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IN VITRO Litsea cubeba (Lour.) PERS DEVELOPMENT UNDER INDOLE-3-BUTYRIC ACID AND 6-BENZYLAMINOPURINE EFFECT VIA STEM NODE CULTURE

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ABSTRACT

Litsea cubeba (Lour.) Pers. is a vital medicinal plant in traditional medicine. Despite its high economic efficiency, *L. cubeba* is grown mainly based on experience; the area is fragmented and small, *L. cubeba* cultivation has relied on methods such as cuttings or grafting, with limited research on *in vitro* tissue culture propagation. This study aims to create a clean *in vitro* bud material source for micropropagation of *L. cubeba* by adding indole-3-butyric acid (IBA) and 6-benzylaminopurine (6-BA) to the cultured medium. Research results show that sterilising *L. cubeba* stems with $HgCl_2$ is more effective than NaClO. After 4 weeks of culture, adding IBA 1.0 mg.L^{-1} gave the best shoot development results. In MS medium supplemented with 6-BA 2.0 mg.L^{-1} gave the best growth results. The bud formation rate reached 100%, the bud colour was green, the buds were fat, and the stem and internodes were divided. The research results serve as a basis for building a process for quickly multiplying the *L. cubeba* from stem sections and creating a source of disease-free seeds to supply to the people.

Keywords: *Litsea cubeba*, 6-BA, IBA, *in vitro*, micropropagation, stem.

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INTRODUCTION

Litsea cubeba (Lour.) Pers. is a tropical and subtropical plant, preferably distributed in southeastern Asia or southern China, Nepal, Japan, Taiwan and Vietnam. It belongs to the genus Litsea, which comprises 622 known species (Agrawal et al., 2011; Han et al., 2013). *L. cubeba* is a member of the Lauraceae family and can be either an evergreen or deciduous tree, typically reaching heights of 5–8 meters. The plant thrives in subtropical regions (Chen et al., 2012) and is capable of growing on poor soils, often found at elevations up to 2,700 m (Saika et al., 2013). It produces white to yellowish flowers that develop into small, pepper-like spherical fruits, 4–5 mm in diameter, which mature from green to black by September. Essential oils can be extracted from various parts of the plant, including its roots, stems, leaves, flowers, and fruits, with yield and composition varying by part (Choudhury et al., 1998; Zhao et al., 2010). The fruit is a significant source of natural citral, widely used as a flavoring in food and cosmetics (Saika et al., 2013). The essential oils derived from *L. cubeba* exhibit numerous bioactive properties; the fruit, in particular, is traditionally used to treat headaches, inflammation, and intoxication (Li et al., 2024). Its antioxidant and antimicrobial properties have garnered research interest, especially concerning its radical scavenging and antibacterial or antifungal activities *in vitro* (Pante et al., 2021). Despite its economic potential, *L. cubeba* cultivation in Vietnam remains largely traditional, with small, fragmented areas of cultivation. This reliance on traditional propagation methods and the limited scale of cultivation has hindered the development of *L. cubeba* as a commercial commodity. As a result, the quality of the plants is inconsistent, they are prone to disease, and there is a risk of genetic degeneration.

Research interest in plant cell culture has been significant since the 1950s, with numerous studies demonstrating its effectiveness in producing biologically active compounds (Mulabagal et al., 2024). One of the primary advantages of plant cell culture is its ability to provide a continuous supply of raw materials for industrial-scale extraction of active ingredients, independent of natural conditions. Additionally, plant cell culture can generate novel compounds and enhance their production by modifying culture conditions (Rajasekaran et al., 1991). The active ingredients produced are free from contaminants such as pesticides, herbicides, or insect repellents, and avoid the variability and fluctuations commonly associated with natural plant products. Endogenous plant hormones play a crucial role in processes related to growth, development, aging, and stress responses. In plant tissue culture, these hormones are vital in regulating organ formation and morphology (Asif et al., 2022). Applying tissue culture technology to propagate *L. cubeba* can produce a large number of uniform, disease-free seedlings. Axillary shoot multiplication, in particular, is a rapid, reliable, and reproducible technique for germplasm conservation and clonal propagation under *in vitro* conditions (Chaudhuri et al., 2007; Behera et al., 2023; Warner et al., 2023). However, the *in vitro* culture conditions for *L. cubeba* have not been extensively studied. This gap in knowledge presents an opportunity to develop *L. cubeba* as a regional specialty crop, offering significant economic benefits and promoting sustainable development in local economies. This study aims to investigate the effects of indole-3-butyric acid (IBA) and 6-benzylaminopurine (6-BA) on the stem growth of *L. cubeba* under *in vitro* conditions, with the goal of establishing a protocol for rapid multiplication from stem sections and creating a disease-free seed source for local cultivation.

