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The Effect of Additional Organic Material Types in the Media on the Growth of Orchid Protocorm Cymbidium bicolor Lindl. in Vitro

Auliya Putri Muanti[1], Ida Ayu Putri Darmawati[1*], Rindang Dwiyani[2], Yuyun Fitriani[1]

[1]Agroecotechnology Study Program, Faculty of Agriculture, Udayana University, Jln. PB. Sudirman Denpasar Bali 80232, **Indonesia**
[2]Master of Agroecotechnology Study Program, Faculty of Agriculture, Udayana University, Jln. PB. Sudirman Denpasar Bali 80232, **Indonesia**

[*]*Corresponding author:* putridarmawati15@gmail.com

ABSTRACT

Cymbidium bicolor Lindl. orchid is one of the types of natural orchids that has high aesthetic and economic value, but its existence in nature is starting to be threatened due to excessive exploitation and habitat destruction. Conservation and propagation efforts in vitro are effective alternative solutions. This study aims to determine the effect of adding organic materials to the media on the growth of protocorms in C. bicolor orchid plants in vitro culture. This research was conducted from November 2024 - March 2025 at the Tissue Culture Laboratory, Faculty of Agriculture, Udayana University. This study used a Completely Randomized Design (CRD) consisting of 8 treatments. The results showed that the provision of organic materials in the form of coconut water, potato porridge, and ambon banana porridge affected the growth of C. bicolor orchid protocorms. The ½ MS + coconut water media treatment had a very significant effect on several variables, namely the fastest shoot emergence time (3.2 DAT), the largest number of roots (3.41 pieces), the longest root length (2.58 cm), and the highest plantlet height (1.70 cm).

Keywords: Coconut water, Cymbidium bicolor Lindl. orchid, organic matter, in vitro culture, protocorm

INTRODUCTION

Indonesia is renowned for its abundant biodiversity, particularly in terms of flora, one of which is orchids (Suwandi, 2021). The Cymbidium bicolor Lindl. orchid, also known as the uncal orchid, is an epiphytic orchid (Cahyanti et al., 2023). Because they depend on host trees, epiphytic orchids are vulnerable to habitat changes caused by human activities such as logging and land conversion. This condition has an impact on orchid populations in nature, including the C. bicolor orchid, which is beginning to be exploited due to its high economic value. Therefore, cultivating the C. bicolor orchid is crucial to reduce pressure on exploitation in nature and maintain its sustainability (Pratama et al., 2021).

The use of in vitro culture techniques has shown high effectiveness in supporting orchid reproduction, especially in Cymbidium bicolor (Solichatun et al., 2020). In vitro culture is an effort to grow plant cells, tissues, or organs under sterile conditions in the laboratory, using synthetic media containing nutrients to form a complete plant (Dwiyani, 2015). Protocorms are the initial structures resulting from orchid seed germination that

are round and yellow, green, or yellowish green in color (Dwiyani, 2015). In the propagation and growth of orchids in vitro, the basic media often used include Vacin and Went (VW), Knudson C, and Murashige and Skoog (MS) (Darmawati and Yuswanti, 2014). MS medium contains high concentrations of essential nutrients, both macro and micro, which function to support optimal plant tissue growth (Hasanah et al., 2014).

Planting media does not only consist ofof macro and micro nutrients, but also contains organic substances as a source of carbon or other organic materials (Widiastoety and Purbadi, 2003). The addition of natural and synthetic organic materials containing essential compounds such as sugar, vitamins, phytoregulators, and amino acids, such as those found in bananas, coconut water, potatoes, and yeast extract, plays a role in accelerating the growth and differentiation of plant cells (Garvita and Handini, 2011). The administration of potato and banana pulp to the moon orchid (Phalaenopsis amabilis) has been shown to stimulate the growth of plantlets through the support of nutrients, vitamins, and carbohydrates as a source of cellular energy (Ningsih et al., 2023). The treatment of a combination of 100 ml/L coconut water and 100 mg/L Ambon banana pulp has been shown to produce the highest number of shoots and leaves, while the use of 150 ml/L coconut water alone is most optimal in stimulating root growth and increasing plant height (Djajanegara, 2010).

