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Influences of Organic Additives on Asymbiotic Seed Germination of *Dendrobium cruentum* Rchb. f. for *In Vitro* Micropropagation

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Abstract

Dendrobium cruentum Rchb. f., a commercial orchid in Thailand, faces the risk of extinction and should be conserved, and propagated for commercial. Therefore, the aim of this research was to identify a suitable VW medium supplemented with organic additives for the micropropagation of *D. cruentum* through asymbiotic seed germination. The VW media supplemented with separate organic additives (coconut water (CW) (100 mL/L), 'Hom Thong' banana homogenate (BH) (1000 mg/L), and potato homogenate (PH) (1000 mg/L) or its combination was determined, and the seed germination rate (%), protocorm formation, and plantlet development were observed. The results showed that a combination of BH and PH resulted in the highest seed germination (94.69 ± 1.55 %) when compared to basal VW (control). Protocorm formation was highest with CW alone (47.38 ± 7.17 %) and a combination of CW and PH (52.13 ± 6.20 %). Additionally, after 10 weeks of culture, supplementation with BH yielded the highest number of plantlets. However, after 24 weeks of culture, each organic additives gave the different developments of healthy plantlets. To investigate suitable potting media for acclimatization of well-grown plantlets in greenhouse conditions, 2 pieces of foam, dried coconut husk, and a combination of peat moss: *Sphagnum* moss: Pumice: Perlite (3:1:1:1) were investigated. The highest survival rate percentage was found in foam-planted plantlets (80 %). Ultimately, the effectively simple mediums containing organic additives and a one-step protocol were provided for the micropropagation of *D. cruentum* via seeds, and the derived information could be used for *D. cruentum* conservation and production.

Keywords: *Dendrobium cruentum Rchb. f.*, Asymbiotic seed germination, Micropropagation, Organic additives, Greenhouse acclimatization

Introduction

Among flowering plants, *Orchidaceae* is the largest family. The orchid members in this family are important economic plants whose flowers and fruits are valuable income producers in several countries including, Thailand [1]. Routinely, orchid seeds are produced in large numbers, but their size is quite small and dust-like within the fruit pod, which lacks storage tissue. These seed characteristics are different from those of seeds of other flowering plants [2]. Seed germination and early development of orchids are correlated with mycorrhizal fungi [3,4]. The absence of mycorrhiza fungi affects orchid seed germination rendering this family vulnerable. To prevent extinction, the unnatural propagation of orchids is essential.

Dendrobium cruentum Rchb. f. belongs to the *Orchidaceae* family and is one of the rare Thai epiphyte orchids of the world. Their native habitat is on the southwest coast of Thailand near the border with Malaysia. The dominant characteristics of this orchid are as follows; the length of their pseudobulbs can reach 30 - 40 cm; 1 - 2 flowers are located on the same apex, and their petals are yellow and green with brick-red color in the lip. The dominant feature of this orchid is that its flowers appear throughout the year [5]. Because of its unique beautiful characteristics, *D. cruentum* has become the most popular ornamental and commercial orchid in Thailand. Moreover, to date, Thailand is one of the largest producers and exporters of *D. cruentum* globally [1]. Currently, the numbers of *D. cruentum* in nature have been greatly decreasing because of illegal overcollection for trade and natural habitat destruction; as a result, the species is at risk of extinction [5]. *D. cruentum* is listed on **Appendix I** of the convention on international trade in endangered species of wild fauna and flora as a 'World Endangered Orchid Species". Based on the abovementioned situation, effective protocols for *D. cruentum* propagation are crucial and urgently needed. Sangdum *et al*. [5] reported that using the inorganic plant growth regulators,

