Effects of BAP, Kinetin, Coconut Water and Activated Charcoal on Growth and Development of *Rhynchostylis gigantea* (Lindl.) Ridl. through Protocorms Culture

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ABSTRACT

Protocorms like bodies (PLBs) of *Rhynchostylis gigantea* (Lindl.) Ridl. were cultured on half NO_3 MS solid medium according to Murashige and Skoog, 1962. The effects of hormones supplemented with different concentrations of BAP (6-benzylaminopurine), Kinetin (6-furfurylaminopurine), Coconut water (CW) and Activated charcoal (AC). Growth and development of protocorms *in vitro* culture were studied. The highest growth and development from BAP 1.0 mg/l, Kinetin 0.5 mg/l, Coconut water 150 ml/l and Activated charcoal 1.0 g/l showed the best result. The growth of protocorms were recorded by color photographs. This experiment was carried out at the Tissue Culture Laboratory, Department of Botany, Dagon University during December to July in 2022 to 2023.

INTRODUCTION

The orchid family (Orchidaceae) is one of the greatest families of flowering plants consisting of 30,000-35,000 species in 850 genera (Hossain, 2013). A checklist of Myanmar orchid flora which consists of 1040 species and 151 genera currently known from Myanmar (Yee Lwin Aung, 2020). The genus *Rhynchostylis* include 4 species; *coelestic*, *gigantea*, *retusa* and *riefer* (Soon, 1980). Orchid species of *Rhynchostylis gigantea* (Lindl.) Ridl. was first described in 1896 John Lindley and is native to Borneo, Myanmar, Thailand, Penisular Malaysia, Laos, Cambodia, Vietnam, China (Hainan) and Philippines (Kew, 2022). Orchid protocorms culture have been tried with various kinds of media by many workers. Plant tissue culture is described as culturing plant seeds, organs, explants, tissues, cells, or protoplasts on a chemically defined synthetic nutrient media under sterile and controlled environmental conditions of light, temperature and humidity (Bhatia, 2015). A wide range of chemically defined synthetic nutrient media Murashige and Skoog (MS) medium are used in the plant tissue culture (Gusain, 2021). Plant tissue culture media should usually include some or all of the following components; macronutrients, micronutrients, vitamins, source of carbon, growth hormones, natural additives and solidifying agents (Fossard,1976). Cytokinin (BAP, Kinetin) is necessary in differentiation and micropropagation of most plant species, it encourage cell division, shoot initiation, callus proliferation and protocorm occurrence (Choi *et al.,* 2011). Coconut water is the effect of cytokinins from naturally additives in orchidaceae culture and it promotes the protocorm occurrence obviously and root development (Yinkai *et al.,* 2020). Activated charcoal is often used in plant tissue culture to stimulate growth and development, absorption of phenolic compound in the orchid culture media and dark conditions for culture media (Sasikarm *et al.,* 2016). The aims and objectives were to investigate a suitable technique in half NO_3 MS solid medium for the effects of various hormones on the protocorms of *Rhynchostylis gigantea* (Lindl.) Ridl. by *in vitro* culture.

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MATERIALS AND METHODS

Collection and Identification of *Rhynchostylis gigantea* (Lindl.) Ridl.

The specimens were collected from Mingalardon Orchid Garden, Mingalardon Township, Yangon region. The morphological and taxonomical studies were made from the collected specimen by using available literature such as Hundley and Chit Ko Ko (1987) and Datta (1970).

Source of Plant Materials

The protocorm like bodies (PLBs) of *Rhynchostylis gigantea* were used as a source plant material for culture. The experiment of *Rhynchostylis gigantea* was carried out at the Tissue Culture Laboratory, Department of Botany, Dagon University during December to July in 2022 to 2023.

Culture Medium

The protocorm like bodies (PLBs) of *Rhynchostylis gigantea* (Lindl.) Ridl. were cultured on half NO_3 MS basal media supplement with 30 g/l of sucrose, 8 g/l of agar for solid media of plant growth hormones: BAP (6-Benzylaminopurine), Kinetin (6-furfurylaminopurine), used of Coconut Water (CW) from natural additives and Activated Charcoal (AC).

Culture of Explants

The initial size and weight of *Rhynchostylis gigantea* (Lindl.) Ridl. PLBs was about 0.5 cm and 30 mg. The PLBs were excised from in a culture chamber. The PLBs were cultured into prepared cultured bottles. The various concentrations of sucrose, plant growth hormones, coconut water and activated charcoal were used and then three replicates were done for each treatment in figure 1.