MATERIALS AND METHODS

This research was conducted in November 2024 - March 2025. located at the Plant Tissue Culture Engineering Laboratory, Faculty of Agriculture, Udayana University, Jl. Moyo Island No. 16X, Pedungan, South Denpasar District, Denpasar City, Bali. The planting material was a 7-month-old protocorm of the Cymbidium bicolor Lindl. orchid after sowing obtained from the Plant Tissue Culture Engineering Laboratory, Faculty of Agriculture, Udayana University. Based on the results of observations under a microscope, the protocorm was in phase 5 referring to the research of Dwiyani et al., (2012) regarding the development stage of the Vanda tricolor orchid embryo after pollination which was characterized by an enlarged embryo size, round shape, and green color. Other materials such as tissue, 70% alcohol, spirits, distilled water, media compactor (gellan gum), MS media, potatoes, bananas, coconut water.

The tools used in this study were a microscope, a Laminar Air Flow Cabinet (LAFC), an autoclave, a refrigerator, an analytical balance, a petri dish, a micropipette, a Bunsen burner, a scapel, a blade, tweezers, a culture bottle, a measuring cup, a beaker glass, a magnetic stirrer, a mortar, a handsprayer, plastic, rubber, latex gloves, a pH indicator, plastic wrap, a pencil, a label, aluminum foil, and a cell phone. The experimental design used in this study was a one-factor Completely Randomized Design (CRD) with eight treatment levels. The treatments include: Bo as control (½ MS without organic matter), Bk (½ MS + 100 g/L potato pulp), Bp (½ MS + 100 g/L Ambon banana pulp), Ba (½ MS + 100 ml/L coconut water), Bak (½ MS + 100 ml/L coconut water + 100 g/L potato pulp), Bap (½ MS + 100 ml/L coconut water + 100 g/L Ambon banana pulp), Bkp (½ MS + 100 g/L potato pulp + 100 g/L Ambon banana pulp), and Bakp (½ MS + 100 g/L potato pulp + 100 g/L Ambon banana pulp + 100 ml/L coconut water). Each treatment was repeated eight times so that there were a total of 64 experimental units.

Stages of implementing cultural research*in vitro*C. bicolor orchid cultivation begins with the sterilization of the room and laboratory equipment to maintain contamination-free conditions. The room is

routinely cleaned and sprayed with 70% alcohol, while tools such as tweezers, scalpels, and petri dishes are washed and sterilized using an autoclave at 121 °C for ± 30 minutes. The Laminar Air Flow Cabinet (LAFC) is also sterilized with alcohol and UV light before being used for the planting process. Next, organic materials are prepared in the form of potato pulp, Ambon banana pulp, and coconut water. Potatoes are boiled, mashed, and weighed, as are ripe Ambon bananas. Coconut water from green dwarf coconuts is filtered and its volume is measured to be used as an additional organic material in the media. The planting medium used is ½ MS enriched with organic materials according to the treatment. The media is mixed with basic components such as ½ MS, sugar, and agar, then added potato pulp, Ambon banana, and coconut water. After being thoroughly stirred and heated, the pH of the medium was adjusted to 5.6–5.8, then transferred into culture bottles and sterilized in an autoclave. Orchid protocorms were then planted into the sterile medium under LAFC using aseptic procedures, with four protocorms per bottle. Afterward, the bottles were capped, labeled, and placed in the incubation room for maintenance. The bottles were neatly arranged on culture racks, and the surrounding area was periodically sterilized using 70% alcohol to reduce the risk of contamination during observation.

