International Journal of Agricultural Technology 2025 Vol. 21(2):767-788
DOI: https://doi.org/10.63369/ijat.2025.21.2.767-788
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effects of sterilizing agents, phenolic compound inhibitors, and plant hormones *in vitro* on lateral bud explant culture of three durian varieties

Wiangsamut, B.[1*], Tilarux, P.[2] and Wattana, K.[1]

[1]Department of Agricultural Technology, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok at Chanthaburi Campus, Chanthaburi, Thailand; [2]Department of Plant Production Technology and Landscape, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok at Chanthaburi Campus, Chanthaburi, Thailand.

Wiangsamut, B., Tilarux, P. and Wattana, K. (2025). Effects of sterilizing agents, phenolic compound inhibitors, and plant hormones *in vitro* on lateral bud explant culture of three durian varieties. International Journal of Agricultural Technology 21(2):767-788.

Abstract The most effective sterilizing agent for achieving sterility in durian lateral bud explants of 'Monthong', 'Kradum Thong', and 'Puangmanee' durian varieties was a 0.3% mercuric chloride ($HgCl_2$) solution for 10 minutes. This treatment resulted in significantly higher contamination-free survival rates ($P < 0.05$) by 70%, 60%, and 50%, respectively, compared to the control treatments. The control treatments involved 30% Clorox (sodium hypochlorite, NaOCl) for 15 minutes followed by 5% Clorox for 10 minutes, resulting in 0% survival. The liquid Woody Plant Medium (WPM) supplemented with Polyvinylpyrrolidone (PVP) at a concentration of 1.0 g/L achieved the most effective reduction in mucilage (phenolic compounds), resulting in 100% effectiveness. PVP acts as an antioxidant, contributing to reduced phenolic compound formation. As a result, the lateral bud explant of durian remained green and clean, and the phenolic compound on the cut surface of the explant effectively dissolved. Notably, this result was significantly better ($p < 0.05$) than using the control liquid medium (WPM). Over 7 months of subculturing on a solid medium once every month, findings revealed a distinct pattern in 'Monthong' durian lateral bud explants. When it cultured on WPM supplemented with 1.0 mg TDZ (Thidiazuron)/L and 10% coconut water, these explants exhibited significantly longer green callus lengths than the rest tested solid media. The combination of a low TDZ level (1.0 mg/L) with coconut water in the WPM solid medium appeared to play a crucial role in promoting callus formation. Interestingly, WPM supplemented with 3.0 mg BA (6-Benzyladenine)/L led to lateral bud explants of 'Kradum Thong' durian splitting into two shoots, each with leaf growth. Conversely, WPM supplemented with 3.0 mg TDZ/L promoted larger shoots with green leaf color. These findings provided valuable insights into optimizing tissue culture conditions for different durian varieties.

Keywords: Callus, Durian, Plant hormone, Phenolic compound, Tissue culture

[*] **Corresponding Author**: Wiangsamut, B.; **Email:** bancha_wi@rmutto.ac.th; timbancha@yahoo.com

Introduction

Durian, scientifically known as *Durio zibethinus* Murr., is a tropical fruit with significant economic importance in Thailand (Wiangsamut *et al*., 2021; Wiangsamut, 2024). It is recognized for its high energy density and is considered one of the most expensive fruits in Southeast Asia (Aziz and Jalil, 2019). Thailand is the leading global producer of durian, cultivating over 100 varieties. However, some economically promising varieties are at risk of genetic mutation and fading from Thai society. Researchers are focusing on disease-free durian propagation using tissue culture techniques to maintain true-to-type characteristics and ensure consistent quality yield. In an experiment involving three durian varieties—'Monthong', 'Kradum Thong', and 'Puangmanee'—the focus was on desirable agricultural traits for consumers. 'Monthong' stood out due to its superior flesh weight, percentage, and thickness compared to other varieties (Wiangsamut and Wiangsamut, 2023). The selection of 'Monthong' for the project was also due to its impressive flowering induction rates of 93.6% to 97% achieved through the application of paclobutrazol. This treatment not only facilitated successful fruit set and high fruit production but also resulted in substantial income. The benefit-cost ratio exceeded 1, suggesting that this approach can deliver a positive net present value to the producers (Wiangsamut and Wiangsamut, 2022). Consumer preference leans toward 'Monthong', with 54% favoring its mild aroma and mildly sweet, crispy yet soft golden-yellow flesh (Wiangsamut and Wiangsamut, 2023). The 'Puangmanee' and 'Kradum Thong' durian varieties are also highly favored for both cultivation and consumption domestically and internationally, attributed to their yellow flesh, exceptional sweetness, and mild aroma (Rojanapornthip, 2018). Tissue culture methods safeguard commercial varieties such as 'Monthong' and native ones like 'Kradum Thong' and 'Puangmanee', enabling rapid multiplication from a single culture. This results in consistent quality durians, offering benefits to farmers and enterprises alike. By 2023, durian cultivation had expanded to 43 provinces in Thailand, with the Eastern region leading in production. The total durian production in the country amounted to 1,246,098 tons, with an average yield of 8.25 tons per hectare (t/ha) (OAE, 2023).

Durian tissue culture propagation research encounters two primary obstacles. Firstly, the explants used for tissue culture are frequently contaminated by durian diseases. These diseases encompass fruit rot instigated by *Lasiodiplodia* sp. (Wiangsamut *et al*., 2023), root and foot rot caused by *Phytophthora palmivora* (Phal *et al*., 2024; Tongon *et al*., 2018), and impairment to water and nutrient-conducting vessels by *Fusarium* sp.

(Pongpisutta *et al*., 2023). Attaining pathogen-free tissue via efficient sterilization and microorganism elimination is imperative for the successful implementation of durian tissue culture. Secondly, the formation of phenolic compounds resulting from the excised durian explant poses challenges. These compounds hinder successful induction rates and the growth of callus and new shoots. This is supported by Jarret *et al*. (1985), who reported that banana tissue cultures often experience excessive blackening (or browning) due to the oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates create a barrier around the tissue, preventing nutrient uptake and hindering growth. Similarly, Permadi *et al*. (2024) stated that browning primarily occurs in response to enzymatic reactions due to explant damage. Left untreated, it can lead to reduced regeneration capacity, hindered callus proliferation, impeded development of adventitious shoots, and, in extreme cases, tissue necrosis. Woody plant species, including trees and shrubs, often have a higher phenolic content in their tissues, which can lead to browning during tissue culture (Hesami *et al*., 2018; Gao *et al*., 2019).

Kongnakorn (1983) conducted a study into the development of 'Durian Nok' embryo in a modified Murashige and Skoog (MS) medium by incorporating 0.2 mg/L of 2,4-dichlorophenoxyacetic acid (2,4-D), finding that the tissue could produce callus. Subsequent transfer of the callus to a medium containing α-naphthaleneacetic acid (NAA) and Kinetin revealed that all concentrations were effective in stimulating callus formation consistently. On the other hand, Namhormchan (1999) explored the cultivation of apical and lateral bud tissues that had been sterilized using mercury in Woody Plant Medium (WPM) supplemented with cytokinin group substances [BA, 2ip (isopentenyl adenine), or kinetin] at varying concentrations of 0, 0.25, 0.5, 1, 2, and 4 mg/L. The study revealed that apical and lateral bud tissues transformed into stipules (leaf ears), with media containing 2ip demonstrating higher vitality induction in apical and lateral buds compared to BA and kinetin; nevertheless, lateral buds did not progress into shoots. All durian varieties release phenolic compounds (or mucilage) when the juvenile parts of the plant are incised. Phenolic compounds (PCs) constitute a chemically diverse group of secondary and reactive metabolites synthesized in plants through the shikimate-phenylpropanoid pathways. These compounds can impede plant growth by internally regulating auxin transport and enzymatic activity, thereby averting tumorigenesis (Rasouli *et al*., 2016). Building upon these initial obstacles, the study aimed to evaluate the effectiveness of different sterilizing agents in achieving sterility for durian lateral bud explants across 'Monthong', 'Kradum Thong' and 'Puangmanee' durian varieties. Moreover, it compared various inhibitors for phenolic compound production to improve the survival rate of

these explants. Additionally, the study explored the efficiency of different types and concentrations of plant hormones in stimulating lateral bud callus and new shoot formation *in vitro*.

