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Effect of Different Cytokinins on *In vitro* Organogenesis in Protocorm-Like Bodies (PLBs) of *Epidendrum* 'Rouge Star No. 8'

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Abstract: The effect of cytokinins is most noticeable in tissue cultures where they are used, to stimulate cell division and control morphogenesis. This study was undertaken to investigate the effects of different types of cytokinins on *in vitro* regulation of organogenesis in tissue culture in PLBs of E*pidendrum* 'Rouge Star No. 8'. Three types of cutokinins including kinetin (Kin), 6-benzylaminopurine (BA) and 2-isopentenyladenine (2ip) were evaluated with various concentrations of 0, 0.1, 1 and 10 mg/L in modified MS medium. These three cytokinins at all concentrations were found significantly enhance organogenesis of PLBs in E*pidendrum* 'Rouge Star No. 8' orchid except 10 mg/L of BA treatment when compared with control. Among these three cytokinins, the highest number of PLBs (14.1) was found at 0.1mg/L kinetin but 100% PLBs induction rate was found at 0.1 mg/L BA. In case of shoot formation, the highest number of shoots per explant (3.0) was observed at 1mg/L BA but the shoot formation rate (66.7%) was highest at 1mg/L kinetin. Results showed that kinetin enhanced root induction when compared with 2ip and BA. In this study, the efficiency of 2ip was observed only in PLBs formation but no satisfactory enhance on shoot and root formation of E*pidendrum* 'Rouge Star No. 8'.

Key words: Kinetin (Kin) · 6-Benzylaminopurine (BA) · 2-Isopentenyladenine (2ip) · Protocorm-Like Bodies (Plbs)

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 [1]. The beauty of flowers, variety of fragrance, brilliance in color and attractive habit has aroused highest admiration among the people throughout the world. Among orchids, *Epidendrum* is the largest genus of orchids with over 1000 species, many of which occur in great abundance in Central America. The *Epidendrum* are sympodial orchids that form owers in clusters on a long in orescence, which come in various shades of orange, yellow, white, light green and tan. *Epidendrum* growers face a number of problems, including the slow rate of sexual and vegetative propagation. Propagation of orchids is a complex process, which involves the environmental (Structural and functional) and physiological changes and may get influenced by the internal and external signaling factors. Tissue culture techniques for micropropagation of orchids are well known for their

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exploitation as a major trade for years in developed countries. In vitro propagation methods have been attempted on shoot-bud or PLB formation from callus derived from roots and leaf tips [2, 3] and oral tissues [4, 5]. Several research reports on the micropropagation of orchids through tissue culture of leaf [6], root tips [7] and lateral buds from young flower stalks [8] 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 [9]. However, the morphogenetic efficiencies were low, few normal plantlets were obtained and establishment of in vitro cultures usually depended on the quality of natural additives such as coconut milk and different plant growth regulators such as auxins, cytokinins etc. To avoid these problems, the multiple PLBs formation technique using different plant growth regulators can be potential solution. Role of cytokinins play a major role in interaction between the plant growth substances and also involves in inhibition of the plant growth substances. Growth regulators have a low importance for germination, but are important for the subsequent development of protocorms [10, 11]. In tissue culture media, cytokinins are one of the most important plant growth regulators for organogenesis. Considering the above problem and scope of solution, the present investigation was undertaken to standardize and to develop a suitable concentration of cytokinin for PLBs multiplication and shoot formation from PLBs of *Epidendrum* Rouge Star No. 8.

MATERIALS AND METHODS

Plant Material and Explants Source: Prtocorm-like bodies (PLBs) of *Epidendrum* Rouge Star No. 8 were used for explants. After PLBs were excised individually, each PLB was used as an ex-plant. Modified Murashige and Skoog [12] medium supplemented with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20 g/L sucrose and 2.2 g/L phytagel (Sigma) were used as a culture medium. Kinetin, BA and 2ip at concentrations of 0, 0.1, 1, 10 mg/L were added to culture media before sterilization. Jars of 250ml (UM culture bottle, AAs one, Japan) with plastic caps containing 30ml of medium were used for culture vessels. The pH of the medium was adjusted to 5.5-5.8 using 0.1mM 2 (N-morpholino) ethanesulfonic acid sodium salt (MES-Na) before autoclaving at 121°C for 15 min. Five explants cultured in one vessel and three vessels were used for each treatment. Cultures were maintained at 25 $\pm$ 1°C under white florescent light (54 µmol / m 2 /s) during 16h photoperiods for 40 days.

