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EFFECT OF VARIOUS GROWTH REGULATORS ON THE PRODUCTION OF PROTOCORM LIKE BODIES IN THREE ORCHID GENERA

G.V.S. SAIPRASAD[1], P. RAGHUVEER, S.KHETRAPAL AND R. CHANDRA

Division of Plant Physiology, Indian Agricultural Research Institute, New Delhi 110 012

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SUMMARY

Efficiency of protocorm like bodies (plbs) production as a function of various growth regulators was assessed in three orchid genera viz. *Dendrobium* 'sonia', *Oncidium*' Gower Ramsay' and *Cattleya leopoldii*. Growth regulators viz. BAP, kinetin, NAA, IAA, 2,4-D and GA_3 were supplemented to MS medium at concentrations of 0.25, 0.5 and 1.0 mg l^{-1}. The number of days taken for initial differentiation was observed to be a function of growth regulator supplemented as well as orchid genera. Maximum number of plbs were produced in BAP 1.0 mg l^{-1}. The relative ranking among plant growth regulators (highest to lowest) for obtaining maximum number of plbs was BAP>Kinetin>NAA>IAA>2,4-D>GA_3. Similar ranking for three orchid genera was *Dendrobium*> *Oncidium*>*Cattleya*.

Key words : **Growth regulators, orchids, protocorm like bodies**

INTRODUCTION

Orchids, the doyen among ornamentals are very distinctive plants. They are known for their long lasting and bewitchingly beautiful flowers which fetch a very high price of about 50 cents to even 50 dollars in both national and international market. Seed propagation of orchid is not successful due to heterozygosity of seed, minute seed size, presence of mycorrhizal association with fungi and also *in vitro* vegetative propagation techniques are time consuming and expensive. Hence, there is an urgent need to develop high volume low cost propagation method i.e. artificial seeds to ensure abundant supply of desired orchid species. To achieve this, a suitable protocol for mass production of protocorm like bodies (plbs) in orchids has to be standardized. Many economically important hybrids develop protocorms slowly in culture (Arditti and Ernst 1993). Inclusion of PGRs was tested as a means of developing efficient multiplication sytem (Prakash *et al.* 1996).

MATERIALS AND METHODS

Three orchid genera viz., *Dendrobium* 'Sonia', *Oncidium* 'Gower Ramsay' and *Cattleya leopoldii* were used in the present study. In *Dendrobium* fractionated plbs explant was used and in case of *Oncidium* and *cattleya* shoot-tip explant was used.

Preparation of shoot-tip explants : The young pseudobulbs and small plants were collected from the greenhouse. The older leaves and dried portions were removed from them and washed thoroughly in running tap water. Then they were cut into 5 cm long shoot-tip segments and washed in detergent (tween-20, 10 drops per 100 ml) and taken to laminar airflow chamber for surface sterilization. These explants were transferred to sterile water in conical flask and surface sterilized with 0.1% $HgCl_2$ and two drops of tween-20 for 180 seconds. These were then given three washes with sterile distilled water at 1, 5 and 10 minutes interval to remove the

[1]Present address and address for correspondence: Division of Biotechnology, I.I.H.R., Hessarghatta Lake Post, Bangalore - 560 089

sterilent from the explants. Then the shoot-tips were isolated carefully along with the leaf primordia after removing the leaf sheath with the help of sterile scalpel. These isolated shoot-tips were inoculated into sterilized nutrient media contained in culture vessels prepared for the experimental studies.

***Preparation of fractionated plb explants*:** Initially shoot-tip explants of *Dendrobium* 'sonia' were cultured on MS medium+BAP 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} for 120 days, which produced several clumps of plb mass. These plb clumps whose apical portion is cut and basal portion is fractionated into 2-3 parts of 0.4-0.5 cms length were used as fractionated plb explants, which were inoculated into sterilized nutrient media.

