Journal of Agricultural Technology 2011 Vol. 7(3): 739-749
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

The study on optimization of growth conditions for *Dendrobium friedericksianum* Rchb.f. seedlings in aseptic culture

Prasertsirivatna, S.* **and Koolpluksee, M.**

Faculty of Agro-industry Technology, Rajamongala University of Technology Tawan-OK, Chanthaburi Campus, Chanthaburi, Thailand.

Prasertsirivatna, S. and Koolpluksee, M. (2011). The study on optimization of growth conditions for *Dendrobium friedericksianum* Rchb.f. seedlings in aseptic culture. Journal of Agricultural Technology 7(3): 739-749.

The study was to examine the optimization of conditions for the growth of *Dendrobium friedericksianum* Rchb.f. seedlings in aseptic culture. It was found that Vacin & Went was the optimal medium for the growth of the orchid. The orchid seeds in the liquid medium before transfer on solid medium for 1 month had 4 days faster germination than the cultivation on the solid medium, although the two patterns of cultivation were non significant in aspect of average dry weight. IBA and 2,4-D at 0.15 mg/l were the best of growth regulators and concentration for stimulating growth of this orchid significantly, right across NAA and kinetin were inhibit growth significantly. But BAP was not affect to growth and development of orchid, even though it was used with 2,-4-D. Besides 2,-4-D affects to orchid development in aspect of biomass, height of shoot and number of root, but not affect to the number of shoot and length of root. The cultivation of orchid seedlings under the conditions of various temperatures and light intensities showed the highest fresh weight on the average at 20 ^{o}C and 4000 luxs. However, the range of the light-exposed times at 12, 14 and 16 hours per day were not significantly affected to the total fresh weight.

Key words: *Dendrobium friedericksianum* Rchb.f, orchid, growth regulator, light intensity, temperature

Introduction

"Leaung chanthaboon" is the name of Local orchid was found in the Eastern of Thailand especially Chanthaburi province. It is in the genus *Dendrobium*, family Orchidaceae, the scientific name is *Dendrobium friedericksianum* Rchb.f. (Sa-ard, *et.al*., 1982). The scientific name of the red brown spot at the lip of flower strain is *D. friedericksianum* Rchb.f. var. *oculatum* S&S. (Paiboon, 1978). "Leaung chanthaboon" is the beautiful orchid and rather expensive, now, there is demanded by both inside and outside the country. Therefore, the amount of this wild orchid was stole from the forest to

*Corresponding author: Saroch Prasertsirivatna; e-mail: prasert853@hotmail.com

sell in the black market, reason to nearly become extinct from the forest of Chanthaburi province. Now, the tissue culture technique is often used to propagate most orchids, because this technique is able to increase the number of orchids in the short time. Therefore, "The Study on Optimization of Conditions for Growth of *Dendrobium friedericksianum* Rchb.f. Seedlings in Aseptic Culture" will make we know to manage the optimal various factors for growth and increase amount of this wild orchid in the forest of eastern Thailand such as the former time.

