

## Effect of Modification Medium on Growth Development of *Dendrobium parishii* *In vitro*

Waranyoo Kaewduangta and Pranee Reamkatog

Department of Agricultural Technology, Faculty of Technology,  
Mahasarakham University, Mahasarakham, Thailand

**Abstract:** The treatments comprise of chemical fertilizer as scale (21-21-21: 2 g/l) and liquid (10 ml/l) supplemented with natural dietary ca. pupa powder 5 g/l, charcoal (2 g/l), brown rice 5 g/l, banana pulp 50 g/l and coconut water 150 ml/l compared with the VW (1949) as control. Complete Randomize Design (CRD) with 31 treatments, 4 replications was used in this experiment, culture for 8 weeks. In this study was undertaken to observe the interactive effects of different media and organic additives on growth development of *Dendrobium parishii*. The result shown that number of pseudobulb was highest in T6 (chemical fertilizer + brown rice). While the healthiest in meaning of plant height was obtained from treatment T20 (chemical fertilizer + pupa powder + coconut water + charcoal). Number of leaves, root length and number of roots were obtained from T12 (chemical fertilizer + pupa powder + banana), T 19 (chemical fertilizer + pupa powder + brown rice + charcoal) and T30 (chemical fertilizer + pupa powder + banana + brown rice + coconut water + Charcoal), respectively.

**Key words:** Modification Medium • Growth Development • *Dendrobium parishii* • *In vitro* Culture

### INTRODUCTION

The Orchidaceae is one of the largest families in the plant kingdom with estimated over 35,000 species represented worldwide [1]. Many of which have developed fascinating characteristics to cope with a wild range of habitats, to attract pollinators and to conserve moisture and nutrients. In Thailand, Orchids are found in all different habitats ranging from hill evergreen forest at 2,565 m. in the north to sea level in the peninsular with approximate a total of 170 genera and 1,230 species of which 150 species are considered endemic to the country [2] and Thailand is one of many countries that is situated in a region with abundance of native wild orchids. *Dendrobium parishii* is a species of Thai's native and found that in the rainforest or mixed deciduous forest, which an important in early development of Thai orchids from Thailand to the world: the thickened stems are usually prostrate or pendulous, to ½ inch (1.3 cm) thick by 12 inches (30 cm) long. They are easy to culture and a good orchid for beginners, the flowers blooming in late spring to early summer, the 2 inch (5 cm) wide rose to lilac purple flowers appear on leafless stems and have pleasant

fragrance [3]. All most of Thai orchid exported (86%) in 2008 were *Dendrobium* [4] and have the volume of exports the orchid in 2008 to 2010 about 86.2, 80.2 and 86.3 million US\$ per year [5], which are high value of all flower plant exported and the based on tissue culture technique of orchid hybrids was studied for a long time, in Year 1998 the government issued a law prohibiting the export wild orchids, which was excluded from propagation by seed culture *in vitro*. Therefore, propagation of orchids using tissue culture techniques has been practiced for more than a century and has resulted in the production of uniform clones in many orchid genera [6], plant inductions and plant regeneration are influenced by many factors, such as genotypes, type of explants and composition of media [7]. Nutrient composition is considered to be major sources of variation in plant tissue culture [8]. Different culture media have been used for efficient plant regeneration in orchid tissue culture. In some research showed that VW medium [9] with organic substrate yielded the highest increase in weight and most numbers of leaves and roots formed in *Aranda* [10]. For this, suitable media and organic additives are needed to be identified for large-scale utilization in orchid tissue

culture. Thus, the present study was undertaken to observe the interactive effects of different media and organic additives with VW on growth development capability of *Dendrobium parishii*'s plantlet under *in vitro* culture.