Culture conditions: The inoculated culture bottles were kept in culture room maintained at a temperature of 25 ± 2°C, photoperiod: 16 h light and 8 h dark. Light intensity was maintained at 50-60 μE $m^{-2} s^{-1}$

Effect of growth regulators: Effect of various growth regulators viz. BAP, Kinetin, NAA, IAA, 2,4-D and GA_3 at concentrations of 0.25, 0.5 and 1.0 mg l^{-1} supplemented to the Murashige and Skoog (1962) basal media, on the production of plbs/multiple shoots and also days taken for initial differentiation, fresh and dry weight in the three orchid genera was studied.

The data was recorded at 120 days after inoculation and was analyzed with completely randomized design with six replications. Each experiment was repeated twice. Mean and standard errors were calculated based on the data obtained from repeated experiments. Mean values were evaluated at p.0.05 level of significance using Duncan's new multiple range test (DMRT).

RESULTS AND DISCUSSION

Days taken for initial differentiation of fractionated plbs/shoot-tip explants was observed to be a function of growth regulators supplemented, as well as orchid genera. In *Dendrobium* where the initial explant is fractionated plbs the days taken for initial differentiation was 5-14. In *Oncidium* and *Cattleya* where shoot-tip was the explant, the range was 11-18 days (Table 1). Number of days taken for initial differentiation of fractionated plbs or shoot-tips was least in MS medium supplemented with 1.0 mg l^{-1} BAP treatment.

Among various plant growth regulators used maximum number of plbs were produced in BAP 1.0 mg l^{-1} treatment in all the three orchid genera (Fig. 1,2 & 3). In general, in all the three orchid genera, 2,4,-D and GA_3 at all the 3 concentrations tested did not produce any plbs (Figs. 1, 2 & 3). However, in *Cattleya* besides 2,4-D and GA_3, NAA and IAA (Fig. 3) also did not assist in the production of plbs. The plbs production ranking among various plant growth regulators (highest to lowest) is BAP > Kinetin > NAA > IAA > 2,4-D > GA_3. This ranking holds good for all the three genotypes.

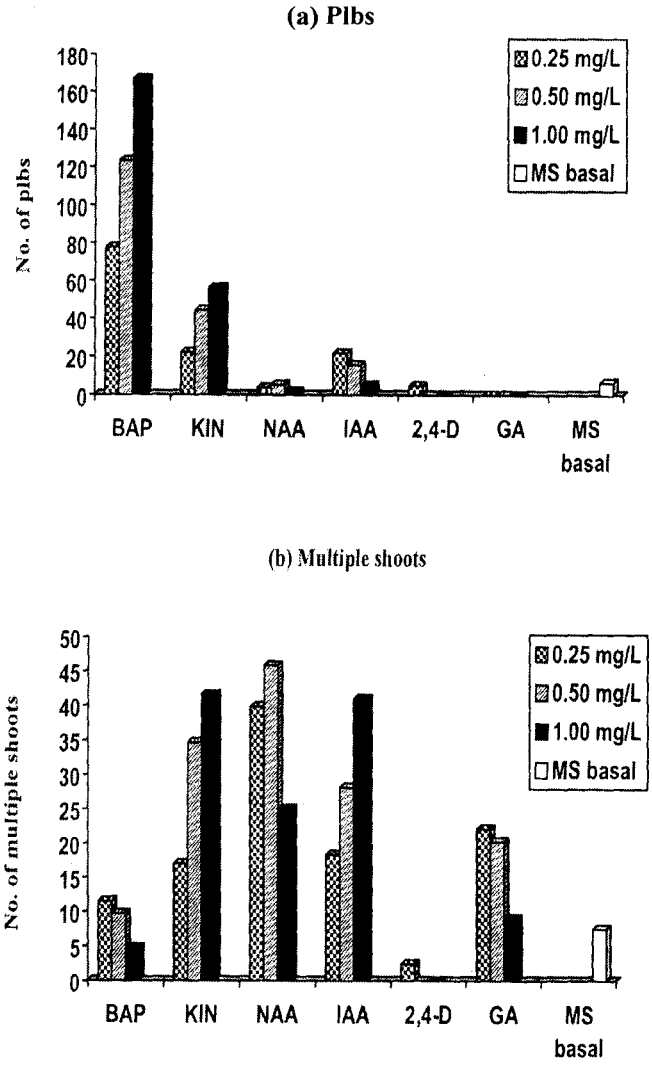

Fig. 1. Effect of various growth regulators supplemented to MS medium on the production of (a) plbs and (b) multiple shoots from fractionated plbs explant of orchid - *Dendrobium* ' Sonia'. Data recorded 120 days after inoculation