The variables observed included the time of shoot emergence, number of shoots, time of leaf emergence, number of leaves, number and length of roots, plantlet height, and seed weight.Total root count, browning percentage, live plantlet percentage, and vitrification percentage. Shoot emergence was recorded through daily visual observation, starting from planting time until small protrusions that develop into leaves appeared, with recording based on days after planting (dap). The number of shoots was counted at the end of the observation period based on explants that had 1–2 leaves. The time to leaf emergence was calculated from the time the shoot developed into a true leaf, while the number of leaves was the total number of fully opened leaves on the plantlet. Root observations included the number of roots growing from the base of the plantlet and the length of the longest root, measured using a millimeter block at the end of the observation. Plantlet height was measured from the base to the highest point of the shoot, and total fresh weight was calculated after the plantlets were cleaned of the media and then weighed using a digital scale. The browning percentage reflects the number of explants that experienced damage in the form of brown or black discoloration, while the live plantlet percentage indicates explants that successfully formed at least one organ (shoot, leaf, or root). These two variables were observed visually and calculated using a percentage formula against the total explants used. In addition, the vitrification percentage was observed to determine explants that experienced physiological changes to become transparent or translucent due to tissue abnormalities. Vitrification was observed at the end of the observation period and calculated as a percentage of the total explants. Data processing was performed using analysis of variance (ANOVA). If there was a significant difference between treatments, further testing was carried out using Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

The addition of various types of organic materials to the culture media showed a real and very significant effect on the growth of orchid protocorms.*Cymbidium bicolor*Lindl. Based on the results of the analysis of variance, the organic matter treatment significantly affected the time of leaf emergence and significantly affected the variables of shoot

number, leaf number, root number, root length, plantlet height, and total fresh weight. However, it did not significantly affect the time of shoot emergence. Meanwhile, the variables of plantlet growth percentage, browning, and vitrification were not analyzed statistically because the data obtained were descriptive in nature (Table 1).

Shoot Emergence Time (dap)

Based on the research results (Table 2), the time for shoots to appearThe fastest growth was shown by the Ba treatment (½ MS + coconut water), which averaged 3.2 days after planting. This indicates that the addition of coconut water as an organic material can accelerate shoot initiation in C. bicolor orchid protocorms (Figure 4.1). Coconut water is a source of natural growth regulators containing auxin and cytokinin, where cytokinin plays an important role in stimulating cell division and shoot induction and proliferation and can help shoot growth and development (Saepudin et al., 2020). This study is in line with the opinion of Tuhuteru et al., (2012) who stated that the addition of coconut water to MS media is very effective in accelerating shoot growth because its cytokinin content is higher than auxin, thus stimulating faster shoot emergence. This indicates that the use of ½ MS media enriched with coconut water can also provide optimal results in accelerating the growth of C. bicolor orchid protocorm shoots.

Table 1. Significance of the Effect of Adding Types of Organic Materials to the Media on Protocorm Growth*C. bicolor Lindl.*

No.	Variables	Treatment
1	Time of emergence of shoots (hst)	ns
2	Number of shoots (fruit)	**
3	Leaf emergence time (dap)	*
4	Number of leaves (blades)	**
5	Number of roots (fruit)	**
6	Root length (cm)	**
7	Plantlet height (cm)	**
8	Total fresh weight (g)	**
9	Percentage of plantlets growing (%)	Td
10	Browning percentage (%)	Td
11	Vitrification percentage (%)	Td

Information :
ns = No significant effect (P≥0.05)
* = Significantly affected (P<0.05)
** = Very significant effect (P <0.01)
td = Not statistically analyzed

Table 2. Effect of Adding Types of Organic Materials to the Media on the Growth of Orchid Protocorms*C. bicolor Against Time of Shoot Appearance, Time of Leaf Appearance, Number of Shoots and Number of Leaves.*

