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GROWTH RESPONSE OF *Melaleuca leucadendron* L. EXPLANTS TO VARIOUS CONCENTRATIONS OF WPM MEDIUM AND THIDIAZURON *IN VITRO*

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ABSTRACT

This study aimed to investigate the effects of Woody Plant Medium (WPM) concentration and Thidiazuron (TDZ) on the in vitro morphogenesis response of *Melaleuca leucadendron* L. leaf explants. The research was conducted from November 2025 to March 2026 at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Sultan Ageng Tirtayasa University. This research used a factorial randomized block design, consisting of three levels of WPM concentration (¼, ½, and full-strength WPM) and three levels of TDZ concentration (0.25, 0.50, and 0.75 mg L^{-1}). Young leaf explants were cultured under controlled conditions (25 ± 2°C, dark incubation). The fastest explant rolling time 1.0 Week After Planting (WAP) was achieved in the combination of ¼ WPM + 0.25 mg L^{-1} TDZ. The results showed that WPM concentration significantly affected explant diameter at 6 weeks after planting (WAP), while TDZ concentration significantly affected explant diameter at both 6 and 8 WAP. A highly significant interaction between WPM and TDZ was observed for explant diameter at 8 WAP. The treatment combination of ¼ WPM and 0.75 mg L^{-1} TDZ produced the highest mean diameter (0,366 cm). A notable finding was the occurrence of direct rhizogenesis in two treatment combinations (¼ WPM + 0.75 mg L^{-1} TDZ and ½ WPM + 0.50 mg L^{-1} TDZ), attributed to the auxin-like activity of TDZ under dark incubation conditions.

Keywords: In vitro culture, *Melaleuca leucadendron* (L.), Thidiazuron, Woody plant medium

INTRODUCTION

Cajuput (*Melaleuca leucadendron* L.) is an economically significant Indonesian plantation species, cultivated primarily for its leaf essential oil, dominated by 1,8-cineole (eucalyptol; 44.76–60.19%) alongside α-terpineol, d(+)-limonene, and β-caryophyllene (Pratiwi & Fitriyani, 2025), with wide use across the pharmaceutical, cosmetic, and traditional medicine industries. However, a persistent supply-demand imbalance exists: national demand reaches approximately 4,500 tons annually, while domestic and imported supply meet only around 2,180 tons (Mumtazy *et al*., 2021). This deficit worsened in 2023, when national cajuput leaf production declined sharply by 34.5%, from 64,133.04 to 41,990.93 tons (Aberth, 2024), rising dependence on imported Chinese eucalyptus oil as a substitute posing significant supply chain risk.

A key upstream constraint is the limited availability of high quality cajuput planting material at scale. Conventional seed-based propagation is structurally weak seeds are exceptionally small (3,000–6,000 seedlings per gram), requiring precise handling and storage (Rimbawanto *et al*., 2017), while high genetic variability in seed derived populations makes it difficult to produce uniform material expressing the superior yield and oil quality traits of selected mother trees preventing reliable mass production of elite clones for high productivity commercial plantations.

Plant tissue culture, based on cellular totipotency, enables regeneration of large numbers of identical plantlets from minimal explant material under aseptic conditions. Within Myrtaceae, Woody Plant Medium (WPM; Lloyd & McCown, 1981) has proven effective, yielding up to 18 shoots explant^{-1} in *Eucalyptus benthamii* (Brondani *et al*., 2012). Among growth regulators, Thidiazuron (TDZ) consistently outperforms conventional cytokinins in recalcitrant woody species (Dewir *et al*., 2018); TDZ at 0.25 mg L^{-1} with 0.1 mg L^{-1} NAA on WPM achieved 43% regeneration from *Eucalyptus grandis* × *E. urophylla* leaf explants (de Oliveira *et al*., 2016). Published reports on in vitro propagation of *M. leucadendron* remain limited, as Myrtaceae protocols are strictly species-specific due to exceptionally high polyphenol and oil content (Assis *et al*., 2018), representing a significant knowledge gap.

To address this knowledge gap, this study evaluated the effect of different WPM concentrations on the early in vitro growth of *Melaleuca leucadendron* leaf explants. The study also assessed the effect of TDZ concentration and examined the interaction between both factors on explant curling time and diameter development. The findings are expected to contribute to the development of an in vitro propagation protocol for *M. leucadendron*.

MATERIALS AND METHODS

This study utilized leaf explants of *Melaleuca leucadendron* L. from a local variety sourced from ERSAM AGRO BIOTECH. The explants were derived from the second youngest leaves (counting from the apical tip) of the eucalyptus plant. The experiment was conducted using a factorial Randomized Block Design (RBD) with two factors and three replicates. The first factor comprised three concentrations of Woody Plant Medium (WPM): W1 = ¼ WPM, W2 = ½ WPM, and W3 = full-strength WPM. The second factor comprised three concentrations of Thidiazuron (TDZ): D0 = 0.25 mg L^{-1} TDZ, D1 = 0.50 mg L^{-1} TDZ, and D2 = 0.75 mg L^{-1} TDZ.

