In Vitro Plantlets production of *Bulbophyllum auricomum*L. cv. Dawei

Han Su Yin Thet[1],Thanda Aye[2]

Abstract

Micropropagation of *Bulbophyllum auricomum* L. was carried out in the tissue culture laboratory, Vegetable and Fruit Research and Development Center (VFRDC). Three different media such as KC, VW and MS were used to select the suitable medium for *Bulbophyllum auricomum* L. Among them, MS medium was suited for *Bulbophyllum auricomum* L. The selected MS medium supplemented with various plant growth regulators and the results showed that the optimum shoot multiplicationwas obtained from 1.5 mg L^{-1} kinetin, the highest bulbs formation in 1.0 mg L^{-1} IAA and the maximum number of roots from MS medium supplement with 0.75 mg L^{-1} IBA.

Keywords: micropropagation, different media, plant growth regulators

Introduction

Orchidaceae is a large family with 25,000 - 30,000 species and 600 - 800 general known from the world (Backer *et al*., 1963). The distribution of Thazin flora are BagoRegion, TanintharyiRegion, Yangon Region, Mon State and Rakhine State in Myanmar (Hundley and Chit KoKo, 1987).Many orchids have ornamental, medicinal used and commercial value. However, most of these commercially important orchid species could not easily be collected from the natural habitat. Hence plant micropropagation is one of the techniques for plants conservation as well as for commercialization(Ahloowalia, 1996).A culture medium is composed of inorganic salt, an iron source, vitamins, amino acids, growth substance (hormones) and carbohydrate.The addition of phytohormones (auxins and cytokinin) or their synthetic counterparts are required either singly or in combination to initiate and maintain cell division. The concentration of hormones may vary for plant tissues (Kumar, 1997).Most of the commercial orchids including *B. auricomum* are successfully propagated by tissue culture (Rao *et al*., 2001).The specific objectives were:to investigate the proper medium,to find out the effects of plant growth regulators on the growth of *Bulbophyllum auricomum* L.

Dr. Thanda Aye, Professor Department of Botany, University of Yangon
Dr. Han Su Yin Thet, Assistant Leature, Department of Botany, University of Yangon

Materials and Methods

In vitro culture of *Bulbophyllum auricomum* L. was carried out in the tissue culture laboratory, Vegetable and Fruit Research and Development Center (VFRDC). The plant materials used in this experiment was the green pods of *B. auricomum* L. Firstly; the plants were collected and grown in the Garden of Yadanar Hall, University of Yangon, for obtaining green pods in support of *in vitro* culture.

Medium selection for micropropagation of *Bulbophyllum auricomum* L.

Before culture, the pods were surface sterilized in 70 % ethanol solution for 5 - 10 seconds. The sterilized pods were flamed and split longitudinal with sterile surgical blade. Then, the sterilized seeds were inoculated in Murashige and Skoog,(MS)1962 (half and full);Knudson C (KC) 1946 (half and full) and Vacin and Went (VW) 1949 (half and full) to find out the suitable medium for seed germination and seedling growth. All media were supplemented with 3% sucrose and solidified with 0.8 % agar. Media were adjusted to pH 5.2 with 1NNaOH or HCl before autoclaving at 121°C and 1.05 kg cm^{-2} for 20 minutes. Three milligram of seeds was cultured in each culture vessel. Six treatments and each treatment had ten replicates were arranged in completely randomized design (CRD).

Achievement of various concentrations of kinetin for shootmultiplication of *Bulbophyllum auricomum* L. plantlets

The developed protocorm from above study were cultured in MS medium for three months. After three months, the explants were cultured in MS medium supplemented with different concentrations of 0.5, 1, 1.5, 2 mg L^{-1} kinetin. Each culture bottle contains 5 explants.Five treatments and each treatment with 10 replicates were arranged in completely randomized design (CRD).

Effect of IAA on bulb formation of *Bulbophyllum auricomum* L.

