

An Effect of Organic Supplements on Stimulating Growth of *Vanda* and *Mokara* Seedlings in Tissue Culture

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Abstract—This study aimed to investigate effect of different organic supplements on growth of *Vanda* and *Mokara* seedlings. *Vanda* and *Mokara* seedlings approximately 0.2 and 0.3 cm. in height were sub-cultured onto VW supplemented with 150 ml/L coconut water, 100 g/L potato extract, 100 g/L ‘Gros Michel’ banana (AAA group) and 100 g/L ‘Namwa’ banana (ABB group). The explants were sub-cultured onto the same medium every month for 3 months. The best medium increased stem height to 0.52 and 0.44 Cm. in *Vanda* and *Mokara* respectively was supplemented with coconut water. The maximum fresh weight of *Vanda* (0.59 g) was found on medium supplemented with ‘Gros Michel’ banana while *Mokara* cultured on medium supplemented with Potato extract had the maximum fresh weight (0.27 g) and number of roots (5.20 roots/shoot) statistically different ($p \leq 0.05$) to other treatments. However, *Vanda* cultured on medium supplemented with ‘Namwa’ banana had the maximum number of roots (3.80 roots/shoot). Our results suggested that growth of different orchid genera was responded diversely to different organic supplements.

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

I. INTRODUCTION

VANDA and *Mokara* (up to date no longer genus *Mokara*, it calls genus *Aranda* now) are the most prevalent monopodial orchids growing general in Thailand as potted plants or as cut flowers. Thus, new varieties are continuously introduced by local breeders. The conventional breeding is the main tool for local breeders; seedpods were produced by crossing between the good parent plants. Therefore, *in vitro* culture techniques for seed germination and micropropagation procedures are very important for producing seedlings and young plants for commercial purpose. Many undefined organic substances such as coconut water (CW), banana, potato and etc. are commonly used for enhancing the growth and development of orchids in tissue culture. Coconut water has been found to be beneficial for development of the immature embryos of *Datura stramonium* [1]. Moreover, MS media [2] supplemented with 5 % of coconut water was able to induce somatic embryogenesis from cereal callus and suspension culture [3]. Potato also found to be beneficial for

another culture of several crops including orchid [4]-[7]. Zhou found that when potato juice was added to the culture media of *Doritaenopsis*, it can recover from hyperhydricity [8]. Banana also general used as organic additive in orchid culture medium. However, the mechanism for stimulating the orchids has not been explained yet.

The objective of this research was to compare the effects of different organic substances on growth and development of *Vanda* and *Mokara* seedlings.

II. MATERIALS AND METHODS

A. Plant Material and Culture Condition

Aseptic seedlings of *Vanda* Tokyo Blue and *Mokara* Aom Yai maintained by monthly subcultures on VW (Vacin and Went, 1949) medium were used as explants for this study. Two mm in height of *Vanda* Tokyo Blue and *Mokara* Aom Yai were cultured on semi-solid VW medium in culture bottles containing 25 ml of medium. The media were supplemented with different concentrations of the homogenates of organic extracts at 15% of coconut water (CW), 10% of potato extract (PE), 10% of ‘Gros Michel’ banana (AAA group) and 10% of ‘Namwa’ banana (ABB group) (w/v). The media were solidified with 2 g/L Phytigel (Sigma). The pH of the VW medium was adjusted to 4.8-5.0. All media were autoclaved at 110 kPa for 15 minutes at 121°C. All cultures were incubated at 25±1°C and under cool-white fluorescent light of 30 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ for 16 hours per day.

B. Preparation of Organic Extracts

The Coconut water was obtained from green coconut at the commercial maturity stage locally sale in Thailand, two cultivars of banana ‘Gros Michel’ (AAA group) and ‘Namwa’ banana (ABB group) and potato were bought at commercial maturity stage from local market as well. The AAA and ABB banana were cut into cubes of 1 cm^3 , 100 g. of each freshly-diced material were ground with 200 ml of liquid VW medium using kitchen blender (Otto) for one minutes. The potato was peeled before being sliced into cubes; 100 g of freshly-diced material were boiled with 200 ml of distilled water and used only the potato extract. These extracts were immediately added to VW medium supplemented with 1 g/L activated charcoal.