Treatment	Observation Variables			
	Shoot Emergence Time (dap)	**Number of shoots (fruit)**	**Leaf Emergence Time (dap)**	**Number of leaves (blades)**
Bo	4.0 (2.12) a	**1.63 (1.45) a**	7.5 (2.81) a	**3.38 (1.95) a**
Ba	**3.2 (1.90) a**	1.25(1.31) a	6.4 (2.62) a	2.88 (1.83) a
Bk	3.3 (1.93) a	1.25 (1.31) a	5.8 (2.42) a	2.63 (1.75) a
Mr.	4.0 (1.80) a	0.50 (0.97) b	3.2 (1.44) b	0.25 (0.82) b
Tank	6.0 (2.39) a	0.88 (1.16) b	7.8 (2.64) b	2 (1.50) b
Bap	6.7 (2.54) a	0.88 (1.16) b	7.5 (2.48) b	0.63 (0.96) b
BKP	5.5 (2.13) a	0.63 (1.03) b	4.2 (1.71) b	0.25 (0.82) b
Bakp	4.2 (1.71) a	0.38 (0.90) b	**1.5 (1.07) b**	0 b

Information: The numbers in the table above are the original data, while the numbers in brackets are transformed data and numbers followed by the same letter show no significant difference in the 5% Duncan test.

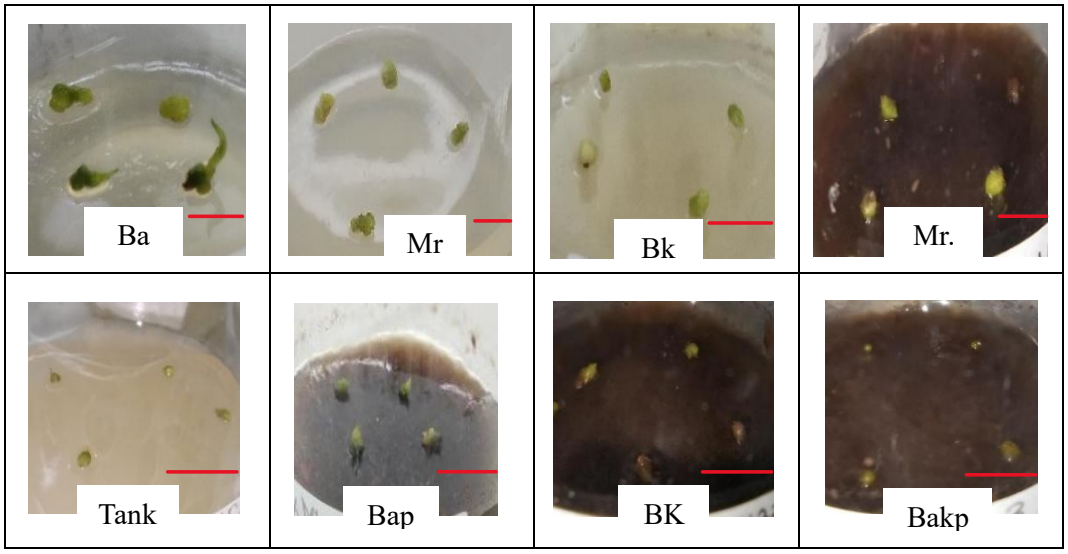

Figure 1. Time of Shoot Emergence for Each Treatment
Scale 1 cm

Number of Shoots (fruit) and Number of Leaves (fruit)

Figure 2 shows that there are treatments capable of growing shoots, with the highest number of shoots being in the Bo treatment (½ MS media without additional organic matter) with an average of 1.63 pieces as shown in Table 2. In addition, the Bo treatment also showed the best results in the leaf number variable, namely the highest number of leaves with an average of 3.38 pieces. Although the control treatment only relies on endogenous plant hormones which are generally less than optimal in stimulating cell division and shoot initiation (Novianti et al., 2022), the results of the study showed that half-concentration MS media (½ MS) was still effective in supporting the growth of Cymbidium bicolor Lindl orchid

shoots. This effectiveness is thought to be due to the nutrient content in the ½ MS media which is sufficient to meet the growth needs of C. bicolor protocorms, even without the addition of organic matter.

The Murashige and Skoog (MS) medium formulation is widely used in tissue culture techniques because it provides essential nutrients, vitamins, carbon sources, plant hormones, amino acids, and solidifying agents needed for explant growth and development (Purwanto et al., 2007). MS media is also characterized by high levels of inorganic salts (Pratama and Nilahayati, 2018), making it capable of supporting the growth of various plant species (Mardin, 2002).

