HAYATI
Journal of Biosciences

Vol. 31 No. 3, May 2024 603-612
DOI:10.4308/hjb.31.3.603-612
ISSN: 1978-3019
EISSN: 2086-4094

In Vitro Medium-Term Storage of Banana Cultivar 'Barangan' Using Alginate-Encapsulation Technique

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ARTICLE INFO

Article history:
Received October 23, 2022
Received in revised form October 24, 2023
Accepted December 8, 2023

KEYWORDS:
nodule-like meristem,
paclobutrazol,
sodium alginate,
thidiazuron

ABSTRACT

In vitro* medium-term storage using the alginate-encapsulation technique is essential for conserving plant genetic resources, preserving vegetatively propagated species by controlling the growth and development of explants, and maintaining plant biodiversity. The study aimed to obtain the optimum combination medium for the encapsulation of bananas and evaluate the viability of the alginate-encapsulated explants after storage. In vitro, medium- term storage of nodule-like meristem of banana cultivar 'Barangan' was performed using sodium alginate, paclobutrazol (PBZ), and Murashige and Skoog (MS) salt. This research consists of 3 stages: (1) Initiation and multiplication of *in vitro* shoots and nodule-like meristems; (2) *In vitro* medium-term storage of banana by encapsulation technique; (3) Regeneration of nodule-like meristem explants after *in vitro* storage. This research showed that the banana's number of nodule-like meristem was optimum on media supplemented with thidiazuron (TDZ) 0.22 mgL^{-1} and indole 3-acetic acid (IAA) 1.75 mgL^{-1}. Encapsulation explant of nodules-like meristems using 3% sodium alginate in full and half-strength MS salt medium supplemented with PBZ 2.5 mgL^{-1} and incubated in liquid MS medium was able to store nodule-like meristem for six months. The color of the explants remains green, and the capsule is not damaged. Sub-culture of nodule-like meristem after storage in MS salt medium containing TDZ 0.22 mgL^{-1} and IAA 1.75 mgL^{-1} showed that nodules-like meristems could regenerate to form new shoots and nodule-like meristem three months after sub-cultured. This research concludes that encapsulated nodule-like meristem was recorded until six months of storage. PBZ was a necessary retardant in minimizing the growth during storage.

1. Introduction

Bananas and plantain (*Musa* spp.) are fruit plants widely consumed in Indonesia. Indonesia is the world's second-largest producer (FAO 2021); production in 2021 was 8.741.147 tons, with an average growth rate over 2020 of 6.28% (SI 2022). Banana cv. Barangan (*Musa acuminata*, AAA genome) is a dessert banana (Valmayor *et al.* 2000) and is mainly produced in the North Sumatra area (Napitupulu 2016). This cultivar is famous for its sweet taste, fragrant aroma, and distinctive aroma (Adhany *et al.* 2018). In Southeast Asia, Barangan has other names: Lakatan (International), Berangan (Malaysia), and Kluai Hom Maew (Thailand) (Valmayor *et al.* 2000).

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Bananas propagate vegetatively through suckers, which result in low genetic diversity. Plants with low genetic diversity often become depleted due to pest and disease attacks, habitat destruction, the spread of alien plants, climate change, and changes in crop patterns. This condition is one of many factors that cause a decrease or even loss of plant genetic resources (Makhful and Nofiarly 2013; Brown and Hodgkin 2015; Begna 2021; Benelli *et al.* 2022). Therefore, conservation of genetic resources is essential for food security and agro-biodiversity. Furthermore, germplasm conservation is essential to protect sources of plant genetic diversity, short-term to long-term preservation, genetic studies, and exchangeable materials as collections (Sakhanokho *et al.* 2013; Anis and Ahmad 2016; Benelli *et al.* 2022).

In vitro preservation of genetic resources can be carried out through (1) development of a tissue

bank, (2) cryopreservation, (3) shoot culture and multiplication, and (4) *in vitro* culture for minimal plant growth (slow growth culture), and root culture of generative reproducing plants for long-term storage (Panis 2009; Anis and Ahmad, 2016; Chauchan *et al.* 2019). *In vitro* technology is currently necessary for plant diversity maintenance strategies, especially for species propagated vegetatively (Keller *et al.* 2006; Khalid and Tan 2017; Benelli *et al.* 2022; Phanomchai *et al.* 2022). According to Benson *et al.* (2011), from 28,000 accessions of plants propagated vegetatively, such as bananas, cassava, potatoes, sweet potatoes, and other tubers, 85% of them were collected *in vitro* with minimal growth and 10% with cryopreservation. The main objective of *in vitro* conservation is to reduce the growth rate by various manipulations, such as using alternatives of gelling agent (isabgol) and carbon source (Agrawal *et al.* 2010; Mohanti *et al.* 2013; El-Bahr *et al.* 2016), plant growth retardant (Indrayanti *et al.* 2019; Mendes *et al.* 2021), and low temperature (Ahmad *et al.* 2012; Chauhan *et al.* 2019). Hence, the germplasm could live longer. One of the *in vitro* conservation techniques that can be used is medium-term storage through the encapsulation technique using sodium alginate.