Figure 1. Initial stage of *Rhynchostylis gigantea* (Lindl.) Ridl.

Culture Condition

All culture bottles were inoculated at 24 ± 1°C under condition illumination from 4-feet fluorescent tubes, (1000 -1200 lux) light intensity and photoperiod of 16/8 hrs (light/dark).

Measurement and Recording of Growth

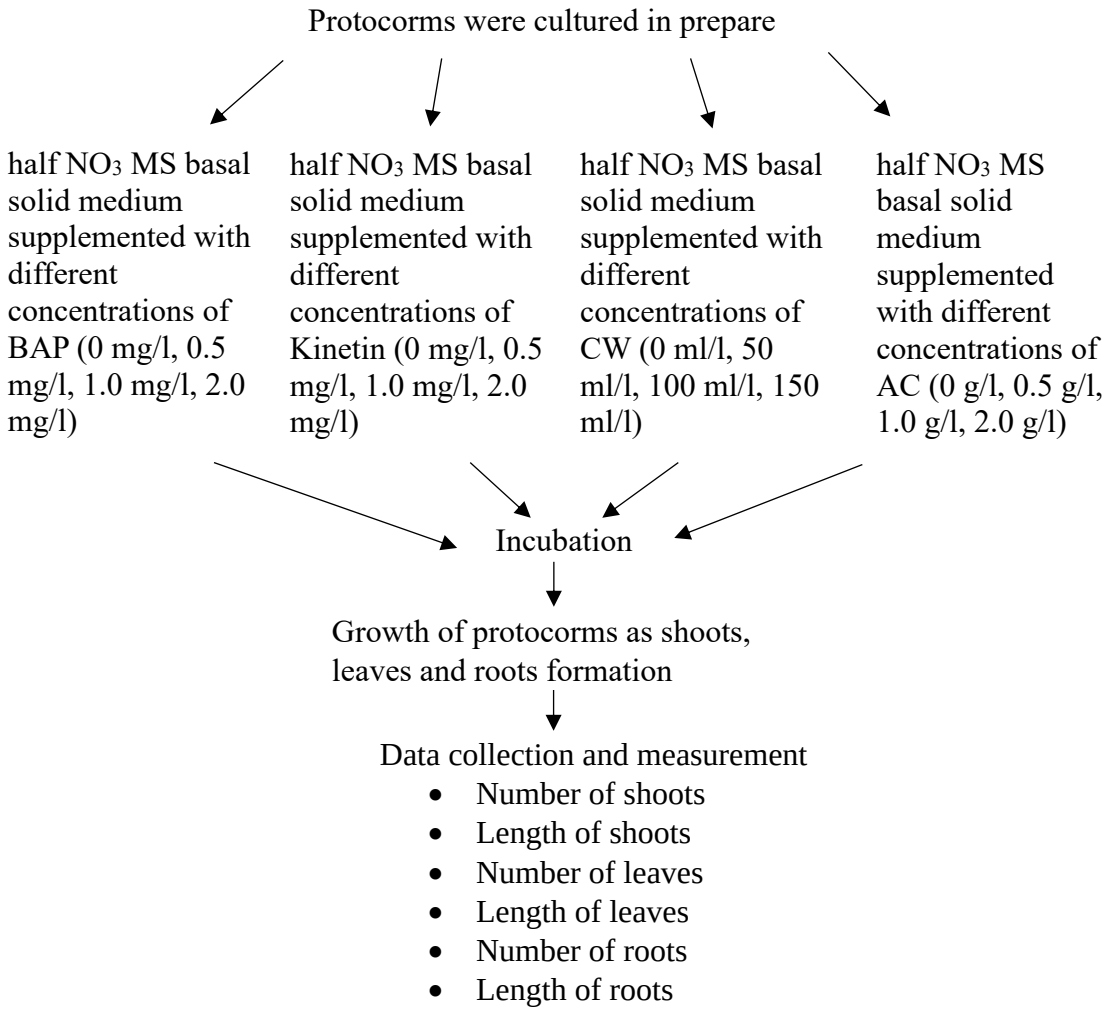
Growth of protocorms were measured by recording fresh weight and dry weight. Growth value is the ratio of final fresh weight to initial fresh weight. The number of shoots, the length of shoots, the number of leaves, the length of leaves, the number of roots and the length of roots were recorded at four months after culture.