naphthaleneacetic acid (NAA) and 6-benzyladenine (BA), successfully promoted the micropropagation of *D. cruentum in vitro* via shoot tips [5]. As inorganic additives, organic additives including coconut water, potato homogenate, banana homogenate, tomato juice, chitosan, peptone, and yeast extract have been widely used in orchid propagation when using tissue culture [6,7]. In addition, organic additives have been effectively used to increase seed germination and seedling regeneration [7]. In the case of orchid seed germination, coconut water (CW), could increase the seed germination in *Rhynchostylis retusa* [8], *Acampe papillosa* [9], *Cypripedium macranthos* [10], *Paphiopedilum wardii* [11] while potato homogenate (PH) could stimulate seed germination in *Vanda roxburgii* [12] and *Dimorphorchis lowii* [13]. Banana homogenate (BH) successfully improved seed germination of *Chloraea gavilu* [14]. Peptone could enhance seed germination of *D. lasianthera* [15], *Epidendrum ibaguense* and *Spathoglottis plicata* [16,17], *D. parishii* [18], *Aerides ringens* [19]. Chitosan improved seed germination of *D. bigibbum* var. compactum, *D. formosum* [20]. Tomato juice (TJ) significantly influences the germination of orchid seeds such as *V. helvola* [21], and *Geodorum densiflorum* [22]. Yeast extract (YE) could increase seed germination of *V. dearie* [23]. Moreover, several reports established the suitable media supplementing organic additives for the propagation of *Dendrobium* orchids *in vitro* [15,20,24-27]. However, the micropropagation of *D. cruentum* via their seeds by using the media supplemented with organic additives has been not determined.

This study aimed to determine the effects of organic additives supplemented into the media for the micropropagation of *D. cruentum* orchids via seed *in vitro*. Ultimately, the nutritionally sufficient media was not only presented but also providing an effective one-step protocol as well. The information gained from this research may be beneficial for *D. cruentum* conservation and the orchid industry.

Materials and methods

Preparation of plant material

Pod (approximately 6 months old) from self-pollination (**Figure 1b**) were used as the source of the explants. The surfaces of the fruits were dipped with 95 % ethanol for 3 - 5 s and fired. The sterilization process was repeated in triplicate. The seeds were then aseptically dissected and used for the next study. The seed viability was tested before performing an experiment by using TTC assay. The seed viability percentage was approximately 95 %.

Figure 1 *Dendrobium cruentum* Rchb.f. inflorescence with a) mature flower and b) pod (6 months old) from self-pollination.

Media preparation and experimental groups

The basal medium used for this study was Vacin and Went (VW) media. the VW media was modified to generate 7 groups of modified VW media (the basal VW used as the control group (VW0)): 1) VW media supplemented with coconut water (CW) (100 mL/L) (VW1), 2) with 'Hom Thong' banana homogenate (BH) (1000 mg/L) (VW2), 3) with potato homogenate (PH) (1000 mg/L) (VW3) and 4) - 7) a combination of CW and BH (VW4), CW and PH (VW5), BH and PH (VW6), and CW, BH and PH (VW7). All media consisted of the same amount of sugar (30 g/L) and the pH of the medium was adjusted to 5.3 before gelling with a 7.5 g/L agar powder. The media were prepared in a 4-fl oz culture bottle. Before use, all media were sterilized by autoclaving at 121 °C, 15 kPa for 15 min.

Determination of seed germination, protocorm formation, and plantlet regeneration

To determine the effect of media supplemented with various organic additives for the development of seed, the protocol was conducted as Mala *et al.*' method [28] with some modifications. Briefly, a spatula was used to sow an approximately equal number of seeds onto the media. Then, the seeds were maintained under standard conditions at 25 ± 2 °C under white fluorescent light at an intensity of 1000 lux for 14 h/day. Each experiment was performed with 12 replicates. The seed germination and stage of seed development were observed from the seeds in the randomly chosen areas in each experiment every week for 3 months using a stereomicroscope. Seed germination was characterized using the parameters described by Pierik *et al*. [29]. Briefly, the seed parameters were classed and given the values as followed:

Seed parameters	**Numbers**
1) seeds containing an embryo but not germinating (swollen)	a
2) swollen seeds, germinating, but not yet rupturing the seed coat	b
3) seeds with embryos just rupturing the seed coat	c
4) seeds with embryos completely out of the seed coat	d

by the letters a, b, c and d indicate the frequency of each class of seeds.

The seed germination percentage was then calculated using the equations as followed:

Seed germination percentage = 100(b + c + d) / (a + b + c + d)

Next, the plantlet formation and development were recorded at 10 and 24 weeks of culture, respectively. The observed parameters included the height of plantlets, the number and length of leaves, and the number, length, and width of roots were investigated from 10 plantlets per experiments, and performed with 3 replicates.

