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Effects of Plant Growth Regulators on Callus Proliferation, Plantlet Regeneration and Growth of Plantlets of *Doritaenopsis* Orchid

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Abstract: Supplement of 2,4-D and BAP into New *Phalaenopsis* (NP) medium was not effective for the growth of callus in *Doritaenopsis* orchid. Among the different combinations of BAP and NAA, 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA highly enhanced PLB formation from calli. For plantlet initiation from PLBs 0.5 mg l⁻¹ BAP was suitable and higher concentration was inhibitory. Similar effect to that of BAP was found for NAA (0.5 mg l⁻¹) and BAP + NAA (0.5 mg l⁻¹ + 0.5 mg l⁻¹) in plantlet initiation. For *in vitro* growth of plantlets, 0.5 mg l⁻¹ of BAP was the best for leaf and shoot growth compared to that of for BAP and NAA. However, for root growth of plantlets 1.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ NAA was the most suitable.

Key words: Callus growth, plantlet regeneration, growth of plantlet, *Doritaenopsis* Orchid, plant growth regulator

Introduction

Doritaenopsis is one of the most popular orchid genera which is an intergeneric hybrid of *Phalaenopsis*. It is an important ornamental plant. Recently, it has been increasing greatly in many countries, specially in developed temperate countries of the world as a pot plant or cut flower. Orchids account for nearly 7% of the total flowering plant species and have been increasing immensely to the international trade in floriculture. Rapid growth, easiness in plantlet regeneration, year round production under control flowering, beauty of flower and long lasting of flower stalk are the advantages in orchids.

Many studies on micropropagation of orchids have been carried out (Fu, 1978; Lin, 1986; Tanaka, 1987; Kobayashi *et al.*, 1991; Ichihashi, 1992). However, there is no practical and economical method of producing plantlets within a short period in *Doritaenopsis*. A feasible propagation method is through callus derived plantlet regeneration (Sagawa, 1990a; Islam and Ichihashi, 1999). If callus could be induced readily, remains stable genetically, grows quickly without changing its nature, produces PLBs easily and finally develops to plantlets it could be an useful method for commercial micropropagation of *Doritaenopsis*.

Reports on callus derived plantlet regeneration and their subsequent growth of *Doritaenopsis* affected with plant growth regulators are not available. Hence, the present

experiment was conducted to investigate the effects of different plant growth regulators on callus growth, PLB initiation from callus, plantlet regeneration and the subsequent growth of plantlets in *Doritaenopsis*.

Materials and Methods

Preparation of callus

Embryogenic calli of *Doritaenopsis* which were developed earlier by culturing flower stalk were maintained by subculturing monthly on NP medium supplemented with 20 g l⁻¹ of sucrose. At the beginning, 1.0 g of source callus was subcultured twice on 20 ml of NP medium supplemented with 20 g l⁻¹ of sucrose in 50 ml flasks at monthly intervals to obtain homogeneous callus. Uniform, friable, translucent, yellowish callus was used as plant material.

Callus culture

Two cultures were performed in callus culture, the first culture (1st 8 weeks) and second culture (2nd 8 weeks) have been indicated as 8 weeks culture and 8 weeks × 2 culture respectively in the subsequent results and discussion. In the first culture, 0.2 g of homogeneous callus was cultured on 20 ml of NP medium supplemented with 2,4-D + BAP for 8 weeks to evaluate their effects on callus growth. For the second culture, 0.2 g of proliferated callus obtained from the first culture was cultured again on the same fresh medium for another 8 weeks with same treatments. In separate cultures, effects of NAA and BAP and PLB initiation from callus were investigated.

Culture of PLB

PLBs obtained from second culture of callus were cultured on 20 ml NP medium supplemented with BAP and NAA for 10 weeks to investigate their effects on plantlet initiation from PLBs. Larger than 2 mm sized, uniform 8 PLBs were used in culture in each flask.

Culture of plantlets

Mini plantlets regenerated from PLBs were culture for 12 weeks on 20 ml NP medium with same supplements used in PLB culture to evaluate their effects on growth of plantlets.