Five months old multiplicated shoots were again cultured in MS medium supplemented with different concentrations of 1, 1.5, 2 mg L^{-1}IAA. Twenty five shoots were cultured in each bottle. There were 4 treatments and each treatment had 10 replicates were arranged in CRD. The medium was changed in every three months.

Rooting of *Bulbophyllum auricomum* L. plantlets in MS medium supplemented with various levels of IBA

Fourteen months old with bulb formation obtained from the previous culture (study 3) were used as the plant materials for the *in vitro* root induction. The cultural bottles each containing MS basal medium supplement with different concentrations of 0.25, 0.50, 0.75, 1.00, 1.25 mg L^{-1}IBAwere used in root induction. The culture condition was maintained the same as in above study. Six treatments, each with 10 replicates were setup in completely randomized design (CRD).

Data collection and statistical analysis

This recorded data from the study 1, 2, 3 and 4 were analyzed using IRRISTAT software developed by International Rice Research Institute (IRRI), LosBaños, Laguna, Philippines.

Results

Medium selection for micropropagation of *Bulbophyllum auricomum* L.

The different media such as MS, KC,and VW were tested for *in vitro* propagation of *Bulbophyllum auricomum* L. Half concentration and full concentration of each medium were prepared in this experiment.When compared the different media, full MS medium had the maximum (630.2 mg) fresh weigh, (56.4mg)dry weight, (8.9)% dry weight per unit of fresh weight, (84) number of protocorm were obtained from Murashige and Skoog (MS) basal medium at 56 days after culture.The statistically analyzed results showed that the growth parameters of media were significant at 0.01 % level (Table 1 and Figure 1 - 4).

Table 1. Growth parameters of *Bulbophyllum auricomum* L. on different media

Treatment	Fresh weight (mg)	Dry weight(mg)	% dry weight per unit fresh weight	Number of protocorm
T1 (½ MS)	430.0	32.5	7.6	56.0
T2 (MS)	**630.2**	**56.4**	8.9	**84.0**
T3 (½ KC)	220.1	28.3	**12.8**	48.0
T4 (KC)	460.2	30.8	6.7	62.0
T5 (½VW)	180.4	22.6	12.5	54.0
T6 (V W)	210.3	25.3	12.1	60.0
F-test	**	**	**	**
5% LSD	37.61	2.99	1.13	3.46
cv %	9.2	8.8	11.6	3.0

**= highly significant.Data were collected 90 days after culture. Data were the mean of 5 replicates.

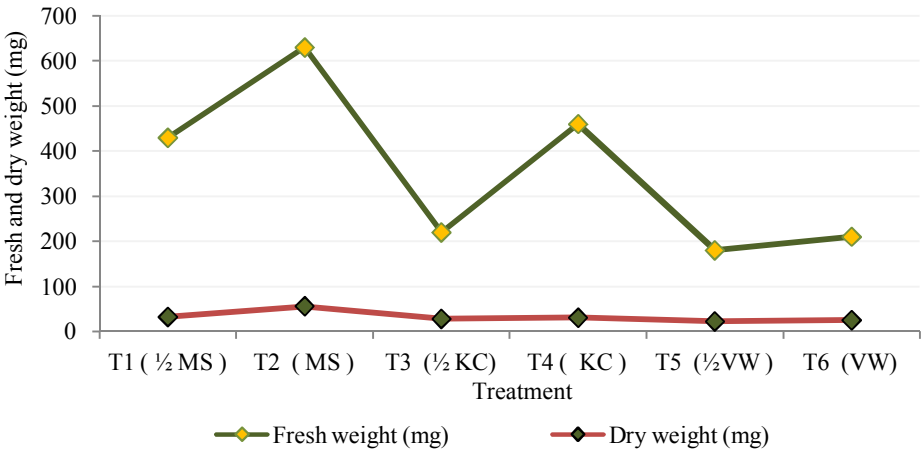

Figure1. Effect of different concentrations of various media on fresh weight and dry weight of *Bulbophyllum auricomum* L.