MATERIALS AND METHODS

Plant material preparation and growth conditions

Healthy *L. cubeba* stems were sourced from a nursery garden in Dak Nong province. Young stem cuttings from the 2nd to the 5th node were isolated from the stem of an 8-month-old plant and then transported to the laboratory, where they were rinsed under running water for 30 minutes, followed by a wash with water and liquid soap to remove

dust and other contaminants. The leaves were then removed, and the stems were cut into single-node segments, selecting nodes two through five, with each segment measuring between 1.0 and 1.5 cm in length for use in the *in vitro* growth experiments.

Unless otherwise noted, all media used in this study contained 0.6% agar and 3% sucrose, with a pH ranging from 5.8 to 6.2. Following inoculation, sterilization, and axillary bud induction experiments were conducted in the dark for two days, after which the cultures were exposed to 12 hours of light per day at an intensity of 2,100–2,500 lux and a temperature of 23–25 ^{o}C. For the proliferation culture test, the axillary buds were immediately placed under the same light and temperature conditions, with 12 hours of light exposure daily at 2,100–2,500 lux and a culture temperature of 23–25 ^{o}C.

Sterilization of explants

In this experiment, young stem segments containing bud points were used as explants. The explants were initially cleaned in a detergent solution to remove dust and debris, followed by a 120-minute rinse under slow-running water to eliminate any remaining residues on the surface. The stem segments were then sterilized using mercuric chloride and sodium hypochlorite at varying concentrations and exposure times, as outlined in Table 1. After sterilization, the segments were rinsed five times with sterile water. Finally, the stem segments were vertically inoculated into a hormone-free Murashige and Skoog (MS) medium (Murashige & Skoog, 1962). Each treatment was repeated three times, with 30 explants per replicate. After 14 days of culture, the rates of contamination, mortality, and survival were recorded.

Table 1. Chemicals characteristics used in sterilization

Treatments	Chemical agents	Concentration (%)	Duration (minute)
T0	Distilled water	0	0
T1	$HgCl_2$	0.5	3
T2			5
T3			7
T4		1.0	3
T5			5
T6			7
T7	NaClO	0.5	3
T8			5
T9			7
T10		1.0	3
T11			5
T12			7

Induction of axillary buds in stem segments

Stem segments with uniform growth characteristics were selected and subjected to the optimal pretreatment and sterilization methods. Following this, the segments were vertically inoculated into MS and B5 media, each supplemented with varying concentrations of 6-benzyladenine (6-BA), indole-3-butyric acid (IBA), and 3% sucrose, as detailed in Table 2. A total of 13 different treatments were applied, with each treatment repeated three times, and each replicate consisting of 30 explants.

Sample collection and analysis parameters

Monitor growth indicators every week, from week 1 to week 4. The measurement was conducted in triplicate. Effects of IBA in combination with 6-BA under *in vitro* shoot multiplication of *L. cubeba* were determined as follows:

$$\text{The frequency of axillary bud induction} = \frac{\text{the number of stems segments that induce axillary buds}}{\text{the number of inoculated stem segments}} \times 100$$

Number of leaves/bud: Count the number of leaves of each bud.

Bud height: Measure the height from the agar surface to the top of the shoot in cm.

Fresh biomass: Stem segments *in vitro* buds in all treatments were weighed immediately after being removed from the test tube to determine fresh biomass in mg.

Table 2. The experiment design investigates the effects of IBA and BA on the growth of explants under *in vitro* culture conditions

Treatments	Medium	Chemical agents	Concerntration ($mg.L^{-1}$)
T13	MS	IBA	0 (control)
T14			0.5
T15			1.0
T16			2.0
T17	B5		0 (control)
T18			0.5
T19			1.0
T20			2.0
T21	MS	6-BA	0.5
T22			1.0
T23			2.0
T24	B5		0.5
T25			1.0
T26			2.0

Micro-morphological characteristics

The iodine green-carmine double staining method was used as follows: the shoots' micro-morphological properties were ascertained per the guidelines provided by the Vietnamese Pharmacopoeia V (Alamgir, 2017). A razor was used to chop and cut the *L. cubeba*'s bud into segments/pieces. After that, the samples were manually sliced using a razor blade into thin, horizontal slices between 10 and 20 µm thick. The thin final cross-sections were then bleached for 10 minutes using 50% (v/v) chloral hydrate after 5.0% (w/v) chloramine-T detergent had been used. Those slices were neutralized with 1.0% (w/v) acetic acid for 2 min before being double stained with 0.3% (w/v) Iodine Green and 1.0% (w/v) Carmine, in which the sample was immersed in Iodine Green for 5s and in Carmine for 10s (until the samples became clearer). After each step, excess bleach, reagents, and dyes were removed using double distilled water. Samples were put on slides with one or two drops of the 50 : 50, (v/v) glycerin-water combination before being covered with a coverslip. The samples were examined and captured on camera using an optical microscope (Labomed, USA) at 4×, 10×, and 40× magnifications.