Media and culture condition

The basal medium (BM) was New *Phalaenopsis* (NP) medium (Ichihashi, 1992). Sucrose 20 g l⁻¹ and gelrite 3 g l⁻¹ were added and the pH was adjusted at 5.6 prior to autoclaving. After dissolving the solidifier, 20 ml of hot medium was dispensed into 100 ml conical flasks. The conical flasks containing the medium were autoclaved at 121°C with 1.16 kg cm⁻² of pressure for 20 minutes. After autoclaving the flasks containing the medium were allowed to cool and explants were cultured. Cultures were maintained in a growth room and allowed to grow at 25±1°C under 16 h photoperiod of 50 μ mol m⁻²s⁻¹ illuminated with fluorescent tubes.

Culture evaluation

After each culture, proliferated callus or PLB were weighed and the number of initiated

PLBs was counted. Data were also recorded on different parameters to estimate growth of plantlets.

Results and Discussion

Growth of callus on media supplemented with different plant growth regulators (PGRs)

In first culture, after 8 weeks, calli on 2,4-D and BAP containing medium remained friable with yellow to green colour (Fig. 1). Different concentration of 2,4-D and BAP in combine did not enhance callus growth. Among three combinations, 1.0 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ BAP allowed callus growth similar to that of control (Fig. 2) where other combinations inhibited callus growth. Rate of callus growth was also similar to that of control (0.23 g d⁻¹ g⁻¹) at 1.0 mg l⁻¹ 2,4-D+0.1 mg l⁻¹ BAP (Fig. 3). Similar growth trend of callus was also found in 8 weeks × 2 culture (Fig. 3 and 4). Different combination specially high auxin and low cytokinin might yield different result and is subjected to further research

Effects of different PGRs on PLB initiation from callus

Different concentration of BAP in combine to NAA affected PLB initiation from callus. In first culture, 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA greatly enhanced PLB formation. This finding agreed with the results of Lin (1986) and Kusumoto (1979). The highest number of PLB (18.33 PLBs/flask) was found at 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA and no PLB was found in control (Table 1). The highest fresh weight of PLB (0.116 g) and fresh weight per PLB (0.007 g) were also recorded at 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA and 3.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA, respectively. Callus PLB ratio was significantly higher (17.1) at 1.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA compared to other treatments (Table 1).

After 8 weeks × 2 culture, the highest and the lowest number of PLB (23.67 and 6.67 PLBs/flask) were recorded at 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA and 1.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA respectively. The fresh weight of PLBs was markedly enhanced with 5.0 mg l⁻¹ BAP+0.1 mg l⁻¹ NAA than other treatments. The highest and lowest callus, PLB ratio were observed at 1.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA and 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA, respectively (Table 1).

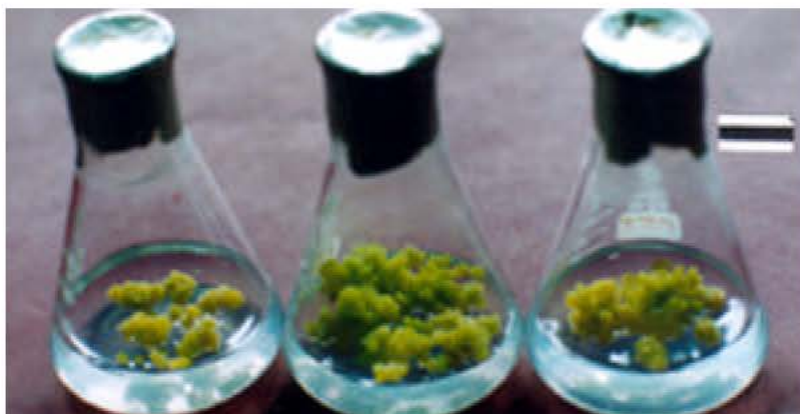


Fig. 1: Showing proliferated callus on the NP medium supplemented with (a) 0.5 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ BAP, (b) 1.0 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ BAP and (c) 1.5 mg l⁻¹ 2,4-D + 0.1 mg l⁻¹ BAP after 8 weeks culture. Scale = 1.0 cm

Table 1: Effects of BAP and NAA on PLB initiation from calli after 8 weeks and 8 weeks × 2 culture