## MATERIALS AND METHODS

The experimental series, A 8-month-old green capsule of *D. parishii*'s that formed after self-pollination was collected and subjected to surface sterilization. The capsule was submersed in 95% ethanol, flamed for few seconds under sterile conditions and then split longitudinally with a sterilized scalpel. The seeds were transferred onto VW media for 6 months and then selected the germinated seedlings of *D. parishii*'s height of plant size approximately 2 cm, remove the trim of leaves and root out the dead, weighting to each seedling from balanced or the most similar for studied on medium modification for *in vitro* culture of *D. parishii*. The treatments comprise of chemical fertilizer as scale (21-21-21: 2 g/l) and liquid (10 ml/l) supplemented with natural dietary ca. pupa powder 5 g/l, charcoal (2 g/l), brown rice 5 g/l, banana pulp 50 g/l and coconut water 150 ml/l compared with the VW (1949) as control. This experimental was carried out in Complete Randomize Design (CRD) with 31 treatments, 4 replications (12 bottle/rep) were culture for 8 weeks. All treatment was used 20 g/l of sucrose and 8 g/l of agar. The cultures were maintained in culture room kept at 25°C with a 12 h photoperiod provided by fluorescent light at 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Thus, the modification medium had 31 treatments: T<sub>1</sub> (VW, 1949), T<sub>2</sub> (the combination of scale and liquid fertilizer), T<sub>3</sub>(T<sub>2</sub> + charcoal), T<sub>4</sub> (T<sub>2</sub> + pupa powder), T<sub>5</sub> (T<sub>2</sub> + banana), T<sub>6</sub> (T<sub>2</sub> + brown rice), T<sub>7</sub>(T<sub>2</sub> + coconut water), T<sub>8</sub> (T<sub>2</sub> + pupa powder + charcoal), T<sub>9</sub> (T<sub>2</sub> + banana + charcoal), T<sub>10</sub> (T<sub>2</sub> + brown rice + charcoal), T<sub>11</sub> (T<sub>2</sub> + coconut water + charcoal), T<sub>12</sub> (T<sub>2</sub> + pupa powder + banana), T<sub>13</sub> (T<sub>2</sub> + pupa powder + brown rice), T<sub>14</sub>(T<sub>2</sub>+ pupa powder + coconut water), T<sub>15</sub> (T<sub>2</sub> + banana + brown rice), T<sub>16</sub> (T<sub>2</sub> + banana + coconut water), T<sub>17</sub> (T<sub>2</sub> + brown rice + coconut water), T<sub>18</sub> (T<sub>2</sub> + pupa powder + banana + charcoal), T<sub>19</sub> (T<sub>2</sub> + pupa powder + brown rice + charcoal), T<sub>20</sub> (T<sub>2</sub>+ pupa powder + coconut water + charcoal), T<sub>21</sub> (T<sub>2</sub> + banana + brown rice + charcoal), T<sub>22</sub> (T<sub>2</sub> + banana + coconut water + charcoal), T<sub>23</sub> (T<sub>2</sub> + pupa powder + banana + brown rice), T<sub>24</sub> (T<sub>2</sub> + pupa powder + banana + coconut water), T<sub>25</sub> (T<sub>2</sub> + pupa powder + brown rice + coconut water), T<sub>26</sub> (T<sub>2</sub> + banana + brown rice + coconut water), T<sub>27</sub> (T<sub>2</sub> + pupa powder + banana + brown rice +

charcoal), T<sub>28</sub> (T<sub>2</sub> + pupa powder + banana + coconut water + charcoal), T<sub>29</sub> (T<sub>2</sub> + banana + brown rice + coconut water + charcoal), T<sub>30</sub> (T<sub>2</sub> + pupa powder + banana + brown rice + coconut water + charcoal) and T<sub>31</sub> (T<sub>2</sub> + pupa powder + banana + brown rice + coconut water + charcoal). This experimental data collected were subjected analysis of variance and means were separated by Duncan's multiple range test (DMRT) at  $p < 0.05$  level of significance. Data were recorded after 8 weeks of culture: handling was following: plant height, leaves number, root number, root length and pseudo bulb number. The experiment was carried out at Tissue Culture Laboratory in Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham, Thailand during the period of October 2008 to February 2009.

## RESULTS AND DISCUSSION

The studied of medium modification on growth development of *D. parishii* under *in vitro* cultured after 8 weeks, selected seedlings that were propagated by using media in the experimental: the plantlets were cultured in medium supplemented with different additive combinations (chemical fertilizer and supplemented with pupa powder, charcoal, brown rice, banana pulp and coconut water compared with the VW as control.) Results have been presented in Table 1 and Fig. 1.

