- Short communication

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Effects of Ethrel on Organogenesis of Protocorm-like bodies in *Dendrobium kingianum In vitro*

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This study attempted to understand the effects of BAP and ethrel on organogenesis of protocorm-like bodies (PLBs) in *Dendrobium kingianum* under white fluorescent lamps *in vitro*. BAP is the most effective cytokinin for multiplication in plant tissue culture. Ethrel regulates many aspects of plant morphogenesis. The highest number of PLBs (13.1) as against in control (8.1) and the highest number of developing shoots (2.1) was recorded in the medium containing 0.1 mg/l BAP combined with 1 mg/l ethrel. Increase in fresh weight showed higher values in same combination. Low concentration of BAP alone increased in the number of PLBs but showed inhibitory effects on shoot formation. On the other hand low concentration of ethrel alone increased both number of PLBs and shoots after 4 weeks *in vitro*.

Dendrobium is popular in the international floriculture industry due to its floriferous flower sprays, wide spectrum of colors, sizes and shapes; year-round availability and long flowering life (Kuehnle 2007, Khosravi et al. 2009). BAP is the most effective cytokinin for multiplication followed by Kn and 2-ip (Hu and Wang 1983, Schuch and Erig 2005). Ethylene is unique among plant hormones; it is a simple hydrocarbon that affects growth, differentiation, and senescence in plants in concentrations as low as 0.01 µl/l (Reid 1995). Ethylene regulates many aspects of plant morphogenesis. Growth and development of cells cultured *in vitro* are largely dependent on the presence of phytohormones. Hence, modification of phytohormone composition and interaction in the nutrient medium has been the primary strategy to manipulate morphogenesis *in vitro*. Research on specific effects of ethylene on cell division is rather limited. From the

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available evidence, it appears that ethylene has mostly inhibitory effects on cell division. In many types of tissue culture ethylene may act as a promoter depending on the species and ethylene concentrations used. The relationship between ethylene compounds and other growth regulators on growth and development of PLBs in *Dendrobium* have not been so far studied.

PLBs of *Dendrobium kingianum* were proliferated in the modified MS (Shimasaki and Uemoto 1990) by transferring to a new medium. After excision of PLB into singles, they were used for explants. This experiment was carried out in floriculture and vegetable science laboratory, Kochi University, Japan. In experiment 1, PLBs were treated for (30, 60, 90, 120 min) with ethrel at 1 and 10 mg/l with and then transferred to MS. In experiment 2, BAP at concentrations of 0, 0.01, 0.1, 1, 10 mg/l were added to culture media before sterilization. PLBs were dipped in ethrel at 0, 1 and 10 mg/l concentrations for 1 hr and combined with BAP. Jars of 250 ml (UM culture bottle, AsOne, 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 0.1 mM 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±1°C under white florescent light (54 µmol/m^2.s) during 16 hrs photoperiods for four weeks. Experimental data were collected by counting the number of PLBs, number of shoots and their fresh weight were measured. The data were statistically analyzed by calculating standard errors of the means (means ± SE) and significant differences assessed by Tukey HSD test ($p \leq 0.05$).

To examine the effects on organogenesis of PLBs of *Dendrobium kingianum,* where PLBs treated with different concentrations of ethrel (0, 1.0, and 10.0 mg/L) alone in different time (0, 30, 60, 90, 120 min) were transferred to the basal medium. The best response for producing the highest number of PLBs was recorded for the medium containing 10 mg/l ethrel (17.7 per explant). PLBs dipped in 30 min, and the highest number of shoot was found (2.1) in same treatment. While the number of PLBs was lowest in the medium containing 10 mg/l ethrel in 120 min (8.9 per explant).

The highest number of shoots (2.1) was recorded in the medium containing 0.1 mg/l BAP combined with 1 mg/l ethrel and was significantly different with other treatments. The lowest rate of shoot formation from PLBs was found (0.5) in 0.1 mg/l BAP + 10 mg/l ethrel medium. The percentage of shoot formation is higher in BAP and ethrel and their combination at low concentration.

