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Effect of alginate on protocorm like bodies (PLBs) formation of *Dendrobium kingianum* cultivar

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ABSTRACT

Mass propagation provides an alternative means of satisfying the demand. Unfortunately, conventional propagation is slow and difficult, suggesting in vitro *methods for mass multiplication may be more appropriate. Supplement of plant growth regulators and biopolysaccarides modified MS medium was effective for the growth and development of protocorm like bodies (PLBs) of Dendrobium kingianum 'Hallelujah'. The results of this experiment revealed that the highest number of PLBs were found in the media containing 0.01mg/L of alginate and that was 17.2/explant whereas control (6.1/explant). The second maximum number of PLBs (16.9) was at concentrations of 0.01mg/L HA9. On the other hand, the high concentration of NAA; HA9 and Alginate showed negative results of PLBs formation in Dendrobium kingianum 'Hallelujah' except BAP. In the case of PLBs formation, 100% PLBs were formed in every low concentration of BAP, NAA and HA9 except alginate.*

Key Words: *Dendrobium, protocorm-like body (PLBs), 6-Benzylaminopurine (BAP), Naphthalene Acetic Acid (NAA), Hyaluronic Acid (HA9) and Sodium alginate.*

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I. Introduction

Orchids are the most beautiful and peculiar type of flower that is also one of the most diverse. The popularity of the orchid flower can be contributed to several factors. These factors include the fragrance of the flowers, their beauty, and their colorful blooms. There are more than 1,000 species of *Dendrobium* orchids and hybrids (Hagsater et al., 1996). They vary in size, bloom color, appearance, and growing requirements. For this reason, it is important to get total care information for a certain type of *Dendrobium* because the care can greatly vary depending on the type of orchid. Orchid growing has not all achieved the transition from a hobby to an industry in many areas. The main reason being that the conventional method of orchid propagation is extremely slow, and the number of propagules produced by these methods is low. Within a limited time, the tissue culture techniques provide a

solution for producing a large number of propagules. Orchid cultivation is one of the most economically significant global nursery industries constituting a multi-billion dollar industry (Hew, 1989; Goh and Kavaljian, 1989; Alam et al., 2002); now with the advent of biotechnology, most desirable and important plants can be cloned using tissue culture as occurs with *Dendrobium*, which accounts for about 80% of the total micropropagated tropical orchids, usually by protocorms (Griesbach, 2003; Saiprasad et al. 2004). PLB cell division arises from the outer cell layers inward, and the frequency of cell division gradually increased with culture time in BA or PGR-free media; frequency of anticlinal cell division was prevalent in the outer three cell layers, whereas periclinal cell division was higher in the inner cell layers (Fujii et al., 1999). Most orchid tissue cultures require PGRs for growth, callus or PLB formation, proliferation and development and even though some early attempts at tissue culture of orchids failed due to the lack of PGRs in the media, other studies involved the successful growth of orchids without the use of PGRs (Morel, 1960; Wimber, 1963). The most commonly used PGRs in orchid tissue culture are auxins and cytokinins. In this experiment, we used naphthalene acetic acid (NAA) as auxin and 6-benzylaminopurine (BAP) as cytokinin. NAA is a synthetic plant hormone in the auxin family and is an ingredient in many commercial plants rooting horticultural products; it is a rooting viceregent and used for the vegetative propagation of plants. It's also used for plant tissue culture. BAP is the first-generation synthetic cytokinin that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division (Siddiqui et al., 2011).