Piyanuch2) 004 (studied the affects of varies factors for growth of *Habenaria rhodocheila* plantlets in the modified medium of Vacin & Went (1949), CMU1, it was found that the embryo cultured at 30 ^{o}C of temperature under the light was larger than the low temperature. Orchid seeds were able to grow at 20 ^{o}C under light or not. The concentration of sugar was not affected to embryo enlargement. Orchid seeds grew in both medium with sugar and without sugar. The highest percentage of seed germination was found in the medium with 1mg/l of BA and 0.1 mg/l of NAA. The largest protocorms was found in the medium with only 1mg/l of BA. The culture under darkness before light did not affect to development of protocorms. The protocorm could not grow to plantlet at 20^{o}C, but could develop at 25 ^{o}C and 3 0^{o}C. The 4% of sucrose was the optimal concentration for development of plantlets and the plantlets could not grow at 8% of sugar. Siriluk2) 001 (reported that the 13.35 g by average of *Dendrobium formosum* pods had seed about 2,032,484; the perfect seeds had about 1,981,619or 97.5%. Seeds were going to grow to plantlets with 4-3leaves on Vacin & Went (1949) medium for the length of 3 months. The 95.2% was the highest rate of germination. The plantlets of *Dendrobium formosum* Roxb. ex Lindl. tend to grow well on the medium with 10g/l of sugar, 100mi/l of coconut water and 2 g/l of activated charcoal. Unchan 2)001 (reported that 52.94% survival of *Dendrobium griffithianum* plantlets was found on the Vacin & Went (1949) medium with 150mi/l of coconut water, 100 g/l of potato extract water, 100 g/l of fragrant banana paste and 1ppm. of NAA. As increased NAA to 5ppm., the percent of survival would decrease to 16.94%. The plantlets with short roots and low survival were found in the medium with 0.1and 0.5 ppm. of -2,4D. Wang *et al.* (2009) reported that *Dendrobium nobile* Lindl. flower developed was deformed under 25 °C but it developed fully when grown in a lower temperature regime (23 °C/18 °C, light/dark) for 45 days. Luo *et al.* (2009) reported that the effects of cytokinins and carbohydrate sources on the conversion of *Dendrobium huoshanense* C.Z. Tang et S.J. Cheng PLBs to shoots depended on their types and concentrations. The best results in terms of shoot development from PLBs occurred on 1/2 MS medium supplemented with 20 μM kinetin and 10 g/l

maltose. Cold pretreatment at 10 °C for 1–2 weeks significantly enhanced the conversion of PLBs to shoots. The developed shoots were rooted on growth regulator-free MS medium supplemented with 8 g/l banana paste to give complete plantlets. And a suitable cold pretreatment (10 °C for 1 week) followed by the use of 20 µM kinetin and 10 g/l maltose in 1/2 MS medium would produce a large number of shoots from PLBs. Previous report stated that the effects of banana extract (BE) and 6-benzylaminopurine (BAP) were evaluated on asymbiotic seed germination and an early differentiation of protocorms and plant regeneration of *Dendrobium lituiflorum* Lindl. High percentage germination was achieved by culturing seeds on modified Knudson C medium supplemented with 10% (v/v) BE. Rapid regeneration was observed within 60 days of culture on 10% (v/v) BE supplemented KC medium where maximum percentage propagules showed development of leaves and root formation. Propagules on BAP supplemented KC medium showed no further development beyond one leaf stage. In another experiment, culture of shoots on 12.5% (v/v) BE supplemented KC medium led to multiplication, shoot elongation as well as vigorous rooting. Shoots cultured on 10 µM BAP supplemented MS medium showed maximum multiplication but these were stunted.

Materials and methods

The optimal basic formula media for growth of "Leaung chanthaboon" was studied by using 4 formula media, as Vacin & Went (Vacin and Went, 1949), White (White, 1963), Knudson (Knudson, 1946) and MS (Murashigs and Skoog, 1962). The 100ml of each medium was prepared and pour to 250 ml flask, and added 1 ml of orchid seed suspension (seeds from 8 months pod in 100 ml sterile distill water). All flasks were shaken by orbital shaker at speed 110 rpm, temperature 25 ^{o}C, light intensity 2,500 luxs., and range exposing under light for 16 hours per day. Seeds were successive cultured in each type of medium for 75 days. After that 5 ml of seeds suspension were sampling for 3 replications and calculated average dry weights.

Pattern of cultivation affect to seed growth was studied by scattering orchid seeds on solid medium and comparing with shaking seeds in liquid medium for 1 month before scattering on solid medium. The experiment was Completely Randomized Design (CRD). There were 2 treatments and 4 replications, the 20 plantlets were sampling by random and were recorded average fresh weights and height of shoots after cultivation for 4 months.

