

THE ESTABLISHMENT OF *Melaleuca cajuputi* (GELAM) CULTURES FROM MATURE SEEDS

ANUAR RASYIDI MOHD NORDIN AND AZIZ AHMAD*

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

*Corresponding author: aaziz@umt.edu.my

<https://doi.org/10.46754/umtjur.v7i2.497>

Submitted: 15 April 2024

Revised: 29 August 2024

Accepted: 8 May 2025

Published: 15 October 2025

Abstracts: *Melaleuca cajuputi*, locally known as Gelam, belongs to the Myrtaceae family. It is used as a source of wood and as a remedy in traditional medicine. Tissue culture is one of the tools to ensure the sustainable supply of resources. However, the micropropagation protocol for this plant species remains limited. Therefore, the objective of the current study was to establish and examine the morphological traits of *M. cajuputi* cultures. To establish the culture, seeds were harvested from the local area, surface sterilised, and subsequently cultured in Murashige and Skoog (MS) medium supplemented with 0 mg/L to 1.5 mg/L of benzylaminopurine (BAP), kinetin (KIN), and thidiazuron (TDZ), respectively. The morphological traits of the cultures developed during the culture period. Results revealed that the highest microbial-free seedlings were obtained from seeds treated with 5.0% (v/v) sodium hypochlorite for 25 minutes. A healthy plantlet with a higher number of leaves (9.6 ± 0.8 /plantlet) and roots (8.7 ± 2.1 /plantlet) was obtained in the phytohormone-free medium, both at full- and half-ionic strength of MS medium after five weeks of culture. Plantlets cultured in a solid medium layered with a liquid medium exhibit better growth compared to those grown solely in a solid medium. The current findings indicate that *M. cajuputi* can easily grow in a culture medium with minimal nutrients. However, further studies should investigate the optimal culture medium for the micropropagation of this plant.

Keywords: Benzylaminopurine, ionic strength, culture medium, micropropagation.

Introduction

The genus *Melaleuca* is a member of the Myrtaceae family and comprises 260 species, primarily distributed in Australia, Southeast Asia, the Southern United States, and the Caribbean, mostly in wetland and coastal ecosystems (Tran *et al.*, 2012). Only a single species, known as *Melaleuca cajuputi* Powell, normally dominates the heath forests found along the coastal regions of Peninsular Malaysia, mainly the coastal corridor of Kelantan and northern Terengganu, as well as Indonesia (Shibli *et al.*, 2013). In Malaysia, the plant species known as “gelam” dominates the Beach Ridges Interspersed with Swales (BRISS) soils on the east coast in low swampy coastal areas, sometimes immediately behind mangroves that may be flooded to a depth of over one metre during the wet season (Doran, 1999). This plant is an evergreen shrub

or usually a single-stem tree that can reach up to 25 metres tall with an extensive root system and 1.2 metres in diameter, or may be reduced to a shrub. Furthermore, the plant can be characterised by the white or greyish papery bark that covers the trunk and branches [Figure 1 (b)]. The leaves are straight or curved and often hairy, and about 5 cm to 10 cm long, 1 cm to 4 cm wide, with 3 to 5 distinct nerves. Meanwhile, the fruits are capsule-like, approximately 3 mm long and 4 mm wide, with thinner valves than most *Melaleuca* species (Figure 1).

M. cajuputi is a multipurpose tree and has a high economic value as a phytoremediator of heavy metals and an aromatic medicinal plant that produces an essential oil mainly 1,8-cineole (Nguyen *et al.*, 2019). Isah *et al.* (2023) suggested that the leaf extract of

the *M. cajuputi* plant should be considered a novel source of natural antioxidants and enzyme inhibitors. The chemical compounds identified in the leaves include 10-methyl anthracene-9-carboxaldehyde, 2-isopropyl-10-methyl phenanthrene, 2-tert-butyl anthracene, β -eudesmol, and α -eudesmol (Isah *et al.*, 2023). To fulfil market demand and ensure the sustainability of the resource, the biotechnology approach may be a useful option. In line with this, plant cell, tissue, and organ culture techniques are promising tools for producing medicinally essential compounds derived from plants (Bapat *et al.*, 2023). Moreover, Liyama, and Cardosa (2021) reported the application of tissue culture techniques in shoot proliferation of the apical segment of *Melaleuca alternifolia*. Nonetheless, the report on the application of

this technique on *M. cajuputi* remains limited. Therefore, the current study aims to establish and examine the morphological traits of in vitro plantlets of *M. cajuputi*. The established in vitro plantlets can be utilised as plant material for further studies on physiology, metabolism, and metabolites produced by the plant species.