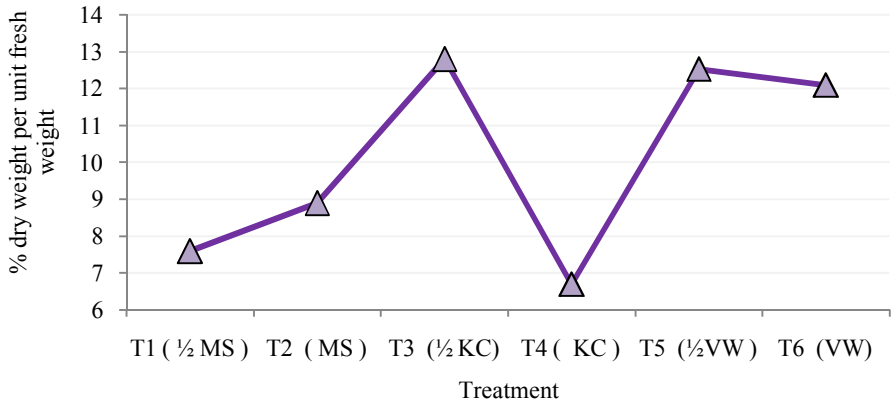

Figure2. Effect of various media on percentage dry weight per unit fresh weight of *Bulbophyllum auricomum*L.

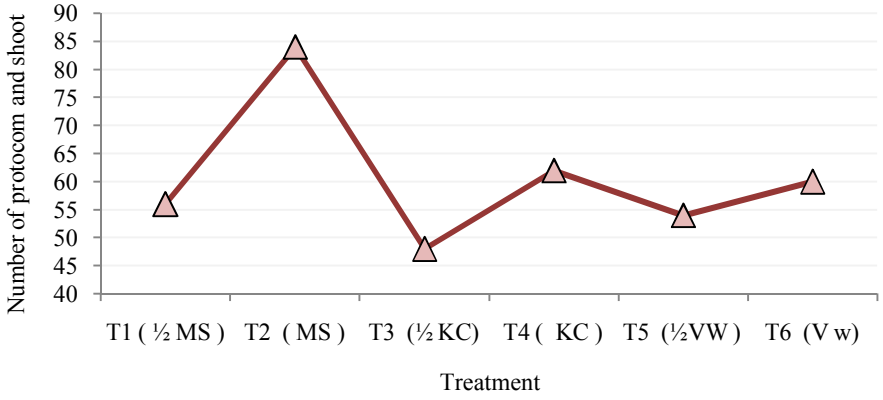

Figure3. Effect of different concentrations of various media on the number of protocorm of *Bulbophyllum auricomum*L.

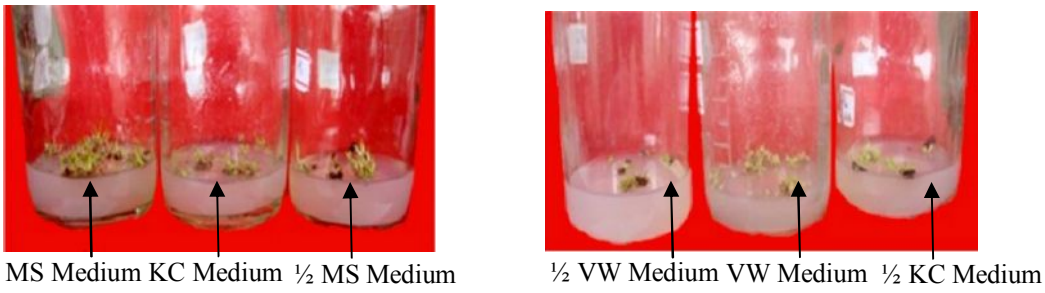

Figure 4. Effect of various media on development of protocorm and shoot formation of *Bulbophyllum auricomum*L.