*Concentration of PGRs into BM	Weight of callus explanted (g)	After 8 weeks culture				After 8 weeks × 2 culture			
		No. of PLB	Fresh wt. of PLB (g)	Fresh wt. per PLB (g)	Callus PLB ratio	No. of PLB	Fresh wt. of PLB (g)	Fresh wt. per PLB (g)	Callus PLB ratio
Control (no PGR)	0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.0 mg l ⁻¹ BAP+0.1 mg l ⁻¹ NAA	0.2	5.00	0.027	0.005	17.10	6.66	0.045	0.006	14.38
3.0 mg l ⁻¹ BAP+0.1 mg l ⁻¹ NAA	0.2	13.33	0.098	0.007	5.87	16.00	0.130	0.007	5.48
5.0 mg l ⁻¹ BAP+0.1 mg l ⁻¹ NAA	0.2	18.33	0.116	0.006	4.70	23.67	0.162	0.006	4.50
LSD at 5%		4.58	0.019	0.006	3.63	6.36	0.019	0.006	2.90

Each treatment had 6 replications

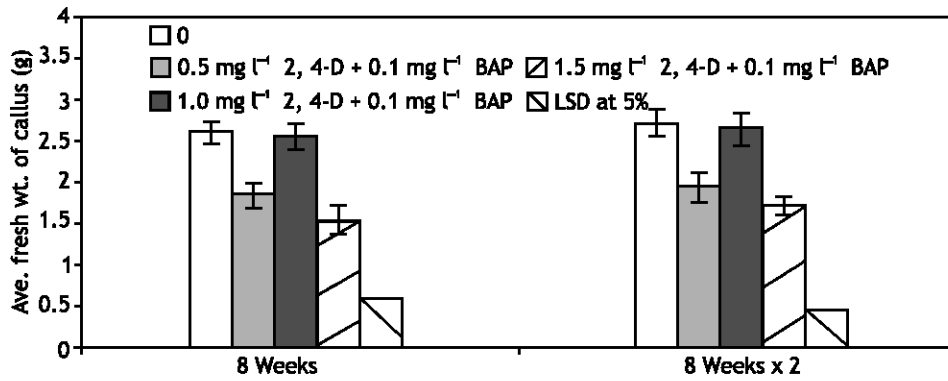


Fig. 2: Effects of different plant growth regulators on callus growth of *Doritaenopsis*. Bars indicate standard deviations

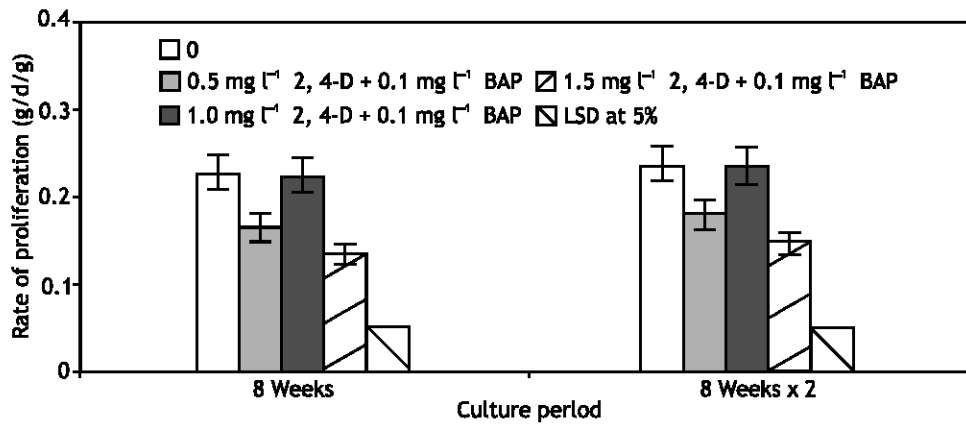


Fig. 3: Effects of different plant growth regulators on rate of callus growth of *Doritaenopsis*. Bars indicate standard deviations

Table 2: Effects of BAP and NAA on the growth of *Doritaenopsis* mini plantlets after 12 weeks culture