In this experiment, we used hyaluronic acid (HA9) and alginate compared with BAP and NAA. In 1934, Karl Meyer and John Palmer described a new polysaccharide isolated from bovine vitreous humor. They found that the substance contained a uronic acid and an aminosugar as named the polysaccharide hyaluronic acid from the hyaloid (vitreous) + uronic acid (Meyer and Palmer, 1934). Alginate is a natural polysaccharide that comprises 30 to 60% of brown algae (on dry weight basis). It is composed of two types of uronic acid: mannuronic acid unit (M) and guluronic acid unit (G). Alginate is known as a marine biopolymer and the use of this is attracting increasing attention as time progress. The chemical composition of alginates varies with the source of origin (algal species and tissue) and the season of harvest (Haug, 1964). As literature for the commercial propagation of Dendrobiums specifically is scarce, due to obvious reasons, a study was conducted to compare the efficiency of different growth regulators and medium supplements for the in vitro propagation of orchid *Dendrobium kingianum* 'Hallelujah' suitable for commercial exploitation.

II. Materials and Methods

Dendrobium kingianum 'Hallelujah' registered in 2014. It's a hybrid of Cherry Love x Millionaire. Prtocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah' were used for explants at the Laboratory of Floriculture & Vegetable Science, Faculty of Agriculture, Kochi University, Japan on April 2019. After PLBs were excised individually, each PLB was used as an ex-plant. Modified Murashige & Skoog 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) was used as a culture medium. BAP; NAA; HA9 and Alginate at concentrations of 0.01; 0.1; 1 and 10 mg/L were added separately to the culture media before sterilization. Jars of 250ml (UM culture bottle, as 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 121ºC for 15 min. Five explants cultured in one vessel and three vessels were used for each treatment. Cultures were maintained at 25±1ºC under white florescent light (54 $\mu molm^{-2}s^{-1}$) during 24 h photoperiods for 42 days. Experimental data were collected by counting the number of PLBs; number of shoots; number of roots and their fresh weight were measured.

III. Results

In this experiment, the effects of BAP, NAA, HA9 and sodium alginate on organogenesis of PLBs in *Dendrobium kingianum* 'Hallelujah' after six weeks were shown in Table 01, at different concentrations. In the case of BAP, the maximum growth rate of PLBs was found in high concentration and the average rate of PLBs was 15.7/explant and percentage was 100% in the media containing 10mg/L BAP compared with control (6.1). We did not find any effect on shoot and root formation using BAP in this experiment. On the other hand, 1mg/L of NAA produced the maximum number of

PLBs (14.9/explant) compared with other concentrations of NAA whereas the lowest number of PLBs found in 10mg/L NAA. The results of HA9 and sodium alginate showed that the highest number of PLBs (17.2/explant) were found in the media where PLBs were cultured in very low concentrations of Alginate and that was 0.01mg/L. PLBs formation rate was maximum (100%) (Figure 01) was found in same concentration. Same concentration of HA9 also produced a high number of PLBs (16.9/explant) compared with BAP and NAA. This experiment results showed that a high concentration of NAA, HA9 and Alginate reduced the number of PLBs and hada negative impact on their formation rate compared with BAP whereas high concentration of BAP (10mg/L) formed 100% of PLBs. Both HA9 and Alginate (Figure 02) in high concentration that means 10mg/L showed negative results on PLBs formation compared with control.

Table 01. Effect of NAA, BAP, hyaluronic acid and sodium alginate on organogenesis of PLBs in *Dendrobium kingianum* 'Hallelujah'.

Treatments(mg/L)		Avg. No. of PLBs	Avg. No. of shoots	Avg. No. of roots	Fresh weight (gm)
Control	0	6.1±0.2	0.07±0.02	0.13±0.05	0.1
BAP	0.01	10±0.3	0.07±0.02	----	0.3
	0.1	9.1±0.2	----	----	0.3
	1	8.5±0.3	----	----	0.3
	10	15.7±0.1	----	----	0.2
Alginate	0.01	17.2± 0.1	----	0.20±0.03	0.3
	0.1	8.9±0.2	0.13±0.03	0.56±0.04	0.2
	1	13.1±0.4	0.20±0.05	0.13±0.03	0.2
	10	4.6±0.3	----	----	0.1
HA9	0.01	16.9±0.5	----	----	0.3
	0.1	11.5±0.3	0.07±0.02	0.13±0.03	0.2
	1	3.5±0.2	----	----	0.07
	10	4.0±0.3	----	----	0.3
NAA	0.01	11.1±0.5	----	----	0.2
	0.1	8.2±0.3	----	----	0.1
	1	14.9±0.3	0.20±0.05	0.33±0.07	0.2
	10	5.3±0.3	----	----	0.09