Materials and Methods

Sources of Plant Material

The mature fruit pot [Figure 1 (e)] was collected from the *M. cajuputi* plant that grows in a swamp located in Jambu Bongkok Forest Reserve, Marang, Terengganu (4° 54' 35" north; 103° 21' 36" east). Concurrently, the seeds released from fruits incubated at 16°C were stored in a bottle in a desiccator for further use as explants.



Figure 1: The morphology of the *M. cajuputi* plant. (a) Complete plant, (b) stem, (c) florescent of flower, (d) flower, and (e) seed pot and seeds

Surface Sterilisation of Seeds and Germination

The surface sterilisation of the explant was conducted under aseptic conditions, where all apparatuses were placed under a laminar airflow hood in the Biotechnology Laboratory at Universiti Malaysia Terengganu. Accordingly, seeds from a few fruit pots were immersed in 20 ml of 70% (v/v) ethanol for three minutes and continuously shaken. Following this, seeds were soaked in 50 ml of Clorox® bleach (containing 5.2% (v/v) sodium hypochlorite) and supplemented with two drops of Tween-20. The soaking period in bleach solutions was assessed at 15, 20, 25, and 30 minutes. The seeds were then rinsed several times with sterilised distilled water, and the floating seeds were discarded.

Afterwards, 100 healthy seeds from each soaking period were transferred to a sterile kitchen towel and placed in sterile petri dishes. A few millilitres of sterile distilled water were added to the kitchen towel paper and the seeds until they were sufficiently moistened. Subsequently, the Petri dishes were placed in a dark condition at room temperature to allow the seeds to germinate for approximately two weeks. The number of fungus-free or contamination-free seedlings was recorded.

Effects of Medium and Phytohormone on Morphological Traits of Seedlings

The treatment media used were solid Murashige and Skoog (MS) basal salt (1962) supplemented with 6-Benzylaminopurine (BAP), Kinetin (KIN), or Thidiazuron (TDZ) at concentrations of 0.0, 0.5, 1.0, and 1.5 mg/L, respectively. In a separate experiment, a modified MS medium containing half-strength MS macronutrients and supplemented with BAP at concentrations of 0.0, 0.5, 1.0, and 1.5 mg/L was used. Note that all media were adjusted to a pH of 5.7 to 5.8 using HCl or NaOH. To solidify, 2.5 g/L Gelrite® was added to the medium. Accordingly, 10 millilitres

of medium was used for each culture tube and sterilised by autoclaving at 121.0°C (105 kPa) for 25 minutes. Subsequently, two weeks of healthy seedlings were randomly selected and aseptically transferred onto treatment media for further growth observation. All cultures were incubated under white light provided by a fluorescent lamp (Phillips, Malaysia) with a photoperiod of 16 hours at a temperature of 25°C. After five weeks of culture, the ten seedlings from each treatment medium were removed from the culture vessel and manually counted for the number of leaves and roots per seedling. The stem length was measured using a ruler.

Statistical Analysis

The significant effect of treatment on the morphological traits of seedlings (leaf number, stem length, and root number) was statistically analysed by One-way Analysis of Variance (ANOVA). The significant differences ($p < 0.05$) were identified using the Post hoc Tukey Honestly Significant Difference (HSD) test in Statistical Package for Social Sciences (SPSS) software.

Results

Surface Sterilisation of Seeds and Germination

Most of the surface-sterilised seeds germinated after two weeks of incubation. Results in Table 1 indicate that the percentage of contamination-free germinated seeds increased with the period of soaking in a Clorox® bleach solution containing 5.2% sodium hypochlorite. In this experiment, seeds treated for 25 minutes and 30 minutes exhibited the lowest contamination rates, with 95% and 96% of the seedlings being contamination-free, respectively. This was followed by 20 minutes and 15 minutes, where 28 and 32 of the seedlings were contaminated (Table 1).