Each experimental unit consisted of 27 leaf explants, totaling 81 explants across all treatments. Observed parameters included explant curling time and explant diameter. Observed parameters included explant curling time and explant diameter. Data were analyzed using Analysis of Variance (ANOVA) based on a factorial Randomized Block Design (RBD). Significant differences among treatments were further analyzed using Duncan's Multiple Range Test (DMRT) at the 5% significance level.

RESULTS AND DISCUSSION

Time of explant curling (WAP)

Curling is among the earliest morphological responses observed after culturing *M. leucadendron* leaf explants, marking the onset of post-initiation growth. Numerically, the fastest response occurred under W1D0 at 1.17 WAP (Figure 2), while W3D0 was slowest at 1.67 WAP, suggesting that higher WPM with low TDZ tended to delay curling, whereas low WPM with low TDZ accelerated it. This is consistent with Fitriani *et al*. (2019), who linked curling onset to early intracellular activity as a physiological response to the in vitro environment.

This response may indicate changes in osmotic adjustment and cell expansion induced by TDZ. TDZ has been shown to remain highly active even at low concentrations (<1 µM), particularly under dark incubation conditions, where early morphogenic responses increased by more than twofold compared with those under light incubation (Novikova & Zaytseva, 2018). The faster curling under ¼ WPM with 0.25 mg L^{-1} TDZ is presumed to reflect nutrient limitation promoting earlier osmotic adaptation, with low dose TDZ sufficient to trigger cell division without toxicity an adaptive capacity essential for successful culture initiation (Liu *et al*., 2024). Observation at 8 WAP (Figure 1) revealed direct rhizogenesis under W1D2 and W2D1 treatments, representing the most notable morphogenic response observed in this study. This response may indicate that TDZ influenced endogenous hormonal balance and promoted root initiation under reduced-strength WPM conditions. Previous studies reported that TDZ can exhibit cytokinin- and auxin-related effects depending on concentration and culture conditions (Dinani *et al*., 2018; Ali *et al*., 2022). In addition, lower WPM salt concentrations may reduce osmotic constraints and support root development, while dark incubation could enhance tissue responsiveness during early morphogenesis (Novikova & Zaytseva, 2018). However, further physiological and hormonal analyses are required to confirm these mechanisms in *M. leucadendron*.

Explant diameter (cm)

Mean diameter declined from 0.330 cm (6 WAP) to 0.267 cm (8 WAP), presumably driven by browning and growth stagnation (Table 1). Baskaran *et al*. (2016) attributed such decline during prolonged culture to phenolic accumulation toxic to tissue unable to enter active proliferation. At 8 WAP, the largest diameters occurred under W1 (0.436 cm) and W2 (0.450 cm), exceeding W3 (0.329 cm), though all remained near or below the initial explant size (≈0.5 cm), indicating stagnation rather than net growth contrasting with *Eucalyptus grandis* × *E. urophylla*, where optimal callus diameter increased 200–400% within four weeks (de Oliveira *et al*., 2016). This underscores that *M. leucadendron* explants remained far from optimal morphogenesis. The larger, more variable diameters at lower WPM concentrations may reflect nutrient balance, since excessively high or low nutrient levels can inhibit growth (Du *et al*., 2026), although species-specific responses also contribute.

Thidiazuron concentration affected explant diameter, with the highest value recorded at 0.25 mg L^{-1} (0.450 cm), decreasing at 0.50 mg L^{-1} (0.286 cm) and slightly increasing at 0.75 mg L^{-1} (0.384 cm). These findings suggest that excessive TDZ concentration may suppress diameter growth, in agreement with Dewir *et al*. (2018), who demonstrated that TDZ stimulates cell division and enlargement at moderate doses but may inhibit normal growth at higher concentrations. This matches Kumar *et al*. (2018), who reported optimal diameter at

0.25 mg L^{-1} TDZ in *Jatropha curcas*. Karataş *et al*. (2017) reported an 89.3% diameter reduction above 0.40 mg L^{-1} from disrupted cytokinin metabolism. W3D1 did not produce the largest explant diameter despite combining higher WPM and TDZ concentrations. This response may be associated with elevated osmotic pressure limiting water uptake, whereas excess TDZ may favor disorganized callus proliferation and oxidative stress rather than coordinated tissue growth (Karataş *et al*., 2017).