Statistical analysis

Statistical analyses were conducted using SPSS software (version 26.0; SPSS Inc., Chicago, IL, USA). Mean comparisons were carried out using either Duncan's multiple range test or the LSD test, with a significance threshold set at 0.05 to identify differences between the treatments and control groups.

RESULTS

Effect of mercuric (II) chloride ($HgCl_2$) and sodium hypochlorite (NaClO) with different soaking times on explant sterilization

After 14 days of cultivation in the MS media, the explants showed different degrees of contamination, survival, and mortality rates after each sterilization treatment (Fig. 1). The $HgCl_2$ treatment demonstrated effective decontamination results at all treatments, as evidenced by a survival rate ranging from 6.67% to 90.00%. At a concentration of 0.5% in 5 minutes, the $HgCl_2$ gave a high percentage of decontamination (90.00%), which was concentrated differently from the remaining treatments (Fig. 1). The transplanted sample has strong vitality, but the stem remains green. NaClO, a common disinfectant in tissue culture, also showed satisfactory results. The results demonstrated that NaClO treatment shows effective decontamination at all treatments, ranging from 6.67% to 66.67%. Within 5 minutes, the highest disinfection result among the treatments was 66.67% at 1.0% NaClO concentration; however, the effectiveness of NaClO is still lower than that of HgCl2. However, as the disinfectant concentration and sterilization time increased, the mortality rate increased significantly (Fig. 1). By combining the analyses of contamination, survival, and mortality, it is inferred that the best way to sterilize the stem segments of *L. cubeba* was to apply a 0.5% mercuric (II) chloride treatment for 5 minutes.

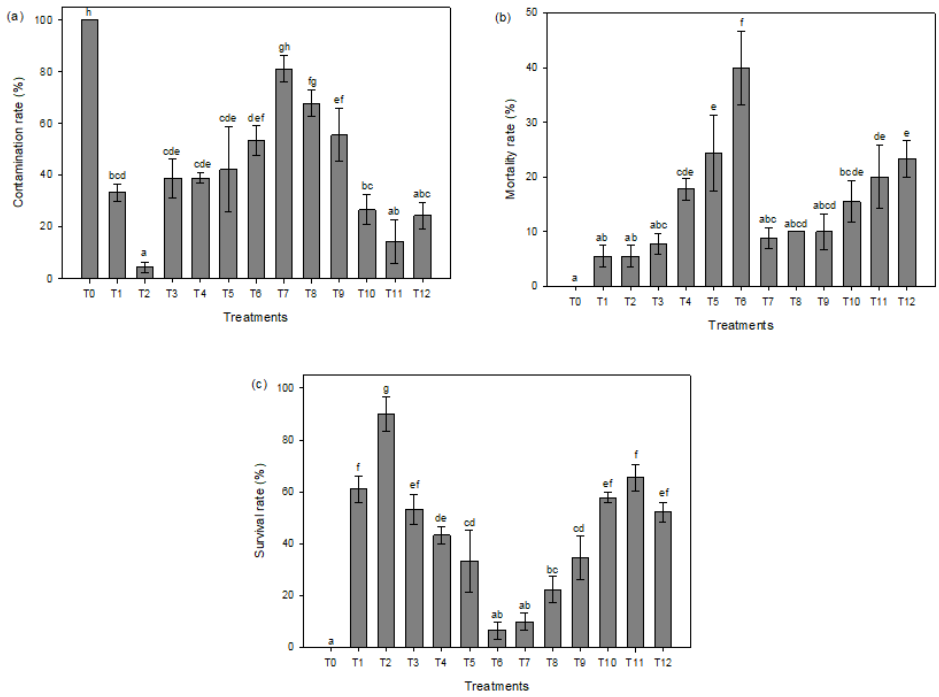

Figure 1. Effects of treatment combinations on contamination rate (a), mortality rate (b), and survival rate (c) of explants. Each bar represents the mean of the observations, and error bars designate the standard deviation

Effect of medium types and indole-3-butyric acid on *in vitro* shoot propagation

A total of six different IBA and medium treatments were set up for axillary bud induction of stem segments. Both the medium and IBA had significant effects on the frequency of axillary bud induction and the mean length of axillary buds, and their interaction also had a significant effect on the mean length of axillary buds (Fig. 2). When IBA was added to MS medium from 0.5 to 2.0 $mg.L^{-1}$, the rate of shoot formation increased from 53.33 (T14) to 100% (T15) and was severely different from the control (T13) ($p < 0.05$). Other indicators, such as the number of

leaves, length, and fresh biomass of explants, increased compared to the control treatment and were significantly different. The monitored traits achieved the best quality at an IBA concentration of 1.0 mg.L^{-1}; the buds were green, and the stems had clear internodes. In the B5 medium, adding IBA to the medium also increased other parameters but was lower than adding IBA to the MS medium (Fig. 2).