Encapsulation technology is an exciting and rapidly developing field of biotechnology research (Sharma *et al.* 2012a; Phanomchai *et al.* 2022). Encapsulated explants can be performed on somatic embryos, shoots, or other meristematic tissues, thereby minimizing the cost of transporting plant material for commercialization and final delivery (Rai *et al.* 2009; Hassanein *et al.* 2011; Sharma *et al.* 2012b; Rihan *et al.* 2017; Phanomchai *et al.* 2022). In addition, it can be stored for a long time without losing viability (Anis and Ahmad 2016), reducing labor and shortening the subculture time (Benelli *et al.* 2022).

The technique of encapsulation has been successfully carried out on Persian violet (Phanomchai *et al.* (2022), Begonia (Sakhanokho *et al.* 2013), and different cultivars of banana such as banana cv. 'Grande Naine' (Hamza 2013), *M. balbisiana* cv. 'Kluai Hin' (Kanchanapoom and Promsorn 2012), cv. 'Hindi' (Hassanaein *et al.* 2011), cv. 'Rasthali (Ganapathi *et al.* 2001). Several horticultural plants have reported the application of paclobutrazol in media Murashige and Skoog's as basal media for a minimal slow growth rate (Chauchan *et al.* 2019; Indrayanti *et al.* 2019; Mendes *et al.* 2021). Paclobutrazol is a retardant that inhibits gibberellin synthesis, a plant growth regulator that influences cell elongation (Desta and Amare 2021; Nagar *et al.* 2021). However, no research has been found regarding the storage of banana cv. 'Barangan' uses encapsulation techniques combined with the basal media formula and paclobutrazol as a retardant. Therefore, further research needs to be conducted to develop this method. This study aimed to obtain the optimum combination medium for the encapsulation of bananas and evaluate the viability of the alginate-encapsulated explants after storage.

2. Materials and Methods

This research was conducted at the Plant Tissue Culture Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta. This research uses experimental methods with a completely randomized design. The explant material used in this study was obtained from the collection of the Tissue Culture Laboratory, UPT Central Seed Center for Agriculture, DKI Jakarta Province. The chemicals used in this experiment are plant growth regulator (thidiazuron, 6-benzylaminopurine, and indole 3-acetic acid), plant growth retardans (paclobutrazol), vitamin, sugar, 3% sodium alginate (Sigma A2033), calcium chloride ($CaCl_2$•$2H_2O$) for encapsulation explant, and incubation media consisting of Murashige Skoog (MS) salt.

2.1. Initiation and Multiplication of *In Vitro* Shoots and Nodule-Like Meristems

This experiment aimed to determine the optimum concentration of Thidiazuron (TDZ), 6-benzylaminopurine (BAP), and indole 3-acetic acid (IAA) for the initiation and multiplication of banana cv. 'Barangan' (*Musa acuminata*, AAA). The objective was to obtain sufficient shoot explants and nodule-like meristems as source material for *in vitro* plant storage. *In vitro* banana shoot explants were grown in Murashige and Skoog (MS), two different media supplemented with BAP 6.5 mg L^{-1} + IAA 1.75 mg L^{-1}, and TDZ 0.22 mg L^{-1} + IAA 1.75 mg L^{-1}, sucrose 30 g L^{-1}, with the pH adjusted to 5.8 and solidified with 7.8 g L^{-1} commercial agar. Banana shoot explants were grown in a culture room with 16 lux lighting at 20°C±2°C for three months and subcultured three times at intervals of four weeks. The parameters measured of the existing shoots were categorized

into three groups: the number of nodules-like meristems, shoots, and leaves.