Data Analysis: Experimental data were collected by counting the number of PLBs, number of shoots; number of roots and their fresh weight were measured. The data were statistically analyzed by calculating standard errors of the means (means $\pm$ SE) and significant differences assessed by Tukey HSD test (P= 0.05).

RESULTS

Effects of Kinetin on Organogenesis in PLBs of *Epidendrum* 'Rouge Star, No.8': The effect of kinetin with different concentrations on organogenesis in PLB cultures of *Epidendrum* 'Rouge Star, No. 8' shown in Table 1. Kinetin at all concentrations enhanced the number of PLBs per explant when compared with control treatment. The rate of PLBs induction was different depending on the concentration of kinetin. Results showed that the highest PLBs induction rate (93.3%) was found at 0.1 and 1 mg/L kinetin and the lowest PLBs induction rate (73.3%) was found at control treatment (Fig. 1). The highest average number of shoot (2.1), shoot formation rate (66.7%; Fig. 2) and the highest number of root (1.3) was found on the medium which supplemented with 1 mg/L kinetin. In addition, 0.1 and 10 mg/L kinetin increased fresh weight also.

Table 1: Effects of Kinetin on organogenesis in PLBs of *Epidendrum* 'Rouge Star, No.8'

Kinetin (mg/L)	Average No. of PLBs	Average No. of Shoot	Average No. of Root	Fresh Weight (g)
0	6.3±1.9 a	0.5±0.4a	0.0±0.0	0.174±0.05
0.1	14.1±2.6a	1.7±0.8a	0.13±0.1	0.382±0.06
1	11.7±2.7a	2.1±0.6a	1.3±0.6	0.288±0.04
10	13.9±1.9a	0.3±0.3a	0.0±0.0	0.316±0.05

Values represent means ±SE followed by the different superscript letters and the same letters are not significantly different at p<0.05 (Tukey).

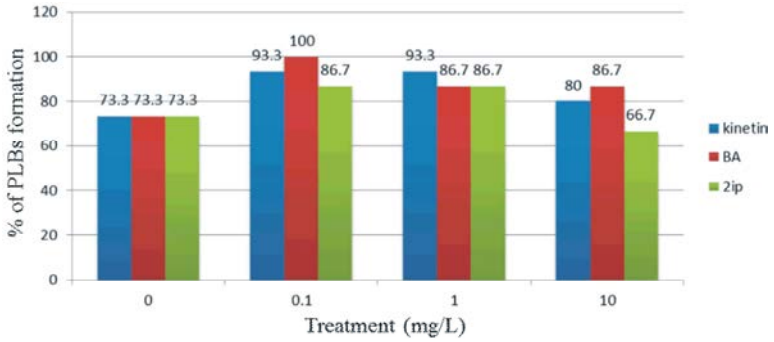

Fig. 1: Effect of different cytokinins (Kin, BA and 2ip) on percentage of PLBs formation in PLB cultures of *Epidendrum* 'Rouge Star, No.8'

