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5-Aminolevulinic Acid Regulates Growth and Development of Protocorm-like Bodies(PLBs) in *Dendrobium kingianum* Cultured *In Vitro*

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Abstract: This study was undertaken to investigate the effects of different chemicals on *in vitro* regulation of organogenesis of tissue culture of *Dendrobium kingianum*. PLBs of *D. kingianum* were explanted on modified MS medium supplemented with various concentration of potassium salt of 1-Napthalene acetic acid (K-NAA), 5-aminolevulinic acid(5-ALA) and N-acetylglucosamine (NAG). The results indicated that the application of K-NAA and NAG at low concentration (0.1 g/L) increased average number of PLBs, shoots and their fresh weight. In case of 5-ALA, PLBs formation rate was 100% at every concentration of 5-ALA and the highest number of PLBs (21.4) was recorded at concentration of 0.1mg/L. While the highest number of shoots per explant (4.0) and the percentage of shoot formation (86.7) were observed at concentration of 1mg/L 5-ALA. A comparative study of effective K-NAA, 5-ALA and NAG found that 5-aminolevulinic acid was the best to enhance both the PLBs induction and shoot formation of *Dendrobium kingianum.*

Key words: Potassium salt (K-NAA) · 5-aminolevulinic acid (5-ALA) · N-Acetylglucosamine (NAG) · Protocorm- like body (PLB) · *Dendrobium kingianum*

INTRODUCTION

The Orchidaceae family has 850 genera, 35,000 species and considered as the largest family of plants and including 7-11 % of flowering plants[1, 2]. Exquisite and perpetual flowers having diverse shapes, forms and colors make them doyen among ornamental plants [3]. Orchids are marketed as the cut flowers as well as a potted floriculture crop in all over the world. Orchids rank second among the top selling cut flowers in the world. Among the various orchid genera, *Dendrobium* is the most popular cut flower orchid in the international orchid trade. *Dendrobium kingianum* is a neat, compact, attractive plant, even when not in bloom and it is easy to grow and rewards one with an array of beautiful, long lasting, fragrant flowers.

1-Naphthaleneacetic Acid, Potassium Salt (K-NAA) has a molecular formula of $C_{12}H_9KO_2$, a molar mass of 224.3 g/mol. K-NAA is a synthetic plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products; it is a rooting agent and used for the vegetative propagation of plants. It is also used for plant tissue culture. N-Acetylglucosamine (*N*-acetyl-D-glucosamine, or GlcNAc, or NAG) is a monosaccharide derivative of glucose. It is an amide between glucosamine and acetic acid. It has a molecular formula of $C_8H_{15}NO_6$, a molar mass of 221.21 g/mol and it is significant in several biological systems. In addition, new studies have reported the effects of chitosan derivatives, N-acetyle-glucosamine on plant growth [4] and in tissue culture on Epidendrum "Rouge Star No.8" [5]. 5-aminolevulinic acid acid (dALA or δ-ALA or

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5-ALA) is the first compound in the porphyrin synthesis pathway, the pathway that leads to heme in mammals and chlorophyll in plants. In plants, production of δ-ALA is the step on which the speed of synthesis of chlorophyll is regulated. Plants that are fed by external δ-ALA accumulate toxic amounts of chlorophyll precursor, protochlorophyllide, indicating that the synthesis of this intermediate is not suppressed anywhere downwards in the chain of reaction. Protochlorophyllide is a strong photosensitizer in plants. ALA application increased the yield of garlic, barely, rice and potato plants by significantly enhancing the photosynthetic capacity and plant biomass [6]. ALA has been also reported to be effective in improving plant growth and crop yields as well as carbon and nitrogen fixing processes [7].

The role of K-NAA. 5-ALA and NAG on growth and development of PLBs in *Dendrobium* have not been so far studied. Therefore, the purpose of this study to investigate the effect of exogenous application of these chemicals on organogenesis of protocorm-like bodies of *in vitro* cultured *Dendrobium kingianum*.

MATERIALS AND METHODS

Plant Material and Explants Source: PLBs of *Dendrobium kingianum* were proliferated in the modified Murashige and Skoog [8] medium by transferring to a new medium. After excision of PLB into singles, they were used for explants.