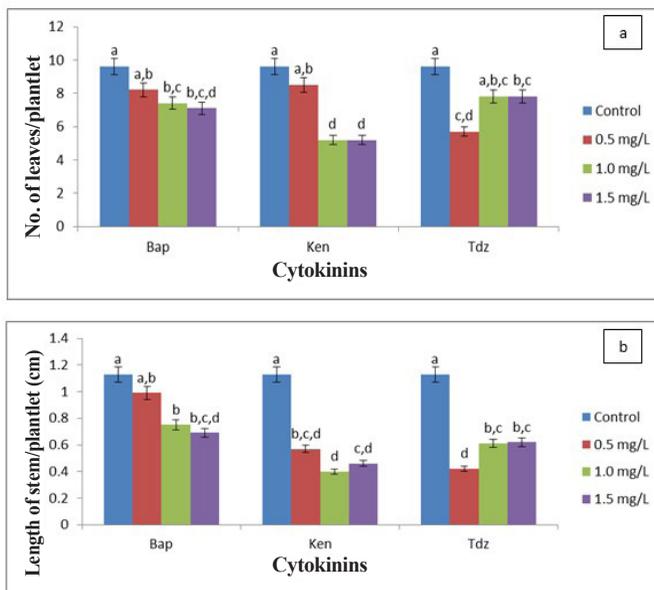
Table 1: Effect of soaking period of seeds in Clorox® bleach on the contamination rate of seedlings after two weeks of culture

Period of Soaking in Clorox® Solution (Min.)	Contaminated Seedlings	Contamination-free Seedlings
15	32	68
20	28	72
25	5	95
30	4	96

Effects of Medium and Phytohormone on the Growth of Seedlings

Figure 3 displays the effects of treatment media used on the growth of *M. cajuputi* seedlings after five weeks of culture. The results revealed that the leaf number, stem length, and root number of the seedlings varied among the treatment media. Notably, the addition of BAP, KIN, and TDZ into the medium did not significantly increase ($p > 0.05$) the leaf number, stem length, and root number of the seedlings (Figure 3). On the other hand, a better growth performance with higher leaf number, stem length, and root number of the *M. cajuputi* seedlings occurred on the phytohormone-free medium (control) compared to the seedlings in phytohormone-containing media (Figure 4).

In terms of leaf number, seedlings cultured in 0.5 mg/L BAP did not significantly differ from seedlings cultured in a medium added with 0.5 mg/L KIN. Results also demonstrated that the lowest leaf number was observed in the seedling culture medium containing 1.0 mg/L and 1.5 mg/L KIN [Figure 3 (a)]. Furthermore, the stem length and root number of *M. cajuputi* seedlings were also significantly decreased ($p < 0.05$) in higher concentrations of BAP, KIN, and TDZ [Figure 3 (b) and (c)]. Table 2 outlines the effects of the modified MS medium on the morphological characteristics of *M. cajuputi* plantlets after five weeks of culture. The results revealed that the seedlings preferred the phytohormone-free medium.



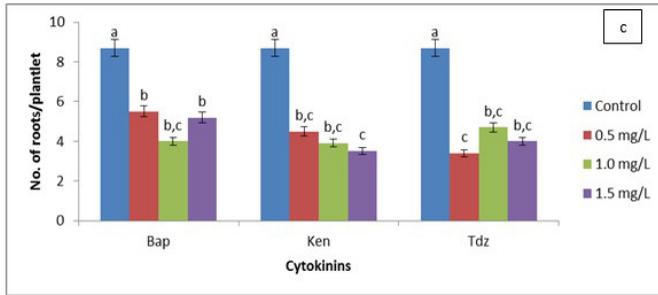


Figure 3: Effects of MS basalt salt medium and concentration of 6-benzylaminopurine (BAP), kinetin (KIN), and thidiazuron (TDZ) on the leaf number per seedling (a), stem length of seedling (b), and root number per seedling (c). *M. cajuputi* after five weeks of culture. The same alphabet indicates no significant difference among treatments tested by Post hoc Tukey Honestly Significant Difference at $p = 0.05$



Figure 4: Effect of different concentrations of 6-benzylaminopurine on the growth of *M. cajuputi* seedlings. After five weeks of culture, control medium (a), 0.5 mg/L of BAP (b), 1.0 mg/L of BAP (c), and 1.5 mg/L of BAP (d)

Table 2: The effect of modified MS medium on the leaf number, stem length, and root number of *M. cajuputi* seedlings compared to MS basalt salt medium after five weeks