Materials and methods

The study was conducted at the Plant Tissue Culture Laboratory, located at the Chanthaburi Campus of Rajamangala University of Technology Tawan-Ok, spanning a duration of one year from September 14, 2021, to September 13, 2022. Lateral branch tips from the durian varieties 'Monthong', 'Kradum Thong' and 'Puangmanee' were collected from trees in the orchards of the University, local farmer orchards, and durian seedlings in nurseries in Khao Khitchakut District and Chanthaburi province, Thailand. Subsequently, three experiments were conducted as outlined below:

First Experiment: Evaluate the efficacy of different sterilizing agents in achieving sterility of durian lateral bud explant samples, aiming to improve the survival rates of these explants

The study used a Completely Randomized Design (CRD) experiment with 9 treatments, each treatment had 5 replications, and each replication had 3 bottles for each type of lateral bud explant and each durian variety, resulting in a total of 405 bottles (135 bottles per durian variety). The lateral bud explant samples were extracted from the juvenile branches in the 'Paysalat leaf' stage (3rd to 5th leaf from the apex) of 'Monthong', 'Kradum Thong', and 'Puangmanee' durian varieties. The term "Paysalat leaf" denotes a leaf that has fully unfurled, displaying a green color that is neither too young nor too mature, and still maintains a soft structural texture. These samples were obtained from cultivation plots and were then subjected to sterilization, comparing two sterilizing agents: Clorox [sodium hypochlorite (NaOCl)] and $HgCl_2$, at various concentrations and durations as follows: 1) 30% Clorox for 15 minutes, then 5% Clorox for 10 minutes—designated as the control treatment; 2) 25% Clorox for 15 minutes, then 5% Clorox for 10 minutes; 3) 20% Clorox for 15 minutes, then 5% Clorox for 10 minutes; 4) 0.4% $HgCl_2$ for 10 minutes; 5) 0.3% $HgCl_2$ for 10 minutes; 6) 0.2 $HgCl_2$ for 10 minutes; 7) 0.15% $HgCl_2$ for 10 minutes; 8) 0.10% $HgCl_2$ for 10 minutes; 9) 0.05% $HgCl_2$ for 10 minutes. Following this, the explants were placed on Woody Plant Medium (WPM) for 2 weeks under fluorescent lighting with an intensity of 1,000 lux for 16 hours each day at a temperature of 25-27 °C. Data collection involved documenting changes in tissue characteristics and the survival rate of the explants, which were further

classified into contamination-free survival rate, infection rate, and contamination-free mortality rate.

Second Experiment: Comparing the different types of inhibitors in order to enhance the survival rate of durian lateral bud explants by inhibiting the formation of phenolic compounds

The study was conducted using a CRD with 15 treatments, each consisting of 5 replications, and 3 bottles per type of lateral bud explant and durian variety. A total of 675 bottles were used, with 225 bottles per durian variety. The WPM liquid media were prepared and phenolic compound (mucilage) inhibitors, such as polyvinylpyrrolidone (PVP), powdered activated charcoal (PAC), and ascorbic acid (AA), were added at different concentrations. The treatments included: 1) WPM Control without PVP, PAC, and AA; 2) WPM+0.5 g PVP/liter (L); 3) WPM+1.0 g PVP/L; 4) WPM+5.0 g Powdered Activated Charcoal(PAC)/L; 5) WPM+10.0 g PAC/L; 6) WPM+1.0 mg Ascorbic Acid(AA)/L; 7) WPM+2.0 mg AA/L; 8) WPM+5.0 g PAC/L+0.5 g PVP/L+1.0 mg AA/L; 9) WPM+5.0 g PAC/L+0.5 g PVP/L+2.0 mg AA/L; 10) WPM+5.0 g PAC/L+1.0 g PVP/L+1.0 mg AA/L; 11) WPM+5.0 g PAC/L+1.0 g PVP/L+2.0 mg AA/L; 12) WPM+10.0 g PAC/L+0.5 g PVP/L+1.0 mg AA/L; 13) WPM+10.0 g PAC/L+0.5 g PVP/L+2.0 mg AA/L; 14) WPM+10.0 g PAC/L+1.0 g PVP/L+1.0 mg AA/L; and 15) WPM+10.0 g PAC/L+1.0 g PVP/L+2.0 mg AA/L. The lateral bud explants, sterilized under optimal conditions in the first experiment, were then transferred to the prepared liquid media and exposed to fluorescent light with an intensity of 1,000 lux for 16 hours per day at a temperature of 25-27 °C. The experiment lasted for one month, during which the percentage of non-phenolic compounds was assessed by observing changes in tissue characteristics and capturing images for all three durian varieties.

Third Experiment: Comparing the efficiency of lateral bud explants, various types and concentrations of plant hormones, in relation to callus formation and the initiation of new shoots across the three durian varieties

The study conducted using a CRD with 18 treatments (TRT), 3 replications, and 5 bottles per replication. A total of 810 bottles were used, with 270 bottles per durian variety. WPM (McCown and Lloyd, 1981) and MS (Murashige and Skoog, 1962) solid media were prepared. Lateral bud explants of ‘Monthong’, ‘Kradum Thong’, and ‘Puangmanee’ durian varieties were grown on MS and WPM solid media. Additionally, both WPM and MS solid

media were supplemented with plant hormones—specifically, BA (6-Benzyladenine) and TDZ (Thidiazuron)—at various concentrations. The cytokinin known as BA (6-Benzyladenine) and BAP (6-Benzylaminopurine) are two names for the same substance (Da Siva, 2012). The 18 treatments (TRT) were: 1) WPM Control as T1; 2) WPM+1.0 mg BA/L as T2; 3) WPM+2.0 mg BA/L as T3; 4) WPM+3.0 mg BA/L as T4; 5) WPM+1.0 mg BA/L+10% coconut water as T5; 6) WPM+1.0 mg TDZ/L as T6; 7) WPM+2.0 mg TDZ/L as T7; 8) WPM+3.0 mg TDZ/L as T8; 9) WPM+1.0 mg TDZ/L+10% coconut water as T9; 10) MS Control as T10; 11) MS+1.0 mg BA/L as T11; 12) MS+2.0 mg BA/L as T12; 13) MS+3.0 mg BA/L as T13; 14) MS+1.0 mg BA/L+10% coconut water as T14; 15) MS+1.0 mg TDZ/L as T15; 16) MS+2.0 mg TDZ/L as T16; 17) MS+3.0 mg TDZ/L as T17; and 18) MS+1.0 mg TDZ/L+10% coconut water as T18. Following the second experiment, lateral bud explants with reduced phenolic compounds were obtained and transferred to culture on MS and WPM solid media to evaluate callus formation and new shoot induction in 'Monthong', 'Kradum Thong', and 'Puangmanee' durian varieties. These explants were then placed under fluorescent lighting with an intensity of 1,000 lux for 16 hours each day at a temperature of 25-27 °C. The experimental results were documented by observing callus length, callus characteristics, new shoot length, and new shoot characteristics. Each experimental set lasted for 7 months in the 'Monthong' and 'Kradum Thong' durian varieties, with subculturing performed monthly. Unfortunately, the lateral bud explants of the 'Puangmanee' durian variety dried up and died within a month after being transferred to culture on both MS and WPM solid media. As a result, it became challenging to find juvenile branches for reculturing, leading to no reculture within the 7-month period.

Data analysis

To identify any statistically significant differences between the treatment means, a one-way analysis of variance (ANOVA) was performed using the Statistix 7 (SXW) software. Duncan's Multiple Range Test (DMRT) was then used for mean comparisons at a 0.05 probability level.

