In vitro growth and multiplication of a hybrid orchid (*Dendrobium alba* × *Ascanda dongtarm)* with different concentration of plant growth regulators

Md. Nurul Islam, Khondokar Md. Nasiruddin[1]and Md. Al Amin[2]

Senior Scientific Officer (SSO), Soil Resource Development Institute (SRDI), Regional Office, Rajshahi, Bangladesh.
[1]Professor, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh
[2]Chief Scientific Officer, Division of Biotechnology, Bangladesh Agriculture Research Institute, Gazipur.
Corresponding author: nurulsrdi78@gmail.com

Abstract

In vitro growth and multiplication of a hybrid orchid was carried out to investigate the effect of different plant growth regulators on development of protocorm like bodies (PLBs) and subsequent plantlets of hybrid orchid. Protocorm like bodies (PLBs) were cultured on Murashige and Skoog (MS) media containing different concentration of NAA (0, 0.5, 1.0, 1.5 and 2.0 mg/l), IAA (0, 0.5, 1.0, 1.5 and 2.0 mg/l) and 0.5 mg/l of Kinetin in all cases were used. Among the different concentration treatments of NAA and IAA; and Kinetin concentration, maximum weight of PLBs (1.37g/explant) were noticed on MS medium supplemented with 1.5 mg/l NAA, and the highest number of plantlets (18.58/explant) were observed on MS medium containing 1.0 mg/l NAA. But maximum PLBs multiplication and or highest number of PLBs (28.50/explant) were found on MS medium supplemented with 2.0 mg/l IAA+0.5 mg/l of Kinetin.

Key words: Hybrid orchid, Protocorm like bodies, In vitro multiplication and growth regulators

Introduction

Orchids are the most fascinating, varied and beautiful of all flowers belong to the family *Orchidaceae*, one of the largest and most diverse plant families which has more than 25,000 species and 700-800 genera (Singa and Voletri, 1995). Among them *Dendrobium alba × Ascanda dongtarm* is an interesting group of hybrid orchid known for their intricately fabricated long lasting colorful flowers. Generally orchids are propagated both vegetative and by sexual means but these processes are very slow. Moreover, distinct variations in offspring are found. Therefore, to get true to the type plant, clonal propagation is the only means. Tissue culture techniques for micropropagation of orchids are well known for their exploitation as a major trade for years in developed countries. Several research reports on the micropropagation of orchids through tissue culture of leaf (Tanaka, 1987), root tips (Kobayashi et al. 1991) and lateral buds from young flower stalks (Ichihashi, 1992) are available. But none of these methods proved effective commercially in producing lots of plantlets in a short period because of low rate of protocorm like bodies (PLBs) formation, low viability of PLBs consuming long times for obtaining PLB and different responses among PLB and hybrids (Tokuhara and Mii, 1993). To avoid these problems, the multiple PLBs formation technique using different plant growth

regulators can be potential solution. Considering the above problem and scope of solution, the present investigation was undertaken to standardize and to develop a suitable combination and concentration of plant growth regulators for PLBs multiplication.

Materials and Methods

The experiment was carried out at the laboratory of Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, during the period from August 2002 to March 2003 to investigate the effect of different plant growth regulators on *in vitro* growth of PLBs of a hybrid orchid. In this experiment, *in vitro* grown one month old PLBs were used as explant and growth medium was Muraskige and Skoog (1962) supplemented by plant growth regulators.

The experiment was laid out in Completely Randomized Design (CRD) with 5 treatments and 4 replications. The treatments combinations were of Naphthalene Acetate NAA (0, 0.5, 1.0, 1.5 and 2.0 mg/l), Indole Acetic Acid (IAA) (0, 0.5, 1.0, 1.5 and 2.0 mg/l) and 0.5 mg/l of Kinetin in all cases were used with treatments.

PLBs were cultured on MS medium supplemented with different concentrations of plant growth regulators NAA and IAA; and their combination by Kinetin concentration. One month old seven *in vitro* PLBs (Plate 1a) were placed in each culture vial. The culture vials were placed in a growth room and allowed to grow at 25±1^{o}C under 16 hour photoperiod illuminated with fluorescent tube of 2000-3000 lux. Data were collected on the effect of different treatments of different parameter such as number of plantlets, number of rooted plantlets, and number of PLBs and weight of PLBs. The data were collected at 30 days interval. The treatment means of analyzed date were compared with LSD values.