Greenhouse acclimatization

The well-grown plantlets with leaves and roots in culture bottles were placed in greenhouse for 1 weeks before performing the experiment. Next, the plantlets were rinsed with running tap water to eliminate the culture media and were then separately transplanted into plastic pots. The plastic pots were divided into 3 groups which were contained the different potting mediums: group 1) two pieces of foam; 2) dried coconut husk; 3) a combination of peatmoss: *Sphagnum* moss: volcano rock: perlite (3:1:1:1). Sixty plantlets were planted in each group. Total plantlets acclimatized in the step were 180 plantlets. The plantlets were watered once a week under a greenhouse environment (ambient temperature 27 to 32 °C under shade nets). The survival rate was evaluated and recorded after acclimatization in greenhouse for 4 weeks.

Experimental design and statistical analysis

The experiments in the greenhouse acclimatization and others were arranged using an RCB and CRD, respectively. The collected data were statistically analyzed using DMRT.

Results and discussion

Effects of media supplemented with organic additives on seed germination, and protocorm formation and development of *D. cruentum* Rchb. f.

In orchids, the effects of organic additives were evaluated on growth and development in several species [6], and could be assumed that the efficacy of organic additives on micropropagation of orchids might be dependent on orchid genotypes and developmental stages. Therefore, this needs to be determined individually for each orchid. The effects of organic additives on asymbiotic seed germination for micropropagation of *D. cruentum* did not be performed, and this was uncovered in this study.

Seed germination

After 2 weeks of culture, the seed germination rate was observed. This study found that most seed germination rates were better than those of the control (VW0) after the addition of organic additives (**Figure 2**). In detail, the highest seed germination rate was detected in the VW6 media in which the seed germination rate percentage was 94.69 ± 1.55. This was followed by VW2-5 media, the seed germination

rate percentages of which were 83.22 ± 1.20, 78.01 ± 3.21, 83.10 ± 1.75, 82.55 ± 2.05, respectively. However, the VW7 media did not significantly affect the seed germination rate versus the control (**Table 1**). These results were similar to previous reports which revealed that the addition of organic additives into media could induce *Dendrobium* seed germination [15,20]. It indicates that all organic additives may provide sufficient endogenous growth regulators requiring the initial stages of seed germination of *D. cruentum*.

Table 1 The germination rates of *D. cruentum* seeds cultured on VW media after 2th weeks of culture.

VW media	Seed germination rate (%)
1) VW0	56.44 ± 5.27^{d}
2) VW1 (CW)	66.17 ± 1.49^{c}
3) VW2 (BH)	83.22 ± 1.20^{b}
4) VW3 (PH)	78.01 ± 3.21^{b}
5) VW4 (a combination of CW and BH)	83.10 ± 1.75^{b}
6) VW5 (a combination of CW and PH)	82.55 ± 2.05^{b}
7) VW6 (a combination of BH and PH)	94.69 ± 1.55^{a}
8) VW7 (a combination of CW, BH and PH)	51.16 ± 3.47^{d}
F-test	*
CV. (%)	2.68

Note: * indicates a significant difference at the 95 % confidence level ($p < 0.05$). Each value represents as the mean ± S.E. CW, BH and PH were coconut water, banana homogenate, and potato homogenate, respectively.