2.2. *In Vitro* Medium-Term Storage of Banana Using Alginate-Encapsulation Technique

Encapsulated banana explants consisted of nodule-like meristems or shoot clumps regenerated from *in vitro* cultures of the Barangan cultivar. The encapsulation technique was carried out aseptically in a Laminar Air Flow Cabinet using the drip method. First, the explants were encapsulated by immersing 5 mm of nodule-like meristem or shoot clump into 3% sodium alginate supplemented with MS salt nutrients (either full or half-strength) and PBZ (at 0 mg L^{-1} or 2.5 mg L^{-1}, depending on the treatment). Each treatment used 5 repeats, each consisting of 5 explants with 5-6 nodules like-meristem or shoots clump). The half-strength MS medium uses half the quantity of macro and micronutrients. The encapsulated explants were transferred to a 100 mM calcium chloride ($CaCl_2 \cdot 2H_2O$) solution and immersed for 30 minutes until the droplets hardened and formed capsules. The resulting capsules were transferred to MS liquid medium (at either full or half-strength) using a sterile spatula for incubation, and the medium was stored in a culture room at 20°C±2°C for medium-term storage.

This experiment was observed up to six months after storage, and the data was recorded based on the encapsulated explants' quality. The number of encapsulated explants that grew or penetrated the capsule (score 3), the number of green explants (score 2), and brownish explants (score 1) were all recorded. The number of encapsulated explants that grew in the liquid medium was then calculated and converted into percentages to determine the effectiveness of the various treatment combinations for *in vitro* medium-term storage of encapsulated explants.

2.3. Regeneration of Encapsulated Explants after Medium-Term Storage

The encapsulated explants were regenerated after six months of *in vitro* storage. This stage was carried out to measure the viability of the explant to grow after the storage period. First, the explant on the green alginate capsule was removed using a scalpel and then cultured on the regeneration medium. The regeneration medium used was MS salts containing TDZ 0.22 mg L^{-1} and IAA 1.75 mg L^{-1}. The explants were stored in a culture room under lighting and maintained at 20°C±2°C. The viability and regeneration of explants were observed for three months. The parameters observed included the number of shoots and the number of nodules-meristems. The data were analyzed using descriptive statistics with standard error (SE) and the Duncan Multiple Range Test (DMRT) at α 0.05.

3. Results

3.1. Initiation and Multiplication of *In Vitro* Shoots and Nodule-Like Meristems

In vitro conservation of banana germplasm through the encapsulation-alginate technique requires sufficient explant material to allow encapsulation. Therefore, the banana shoot explants were growing in MS medium to compare the effectiveness of TZD or BAP to induce multiple nodule-like meristems and shoots of banana explants. This research shows several new shoots growing on the fifth day after culture. After one month of culture, the existing shoots were categorized into three groups: shoots with fully opened leaves, shoots with unopened leaf primordia, and nodules-like meristems, which are protrusions of shoots (Figure 1).

Explants grown on MS media supplemented with 0.22 mg L^{-1} TDZ and 1.75 mg L^{-1} IAA produced a significantly higher number of nodule-like meristem (6.42±0.76) and shoots (6.32±0.91) per explant than those grown on med5a containing 6.5 mg L^{-1} BAP and 1.75 mg L^{-1} IAA after three months of culture (Table 1). The number of leaves per explant did not differ significantly between the two treatments, although leaves produced from additional BAP were phenotypically wider (Figure 1).

3.2. *In Vitro* Medium-Term Storage of Banana Using Alginate-Encapsulation Technique

In vitro storage of bananas using 3% sodium alginate for six months showed that the lowest percentage (<16.70%) of encapsulated explants growing outside of the capsule and the explant remained green was obtained from encapsulated explants with 2.5 mg L^{-1} paclobutrazol (PBZ) and incubated in full strength (A_4L_1) and a half-strength MS liquid medium (A_3L_2 and A_4L_2) (Table 2). Therefore, it is concluded that this treatment is the best combination for *in vitro* medium-term storage of bananas. The score is 2.00–2.16, meaning

