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An Effect of Organic Supplements on Stimulating Growth of *Dendrobium* Protocorms and Seedlings

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***Abstract*—**This study was aimed to investigate the effect of various organic supplements on growth and development of *Dendrobium discolor*'s protocorms and seedlings growth of *Dendrobium* Judy Rutz. Protocorms of *Dendrobium discolor* with 2.0 cm. in diameter and seedlings of *Dendrobium* Judy Rutz at the same size (0.5 cm. height) were sub-cultured on Hyponex medium supplemented with cow milk (CM), soy milk (SM), potato extract (PE) and peptone (P) for 2 months. The protocorms were developed to seedlings in all treatments after cultured for 2 months. However, the best results were found on Hyponex medium supplemented with P was the best in which the maximum fresh and dry weight and maximum shoot height were obtained in this treatment statistically different ($p \leq 0.05$) to other treatments. Moreover, Hyponex medium supplemented with P also stimulated the maximum mean number of 5.7 shoots per explant which also showed statistically different ($p \leq 0.05$) when compared to other treatments. The results of growth of *Dendrobium* Judy Rutz seedlings indicated the medium supplemented with 100 mL/L PE enhanced the maximum fresh and dry weigh per explants with significantly different ($p \leq 0.05$) in fresh weight from other treatments including the control medium without any organic supplementation. However, the dry weight was not significantly different ($p \leq 0.05$) from medium supplemented with SM and P. There was multiple shoots induction in all media with or without organic supplementation ranging from 2.6 to 3 shoots per explants. The maximum shoot height was also obtained in the seedlings cultured on medium supplemented with PE while the longest root length was found in medium supplemented with SM.

***Keywords*—**Fresh weight, *in vitro* propagation, orchid, plant height.

I. Introduction

DENDROBIUM is the most popular orchid for cut flower trade in Asia. The environmental conditions required for the growth, development and culture of *Dendrobium* orchids are adequately available throughout the year in Thailand. In Thailand, micropropagation of orchids is the most frequently used for producing a good quality seedling as well as young plants [1], [2].When mass propagation of new hybrids are needed within a short period of time, tissue culture is the only method to fulfill that objective [3]. Many studies on micropropagation of orchids have been carried out [4]–[8]. Tokuhara and Mii [9] reported that the appropriate combination and concentrations of hormones, organic additives and the composition of macro and micro elements in the culture medium were very importance for micro-propagation of *Dendrobium* for commercial mass propagation.

Arditti [10] suggested that complex growth supplements have the ability to influence *in vitro* regeneration, multiplication of protocorm-like bodies (PLBs) and growth of orchid seedlings since than a variety of organic growth supplements such as apple juice, banana homogenate, casein hydrolysate, coconut water, corn extract, tomato juice, peptone, yeast extract etc. were tested for promoting growth and development of *in vitro* cultures. Growth rate of the tissues can be increased by the addition of organic supplements and plant extracts [11].

Effects of organic growth additives are tested in a large number of orchids such as *Paphiopedilum* species[12], *Vanda* hybrids [13], *Acampe praemorsa* [14], *Aranda* [15], *Cattleya*, *Encyclia*, *Oncidium* and *Stanhopea* [16], *Dendrobium* species [17], *Geodorum densiflorum* [18], *Doritaenopsis* [19], *Cypripedium formosanum* [20], *Dendrobium* hybrid [21], *Phalaenopsis gigantean* [22], *Dendrobium* species [23] and in *Zygopetalum mackayi* [24].

The objective of the present work is to examine the hypothesis that organic growth supplements might affect growth of *D.* Judy Rutz seedlings and *D. discolor* protocorms without the use of any growth regulators.