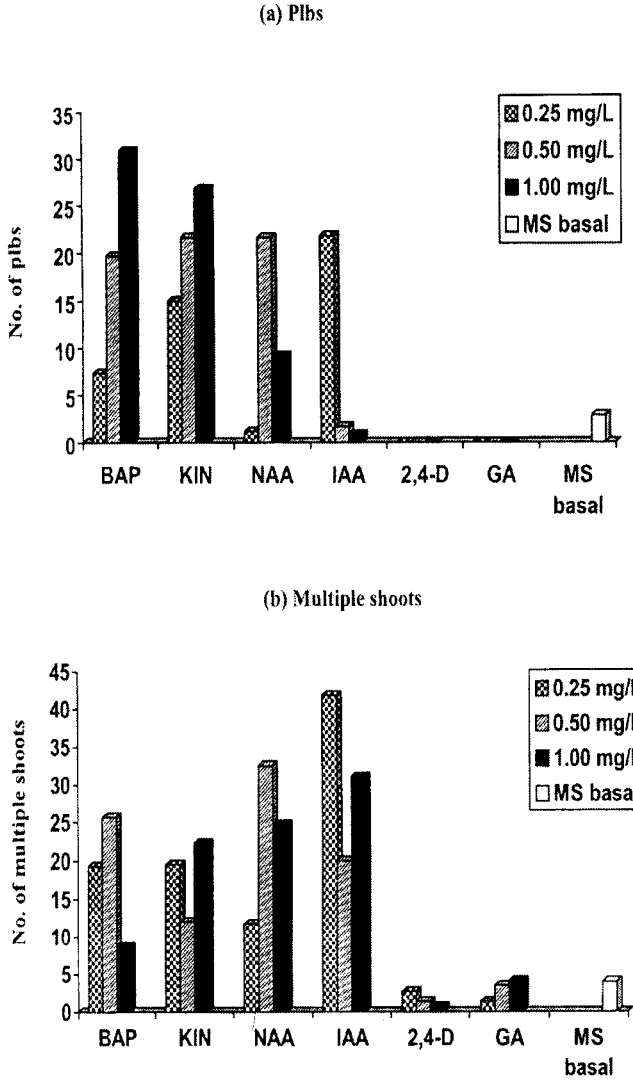

Fig. 2. Effect of various growth regulators supplemented to MS medium on the production of (a) plbs and (b) multiple shoots from shoot-tip explant of orchid - *Oncidium* ' Gower Ramsay'. Data recorded 120 days after inoculation