Values represent means ± SE

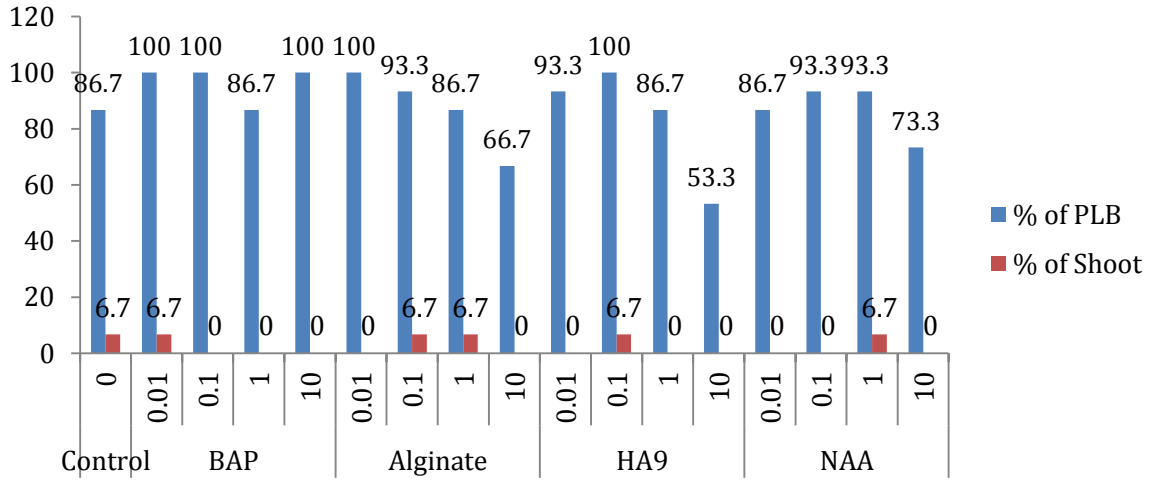

Figure 01. Effect of NAA, BAP, HA9 and sodium alginate on percentage of PLBs and shoot formation of *Dendrobium kingianum* 'Hallelujah'.

Percentage of PLB/shoot formation (%) = [(Number of cultured explants with new PLBs or shoots) / (Total number of cultured explants)] x 100.

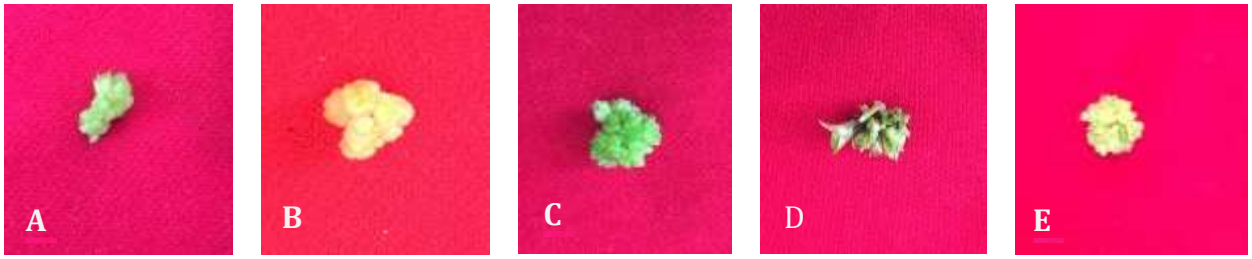

Figure 02. Effect of NAA, BAP, HA9 and sodium alginate PLBs of *Dendrobium kingianum* 'Hallelujah' A: Control; B: 0.01mg/L HA9; C: 0.01mg/l sodium alginate D: 1mg/L NAA and E: 10mg/L sodium alginate.