Results

Sterilizing agents for durian lateral bud explant

The finding revealed that the type, concentration, and duration of sterilization agents had significantly ($P < 0.05$) influenced the contamination-

free survival rates, infection rates, and contamination-free mortality rates for all three durian varieties over a 14-day period (Tables 1-3). Lateral bud explants from all three durian varieties that were submerged in a 0.3% $HgCl_2$ solution for 10 minutes demonstrated significantly higher contamination-free survival rates compared to the control treatments (30% Clorox for 15 minutes, followed by 5% Clorox for 10 minutes) (Table 1). These contamination-free survival rates were 70% (‘Monthong’), 60% (‘Kradum Thong’), 50% (‘Puangmanee’), and 0% for the control treatments across all three durian varieties. Similarly, lateral bud explants from ‘Monthong’, ‘Kradum Thong’, and ‘Puangmanee’ that were submerged in a 0.3% $HgCl_2$ solution for 10 minutes exhibited significantly ($P < 0.05$) lower infection rates than the control treatments. Across all three durian varieties, the infection rates remained steady at 30%, whereas the control treatments exhibited 100% infection rate for each of the durian varieties (Table 2).

The lateral bud explants of ‘Kradum Thong’ and ‘Puangmanee’ submerged in a 0.4% $HgCl_2$ solution for 10 minutes exhibited significantly higher contamination-free mortality rates ($P < 0.05$) in comparison to the control treatments, with the exception of ‘Monthong’ (Table 3). Specifically, the contamination-free mortality rates were recorded at 70% for both ‘Kradum Thong’ and ‘Puangmanee’, while the control treatments for these durian varieties exhibited 0% contamination-free mortality rates (Table 3). Conversely, lateral bud explants of ‘Monthong’, ‘Kradum Thong’, and ‘Puangmanee’ submerged in various rest treatments (including 25% Clorox for 15 minutes followed by 5% Clorox for 10 minutes, 20% Clorox for 15 minutes followed by 5% Clorox for 10 minutes, 0.3% $HgCl_2$ for 10 minutes, 0.2% $HgCl_2$ for 10 minutes, 0.15% $HgCl_2$ for 10 minutes, 0.10% $HgCl_2$ for 10 minutes, and 0.05% $HgCl_2$ for 10 minutes) did not exhibit any significant differences ($P \geq 0.05$) when compared to the control treatment (Table 3).

Through the process of sterilizing lateral bud explants from all three durian varieties using varying concentrations and durations of Clorox solution, it was consistently found that a survival rate of 0%, an infection rate of 100%, and a contamination-free mortality rate of 0% were achieved. Similar results were observed when using lower concentrations of mercuric chloride solution (beginning at 0.15%) for a duration of 10 minutes (Tables 1-3). Based on the result, the optimal concentration and duration for sterilizing lateral bud explants from all three durian varieties was a 0.3% $HgCl_2$ solution for 10 minutes. This treatment led to the highest contamination-free survival rate, showing a significant ($P < 0.05$) difference compared to the other treatments that were tested.

Table 1. Contamination-free survival rate (%) of lateral bud explants from three durian varieties on WPM solid medium following a 2-week sterilization

Types of sterilizing agents, concentration, and duration of sterilization	Contamination-free survival rate (%)		
	'Monthong'[1]	**'KradumThong'**[1]	**'Puangmanee'**[1]
30% Clorox for 15 min followed by 5% Clorox for 10 min (Control treatment)	0^{b}	0^{b}	0^{b}
25% Clorox for 15 min followed by 5% Clorox for 10 min	0^{b}	0^{b}	0^{b}
20% Clorox for 15 min followed by 5% Clorox for 10 min	0^{b}	0^{b}	0^{b}
0.4% $HgCl_2$ for 10 min	60^{a}	30^{ab}	30^{ab}
0.3% $HgCl_2$ for 10 min	70^{a}	60^{a}	50^{a}
0.2% $HgCl_2$ for 10 min	30^{ab}	40^{ab}	40^{ab}
0.15% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
0.10% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
0.05% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
F-test	*	*	*

[1]/ The different letters in the same column are significantly different ($p < 0.05$) through Duncan's Multiple Range Test (DMRT); A single star (*) in each column indicates statistical significance ($p < 0.05$); "min" refers to minutes

Table 2. Infection rate (%) of lateral bud explants from three durian varieties on WPM solid medium following a 2-week sterilization

Types of sterilizing agents, concentration, and duration of sterilization	Infection rate (%)		
	'Monthong'[1]	**'Kradum Thong'**[1]	**'Puangmanee'**[1]
30% Clorox for 15 min followed by 5% Clorox for 10 min (Control treatment)	100^{a}	100^{a}	100^{a}
25% Clorox for 15 min followed by 5% Clorox for 10 min	100^{a}	100^{a}	100^{a}
20% Clorox for 15 min followed by 5% Clorox for 10 min	100^{a}	100^{a}	100^{a}
0.4% $HgCl_2$ for 10 min	20^{cd}	0^{d}	0^{d}
0.3% $HgCl_2$ for 10 min	30^{bcd}	30^{bcd}	30^{bcd}
0.2% $HgCl_2$ for 10 min	70^{ab}	60^{abc}	50^{bc}
0.15% $HgCl_2$ for 10 min	100^{a}	100^{a}	100^{a}
0.10% $HgCl_2$ for 10 min	100^{a}	100^{a}	100^{a}
0.05% $HgCl_2$ for 10 min	100^{a}	100^{a}	100^{a}
F-test	*	*	*

[1]/ The different letters in the same column are significantly different ($p < 0.05$) through Duncan's Multiple Range Test (DMRT); A single star (*) in each column indicates statistical significance ($p < 0.05$); "min" refers to minutes

Table 3. Contamination-free mortality rate (%) of lateral bud explants from three durian varieties on WPM solid medium following a 2-week sterilization

Types of sterilizing agents, concentration, and duration of sterilization	Contamination-free mortality rate (%)		
	'Monthong'[/1]	**'Kradum Thong'**[/1]	**'Puangmanee'**[/1]
30% Clorox for 15 min followed by 5% Clorox for 10 min (Control treatment)	0^{b}	0^{b}	0^{b}
25% Clorox for 15 min followed by 5% Clorox for 10 min	0^{b}	0^{b}	0^{b}
20% Clorox for 15 min followed by 5% Clorox for 10 min	0^{b}	0^{b}	0^{b}
0.4% $HgCl_2$ for 10 min	20^{b}	70^{a}	70^{a}
0.3% $HgCl_2$ for 10 min	0^{b}	10^{b}	20^{b}
0.2% $HgCl_2$ for 10 min	0^{b}	0^{b}	10^{b}
0.15% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
0.10% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
0.05% $HgCl_2$ for 10 min	0^{b}	0^{b}	0^{b}
F-test	ns	*	*

[1]/ The different letters in the same column are significantly different ($p < 0.05$) through Duncan's Multiple Range Test (DMRT); An 'ns' in a column indicates non-significance ($p \geq 0.05$); A single star (*) in each column indicates statistical significance ($p < 0.05$); "min" refers to minutes

Phenolic compound inhibitors

On studying the effects of WPM liquid medium supplemented with ascorbic acid (AA) (referred to as WPM+AA) and the WPM liquid medium supplemented with Polyvinylpyrrolidone (PVP) (referred to as WPM+PVP) on 'Monthong' durian lateral bud explants for a week, it was observed both aforementioned media significantly ($P < 0.05$) reduced mucilage (phenolic compounds) in comparison to the WPM liquid medium alone [denoted as WPM Control] (Figure 1). Similar results were observed when comparing it to the WPM liquid medium supplemented with powdered activated charcoal (PAC) (data not shown). Over a period of 1 month, it was revealed that the WPM+1.0 g PVP/L (WPM liquid medium supplemented with PVP at a concentration of 1.0 g/L) achieved the most effective mucilage reduction of 100% across 'Monthong', 'Kradum Thong', and 'Puangmanee' durian varieties (Table 4). This medium effectively dissolved mucilage in the cut area of the durian explant, and the measured value was significantly better ($P < 0.05$) than the WPM liquid medium without any additives [WPM Control] (Table 4). Using ascorbic acid (AA) was the next most effective choice for dissolving mucilage in the cut area of durian explant, although it was not as effective as PVP. On the other hand, powdered activated charcoal (PAC) did not effectively dissolve

mucilage but rather aggregated it, even when used in combination with PVP or ascorbic acid (Table 4). Therefore, it is recommended to culture the lateral buds of all three durian varieties in WPM liquid medium supplemented with PVP at a concentration of 1.0 g/L (WPM+1.0 g PVP/L) over a month (Table 4 and Figure 2) in order to reduce mucilage (phenolic compounds).