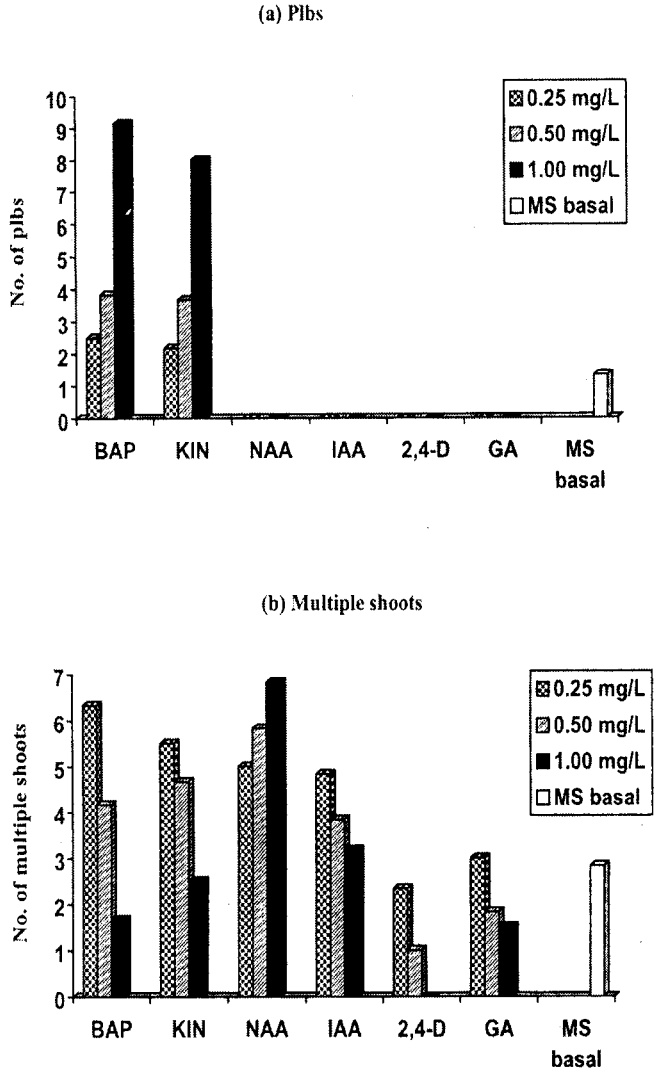

Fig. 3. Effect of various growth regulators supplemented to MS medium on the production of (a) plbs and (b) multiple shoots from shoot-tip explant of orchid - *Cattleya leopoldii*. Data recorded 120 days after inoculation

Maximum number of multiple shoots was observed in NAA treatments in both *Dendrobium* (Fig. 1) and *Cattleya* (Fig. 3) and IAA treatment in *Oncidium* (Fig. 2). In some treatments, inverse relationship was observed between number of plbs and multiple shoots. In case of auxin treatments (i.e., NAA and IAA), an increase in multiple shoots was associated with decrease in the number of plbs. This was also observed to be a function of concentration of the auxins (IAA/NAA), whereas in case of BAP treatments an increase in plbs was observed to be associated with the decrease in the number of multiple shoots. Thus, decrease in number of multiple shoots was observed with increase in BAP concentrations. In case of kinetin treatments number of plbs produced were more or less equal to the number of multiple shoots. No such positive or negative relationship was observed among 2,4-D and GA_3 treatments. The above mentioned response was more or less similar for all the three orchid genera studied (Fig. 1, 2, & 3).

Table 1. Effect of various growth regulators supplemented to MS medium on days taken for initial differentiation (I.D.), fresh and dry weight from fractionated plbs explant of orchid - *Dendrobium* and shoot-tip explant of orchids - *Oncidium* and *Cattleya*. Data recorded 120 days after inoculation.