**Height of Shoot:** The combined effect of different additive combinations of modification medium on shoot height showed significantly differences. The highest shoot height was obtain from treatment 20<sup>th</sup> (chemical fertilizer + pupa powder + coconut water + charcoal) but are not significant from treatment 12<sup>nd</sup> (T<sub>2</sub> + pupa powder + banana), treatment 18<sup>th</sup> (T<sub>2</sub> + pupa powder + banana + charcoal) and treatment 19<sup>th</sup> (T<sub>2</sub> + pupa powder + brown rice + charcoal)

**Number of Leaves:** The number of leaves produced per plantlet showed significantly variation at various combination of modification medium. The highest number of leaves was obtain from treatment 12<sup>nd</sup> (T<sub>2</sub> + pupa powder + banana) but are not significant with treatment 13<sup>rd</sup> (T<sub>2</sub> + pupa powder + brown rice), treatment 14<sup>th</sup> (T<sub>2</sub> + pupa powder + coconut water), treatment 17<sup>th</sup> (T<sub>2</sub> + brown rice + coconut water), treatment 18<sup>th</sup> (T<sub>2</sub> + pupa powder + banana + charcoal), treatment 19<sup>th</sup> (T<sub>2</sub> + pupa powder + brown rice + charcoal) and treatment 20<sup>th</sup> (T<sub>2</sub> + pupa powder + coconut water + charcoal).

Table 1: The growth development of *Dendrobium parishii* on the formulation of Modification Medium for 8 weeks after cultured.

Modification Medium	Height (cm)	Leaf no. (leaves)	Root no. (root)	Root length (cm)	Pseudo bulb no. (%)
T1	0.75ij	4.25g	1.00h	0.24j	0d
T2	0.81fghi	4.63fg	4.88efg	0.54hi	0d
T3	0.85defgh	5.63c	5.50abc	0.58fgh	37.5b
T4	0.86defg	5.38cd	5.50abc	0.59efgh	25b
T5	0.86defg	5.5c	5.50abc	0.56ghi	0d
T6	0.88cdefg	5.5c	5.75ab	0.55ghi	62.5a
T7	0.88cdefg	5.25cde	5.75ab	0.56ghi	25b
T8	0.86defg	5.25cde	5.63abc	0.55ghi	25b
T9	0.86defg	5.63c	5.75ab	0.56ghi	25b
T10	0.88cdefg	5.63c	5.38bcd	0.56ghi	25b
T11	0.88cdefg	5.63c	5.63abc	0.58fgh	0d
T12	0.96ab	7.63a	5.50abc	0.74a	0d
T13	0.91abcd	7.38ab	5.25cde	0.71abc	0d
T14	0.95abc	7.25ab	5.50abc	0.73ab	0d
T15	0.9abcde	7b	5.38bcd	0.71abc	0d
T16	0.93abcd	7b	5.63abc	0.74a	50ab
T17	0.89bcdef	7.25ab	5.50abc	0.70abc	12.5c
T18	0.96ab	7.13ab	5.63abc	0.70abc	0d
T19	0.96ab	7.25ab	5.50abc	0.75a	0d
T20	0.98a	7.38ab	5.63abc	0.74a	12.5c
T21	0.93abcd	6.88b	5.75ab	0.74a	12.5c
T22	0.89bcdef	7.38ab	5.25cde	0.73ab	0d
T23	0.64k	5.63c	5.00def	0.66cd	37.5b
T24	0.78hi	5.25cde	4.88efg	0.64de	0d
T25	0.81fghi	5.5c	4.50g	0.63def	0d
T26	0.8ghi	5.5c	4.75fg	0.68bcd	0d
T27	0.65k	5.5c	4.88efg	0.68bcd	25b
T28	0.8ghi	5.75c	4.75fg	0.60efg	0d
T29	0.83efghi	5.63c	4.75fg	0.63def	12.5c
T30	0.68jk	4.75efg	5.88a	0.51i	0d
T31	0.66k	4.88def	5.88a	0.51i	0d
F-test	**	**	**	**	**
LSD	0.04	0.3	0.24	0.03	25
CV (%)	10.08	9.9	9.09	9.53	15.09