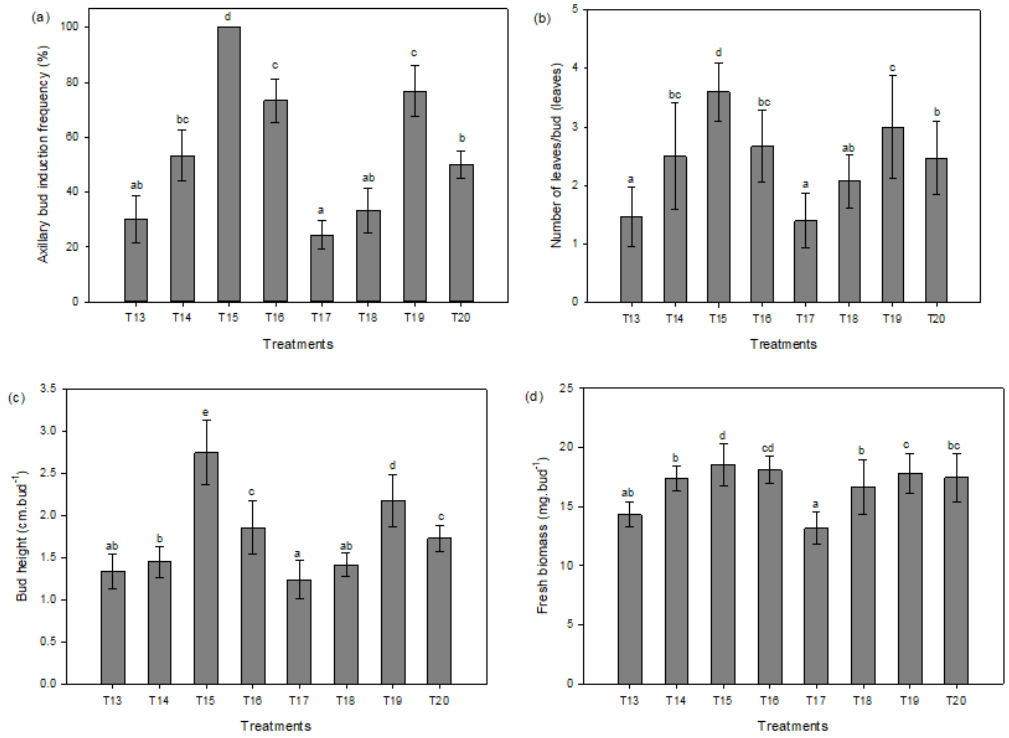

Figure 2. Effects of medium types and indole-3-butyric acid on axillary bud induction frequency (a), number of leaves/bud (b), bud height (c), and fresh biomass (d) of shoots. Each bar represents the mean of the observations, and error bars designate the standard deviation

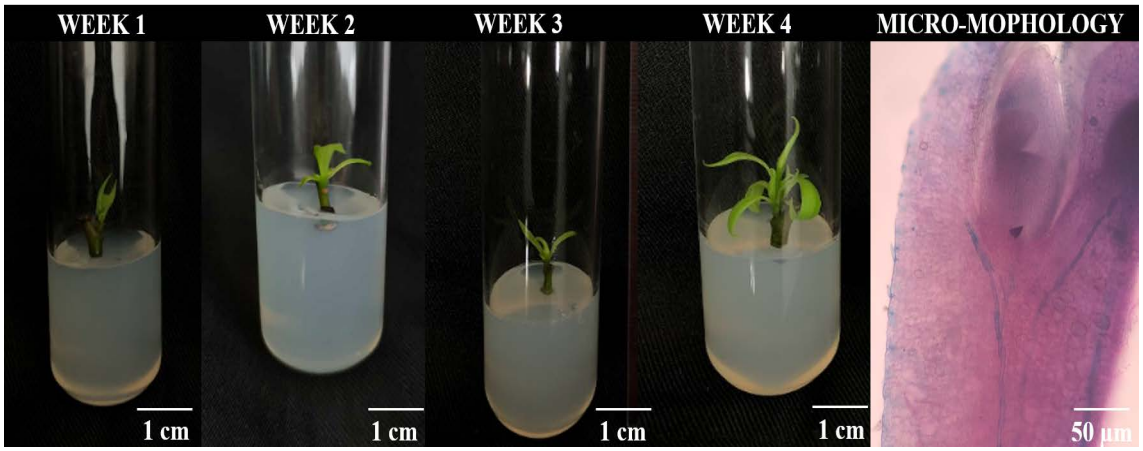

Figure 3. Bud development of the stem node of *Litsea cubeba* after 4 weeks under *in vitro* conditions in MS medium supplemented 1.0 mg.L^{-1} IBA