The influence of growth regulators to protocorm development was studied in 2 experiments as follows:-

The optimal type and concentration of growth regulators for protocorm development were investgateed by using 3 types of auxin (IBA, 2,4-D and NAA) and 2 types of cytokinin (kinetin and BAP) at the 5 levels of concentration (0.05, 0.10, 0.15, 0.20, 0.30 mg/l) in CRD of experiment. The protocorms were cultivated in liquid medium of Vacin & Went and the medium without growth regulator served as control. The 100 ml of liquid medium in 250 ml flask was prepared as a replications and each treatment was 3 replications. 1 ml of orchid seed suspension (seeds from 8 months pod in 100 ml sterile distill water) in each flask was added. All flasks were shaken by orbital shaker at speed 110 rpm., temperature 25 ^{o}C, light intensity 2500 luxs., and range exposing under light for 16 hours per day. Seeds were successive cultured in each type of medium for 2 months. After that, 5 ml of seed suspension was sampling for 3 replications and calculated average dry weights.

The result from previous experiment showed the optimal type of growth regulators and level of concentration was used for the next experiment. The best of auxin and cytokinin for growth of orchid seeds were selected for using in the experiment. The experiment was investgated the relationships of auxin and cytokinin which affected to put out shoot and root of protocorms. The 30 ml of Vacin & Went medium with 150 g/l of paste banana and 0.7% agar in 200 ml bottle were used to cultivate protocorms. Factorial experiment in CRD with 3 replications was designed for this experiment. The 10 plantlets which age 5 months, each of them with 1 shoot, height about 0.5 cm. and without root were transplanted in a bottle. One bottle represented one replication. The average of total weight, number of shoots and roots, height of shoots and length of roots were recorded at 4 months of cultivation.

The influence of light and temperature to growth of orchid plantlets was designed by Split Plot in CRD with 5 replications. The 1 ml of seed suspension which shaken in Vacin & Went liquid medium for 2months were blew in the same solid medium in the bottle. One bottle represented one replication. All bottles were exposed in the condition of various temperature and light intensity. The exposing under the light was 16 hours per day. The temperature level at 20, 25and 30 ^{o}C was main plot and the light intensity at1000 , 2000, 3000and 4000 luxs. was sub plot. The total fresh weights were recorded at 4 months of cultivation.

The influence of time lenght per day for orchid receiving to growth of orchid plantlets was studied by CRD experiment. The plantlets age of 6 months with height about 0.5-0.6 cm., total weight about 0.1 g, were grew in Vacin & Went solid medium. All bottles were exposed 25^{o}C and 3000 luxs of light intensity. There were 3 treatments and 10 replications. Each treatment was

exposed under the light for 12, 14 and 16 hours per day respectively. The total fresh weight was recorded at 3 months of cultivation.

The data were computed Analysis of Variance. Treatment means was compared by Leasf Significant different Test (LSD).

Results and discussion

The comparison of optimal basic formula media for growth of "Leaung chanthaboon"

Orchid seeds of 8 month pod were successively cultured for 75 days in liquid media of 4 basic formulas as follows:- Vacin & Went, White, Knudson and MS. The dry weight was determined and shown in Table 1. It was found that the dry weight of orchid biomass from Vacin & Went medium was highest 5.23)mg/5ml) and significantly different from other treatments. The Vacin & Went medium was selected to use in other experiments. This result agreed with others reported that the Vacin & Went is the popular formula for culturing seed and tissue of orchid genus *Dendrobium*. (Piyanuch,2 004; Sirilux, 2001; Unchan, 2001(

Table 1. The dry weight)mg/5 ml) of "Leaung chanthaboon" seeds which cultured in different formula of liquid media**.**

Media	Dry weight (mg/5ml)
White	0.98
Vacin & Went	5.23
Knudson	1.06
MS	2.01
F-test	**
CV) %)	15.37
LSD 0.05	1.1593
LSD 0.01	2.1620

/1** = significant at the level 0.01

Pattern of cultivation affected to seed growth

The total fresh weight of seed growth was compared between culturing on solid medium and in liquid medium for 1 month before scattered on solid medium, the result was shown in Table 2.

Table 2. Height of shoots and total fresh weights of 4 month plantlets which cultured on solid medium and in liquid medium for 1 month before scattered on solid medium.