In all column, mean followed by the same letters are not significantly different at the  $p < 0.05$  level of significance  
 \*\* are significantly different at the  $p < 0.05$  level of significance

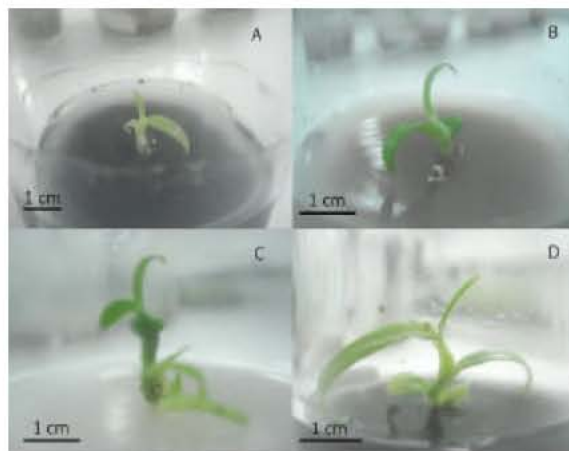


Fig. 1: The growth development of *Dendrobium parishii* on the formulation of Modification Medium for 8 weeks after cultured. A: Vacin and Went (1949), B: T<sub>2</sub> (the combination of scale + liquid fertilizer), C: T<sub>6</sub> (the combination of scale + liquid fertilizer + brown rice) and D: T<sub>20</sub> (the combination of scale + liquid fertilizer + pupa powder + coconut water + charcoal)

**Number of Roots per Plantlet:** The number of roots produced per plantlet showed significant variation at various modifications medium. In case of studies the highest number of roots was obtained from treatment 30<sup>th</sup> (T<sub>2</sub> + pupa powder + banana + brown rice + coconut water + charcoal) and treatment 31<sup>st</sup> (T<sub>2</sub> + pupa powder + banana + brown rice + coconut water + charcoal) but are not significant with treatment 6<sup>th</sup> (T<sub>2</sub> + brown rice), treatment 7<sup>th</sup> (T<sub>2</sub> + coconut water) and treatment 9<sup>th</sup> (T<sub>2</sub> + banana + charcoal).

**Length of Root:** The length of root was found statistically significantly on different modification medium. The highest root length was obtained from treatment 12<sup>nd</sup> (T<sub>2</sub> + pupa powder + banana), treatment 16<sup>th</sup> (T<sub>2</sub> + banana + coconut water), treatment 19<sup>th</sup> (T<sub>2</sub> + pupa powder + brown rice + charcoal), treatment 20<sup>th</sup> (T<sub>2</sub> + pupa powder + coconut water + charcoal) and treatment 21<sup>st</sup> (T<sub>2</sub> + banana + brown rice + charcoal) are similar.

**Number of Pseudo Bulb per Plantlet:** Statistically significant variation was noticed on pseudo bulb number of plantlet with different modification medium. The highest pseudo bulb number of plantlet was obtained from treatment 6<sup>th</sup> (T<sub>2</sub> + brown rice) but is not significant with treatment 16<sup>th</sup> (T<sub>2</sub> + banana + coconut water). That the interaction of different modification medium and organic additives showed significant effect on the number of pseudobulb and growth development. Partially support result showed by report, where they observed banana pulp with VW medium significantly increased the leaf number of *Dendrobium nobile* [11] and the reported of modified media for *Rhynchostylis gigantea* (Lindl) Ridl were used to replace synthetic chemicals that were used to prepare tissue culture media. The result showed that, the formulation of modified-media; bamboo-charcoal, pupa powder, banana pulp, unpolished rice and coconut water showed the highest of plant growth and development [12].

The results from the present studied suggest that modification medium with natural additive combination are in agreement with the reported a large number of complex additives like coconut water, banana pulp, peptone, tomato juice, slap honey and beef extract can be very effective in providing undefined mixture of organic nutrients and growth factors. For *in vitro* growth of PLBs and seedlings, some complex organic additives were reported satisfactory while some were unsatisfactory and even inhibitory and similar with the studies suitable media and organic additives are needed to be identified for large-

scale utilization in orchid tissue culture, the study was undertaken to observe the interactive effects of different media and organic additives on formation of PLBs and their subsequent plantlet regeneration capability of *Dendrobium* orchid [13]. Except the length of shoots and leaves, the highest values of all parameters were obtained from the interaction of ½MS medium with Sabri banana pulp. However, the longest shoots were found in KC medium with Sabri banana pulp and the longest leaves were found in both KC and ½ MS media with Sabri banana pulp [14].

