The higher the score, more callus formed. Medium with TDZ 1 mg.L<sup>-1</sup> had a high percentage of callus formed from very few to abundance. Meanwhile, callus formed on medium with 3 mg.L<sup>-1</sup> TDZ+ 0.2 mg.L<sup>-1</sup> 2,4-D tend to have very few calluses.

In generally, the callus initiate formed in the living tissue with injured area, such as the four sides of leaf cutting, and the incision on the adaxial side of the leaf. Furthermore, the absorption of these nutrients can encourage the occurrence of cell division that continues to lead to callus formation [16, 17]. The amount of nutrients absorbed causes the callus to enlarge and sometimes even cover the entire explant surface. Nevertheless, there are also explants that only form a small amount of callus in some parts of the explant or even do not form callus at all. This can be caused by the absorption ability of GPRs and different nutrients in each explant. In addition, browning (browning) and imbalance of auxin and cytokinin can also cause callus formation in explants [16].
3.2. Adventitious root and shoot

Adventitious root was induced by treatment M2 (0.1 mg.L\(^{-1}\) 2,4-D) (figure 2a) and M3 (0.2 mg.L\(^{-1}\) 2,4-D) (figure 3). The use of 2,4-D alone is expected to induce root formation in explants. The 2,4-D is known to play a role in root initiation at low concentrations [14]. Otherwise, the use of cytokines in higher concentrations (1–10 mg.L\(^{-1}\)) can induce adventitious shoot formation, but root formation is generally inhibited [17].

Figure 2b. showed that adventitious shoots were formed in M4 containing 1 mg.L\(^{-1}\) TDZ, M7 containing 2 mg.L\(^{-1}\) TDZ and M10 containing 3 mg.L\(^{-1}\) TDZ. High concentrations of cytokinin effectively stimulate adventitious shoot formation. Moreover, TDZ is a phenyl-urea class cytokinin, which induces shoots more efficiently compared with cytokines such as zeatin, BAP and kinetin [18]. The process of shoot induction in in vitro culture is influenced by several factors, including the expansion conditions and the addition of PGRs with the right [19]. A high cytokinin concentrations are known to be effective in triggering shoots growth. Meanwhile, TDZ is a synthetic phenyl-urea type cytokinin that has a better ability to induce shoots among other type of cytokinin such as zeatin, and kinetin [18].

![Figure 2. Adventitious root (a) formed in medium M2 (0.1 mg.L\(^{-1}\) 2,4-D) and adventitious shoot (b) formed in M4 (1 mg.L\(^{-1}\) TDZ). Arrows show the positions of adventitious shoots and root.](image)

![Figure 3. Percentage of M. malabathricum leaf explants with adventitious root.](image)
4. Conclusion
Leaf explants of *M. malabathricum* responded to treatment by forming callus, adventitious root or adventitious shoot form after 15 days of culture. The callus was formed in all media treatment after 4 weeks and adventitious root was in media containing induced 2,4-D (0.1 and 0.2 mg.L\(^{-1}\)) and adventitious shoot was formed in medium containing TDZ 1, 2 and 3 mg.L\(^{-1}\) respectively.

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