Results and Discussion

Plantlet regeneration from PLBs renders the unique facilities of reproducible protocol in orchids. In this study, three experiments were conducted with a cross between *Dendrobium alba* × *Ascanda dongtarm*, *in vitro* PLBs were used for multiplication of PLBs and plantlet production with different plant growth regulators were investigated and presented below.

Experiment 1: *In vitro* growth of PLBs supplemented with NAA in MS medium

Different concentrations of NAA on *in vitro* growth of PLBs at different days after inoculation have been presented in Table 1 & Plate 1b. The highest number of PLBs (18.58/explant) was observed in 1.0 mg/l NAA at 90 days after inoculation (DAI). The maximum weight of PLBs (1.37/explant), rooted plantlets (3.33/explant) were obtained from 1.5 mg/l NAA at 90 DAI. Pathania et al. (1998) observed that Knudson C medium supplemented with NAA at 0.4 mg/l was the best for multiplication of PLBs which was closed to the investigation. While the highest number of plantlets (15.67/explant) was found with 1.0 mg/l NAA, whereas the lowest (6.33/explant) was observed in 0.0 mg/l NAA at 90 DAI (Figure 1).

Table 1: *In vitro* growth of PLBs at different days after inoculation under the supplementation of different concentrations of NAA

NAA Conc. (mg/l)	Weight of PLBs (g/explant)			Number of PLBs/explant			Number of rooted plantlets/explant		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
0	0.28	0.66	1.07	3.32	5.08	9.67	0.33	0.62	1.00
0.5	0.31	0.83	1.13	4.67	5.42	11.32	0.42	0.64	1.33
1.0	0.38	0.93	1.22	6.33	11.15	**18.58**	0.48	0.66	1.67
1.5	0.55	1.05	**1.37**	5.33	10.21	14.67	1.33	2.02	**3.33**
2.0	0.42	0.96	1.28	4.33	8.50	12.67	0.95	1.33	2.67
LSD(0.05)	0.049	0.049	0.015	0.049	0.244	0.319	0.049	0.049	0.015

DAI = Days After Inoculation

Experiment 2. *In vitro* growth of PLBs supplemented with NAA and Kinetin (Kn) in MS medium

Effect of combination of NAA and 0.5 mg/l of Kinetin on *in vitro* growth of PLBs at different days after inoculation have been presented in Table 2 & Plate 1c. The highest multiplied PLBs (16.33/explant) and the maximum weight of PLBs (1.15g/explant) were obtained from 2.0 mg/l NAA + 0.5 mg/l Kinetin. The highest rooted plantlets numbers (4.34/explants) were found with 1.0 mg/l NAA + 0.5 mg/l Kinetin at 90 DAI. Highest number of plantlets (18.33/explant) was found with 1.0 mg/l + 0.5 mg/l kn, whereas the lowest (7.67/explant) was obtained with 0 mg/l NAA+ 0 mg/l Kn at 90 DAI (Figure 2).

The present findings is partially supported by Rahman (2001) who found that the highest fresh weight of PLBs (0.116g) with the supplementation of 5.0 mg/l BAP +0.1mg/l NAA in *Doritaenopsis* orchid. The present findings also partially agreed with the report of Tokuhara and Mii (1993) where they showed that the highest rate of PLB formation occurred with 0.1 mg/l NAA and 2.0 mg/l IBA.