Figure 1. The level of phenolic compounds (mucilage) in 'Monthong' lateral bud explants cultured in WPM liquid medium supplemented with inhibitors [ascorbic acid (AA) and polyvinylpyrrolidone (PVP)] monitored for 7 days: WPM Control (a); WPM+AA (b); WPM+PVP (c)

Table 4. Percentage of non-phenolic compounds after a month, that was detected in lateral bud explants from three durian varieties in WPM liquid medium measured in 15 experimental sets following the addition of mucilage-inhibiting agents

Types of Mucilage-Inhibiting Agents and Concentration	**Non-phenolic compounds (%)**		
	'Monthong'[1/]	**'Kradum Thong'**[1/]	**'Puangmanee'**[1/]
WPM Control	30.0±2.9^{e}	30.3±1.5^{d}	26.7±1.7^{c}
WPM+0.5 g PVP/L	61.7±1.7^{g}	42.7±1.5^{e}	40.3±0.9^{e}
WPM+1.0 g PVP/L	100.0±0.0^{h}	100.0±0.0^{g}	100.0±0.0^{g}
WPM+5.0 g PAC/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
WPM+10.0 g PAC/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
WPM+1.0 mg AA/L	51.3±2.4^{f}	31.7±1.5^{d}	30.3±1.5^{d}
WPM+2.0 mg AA/L	60.3±0.9^{g}	50.7±1.8^{f}	66.1±3.8^{f}
WPM+5.0 g PAC/L+0.5 g PVP/L+1.0 mg AA/L	12.3±0.9^{b}	11.3±0.9^{b}	14.0±0.6^{b}
WPM+5.0 g PAC/L+0.5 g PVP/L+2.0 mg AA/L	11.3±0.9^{b}	13.3±0.7^{b}	12.3±0.9^{b}
WPM+5.0 g PAC/L+1.0 g PVP/L+1.0 mg AA/L	21.7±1.5^{d}	18.7±2.7^{c}	12.0±0.6^{b}
WPM+5.0 g PAC/L+1.0 g PVP/L+2.0 mg AA/L	16.7±0.9^{c}	14.7±0.3^{b}	15.7±0.9^{b}
WPM+10.0 g PAC/L+0.5 g PVP/L+1.0 mg AA/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
WPM+10.0 g PAC/L+0.5 g PVP/L+2.0 mg AA/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
WPM+10.0 g PAC/L+1.0 g PVP/L+1.0 mg AA/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
WPM+10.0 g PAC/L+1.0 g PVP/L+2.0 mg AA/L	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
F-test	*	*	*

[1/] The different letters in the same column are significantly different ($p < 0.05$) through Duncan's Multiple Range Test (DMRT); A single star (*) in each column indicates statistical significance ($p < 0.05$)

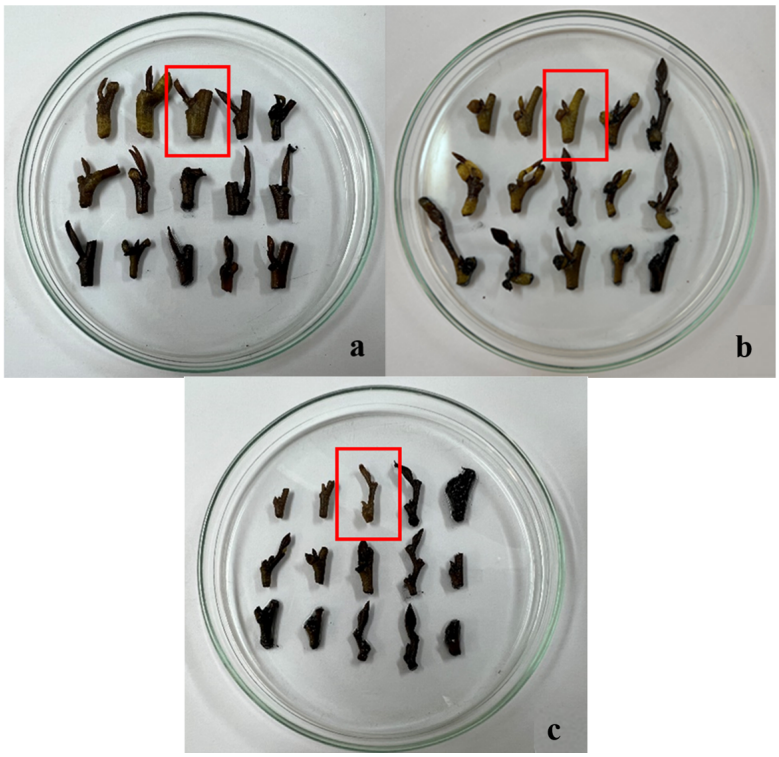

Figure 2. Lateral bud explants treated with mucilage-reducing agents in all 15 experimental sets for each variety: 'Monthong' (a); 'Kradum Thong' (b); and 'Puangmanee'(c); lateral bud explants in the petri dishes marked by red rectangles were treated with Woody Plant Medium (WPM) liquid medium supplemented with 1.0 g PVP/L, symbolized as WPM+1.0 g PVP/L

Callus formation

During the 2-week period, the sterile durian lateral bud explants of 'Monthong' and 'Kradum Thong' cultured on WPM Control solid medium, maintained their cleanliness and viability (Figure 3). Despite the absence of callus formation in a short timeframe, 'Monthong' displayed a larger lateral bud explant compared to 'Kradum Thong' due to their genetic differences (Figure 3). In the third month, lateral bud explants of 'Monthong' cultured on solid medium with T15 exhibited the significantly longest callus length at 1.65±0.49 cm ($P < 0.05$), followed by T18 (1.25±0.35 cm), T9 (0.90±0.07 cm), and T17 (0.85±0.21 cm). Notably, these callus formations were considered longer than that of T1, which measured 0.55±0.07 cm (Table 5). Meanwhile, callus formations in treatments T2, T3, T4, T5, T6, T7, T8, T10, T11, T12, T13, T14, and T16 were not significantly different ($P \geq 0.05$) from the control treatment

(T1), based on callus length values (Table 5). By the seventh month, only lateral bud explants of **'**Monthong' cultured on solid medium with T9 exhibited a significantly larger green callus length than the WPM Control (Figures 4c and 4a). It was observed that the lateral bud explants on the WPM Control transitioned from green callus to brown callus color and eventually began to dry up (Figure 4a). In contrast, the callus of 'Monthong' cultured on solid medium with T15 changed from green to brown color and then started to dry up, resembling the WPM Control (Figures 5c and 4a), while the lateral bud explant cultured on T10 exhibited the development of a new shoot (Figure 5a).

New shoot induction

In the third month, the T14 solid medium formula led to new shoot induction with leaf growth, resulting in an average length of 1.00±0.42 cm from the 'Monthong' lateral bud explant (Table 5, Figures 4a and 5b). This length was significantly ($P < 0.05$) longer than that of T1 (WPM Control), which had an average length of 0.05±0.07 cm with no leaf growth. Additionally, the T4 solid medium induced new shoot growth with leaf development that did not exhibit significant differences ($P \geq 0.05$) compared to T1. However, its length was 0.85±1.06 cm longer than that of T1 (Table 5, Figures 4a and 4b). The solid media that were left (T2, T3, T5, T6, T7, T8, T9, T10, T11, T12, T3, T15, T16, T17, and T18) showed no significant differences ($P \geq 0.05$) in new shoot induction when compared to T1 (Table 5). By the seventh month, some of the emerged leaves from the lateral bud explant fell, and the explant size decreased on T4 solid medium. Conversely, the lateral bud explant on T1 dried up and eventually died (Figures 4a and 4b). Meanwhile, the emerged leaf of the lateral bud explant on T14 (MS+1.0 mg BA/L+10% coconut water) solid medium also fell, dried up, and eventually died, similar to T1 (Figures 4a and 5b).