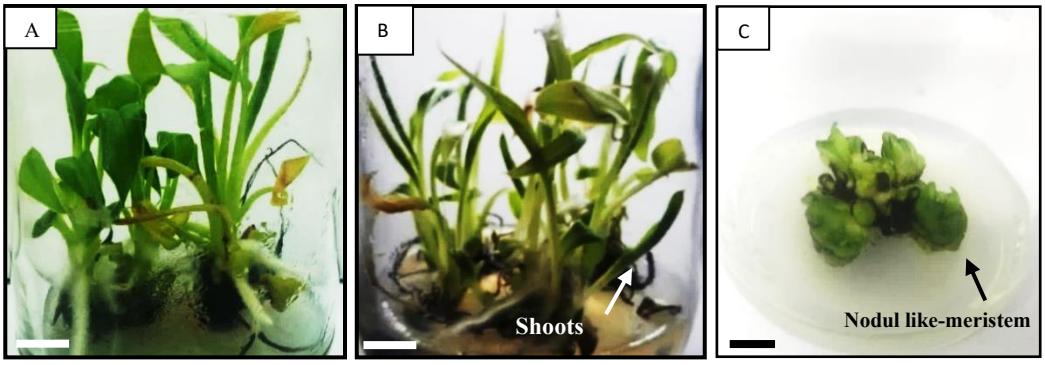

Figure 1. Representatives of banana shoots and leaves after 2 months of culture on MS medium supplemented with (A) BAP and IAA (A), (B) TDZ and IAA. (C) Media with TDZ produced more nodules and shoots after 3 months of culture (white and black scale bar represented 1 cm)

Table 1. Effect of plant growth regulator on the average number of the nodule-like meristem, shoots, and leaves number per explant of banana cv. 'Barangan'

MS medium supplemented	Nodule-like meristem		Shoots numbers		Leaves numbers	
	1 month	3 month	1 month	3 month	1 month	3 month
BAP 6.5 mg L^{-1} + IAA 1.75 mg L^{-1}	1.35^{a}±0.27	4.47^{a}±0.64	1.60^{a}±0.30	2.45^{a}±0.41	1.52^{a}±0.21	4.17^{a}±0.42
TDZ 0.22 mg L^{-1} + IAA 1.75 mg L^{-1}	2.45^{b}±0.34	6.42^{b}±0.76	3.10^{b}±0.47	6.32^{b}±0.91	1.37^{a}±0.18	4.15^{a}±0.38

The numbers followed by the same letter in the same column are not significantly different based on the T-test independent sample at the 5% level

Table 2. The percentage (%) of encapsulating explants of banana growing through the alginate capsule in incubation medium at 2 to 6 months after *in vitro* storage

Code	Treatment of *in vitro* storage	Percentage (%) of encapsulated explant growing in incubation media at the age		
		2 months	4 months	6 months
A_1L_1	Encapsulation of explants using sodium alginate + MS + PBZ 0 mg L^{-1} and incubated in MS liquid medium	11.10	27.80	33.30
A_2L_1	Encapsulation of explants using sodium alginate + ½MS + PBZ 0 mg L^{-1} and incubated in MS liquid medium	0.00	11.10	33.30
A_3L_1	Encapsulation of explants using sodium alginate + MS + PBZ 2.5 mg L^{-1} and incubated in MS liquid medium	0.00	33.30	50.00
A_4L_1	Encapsulation of explants using sodium alginate + ½MS + PBZ 2.5 mg L^{-1} and incubated in MS liquid medium	0.00	5.60	16.70
A_1L_2	Encapsulation of explants using sodium alginate + MS + PBZ 0 mg L^{-1} and incubated in ½ MS liquid medium	0.00	0.00	0.00
A_2L_2	Encapsulation of explants using sodium alginate + ½MS + PBZ 0 mg L^{-1} and incubated in ½ MS liquid medium	0.00	5.60	5.60
A_3L_2	Encapsulation of explants using sodium alginate + MS + PBZ 2.5 mg L^{-1} and incubated in ½ MS liquid medium	0.00	5.60	16.70
A_4L_2	Encapsulation of explants using sodium alginate + ½ MS + PBZ 2.5 mg L^{-1} and incubated in ½ MS liquid medium	0.00	5.60	11.10

the explant remains green and does not germinate through the capsule (Table 3; Figure 2).

This study also showed that encapsulation of explants in full-strength MS nutrition and PBZ 2.5 mg L^{-1} and incubated in MS liquid medium (A_3L_1) has the highest percentage (33.3% and 50.0%) of encapsulating explants growing through the capsule at six months of *in vitro* storage (Table 2; Figure 2). Furthermore, this showed that alginate encapsulated with the full strength of MS nutrient concentration did not inhibit growth. Therefore, this encapsulation nutrition formulation had a good composition for growth, not storage.