## CONCLUSION

Considering the above results, effect of modification medium on growth development of *D. parishii* under *in vitro* culture shown that number of pseudo bulb was highest (62.5%) in treatment 6<sup>th</sup> (chemical fertilizer + brown rice). While the healthiest in meaning of plant height, number of leaves and root length was obtained from treatment 20<sup>th</sup> (chemical fertilizer + pupa powder + coconut water + charcoal) are 0.98 cm/plantlet, 7.38 leaves/plantlet and 0.74 cm/plantlet respectively.

## ACKNOWLEDGEMENT

This research was supported by Mahasarakham University, Mahasarakham, Thailand. Hereby authors would like to thank them for their support and also thank the staff at Department of Agricultural Technology, Faculty of Technology, Mahasarakham University for facility of this experiment.

## REFERENCES

1. Dressler, R.L., 1993. Phylogeny and Classification of the Orchid Family. Dioscorides Press, Portland, Oregon.
2. Nanakorn, W. and S. Indharamusika, 1999. Ex-situ Conservation of Native Thai Orchids at Queen Sirikit Botanic Garden. Queen Sirikit Botanic Garden, P.O. Box 7, Mae Rim, Chiang Mai, 50180 Thailand. Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization, 23-27 November 1997, Phuket, Thailand. Other presentations are published in Pure Appl. Chem., 70: 11.
3. Queen Sirikit Botanic Garden, 1998. Thai Orchid. Queen Sirikit Botanic Garden, P.O. Box 7, Mae Rim, Chiang Mai, 50180 Thailand.

4. Kasikorn Research Center, 2008. Reports/Ratings. Business Brief, 14(2141): 18 April 2008.
5. The former Ministry of Commerce, 2009. Thailand Trading Report. Web Service. < [http://www2.ops3.moc.go.th/export/recode\\_export\\_rank/report.asp](http://www2.ops3.moc.go.th/export/recode_export_rank/report.asp)>15 July 2010.
6. Arditti, J., 2008. Micropropagation of orchids, 2nd edn. Blackwell Publishing Ltd, Maiden, MA, USA.
7. Jain, R.K., 1997. Effects of some factor on plant regeneration from indica rice cells and protoplasts: A review. Indian J. Biol., 35: 323-331.
8. Khanna, H.K. and S.K. Raina, 1998. Genotype X culture media interaction effects on regeneration response of three *indica* rice cultivars. Plant Cell Tissue Organ Cult., 52(3): 145-153.
9. Vacin, E.F. and F.W. Went, 1949. Some pH Changes in nutrient solutions. Bot. Gaz., 110: 605-613.
10. Lim-Ho, C.L., G.C. Lee and L.K. Phua, 1985. Clonal propagation of orchids from flower buds. Proc. 50<sup>th</sup> Asian Orchid Cong. A.N. Rao (ed.). 1984, Singapore. pp: 90-110.
11. Suddep, R., P.K. Rajeevan, P.K. Valsalakumari and C.K. Geetha, 1997. Influence of organic supplements on shoot proliferation in *Dendrobium*. J. Hort., 3(1-2): 38-44.
12. Kaewkhiew, P. and W. Kaewduangta, 2010. Natural Additives Modification Medium: Growth of *Rhynchosyilis gigantean* by Tissue Culture Technique. Asian J. Plant Sci., 9(8): 498-501.
13. Arditti, J., 1967. "Orchid Biology: Reviews and Perspective". Cornell University Press, Ithaca, New York. pp: 114-1255.
14. Aktar, S., K.M. Nasiruddin and K. Hossain, 2008. Effects of different media and organic additives interaction on *in vitro* regeneration of *Dendrobium* orchid. J. Agric. Rural, 6: 69-74.