When culturing lateral bud explants of the 'Kradum Thong' durian variety across 18 different solid media (T1-T18), it was noted that there was an absence of callus formation. Nevertheless, there was evidence of new shoot induction taking place (Figures 6 and 7). During the third month, only four solid media formulations—T4, T8, T14, and T16—demonstrated significantly better induction of new shoots with leaf growth compared to T1 (Figures 6a, 6b, 6c, 7b, and 7c). This pattern persisted until the seventh month, when these same four media formulations surpassed the control, promoting vigorous shoot development. Notably, T4 led to lateral bud explants dividing into two shoots with leaf growth, while T8 encouraged the growth of larger shoots with green leaf color (Figures 6b and 6c). Despite the initial observation in the 'Kradum Thong' durian variety where the T10 solid medium triggered new shoot

formation with more extensive leaf growth than T1, by the third month of tissue culture, the emerging leaf dried out, while the lateral bud explant remained viable (Figures 6a and 7a). By the seventh month, the rate of new shoot induction was slower than that of T1 (Figures 6a and 7a).

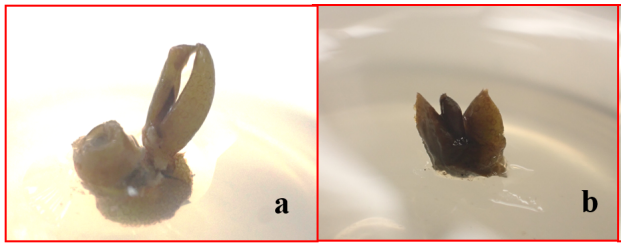

Figure 3. Condition of sterile durian lateral bud explants following 2 weeks of culture on solid WPM Control medium: 'Monthong' (a) and 'Kradum Thong' (b)

Table 5. Callus and new shoot lengths of 'Monthong' durian lateral bud explants induced by WPM and MS solid media supplemented with different concentrations of BA and TDZ over a 3-month period

Types of solid media supplemented with different concentrations of plant hormones	**Callus length[/1] (cm)**	**New shoot length[/1] (cm)**
T1 (WPM Control)	0.55±0.07^{abc}	0.05±0.07^{b}
T2 (WPM supplemented with 1.0 mg BA/L)	0.30±0.07^{abc}	0.10±0.14^{ab}
T3 (WPM supplemented with 2.0 mg BA/L)	0.70±0.42^{bc}	0.00±0.00^{b}
T4 (WPM supplemented with 3.0 mg BA/L)	0.55±0.35^{abc}	0.85±1.06^{ab}
T5 (WPM supplemented with 1.0 mg BA/L and 10% coconut water)	0.50±0.07^{abc}	0.00±0.00^{b}
T6 (WPM supplemented with 1.0 mg TDZ/L)	0.25±0.07^{ab}	0.00±0.00^{b}
T7 (WPM supplemented with 2.0 mg TDZ/L)	0.30±0.07^{abc}	0.00±0.00^{b}
T8 (WPM supplemented with 3.0 mg TDZ/L)	0.55±0.07^{abc}	0.50±0.70^{ab}
T9 (WPM supplemented with 1.0 mg TDZ/L and 10% coconut water)	0.90±0.07^{cd}	0.00±0.00^{b}
T10 (MS Control)	0.25±0.07^{ab}	0.00±0.00^{b}
T11 (MS supplemented with 1.0 mg BA/L)	0.65±0.07^{bcd}	0.00±0.00^{b}
T12 (MS supplemented with 2.0 mg BA/L)	0.25±0.35^{ab}	0.30±0.42^{ab}
T13 (MS supplemented with 3.0 mg BA/L)	0.30±0.42^{abcd}	0.00±0.00^{b}
T14 (MS supplemented with 1.0 mg BA/L and 10% coconut water)	0.00±0.00^{a}	1.00±0.42^{a}
T15 (MS supplemented with 1.0 mg TDZ/L)	1.65±0.49^{e}	0.35±0.07^{ab}
T16 (MS supplemented with 2.0 mg TDZ/L)	0.45±0.21^{abc}	0.65±0.77^{ab}
T17 (MS supplemented with 3.0 mg TDZ/L)	0.85±0.21^{bcd}	0.00±0.00^{b}
T18 (MS supplemented with 1.0 mg TDZ/L and 10% coconut water)	1.25±0.35^{de}	0.00±0.00^{b}
F-test	*	*

[1]/ The different letters in the same column are significantly different ($p < 0.05$) through Duncan's Multiple Range Test (DMRT); A single star (*) in each column indicates statistical significance ($p < 0.05$)

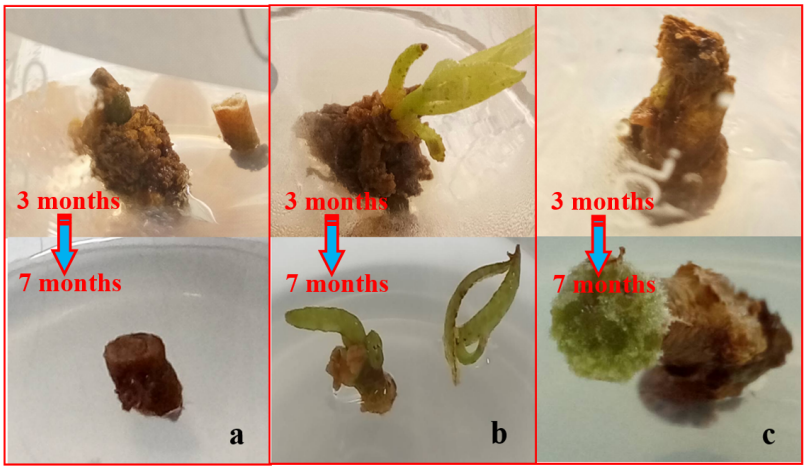

Figure 4. Callus formation and new shoot induction of 'Monthong' durian on WPM solid media supplemented with BA and TDZ from the 3rd to 7th month: WPM Control (a); WPM supplemented with 3.0 mg BA/L (b) and; WPM supplemented with 1.0 mg TDZ/L and 10% coconut water (c)

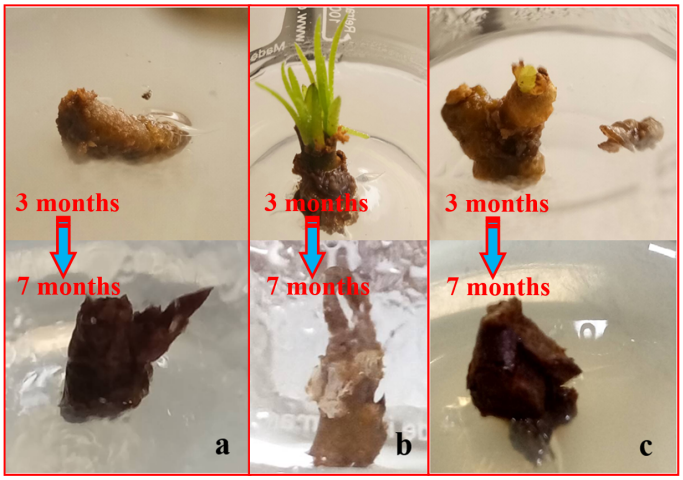

Figure 5. Callus formation and new shoot induction of 'Monthong' durian on MS solid media supplemented with BA and TDZ from the 3rd to 7th month: MS Control (a); MS supplemented with 1.0 mg BA/L and 10% coconut water (b) and; MS supplemented with 1.0 mg TDZ/L (c)