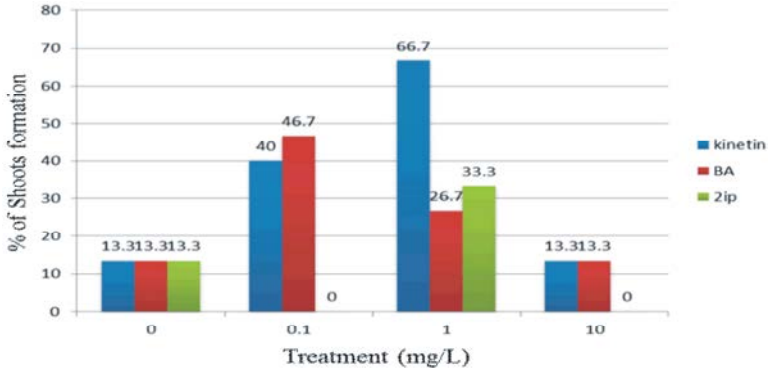

Fig. 2: Effect of different cytokinins (Kin, BA and 2ip) on percentage of shoot formation in PLB cultures of *Epidendrum* 'Rouge Star, No.8'

Table 2: Effects of 6-benzylaminopurine on organogenesis in PLBs of *Epidendrum* 'Rouge Star, No.8'

BA (mg/L)	Average No. of PLB	Average No. of Shoot	Average No. of Root	Fresh Weight (g)
0	6.3±1.9a	0.5±0.4a	0.0±0.0	0.174±0.05
0.1	7.5±1.9a	1.5±0.6a	0.13±0.09	0.190±0.03
1	9.1±2.4a	3.0±0.8a	0.0±0.0	0.165±0.04
10	3.6±0.6a	0.2±0.1a	0.0±0.0	0.083±0.02

Values represent means ±SE followed by the different superscript letters and the same letters are not significantly different at p<0.05 (Tukey).

Table 3L Effects of 2 isopentenyl-adenine on organogenesis in PLBs of *Epidendrum* 'Rouge Star, No.8'

2ip (mg/L)	Average No. of PLB	Average No. of Shoot	Average No. of Root	Fresh Weight (g)
0	6.3±1.9a	0.5±0.4a	0.0±0.0	0.174±0.05
0.1	9.4±2.4a	0.0±0.0b	0.0±0.0	0.205±0.05
1	14.0±3.9a	1.5±0.7a	0.0±0.0	0.363±0.09
10	9.1±2.9a	0.0±0.0b	0.0±0.0	0.206±0.05

Values represent means ±SE followed by the different superscript letters and the same letters are not significantly different at p<0.05 (Tukey).

Effects of 6-benzylaminopurine on Organogenesis in PLBs of *Epidendrum* 'Rouge Star, No.8': The effect of BA with different concentrations on organogenesis of PLB cultures in *Epidendrum* 'Rouge Star, No. 8' shown in Table 2. The highest number of PLBs per explants (9.1) was recorded on the medium supplemented with 1 mg/L BA compared with other treatments. But the rate of PLBs formation (100%) was highest at 0.1 mg/L BA (Fig 1). On the other hand, the maximum number of shoots per explant (3.0) and the percentage of shoot formation rate (46.7; Fig 2) was found at 1 mg/L and 0.1 mg/L BA respectively. In addition, highest fresh weight (0.19g) was observed at 0.1 mg/L BA treatment and found that root was formed only at the same concentration of BA.

But high concentration (10 mg/L) of BA had negative effect on growth and development of PLBs. Effects of low concentration of BA has no significant difference with high concentration treatment on organogenesis of PLBs in *Epidendrum cymbidium in vitro*.

Effects of 2 Isopentenyl-adenine on Organogenesis in Plbs of *Epidendrum* 'Rouge Star, No.8': The effect of 2ip with different concentrations on organogenesis of PLB cultures in *Epidendrum* 'Rouge Star, No. 8' shown in Table 3. The highest number of PLBs (14) was recorded in the media containing with 1mg/L 2ip. Increase in fresh weight showed higher values in same combination. The maximum PLBs induction rate (86.7%) was found in both at 0.1 and 1 mg/L 2ip (Fig 1). This cytokinin has very little effect on shoot formation in *Epidendrum* orchid. In addition, results showed that no root was formed in any concentration of 2ip treatment. 1 mg/L 2ip induced shoot formation (1.5,) and was significantly different with 0.1 and 10 mg/L 2ip treatment.