Morphological Parameter	Concentration of BAP	Half-strength MS Macronutrients
Leaf number	0.0	9.6 ± 0.8 ^a
	0.5	7.5 ± 2.9 ^b
	1.0	6.6 ± 3.69 ^b
	1.5	8.7 ± 1.42 ^{ab}
Stem length	0.0	1.13 ± 0.21 ^a
	0.5	0.83 ± 0.37 ^{bc}
	1.0	0.72 ± 0.43 ^{abc}
	1.5	0.77 ± 0.14 ^{abc}
Roo number	0.0	8.7 ± 2.1 ^a
	0.5	4.1 ± 1.1 ^{abc}
	1.0	3.6 ± 2.8 ^{bc}
	1.5	4.3 ± 1.2 ^{abc}

The values above are mean \pm SD and were calculated based on 10 replicates in each group. Different alphabet indicates that the values of mean \pm SD have a significant difference at ($p < 0.05$).

Discussion

In the present study, seeds from mature pots were used as explants to establish the in vitro culture of *M. cajuputi* seedlings. Although a pure Clorox® bleach solution was used, a small number of seeds remained contaminated. Therefore, to obtain 100% contaminant-free *M. cajuputi* seeds, a longer soaking period (> 30 minutes) in Clorox® bleach solution may be applied. Accordingly, a higher concentration of disinfection is used due to the physical characteristics and source of the explant, as disinfectant activity varies among different plant species. In this study, sodium hypochlorite is a corrosive reagent that can penetrate deeper into cells and clean seeds of dirt and contaminant agents. Often, the outer surface of plant organs collected from the field is inhibited by microorganisms such as bacteria and fungi, which can cause contamination of the cultures.

Mihovilovic *et al.* (2024) stated that the use of sodium hypochlorite and plant preservatives can reduce seed-borne pathogen contamination, such as *Alternaria*, *Bipolaris*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Tilletia*, and *Ustilago* species when establishing in vitro culture of seeds. Hence, explants used for culture establishment must be thoroughly washed with disinfectant before being inoculated into a culture medium rich in nutrients. The disinfectant of the explant depends on the type of plant organ used as an explant (Lal *et al.*, 2023a). Other disinfectants that can be used in the surface sterilisation of plant organs are mercury chloride (Lal *et al.*, 2003b).

In most cases, plant seed germination is increased under dark conditions and with temperatures from 25 degrees to 30 degrees. During the germination process, light is not required as the photosynthesis process does not occur during this stage. At the same time, the

survival of the seedling depends solely on the nutrients stored in the endosperm of the seeds (Martínez-Ballesta *et al.*, 2020). Note that the presence of light can harden all parts of the seeds due to the decomposition of carbonic acid gas, which expels oxygen and fixes carbon, thereby slowing down the germination process or inhibiting seed germination in some species (Washa, 2015).

In this study, the MS basalt salt was supplied with three types of phytohormones, namely BAP, KIN, and TDZ, at varying concentrations to enhance the growth and proliferation of *M. cajuputi* seedlings under in vitro conditions. Nonetheless, the results contradicted the previously published report on *M. alternifolia* species (Chen *et al.*, 2016), which used full and half-strength MS media added with 0.6 mg/L BAP and 0.1 mg/L NAA. However, the use of full-strength MS medium and modified MS medium with half-strength micronutrients supplied with phytohormone failed to enhance the growth performance of seedlings after five weeks of culture.

On the other hand, the phytohormone-free media was most suitable to facilitate the growth of seedlings. Phytohormones, particularly cytokinins, are commonly applied in plant organ cultures to enhance the growth and proliferation of explants, including those of Myrtaceae species (Thakur *et al.*, 2024). In some cases, the use of cytokinin combined with auxin may be beneficial for enhancing the growth and proliferation of cultures, particularly those of woody species, including *M. alternifolia* (List *et al.*, 2006; Chen *et al.*, 2016; Liyama & Cardosa, 2021).

Conclusions

The in vitro plantlets of *M. cajuputi* were successfully established in both phytohormone-free and phytohormone-containing media. Correspondingly, seeds soaked for an extended period, up to 30 minutes, produced the highest number of contamination-free seedlings. In addition, the phytohormone-free medium of MS basalt salt and modified MS medium were

revealed to be the most preferred medium for the growth of *M. cajuputi* seedlings. Thus, further study should be conducted to determine the most suitable medium for the proliferation of this plant species.