Achievement of various concentrations of kinetin for shoot multiplication of *Bulbophyllum auricomum*L. plantlets

After ninety days, *in vitro* multiplicated *Bulbophyllum auricomum* L. shoots were cultured in MS medium supplemented with different concentrations of kinetin. Five explants were inoculated per bottle.Plantlets were sub-cultured in every 2 - 3 weeks. This procedure was repeated 3 times as long as the source plantlets appear healthy. The new shoots formation were formed 30 days after culture in all treatments.When compared the various concentrations of treatments,1.5 mg L^{-1}kinetin possessed the highest 720.30 mg fresh weight, 60.2 mg dry weight , 8.4 percentage of dry weight per unit of fresh weight,7.43 growth value,33.90 number of shoot, number of leaf per plant 2.0 and leaf length1.71 cm.The statistically analyzed results showed that 1.5 mg L^{-1} kinetin had highly significantly affects in the culture of *Bulbophyllum auricomum*L. (Table 2, Figure 5 - 8).

Table 2. Effect of various concentrations of kinetin on the growth of *Bulbophyllum auricomum* L. plantlets

Treatment	Fresh weight (mg/bottle)	Dry weight (mg/bottle)	% dry weight / unit fresh weight	Growth value	Number of shoot / Treatment	Number of leaf/plant	Leaf length (cm)
0 mg L^{-1}Kinetin	520.61	35.6	6.8	5.37	16.86	2.0	1.59
0.5 mg L^{-1}Kinetin	613.39	42.5	6.9	6.32	21.93	2.0	1.65
1.0 mg L^{1}Kinetin	691.00	50.8	7.4	7.12	27.69	2.0	1.65
1.5 mg L^{-1} Kinetin	**720.30**	**60.2**	**8.4**	**7.43**	**33.90**	2.0	**1.71**
2.0 mg L^{-1} Kinetin	700.60	58.3	8.3	7.22	29.67	2.0	1.63
F-Test	**	**	**	ns	**	ns	ns
5% LSD	26.45	2.47	0.57	0.27	2.36	0	0.17
cv %	3.1	3.7	5.5	3.1	7.1	0	19.8

**= highly significant, ns = non significant. Ninety seven milligrams of seeds were cultured in each culture vessel. Data were collected 90 days after culture. Data were the mean of 5 replicates.

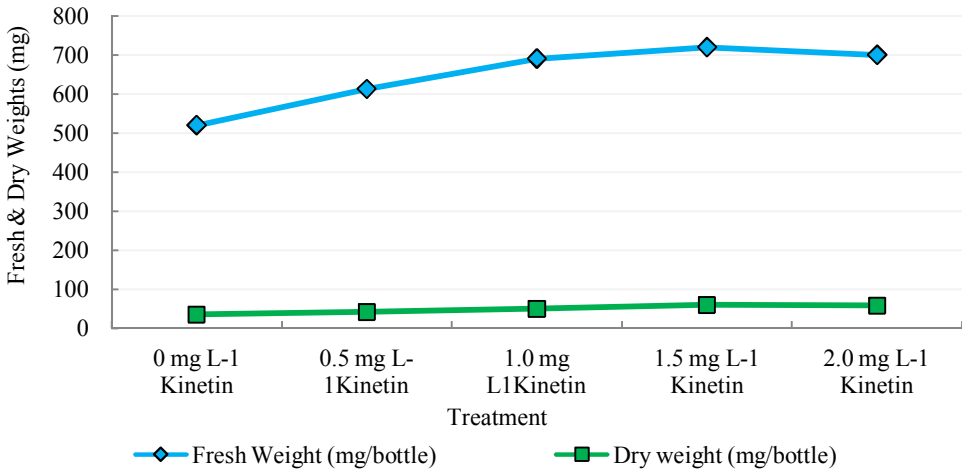

Figure 5. Effect of different concentrations ofkinetin on fresh weight and dry weight of plantlets