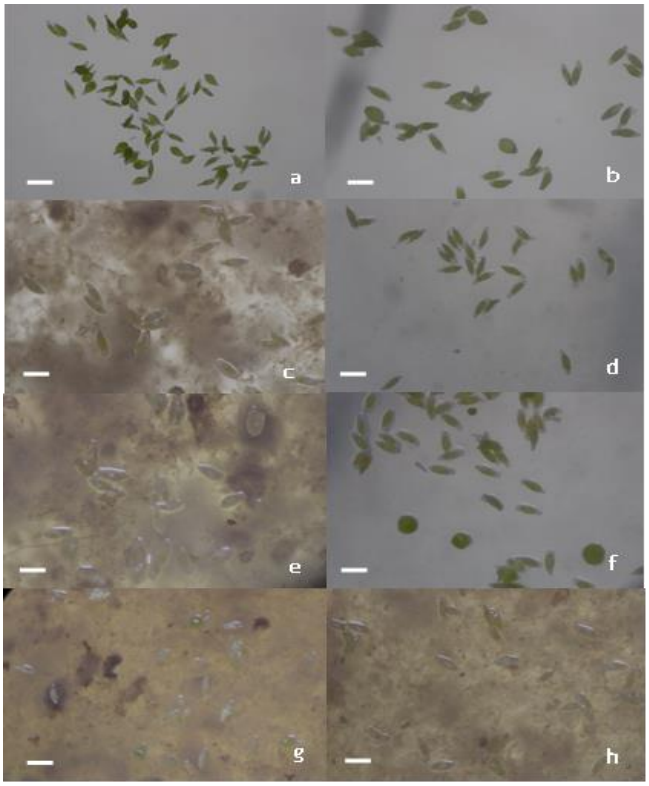

Figure 2 Seed germination of of *D. cruentum* after the 2 weeks of culture on several VW media (scale bar = 0.5 mm) consisting of a) VW0, b) VW1, c) VW2, d) VW3, e) VW4, f) VW5, g) VW6 and h) VW7.

Protocorm and seedling formations

The protocorm was formed at 3th - 4th weeks after culture. Until the 5th weeks of culture, papillae occurred on the surface, and at the 7th - 8th weeks of culture, the cotyledon was formed. In the 10th weeks of culture, the protocorm formation percentage and the plantlet formation were observed. The results showed that some media only whose protocorm and seedling formation was better than that of the control after the addition of organic additives (**Figure 3**). In detail, the VW1 and VW5 media significantly affected the protocorm formation percentages when compared to the control, whereas the other media did not. In addition, when observing the shoot, leaf, and rhizoid formation of protocorm, the results showed that some media significantly affected the observed morphologies. The VW1 and VW3 medium significantly increased the number of protocorms presenting shoots from 30.12 ± 4.10 and 36.00 ± 5.71 protocorms, respectively. The VW2 media significantly increased the number of protocorms containing shoots and rhizoids with values of 58.00 ± 6.11 protocorms. Moreover, VW2 media yielded the highest numbers of seedlings (protocorm containing shoots, leaves, and rhizoids), followed by VW6 media with values of 8.50 ± 1.64 and 4.88 ± 0.89 seedlings, respectively (**Table 2**). However, in the 20th weeks of culture, protocorm derived from most media could be transformed into the seedlings except for VW7 (data not shown).