3.3. Regeneration of Nodule-Like Meristem Explants after *In Vitro* Storage

The encapsulated banana cv. Barangan explants with green vigor after *in vitro* storage were removed using a scalpel. The explants were subcultured into a regeneration medium supplemented with TDZ 0.22 mg L^{-1} and IAA 1.75 mg L^{-1}. After three months of culturing, the nodule-like meristem proliferated and multiplied even though the cultures had been stored for six months (Figure 3). The number of shoots and nodules-like meristems increased until three months of culture. It also showed that the effect of *in vitro* storage and PBZ as a growth retardant in encapsulated

Table 3. The average quality alginate-encapsulated explant score after six months of *in vitro* storage in a liquid medium

Alginate encapsulated media	Incubation in liquid media	
	(L_1) full-strength MS liquid media	(L_2) half-strength (½) MS liquid media
(A_1) MS + PBZ 0 mg L^{-1}	1.50±0.15	1.58±0.23
(A_2) ½MS + PBZ 0 mg L^{-1}	1.83±0.71	1.50±0.23
(A_3) MS + PBZ 2.5 mg L^{-1}	1.75±0.13	2.08±0.26
(A_4) ½MS + PBZ 2.5 mg L^{-1}	2.00±0.24	2.16±0.24

Score 1.0 - ≤ 2.0 the explant browning, Score ≥ 2.0 - ≤ 3.0 the explant remained green and did not grow through the capsule

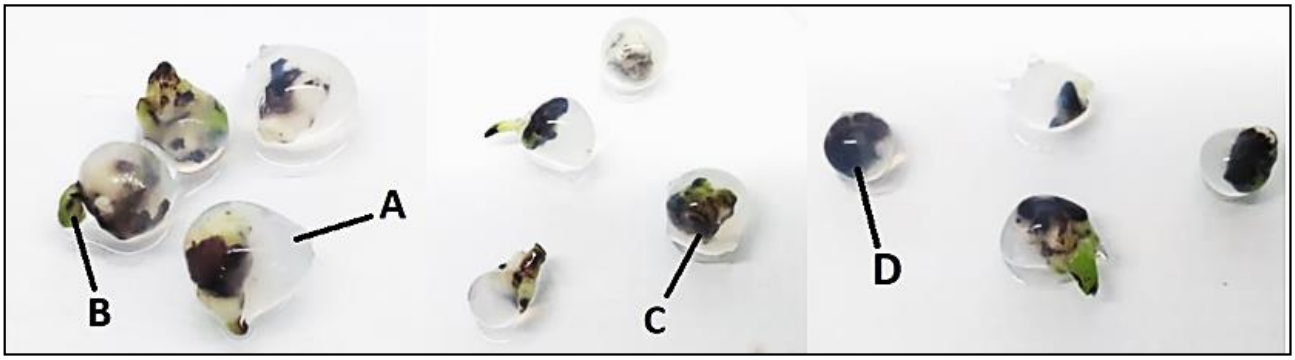

Figure 2. Scoring of explant quality during the storage period of encapsulated banana explants. (A) Alginate capsule, (B) Green explants grew or penetrated the capsule-score 3, (C) Green explant-score 2, (D) brownish explant-score 1. 1 (black scale bar represented 1 cm)

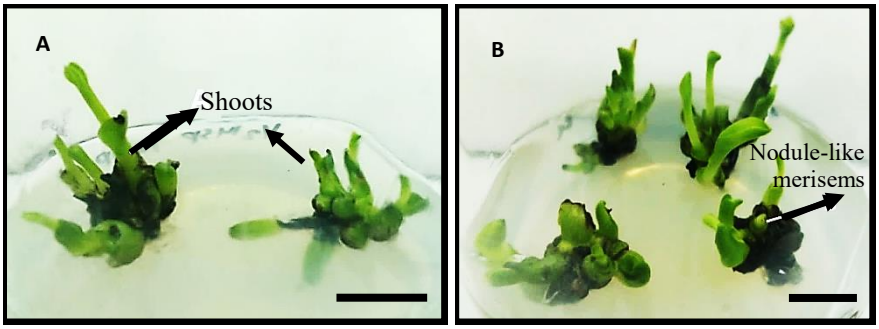

Figure 3. Representative figure of encapsulating explant that regenerated to form shoots and nodule-like meristem after subculturing into fresh media supplemented with a plant growth regulator. (A) shoots regenerated from encapsulated explant A_3L_1, (B) Nodule-like meristem regenerated from encapsulated explant A_4L_1.(black scale bar represented 1 cm)

explants was lost after they were regenerated into a fresh medium. However, encapsulated and incubated explants in full and half-strength MS liquid medium showed different plant growth responses. This study showed that explant encapsulation treatments incubated with half-strength MS liquid medium with better storage ability with explant remain green (A_3L_2 and A_4L_2) (Table 3) tend to have slower regeneration than treatments with lower storage ability (A_2L_1, A_3L_1, and A_1L_1,) (Table 4 and 5).