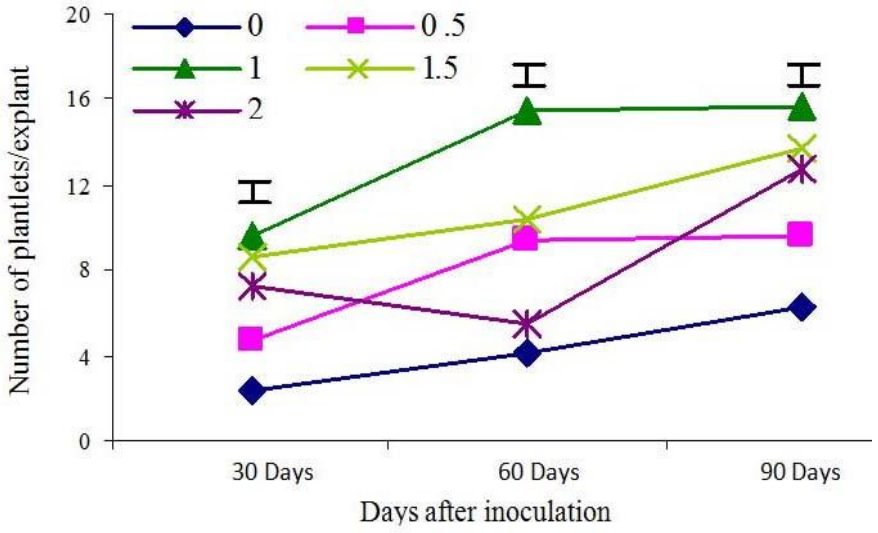

Figure 1. Effect of different concentration of NAA on *in vitro* number of plantlets per explant at different days after inoculation (DAI). Vertical bars represent LSD (0.05) values.

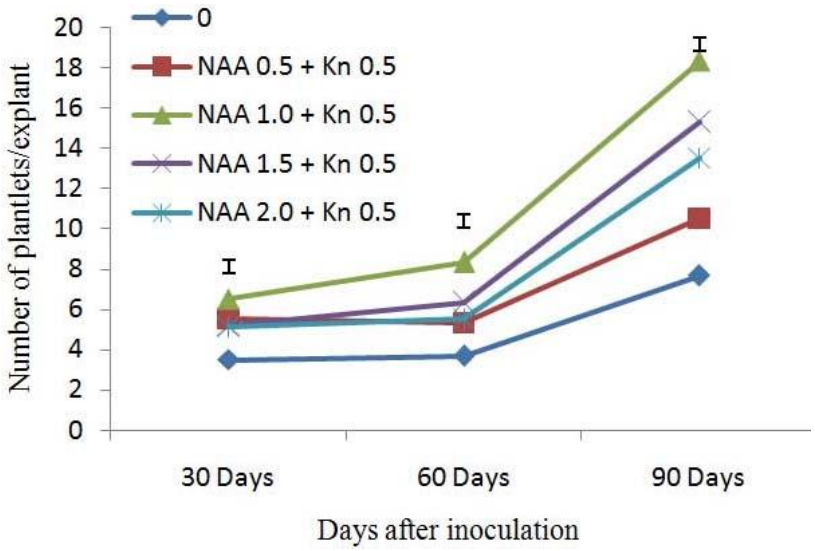

Figure 2. Effect of different concentration of NAA with 0.5 mg/l Kn on in vitro number of plantlets per explant at different DAI. Vertical bars represent LSD (0.05) values.

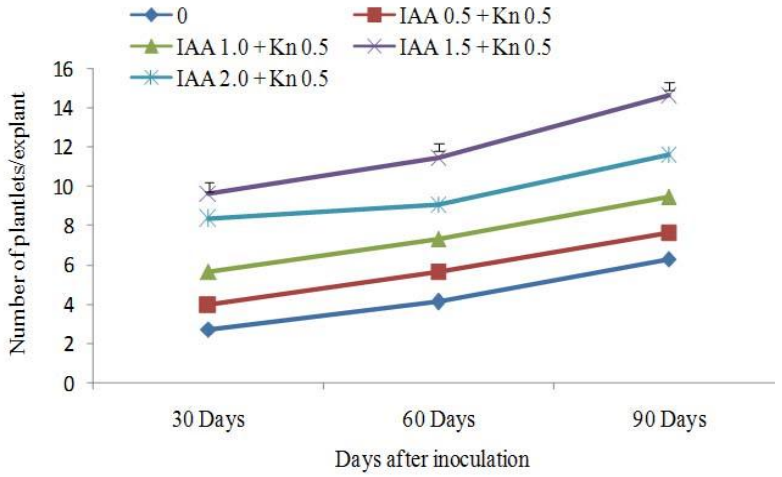

Figure 3. Effect of different concentration of IAA 0.5 mg/l Kn on in vitro number of plantlets per explant at different DAI. Vertical bars represent LSD (0.05) values.