Figure 2. The highest number of shoots in the treatments Control
Scale 1.5 cm

This is in contrast to the Bakp combination treatment (½ MS + coconut water + potato + banana) which showed the lowest number of shoots on average 0.38 pieces and the number of leaves 0 pieces (Table 2). The addition of three types of organic materials in the Bakp treatment is thought to cause an excessive increase in auxin levels, thereby inhibiting shoot formation. According to Fereol et al., (2002), in the process of organogenesis, the combination of high cytokinin levels with low auxin is very substantial to stimulate the formation of shoots and leaves, because too high auxin levels can actually inhibit shoot growth.

Leaf Emergence Time (dap)

The results of the analysis of variance showed that the fastest leaf emergence time was an average of 1.5 hst by the combination treatment of two materials.The organic fertilizer, namely Bakp (½ MS + coconut water + potatoes + Ambon bananas), was significantly different from almost all other treatments (Table 2). Conversely, the treatment with the slower leaf emergence time was the Bo treatment (½ MS) or control. This combination treatment accelerated leaf emergence time because it provided complete nutrition, natural hormones, and optimal energy for protocorm growth compared to other treatments that only used one or two ingredients. The meristematic tissue in the protocorm responds optimally to the combination of complete nutrients from ½ MS medium and natural hormones and energy sources from coconut water, bananas, and potatoes. This accelerates leaf emergence in Cymbidium bicolor Lindl plantlets.

Number of roots (fruit), Root Length (cm) and Plantlet Height (cm)

Figure 3. The Ba media treatment (½ MS + coconut water) had a significant effect on three growth variables, namely the largest number of roots with an average of 3.41 pieces, the longest root length reaching 2.58 cm, and tinThe highest plantlet height was 1.70 cm on average (Table 3). These results indicate that coconut water can support optimal root and plantlet growth. The natural plant growth regulators (PGRs) in coconut water, such as auxins, cytokinins, and other vitamins, are thought to play a role in stimulating root formation and increasing plantlet height. This finding aligns with the research of Srilertari and Suwardi (2020), which stated that adding coconut water to plant culture media,

especially nutrient-rich MS media, can significantly increase plantlet height, root length, and root number. The higher cytokinin content compared to auxin in coconut water causes the cell division process in explants to be more directed towards the formation of lateral shoots, due to the high cytokinin-to-auxin ratio.

Table 3. Effect of Adding Types of Organic Materials to the Media on the Growth of Orchid Protocorms*C. bicolor Lindl. On Root Number, Root Length, Plantlet Height, and Total Fresh Weight.*

Treatment	Observation Variables			
	Number of Roots (fruit)	**Root Length (cm)**	**Plantlet Height (cm)**	**Total Fresh Weight (g)**
Bo	3.18 (1.56) a	1.56 (1.41) a	1.30 (1.33) a	0.06 (0.75) a
Ba	**3.41 (1.31) a**	**2.58 (1.71) a**	**1.70 (1.48) a**	0.06 (0.75) a
Bk	2.15 (1.05) b	1.02 (1.13) b	1.45 (1.37) a	**0.10 (0.77) a**
Mr.	0.05 (0.77) b	0.05 (0.74) b	0.14 (0.79) b	0.01 (0.71) b
Tank	1.06 (1.01) b	0.81 (1.08) b	0.66 (1.04) a	0.02 (0.72) b
Bap	0.19 (0.97) b	0.19 (0.82) b	0.33 (0.90) b	0.02 (0.72) b
BKP	0.09 (0.77) b	0.09 (0.76) b	0.28 (0.87) b	0.01 (0.71) b
Bakp	0.04 (0.77) b	0.04 (0.73) b	0.01 (0.71) b	0.01 (0.71) b

Information: The values listed in the table are raw data, while the numbers in parentheses are the transformed results. Similarity in letters in numbers indicates that the difference is not significant according to Duncan's test at the 5% level.