*Concentration of PGRs into BM	No. of leaf/plantlet	Length of leaf (cm)	Width of leaf (cm)	Length of shoot (g)	Fresh wt. of shoot (g)	Dry wt. of shoot (g)
Control (no PGR)	5.0	1.25	0.50	1.55	0.092	0.004
0.5 mg l ⁻¹ BAP	6.0	2.25	0.85	2.55	0.352	0.015
1.0 mg l ⁻¹ BAP	5.5	1.35	0.60	2.15	0.126	0.006
2.0 mg l ⁻¹ BAP	3.5	1.60	0.60	2.15	0.085	0.004
0.5 mg l ⁻¹ NAA	4.5	1.55	0.60	2.15	0.091	0.004
1.0 mg l ⁻¹ NAA	4.5	1.45	0.53	2.15	0.148	0.008
2.0 mg l ⁻¹ NAA	4.0	1.25	0.50	2.15	0.119	0.006
0.5 mg l ⁻¹ BAP+	4.0	1.45	0.79	2.15	0.099	0.005
0.5 mg l ⁻¹ NAA						
1.0 mg l ⁻¹ BAP+	5.0	2.05	0.75	1.95	0.107	0.005
1.0 mg l ⁻¹ NAA						
2.0 mg l ⁻¹ BAP+	6.0	1.30	0.80	2.65	0.214	0.010
2.0 mg l ⁻¹ NAA						
LSD at 5%	0.762	0.323	0.132	1.95	0.054	0.017

Table 2: Continue

*Concentration of PGRs into BM	No. of root per plantlet	Length of root (cm)	Diameter of root (mm)	Fresh wt. of root (g)	Dry wt. of root (g)	Root-shoot ratio (freshwt.)
Control (no PGR)	3.0	1.35	1.90	0.047	0.003	0.514
0.5 mg l ⁻¹ BAP	3.0	1.45	3.00	0.076	0.006	0.220
1.0 mg l ⁻¹ BAP	3.0	1.25	3.00	0.069	0.005	0.549
2.0 mg l ⁻¹ BAP	2.0	0.95	2.50	0.021	0.002	0.246
0.5 mg l ⁻¹ NAA	3.5	3.25	3.00	0.139	0.007	1.530
1.0 mg l ⁻¹ NAA	3.5	1.60	3.00	0.092	0.006	0.621
2.0 mg l ⁻¹ NAA	2.5	1.45	2.50	0.074	0.006	0.621
0.5 mg l ⁻¹ BAP+	2.5	3.00	2.75	0.190	0.009	1.790
0.5 mg l ⁻¹ NAA						
1.0 mg l ⁻¹ BAP+	3.0	3.50	2.75	0.203	0.010	2.040
1.0 mg l ⁻¹ NAA						
2.0 mg l ⁻¹ BAP+	40	2.30	2.50	0.163	0.008	0.760
2.0 mg l ⁻¹ NAA						
LSD at 5%	0.539	0.353	0.508	0.017	0.017	0.054

Each treatment had 6 replications

Effects of different plant growth regulators on plantlet regeneration from PLBs

The BAP supplemented medium at 0.5, 1.0 and 2.0 mg l⁻¹ initiated plantlets from PLB to 66.63, 62.5 and 50% respectively which was significantly higher over control (Fig. 4). The best effect was observed with the lowest level of BAP. The present findings agreed with the report of Vij *et al.* (1994). They showed that cytokinins favoured plantlet regeneration via formation of protocorm like bodies in *Vanda Kaseem's Delight* "Tom Boykin".

The NAA in medium significantly stimulated plantlet initiation but was less effective than BAP. The highest plantlet initiation (58.25%) was found with 0.5 mg l⁻¹ NAA where the higher concentration found inhibitory (Fig. 4). However, the plantlet initiation was higher in NAA supplemented medium than that of control.