C. Experimental Design and Data Analysis

Experiments were performed in a completely randomized design. Each treatment had 10 replicates consisting of 4

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explants per culture bottle and sub-cultured monthly. Plant height, fresh weight, number of leaves and number of roots from explants was evaluated after 12 weeks of culture. The data were analyzed for statistical significance using the analysis of variance, and mean separation was done using SPSS program ver.17.0 and Duncan's multiple range test (DMRT), respectively at a significance level of $P < 0.05$.

III. RESULTS

A. Plant Growth and Development

The growth of *Vanda* and *Mokara* seedlings was established on semi-solid VW medium supplemented with potato extract, coconut water, AAA and ABB banana. The results showed that different orchid genus responded diversely to each organic supplement. A single seedling of *Vanda* was greatly developed into clump with 2-3 new shoots on VW medium supplemented with AAA and ABB banana. The media supplemented with AAA banana gave the maximum average of 2.7 shoots (Table I; Fig. 1 (d)) statistically different to other treatments except the treatment supplemented with ABB banana (Table I; Fig. 1 (e)). VW without organic supplement or VW supplemented with CW had no effect on shoot

induction of *Vanda* seedling (Table I; Figs. 1 (a) and (b)) while VW supplemented with PE showed little effect on shoot induction (Table I; Fig. 1 (c)). VW medium supplemented with CW gave the maximum plant height approximately 0.52 cm. significantly than other treatments (Table I; Fig. 1 (b)). The maximum root number was found in VW medium supplemented with ABB banana (Table I; Fig. 1 (e)). However, the maximum fresh weight was 0.50 and 0.50 g. found in VW medium supplemented with AAA and ABB banana respectively (Table I; Figs. 1 (d) and (e)). *Mokara* seedling was responded differently to the above organic supplements. The results showed the maximum fresh weight was 0.27 g found in VW medium supplemented with PE significantly different to other treatment (Table II; Fig. 2 (c)). Moreover, VW medium supplemented with PE also had a great effect on multiple shoot and root induction. The average of 3.3 shoots and 5.2 roots were obtained in VW medium supplemented with PE significantly different to other treatments (Table II; Fig. 2 (c)). There was no statistically different in plant height among different treatments (Table II; Fig. 2).

TABLE I
EFFECT OF DIFFERENT ORGANIC SUBSTANCES ON GROWTH AND DEVELOPMENT OF *VANDA* TOKYO BLUE AFTER 12 WEEKS OF CULTURE

Organic substance	Plant height (cm)	Fresh Weight (g)	No. of leaves	No. of roots	No. of shoots
none	0.28c	0.15c	4.6	1.8c	1.0b
Coconut water	0.52a	0.35b	5.2	2.2bc	1.0b
Potato extract	0.38b	0.45ab	5.5	2.6b	1.3b
AAA banana	0.34b	0.59a	5.2	2.8b	2.7a
ABB banana	0.34b	0.50a	5.3	3.8a	2.5a
F-test	*	*	ns	*	*

Remark: * = significant difference at 95% level of confidence, ns = no statistical difference; mean value followed by the same letter using DMRT

TABLE II
EFFECT OF DIFFERENT ORGANIC SUBSTANCES ON GROWTH AND DEVELOPMENT OF *MOKARA* AOM YAI AFTER 12 WEEKS OF CULTURE

Organic substance	Plant height (cm)	Fresh Weight (g)	No. of leaves	No. of roots	No. of shoots
none	0.38	0.10c	5.2	2.2c	1c
Coconut water	0.44	0.19b	5.9	3.2b	1.8b
Potato extract	0.38	0.27a	5.5	5.2a	3.3a
AAA banana	0.38	0.15bc	5.2	2.0c	1c
ABB banana	0.42	0.16bc	5.3	2.4c	1.3bc
F-test	ns	*	ns	*	*

Remark: * = significant difference at 95% level of confidence, ns = no statistical difference; mean value followed by the same letter using DMRT