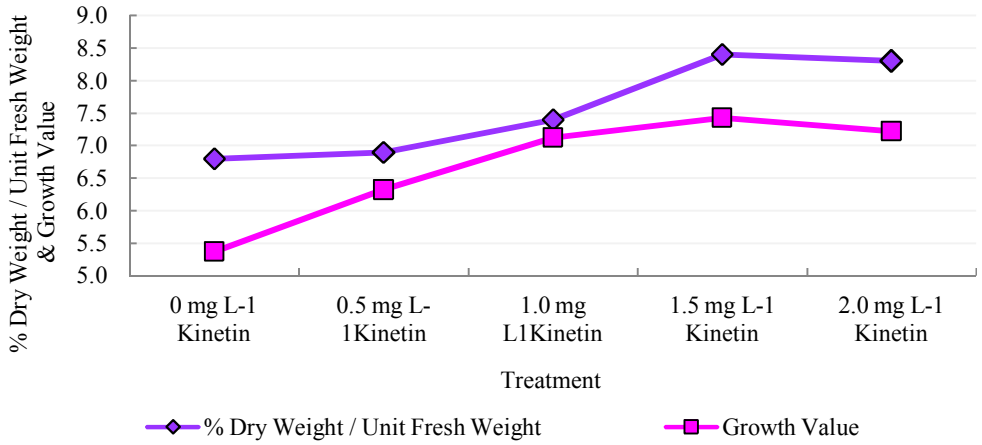

Figure 6. Effect of various concentrations ofkinetin on percentage dry weight per unit of fresh weight and growth value of plantlets

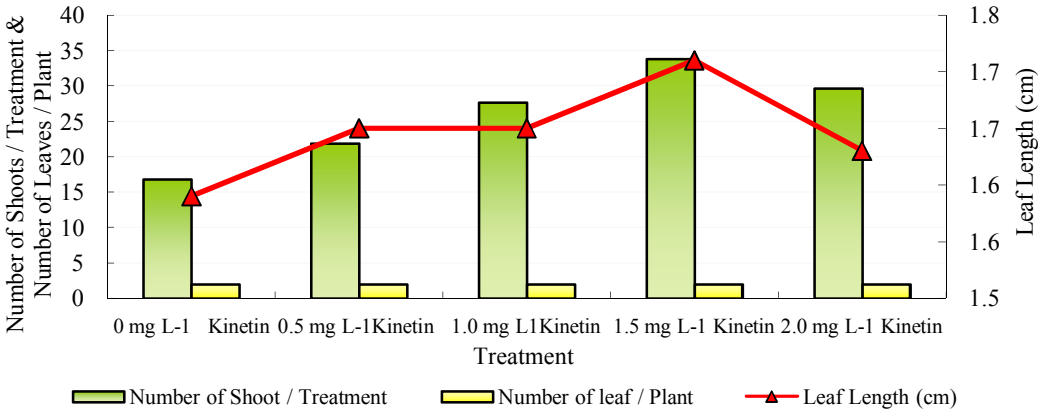

Figure 7. Effect of various concentrations ofkinetin on number of shoot per treatment, number of leaves per plant and leaf lengthof plantlets

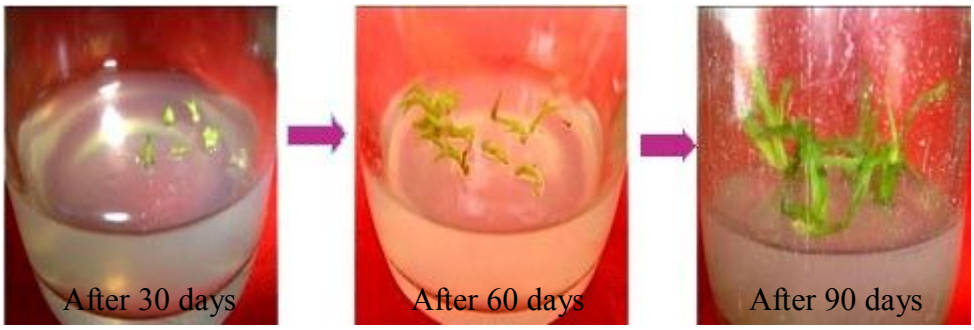

Figure 8. Development of shoot formation by various kinetin treatments

Effect of IAA on bulb formation of *Bulbophyllum auricomum* L.