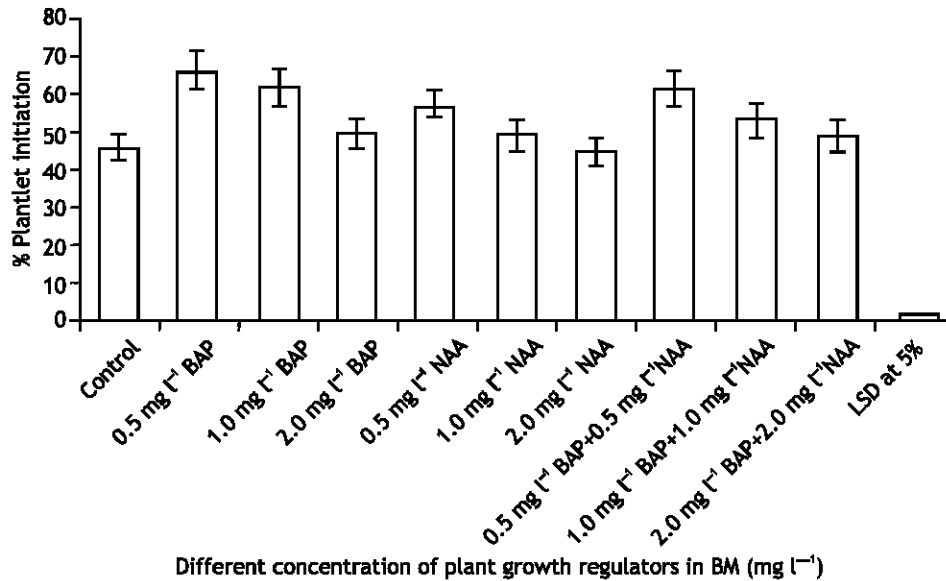


Fig. 4: Effects of different plant growth regulators on plantlet initiation from PLB after 10 weeks culture

The BAP and NAA in combine supplemented into medium were almost similar to that of BAP alone. In this case, the highest plantlet initiation (62.50%) was recorded at 0.5 mg l⁻¹ BAP+0.5 mg l⁻¹ NAA (Fig. 4). This result also revealed that lower concentration of BAP with lower concentration of NAA promoted plantlet initiation than higher concentration. These findings agree with the results of Kukulczanta *et al.* (1989), who reported that plantlet regeneration and organogenesis in Cambria orchid were greatest on MS medium containing 0.2 ppm BA, 0.2 ppm NAA and 1000 ppm peptone.

From the above findings, it might be concluded that only lower level of BAP (0.5 mg l⁻¹) might be recommended for plantlet regeneration from PLB in comparison to that of NAA as well as BAP and NAA in combine.

Effects of different PGRs on the subsequent growth of mini plantlets

The BAP and NAA supplemented NP medium affected the subsequent growth of mini plantlets differently. Number of leaf/plantlet was significantly influenced at lower level of BAP (0.5 mg l⁻¹) and higher level of BAP + NAA in combine (2.0 mg l⁻¹ BAP + 2.0 mg l⁻¹ NAA) than other treatments. Similar effect was found in width of leaf (Table 2; Fig. 5).

The BAP (0.5 mg l⁻¹) and BAP + NAA (1.0 mg l⁻¹ of each) supplemented BM greatly enhanced length of leaf than that of control and other treatments. Length of shoot was also promoted like length of leaf (Table 2). The highest fresh weight and dry weight of shoot was obtained at 0.5 mg l⁻¹ BAP over other treatments. The lowest fresh and dry weight of shoot was found at high level of BAP and at low level of NAA.