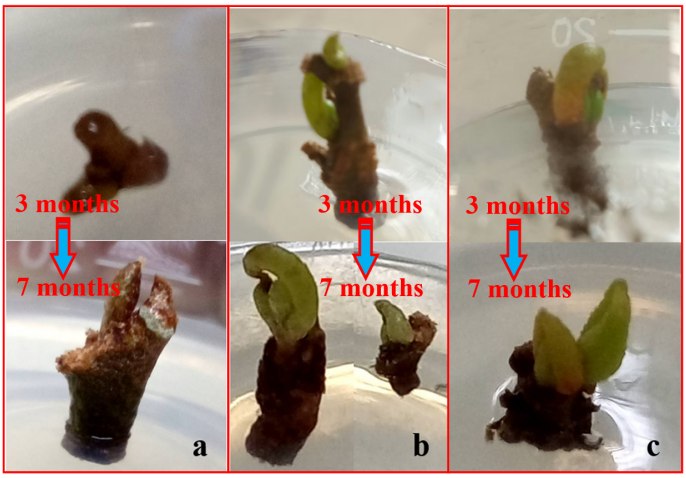

Figure 6. Callus formation and new shoot induction of 'Kradum Thong' durian on WPM solid media supplemented with BA and TDZ from the 3rd to 7th month: WPM Control (a); WPM supplemented with 3.0 mg BA/L (b) and; WPM supplemented with 3.0 mg TDZ/L (c)

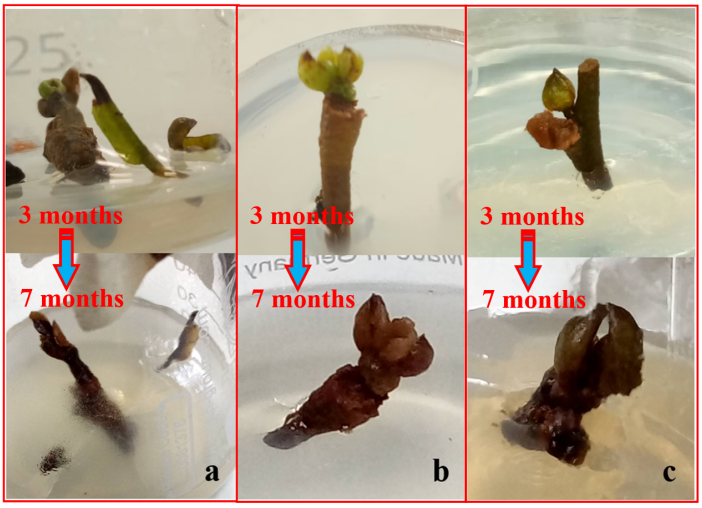

Figure 7. Callus formation and new shoot induction of 'Kradum Thong' durian on MS solid media supplemented with BA and TDZ from the 3rd to 7th month: MS Control (a); MS supplemented with 1.0 mg BA/L and 10% coconut water (b) and; MS supplemented with 2.0 mg TDZ/L (c)

Discussion

Sodium hypochlorite (NaOCl), commonly known as Clorox, used for disinfection in tissue culture, was found to be less effective than mercuric chloride in eliminating microbial contamination in this study. Specifically, when NaOCl was used at concentrations of 30% for 15 minutes followed by 5% for 10 minutes, 25% for 15 minutes followed by 5% for 10 minutes, or 20% for 15 minutes followed by 5% for 10 minutes, it resulted in 100% total infection rates across all three durian varieties. Consistent with the findings of Samiei *et al*. (2017), NaOCl was less effective than mercuric chloride ($HgCl_2$) in eliminating microbial contamination in *Dionysia tapetodes* explants. When NaOCl was used at a concentration of 3% for 15 minutes, it resulted in a 75% total infection rate. In such instances, mercuric chloride is commonly employed. However, it's important to note that while mercuric chloride effectively eliminates bacteria, there is an increased tissue mortality rate due to exposure to its high concentration. The results revealed that lateral bud explants of 'Kradum Thong' and 'Puangmanee' when submerged in a 0.4% $HgCl_2$ solution for 10 minutes, exhibited significantly higher contamination-free mortality rates ($P < 0.05$) compared to the control treatments. The control treatments involved using 30% Clorox for 15 minutes, followed by 5% Clorox for 10 minutes. Notably, this trend did not hold for the 'Monthong' variety. Interestingly, lateral bud explants of 'Monthong' displayed a higher level of tolerance towards higher concentrations of $HgCl_2$ compared to those of 'Kradum Thong' and 'Puangmanee.' Moreover, larger lateral bud explants, such as those of 'Monthong,' demonstrated enhanced resistance against the damaging effects of sterilizing agents. The findings are consistent with the genetic characteristics of these varieties: 'Monthong' possesses the largest lateral bud size, followed by 'Kradum Thong' and 'Puangmanee'. Consequently, smaller lateral bud explants are more vulnerable to drying out and death when subjected to sterilization through immersion in a 0.4% $HgCl_2$ solution for a duration of 10 minutes, as indicated by the value of contamination-free survival rate. In a study akin to that of Samiei *et al*. (2017), the most effective sterilization treatment for *Dionysia tapetodes* explants involved the application of 0.1% $HgCl_2$ for 4 minutes, resulting in 91.6% healthy explants. However, a higher 0.2% $HgCl_2$ concentration was found to be toxic to the explant tissues, resulting in 41.66% necrosis. Similarly, mercuric chloride is recognized for its high toxicity to plant tissues (Kunasakdakul *et al*., 2021). Nevertheless, the results suggest that lateral bud explants from all three durian varieties, when submerged in 0.3% $HgCl_2$ for 10 minutes, displayed significantly higher contamination-free survival rates compared to treatment

with submersion in 30% Clorox for 15 minutes, followed by 5% Clorox for 10 minutes. Similar result to that of Kermanee (1993) who recommended the use of relatively low concentration of $HgCl_2$, approximately 0.1-1.0%, applied for 2-10 minutes when employed for cleaning tissue samples prior to culturing to ensure they are free from fungi and bacteria. The effectiveness of pathogen eradication by NaOCl and $HgCl_2$ is dependent on multiple factors beyond the type and concentration of the disinfectant, including the decontamination duration. The type of tissue also plays a crucial role. In situations where the plant tissue is thick and robust, a higher concentration of disinfectant and longer soaking time might be required. Conversely, for fragile and thin plant tissue, a lower concentration and shorter exposure time may be sufficient (Kitwicharn, 2004). It's essential to take into account these factors when formulating decontamination procedures. The choice of disinfectant type and concentration, along with the decontamination duration, plays a crucial role in the treatment of various plant tissues. This study delved into the effectiveness of using $HgCl_2$ to decontaminate lateral bud explants from 'Monthong', 'Puangmanee', and 'Kradum Thong' durian varieties. The application of a 0.3% $HgCl_2$ solution for 10 minutes yielded the highest rates of contamination-free survival for the lateral bud explant of these durian varieties. Conversely, lower concentrations of $HgCl_2$ resulted in increased infection rates in lateral bud explants across all three durian varieties. In summary, the results indicate that treating with a 0.3% $HgCl_2$ solution for 10 minutes produced the highest survival rates: 70% for 'Monthong', 60% for 'Kradum Thong', and 50% for 'Puangmanee', all while maintaining contamination-free lateral bud explants.

During the one-month period, the result indicated that the most effective reduction in phenolic compounds was achieved when using a liquid medium of Woody Plant Medium supplemented with 1.0 g polyvinylpyrrolidone per liter (L). This particular treatment exhibited a 100% effectiveness rate across 'Monthong', 'Kradum Thong', and 'Puangmanee' durian varieties. Polyvinylpyrrolidone (PVP), recognized for its antioxidant properties, played a role in the reduction of phenolic compounds. Notably, inclusion of PVP as phenolic compound reducing agent did not have any negative impact on the lateral bud explant of durian, as they maintained their green and clean appearance. There was no evidence of browning in plant tissue culture observed during the 1-month period. Browning is a process caused by the oxidation of phenolic compounds through the action of polyphenol oxidase (PPO), leading to the production of dark pigments. This finding is consistent with the study of He *et al*. (2005), which indicated that PVP exhibited the highest efficacy as an anti-browning agent for *P. suffruticosa* 'Luo Yang Hong'. Adding PVP to the medium reduced PPO activity in the petal explants of *Paeonia lactiflora* for the

first two weeks, leading to a 95% suppression of browning (Cai *et al*., 2020). The PPO is a very important enzyme for the formation of the browning compound quinine (Toivonen and Brummell, 2008; Zhu *et al*., 2009). Overall, the results suggest that submerging durian lateral bud explants in a WPM liquid medium supplemented with 1.0 g PVP/L was effective in alleviating browning issues. This treatment achieved 100% effectiveness across all three durian varieties ('Monthong', 'Kradum Thong', and 'Puangmanee') over a 1-month period.