II. Materials and Methods

A. Plant Material and Culture Condition

D. Judy Rutz's seedlings (0.5 cm in height with 0.03 g fresh weight) and protocorms of *D. discolor* (0.2 cm in height and 0.003 g fresh weight) were obtained from 24-week old and 10-week old of *D.* Judy Rutz's and *D. discolor in vitro* seed cultures. The seedlings and protocorms were maintained for 8-12 weeks on H medium which composed of 0.35% (w/v) Hyponex fertilizer (6.5-6-19), 2.0% (w/v) sucrose, 0.2% (w/v) phytagel and also 0.5 g/l activated charcoal were used as control. The complex growth supplements such as 150 ml/l milk, 2.0 g/l milk powder (Carnation), 2.0 g/l organic soy powder, 100 ml/l potato extract, 2.0 g/l peptone, and 100 ml/l potato extract with 2.0 g/l peptone were used individually in the medium. Cow milk, milk powder (Carnation), organic soy powder and potato were bought from a local market. Potato was peeled and cut into pieces about 1 cm^3 in size. One hundred grams of freshly diced potato was boiled for 20 min in 500 ml of water and the hot supernatant was filtered. The

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pH of medium was adjusted to 5.4 after adding the organic growth supplements. The medium was autoclaved at 121°C at pressure of 1.1 kg/cm^2 for 15 min.

B. Inoculations and Inoculation Conditions

The inoculations were done under aseptic conditions in a laminar air flow cabinet. All the cultures were incubated at 25 ± 2°C under 16 h photoperiod of 3,500 lux light intensity (Fluorescent tubes 40 W; Philips).

C. Observation and Statistical Analysis

The effect of organic supplements was tested on growth (fresh and dry weight of single plant) number and length of shoots and roots in H medium (Hyponex medium) for 8-12 weeks. The data were recorded. The results were tested using one-way ANOVA test and were analyzed using the Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

III. Results

D. Judy Rutz seedlings and *D. discolor* protocorms were cultured in H medium supplemented with different organic supplements (milk, milk powder, soy powder, potato, peptone and potato with peptone).

Fresh weight: The results showed significant difference in the fresh weight of *D.* Judy Rutz after inoculation. The maximum fresh weight (0.578 g) of *D.* Judy Rutz was obtained from H medium containing potato extract with 2.0 g/l peptone significantly different than other treatments (Table I, Fig. 1 (a)). The fresh weights of *D.* Judy Rutz in H medium containing milk powder, organic soy powder, or peptone medium were not significantly compared to the control. However, the minimum fresh weight of single plantlet (0.179 g) was found significantly lower in H medium containing milk medium than the control.

For protocorms of *D. discolor*, the fresh weight in all organic supplement treatments showed significant difference with control after inoculation. The fresh weight 0.182, 0.161, 0.087, 0.062 and 0.030 g of *D. discolor* were obtained from H medium containing potato extract with 2.0 g/l peptone, 2.0 g/l peptone, potato extract, organic soy powder and milk powder, respectively (Table II, Fig. 1 (b)).

***Dry weight*:** The dry weight of *D.* Judy Rutz in organic supplement treatments showed significant difference with the control after inoculation. The maximum dry weight 0.0351 g of *D.* Judy Rutz was obtained from H medium containing potato extract with 2.0 g/l peptone (Table I, Fig. 2 (a)). The dry weights of *D.* Judy Rutz in H medium containing milk powder, organic soy powder, or peptone medium were not significantly compared to the control. However, the minimum dry weight of single plantlet (0.0126 g) was found significantly lower in H medium containing milk medium than the control (Table I, Fig. 2 (a)).

For protocorms of *D. discolor*, the dry weight in organic supplement treatments showed significant difference with the control after inoculation. The maximum dry weight 0.0130 and 0.0126 g of *D. discolor* were obtained from H medium containing potato extract with 2.0 g/l peptone and 2.0 g/l peptone alone, respectively (Table I, Fig. 2 (b)). The dry weight of *D. discolor* in H medium containing milk powder (0.0026 g), organic soy powder (0.0046 g), or potato extract (0.0069 g) medium were significantly when compared to the control (0.0011 g) (Table II, Fig. 2 (b)).

***Number of shoots*:** The shoots numbers of *D.* Judy Rutz in H medium containing milk (3.1 shoots/explant) (Fig. 5 (b)), milk powder (2.8 shoots/explant) (Fig. 5 (c)), potato extract (3.0 shoots /explant) (Fig. 5 (e)), peptone (2.8 shoots/explant) (Fig. 5 (f)) and potato extract with peptone (3.3 shoots /explant) (Fig. 5 (g)) were not significantly compared to the control (3.0 shoots/ explant) (Fig. 5 (a)). However, the minimum numbers of shoots (2.6 shoots/explant) was found significantly lower in H medium containing organic soy powder medium than the medium supplemented with potato extract and peptone (Table I, Fig. 3 (a)).