In the treatments of this study, when observing the micro-morphological characteristics of the stem node *in vitro* after 4 weeks of culture on different treatments, it was shown that each stem segment *in vitro* only produces 1 bud was obtained and the buds developed well after each week of culture (Fig. 3).

Effect of medium types and 6-benzylaminopurine on *in vitro* shoot propagation

Following 28 days of cultivation, medium and 6-BA treatments had significant effects on the frequency of axillary bud induction and the mean length of axillary buds (Fig. 4). The research results indicate that adding 6-BA to the MS medium increased the rate of shoot formation compared to the control treatment. The rate of shoot formation was highest at the concentration levels of 1.0 $mg.L^{-1}$ and 2.0 $mg.L^{-1}$ reached 100% and had a statistical difference compared to the remaining treatments ($p < 0.05$). There were statistical differences between the remaining treatments for the traits monitored at the highest concentration level of 2.0 $mg.L^{-1}$ ($p < 0.05$). B5 medium supplemented with 6-BA at different concentrations also gave similar results at 2.0 mg.L-1 provided the best shoot development parameters, but it was lower when compared to MS medium with a 6-BA supplement (Fig. 4).

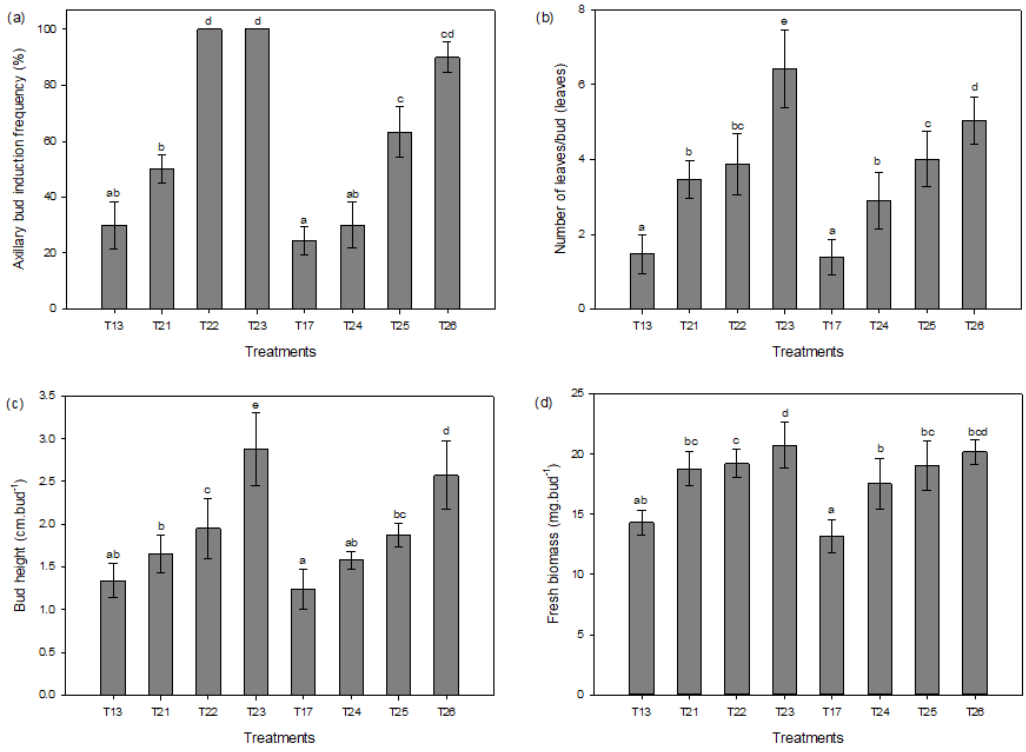

Figure 4. Effects of medium types and 6-benzylaminopurine on axillary bud induction frequency (a), number of leaves/bud (b), bud height (c), and fresh biomass (d) of shoots. Each bar represents the mean of the observations, and error bars designate the standard deviation

Similar to the results of the micro-morphological characteristics survey of the stem nodes *in vitro* supplemented with IBA, after 4 weeks of culture in the medium supplemented with 6-BA, it was shown that each stem node *in vitro* produced only 1 bud and these buds developed well after each week of culture (Fig. 5).

