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Natural Additives Modification Medium: Growth of *Rhynchostylis gigantea* by Tissue Culture Technique

P. Kaewkhiew and W. Kaewduangta
Department of Agricultural Technology, Faculty of Technology,
Mahasarakham University, Mahasarakham, 44150, Thailand

Abstract: *Rhynchostylis gigantea* (Lindl) Ridl is native Thailand. It was difficult to propagate under natural conditions. Tissue culture technique is a way that had high potential to solve this problem. This experiment Vacin and Went was conducted to test modified medium for *R. gigantea*. The modified media were applied as the combination of scale and liquid fertilizer. In addition modification medium, those media were mixed with pupa powder, bamboo-charcoal, potato, sweet potato, unpolished rice, banana pulp and coconut water as supplementary compounds. The modified media were used to replace synthetic chemicals that were used to prepare tissue culture media. The result showed that, the formulation of modified-media; 2 g L⁻¹ of bamboo-charcoal, 5 g L⁻¹ of pupa powder, 50 g L⁻¹ of banana pulp, 50 g L⁻¹ of unpolished rice and 150 mg L⁻¹ of coconut water showed the highest of plant growth and development, plant height (8.05 cm), fresh weight (1.753 g), number of leaf (5 leaf) and root number (3.9 roots). After transplanted to green house for 6 weeks, all of modified media did not have statistically significant of the number of survival plants and chlorophyll content. Thus, the natural modified media had the potentiality to be applied as *R. gigantea* cultural media to replace the synthetic chemical media.

Key words: *Rhynchostylis gigantea*, natural additive, modification medium, tissue culture technique, scale and liquid fertilization, synthetic chemical

INTRODUCTION

Thailand is one of the worlds orchid exporters. However, the orchid propagation technique is the one most important support for the mass orchid production. The volume of exports of orchid by the new way of breeding technique is limited due to some species of orchid, especially wild species that are difficult to propagate and time consuming; this is evidenced by the year 2002 with exports about 94,765 plants. The top 5 orchids are *Rhynchostylis gigantea* (Lindl.) Ridl, *Grammatophyllum scriptum*, *Pecteilis sagarikii*, *Ascocentrum miniatum* and *Dendrobium chrysotoxum*. In the year 1996, the export market of orchid cut flower valued about 2,539 million baht and orchid trees at 447 million baht (Thammasiri, 2007) and in 2005 to 2009 about 2,539, 2,921.44, 2,944.71, 2,833.80 and 2,738.33 million baht per year (The Former Ministry of Commerce, 2009). Based on studies of tissue culture techniques of orchid hybrids for a long time, the government in 1998 passed a law prohibiting the exports of wild orchid which excluded the propagation by seed culture *in vitro*. Different culture media have been used for efficient plant regeneration of

orchid *in vitro* culture. Thus, Lim-Ho *et al.* (1982) showed that VW medium (Vacin and Went, 1949) with organic substrate yielded the highest increase in weight and most numbers of leaves and roots in Aranda orchid. And Charoendee *et al.* (2001) were studied on seed development of *Dendrobium formosum* Roxb. On modified VW medium supplemented with coconut water, potato extract, sucrose and blended banana, the result showed seed can developed to leaves, seedling which has root and the highest germination percentage. *Rhynchostylis gigantea* is in *Rhynchostylis* order and Orchidaceae family, this is a species of Thai's native and found that in the rainforest or mixed deciduous forest, which is important in early development of Thai orchids from Thailand to the world: with the characteristic bouquet of beautiful flowers cylindrical prominent curve, each bouquet has more than 50 flowers, petal are white with purple points, has strong aroma when the bouquet of flowers are full bloom and flowers bloom in mid-winter period that is December to February (Udorn *et al.*, 2006). Thus, the study was aimed to compare the potentiality of modified media and Vacin and Went media for *in vitro* culture of *R. gigantea*.