CONCLUSION

1. The ¼ WPM medium concentration treatment had a significant effect on explant diameter at 6 WAP, with a mean explant diameter of 0.367 cm.
2. The TDZ concentration treatment had a significant effect on explant diameter. A TDZ concentration of 0.25 mg L^{-1} produced the highest explant diameter growth at 6 WAP and 8 WAP, with mean explant diameters of 0.405 cm and 0.319 cm, respectively.
3. A significant interaction effect was observed between WPM medium concentration and TDZ concentration on explant diameter at 8 WAP. The combination of ¼ WPM and 0.75 mg L^{-1} TDZ produced the numerically highest value (0.366 cm), although it was not significantly different from several other treatment combinations based on DMRT.

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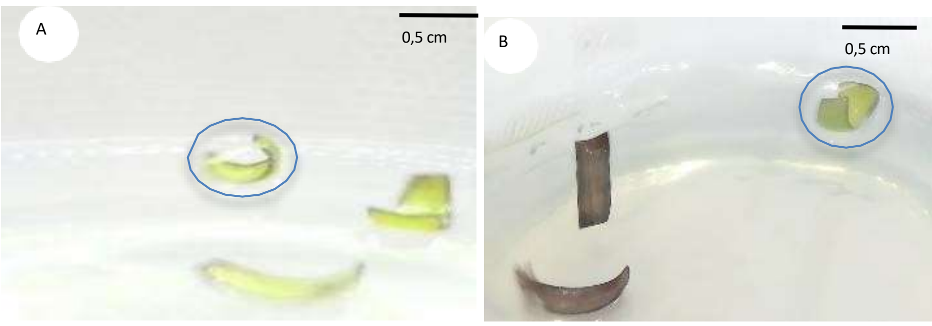
Table 1. Effect of WPM medium and thidiazuron concentrations on the diameter of *Melaleuca leucadendron* L. leaf explants at 6 and 8 WAP under in vitro conditions.

WPM concentration	Thidiazuron concentration			Mean
	D_0	D_1	D_2	
		cm		
		6 WAP		
W_1	0,436	0,280	0,384	0,367a
W_2	0,450	0,272	0,367	0,363a
W_3	0,329	0,286	0,167	0,261b
Mean	0,405a	0,279b	0,306b	0,330
		8 WAP		
W_1	0,331a	0,100b	0,366a	0,266
W_2	0,309a	0,270a	0,315a	0,298
W_3	0,316a	0,271a	0,127b	0,238
Mean	0,319a	0,214ab	0,269b	0,267

Notes: Values followed by the same letter within each row are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.



Notes :
A. W_1D_2 (¼ WPM + 0,75 mg L^{-1} TDZ)   B. W_2D_1 (½ WPM + 0,50 mg L^{-1} TDZ)

Figure 1. Curling growth of *Melaleuca leucadendron* L. leaf explants at 8 WAP

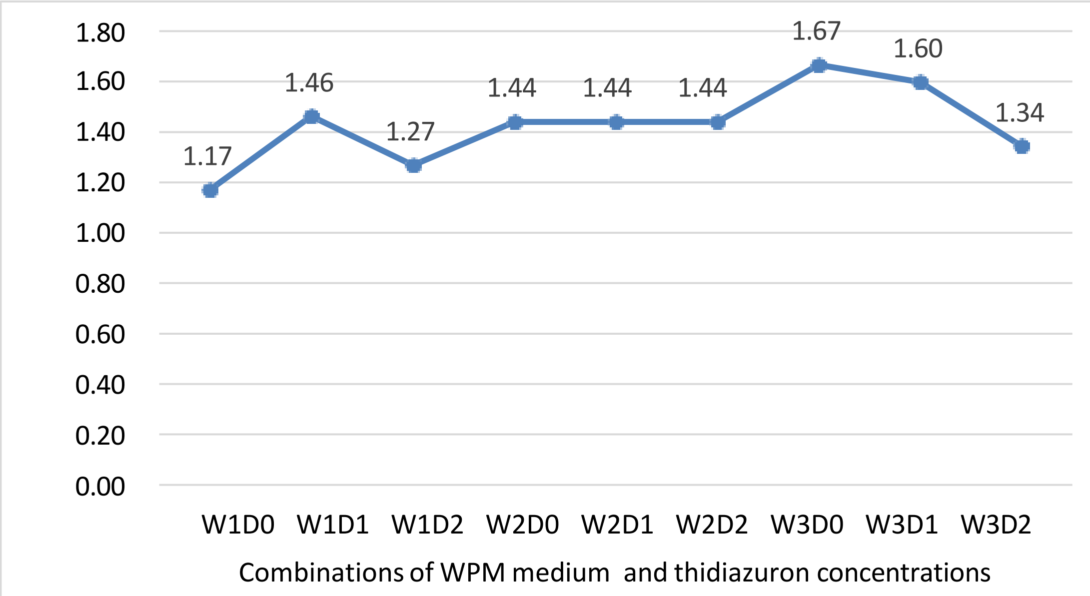


Figure 2. Time to explant curling of *Melaleuca leucadendron* L. leaf explants under different combinations of WPM medium and thidiazuron concentrations during 8 WAP