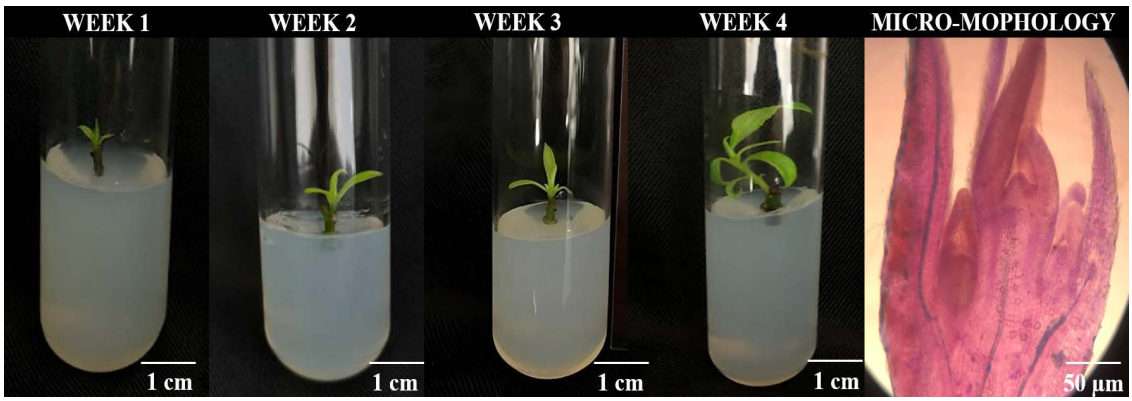

Figure 5. Bud development of the stem node of *Litsea cubeba* after 4 weeks under *in vitro* conditions in MS medium supplemented 2.0 mg.L^{-1} 6-BA

DISCUSSION

Selecting the appropriate explant from a healthy, vigorous plant is crucial in the initial stages of plant tissue culture (Pan et al., 2017). In this study, shoot stem segments from young, healthy, pest- and disease-free green branches of *L. cubeba* were used as explants. It is well-established that shoot-tip explants are particularly vulnerable during sterilization due to their delicate nature, often resulting in low survival rates (Ye, 2004). However, the ideal explant may differ among plant varieties and depending on the specific objectives of the culture. Establishing an aseptic propagation system in plant tissue culture requires effective sterilization of explants (Ahmad et al., 2016). In this study, explants of *L. cubeba* were disinfected using $HgCl_2$ and NaClO. The findings indicate that the duration and concentration of NaClO treatment significantly influenced the contamination rate, viability, and mortality of the explants. Although NaClO, a milder sterilizing agent, should be used more frequently to manage contamination, $HgCl_2$ proved to be more effective due to its strong bactericidal properties, resulting in higher decontamination success. The experiments demonstrated that $HgCl_2$ consistently provided effective sterilization across all tested concentrations. However, increasing the concentration and exposure time of both $HgCl_2$ and NaClO had more detrimental effects on the explants. Higher concentrations and prolonged soaking times led to greater damage to the stem segments, slowed growth recovery, and, in some cases, inhibited regrowth altogether. Previous studies by Srivastava et al. (2010) and Rihan et al. (2014) also reported increased shoot mortality with higher concentrations and extended exposure to $HgCl_2$.

PGRs are crucial to the growth and development of plant tissues or organs in laboratory settings (Bhojwani & Dantu, 2013). Using the right cytokinins along with other growth hormones can greatly speed up the process of activating and growing axillary buds in explants (Liang et al., 2020; Chen et al., 2020). The predominant hormone for initiating culture and promoting proliferation is 6-BA. In the current study, an increase in the overall concentration of 6-BA and IBA enhanced the induction and proliferation of axillary buds. Simultaneously, the results showed that the concentration of 6-BA had a more significant effect on axillary buds induction than IBA with different types of medium. An appropriate concentration of 2.0 mg.L^{-1} 6-BA contributes to the elongation of axillary buds. Ho et al. (2021) also found that 6-BA promoted axillary bud induction in *Acacia confusa* nodal sections. When the MS medium was supplemented with 2.0 mg.L^{-1} 6-BA, it emerged as the optimal medium, with the mean axillary bud length reaching its peak at 2.88 cm. Compared to the B5 medium, the appropriate concentration of 6-BA maximized

the effectiveness of the MS medium in promoting axillary bud growth. The high levels of inorganic salts, such as nitrogen and potassium, in the MS medium, fulfill the nutritional needs for rapid growth and support the culture's development and long-term viability.