MATERIALS AND METHODS

The experiment was carried out at Tissue Culture Laboratory and Green House in Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham, Thailand during the period of October 2008 to February 2009. In the first experimental series, studied on medium modification for *in vitro* culture of *Rhynchosstylis gigantean*, selected seedlings that were propagated by using media in this study. The cultures were maintained in culture room kept at 25°C with a 12 h photoperiod provided by white fluorescent light at 60 $\mu\text{mol}/\text{m}^2/\text{sec}$ for 8 weeks. Then selected a height of plant size approximately 2 cm, remove the trim of dead leaves and roots, weighing each seedling from balanced or the most similar and were cultured on Vacin and Went (Vacin and Went, 1949) plant growth regulators (PGRs)-free media (as control), the modified media were applied as the combination of scale (2 g L⁻¹) and liquid (10 ml L⁻¹) fertilizer, in addition, those media were mixed with pupa powder (5 g L⁻¹), bamboo-charcoal (2 g L⁻¹), potato (50 g L⁻¹), sweet potato (50 g L⁻¹), unpolished rice (50 g L⁻¹), banana pulp (50 g L⁻¹) and coconut water (150 ml L⁻¹) as supplementary compounds. All treatment was used 20 g L⁻¹ of sucrose and 8 g L⁻¹ of agar. Thus, the natural modified media had 13 treatments : VW, M₁ (the combination of scale and liquid fertilizer), M₂ (M₁ with bamboo-charcoal), M₃ (M₂ with pupa powder), M₄ (M₂ with banana pulp), M₅ (M₃ with banana pulp), M₆ (M₅ with potato), M₇ (M₅ with sweet potato), M₈ (M₅ with unpolished rice), M₉ (M₅ with coconut water), M₁₀ (M₆ with coconut water), M₁₁ (M₇ coconut water) and M₁₂ (M₈ coconut water). This experiment was carried out in Complete Randomize Design (CRD) with 4 replications, data were analyzed using the analysis of variance and Duncan's multiple range test at p<0.05 level of significance. Data were recorded after 8 weeks of culture. Data handling was as follows; plant height, fresh weight, number of leaf and root number. In the second experiment, the result in the first experiment showed that, the formulation of modified-media; showed the highest of plant growth and development, plantlet were transplanted to green house and were used Complete Randomize Design (CRD), data were analyzed using the analysis of variance and Duncan's multiple range test at p<0.05 level of significance. Each experiment was 4 replicated. Data were recorded after 6 weeks of transferred. Data handling: leaf number, leaf length, leaf width, size of stem and chlorophyll content.

RESULTS AND DISCUSSION

The studied on medium modification for *in vitro* cultured of *R. gigantean*, selected seedlings that were propagated by using media in the first experiment showed on Table 1, the formulation of modified-media M₁₂: 2 g L⁻¹ of bamboo-charcoal, 5 g L⁻¹ of pupa powder, 50 g L⁻¹ of banana pulp, 50 g L⁻¹ of unpolished rice and 150 mg L⁻¹ of coconut water showed the highest of plant growth and development, plant height (8.05 cm), fresh weight (1.753 g), number of leaf (5 leaves) and root number (3.9 roots). The result on M₁ (the combination of scale and liquid fertilizer) showed, growth induction in cultured at the less: plant height (4.95 cm), fresh weight (0.613 g), number of leaf (3.80 leaf) and root number (3.20 roots). The resulted that present depend on minimum natural additive, partially support result showed by Lim-Ho *et al.* (1982) they found similar resulted in *Aranda* orchid. Kalpona *et al.* (2000) observed VW medium supplemented with a combination of 3% banana pulp and 10% coconut water was more effective and enhanced the production of *Dendrobium* orchid and in *D. nobile* reported by Suddep *et al.* (1997), they observed banana pulp with VW medium significantly increased the leaf number. Similar reported by Charoendee *et al.* (2001) were study on seed development of *Dendrobium formosum* Roxb. On modified VW medium supplemented with 150 ml L⁻¹ coconut water, 50 ml L⁻¹ potato extract, 20 g L⁻¹ sucrose and 100 g L⁻¹ banana pulp, the result showed seed can developed to 3-4 leaves seedling which has root and the highest germination percentage (95.20%). Yesmin (2005) showed

Table 1: The growth of *Rhynchosstylis gigantea* (Lindl.) Ridl on the formulation of modified medium for 8 weeks after cultured

Modified medium	Height (cm)	Weight (g)	Leaf No. (leaves)	Root No. (root)
VW	7.85ab	1.730a	4.70ab	3.90a
M ₁	4.95h	0.613e	3.80de	3.20c
M ₂	5.02gh	0.649de	3.60e	3.20c
M ₃	5.94e	0.717d	3.90de	3.30c
M ₄	6.42d	0.829c	4.00de	3.40bc
M ₅	6.38d	0.842c	4.00de	3.20c
M ₆	6.89c	0.837c	4.00de	3.10c
M ₇	5.32fg	0.864c	4.30bcd	3.30c
M ₈	7.66b	1.694a	4.60abc	3.80ab
M ₉	7.12c	1.009b	4.10cde	3.40bc
M ₁₀	7.06c	1.053b	4.10cde	3.30c
M ₁₁	5.48f	1.055b	4.10cde	3.30c
M ₁₂	8.05a	1.753a	5.00a	3.90a
F-test	**	**	**	**
CV (%)	5.98	27.61	12.97	12.81

In all column, mean followed by the same letters are not significantly different at the p<0.05 level of significance and data were analyzed using the analysis of variance and Duncan's multiple range test. ** are significantly different at the p<0.05 level of significance