For protocorms of *D. discolor*, the maximum shoot numbers 5.7 shoots/explant (Fig. 6 (f)) was found in H medium containing 2.0 g/l peptone and potato extract with 2.0 g/l peptone significantly compared to other treatments (Table II). The shoots numbers of *D. discolor* in H medium containing milk powder (1.3 shoots/explant), organic soy powder (1.3 shoots/explant) and potato extract (1.5 shoots/explant) were not significantly compared to the control (1.0 shoots/explant) (Table II, Fig. 6).

***Number of roots*:** The roots numbers of *D.* Judy Rutz in H medium containing milk (4.5 roots/explants), milk powder (5.5 roots/explants), organic soy powder (4.8 roots/explants), potato extract (5.4 roots/explant), peptone (4.9 roots/explant) and potato extract with peptone (6.4roots /explants) were not significantly compared to the control (5.9 roots/explant) (Table I, Fig. 3 (a)).

For protocorms of *D. discolor*, the maximum root numbers 5.5 roots/explants was obtained from H medium containing potato extract with 2.0 g/l peptone (Table I, Fig. 3 (a)). The control treatment had the lowest root number significantly different than other treatments added with organic supplements (Table II, Fig. 3 (b)).

***Length of shoot*:** The highest (1.60 cm) and lowest (0.89 cm) shoot lengths of *D.* Judy Rutz were obtained from potato extract with peptone and milk, respectively (Table I, Fig. 4 (a)). The shoot lengths of *D.* Judy Rutz in H medium containing milk powder (1.20 cm), organic soy powder (1.29 cm), potato extract (1.44 cm) and peptone (1.26 cm) were not significantly compared to the control (1.36 cm) (Table I, Fig. 4 (a)).

For protocorms of *D. discolor*, the maximum shoot lengths 0.96 cm was obtained from potato extract with 2.0 g/l peptone. The shortest shoot lengths 0.41 cm of *D. discolor* was found in H medium supplemented with milk powder, but not significantly different when compared to the control (0.43 cm) (Table II, Fig. 4 (b)).

***Length of root*:** The longest root lengths of *D.* Judy Rutz1.67 cm was found in H medium containing organic soy powder significantly different to other treatments including the control (Table I, Fig. 4 (a)). However, the shortest roots length

of *D.* Judy Rutz in was found in H medium containing cow milk (0.84 cm) (Table I, Fig. 4 (a)).

For protocorms of *D. discolor*, the longest (0.93 cm) roots length was obtained from potato extract with 2.0 g/l peptone medium (Table II, Fig. 4 (b)).The shortest roots length of *D. discolor* (0.12 cm) was found in H medium supplemented with milk powder significantly lower than other treatment including the control (Table II, Fig. 4 (b)).

TABLE I
EFFECTS OF HYPONEX MEDIUM SUPPLEMENTED WITH DIFFERENT ORGANIC SUPPLEMENTS ON GROWTH OF *D* .JUDY RUTZ IN TISSUE CULTURE FOR 8 WEEKS