4. Discussion

4.1. Initiation and Multiplication of *In Vitro* Shoots and Nodule-Like Meristems

This study showed that explants grown on media containing thydiazuron and Indole 3-acetic acid (IAA) were more effective and efficient in producing shoots and nodule-like meristem than BAP and IAA (Table 1). The higher number of nodule-like meristem and shoots produced from MS media supplemented with 0.22 mg L^{-1} TDZ and 1.75 mg L^{-1} IAA. The proliferation and multiplication of explants were influenced by the type of cytokinin used, the concentration of PGR, and the character of the banana genotype cultivar (Arinaitwe *et al.* 2000; Agbadje *et al.* 2021). Cytokinins are always used with auxin to promote cell division, shoot proliferation, and differentiation of adventitious shoots. In addition, the cytokinins can mediate lateral bud release from apical dominance (Shirani *et al.* 2009; Le Bris 2017; Pai and Desai 2018). Thidiazuron (TDZ) is a phenyl urea substitute that acts as a plant growth regulator to induce the regeneration of recalcitrant species in plant tissue culture (Erland *et al.* 2020; Dinani *et al.* 2021). TDZ is a potential synthetic growth regulator that exhibits auxin and cytokinin (CK)-like effects on plants, including a proliferation of

Table 4. Regeneration of explant after six months of *in vitro* storage to the average number of nodule-like-meristem grows on MS solid media supplemented with TDZ and IAA at 1 to 3 months after culturing

Treatment of *in vitro* storage		The average number of nodules-like meristems grows		
Alginate encapsulated media (A)	Incubation liquid media (L)	1 month after subculture	2 month after subculture	3 month after subculture
(A_1) MS + PBZ 0 mg L^{-1}	(L_1) full-strength MS liquid media	0.75±0.48	1.75±1.03	3.50±1.17
(A_2) ½MS + PBZ 0 mg L^{-1}		2.75±0.48	4.00±0.40	5.50±0.64
(A_3) MS + PBZ 2.5 mg L^{-1}		2.75±1.10	4.50±1.70	6.75±1.05
(A_4) ½MS + PBZ 2.5 mg L^{-1}		2.00±0.41	3.50±0.86	5.50±1.44
(A_1) MS + PBZ 0 mg L^{-1}	(L_2) half-strength (½) MS liquid media	1.00±0.70	1.75±1.03	2.75±1.70
(A_2) ½MS + PBZ 0 mg L^{-1}		1.00±0.40	1.75±0.85	3.00±1.08
(A_3) MS + PBZ 2.5 mg L^{-1}		0.75±0.48	1.25±0.75	3.00±1.78
(A_4) ½MS + PBZ 2.5 mg L^{-1}		1.50±0.95	2.00±1.41	2.50±1.89

Table 5. Regeneration explant after 6 months of in vitro storage to the average number of shoots grows on MS solid media supplemented with TDZ and IAA at 1 to 3 months after culturing

Treatment of *in vitro* storage		The average number of shoots grows		
Alginate encapsulated media (A)	Incubation liquid media (L)	1 month after subculture	2 month after subculture	3 month after subculture
(A_1) MS + PBZ 0 mg L^{-1}	(L_1) full-strength MS liquid media	1.00±0.70	2.25±1.43	3.25±1.88
(A_2) ½MS + PBZ 0 mg L^{-1}		1.00±0.41	1.50±0.29	2.25±0.25
(A_3) MS + PBZ 2.5 mg L^{-1}		2.25±1.03	3.00±1.22	6.50±1.21
(A_4) ½MS + PBZ 2.5 mg L^{-1}		5.75±1.31	9.50^{c}±1.60	11.00±1.94
(A_1) MS + PBZ 0 mg L^{-1}	(L_2) half-strength (½) MS liquid media	1.00±0.40	2.00±0.81	3.00±1.08
(A_2) ½MS + PBZ 0 mg L^{-1}		1.00±0.57	1.25±0.75	1.50±0.86
(A_3) MS + PBZ 2.5 mg L^{-1}		0.75±0.47	1.50±0.86	1.50±0.87
(A_4) ½MS + PBZ 2.5 mg L^{-1}		0.75±0.48	1.00±0.57	2.75±1.54

adventitious shoots at low concentrations, somatic embryogenesis at high concentrations, an increase in photosynthetic activity, and an increase in uptake of sugars from the culture media (Erland *et al.* 2020; Dinani *et al.* 2021).