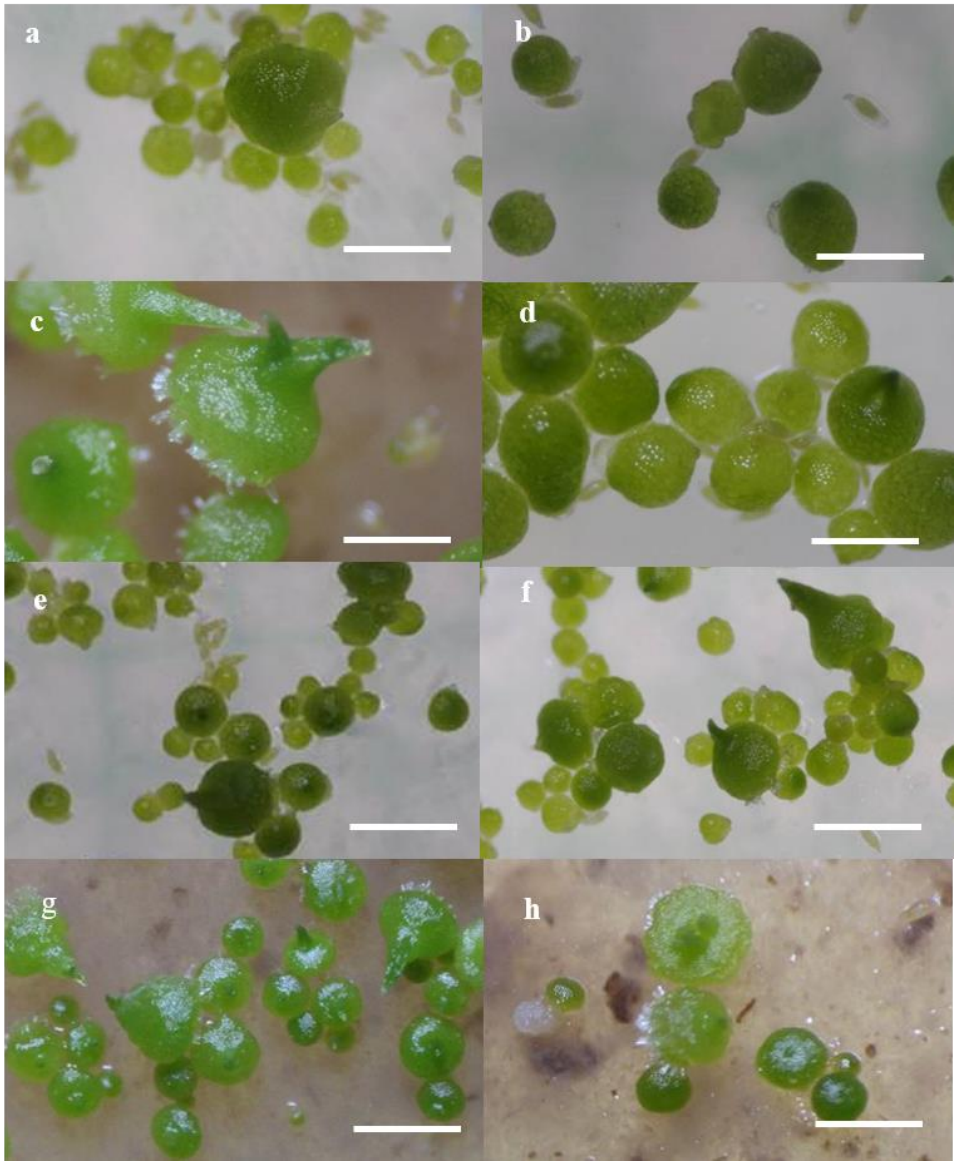

Figure 3 *D. cruentum* protocorms after the 10th weeks of culture on several VW media (scale bar = 1 mm) consisting of a) VW0, b) VW1, c) VW2, d) VW3, e) VW4, f) VW5, g) VW6 and h) VW7.

Table 2 Effects of the VW media on protocorm and seedling formation of *D. cruentum* after the 10th weeks of culture.

VW media	Protocorm formation (%)	Number of protocorm containing shoot	Number of protocorm containing shoot and rhizoid	Number of protocorm containing leaf, shoot, and rhizoid
1) VW0	28.75 ± 6.00^{bc}	2.50 ± 0.46^{c}	1.63 ± 0.32^{d}	0.00 ± 0.00^{c}
2) VW1	47.38 ± 7.17^{a}	30.12 ± 4.10^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
3) VW2	12.75 ± 1.42^{c}	3.63 ± 0.56^{c}	58.00 ± 6.11^{a}	8.50 ± 1.64^{a}
4) VW3	29.38 ± 5.79^{bc}	36.00 ± 5.71^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
5) VW4	39.00 ± 7.45^{ab}	5.38 ± 0.89^{c}	20.75 ± 3.83^{c}	0.00 ± 0.00^{c}
6) VW5	52.13 ± 6.20^{a}	15.13 ± 2.63^{b}	9.38 ± 1.62^{d}	0.00 ± 0.00^{c}
7) VW6	14.00 ± 3.19^{c}	2.88 ± 0.44^{c}	39.25 ± 5.35^{b}	4.88 ± 0.89^{b}
8) VW7	19.75 ± 2.35^{c}	3.63 ± 0.50^{c}	4.50 ± 1.45^{d}	0.38 ± 0.18^{c}
F-test	*	*	*	*
CV. (%)	8.23	14.68	16.48	22.80

Note: * indicates a significant difference at the 95 % confidence level ($p < 0.05$). Each value represents as the mean ± S.E.

Overall, it could be concluded that banana homogenate significantly accelerated the seedlings formation from *D. cruentum* protocorms. These results were similar to a few previous reports that revealed that coconut water, banana homogenate and potato homogenate could stimulate the growth of protocorms in various orchids [26,30,31] including *Dendrobium* orchids. In 'Earsakul' *dendrobium*, the greatest of total PLB (protocorm like body) fresh weight, the increased PLB number, and growth rates were obtained when using VW medium supplementing with tomato (*Solanum lycopersicum*) and 'Khai' banana (*Musa acuminata*) [27]. The 'Alya Pink' *dendrobium* PLB proliferation was affected when using coconut water and glucose whereas banana and tomato homogenate were not effective in this case [25].