Culture Medium and Culture Conditions: MS medium supplemented with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20gm/L sucrose and 2.2 gm/L phytagel (Sigma) were used as a culture medium. Potassium salt(K-NAA; Sigma, USA) at concentrations of 0, 0.1, 1, 10 mg/L, N-acetyl-glucosamine (NAG) and 5-aminolevulinic acid (ALA) at concentrations of 0, 0.01 0.1, 1, 10 mg/L added to culture media before sterilization. Five explants cultured in one vessel and three vessels were used for each treatment. 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 ±1°C under white light emitting diodes during 16 h photoperiods for 30 days.

Data Analysis: 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 = 0.05).

RESULTS AND DISCUSSION

Effect of K-NAA on Organogenesis in PLBs of *Dendrobium Kingianum:* The effect of K-NAA with different concentrations on organogenesis in PLB cultures of *Dendrobium kingianum* are shown in Table 1. The highest number of PLBs per explant (18.3) and 100% PLBs formation rate was recorded MS medium supplemented with 0.1 mg/L K-NAA and showed significantly different with control and high concentration of K-NAA treatment. The maximum percentage of shoot formation rate (80.0) was recorded in control treatment, whereas the highest fresh weight (0.287g) after 30 days was observed at concentration of 0.1mg/L of K-NAA treatment compared with other treatments.

Effect of 5-Aminolevulinic Acid (5-ALA)on Organogenesis in PLBs of *Dendrobium Kingianum:* The 5-aminolevulinic acid at every concentration enhanced the growth of PLBs and shoot formation when compared to control as shown in Table 2. 5-ALA clearly

Table 1: Effect of K-NAAon organogenesis in PLBs of *Dendrobium kingianum*

	PLB		Shoot		
Treatment (mg/L)	No.	Rate (%)	No.	Rate (%)	Fresh Weight (gm)
Control	11.1±0.6b	86.7	2.5±0.1a	80.0	0.257±0.01a
0.1	18.3±0.7a	100	1.5±0.1a	73.3	0.287±0.01a
1	7.9 ±0.3b	93.3	0.4±0.3b	20.0	0.082±0.00b
10	2.7 ±0.1c	73.3	0.0±0.0b	00.0	0.041±0.00b

Values represent means ±SE followed by the different superscript letters show significant differences by Tukey HSD test (P□0.05)

Table 2: Effect of 5-ALA on organogenesis in PLBs of *Dendrobium kingianum*

	PLB		Shoot		
Treatment (mg/L)	No.	Rate (%)	No.	Rate (%)	Fresh Weight (gm)
Control	11.1±0.6b	86.7	2.5±0.1a	80.0	0.257±0.01b
0.01	20.9±0.5a	100	2.1±0.1a	66.7	0.393±0.01a
0.1	21.4±0.7a	100	0.7±0.1b	53.3	0.292±0.01a
1	17.9±0.6a	100	4.0±0.2a	86.7	0.312±0.01a
10	14.1±0.5b	100	2.6±0.2a	53.3	0.306±0.00a

Values represent means ±SE followed by the different superscript letters show significant differences by Tukey HSD test (P□0.05)

Table 3: Effect of NAG on organogenesis in PLBs of *Dendrobium kingianum*

	PLB		Shoot		
Treatment (mg/L)	No.	Rate(%)	No.	Rate(%)	Fresh Weight (gm)
Control	11.1±0.6a	86.7	2.5±0.1b	80.0	0.257±0.01a
0.01	13.2±0.6a	100	3.4±0.2a	73.3	0.262±0.01a
0.1	14.7±0.5a	100	5.1±0.2a	93.3	0.335±0.01a
1	12.5±0.6a	93.3	4.9±0.2a	86.7	0.236±0.01a
10	12.3±0.5a	93.3	3.8±0.2a	80.0	0.297±0.00a

Values represent means ±SE followed by the different superscript letters show significant differences by Tukey HSD test (P□0.05)

stimulated PLBs growth and induced shoot formation. The most effective concentration in terms of the average number of PLBs, PLBs induction rate, average number of shoot, shoot formation rate and fresh weight was 0.1mg/L compared to the control. The highest number of PLBs per explant (21.4) was recorded MS medium supplemented with 0.1 mg/L 5-ALA and showed significantly different with control and high concentration of 5-ALA treatment.. The maximum percentage of shoot formation rate (86.7) and highest fresh weight (0.393g) was observed with concentration of 1mg/L and 0.01mg/L 5-ALA treatment which significantly different with other treatment. In case of 5-ALA, the percentage of PLBs formation rate was 100% in every concentration compared with control.