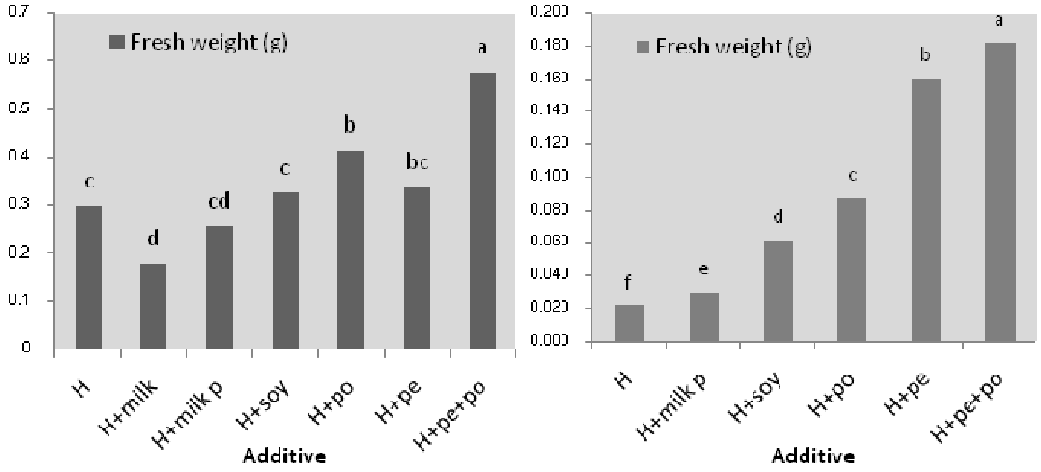
Additive	Fresh weight (g)	Dry weight (g)	Number of shoots/explants	Number of roots/explants	Length of shoot (cm)	Length of root (cm)
H	0.299 ± 0.0258^{c}	0.0192 ± 0.00199^{c}	3.0 ± 0.18^{ab}	5.9 ± 0.55^{ab}	1.36 ± 0.059^{bc}	1.04 ± 0.059^{de}
H+milk	0.179 ± 0.0122^{d}	0.0126 ± 0.00083^{d}	3.1 ± 0.22^{ab}	4.5 ± 0.33^{b}	0.89 ± 0.038^{d}	0.84 ± 0.044^{e}
H+milk p	0.258 ± 0.0192^{cd}	0.0176 ± 0.00133^{cd}	2.8 ± 0.19^{ab}	5.5 ± 0.54^{ab}	1.20 ± 0.059^{c}	1.12 ± 0.068^{cd}
H+soy	0.326 ± 0.0288^{c}	0.0227 ± 0.00199^{bc}	2.6 ± 0.13^{b}	4.8 ± 0.38^{b}	1.29 ± 0.047^{bc}	1.67 ± 0.100^{a}
H+po	0.416 ± 0.0292^{b}	0.0261 ± 0.00208^{b}	3.0 ± 0.19^{ab}	5.4 ± 0.54^{ab}	1.44 ± 0.057^{b}	1.46 ± 0.067^{b}
H+pe	0.340 ± 0.0314^{bc}	0.0208 ± 0.00218^{bc}	2.8 ± 0.16^{ab}	4.9 ± 0.38^{b}	1.26 ± 0.049^{c}	1.15 ± 0.072^{cd}
H+pe+po	0.578 ± 0.0457^{a}	0.0351 ± 0.00265^{a}	3.3 ± 0.27^{a}	6.4 ± 0.48^{a}	1.60 ± 0.064^{a}	1.32 ± 0.070^{bc}

Means ± Std. Error in a column with similar superscripts are not significantly different at $p \leq 0.05$
Where, H = Hyponex,p = powder, soy = Organic Soy Powder, po = potato extract, pe = peptone.

TABLE II
EFFECTS OF HYPONEX MEDIUM SUPPLEMENTED WITH DIFFERENT ORGANIC SUPPLEMENTS ON GROWTH OF *D .DISCOLOR* IN TISSUE CULTURE FOR 12 WEEKS

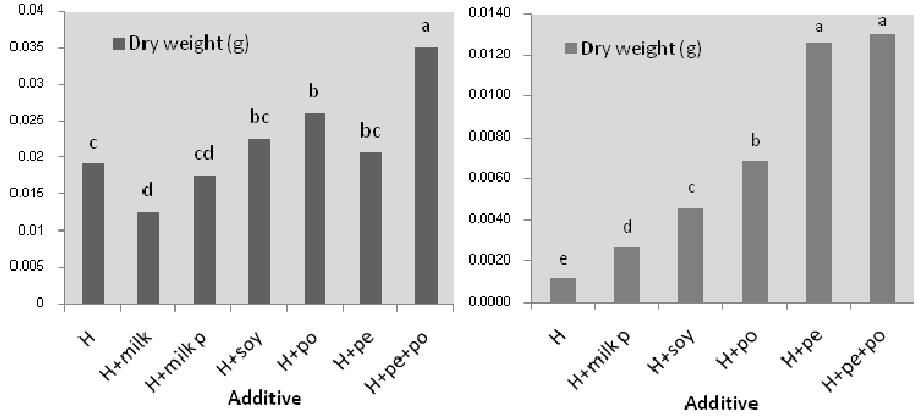
Additive	Fresh weight (g)	Dry weight (g)	Number of shoots/explants	Number of roots/explants	Length of shoot (cm)	Length of root (cm)
H	0.022 ± 0.0007^{f}	0.0011 ± 0.00003^{e}	1.0 ± 0.00^{c}	1.0 ± 0.00^{d}	0.43 ± 0.008^{e}	0.62 ± 0.029^{b}
H+milk	0.000 ± 0.0000^{g}	0.0000 ± 0.00000^{f}	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}	0.00 ± 0.000^{f}	0.00 ± 0.000^{e}
H+milk p	0.030 ± 0.0002^{e}	0.0026 ± 0.00002^{d}	1.3 ± 0.12^{c}	1.9 ± 0.19^{c}	0.41 ± 0.008^{e}	0.12 ± 0.011^{d}
H+soy	0.062 ± 0.0028^{d}	0.0046 ± 0.00011^{c}	1.3 ± 0.13^{c}	3.1 ± 0.21^{b}	0.61 ± 0.010^{d}	0.59 ± 0.029^{b}
H+po	0.087 ± 0.0021^{c}	0.0069 ± 0.00012^{b}	1.5 ± 0.13^{c}	3.5 ± 0.27^{b}	0.70 ± 0.006^{c}	0.47 ± 0.019^{c}
H+pe	0.161 ± 0.0030^{b}	0.0126 ± 0.00024^{a}	5.7 ± 0.67^{a}	3.5 ± 0.32^{b}	0.80 ± 0.012^{b}	0.48 ± 0.015^{c}
H+pe+po	0.182 ± 0.0026^{a}	0.0130 ± 0.00019^{a}	3.6 ± 0.47^{b}	5.5 ± 0.31^{a}	0.96 ± 0.016^{a}	0.90 ± 0.025^{a}