During the 7-month period of subculturing on solid medium once a month, the results revealed a distinct pattern in the lateral bud explants of 'Monthong' durian. When these explants were cultured on (WPM) supplemented with 1.0 mg TDZ/L and 10% coconut water, they displayed notably longer green callus lengths compared to all 18 tested solid media in this study. The combination of a low TDZ level with coconut water in the WPM solid medium appeared to have a significant impact on callus formation. Interestingly, the findings contradict those of Huetteman and Preece (1993), who proposed that low levels of TDZ promote axillary shoot proliferation in woody plant species, while higher levels may hinder it. Conversely, Shirani *et al*. (2010) observed that higher concentrations of TDZ impede the growth and development of the regenerants. These conflicting effects underscore the importance of precise TDZ levels for successful tissue culture protocols. In this study, it was found that higher concentrations of TDZ in WPM solid medium, specifically WPM supplemented with 3.0 mg TDZ/L, led to a significant increase in the induction of new shoots with leaf growth in 'Kradum Thong' durian over a 3-month period compared to the control (WPM alone without TDZ added). This trend persisted up to the seventh month, with the medium containing TDZ outperforming the control by promoting robust shoot and larger shoots with green leaves. Conversely, when 'Kradum Thong' durian was cultivated on MS solid medium supplemented with 2.0 mg TDZ/L, smaller shoots were observed. This observation is consistent with previous studies by Fiola *et al*. (1990) and Malik and Saxena (1992), which demonstrated that TDZ effectively breaks lateral bud dormancy and stimulates shoot development in various plant species. TDZ has been shown to trigger or boost various biological processes within cells, as demonstrated by Guo *et al*. (2011). TDZ was categorized as a cytokinin due to its induction of natural cytokinin-like responses. Subsequently, an increase in endogenous auxin, ethylene, and ABA was recorded in response to TDZ treatment (Yip and Yang, 1986; Murthy *et al*., 1995; Murthy *et al*., 1998). Ethylene, a harmful by-product of TDZ-induced metabolic processes, has been observed (Hutchinson *et al*., 1997). It has been suggested that TDZ induces a response similar to auxin, since applying auxin

increases ethylene production. It is probable that TDZ treatment does not directly induce leaf abscission but instead arises from the auxin responses mediated by TDZ. During the 7-month period of the study, a number of leaves that had sprouted from lateral bud explant of 'Monthong' variety eventually fell off. The reduction in size of the explant was linked to the presence of dead bark on the lateral bud explant, which was eliminated during each subculture on WPM supplemented with 3.0 mg BA/L. Consequently, the lateral bud explant ended up being smaller, with only a few leaves remaining.

During the 3-month period, it was observed that the addition of 3.0 mg BA/L to WPM and 1.0 mg BA/L and 10% coconut water to MS medium notably enhanced the induction of new shoots with leaf growth in 'Kradum Thong' durian compared to the control (WPM without BA supplementation). This positive effect was still evident in the seventh month, with the same two media formulations continuing to outperform the control, resulting in robust shoot development. The lateral bud explants of 'Kradum Thong' durian cultured in WPM supplemented with 3.0 mg BA/L exhibited an intriguing phenomenon by splitting into two shoots, each with leaf growth. A similar outcome was observed by Mondal *et al*. (2015) in their study on the effect of coconut water and ascorbic on the micropropagation of banana variety Dwarf Cavendish. The shoot tip was cultured on MS medium supplemented with BAP (Benzyleaminopurine) at a concentration of 5.0 mg/L, along with varying concentrations of coconut water (0, 50, 100, 150, and 200 mL/L) and ascorbic acid (0, 25, 50, 75, and 100 mg/L). An increase in the concentration of coconut water up to 100 mg/L and ascorbic acid up to 50 mg/L resulted in a significant enhancement in the frequency of explants exhibiting shoot regeneration, the number of shoots regenerated per explant, and the length of shoots, respectively. Ascorbic acid, known for its role as an antibrowning agent for fresh-cut potatoes (Xu *et al*., 2022), also acts as an antioxidant/antibrowning agent (Davis *et al*., 1974; Slavova, 1982; Gupta, 1986). The browning in plant tissue, attributed to polyphenol oxidase (Bar-NUN and Mayer, 1983), and the formation of strongly oxidising quinines, which cause poor growth, can be inhibited by ascorbate (Wickers *et al*., 1984).

In conclusion, the most effective concentration and duration for sterilizing lateral bud explants in the three durian varieties, 'Monthong', 'Kradum Thong', and 'Puangmanee', is a 0.3% mercuric chloride solution applied for 10 minutes. The treatment led to the highest contamination-free survival rate, which was significantly different from the other treatments tested ($P < 0.05$). Moreover, the liquid WPM supplemented with PVP at a concentration of 1.0 g/L successfully achieved 100% reduction in mucilage across all three durian varieties, ensuring that lateral bud explants remained

healthy and free from contamination. PVP also played a role as an antioxidant, aiding in the reduction of phenolic compound formation. Additionally, after a 7-month period of subculturing on solid medium once a month, only lateral bud explants of 'Monthong' durian cultured on WPM supplemented with 1.0 mg TDZ/L and 10% coconut water exhibited significantly longer green callus lengths compared to all 18 tested solid media. Lower TDZ levels (1 mg TDZ/L) combined with 10% coconut water in WPM solid medium promoted callus growth in 'Monthong' lateral bud explants. In terms of shoot development, WPM supplemented with 3.0 mg BA/L resulted in lateral bud explants of 'Kradum Thong' durian splitting into two shoots with leaf growth, while WPM supplemented with 3.0 mg TDZ/L promoted larger shoots with green leaf color. These results offer valuable insights for optimizing explant sterilization and growth conditions in durian tissue culture propagation.

Acknowledgements

The authors wish to extend their appreciation to the National Research Council of Thailand (NRCT), under the Plant Genetic Conservation Project initiated by Her Royal Highness Princess Maha Chakri Sirindhorn, for the funding assistance granted for this study.

References

Aziz, N. A. A. and Jalil, A. M. M. (2019). Bioactive compounds, nutritional value, and potential health benefits of indigenous durian (*Durio Zibethinus* Murr.): A review. Foods, 8:96.

Bar-NUN, N. and Mayer, A. M. (1983). Suppression of catechol oxidase of norleucine in plant suspension cultures. Phytochemistry, 22:1329-1333.

Cai, X. Wei, H., Liu, C., Ren, X., Thi, L. T. and Jeong, B. R. (2020). Synergistic effect of NaCl pretreatment and PVP on browning suppression and callus induction from petal explants of *Paeonia lactiflora* Pall. 'Festival Maxima'. Plants, 9:346.

Da Siva, J. A. T. (2012). Is BA (6-Benzyladenine) BAP (6-Benzylaminopurine)? The Asian and Australasian Journal of Plant Science and Biotechnology, 6 (Special Issue 1): 121-124.

Davis, D. G., Dusbabek, K. E. and Hoeraug, R. A. (1974). *In vitro* culture of callus tissues and cell suspensions from okra (*Hibiscus esculentus* L.) and cotton (*Gossypium hirsutum*). *In Vitro* (*Rockville*), 9:395-398.

Fiola, J. A., Hassan, M. A., Swartz, H. J., Bors, R. H. and McNicols, R. (1990). Effect of thidiazuron, light influence rates, and kanamycin on *in vitro* shoot organogenesis from excised Rubus cotyledons and leaves. Plant Cell, Tissue and Organ Culture, 20:223-228.

Gao, J., Zhang, P., Xue, J., Xue, Y., Wang, S. and Zhang, X. (2019). Advances in phenolic substances and their effects on browning in woody plant tissue culture. Acta Horticulturae Sinica, 46:1645-1654.

Guo, B., Abbasi, B. H., Zeb, A., Xu, L. L. and Wei, Y. H. (2011). Thidiazuron: A multi-dimensional plant growth regulator. African Journal of Biotechnology, 10:8984-9000.