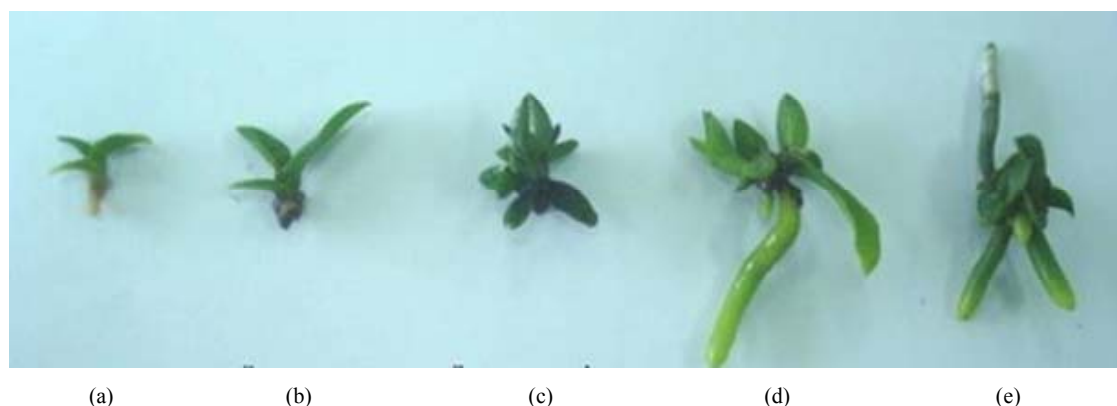


Fig. 1 Growth of *Vanda* Tokyo Blue after 12 weeks of culture on VW medium supplemented with different organic substances (a) = Control without organic supplement, (b) = CW, (c) = PE, (d) = AAA banana and (e) = ABB banana

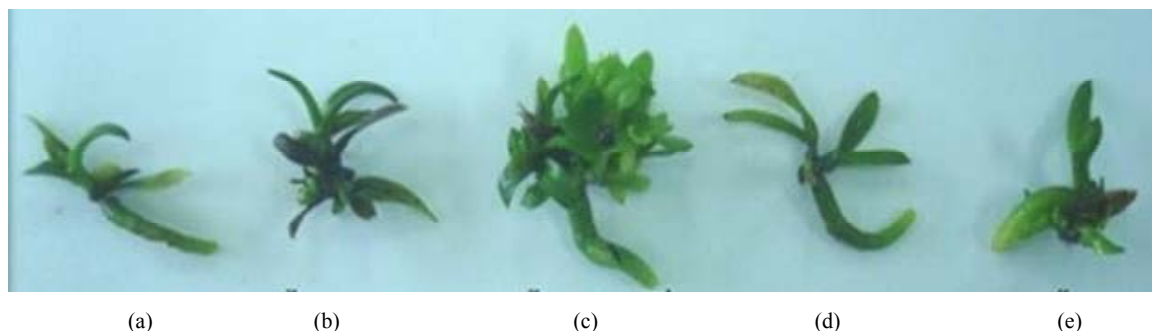


Fig. 2 *Mokara Aom Yai* after 12 weeks of culture on VW medium supplemented with different organic substances (a) = Control without organic supplement, (b) = CW, (c) = PE, (d) = AAA banana and (e) = ABB banana

IV. DISCUSSIONS

In this study, banana homogenized either AAA or ABB cultivars gave the best results for *Vanda* growth and development. The result was agreed with *Dendrobium* orchid PLBs had the maximum fresh weight when cultured on half-strength MS supplemented with banana [9]. The enhancing effect of banana on growth and development of orchid might due to banana pulp contained high level of iron, potassium, vitamin B6 and B2 and tryptophan [10]. Banana also had ability to stabilize the pH of the medium by acting as antacid to neutralize acidity condition [10]. Many studies indicated that banana extract added to orchid culturing media promoted PLBs proliferation and growth [9]-[12]. Mas (AA) Banana pulp at the concentration of 10% increased the proliferation rate of *Phalaenopsis violacea*'s PLBs and had a potential to substitute sucrose in half-strength MS semi-solid medium [10].

The response of *Mokara* seedlings in terms of growth and development was significantly enhanced in VW medium supplemented with PE. The results showed the maximum fresh weight, number of roots and shoots were obtained when PE was added to the medium. In contrast with *Vanda*, shoot multiplication of *Mokara* was greater in VW medium supplemented with PE than banana. Vitamin B6 obtained from PE induced PLBs proliferation due to the production of essential amino acid [13].

V. CONCLUSION

This study was concluded that organic supplements added into culture medium help in producing shoot multiplication and root induction and regeneration. However, different orchid genus responded diversely to each organic supplement. Thai local banana cv. Namwa (ABB) could be able to use as organic supplementary in sub-culture medium for growth and development of *Vanda* Tokyo Blue seedlings. Whereas, PE was the best organic substance to enhance growth and development of *Mokara Aom Yai* seedlings during sub-culture period.

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