Table 4.The Effect of Adding Types of Organic Materials to the Media on the Growth of C. bicolor Lindl. Orchid Protocorms on the Percentage of Browning, Plantlet Survival and Virtification.

Treatment	Observation Variables		
	Browning Percentage (%)	**Percentage of Live Plantlets (%)**	**Vitrification Percentage (%)**
Bo	0	100	0
Ba	0	100	0
Bk	0	100	0
Mr.	**12.50**	87.50	0
Tank	0	100	0
Bap	0	100	0
BKP	**12.50**	75	**12.50**
Bakp	0	87.50	**12.50**

Description: Columns filled with 0 indicate that the explant did not experience browning and virification.

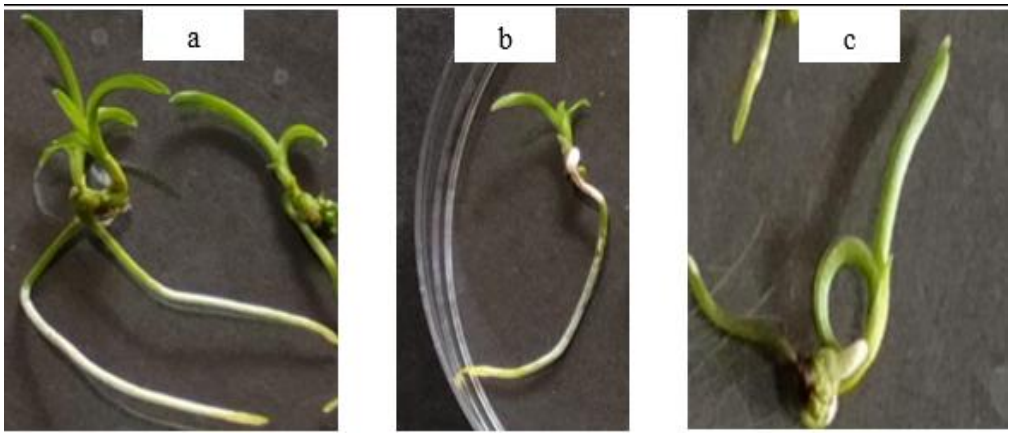

Figure 3. Ba Treatment (½ MS + Coconut Water) (a) Highest Number of Roots, (b) Longest Root Length, and (c) Highest Plantlet Height.

Total Fresh Weight (g)

From the research results, it was found that the treatment of ½ MS media with the addition of potato organic material (½ MS + Potato) produced the highest average total fresh weight, which was 0.10 g, compared to other treatments (Table 3). Based on the analysis of variance, the potato treatment had a very significant effect on the total fresh weight variable and provided the best results among all treatments. This is in line with the research of Ambarwati et al., (2024) which showed that the addition of potato organic material to the in vitro culture media of Coelogyne cross-bred orchids had a very significant effect on the total fresh weight variable and produced the best results compared to other organic materials such as tomatoes and carrots. According to Emila and Mayta, (2024) the content of carbohydrates, vitamins B1, B6, C, and minerals such as K, Fe, and Mg in potatoes plays an important role in supporting metabolism and cell division increasing the fresh weight of plants.

Browning Percentage (%)

The results of the descriptive analysis show that the pressThe browning percentage in C. bicolor orchid protocorms differed between treatments. The Bo, Ba, Bk, Bak, Bap, and Bakp treatments showed no browning symptoms (0%), while the Bp and Bkp treatments showed a browning percentage of 12.50% (Figure 4). This finding indicates that the Bp and Bkp treatments have the potential to trigger browning in protocorms. Browning is a condition of plant tissue browning caused by the accumulation of oxidized phenolic compounds due to injury to the explant, which can inhibit growth, development, and even cause explant death (Sulichantini et al., 2023). According to Helena et al., (2022), oxidation of phenolic compounds by the PPO enzyme in banana tissue can cause explants to experience blackish browning, which if left untreated, can inhibit regeneration and lead to explant death.