Method of Media Preparation

According to Murashige and Skoog (1962), method of medium preparation applied in the present study is as follows:

- 50 ml of stock solutions were poured into 1000 ml beaker.
- Sugar (30 g/l of sucrose) were added and stirred.
- The required amount of distilled water was added to fulfill the 1L media concentration.
- pH value of the media was measured and adjusted (5.8) with NaOH and HCL.
- The solution was gently heated and stirred continuously until boiled.
- Agar powder 8 g/l was slowly added and stirred to dissolved the agar and then the media became amber coloured.
- Then 20 ml of the medium was poured into 150 ml size culture bottles.
- The culture bottles were covered and sterilized by autoclaving at 121°C (1.5kg/cm^2) pressure for 15 mins.
- Then the bottles were cooled and used.

Micropropagation of protocorm like bodies *Rhynchostylis gigantea* (Lindl.) Ridl. in MS solid medium



Word diagram of experiments 1, 2, 3 and 4

Table 1. Different concentration of BAP supplemented to solid medium

Treatment	Basal Medium (solid)	Hormone BAP concentration (mg/l)
T_0	half NO_3 MS	0
T_1	half NO_3 MS	0.5
T_2	half NO_3 MS	1.0
T_3	half NO_3 MS	2.0

Table 2. Different concentration of Kinetin supplemented to solid medium

Treatment	Basal Medium (solid)	Hormone kinetin concentration (mg/l)
T_0	half NO_3 MS	0
T_1	half NO_3 MS	0.5
T_2	half NO_3 MS	1.0
T_3	half NO_3 MS	2.0

Table 3. Different concentration of Coconut Water (CW) supplemented to solid medium

Treatment	Basal Medium (solid)	Coconut Water concentration (ml/l)
T_0	half NO_3 MS	0
T_1	half NO_3 MS	50
T_2	half NO_3 MS	100
T_3	half NO_3 MS	150

Table 4. Different concentration of Activated Charcoal (AC) supplemented to solid medium

Treatment	Basal Medium (solid)	Activated Charcoal concentration (g/l)
T_0	half NO_3 MS	0
T_1	half NO_3 MS	0.5
T_2	half NO_3 MS	1.0
T_3	half NO_3 MS	2.0

RESULT

Morphological Characters of *Rhynchostylis gigantea* (Lindl.) Ridl.

Scientific name - *Rhynchostylis gigantea* (Lindl.) Ridl.
Family - Orchidaceae
English name – Foxtail Orchid
Myanmar name – Kyaung-mi-nantha

Monopodial epiphytes, pseudobulbs not present, stems erect, stout, leaves alternate, apex obtuse, unequally bilobed, thick leathery leaves, dark green, inflorescences axillary, racemes, flower thickly textured, white spotted with purplish red, fragrant blooms, sepals similar, obtuse, petals oblong, smaller than sepals, apex obtuse in figure 2.

Figure 2. Habit of Rhynchostylis gigantea (Lindl.) Ridl.

Table 5. Mean values of *Rhynchostylis gigantea* (Lindl.) Ridl. cultured on ½ NO_3 MS solid medium with different hormone concentrations of BAP

Three Months Data				
Growth parameter	Treatment (BAP)			
	0 mg/l (T_0)	0.5 mg/l (T_1)	1.0 mg/l (T_2)	2.0 mg/l (T_3)
Number of shoots	7.33	13.33	**22.66**	16.66
Length of shoots (cm)	0.31	0.43	**0.53**	0.41
Number of leaves	1.33	10.03	**50.04**	7.71
Length of leaves (cm)	0.33	0.63	**1.43**	0.82
Number of roots	0.31	1.33	**9.33**	1.01
Length of roots (cm)	0.11	0.33	**0.93**	0.24

In experiment-1, after three months culture, 1.0 mg/l (T_2) medium gave the best result of PLBs growth and development while 0 mg/l (T_0) medium gave the lowest result.

Figure 3. Different concentrations of BAP after three months culture in *Rhynchostylis gigantea* (Lindl.) Ridl.

Table 6. Mean values of the *Rhynchostylis gigantea* (Lindl.) Ridl. cultured on half NO_3 MS solid medium with different hormone concentrations of Kinetin

Three Months Data				
Growth parameter	Treatment (Kinetin)			
	0 mg/l (T_0)	0.5 mg/l (T_1)	1.0 mg/l (T_2)	2.0 mg/l (T_3)
Number of shoots	7.33	**36.66**	13.33	12.02
Length of shoots (cm)	0.31	**0.62**	0.52	0.43
Number of leaves	1.33	**33.33**	15.01	8.02
Length of leaves (cm)	0.33	**1.33**	0.91	0.72
Number of roots	0.31	**6.72**	3.72	2.01
Length of roots (cm)	0.11	**0.93**	0.62	0.52

In experiment-2, after three months culture, the maximum number of shoots, leaves and roots found the medium supplemented with kinetin 0.5 mg/l (T_1) while 0 mg/l (T_0) medium gave the lowest result.