Ahanhanzo *et al.* (2010) reported that BAP was more effective in stimulating the formation of explant morphology, especially in the leaf formation of yam. In this study, a high concentration of BAP (6.5 mg L^{-1}) reduces the shoot formation in banana cv. 'Barangan' (Table 1). This result was in line with Cruz-Rosero *et al.* (2016), who reported that increasing BAP doses inhibited shoot multiplication and elongation of the Orito banana cultivar. Sipen and Davey (2012) reported that a medium supplemented with BAP at 6 mg L^{-1} without IAA produced a maximum of 5 shoots per original shoot tips of banana cv. 'Berangan' after 1 month of culture. According to Jafari *et al.* (2013), the highest concentration of BAP (7.43 mg L^{-1}) simultaneously increased the formation of abnormal banana shoots. 'Berangan'.

Thydiazuron (TDZ) is the best hormone in shoot multiplication, producing more axillary proliferation (Dewir *et al.* 2018; Pai and Desai 2018). Using urea-type cytokinins like TDZ at a lower concentration (0.1 µM) has more activity than adenine or purine types such as BAP (Pai and Desai 2018). Effect TDZ in various banana cultivars shows that TDZ at low concentrations (0.05–0.1 µM) could stimulate shoot proliferation of banana cv. 'Topala', 'Gros-Michel', and 'Pélipita' from Afrika (Youmbi *et al.* 2006). TDZ at concentration 0.1 mg L^{-1} produces the highest number of banana shoots cv. 'Radja Bulu' (Sukmadjaya *et al.* 2007) is the highest number of plantlets produced and plant height banana cv. 'Saba' and 'Susu' (Rai *et al.* 2019).

In diploid bananas (*M. acuminata*-AA and AB) from South India, the maximum number of shoots from male inflorescence explant were obtained in varying concentrations of TDZ (0.45-13.5 µM) (Smitha *et al.* 2014). At banana cv. Poovan (AAB), the maximum shoot multiplication was obtained in 0.5 mg L^{-1} TDZ and BAP 3 mg L^{-1} (Sivakumar and Visalakshi 2021). According to Roostika *et al.* (2019), BAP 5 mg L^{-1}, TDZ 0.1 mg L^{-1}, and PVP 300 mg L^{-1} produce the highest number of shoots and the number of wild banana leaves (*M. acuminata* ssp. Sumatrana). The highest number of shoots and roots with maximum root elongation (16.5 cm) of banana cv. 'Grande Naine' was recorded in 0.5 mg L^{-1} TDZ (Subrahmanyeswati *et al.* 2022).

4.2. *In Vitro* Medium-Term Storage of Banana Using Alginate-Encapsulation Technique

Encapsulation is a technique of coating explants with a particular material so that the explants are not easily damaged during storage. *In vitro* storage with minimal growth by encapsulation using sodium alginate in Murashige and Skoogs Medium is necessary to reduce the constraints of banana preservation. Sodium alginate and calcium chloride are the best combinations for encapsulation explants because these ions are non-destructive, inexpensive, easy to use, and result in the high conversion of encapsulated embryos to plants (Sharma *et al.* 2012a; Reyes *et al.* 2017; Phanomchai *et al.* 2022). It is also cost-effective for clonal propagation systems (Ahmad *et al.* 2012). In this study, encapsulation explant using 2.5 mg L^{-1} paclobutrazol (PBZ) and incubated in full strength and a half-strength MS liquid medium (Table 2) could store the explant for 6 months.

A previous study reported that PBZ at 2.5 mg L^{-1} and 5.0 mg L^{-1} could support the quality of banana cv. Kepok leaves remain green for six months of *in vitro* storage (Indrayanti *et al.* 2019). It is predicted that paclobutrazol suppresses chlorosis and necrosis in the encapsulated explant. PBZ has been associated with effects on the photosynthetic capacity of plants (Xia *et al.* 2018; Nagar *et al.* 2021). In the Citrus explant, the number of green leaves after *in vitro* storage is essential to establish vigor and was also affected by increased PBZ concentration (Mendes *et al.* 2021).