Effects of media supplemented with organic additives on the *D. cruentum* seedlings growth

The growth of seedlings was observed after the 24th weeks of culture. The results found that the growth of seedlings in some media was better than that of the control (**Figure 4**). VW2 media significantly affected the stem height of seedlings, with a height of 6.90 ± 0.90 cm. In addition, VW6 media yielded the highest root length, followed by that in VW2, with root lengths of 10.51 ± 2.31 and 7.05 ± 0.85 mm, respectively. Moreover, VW4 media significantly supported the best root width increment. In the case of leaves, VW1 and VW3 yielded the highest leaf numbers with the same numbers of 3.30 ± 0.34 leaves. VW2 and VW6 gave the highest leaf length with the length of 2.56 ± 0.53 mm and 2.24 ± 0.29 mm, respectively. In contrast, the root number was not influenced by the addition of organic additives when compared to the control (**Table 3**).

Table 3 Effect of the VW medium on *D. cruentum* seedling development after the 24 weeks of culture.

VW media	Stem height (cm)	Number of leaves	Leaf length (mm)	Number of roots	Root length (mm)	Root width (mm)
1) VW0	4.55 ± 0.31^{bc}	2.80 ± 0.20^{ab}	1.21 ± 0.19^{bc}	3.00 ± 0.30^{a}	2.69 ± 0.45^{de}	0.49 ± 0.02^{c}
2) VW1	5.56 ± 0.41^{ab}	3.30 ± 0.34^{a}	1.12 ± 0.11^{bc}	1.50 ± 0.27^{c}	2.62 ± 0.42^{de}	0.45 ± 0.05^{c}
3) VW2	6.90 ± 0.90^{a}	2.30 ± 0.15^{bc}	2.56 ± 0.53^{a}	2.80 ± 0.20^{ab}	7.05 ± 0.85^{b}	0.86 ± 0.66^{ab}
4) VW3	4.05 ± 0.24^{cd}	3.30 ± 0.34^{a}	1.30 ± 0.11^{bc}	1.50 ± 0.27^{c}	2.62 ± 0.42^{de}	0.45 ± 0.05^{c}
5) VW4	3.60 ± 0.23^{cd}	2.40 ± 0.22^{bc}	1.35 ± 0.22^{bc}	2.30 ± 0.21^{ab}	6.58 ± 0.74^{dc}	1.15 ± 0.30^{a}
6) VW5	5.25 ± 1.07^{bc}	2.20 ± 0.15^{c}	0.93 ± 0.13^{c}	2.10 ± 0.23^{bc}	4.21 ± 0.54^{cd}	0.60 ± 0.55^{bc}
7) VW6	2.70 ± 0.21^{c}	2.10 ± 0.23^{c}	2.24 ± 0.29^{a}	2.10 ± 0.31^{bc}	10.51 ± 2.31^{a}	0.84 ± 0.81^{ab}
8) VW7	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{d}
F-test	*	*	*	*	*	*
CV. (%)	7.04	5.89	8.06	6.74	10.43	8.94

Note: * indicates a significant difference at the 95 % confidence level ($p < 0.05$). Each value represents as the mean ± S.E.

These results were similar to a few previous reports that revealed that the organic additives could promote the growth of seedling in another *Dendrobium*. By this, MS individually supplemented with coconut water and different types of homogenized bananas could promote the growth of *D. farmeri* and *D. griffithianum* seedling [32].