Table 2. *Invitro* growth of PLBs at Different Days after Inoculation (DAI) under the supplementation of different concentrations of NAA and Kinetin

NAA + Kn (mg/l)	Weight of PLBs (g/explant)			Number of PLBs/explant			Number of rooted plantlets/explant		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
0 + 0	0.20	0.42	0.52	4.60	8.50	5.33	0.34	0.99	1.64
0.5 + 0.5	0.32	0.54	0.70	5.31	9.50	9.67	0.48	0.69	2.36
1.0 + 0.5	0.42	0.72	0.88	8.50	9.47	11.33	0.95	2.00	**4.34**
1.5 + 0.5	0.43	0.67	0.95	9.42	10.40	12.33	0.22	0.46	1.64
2.0 + 0.5	0.54	0.82	**1.15**	10.40	12.67	**16.33**	0.41	0.34	1.34
LSD(0.05)	0.015	0.015	0.015	0.069	0.402	0.419	0.015	0.049	0.049

DAI = Days After Inoculation

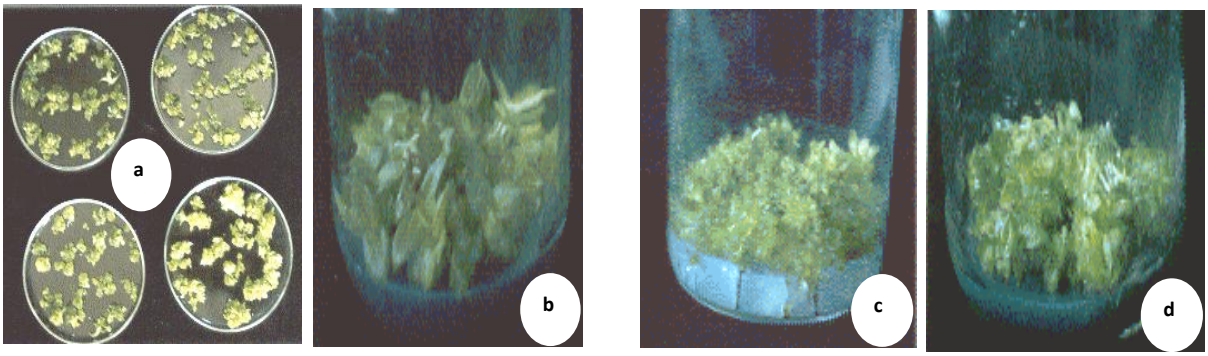

Plate 1. PLB growth of hybrid orchid on MS meidum a) *invitro* PLBs growth of hybrid orchid on MS medium; b) PLB formation and multiplication with 1.5 mg/l NAA at 90 days after inoculation; c) PLB formation and multiplication with 1.5 mg/l NAA + 0.5 mg/l Kinetin at 90 days after inoculation; d) PLB formation and multiplication with 2.0 mg/l IAA + 0.5 mg/l Kinetin at 90 days after inoculation

Experiment 3. *In vitro* growth of PLBs supplemented with IAA and Kinetin (Kn) in MS medium

Effects of different concentrations of IAA and Kinetin on in vitro growth of PLBs at different days after inoculation have been presented in Table 3 and Plate 1d. The highest multiplied PLBs (28.50/explant), with the maximum weight (1.17g/explant) were observed on MS medium containing 2.0 mg/l IAA + 0.5 mg/l Kinetin and 0.5 mg/l IAA +0.5mg/l Kinetin respectively. The highest number of rooted plantlets (3.49/explant) was noticed in 0.5 mg/l IAA + 0.5 mg/l Kinetin supplemented treatment concentrations.

Table 3. Different concentrations of IAA and Kinetin on in vitro growth of PLBs at different days after inoculation

IAA + Kn (mg/l)	Weight of PLBs (g/explant)			Number of PLBs/explant			Number of rooted plantlets/explant		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
0 + 0	0.20	0.66	0.94	8.67	9.67	10.67	0.09	0.15	0.35
0.5 + 0.5	0.35	0.80	1.00	10.28	14.47	15.50	0.95	2.5	**3.49**
1.0 + 0.5	0.31	0.86	0.97	12.67	19.50	22.67	0.41	0.67	1.69
1.5 + 0.5	0.42	0.93	0.99	13.33	20.50	25.67	0.11	1.98	3.31
2.0 + 0.5	0.55	1.05	**1.17**	16.50	23.50	**28.50**	0.50	1.23	1.96
LSD(0.05)	0.049	0.049	0.049	0.381	0.361	0.341	0.138	0.234	0.223

DAI= Days After Inoculation

The highest number of plantlets (14.67/explant) was obtained from 1.5 mg/l IAA +0.5 mg/l Kn (Figure 3), whereas the lowest number of plantlet (6.33/explant) was observed at 0 mg/l IAA+0 mg/l Kn at 90 DAI. Kusumoto (1979) showed that protocorm proliferation was stimulated effectively by 5 mg/l BA and 0.1 mg/l NAA which was closed to the present investigation.