Gupta, P. P. (1986). Eradication of mosaic disease and rapid clonal multiplication of bananas and plantains through meristem tip culture. Plant Cell, Tissue and Organ Culture, 6:33-40.

He, S. L., Chen, X. L., Chen, L., Kong, D. Z. and Ren, N. H. (2005). The preliminary research of preventing browning of peony petiole *in vitro* culture. (in Chinese). Henan Science, 23:47-50.

Hesami, M., Tohidfar, M., Alizadeh, M. and Daneshvar, M. H. (2018). Effects of sodium nitroprusside on callus browning of *Ficus religiosa*: an important medicinal plant. Journal of Forestry Research, 31:789-796.

Huetteman, A. and Preece, E. J. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture, 33:105-119.

Hutchinson, M. J., KrishnaRaj, S. and Saxena, P. K. (1997). Inhibitory effect of GA_3 on the development of thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium* x *hortorum* Bailey) hypocotyl cultures. Plant Cell Reports, 16:435-438.

Jarret, R. L., Rodriguez, W. and Fernandez, R. (1985). Evaluation, tissue culture propagation, and dissemination of 'Saba' and 'Pelipita' plantains in Costa Rica. Scientia Horticulturae, 25:137-147.

Kermanee, P. (1993). Plant cultivation techniques. Odean Advertising Publishing House, Bangkok. 158 p.

Kitwicharn, B. (2004). Plant tissue culture technology. 2nd printing. Klang Nana Witthaya Printing Press, Khon Kaen. 207 p.

Kongnakorn, P. (1983). Cultivation of 'Durian Nok' embryos in test tubes. (Master's Special Problem). Department of Horticulture. Kasetsart University, Kamphaeng Saen Campus.

Kunasakdakul, S., Tappatat, Y., Nontasawatsri, C., Kunasakdakul, K., Charoensub, R., Wannaprasert, P., Chuntaratin, P. and Ray, L. O. (2021). Plant tissue culture work base. Kaenchan Printing Center. Bangkok. 508 p.

Malik, K. A. and Saxena, P. K. (1992). Regeneration in Phaseolus vulgare L., high frequency induction of direct shoot formation in intact seedlings by N6- benzylaminopurine and thidiazuron. Planta, 186:384-389.

McCown, B. H. and Lloyd, G. (1981). Woody plant medium (WPM)—A mineral nutrient formulation for microculture of woody plant species. HortScience, 16:453-453.

Mondal, S., Ahirwar, M. K., Singh, M. K. and Singh, R. P. (2015). Effect of coconut water and ascorbic acid on shoot regeneration in banana variety Dwarf Cavendish. International Journal of Bio-resource, Environment and Agricultural Sciences, 1:65-69.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum., 15:473- 497.

Murthy, B. N. S., Murch, S. J. and Saxena, P. K. (1995). Thidiazuron-induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogea*): Endogenous growth regulator levels and significance of cotyledons. Physiologia Plantarum, 94:268-276.

Murthy, B. N. S., Murch, S. J. and Saxena, P. K. (1998) Thidiazuron: A potent regulator of *in vitro* plant morphogenesis. In Vitro Cellular & Developmental Biology - Plant, 34:267-275.

Namhormchan, S. (1999). Sterile cultivation of durian. (in Thai). (Master's Thesis). Horticulture, Graduate School, Kasetsart University. 71 p.

Office of Agricultural Economics (OAE) (2023). Durian. Retrieved from https://mis-app.oae.go.th/product/ทุเรียน

Permadi, N., Akbari, S. I., Prismantoro, D., Indriyani, N. N., Nurzaman, M., Alhasnawi, A. N., Doni, F. and Julaeha, E. (2024). Traditional and next-generation methods for browning control in plant tissue culture: Current insights and future directions. Current Plant Biology, 38:100339.

Phal, P., Soytong, K. and Poeaim, S. (2024) A new nanofibre derived from *Trichoderma hamatum* K01 to control durian rot caused by *Phytophthora palmivora*. Asian Journal of Agriculture and Biology, 2024:2023182.

Pongpisutta, R., Keawmanee, P., Sanguansub, S., Dokchan, P., Bincader, S., Phuntumart, V. and Rattanakreetakul, C. (2023). Comprehensive investigation of die-back disease caused by *Fusarium* in durian. Plants, 12:3045.

Rasouli, H., Farzaei, M. H., Mansouri, K., Mohammadzadeh, S. and Khodarahmi, R. (2016). Plant cell cancer: May natural phenolic compounds prevent onset and development of plant cell malignancy? A Literature Review. Molecules, 21:1104.

Rojanapornthip, J. (2018). Amazingly delicious Thai durian. Available at https://www.technologychaoban.com/agricultural-technology/article_53269 (May 24, 2024).

Samiei, L., Aghdaei, M. and Vessal, S. (2017). Control of *in vitro* contamination in *Dionysia tapetodes* for effective micropropagation. Journal of Ornamental Plants, 7:221-228.

Shirani, S., Mahdavi, F. and Maziah, M. (2010). Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot-tips. African Journal of Biotechnology, 8:5755-5761.

Slavova, J. V. (1982). Organogenesis of sugar beet callus cultures. Fiziol Ras (Sofia), 7:71-77.

Toivonen, P. M. A. and Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biology and Technology, 48:1-14.

Tongon, R., Soytong, K., Kanokmedhakul, S. and Kanokmedhakul, K. (2018). Nano-particles from *Chaetomium brasiliense* to control *Phytophthora palmivora* caused root rot disease in durian var. 'Montong'. International Journal of Agricultural Technology, 14:2163-2170.

Wiangsamut, B. (2024). Thai durian varieties, pests, diseases, and flowering induction. E-Book, Faculty of Agro-Industrial Technology, Rajamangala University of Technology Tawan-Ok, Chanthaburi Campus. 349 p. Retrieved from https://agro.rmutto.ac.th/?p=6047

Wiangsamut, B. and Wiangsamut, M. E. L. (2022). Effects of paclobutrazol on flowering of juvenile durian trees cv. 'Monthong' and its costs and returns of production. International Journal of Agricultural Technology, 18:2315-2328.

Wiangsamut, B. and Wiangsamut, M. E. L. (2023). Assessment of natural fruit ripening and fruit quality of three elite durian cultivars for overland export. Trends in Sciences, 20:4647.

Wiangsamut, B., Thongkamngam, T. Anutrakunchai, S., Wiangsamut, M. E. L., Poonnapirom1, N., Noiyeam, P., Chaaum, P., Suksupkit, L. and Kasang, R. (2023). Application of mangosteen peel fermented solution, *Trichoderma harzianum*, and sodium bicarbonate mixed with potassium permanganate for fruit rot control of durian cv. 'Monthong'. Rambhai Barni Rajabhat Agricultural Journal, 1:36-49.

Wiangsamut, B., Wiangsamut, M. E. L., Wattana, K., Bamrung, P. and Charroenmoon, K. (2021). Effect of ethephon on fruit ripening and fruit components of durian cv. 'Monthong' after harvest. International Journal of Agricultural Technology, 17:2021-2034.

Wickers, H. J., Peetsma, G. J., Malingré, T. M. and Huizing, H. J. (1984). Purification and properties of a phenol oxidase derived from suspension cultures of *Mucuna pruriens*. Planta, 162:334-341.

Xu, Y., Wang, D., Zhao, W., Zheng, Y., Wang, Y., Wang, P., Ma, Y. and Zhao, X. (2022). Low frequency ultrasound treatment enhances antibrowning effect of ascorbic acid in fresh-cut potato slices. Food Chemistry, 380:132190.

Yip, W. K. and Yang, S. F. (1986). Effect of thidiazuron, a cytokinin–active ureaderivative, in cytokinin-dependent ethylene production system, Plant Physiology, 80:515-519.

Zhu, L. Q., Zhou, J., Zhu, S. H. and Guo, L. H. (2009). Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. Food Chemistry, 114:174-179.

(Received: 10 July 2024, Revised: 6 March 2025, Accepted: 12 March 2025)