Percentage of Live Plantlets (%)

This is in line with the results of the descriptive analysis of the percentage of live plantlets variable, where most treatments showed a high survival rate. Table 4 shows the Bo, Ba, Bk, Bak, andBap showed a 100% live plantlet percentage, indicating that organic materials such as coconut water, potatoes, and their combinations were able to optimally support protocorm viability. Conversely, a decrease in the live plantlet percentage occurred in the Bp (87.50%) and Bkp (75%) treatments, both of which showed browning

(Table 4). This confirms the hypothesis that the high phenolic compound content in bananas triggers browning, which then negatively impacts plantlet viability. These findings support previous research that PPO enzyme activity and phenolic compound accumulation in tissue can reduce explant viability in in vitro culture (Sulichantini et al., 2023; Helena et al., 2022).

Vitrification Percentage (%)

The highest vitrification percentage was found in the Bkp (½ MS + potato + banana) and Bakp (½ MS + coconut water + potato + banana) treatments, namely raverage of 12.50%. Meanwhile, other treatments did not experience vitrification with an average of 0% (Table 4). The conditions that occurred in the explants experienced a change in color in the protocorm to clear/white. The changes that occurred in the explants caused suboptimal growth as shown in (Figure 5). According to Dwiyani (2015) vitrification occurs when the water content in plant cells is excessive, which results in damage to physiological functions and is characterized by organs that appear transparent. One of the main causes of vitrification in plant tissue culture is an imbalance of growth hormones, especially high concentrations of cytokinins (BAP) in the culture medium (Zulkarnain, 2018).

Figure 4. Browning that occurs in Treatment Bp (½ MS+Banana Porridge)
Scale 1.5 cm

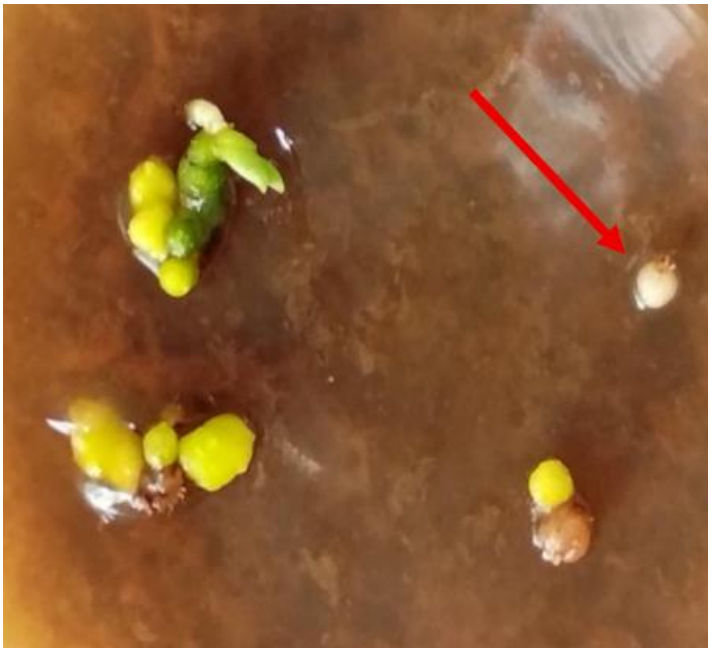

Figure 5. Vitrification in Treatment

CONCLUSION

Based on in vitro research, it was found that the addition of organic materials to the culture medium significantly affected various aspects of the growth of Cymbidium bicolor Lindl. protocorms, including the number of shoots, the number of leaves, roots, root length, plantlet height, total fresh weight, and leaf emergence time. Coconut water was the most effective organic material in supporting protocorm growth. The Ba treatment (½ MS + coconut water) resulted in the fastest shoot emergence time, the highest number of roots, the longest root length, and the highest plantlet height. Based on these results, it is recommended to use ½ MS media with the addition of coconut water as the sole organic material to support the optimal growth of C. bicolor orchid protocorms because coconut water has been proven to accelerate shoot growth and support root formation and overall plantlet development. In addition, further research is needed by regulating the concentration of each organic material specifically to avoid negative effects from excessive combinations, as seen in the Bakp treatment.

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