Means ± Std. Error in a column with similar superscripts are not significantly different at $p \leq 0.05$
Where, H = Hyponex, p = powder, soy = Organic Soy Powder, po = potato extract, pe = peptone.



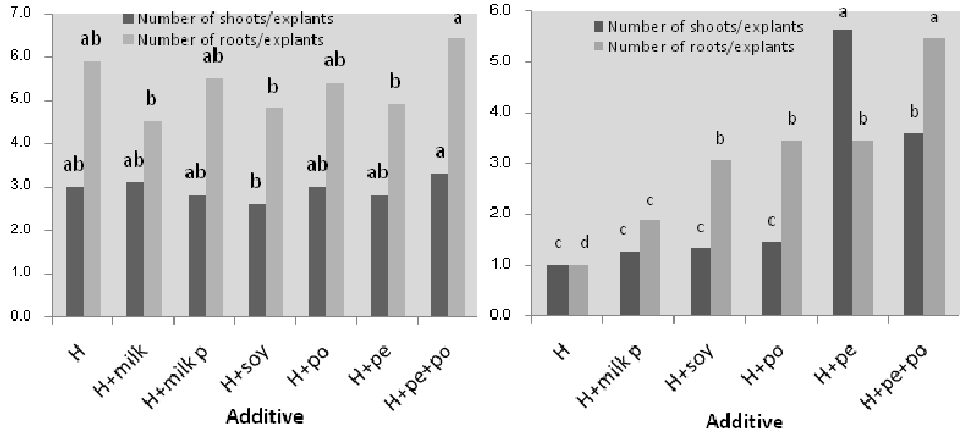
(a) (b)

Fig. 1 Effects of Hyponex medium supplemented with different organic supplements on fresh weight of *D.* Judy Rutz seedling for 8 weeks (a) and *D. discolor* protocorm for 12 weeks (b)



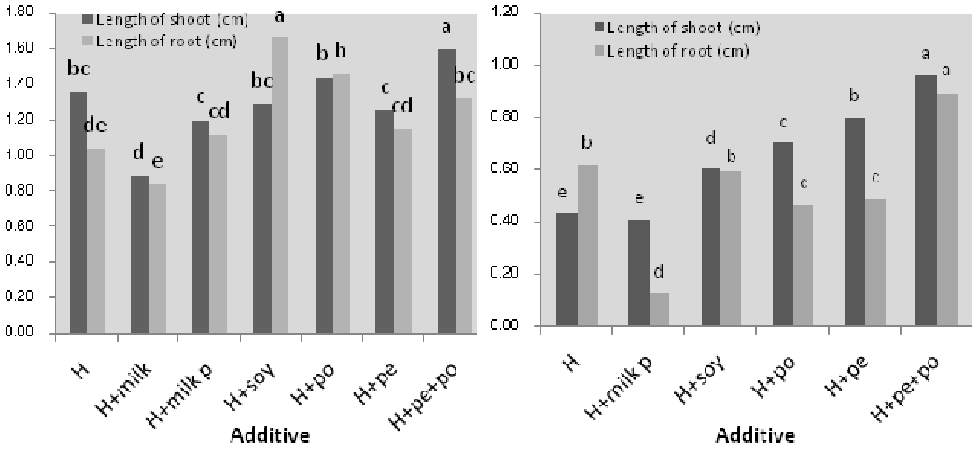
(a) (b)