In contrast, the B5 medium is characterized by lower ammonium levels and higher concentrations of nitrate and thiamine hydrochloride (Lin et al., 2021). The study results indicate that the effectiveness of shoot growth in treatments supplemented with IBA is lower than that with 6-BA. IBA, an auxin-type plant growth regulator, promotes cell elongation and division by relaxing the cell wall, which increases protoplasm volume and mass, depending on its concentration and the specific tissue it influences (Cao et al., 2023). At low concentrations, auxin encourages bud growth (Cao et al., 2023). However, as the concentration of auxin increases beyond a certain threshold, it can inhibit bud growth or induce dormancy (Wei et al., 2022). When IBA was incorporated into the explant culture, it accelerated axillary bud growth and created favorable conditions for bud explant development. Additionally, IBA facilitates tissue shoot elongation, contributing to the formation of a complete plant during propagation (El-Banna et al., 2023). While auxins are known to stimulate stem elongation and lateral root formation, they also inhibit axillary bud formation and growth by reinforcing apical dominance (Cao et al., 2023).

CONCLUSION

This study successfully established an initial *in vitro* propagation method for *L. cubeba* by exploring key processes, including explant sterilization, axillary bud induction, and proliferation. Explants were sterilized using 0.5% HgCl2 for 5 minutes and subsequently inoculated onto MS medium supplemented with either 1.0 $mg.L^{-1}$ IBA or 2.0 $mg.L^{-1}$ 6-BA to promote axillary bud sprouting. While the buds exhibited healthy growth, the induction of new bud formation was less effective. The findings of this experiment offer technical support for the rapid propagation of *L. cubeba*. However, further research is needed to optimize bud production by adjusting the composition of the culture medium, experimenting with different hormones, varying hormone concentration ratios, and exploring other growth conditions.

REFERENCES

Agrawal N., Choudhary A. S., Sharma M. C. & Dobhal M. P., 2011. Chemical Constituents of Plants from the Genus Litsea. *Chemistry & Biodiversity*, 8(2): 223–243.

Ahmad I., 2016. *In vitro* surface sterilization of the shoot tips of *Bougainvillea spectabilis* Willd. *Pure and Applied Biology*, *5*(4): 45–65.

Alamgir A. N. M., 2017. Pharmacognostical Botany: Classification of medicinal and aromatic plants (MAPs), botanical taxonomy, morphology, and anatomy of drug plants. In *Progress in Drug Research*: 177–293.

Asif R., Yasmin R., Mustafa M., Ambreen A., Mazhar M., Rehman A., Umbreen S. & Ahmad M., 2022. Phytohormones as Plant Growth Regulators and Safe Protectors against Biotic and Abiotic Stress. In *Intech Open eBooks*.

Behera S., Kar S. K., Monalisa K., Mohapatra S., Meher R. K., Barik D. P., Panda P. C., Naik P. K. & Naik S. K., 2023. Assessment of genetic, biochemical fidelity, and therapeutic activity of *in vitro* regenerated Hedychium coronarium. *In Vitro Cellular & Developmental Biology - Plant*, 59(5): 602–620.

Bhojwani S. S. & Dantu P. K., 2013. Plant Tissue Culture: An Introductory text. In *Springer eBooks*.

Cao D., Chabikwa T., Barbier F., Dun E. A., Fichtner F., Dong L., Kerr S. C. & Beveridge C. A., 2023. Auxin-independent effects of apical dominance induce changes in phytohormones

correlated with bud outgrowth. *Plant Physiology*, 192(2): 1420–1434.

Chaudhuri R. K., Pal A. & Jha T. B., 2007. Production of genetically uniform plants from nodal explants of *Swertia chirata* Buch Ham. Ex Wall.—an endangered medicinal herb. *In Vitro Cellular & Developmental Biology - Plant*, 43(5): 467–472.

Chen C., Tseng Y., Chu F., Wen T., Cheng W., Chen Y., Tsao N. & Wang S., 2012. Neuropharmacological activities of fruit essential oil from *Litsea cubeba* Persoon. *Journal of Wood Science*, 58(6): 538–543.

Chen S., Xiong Y., Yu X., Pang J., Zhang T., Wu K., Ren H., Jian S., Da Silva J. a. T., Xiong Y., Zeng S. & Ma G., 2020. Adventitious shoot organogenesis from leaf explants of *Portulaca pilosa* L. *Scientific Reports*, 10(1).

Choudhury S., Ahmed R., Barthel A. & Leclercq P. A., 1998. Composition of the Stem, Flower and Fruit Oils of *Litsea cubeba* Pers. from Two Locations of Assam, India. *Journal of Essential Oil Research*, 10(4): 381–386.

El-Banna M. F., Farag N. B., Massoud H. Y. & Kasem M. M., 2023. Exogenous IBA stimulated adventitious root formation of *Zanthoxylum beecheyanum* K. Koch stem cutting: Histo-physiological and phytohormonal investigation. *Plant Physiology and Biochemistry*, 197: 107639.