DISCUSSION

In tissue culture media, more commonly cytokinins are: BA (6-benzylaminopurine), Kinetin and 2ip (Isopentenyl-adenine). This study was done to investigate the effectiveness of these common cytokinins and to determine the best one and their optimum levels. These three cytokinins are concerned with cell division, modification of apical dominance, shoot differentiation, etc. These compounds are also used for shoot proliferation by release of auxiliary buds from apical dominance. The effect of cytokinin on tissue or organ cultures can vary according to the particular used, the type of culture, the variety of plant from which it was derived.

In this study, the numbers of PLBs were higher in kinetin and 2ip than BA treatment. In contrast, PLBs induction rate was 100% in case of BA treatment. High concentration of BA showed negative effect on PLBs number. A positive effect of cytokinins(e.g. BA or KIN) on orchid development was reported by Rasmussen [13]. Subculture of the tissue onto a medium containing a cytokinin can then cause the cells to divide synchronously after a lag period [14]. Hu and Wang [15] observed differences among the cytokinins and benzylaminopurine induced high multiplication rates while kinetin and 2ip stimulated only plant growth. This result are similar with present study because the present research confirmed that BA is more effective on shoot proliferation than Kin and 2ip. BA gives a high rate of shoot proliferation in Gerbera, but the best shoot quality is obtained using 5-10 mg/L kinetin [16, 17]. Among the benzylaminopurine, kinetin and coconut water, BA influences shoot proliferation by stimulating quick cell divisions to induce large number of multiple shoots [18-20]. Increased fresh weight shows maximum values in present investigation on the medium supplemented with 0.1 mg/L kinetin. Less fresh weight might be due to the reason that Kin shows less effectiveness than BAP in order to trigger the enzymes responsible for enhancement of vegetative growth [21]. Effects of kinetin on of *Epidendrum* 'Rouge Star, No.8' fresh weight are similar with Bennet[21] result. The differential growth response is a common phenomenon that has been observed in most plant tissues [22, 23]. Harvais [24] also found differences in protocorm growth of C. reginae in response to cytokinins and kinetin and BA in that order, were thw most suitable with 2iP. The reasons for differential growth responses to cytokinins is not completely understood but may be due to differences in their metabolism [25, 26].

CONCLUSION

Micropropagation of plants has become a significant technique to reproduce and make the availability of orchids that is otherwise difficult to propagate traditionally by seed or vegetative. This research showed that the choosing an appropriate of concentration of Kinetind, BA and 2ip was effective on traits of organogenesis of *Epidendrum* and on the basis of results concluded that comparatively low concentration of BA, kinetin and 2ip were more effective to promote the organogenesis in PLB cultures of *Epidendrum* 'Rouge Star, No.8'.

REFERENCES

1. Singh, B. and S.R. Voletri, 1995. Research and Achievements. Div. Floriculture and Landscaping. Indian Agricultural Research Institute, New Delhi, pp: 15.
2. Churchill, M.E., E.A. Ball and J. Arditti, 1972. Tissue culture of orchids-II. Methods for root tips. Am. Orchid Soc. Bull. 41: 726-730.
3. Stewart, J. and J. Button, 1978. Development of callus and plantlets from Epidendrum root tips cultured in vitro. Am. Orchid Soc. Bull, 47: 607-613.