There was significant interaction between BAP and ethrel combinations in the culture medium on the multiplication and growth on the number of PLBs and fresh weight. The highest number of PLBs (13.1) was recorded in the

Table 1. Effects of Ethrel(time) on PLBs of *Dendrobium kingianum*.

Ethrel (mg/l)	Time (min)	No. of PLBs	No. of shoot	Fresh weight (gm)
0	0	11	1.0	0.142
1	0	11.3	1.0	0.116
1	30	14.1	1.5	0.129
1	60	12.9	0.6	0.076
1	90	15.4	1.2	0.126
1	120	9.5	0.5	0.084
10	0	9.2	1.1	0.070
10	30	17.7	2.1	0.265
10	60	11.9	1.1	0.103
10	90	9.9	0.9	0.123
10	120	8.9	0.9	0.102

Table 2. Effect of BAP and ethrel on rate of PLBs formation and their fresh weight of *Dendrobium kingianum*.

Treatment (mg/l) BAP	Ethrel	Average No. of PLBs	Fresh weight (g)	Average No. of Shoots
0	0	8.1 ± 0.9 ^{b}	0.094 ± 0 .01 ^{ab}	1.3 ± 0 .49 ^{a}
0.1	0	9.4 ± 1.1^{ab}	0.104 ± 0 .01^{a}	1 ± 0.26 ^{a}
1	0	8.7 ± 1.2 ^{b}	0.096 ± 0 .01 ^{ab}	1.1 ± 1.2^{a}
10	0	6.5 ± 0.8^{b}	0.076 ± 0 .01^{b}	1.5 ± 0.0 ^{a}
0	1	11.2 ± 1.3 ^{a}	0.132 ± 0.02^{a}	1.9 ± 0.4 ^{a}
0.1	1	13.1 ± 2.1 ^{a}	0.148 ± 0.03 ^{a}	2.1 ± 0.7 ^{a}
1	1	5.7 ± 0.5 ^{b}	0.066 ± 0.01 ^{b}	0.6 ± 0.3 ^{b}
10	1	7.6 ± 0.7 ^{b}	0.073 ± 0.01 ^{b}	0.7 ± 0.3 ^{b}
0	10	10 ± 1.9^{a}	0.099 ± 0.03 ^{a}	1.7 ± 0.6 ^{a}
0.1	10	9.3 ± 1.1 ^{ab}	0.093 ± 0.01 ^{ab}	0.7 ± 0.2 ^{b}
1	10	7.7 ± 0.8 ^{b}	0.079 ± 0.01 ^{b}	0.5 ± 0.2 ^{b}
10	10	8.3 ± 0.8 ^{b}	0.078 ± 0.01 ^{b}	0.9 ± 0.3 ^{ab}

Values represent mean ± SE followed by the different superscript letters show significant differences at $p \leq 0.05$ by Tukey HSD test. Average number of PLBs: number of PLB/one PLB explants.

medium containing 0.1 mg/l BAP combined with 1 mg/l ethrel and was significantly different with control (8.1) and other treatments. Increase in fresh weight showed higher values in same combination. Whereas lowest number of

PLBs (5.7) indicated on the medium supplemented with 1 mg/l BAP and PLBs treated with 1 mg/l ethrel. Low concentration of BAP alone increase the number of PLBs. On the other hand low concentration of ethrel alone increased both number of PLBs and fresh weight after 4 weeks.