Patterns of cultivation	Height of shoots	Total fresh weight
Culture on solid medium	0.95	0.50
Culture in liquid medium for 1 month before	0.97	0.63
F-test[/1]	ns	ns
CV)%)	0.06	0.16

[/1]ns =non significant

The difference of height and total fresh weight average between 2 patterns of cultivations were tested by that was non-significantly differenceest, it was found that the differences were non significant. However, seeds cultivation in liquid medium should be grow well, because around of seeds could contact the medium, they would to absorb medium faster than those on solid medium. Addition to shaking made to increase oxygen in the liquid medium and increase respiratory rate of seeds. We found that the seeds which culture in liquid medium before 1 month could germinate faster than solid medium cultivation about 4 days.

At the 7 days of seed cultivation in liquid medium, the germinating seeds were counted by modified Breed's method. (Nonglux and Preecha, 1988) It was found that all perfected seeds germinated, except lean seeds about 9.52 % of total seeds (figure 1(3)). The growing rate of "Leaung chanthaboon" seeds were about 90.48 %. The development of seed growth was observed under microscopic field, and the perfected seeds could grow faster than lean seeds. (Fig. 1)

Growth regulator influence to protocorm growth

The type and concentration level of growth regulators were compared the growth of orchid, in order to select the optimal type and level concentration of growth regulator for protocorm growth. The 3 auxins of IBA, 2, 4-D and NAA at 0.15 mg/l of concentration, the average dry weight of biomass from IBA and 2, 4-D flasks were significantly (P=0.01) higher than that control flask. At 0.05 mg/l of concentration, the average dry weight of biomass from 2,4-D flask was also significantly (P=0.05) higher than that control flask but that from IBA was non significant different when compared with control. In the opposite, NAA was significantly (P=0.01) affected to protocorm growth as lower than control (Table 4) 2, 4-D, the representative of auxins was selected to use in the other experiment. 2 cytokinins of kinetin and BAP, It was found that kinetin affected

to protocorm growth as significantly (P=0.01) lower than control which similar to NAA (Table 4). But the average dry weight from BAP flask was non significant different when compared with control flask. Thus, cytokinin was not necessary for tissue culture of "Leaung chanthaboon". But BAP was selected to use for studying the relationship between auxin and cytokinin which affected to growth of this orchid.

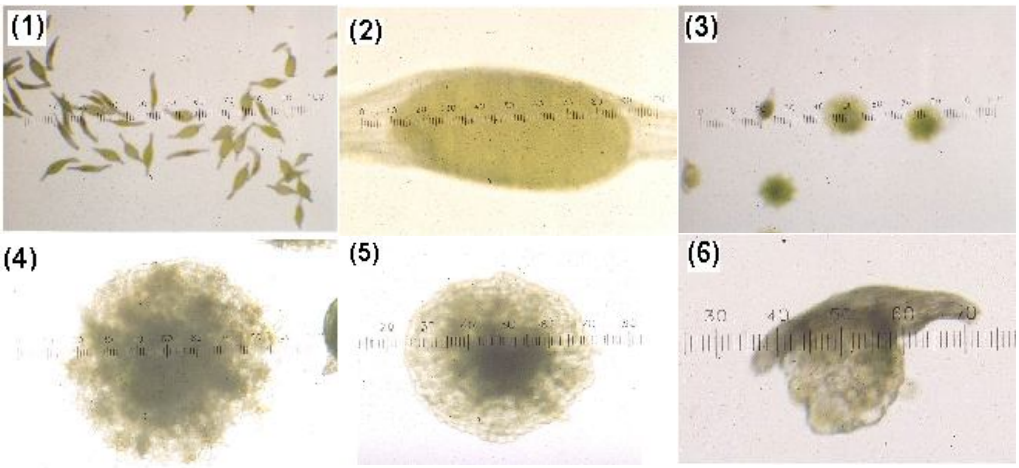

Fig. 1. Seeds of "Leaung chanthaboon" from 8 month pod. (1) and (2) seeds from pod before culturing in the liquid medium (1 scale = 29 and 4 μ respectively.)., (3)after culturing in Vacin & Went liquid medium for 7 days, the perfect seed was germinated before. (1 scale = 29 μ) .,)4) and (5) the enlarge photo of perfect seed, there had amount of hairs. (1 scale = 10 μ).,)6) imperfect seed was going to release from seed coat. (1 scale = 10 μ).