Summary and Conclusion

Effect of growth regulator NAA: Among the different treatment concentration of NAA, highest number of PLBs (18.58/explant) was found with 1.0 mg/l NAA after 90 days of incubation (DAI). While maximum weight (1.37g/explant) of PLB and rooted plantlets number (3.33/explant) was obtained from 1.5 mg/l NAA after 90 DAI (Table 1). But plantlets number found highest (15.67/explant) with 1.0 mg/l of NAA after 90 DAI (Figure 1).

Effect of growth regulator NAA and Kinetin (Kn): Highest number of PLBs (16.33/explant) and maximum weight of PLBs (1.15g/explant) was observed with 2.0 mg/l NAA + 0.5 mg/l Kn. But the highest rooted number of plantlets (4.34/explant) was obtained from 1.0 mg/l NAA + 0.5 mg/l Kn (Table 2). While highest number of plantlets development was found with 1.0 mg/l NAA + 0.5 mg/l Kn treatment concentration (Figure 2).

Effect of growth regulator IAA and Kn: Highest number of PLBs (28.50/explant) multiplication with a maximum weight of 1.17g/explant was found with 2.0 mg/l IAA + 0.5 mg/l Kn. But the highest number of rooted plantlets was observed with 0.5 mg/l IAA + 0.5 mg/l Kn treatment concentration (Table 3). While the highest number of plantlets (14.67/explant) was found with 1.5 mg/l IAA + 0.5 mg/l Kn (Figure 3).

From the above treatments, it was revealed that for maximum weight of PLBs with 1.5 mg/l NAA is the best, while for rooted number of plantlets were found best with 1.0 mg/l of NAA concentration. But for maximum PLBs multiplication of the studied cross hybrid orchid (*Dendrobium alba* × *Ascanda dongtarm*) plant growth regulator combination 2.0 mg/l NAA + 0.5 mg/l Kinetin was found suitable among all other treatment.

Acknowledgement

The author acknowledges the contribution of staff from Division of Biotechnology, Bangladesh Agriculture Research Institute and all supports to conduct this research in their Plant Tissue Culture laboratory, Gazipur, Bangladesh. The authors convey their gratitude to Md. Noor-E-Alam Siddique, Senior Scientific Officer, Ministry of Agriculture, for adequate review and critique of this article.

References

Ichihashi S. 1992. Micropropagation of Phalaenopsis though the culture of lateral buds from young flower stalks. Lindleyana, 7(4): 208-215.

Kobayashi M, Komatuda M and Yonai S. 1991. Studies on the vegetative propagation of Phalaenopsis through root tip culture. Abstr., Japan Soc. Hort. Sci., 59(2): 664-665.

Kusumoto M. 1979. Effects of combinations of growth regulators and of organic supplements on the proliferation and organogenesis of cattleya protocorm like bodies cultured in vitro. J. Japan Soc. Hort. Sci., 47(4): 502-510. Murashige T., Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 15: 473-497.

Pathania NS, Sehqal OP, Debojit P, Dilta BS and Paul D. 1998. Studies on micropropagation in Dendrobium cv. Sonia. J. Orchid Soc. India, 12(1-2): 35-38.

Rahman ARMM. 2001. Studies on the improvement of in vitro plantlet regeneration in Doritaenopsis and Cattleya Orchids. MS thesis. Department of Crop Botany, Bangladesh Agricultural University, Mymensingh.

Singh B and Voletri SR. 1995. Research and Achievements. Div. Floriculture & Landscaping. Indian Agricultural Research Institute, New Delhi. p. 15.

Tanaka M. 1987. Studies on the clonal propagation of Phalaenopsis through *in vitro* culture. Mem. Fac. Agric., Kagawa Univ., 49: 1-85.

Tokuhara K and Mii M. 1993. Micropropagation of Phalaenopsis by culturing shoot tips of flower stalk buds. Plant Cell Rep. 13(1): 7-11.

Citation for this article:

M N Islam, K M Nasiruddin and M Al Amin, 2014. *In vitro* growth and multiplication of a hybrid orchid (*Dendrobium alba* × *Ascanda dongtarm)* with different concentration of plant growth regulators. Journal of Bioscience and Agriculture Research 01(01): 27-33.