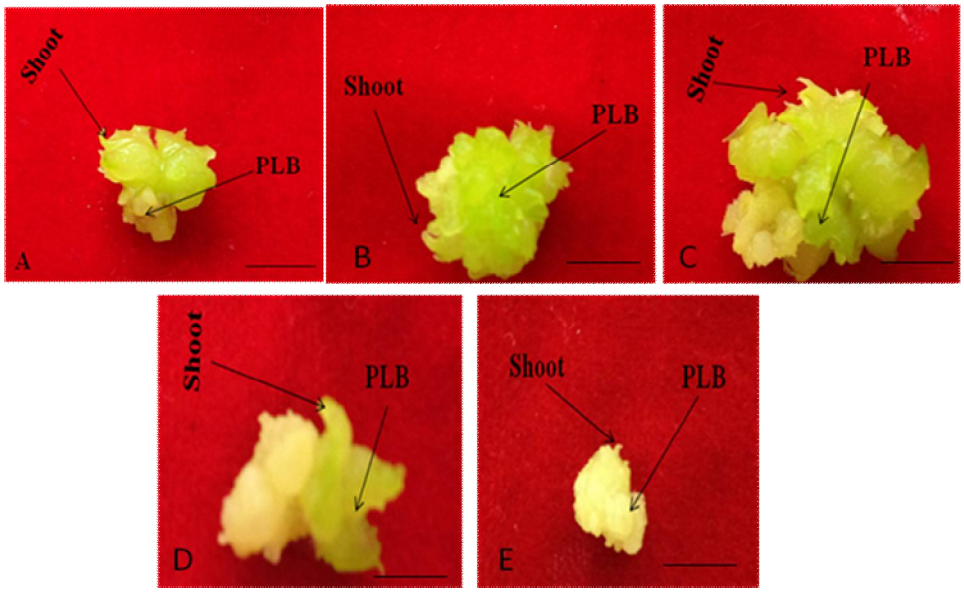

Fig. 1. Effect of BAP and ethrel on organogenesis in PLB cultures of *D. kingianum.* A: BAP 0 mg/l + ethrel 0 mg/l, B: BAP 0 mg/l + ethrel 1 mg/l, C: BAP 0.1 mg/l + ethrel 1 mg/l , D: BAP 1 mg/l+ ethrel 10 mg/l, E: BAP 10 mg/l + ethre1 l0 mg/l. Bars: 1cm.

BAP is widely used for micropropagation of orchids because of its ability to induce organogenesis. It can be postulated that, BAP at low concentrations promote the activity of ethrel by PLB cultures. This result indicates that there is a synergistic effect between BAP and ethrel with respect to growth regulation. The effect of ethylene on *in vitro* morphogenesis, as with other phytohormones, depends on its concentration in and around the cultured tissues , as well as their sensitivity to it (Thorpe 1994) There is often a critical ethylene concentrations at which morphogenesis is affected, concentrations above or below this level being inhibitory or ineffective, respectively (Hughes 1981, Biddington 1992). Successful *in vitro* regeneration nearly always depends on the use of phytohormones and most effects of ethylene on tissue cultures might be said to involve some interaction with phytohormone. Ethylene stimulates *in vitro* bulblet formation in tulip (Alderson et al. 1986). In Crocus, it causes corm formation synergistically with cytokinins (Plessner et al. 1990). BAP and ethrel combinations than lower concentrations. Low concentrations of ethrel stimulate

BAP activity but its high concentrations (10 mg/l) drastically reduce the activity of BAP resulting lower rate of shoot formation. In tomato cotyledonary explants, shoot formation was also enhanced by ethylene concentrations, within limits, while excessive ethylene application reduced shoot differentiation (Mebsuali-Sodi et al. 1990). In an early study, Mac-Kenzie and Street (1970) found no significant change in the final cell number in sycamore cell suspension cultures when ethrel was added up to 10 µM. However, higher concentrations of ethrel inhibited growth and caused cell lysis. Ethylene enhances organogenesis and organ development *in vitro* culture system.

The result of this experiment concluded that low concentrations of BAP and ethrel has regulatory effect on organogenesis of PLBs *Dendrobim kingianum* in every aspects of growth and development terms. More research is needed to understand the diverse ways in which concentrations of ethylene and phytohormones has been reported to influence *in vitro* growth and development of PLBs of *Dendrobim kingianum*.

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