The best auxin and cytokinin from previous experiment were selected to use in this experiment which investigated the influence of relationship between auxin and cytokinin affected to protocorm development. Table 5 showed the good 2,-4-D gave significantly better in the number of root, height of shoot and total fresh weight but length of root and number of shoot were non significantly different. Plantlet with a lot of roots could increase water and mineral absorption affected to increase growth and development. Growth and development of orchids the type and concentration level of growth regulators are different used that, depended on species of plants. Prazak (2001) studied the propagation of *Dendrobium kingianum* Bidwill by using shoot on MS medium, it was found that BA 2.0 mg/l + IAA 0.5 mg/l well stimulated to shoot growth but only IAA or NAA 0.5 mg/l would stimulate to a lot of shoots growing out. The mixture of NAA (0.5, 1.0 mg/l) and BA (0.5, 1.0, 2.0 mg/l) increased the length of shoot. In addition to the mixture of NAA (0.5, 1.0 mg/l) and kinetin (0.5, 1.0, 2.0 mg/l) gave more increased in length of root than that kinetin only

and BAP did not influent to orchid growth. The result of BAP was similar to the previous experiment. Even though using BAP with 2, 4-D was probably unaffected to growth development of "Leaung chanthaboon".

Table 3. Comparison of growth regulators at the different of concentration affected to protocorm growth by determining average dry weight (g/100 ml medium).

Growth regulators	Concentration)mg/l(				
	0.05	**0.10**	**0.15**	**0.20**	**0.30**
control	0.60	0.60	0.60	0.60	0.60
IBA	0.56	0.62	0.92	0.71	0.65
2,4-D	0.81	0.72	1.08	0.65	0.54
NAA	0.38	0.49	0.33	0.58	0.58
Kinetin	0.21	0.42	0.6	0.47	0.54
BAP	0.38	0.46	0.51	0.55	0.5
F-test[/1]	**	NS	**	NS	NS
CV (%)	19.25	20.46	17	16.64	15.98
LSD 0.05	0.1679	-	0.2026	-	-
LSD 0.01	0.2354	-	0.2841	-	-

[/1]ns = non significant ** = significant at the level 0.01

Table 4. Comparison of growth regulator concentration levels affected to protocorm growth by determining average dry weight (g/100 ml medium).

Concentrations)mg/l(	Growth regulators				
	IBA	**2,4-D**	**NAA**	**kinetin**	**BAP**
0.00	0.60	0.60	0.60	0.60	0.60
0.05	0.56	0.81	0.38	0.21	0.38
0.10	0.62	0.72	0.49	0.42	0.46
0.15	0.92	1.08	0.33	0.6	0.51
0.20	0.71	0.65	0.58	0.47	0.55
0.30	0.65	0.54	0.58	0.54	0.5
F-test[/1]	*	**	**	**	NS
CV (%)	14.78	16.63	15.97	20.69	21.96
LSD 0.05	0.1788	0.216	0.1392	0.1731	-
LSD 0.01	-	0.3028	0.1952	0.2426	-

[/1]ns = non significant, * = significant at the level 0.05, ** = significant at the level 0.01

Table 5. The influence of growth regulators to protocorm development.