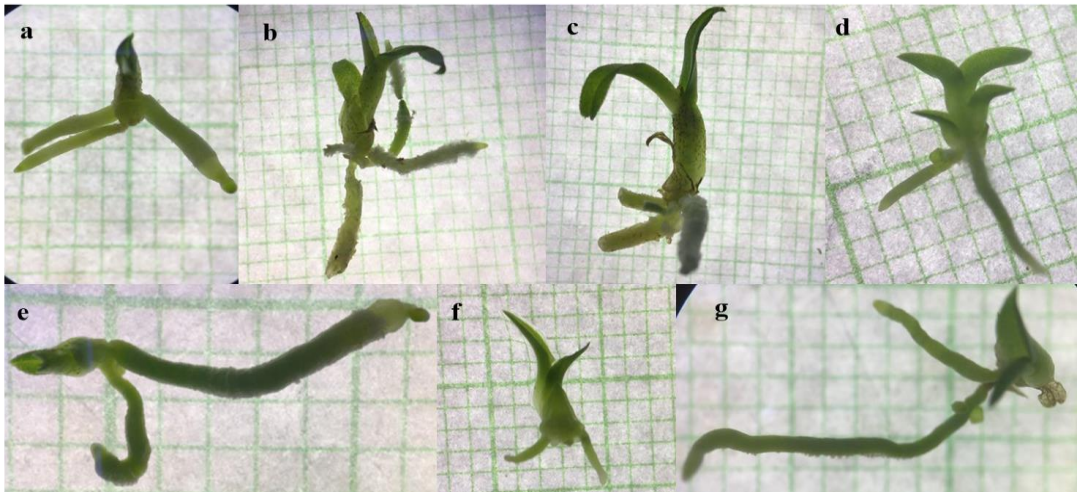

Figure 4 Seedlings of *D. cruentum* cultured on several VW media consisting of VW0 (a), VW1 (b), VW2 (c), VW3 (d), VW4 (e), VW5 (f), and VW6 (g) after the 24th weeks of culture.

It is well-known that coconut water is routinely used as growth regulators in plant tissue culture because it contains cell division-inducing substances including major cytokinin, zeatin, zeatin ribosides, indoleacetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA). In addition, coconut water also contains the substances that could facilitate germination including soluble sugars, a natural carbon source, amino acids, vitamins (thiamin, pyridoxine, ascorbic acid), minerals [33,34] and various organic ions (phosphorus, magnesium, potassium, calcium, iron, and manganese [35]. Banana homogenate contains the high levels of some valuable organic and inorganic substances, particularly carbohydrates which supply energy to plants during the early stages of *in vitro* cultivation, natural growth regulators, such as zeatin, gibberellin, and indole-3-acetic acid (IAA) [36-39], and the high levels of substances involving in seed germination such as potassium, manganese, calcium, sodium, iron, zinc, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, ascorbic acid, folic acid [39]. In addition, banana homogenate had high amounts of antioxidant compounds (ascorbic acid, phenolic, and carotenoid content) [40,41]. Sugar and antioxidants play an integral role in the proliferation of healthy PLBs, and the antioxidants could also prevent the browning of PLB culture [42]. Potato homogenate contains substances facilitating seed germination and development including carbohydrates and amino acids, vitamins (C, B1, and B6), and mineral elements (potassium, iron, and magnesium) [43,44]. All of these results supported why seed germination, protocorm formation and seedling development of *D. cruentum* were high in media containing organic additives as well as other orchids and plants [6-23].

3. Acclimatization of seedlings in greenhouse

Because of *Dendrobium* is an epiphytic orchid, therefore, the proper substratum should contain the good water holding capacity and good drainage. In addition, other properties of proper substratum such as stability, weight, easy availability, costs and consistency are also important [45]. Thus, in this research, foam, dried coconut husk, and the combination of peat moss: *Sphagnum* moss: pumice: perlite (3:1:1:1) were used as the potting medium based on the properties mentioned above.

After maintenance of the seedlings *in vitro* for 4 months, the healthy seedlings were transferred to acclimatize in greenhouse conditions. The results showed that seedlings derived from our experiments were successfully transferred and acclimatized under greenhouse conditions (**Figure 5**). The highest survivability was obtained when using 2 pieces of foam (80 %) followed by dried coconut husk (65 %), and the combination of peat moss: *Sphagnum* moss: pumice: perlite (3:1:1:1) (50 %) (**Table 4**). In addition, we found that seedlings planted on foam and dried coconut husk seemed to continually grow better than those of seedlings cultured on the combination of peat moss: *Sphagnum* moss: pumice: perlite.

Table 4 Survival rate of seedlings in greenhouse for 4th weeks.

Potting medium	Survival rate (%)
1) 2 pieces of foam	80
2) dried coconut husk	65
3) A combination of peat moss: *Sphagnum* moss: Pumice: Perlite (3:1:1:1)	50
F-test	*
C.V. (%)	3.20

Note: * indicates a significant difference at the 95 % confidence level ($p < 0.05$).