Acknowledgements

The author thanks Universiti Malaysia Terengganu for providing the platform to conduct the study.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

- Bapat, V. A., Kavi Kishor, P. B., Jalaja, N., Jain, S. M., & Penna, S. (2023). Plant cell cultures: Biofactories for the production of bioactive compounds. *Agronomy*, *13*, 858. <https://doi.org/10.3390/agronomy13030858>
- Chen, B., Li, J., Zhang, J., fan, H., & Wu, L. (2016). Improvement of the tissue culture techniques for *Melaleuca alternifolia*. *Journal of Forest Research*, *27*, 1265-1269. DOI:10.1007/s11676-016-0301-7
- Doran, J. C. (1999). *Melaleuca cajuputi* Powell. In Oyen, L. P. A., & Nguyen Xuan Dung (Eds.), *Plant resources of South-East Asia. No. 19: Essential oil plants* (pp. 126-131). Bogor, Indonesia: Prosea Foundation.
- Isah, M., Zengin, G., Wan Abdul Wahab, W. N. A., Abdullah, H., Sul'ain, M. D., Uba, A. I., Wan Ishak, W. R., & Jamil, S. (2024). Antioxidant, enzyme inhibition, toxicity, and molecular docking analysis of *Melaleuca cajuputi* leaf extract and fractions. *Natural Resources and Human Health*, *4*(1), 89-97. <https://doi.org/10.53365/nrfhh/176775>
- Lal, M., Jamwal, M., Bakshi, P., Sharma, N., Sood, Y., Jasrotia, A., Sharma, A., Sharma, S., & Sharma, R. (2023a). Establishing surface sterilisation protocol for clonal apple rootstock MM106. *Journal of Applied and Natural Science*, *15*(4), 1654-1659.
- Lal, M., Jamwal, M., Sood, Y., Bakshi, P., Sharma, N., Sharma, S., & Kumar, S. (2023b). Micropropagation of fruit crops: A review. *Plant Science Today*, *10*, 108-117.
- List, S. E., Brown, L., & Walsh, K. B. (1996). A micropropagation protocol for *Melaleuca alternifolia* (tea tree). *Animal Production Science*, *36*(6), 755-760.
- Liyama, C. M., & Cardoso, J. C. (2021). Micropropagation of *Melaleuca alternifolia* by shoot proliferation from the apical segment. *Trees*, *35*, 1497-1509.
- Martínez-Ballesta, M. C., Gilabert C. E., Conesa, E., Ochoa, J., Vicente, M. J., Franco, J. A., Bañon, S., Martínez, J. J., & Fernández, J. A. (2020). The importance of ion homeostasis and nutrient status in seed development and germination. *Agronomy*, *10*(4), 504. <https://doi.org/10.3390/agronomy10040504>
- Mihovilović, A. B., Kereša, S., Lazarević, B., intarić, S. T., Martinko, K., Marković, Z., Turkalj, K., & Jerčić, I. H. (2024). The use of sodium hypochlorite and plant preservative mixture significantly reduces seed-borne pathogen contamination when establishing in vitro cultures of wheat (*Triticum aestivum* L.) seeds. *Agriculture*, *14*, 556. <https://doi.org/10.3390/agriculture14040556>
- Murashige, T., & Skoog, F. (1962). A reverse medium for rapid growth and bioassays with Tobacco tissue culture. *Physiological of Plant*, *15*, 473-478.
- Nguyen, H. T. H., Rimawanto, A., Prastyono, P., Kartikawati, N., & Wu, H. X. (2019). Genetic improvement for essential oil yield and quality in *Melaleuca cajuputi*. *Industrial Crops and Products*, *137*. DOI: 10.1016/j.indcrop.2019.05.061
- Shibli, N. M., Nik, M. M., Noor, A. M. S., & Arifin, A. (2010). Assessment of *Melaleuca cajuputi* as a heavy metals phytoremediator for sewage sludge contaminated soil. *American Journal of Applied Sciences*, *10*(9), 1087-1092
- Thakur, S., Shruti, Hashimi, S., Mishra, S., Ekka, S. K., Kushwaha, A., & Kujur, R. (2024). A review of plant cell tissue culture. *Asian Journal of Biology*, *20*(2), 14-18.

- Tran, D. B., Dargusch, P., Moss, P., & Hoang, T. V. (2012). An assessment of potential responses of the *Melaleuca* genus to global climate change. *Mitigation and Adaptation Strategies for Global Change*, 1-7.
- Washa, B. W. (2015). Potential of the dark as a factor affecting seed germination. *International Journal of Science and Technology*, 5(2). <http://www.ejournalofsciences.org>