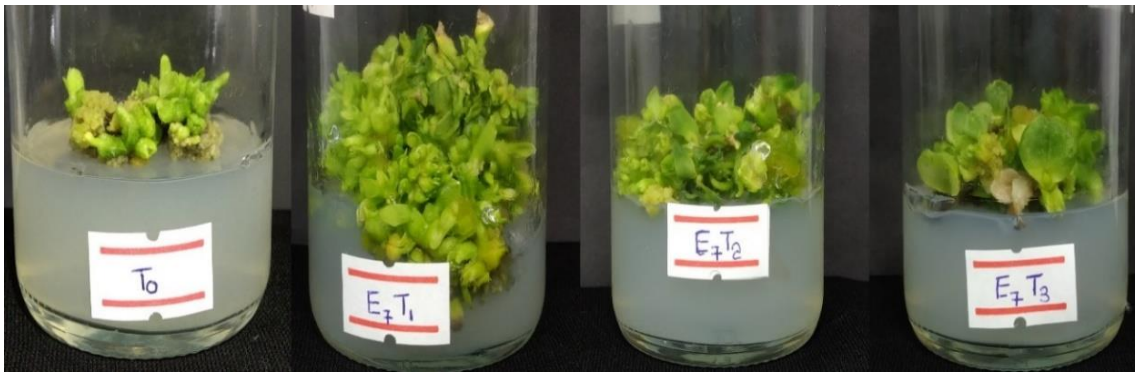


Figure 4. Different concentrations of BAP after three months culture in *Rhynchostylis gigantea* (Lindl.) Ridl.

Table 7. Mean values of *Rhynchostylis gigantea* (Lindl.) Ridl. cultured on ½NO_3 MS solid medium with different hormone concentrations of Coconut water (CW)

Three Months Data				
Growth parameter	Treatment (Coconut water)			
	0 ml/l (T_0)	50 ml/l (T_1)	100 ml/l (T_2)	150 ml/l (T_3)
Number of shoots	7.33	16.73	19.33	**50.03**
Length of shoots (cm)	0.31	0.33	0.42	**0.52**
Number of leaves	1.33	3.33	6.73	**20.03**
Length of leaves (cm)	0.33	0.73	0.81	**1.03**
Number of roots	0.31	0.71	1.33	**2.03**
Length of roots (cm)	0.11	0.33	0.73	**2.43**

In experiment-3, after three months culture, 150 ml/l (T_3) of culture medium gave the number of shoots multiplication and leaves and roots well-developed.

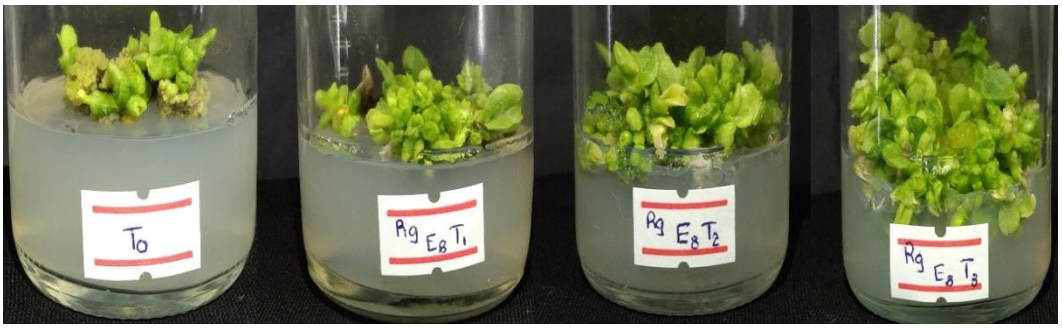


Figure 5. Different concentrations of Coconut water (CW) after three months culture in *Rhynchostylis gigantea* (Lindl.) Ridl

Table 8. Mean values of *Rhynchostylis gigantea* (Lindl.) Ridl. cultured on half NO_3 MS solid medium with different hormone concentrations of Activated Charcoal (AC)

Three Months Data				
Growth parameter	Treatment (Activated charcoal)			
	0 g/l (T_0)	0.5 g/l (T_1)	1.0 g/l (T_2)	2.0 g/l (T_3)
Number of shoots	7.33	20.03	**56.72**	13.33
Length of shoots (cm)	0.31	0.52	**0.63**	0.43
Number of leaves	1.33	3.33	**18.71**	2.74
Length of leaves (cm)	0.33	0.83	**1.14**	0.73
Number of roots	0.31	1.33	**1.74**	1.04
Length of roots (cm)	0.11	0.43	**0.53**	0.32

The result of the last experiment after three months cultue, 1.0 g/l (T_2) of culture medium gave the highest levels of shoots, leaves and roots.