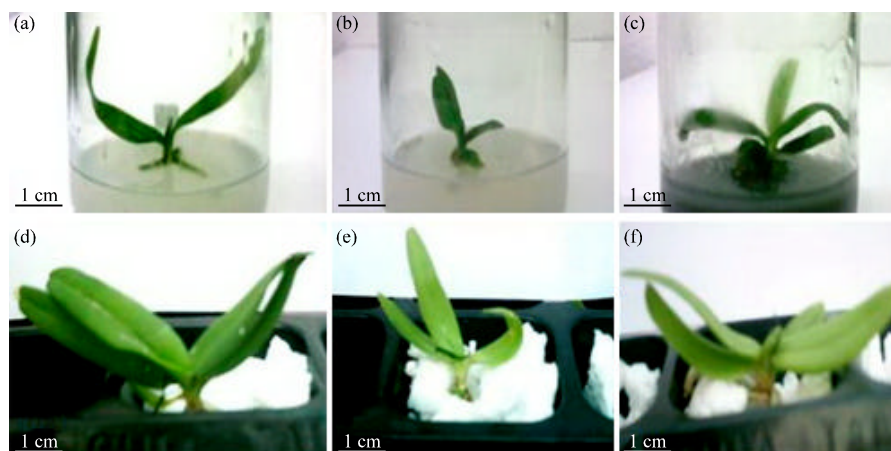


Fig. 1: *Rhynchostylis gigantea* (Lindl.) Ridl in medium containing for 8 weeks after cultured. (a): VW media, (b): M1 media and (c): M12 media. Then (d, e and f) are *R. gigantea* under green house for 6 weeks after transplanted from a, b and c

Table 2: The growth of *Rhynchostylis gigantea* (Lindl.) Ridl under green house for 6 weeks after transplanted

Modified medium	Leaf No. (leaves)	Leaf length (cm)	Leaf width (cm)	Size of stem (cm)	Chlorophyll content (Spad unit)
VW	4.25	5.23a	1.05ab	0.53a	48.73a
M ₁	3.25	3.43b	0.81b	0.43b	15.63d
M ₈	3.5	3.99b	0.96ab	0.43b	25.49c
M ₁₂	3.5	5.22a	1.11a	0.51a	28.10b
F-test	ns	**	*	*	**
C.V. (%)	18.68	12.29	16.02	10.10	3.53

In all column, means followed by the same letters are not significantly different at the $p < 0.05$ level of significance and data were analyzed using the analysis of variance and Duncan's multiple range test. ns are not significantly different at the $p < 0.05$ level of significance. **are significantly different at the $p < 0.05$ level of significance

that VW medium supplemented sabri banana and charcoal was the best for shoot production of *Dendrobium* hybrid orchid. After 8 weeks were transplanted to the second experimental, the result in the first experimental showed that, the formulation of modified-media; showed the highest of plant growth and development, plantlet were transplanted to green house.

After transplanted to green house condition for 6 weeks, all of modified media (VW, M₁, M₈ and M₁₂) showed the leaf number, leaf length, leaf width, size of stem and chlorophyll content at 4.25 leaves, 5.23 cm, 0.53 cm and 48.73 Spad Unit, M₁ showed: 3.25 leaves of the leaf number, 3.43 cm of leaf length, 0.81 cm of leaf width, 0.43 cm of size of stem and 15.63 Spad Unit of chlorophyll content. M₈ showed: 3.5 leaves of the leaf number, 3.99 cm of leaf length, 0.96 cm of leaf width, 0.43 cm of size of stem and 25.49 Spad Unit of chlorophyll content and M₁₂ showed 5.22 leaves of the leaf number, 3.43 cm of leaf length, 1.11 cm of leaf width, 0.51 cm of size of stem and 28.10 Spad Unit of chlorophyll content

(Table 2). All treatment data were analyzed using the analysis of variance did not had statistically significant of the leaf number but had statistically significant of leaf length, leaf width, size of stem and chlorophyll content. The data from M₁₂ are the best either nor Spad Unit of chlorophyll content (Fig. 1a-f). Thus, if we need higher chlorophyll content, we should spray solution of fertilizer when we are planting. This experiment showed the natural modified media had the potentiality to apply as *R. gigantea* cultural media to replace the synthetic chemical media. The protocol developed in this study can be applied with other types of orchids as appropriate. It is also important for growth period propagation of orchid.

CONCLUSION

The best formulation of modified-media is M₁₂; 2 g L⁻¹ of bamboo-charcoal, 5 g L⁻¹ of pupa powder, 50 g L⁻¹ of banana pulp, 50 g L⁻¹ of unpolished rice and 150 mg L⁻¹ of coconut water showed the highest of plant growth and development, plant height (8.05 cm), fresh weight (1.753 g), number of leaf (5 leaves) and root number (3.9 roots). After transplanted for 6 weeks, all of modified media did not had statistically significant of the number of survival plants and chlorophyll content. Thus, the natural modified media had the potentiality to apply as *R. gigantea* cultural media to replace the synthetic chemical media.

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