Paclobutrazol (PBZ) is one of the members of the triazole family that has growth-regulating properties and affects plant growth and development (Arteca 1996). The growth-regulating character of paclobutrazol is mediated by changes in levels of plant hormones, including gibberellins (GA), cytokinins (CK), and abscisic acid (ABA). PBZ inhibits GA biosynthesis, whereas cell division still occurs, but the newly formed cells cannot undergo cell elongation (Desta and Amare 2021; Nagar *et al.* 2021). In this study, alginate encapsulated explant supplemented with PBZ could retard the nodule-like meristem germinating in a liquid MS medium for six months (Table 3), and the explant remained green.

4.3. Regeneration of Nodule-Like Meristem Explants after *In Vitro* Storage

At the storage periods, incubation of encapsulated explant in a half-strength liquid MS medium causes inhibition of growth of the explant after storage (A_3L_2 and A_4L_2). As a result, the viability of the explant regenerating to form a new nodule meristem and the shoot was slow. Therefore, they are predicted to need more time to grow in regeneration media (Tables 2, 4, and 5). Furthermore, plant cell growth and development depend on nutrient availability in incubation media. If the nutrient is reduced, the cells are less active in dividing. In this study, the encapsulated explants using sodium alginate in half-strength MS nutrition with 2.5 mg L^{-1} PBZ and incubated in a full-strength liquid medium (A_4L_1) produced the highest number of shoots (11.00±2.94) and nodules-meristem (5.50±0.64) during the three months of the regeneration period (Table 4 and 5). Phenotypically, the paclobutrazol treatment showed more vigorous and shoot greens (Figure 3).

PBZ on a growing medium could give a more prolonged absorption time, giving more active ingredient absorption (Desta and Amare 2021). Physiologically, retardants are reported to support the formation of chlorophyll so that the culture looks greener and more vigorous (Xia *et al.* 2018; Mendes *et al.* 2021). In addition, the amount of chlorophyll formed can increase the efficiency of photosynthesis so that the growth of the culture becomes more vigorous and stimulated (Roostika *et al.* 2012). According to Xia *et al.* (2018), applying PBZ significantly increased photosynthetic and transpiration rate and water use efficiency (WUE). However, it decreased intercellular CO_2 concentration (Ci) compared to controls.

The common condition for *in vitro* storage through alginate encapsulation was the minimum growth rate of the encapsulated explant. The ability of encapsulated explants to grow after storage without any differences in genetic and physiological characteristics is essential for *in vitro* storage. Therefore, the treatment applied during the storage period is essential to maintain the ability of explants to grow in the regeneration medium. In this study, the treatment during storage did not reduce the viability. All encapsulated explants could regenerate into shoots and nodule-like meristem in different characters concerning the number of shoots and leaves. The best combination was obtained from the A_4L_1 treatment (Table 3; Figure 3 and 4), and it was concluded that PBZ was a necessary retardant in minimizing the growth of encapsulating explants during storage.

The high ability of the culture to regenerate after storage is very beneficial because it can extend the shelf life of the culture. The most desirable results of encapsulated explants were the ability of the explants to maintain viability in the capsule and grow after a sufficiently long storage period (Ahmad *et al.* 2012; Rihan *et al.* 2017; Mendes *et al.* 2021). Maintaining viability and regeneration after the storage is necessary for germplasm storage, exchange, or field applications.

The formulation of alginate encapsulation and incubation liquid media obtained in the study is very promising for medium-term *in vitro* storage of other banana cultivars. The limitation of this encapsulation technique is that nodule-like meristems and banana shoots are used as a source of explants. Most are unevenly sized, which makes getting the same capsule size challenging. The best concentration to produce nodule-like meristem combines TDZ 0.22 mg L^{-1} and IAA 1.75 mg L^{-1}. Explant of nodules-like meristems encapsulating using 3% sodium alginate in full and half-strength MS salt medium supplemented with PBZ 2.5 mg L^{-1} and incubated in full-strength liquid MS medium (A_4L_1) is the best combination for *in vitro* storage of banana cv. 'Barangan' explants for six months. The color of the explants remains green, and the capsule is not damaged. Regeneration of encapsulated explant after storage in MS salt medium containing TDZ 0.22 mg L^{-1} and IAA 1.75 mg L^{-1} showed that nodules-like meristems could regenerate to form new shoots and nodule-like meristem three months after sub-cultured. It also concluded that the alginate encapsulation technique with the addition of paclobutrazol could conduct the medium-term storage of banana explant.

Acknowledgements

This research was funded by National Collaborative Research-BLU Univeristas Negeri Jakarta in 2022. No: 12 /PKN/LPPM/IV/2022.

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