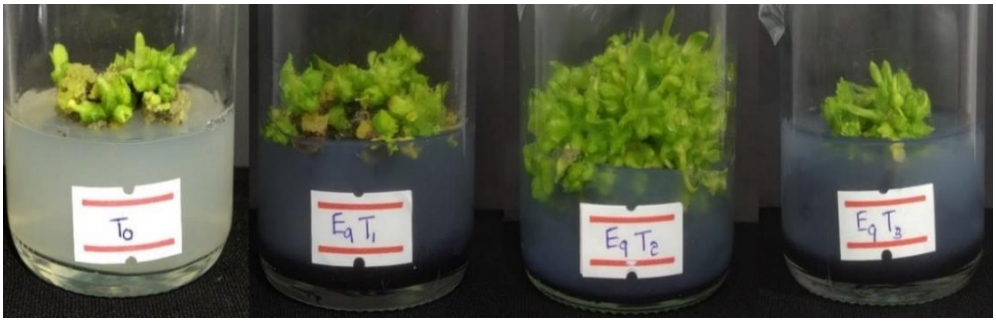


Figure 9. Different concentrations of Activated charcoal (AC) after three months culture in *Rhynchostylis gigantea* (Lindl.) Ridl.

DISCUSSIONS AND CONCLUSION

In this experiment, the morphological study, *Rhynchostylis gigantea* (Lindl.) Ridl. leaves are simple, alternate, obtuse, unequally bilobed, inflorescence axially, racemes, white with purplish-red colour. This finding was in agreement with Datta (1970) Hundley and Chit Ko Ko (1987).

In experiment-1, half NO_3 MS solid medium supplemented with different concentrations of BAP (0 mg/l, 0.5 mg/l, 1.0 mg/l, 2.0 mg/l) were used. After three

months culture, 1.0 mg/l (T_2) medium gave the best result of PLBs growth and development and 0 mg/l (T_0) medium gave the lower result. This observation agreed with the results of Tran (2019) who reported that BAP 1.0 mg/l formation and proliferation of callus suspension in MS medium. Sasikarm *et al.,* (2016) reported that 1-2 mg/l BAP induced shoots and PLBs formation, but 4 mg/l BAP decreased shoot formations.

In expriement-2, half NO_3 MS solid medium with different concentrations of kinetin (0 mg/l, 0.5 mg/l, 1.0 mg/l, 2.0 mg/l) were used. After three months, the maximum number of shoots, leaves and roots found the medium supplemented with kinetin 0.5 mg/l (T_1). This finding agreed with Tran, (2019) reported that MS medium supplemented with kinetin 0.5 mg/l rapid PLBs growth and highest proliferation rate.

In experiment-3, half NO_3 MS solid medium with different concentrations of coconut water (0 ml/l, 50 ml/l, 100 ml/l, 150 ml/l) were used. The result of the experiment after three months culture, 150 ml/l (T_3) of culture medium gave the number of shoots multiplication and leaves and roots well-developed. This result agreed with Kaewkhiew *et al.,* (2010) have postulated that 150 ml/l of coconut water showed the highest of plant height, fresh weight, number of leaf and number of roots.

The last experiment, half NO_3 MS solid medium with different concentrations of activated charcoal (0 g/l, 0.5 g/l, 1.0 g/l, 2.0 g/l) were used. The result of the last experiment after three months culture, 1.0 g/l (T_2) of culture medium gave the highest levels of shoots, leaves and roots. This result agreed with Aung Htay Naing *et al.,* (2010) reported that activated charcoal 1 g/l medium showed distinctly superior growth response than without AC.

In all experiments, BAP 1.0 mg/l (T_2) is the best growth and development from protocorms to plantlets of *Rhynchostylis gigantea* (Lindl.) Ridl. It is concluded that the attractive purplish-red flower color of *R. gigantea* make it a popular commercial orchid with various extinction reasons so not only for commercial reasons but also for conservation with plant tissue culture technique.

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