The shoots obtained from the above study were used as the plant materials for the bulb formation of *Bulbophyllum auricomum* L. There were four treatments, each treatment with ten replicates.Twenty five shoots were cultured in each bottle. The shoots were cultured in MS medium supplemented with different concentrations of indole acidic acid (IAA).The bulb formation were started 60 days after culture in 1.0 mg L^{-1} IAA added MS medium. The highest number of bulbs formation (181) was obtained 1.0 mg L^{-1}IAA added MS basal medium followed by (144) in 1.5 mg L^{-1}IAA and (142) in 2.0 mg L^{-1}IAA then the least number of bulb (56) from MS basal medium. The statistically analyzed results showed that 1.0 mg L^{-1}IAA had significant affects in the bulb formation of *Bulbophyllum auricomum* L. (Table 3 and Figure9 -10).

Table 3. Bulbformation of *Bulbophyllum auricomum* L. in various concentrations of IAA treatments

Treatment	Number of bulbs
Control	56
MS + IAA 1.0 mg L^{-1}	**181**
MS + IAA 1.5 mgL^{-1}	144
MS + IAA 2.0 mg L^{-1}	142
F-test	*
5%LSD	24.33
cv %	17.4

* = significant

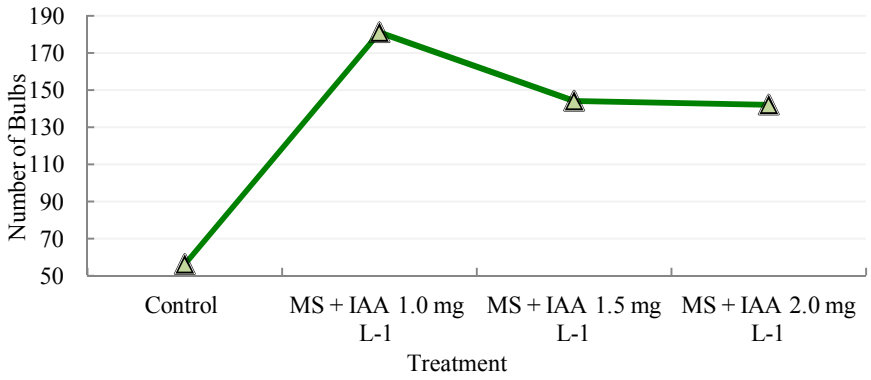

Figure 9. Various concentrations of IAA effect on bulb formation of *Bulbophyllum auricomum* L.

1.0 mg L^{-1} IAA 1.5 mg L^{-1} IAA 2.0 mg L^{-1} IAA

Figure 10. Various concentrations of IAA effect on bulb formation of *Bulbophyllum auricomum* L.

Rooting of *Bulbophyllum auricomum* L. shoots in MS medium supplemented with various concentrations of IBA

The multiplicated shoots of *Bulbophyllum auricomum* L. were used as the planting materials for *in vitro* root induction.Each cultural bottle contained 5 plantlets. Data were collected 6 weeks after culture.The statistical results of root length on IBA treatments showed that all treatments were significant at 0.01 % level. The highest number of roots 3.2 and the longest roots length 1.5 cmwere obtained on 0.75 mg L^{-1}IBA added MS medium (Table 4 and Figure 11 - 12).