Fig. 2 Effects of Hyponex medium supplemented with different organic supplements on dry weight of *D.* Judy Rutz seedling for 8 weeks (a) and *D. discolor* protocorm for 12 weeks (b)



(a) (b)

Fig. 3 Effects of Hyponex medium supplemented with different organic supplements on shoot and root number of *D.* Judy Rutz seedling for 8 weeks (a) and *D. discolor* protocorm for 12 weeks (b)



(a) (b)

Fig. 4 Effects of Hyponex medium supplemented with different organic supplements on shoot and root length of *D.* Judy Rutz seedling for 8 weeks (a) and *D. discolor* protocorm for 12 weeks (b)

Fig. 5 Seedling of *D.* Judy Rutz on Hyponex medium (a), and H medium supplemented with cow milk (b), milk powder (c), organic soy powder (d), potato (e), peptone (f) and peptone + potato (g) for 8 weeks

Fig. 6 Protocorm of *D. discolor* on Hyponex medium (a) and H medium supplemented with milk powder (b), organic soy powder (c), potato (d), peptone (e) and peptone + potato (f) for 12 week

IV. Discussions

In this experiment revealed that H medium supplemented with peptone and potato extract was the best medium for growth and development of *Dendrobium* seedling and protocorm stages. Earlier Arditti [25] made similar conclusions indicating the beneficial effects of organic growth supplements on growth and differentiation of protocorms and further seedling development. It was also earlier reported by Chen and Chang [26] that at an efficient concentration, organic and inorganic nitrogen sources can promote the growth of explants. In experiment, potato extract and potato extract with peptone medium proved beneficial for development of healthy shoots growth of *D.* Judy Rutz seedlings and *D .discolor* protocorms.

According to potato contains carbohydrates, protein, fat, several vitamins, phenolic compounds and low levels of some amino acids and fatty acids. Any of these or other substances, yet unknown, single or in combination might be factor(s) that enhance(s) seedling growth compared to the control.

Peptone being water soluble protein hydrolysate with very high amino acid content, thus it promotes plant growth in tissue culture. Peptone is also known to have stimulated callus growth in *Phalaenopsis*, *Doritaenopsis*, and *Neofinetia* [27]. It also enhanced seedling growth in *Paphiopedilum*, *Phaius*, and *Vanda* [28]. In *Peristeria elata* peptone induced early and healthy growth of seedlings [29].

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Milk is an emulsion or colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals [30]. In this experiment found that protocorm of *D. discolor* cultured on to Hmedium supplemented with cow milk were deceased. Similar result were found in seedlings of *D.* Judy Rutz cultured on H medium containing milk significantly lower growth and development when compared to other treatment, probably because fat can interfere with absorption of nutrients for plants.

Soy milk is a beverage made from soybeans. It is a stable emulsion of oil, water, and protein. Soy milk contains about the same proportion of protein as cow's milk: around 3.5%; also 2% fat, 2.9% carbohydrate, and 5% ash [31].

Supplementations of organic growth adjuncts in orchid culture medium is simple, practical, beneficial and a conventional method to improve media used for commercial production [27]. In the present studies, the used organic supplements contain amino acids, proteins, carbohydrates, vitamins, phenolic acids and organic compounds. Any of these component(s) could be responsible for promoting growth and development of the cultures. Further studies are required to determine which factor(s) is responsible for promoting effect of these organic additives.

V. Conclusion

From the above findings, it may be concluded that H medium containing potato extract with peptone performed best for *in vitro* growth of *Dendrobium* orchid comparison than that of other organic supplement stimulants. The protocol developed in this study may be useful for regeneration the orchid species. It is also important factor for shortening of growth period and rapidly propagates of orchid.

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