Han X., Wang Y., Chen Y., Lin L. & Wu Q., 2013. Transcriptome Sequencing and Expression Analysis of Terpenoid Biosynthesis Genes in *Litsea cubeba*. *PLoS ONE*, 8(10): e76890.

Ho W., Huang Y., Huang W., Huang Y. & Chung J., 2021. Effective in vitro culture using dormant bud of nodal sections from a mature Acacia tree. *In Vitro Cellular & Developmental Biology - Plant*, 58(3): 437–446.

Li G., Li Z. & Wang Y., 2024. The genus Litsea: a comprehensive review of traditional uses, phytochemistry, pharmacological activities and others studies. *Journal of Ethnopharmacology*, 334: 118494.

Liang H., Xiong, Y., Guo, B., Yan, H., Jian, S., Ren, H., Zhang, X., Li, Y., Zeng, S., Wu, K., Zheng, F., Da Silva, J. a. T., Xiong, Y., & Ma, G., 2020. Shoot organogenesis and somatic embryogenesis from leaf and root explants of *Scaevola sericea*. *Scientific Reports*, *10*(1).

Lin J., Zou J., Zhang B., Que Q., Zhang J., Chen X. & Zhou W., 2021. An efficient *in vitro* propagation protocol for direct organogenesis from root explants of a multi-purpose plant, *Broussonetia papyrifera* (L.) L'Hér. Ex Vent. *Industrial Crops and Products*, 170: 113686.

Mulabagal V. & Tsay H. S., 2004. Plant cell cultures- an alternative and efficient source for the production of biologically important secondary metabolites. *International Journal of Applied Science and Engineering*, 2(1): 29–48.

Murashige T. & Skoog F., 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, 15(3): 473–497.

Pan M., Fu R. K., Yang J., Qi H.S., Huang S., Jiang D. Q., Wang J. F. & Yun Y., 2017. Seeds Sterile Rapid Propagation of *Bougainvillea glabra*. Mol. *Plant Breed*, 15: 1894–1899.

Pante G. C., Castro J. C., Lini R. S., Romoli J. C. Z., De Almeida R. T. R., Garcia F. P., Nakamura C. V., Pilau E. J., De Abreu Filho B. A. & Machinski M., 2021. *Litsea cubeba* essential oil: chemical profile, antioxidant activity, cytotoxicity, effect against *Fusarium verticillioides* and fumonisins production. *Journal of Environmental Science and Health Part B*, 56(4): 387–395.

Rajasekaran T., Ravishankar G. A. & Venkataraman L. V., 1991. Influence of nutrient stress on pyrethrin production by cultured cells of pyrethrum (*Chrysanthemum cinerariaefolium*). *Current Science*, 33(9): 1417–1419.

Rihan H. Z., Al-Issawi M., Al-Swedi F. & Fuller M. P., 2012. The effect of using PPM (plant preservative mixture) on the development of cauliflower microshoots and the quality of artificial seed produced. *Scientia Horticulturae*, 141: 47–52.

Saikia A. K., Chetia D., D'Arrigo M., Smeriglio A., Strano T. & Ruberto G., 2013. Screening of fruit and leaf essential oils of *Litsea cubeba* Pers. from north-east India - chemical composition and antimicrobial activity. *Journal of Essential Oil Research*, 25(4): 330–338.

Srivastava N., Kamal B., Sharma V., Negi Y. K., Gupta S., Singh S. B. & Garhwal P., 2010. Standardization of Sterilization Protocol for Micro propagation of *Aconitum heterophyllum*- An Endangered Medicinal Herb. *Academic Arena, 2*(6): 37–42.

Warner J., Camacho-Solís Y. & Jiménez V. M., 2023. Direct and indirect *in vitro* regeneration of *Vanilla odorata* C. Presl. and *V. pompona* Schiede, two aromatic species with potential relevance for future vanillin production. *In Vitro Cellular & Developmental Biology - Plant*, 59(5): 621–636.

Wei J., Yang Q., Ni J., Gao Y., Tang Y., Bai S. & Teng Y., 2022. Early defoliation induces auxin redistribution, promoting paradormancy release in pear buds. *Plant Physiology*, 190(4): 2739–2756.

Ye D. Y., 2004. *Study on Technique System of the Tissue Culture in Bougainvillea glabra* [Doctoral dissertation, Sichuan Agriculture University].

Zhao O., Zhou J. and Ban D., 2010. Analysis of volatile oil from different parts of *Litsea cubeba. Journal of Chinese Medicinal Materials,* 33(9): 1417–1419.