Table 4. Root formation, number of roots, roots length from different IBA treatments

Treatment	Number of root	Root length (cm)
Control	0	0
MS + IBA0.25 mg L^{-1}	0.9	1.3
MS + IBA 0.50 mgL^{-1}	1.6	1.2
MS + IBA 0.75 mg L^{-1}	**3.2**	**1.5**
MS + IBA 1.0 mgL^{-1}	2.5	1.3
MS + IBA 1.25 mgL^{-1}	2.1	1.1
F-test	**	**
5%LSD	1.16	0.1
cv %	14.8	15.3

**= highly significant

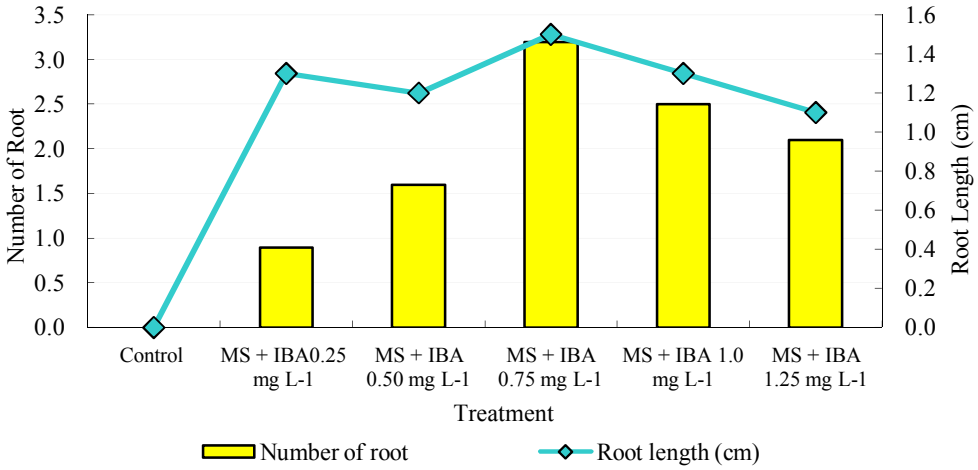

Figure 11. Effect of various concentrations of IBA on number of rootsand root length of *Bulbophyllum auricomum* L. plantlets

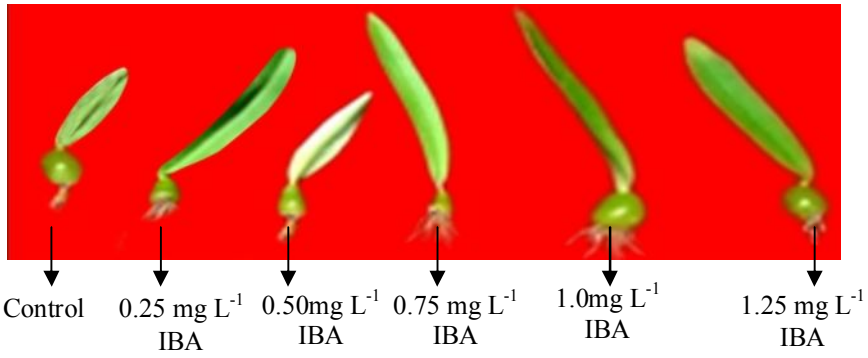

Figure 12. Effect of different concentrations of IBA on rooting *Bulbophyllum auricomum* L. plantlets