4. Stewart, J. and J. Button, 1976. Rapid vegetative multiplication of Epidendrum O'brienianum in vitro and in the greenhouse. Am. Orchid Soc. Bull, 45: 922-930.
5. Kuhn, L., 1981. The Eric Young micropropagation center. Am. Orchid Soc. Bull, 50: 47-50.
6. Tanaka, M., 1987. Studies on the clonal propagation of Phalaenopsis through in vitro culture. Mem. Fac. Agric., Kagawa Univ., 49: 1-85.
7. Kobayashi, M., M. Komatuda and S. Yonai, 1991. Studies on the vegetative propagation of Phalaenopsis through root tip culture. Abstr., Japan Soc. Hort. Sci., 59(2): 664-665.
8. Ichihashi, S., 1992. Micropropagation of Phalaenopsis though the culture of lateral buds from young flower stalks. Lindleyana, 7(4): 208-215.
9. Tokuhara, K. and M. Mii, 1993. Micropropagation of Phalaenopsis by culturing shoot tips of flower stalk buds. Plant Cell Rep., 13(1): 7-11.
10. Stewart, J., 1989. Orchid propagation by tissue culture-pat, present and future. In: Pritchard, H.W.(ed.): Modern Methods in Orchid Conservation. Cambridge University Press. Cambridge, pp: 87-102.
11. De Pauw, M.A., W.R. Rempjery and C.E. Palmer, 1995. The cytokinin Preference for in vitro germination and protocorm growth of Cypripedium candidum. Ann. Bot., 75: 267-275.
12. Shimasaki, K. and S. Uemoto, 1990. Micropropagation of aterrestrial Cymbidium species using rhizomes developedfrom seeds and pseudobulbs. Plant Cell Tissue Organ Cult.22:237-244.
13. Rasmussen, H.N., 1995. Terrestrial Orchids: from Seed to Mycotrophic Plant. Cambridge University Press, Cambridge, UK.
14. Jouanneau, J.P., 1971. Controle par les cytokinines de la synchronization des mitoses dans les cellules de tabac. Exp. Cell. Res., 67: 329-337.
15. Hu, C.Y. and P.J. Wang, 1983. Meristem, shoot tip and bud cultures. In: Evans D>A., Sharp W.R., Ammirato P.V; Yamada Y. Handbook of plant cell culture:techniques for propagation and breeding. New York. Macmillan, pp: 117-227.
16. Pierik, R.L.M., H.H.M. Strrgmans, J.A.M. Verhaegh and A.N. Wouters, 1982. Effect of cytokinin and cultivar on shoot formation of Gerbera jamesonii in vitro. Neth. J. Agric. Sci., 30: 341-346.
17. Hempel, M., B. Petos-Witkowska and J. Tymoszuk, 1985. The influence of cytokinins on multiplication and subsequent rooting of Gerbera in vitro. Acta Hortic., 167: 301-305.
18. Yakimova, E., V.K. Toteva, I. Groshkoff and G. Ivanova, 2000. Effect of BA and CPPU on protease and alpha-amylase activity of in vitro cultured explants of Rosa hybrid L. Bulg. J. Plant Physiol., 26: 39-47.
19. Roy, J. and N. Banerjee, 2002. Induction of callus and plant regeneration from shoot tip explants of Dendrobium densiflorum (Lam.) Schltr. Sci. Hortic., 94: 181-192.
20. Ronzhina, E.S., 2003. Effect of 6-benzylaminopurine on the structure of photosynthetic apparatus of Faba bean(Vicia faba L.) Appl. Biochem. Microbiol., 39: 411-417.
21. Bennet, I.J., J.A. McComb, C.M. Tonkin, D.A.J. McSaviid, 1994. Alternating cytokinins in multiplication medium stimulates in vitro shoot growth and rooting of Eukalyptus globules Labill. Ann. Bot., 74: 53-58.
22. Bosse, C.A. and J. Van Staden, 1989. Cytokinins in cut carnation flowers. V. Effects of cytokinin type, concentration and mode of application on flower longevity. Journal of Plant B. Physiology, 135: 155-159.
23. Van der Krieken, W.M., A.F. Croes, M.J.M Smulders and G.J. Wullems, 1990. Cytokinins and flower bud formation in vitro in tobacco. Plant Physiology, 92: 565-569.
24. Harvais, G., 1982. An improved culture medium for growing the orchid Cypripedium reginae axenically. Canadian Journal of Botany, 60: 2547-2556.
25. Horgan, R., 1984. Cytokinins. In: Wilkuns MB, ed. Advanced Plant Physiology. Marshfield: Pitman, pp: 53-77.
26. Mok, M.C., D.W.S. Mok and J.E. Turner, 1985. Reversed activities of cytokinin bases and ribonucleosides in callus tissues of *Phaseolus lunatus* L. Journal of Plant B. Physiology, 121: 273-280.