IV. Discussion

The application of auxin and cytokinin, especially NAA and BAP, resulted in variable patterns with the formation and proliferation of PLBs. Prasertsirivatna and Koolpluksee (2011) reported that the exogenous application of cytokinins did not affect the growth of *D. friedericksianum* since the growth of PLBs was slower than that in cytokinin added media. Alternatively, the high concentration of cytokinin inhibited PLB proliferation from leaf segments of Aranda Wan CharkKuan 'Blue' × V. coeruleaGrifft. ex. Lindl (Gantait and Sinniah, 2012). The results of this experiment showed opposite findings in the case of BAP on PLBs of *Dendrobium kingianum* 'Hallelujah'. Many have reported that PLBs formation was inhibited in the PGRs-free medium as observed Cymbidium (Pant and Pradhan, 2010), and Phalaenopsis (Niknejad et al., 2011) in which low concentrations ranging from 0.5 to 5 mg.L-1 were required for PLBs multiplications. (Mohanty et. al., 2012) reported that optimal concentrations of auxins (NAA or IBA) for PLB generation with yield decreasing at higher levels. We found the same results using NAA and his experiment also proved that PGRs improve the growth and development of PLBs in *Dendrobium*. The purpose of the present study was to see the effects of sodium alginate on organogenesis of PLB cultures in *Dendrobium kingianum*'Hallelujah' compared with PGRs and HA9. Hyaluronic acid has newly introduced growth chemicals in plant tissue culture. Its osmotic activity is non-ideal and disproportionately high with its molecular weight. For this and other reasons, it is capable of profound effects on the distribution and movement of water and plays a major part in water homeostasis (Comper and Laurent, 1978).

Application of HA9 resulted in significant promotion of PLBs and shoot formation, compared to the control phase and this new PLB formation occurred within a very short time. Hyaluronic acid shortens the adaptation period of cells on the material surface, and then cells enter the normal cell cycle quickly (Milelle et al., 2002). Recently, in orchid tissue culture HA worked as a plant growth regulator in *Cymbidium dayanum* (Nahar et al., 2012), *Cymbidium insigne and Cymbidium finlaysonianum* under white fluorescent light *in vitro* condition. Otherwise, hyaluronic acid shows high solubility in culture medium. Habiba et. al. (2014) revealed HA9 can be a new plant growth regulator like BA for *Dendrobium* species. We found the same result like low concentration of HA9 increases number of PLBs and formation rate was 100% on PLBs of *Dendrobium kingianum* 'Hallelujah'. On the other hand, the present study revealed the effects of another polysaccharide: Alginate. Habiba et al. (2017) firstly used these chemicals in orchid micropropagation and found a regulatory effect on *Dendrobium*. Actually, in plants, Alginate used as a soil fertilizer. It helps to improve the water-holding characteristics of soil and help the formation of crumb structure. They do this because the alginic acid in the seaweed combines with metallic radicals in the soil to form a polymer with greatly increased molecular weight, of the type known as cross-linked. Sodium alginate absorbs water quickly which makes it useful as an additive in dehydrated cells and tissues in plants. In the present experiment, we found very good impacts of sodium Alginate on PLBs in *Dendrobium kingianum* 'Hallelujah' because our study showed that very low concentrations of sodium alginate with modified MS medium produced maximum number of PLBs within a short period. The present result revealed that like PGRs and newly declared plant growth regulator of HA9 if sodium alginate added to modified MS media in low concentration, it acts as a plant growth regulator to induce PLBs formation.

V. Conclusion

From the above results of this experiment, we found that high concentration of commonly used plant growth regulators (BAP and NAA) increased the number of PLBs in *Dendrobium kingianum* 'Hallelujah'. On the other hand, low concentration of HA9 and Alginate showed regulatory effects on organogenesis of this orchid. More research is needed to understand the response of alginate on PLBs in *Dendrobium kingianum* 'Hallelujah'.

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