Effect of N-acetyl-glucosamine (NAG) on organogenesis in PLBs of *Dendrobium kingianum:* The N-acetyl-glucosamine (NAG) at every concentration enhanced the growth of PLBs and shoot formation compared to control as shown in Table 3. NAG clearly stimulated PLBs growth and induced shoot formation.The most effective concentration in terms of the average number of PLBs, PLBs induction rate, average number of shoot, shoot formation rate and fresh weight was 0.1mg/L compared to the control. Results showed that the highest PLBs induction rate (100%) was found at 0.01mg/l and 0.1mg/L NAG, whereas the lowest PLBs induction rate (86.7%) was found without NAG. Shoot formation rate was highest at concentration of 0.1 mg/L NAG (93.3%) and the lowest shoot formation was found without NAG (80%). The media supplemented with NAG at 0.1mg/L produced higher fresh weight of PLB. However, all the of four tested doses of NAG showed no significant effect on the average number of PLBs per explants.

DISCUSSION

This study showed that low concentration of these three chemicals regulates promotive activity and increased the average no. of PLBs and their percentage rate whereas at high concentrations reduce the rate of PLBs and their fresh weight of *Dendrobium kingianum* except in 5-ALA. In this study we used K-NAA as auxin was to determine the role on organogenesis of PLBs in *Dendrobium kingianum*. Generally K-NAA used as for rooting induction and several researches has done on root formation and acclimatization of plants using K-NAA. This study results showed that low concentration of K_NAA has promotive effect on PLBs formation but in case of shoot formation K-NAA showed negative impact at every concentration in PLBs of *D. kingianum*. On the other hand, low concentration of 5-ALA (0.1mg/ L) supplementation in culture media enhanced the highest number of PLBs and PLBs formation rate was 100% in every concentration of 5-ALA whereas highest formation of shoot was found at 1mg/L of 5-ALA of *Dendrobium kingianum in vitro*. ALA is a potential plant growth regulator in stress conditions, being an essential biosynthetic precursor of tetrapyrrole compounds such as heme, cytochromes and chlorophyll [9]. In plants ALA is synthesized from glutamate in a reaction involving a glutamyl-tRNA

intermediate and requiring ATP and NADPH as cofactors; its formation is the rate limiting step in chlorophyll biosynthesis [10, 11]. ALA is found in all plants and its concentration is regulated at low concentrations (60µmol) *in vivo* [9, 12]. Unfortunately, commercial ALA is too expensive for many common agricultural applications. Recently it was found that low concentrations of ALA had a promotive effect on growth and yield of several crops and vegetables [9, 13-15]. During 50 days of culture, there was no malformation observed in regenerated shoots. High concentration of exogenous ALA can be used as non-polluting, non-residual photosensitive herbicides; in low concentration, it can regulate plant growth and development, increase productivity and enhance plant resistance [15]. The present study also confirmed that ALA could work as plant growth regulator in *Dendrobium kingianum*. ALA has the ability to stimulate PLBs proliferation of *Dendrobium in vitro*. This research indicated that low concentration of 5-ALA enhanced the number of PLBs and comperatively high concentration enhanced shoot formation of *Dendrobium kingianum.* NAG also acts as plant growth stimulator like K-NAA and 5-ALA. Low concentration of NAG (0.01 & 0.1mg/ L) supplementation in culture media enhanced the highest formation of PLBs and shoot of *Dendrobium kingianum in vitro*. Similar results were found by the application of NAG at every concentration enhanced the growth of PLBs and shoot formation in *Epidendrum* 'Rough Star No. 8' and *Cymbidium dayanum in vitro* [5, 16]. Application of NAG at every concentration enhanced the growth of PLBs and shoot formation in *Dendrobium kingianum.* These findings indicated that if NAG added to culture media acts as plant growth stimulator to induce PLBs and shoot formation of *Dendrobium kingianum*. However, possibility of avoiding mutation of cultures in the presence of these chemicals should be confirmed for the establishment of micropropagation system in long span.

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 these three chemicals was effective on traits of organogenesis of *Dendrobium kingianum* and on the basis of results concluded that comparatively low concentration of K-NAA, 5-ALA and NAG were more effective to promote the organogenesis in PLB cultures of *Dendrobium kingianum* in vitro. The result of this experiment concluded that among all these three chemicals 5-ALA showed more regulatory effect on organogenesis of PLBs *Dendrobim kingianum* in evry 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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