Total weight (g)					Height of shoot (mm.)					Number of root				
BAP (mg/l)	concentration of 2,4-D (mg/l)			average	BAP (mg/l)	concentration of 2,4-D (mg/l)			average	BAP (mg/l)	concentration of 2,4-D (mg/l)			average
	0	0.10	0.15			0	0.10	0.15			0	0.10	0.15	
0	0.64	0.72	0.92	0.76a	0	11.14	8.95	9.71	9.93	0	4.27	4.47	5.43	4.72
0.15	0.82	0.79	0.91	0.84a	0.15	10.93	10.17	10.29	10.46	0.15	4.27	5.20	4.77	4.75
0.20	0.73	0.75	0.94	0.81a	0.20	11.16	9.74	10.33	10.41	0.20	4.40	4.43	5.80	4.88
Average	0.73	0.75	0.93	0.80	average	11.08	9.62	10.11	10.27	average	4.31	4.70	5.33	4.78
	treatment	BAP(A)	2,4-D(B)	AxB		treatment	BAP(A)	2,4-D(B)	AxB		treatment	BAP(A)	2,4-D(B)	AxB
F-test	ns	ns	**	ns	F-test	ns	ns	**	ns	F-test	**	ns	**	ns
CV(%)	16.20	$LSD_{0.05}$=0.13, $LSD_{0.01}$=0.18			CV(%)	9.00	$LSD_{0.05}$=0.91, $LSD_{0.01}$=1.25			CV(%)	10.40	$LSD_{0.05}$=0.49, $LSD_{0.01}$=0.68		

Number of shoot					Length of root (mm.)				
BAP (mg/l)	concentration of 2,4-D (mg/l)			average	BAP (mg/l)	concentration of 2,4-D (mg/l)			averag
	0	0.10	0.15			0	0.10	0.15	
0	1.67	1.97	1.87	1.83	0	10.71	11.36	9.77	10.61
0.15	1.54	1.70	1.67	1.63	0.15	11.87	10.32	11.06	11.09
0.20	1.70	1.67	1.97	1.78	0.20	9.84	10.47	9.64	9.98
average	1.63	1.78	1.83	1.75	average	10.81	10.72	10.16	10.56
	treatment	BAP(A)	2,4-D(B)	AxB		treatment	BAP(A)	2,4-D(B)	AxB
F-test	ns	ns	ns	ns	F-test	ns	ns	ns	ns
CV(%)	10.40				CV(%)	18.90			

ns = non significant
* = significant at the level 0.05
** = significant at the level 0.01

Influence of light and temperature for growth of "Leaung chanthaboon"

The comparison of light intensity and temperature at different levels for growth of this orchid was shown in Table 6 .It was found that both light intensity and temperature were the most affected to growth of plantlets, especially at low temperature (20^{o}C) and high intensity of light (4000 luxs) that bshowed significantly highest in total fresh weight of plantlets. That is opposite to the nature, if the light intensity is high, the temperature is probably high. Thus, the growth rate of "Leaung chanthaboon" is rather slow in the nature especially at the first 3 years. This result similar to the several orchid (*Dendrobium*) agriculturists' reports which may be introduced to grow orchids under high light intensity and rather low temperature. For instant, Anonymous (2005A) introduced to grow orchid (*Dendrobium*) under the condition of light intensity 20,30-000, 000luxs, the temperature during the day should be ranged 21-32 ^{o}C, and at night should be ranged 13-17 ^{o}C. Anonymou (2005b) introduced to grow *Dendrobium moniliforme* under the light intensity of 25,35-000, 000 luxs., temperature 18-29 ^{o}C at daytime and should not lower than 8 ^{o}C at night.

Table 6. Influence of light intensity and temperature affected to total fresh weight of"Leaung chanthaboon".

Light)luxs(/temp.(°C(	Total fresh weight (g)			Average
	20	25	30	
1000	0.59b	0.30b	0.48a	0.45
2000	0.33b	0.41b	0.54a	0.43
3000	0.34b	0.44b	0.64a	0.48
4000	1.57a	0.85a	0.48a	0.97
		Temp.	light	Temp. x light
F-test/1		*	**	**
CV (%)		29.7	40.1	
		2M at each S	2S at each M	
LSD 0.05		0.29	0.30	
LSD 0.01		0.39	0.40	

* /1= significant at the level 0.05
** = significant at the level 0.01

Influence of time length for growth of "Leaung chanthaboon"

The comparison of time length affected to growth of orchid plantlets was shown in Table 7. It was found that the average total fresh weight of all treatments were not significantly different. It was observed the average total fresh weight of plantlets tended to increase, if they were treated with long time length of light per day. Because the time of this experiment (3 months) may be not long enough to observe the different of total fresh weight.