Discussion and Conclusion

The seeds were cultured in the different media (½ MS, MS, ½ KC, KC, ½ VW and VW) for *in vitro* propagation of *Bulbophyllum auricomum* L.The best results were obtained from Murashige and Skoog (MS) basal medium. The nitrogen content of MS medium was 7 times higher than that of VW and 3 times than KC. Nitrogen enhanced the plant growth especially is vegetative phase. The higher the nitrogen content, the higher the plant growth was observed in this experiment.The orchid seeds germination and seedlings growth required a sound balanced of mineral nutrients (Arditti, 1992). The favorable salt concentrations were varied in different species (Yates and Curtis, 1949) and different species have noticeable preferences of growth regarding to the composition of culture medium (Withner, 1942).After 3 months, the collected shoots from above studywere cultured in MS medium supplemented with different concentrations of Kinetin for shoot multiplication of *Bulbophyllum auricomum*L.The new shoot formations were formed at 30 days after culture in all treatments.Among treatments,the highest results were obtained on Kinetin 1.5 mgL^{-1}added MS medium.A single shoot was developed into multiple shoots on MS medium supplemented with cytokinin. It is generally believed that kinetin is the most active plant growth regulators in stimulating shoot multiplication (Huettemon and Preece, 1993).Five month multiplicated shoots were again cultured in MS medium supplement with different concentrations of IAA for the bulbs formation. Three months after culture, the bulbs were formed in 1.0 mg L^{-1} IAA added to MS medium. Among *in vitro* bulbs formation treatments, the highest results of bulbs development were obtained 1.0 mg L^{-1} IAA. The addition of 1.0 mg L^{-1} IAA to the basal MS medium gave the development of bulbs in *Bulbophyllum*(Hasegawa, 1980).Root initiation from the base of the bulb was observed two months after culture.Among*invitro* root induction treatments, the best results of root development were obtained onMS medium supplement with 0.75 mg L^{-1}IBA.One of the auxin, IBA also exerted better response to longer root length. Hedge and Saikia (2001) found that the longest root length in *Dendrobium* sp. was obtained from 0.75mg L^{-1}IBA added MS medium. Similarly the promotive effect of auxin on root initials was observed by Kumar (1997). Among three different media which were applied in this research, MS medium was suitable for *in vitro* propagation of *Bullbophyllum auricomum* L. Various concentrations of kinetin were used for shoot multiplication. Among them, 1.5 mg L^{-1}kinetininduced the optimum shoot multiplication. Among IAA, 1.0 mg L^{-1} IAA improved the bulbs formation and 0.75 mg L^{-1} IBA for root formation were suggested for *Bullbophyllum auricomum* L.

Acknowledgements

I wish to express my deepest gratitude to Dr. Aye Pe, Professor and Head, Department of Botany, University of Yangon, for his permission. I am thankful to Vegetable and Fruit Research and Development Center (VFRDC) for allow me to use the departmental facilities to do this research work. I am thankful to Department of Botany, Dagon University and Chonbuk National University Korea for active participation me to present my research paper in 1st Myanmar-Korea Conference on "Useful Plants and Biotechnology".

References

Ahloowalia, B. S. 1996. **Waston module, A new system of plant micropropagation.** BioLink 2: 17.

Arditti, J. 1992. **Fundamentals of orchid biology.** New York: John Wiley and Sons, Inc.

Backer, C. A., R. C. Bakhuizer and J. V. D. Brink. 1963. **Flora of Java, Vol. III**. N.V.P. Noord Hoff. Greningen. Netherland.

Hasegawa, P. M. 1980. **Factors affecting studies on some important flower crops**. International tips. J. Am. Soc. Hortic. Sci. 105 (2): 216 - 220.

Hegde, S. N. and D. K. Saikia. 2001. **Cultivation of *Dendrobium* in Arunachal Pradesh.** State Forest Research Institute, Arunachal Pradesh, India.

Huettemon, C. A. and J. E. Preece. 1993. **Thidiazuron: a potent cytokinin for woodyplant tissue culture**. Plant cell. Tissue Org. Cult. 33: 105 - 119.

Hundley, H. G. M. and Chit KoKo. 1987**. List of Trees, Shrubs, Herbs and PrincipalClimbers of Myanmar.** (4thed). Yangon: Government Printing Press.

Kumar, K. D. 1997. **An introduction to plant tissue culture.** Department of Botany, Borasai Govt. College, West Bengal India: New Central Book Agency (p) Ltd.

Rao, A. N., N. Ahmed and S. N. Hegde. 2001. **Culture notes from Tipi-4: *In vitro* culture of some rare and endangered archids of Arunachal Pradesh**. Orchid Research Centre, SFRI. Tipi, Forest Department, Arunachal Pradesh.

Withner, C. L. 1942. **Nutrition experiments with orchid seedlings**. Amer. Orch. Soc. Bulletin. 11: 112 - 114.

Yates, R. and J. T. Curtis. 1949. **The effect of sucrose and other factors on the shoot-root ratio of orchid seedlings.** Amer. J. Bot. 36: 390 - 396.