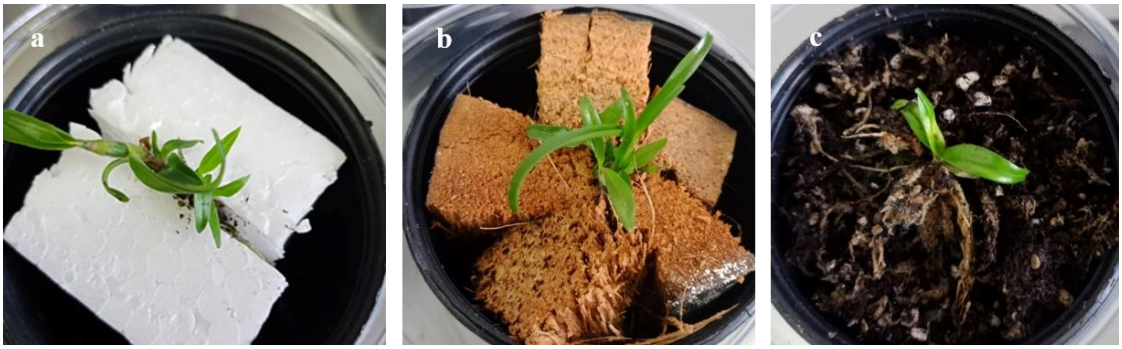

Figure 5 The transplanted seedlings that is grown in a) 2 pieces of foam, b) dried coconut husk, and c) a combination of peatmoss: *Sphagnum* moss: Pumice: Perlite (3:1:1:1) after the 4th weeks of planting under greenhouse conditions.

Generally, the substrates that contained a high-water absorption capacity such as coconut husk, *Sphagnum* moss and perlite are use in a single or combined with other substrates for acclimatization of *Dendrobium* in the greenhouse [45]. However, Evans *et al*. [46] reported that using *Sphagnum* moss and coconut coir-based substrates may encounter fungus gnats, an important pest in plantlet production.

From our results, the different survival rates after acclimatization in the greenhouse might reflect the different water adsorption of the potting mediums. Coconut husk and the mixed potting medium (Peat moss: *Sphagnum* moss: Pumice: Perlite (3:1:1:1)) were high-water absorption capacity materials resulting in unsuitable environment for seedling growth, i.e., the excess level of water adsorption, high moisture retention. Moreover, fungus gnats might occur in both groups, and affect the survivability of seedlings during acclimatization in the greenhouse. However, this hypothesis needs to be investigated further.

Several previous reports indicated that the survival rate of *in vitr*o cultured *Dendrobium* orchid seedlings in the greenhouse was varied. For example, the highest survival rate of *Dendrobium* 'Gradita 31' (100 %) was occurred when using a combination potting medium of wood charcoal and *C. rumphii* bulk (1:1, v/v) [47]. In addition, using a combination of peat moss, wood charcoal, and bricks (1:1:1) in small plastic pots (7.5 cm in diameter) gave the highest survival rate of *D. nobile* seedlings with up to 95 % [48]. In the case of *D. lasianthera* seedlings, the highest survivability under greenhouse conditions (> 90 %) was obtained when the mixture of coconut fiber and *Sphagnum* moss (3:1) was used [15]. Using a combination of sand, brick or tile, charcoal pieces, and coir fiber (1:4:4:2) could provide an 80 % survival rate for *D.* 'Sonia' [49]. Over 60 % of *D. longicornu* seedlings survivability was derived from using a combination of crushed brick and charcoal, shredded bark, and moss [50]. However, the seedlings derived from this research were healthy and could acclimatize under greenhouse conditions.

Conclusions

In this study, the micropropagation of *D. cruentum* Rchb. f. in media supplemented with organic additives was evaluated. Overall, it could be concluded that media supplemented with organic additives yielded better observed parameters than a medium without organic additives. Based on these results, a combination of banana and potato homogenates was determined to be the best for seed germination rate. Coconut water and its combination with potato homogenate were suitable for protocorm formation. Interestingly, banana homogenate yielded the highest number of protocorm-derived seedlings. Moreover, supplementation with organic additives positively affected seedling growth. However, supplementation with banana homogenate gave the best results of the most observed growth parameters. In addition, the healthy plantlets could acclimatize in the greenhouse with a high survival rate (80 %) when using the foam as the potting medium. Therefore, the VW media supplemented with organic additives instead of PGRs provided the sufficiently nutritional, practical, beneficial, and convenient media for *D. cruentum* micropropagation *in vitro* through seeds in a one-step protocol. Finally, it could reduce the cost and be helpful for *D. cruentum* species conservation and commercial applications.

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