Table 7. Time length of light affected to total fresh weight of "Leaung chanthaboon".

Range of time light (hours/day)	Average total fresh weight (g)
12	0.17
14	0.18
16	0.18
F-test	ns
CV (%)	19.0

The study was to examine the optimization of conditions of *Dendrobium friedericksianum* Rchb.f. seedlings growth in aseptic culture. It was found that during 4 formulas mediums for instant Vacin & Went, White, Knudson and MS. Vacin & Went gave the best medium of the growth of the orchid. The average dry weight of orchid seeds cultivated in the liquid medium before transfer on solid medium for 1 month was not different from solid medium but 4 days faster

germination than the cultivation on the solid medium. IBA and 2,4-D at 0.15 mg/l were the best growth regulators and concentration for stimulating growth of this orchid NAA and kinetin were inhibited the growth. But BAP was not affect the growth and development of orchid, even though it was used with 2,-4-D. Besides 2,-4-D affected to orchid development in aspect of biomass, height of shoot and number of root, but not affect to the number of shoot and length of root. The cultivation of orchid seedlings under the conditions of various temperatures and light intensities showed the highest fresh weight on the average at 20 ^{o}C and 4000 luxs. However, the range of the light-exposed times at 12, 14 and 16 hours per day were not significantly affected the total fresh weight, but total fresh weight tended to increase if the length of exposed time was longer.

References

Anonymous. (2005a). Dendrobium culture: http://www.cityfloral greenhouse.com/tips/orchid_ dendrobium.htm.

Anonymous. (2005b). Culture of *Dendrobium moniliforme* - the Japanese Stone Orchid: http://newworldorchids. com/pages/denculture.htm.

Boonkead, S., Sadakorn, C. and Sadakorn, T. (1982). Botanical name of plants in Thailand. Forestry book printing fund. Faculty of forestry. Kasetsart university. 258 p.

Knudson, L. (1946). A new nutrient solution for the germination of orchid seed. Amer. Irch. Soc. Bull., 14 (3), 214-217.

Luo, J.P., Christoph, W. and Brigitte, K. (2009). Enhanced micropropagation of *Dendrobium huoshanense* C.Z. Tang et S.J. Cheng through protocorm-like bodies: The effects of cytokinins, carbohydrate sources and cold pretreatment. Scientia Horticulturae 123(2): 258-262.

Murashigs, L. and Skoog, F. (1962). A revised mendium for rapid growth and bioassays with tobacco tissue culture. Physiol.Plant 15: 473-497.

Paireepairit, P. (1978). Orchid book for the Beginner. Artorn Printing. Bangkok. 434 p.

Piyatragoon, P. (2004) .The Factors affected to Germination and Development of *Habenaria rhodocheila* Hance Plantlets. Master of Science Thesis. Horticulture Department Chieng Mai University.

Prazax, R. (2001). Micropropagation of *Dendrobium kingianum* Bidwill orchid. Biotechnologia2 (53): http://www.biotechnologia.pl/ biotechnologia/15/53/221.

Sirilux chareandee .(2001) .The Consevation and Propagation of Dendrobium *Dendrobium formosum* Lind. Master Thesis. Botany Department, Science Faculty. Kasetsart University.

Tongchang, U .(2001) .Effect of 2,4-D, NAA and paclobutazol to Growth of *Dendrobium griffiththianum* Lindl. Plantlets. Special Problem. Horticulture Department, Kasetsart University .

Vacin, E.F. and Went, F.W. (1949). Some pH changes in nutrient solutions. Bot. Gaz. 110: 605-613.

Wang, Z.H., Wang, L. and Ye, Q.S. (2009). High frequency early flowering from *in vitro* seedlings of *Dendrobium nobil*. Scientia Horticulturae 122(2): 328-331.

White, P.R. (1963). The Cultivation of Plant and Animal Cells. 2nded. Ronald Press Co., New York.

(Received 23 November 2010; accepted 7 March 2011)