Growth regulators (mg l^{-1})	*Dendrobium* Days taken for I.D.	*Dendrobium* Fw per culture (g)	*Dendrobium* Dw per culture (g)	*Oncidium* Days taken for I.D.	*Oncidium* Fw per culture (mg)	*Oncidium* Dw per culture (mg)	*Cattleya* Days taken for I.D.	*Cattleya* Fw per culture (mg)	*Cattleya* Dw per culture (mg)
BAP 0.25	7.33 fgh	20.59 g	0.80f	11.83 hij	2241.33 e	152.98fg	13.67 def	734.81 c	54.14cd
BAP 0.50	6.33 ij	31.54 c	1.16c	11.50 ij	2034.33 ef	136.78fgh	12.67 gh	223.27 fgh	15.15fgh
BAP 1.00	5.00 k	35.85 a	1.33a	11.00j	681.66 efg	30.87i	12.33h	154.35 ghi	12.84ghi
Kinetin 0.25	7.50 efg	26.70 e	1.04d	12.33 fghi	3923.67 d	254,96de	14.33 cd	333.54 ef	23.87fg
Kinetin 0.50	6.67 ghi	33.86 b	1.24b	11.83 ghij	4248.83 d	300.60cde	13.50 defg	550.51d	40.47e
Kinetin 1.00	5.50 jk	36.44a	1.36a	11.67hji	6481.83 c	347.96cd	12.67gh	252.94 efg	16.89fg
NAA 0.25	7.83 ef	20.37 g	0.95 e	12.83 efg	5566.83 cd	364.95c	13.67 def	743.44 c	60.85c
NAA 0.50	6.17 ij	28.23 d	1.07 d	12.17 fghi	12283.50 a	640.39 a	13.33 efg	886.23 b	78.65 b
NAA 1.00	6.83 ghi	22.13 f	0.90e	12.33 fghi	9808.67 b	519.20b	13.00 fgh	1260.82 a	109.80 a
IAA 0.25	8.83d	18.85 h	0.80f	12.00 ghi	10524.83 b	526.88b	14.00 cde	575.92 d	46.50de
IAA 0.50	8.33 de	23.35 f	0.91 e	12.50 efgh	4350.17 d	234.95 ef	14.17 cde	353.20 e	26.65f
IAA 1.00	6.50 hi	26.35 e	1.06d	12.67 efgh	5448.00 cd	362.33c	14.67 c	332.0.3 ef	22.16fg
2,4-D 0.25	9.83 c	1.87 jk	0.18h	13.83cd	248.87 fg	23.83i	14.67 c	126.99 hij	10.81ghi
2,4-D 0.50	11.50 b	0.69 kl	0.07 j	15.67 b	160.68 fg	14.18i	16.17 b	51,75 ij	2.25hi
2,4-D 1.00	14.00 a	0.14 l	0.0lk	16.67 a	83.52 g	7.28i	18.33 a	17.58 j	1.14i
GA_3 0.25	11.33b	3.47i	0.28g	13.33 cde	196.17fg	15.44i	14.00cde	174.10gh	15.73fg
GA_3 0.50	10.17c	2.74 ij	0.25g	13.00def	606.50 efg	47.34 hi	13.67 def	149.75 ghi	13.41 fghi
GA_3 1.00	8.83 d	1.49 jkl	0.12ij	12.67 efgh	1560.83 efg	110.53ghi	12.83fgh	124.85hij	10.63ghi
MS (basal) Control	11.83 b	1.98 jk	0.17 hi	14.00c	741.61 efg	61.27 ghi	16.00 b	151.64 ghi	20.38 fg
C.D. (at 5% P)	0.911	1.437	0.055	0.866	1698.342	97.055	0.767	115.068	11.950

Means followed by common letter within the column are non-significant at 5%.

Fresh weight observed was related to the total mass of plbs and multiple shoots. Maximum fresh weight was observed in kinetin treatments of *Dendrobium* and in NAA treatments of *Oncidium* and *Cattleya* (Table 1). Dry weight pattern was similar to that of fresh weight (Table 1).

Many orchid species were shown to require auxins and/or cytokinins for plb formation and plantlet development (Arditti and Ernst 1993). Plb formation was shown to be not only dependent on auxin : cytokinin ratio but also orchid genera and species. Thus, while NAA/BAP ratio of 12.2 was found optimum for *Spathoglottis plicata* (Teng *et al.* 1997), a ratio of 0.12 was observed to be good for *Phalaenopsis* leaf culture (Tanaka and Sakanishi 1985) and 0.42 for *Dendrobium antennatum* Lindley. (Kukulczanka and Wojciechowska 1983). There are some reports which indicate BAP alone is required for optimum plb formation and this requirement is also species specific. For instance, in various species of *Vanda*, 0.44-4.44 μM BA was applied (Sharma and Chaturvedi 1988), in *Cymbidium grandiflorum* - Griff. 4.44 μM BA (Gu *et al.* 1987), whereas in *Phalaenopsis*, 44.44 μM BA was employed (Tanaka and Sakanishi 1980).

In the present study, cytokinin treatments (BAP and kinetin) resulted in production of more number of plbs in all the three orchid genera, which would help in production of artificial seeds (a high volume low cost propagation method) to ensure abundant supply of desired orchid species. Auxin treatments (NAA and IAA) resulted in the production of more number of multiple shoots rather than plbs, which may help in micropropagation of desired orchid species. GA_3 and 2,4-D treatments resulted in the production of etiolated multiple shoots and callus respectively.

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