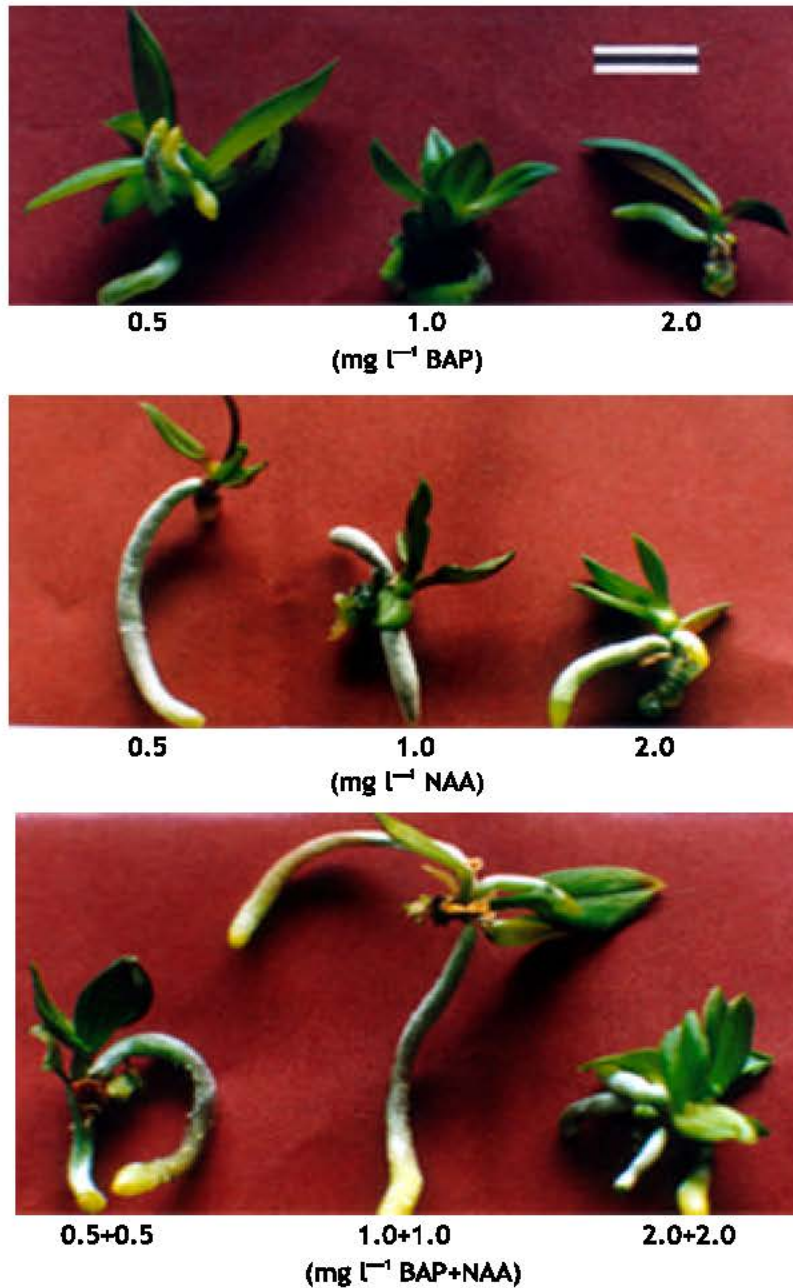


Fig. 5: Showing the growth of *Doritaenopsis* plantlets *in vitro* on NP medium supplemented with (a) BAP, (b) NAA and © BAP and NAA in combine after 12 weeks culture. Scale = 1.0 cm

Number of root/plantlet was markedly enhanced at 2.0 mg l⁻¹ BAP + 2.0 mg l⁻¹ NAA where only 2.0 mg l⁻¹ BAP supplement significantly decreased root number over control. Lower levels (0.5 - 1.0 mg l⁻¹) of BAP and NAA were supported root diameter. The highest length of root as

well as highest fresh and dry weight of root were found at 1.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ NAA and their lowest values were obtained at 2.0 mg l⁻¹ BAP. Root growth was supported at 1.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ NAA than shoot but 0.5 mg l⁻¹ BAP enhanced shoot growth over root (Table 2; Fig. 5).

It might be conclude that supplement of PGRs into BM is not effective for callus growth of *Doritaenopsis*. For PLB production from callus, 5.0 mg l⁻¹ BAP + 0.1 mg l⁻¹ NAA is suitable. Plantlets is effective at 0.5 ml⁻¹ of BAP for the subsequent growth of plantlets *in vitro* 0.5 mg l⁻¹ BAP or 1.0 mg l⁻¹ BAP + 1.0 mg l⁻¹ NAA to be supplemented into BM regeneration from PLB.

Addition of PGRs into culture medium is apparently effective for the growth of explants. In callus growth of *Doritaenopsis*, PGRs were not promotive for the proliferation of callus but inhibitory. However, these were enhancing for the growth of PLBs and plantlets.

The present study indicated that, in callus derived PLB and plantlet regeneration stages, different supplement of auxin and cytokinin is needed for the growth of explants. Requirement of PGR supplement during *in vitro* regeneration may vary among different species. Therefore, in callus growth and subsequent plantlet regeneration, selection of the PGRs and their concentration are very important. In callus derived plantlet regeneration somaclonal variation in plantlets is a major concern. Supplement of PGR will enhance the risk of mutation. Low concentration of PGR might reduce the risk. Further research is required to estimate the risk of mutation in callus derived plantlet regeneration.

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