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Effect of Hyaluronic Acid (HA) on Organogenesis in Protocorm-Like Bodies(PLBs) of *Phalaenopsis* 'Fmk02010' Cultured *in vitro*

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Abstract: *Phalaenopsis* orchids have high economic value in the floriculture industry as cut flowers and even more as potted plants. The aim of this study is to integrate findings on the regulatory function of Hyaluronic acid (HA) on growth and development of protocorm-like bodies(PLBs) in *Phalaenopsis* 'Fmk02010'. Results of this study revealed that low concentration of HA in modified MS medium increased maximum formation of PLB within short duration of culture. In *Phalaenopsis*, 100% PLB formation rate was observed on all the medium containing HA9 or HA12 except high concentration after six weeks of culture. In, *Phalaenopsis,* 93.3% shoot formation rate was observed on media containing 0.1 mg/L HA12. Based on our present study, it could be concluded that low concentration of Hyaluronic acid added with culture medium, rapidly increased the formation rate of PLBs and shoots *in vitro* culture of *Phalaenopsis* 'Fmk02010'.

Key words: Hyaluronic acid · Protocorm-like bodies(PLBs) · *Phalaenopsis*

INTRODUCTION

ORCHIDS, the most beautiful flower occupy top position among all the flowering plant and *Phalaenopsis* are the most important and popular orchids in horticulture. The family Orchidaceae is one of the largest and most diversified among the angiosperms, comprising about 700 genera and 25,000 species of terrestrial plants, as well as epiphytes, lithophytes and saprophytes [1, 2]. Today, orchids are produced and traded worldwide. *Phalaenopsis* orchids have high economic value in the floriculture industry as cut flowers and even more as potted plants. Because of the large, colourful and durable flowers, as well as their adaptability to room conditions, they are the most popular orchid genus in the horticultural industry. *Phalaenopsis* is a monopodial epiphytic orchid, which is difficult to propagate vegetatively. The characteristics of seedlings are not uniform and propagation through tissue culture has been desired. Several tissue culture techniques have been developed for *Phalaenopsis* orchids, including the culture of flower stalks with axillary buds, meristems, flower stalks explants, internodal segments of flower stalks [3, 4]. leaf segments [5] and root tips [3, 6]. Due to difficulties in regeneration to protocorms and then to plants, all the procedures mentioned above have been inadequate for meeting commercial needs for vegetative propagation. Some of these methods gave a lot of protocorms, but these developed slowly or poorly to vital plants. However, in vitro culture technologies are still a challenge because of the slow growth of plantlets, low multiplication rate, poor rooting and somaclonal variation. In addition to being a structural component of homogeneous polysaccharides like chitin, Hyaluronic acid (HA) is a linear heteropolysaccharide that is composed of repeating D-glucuronic acid and *N*-acetyl-glucosamine (GlcNAc)

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residues. HA has been used in the applications in cosmetic, food, healthcare and pharmaceutical fields [7, 8, 9]. HA plays an important role in the interaction with extracellular matrix components, cell adhesion and migration, regulation of protein secretion, gene expression and cell proliferation and differentiation [10].

To date, there has a few report of HA act as a plant growth stimulator in some plant species including in orchid species [11, 12]. Therefore, the objective of this section was investigated the effect of various type and concentration of Hyaluronic acid on organogenesis of *Phalaenopsis* 'Fmk02010' *in vitro* culture.

MATERIALS AND METHODS

Plant Materials and Culture Conditions: PLBs of *Phalaenopsis* 'Fmk02010' were proliferated in the modified MS [13] medium by transferring to a new medium. After excision of PLB into singles, they were used for explants. Modified MS medium supplement with 412.5 mg l^{-1} ammonium nitrate, 950 mg l^{-1} potassium nitrate, 20 g l^{-1} sucrose and 2 g l^{-1} Phytagel (Sigma). Modified MS medium was adjusted to pH 5.5- 5.8 with 1 mM 2-(N-morpholino) ethanesulfonic acid sodium salts (MES-Na) before autoclaving at 121°C for 15 min at 1.5 Kgf cm^{-2}. 250 ml of UM culture bottles (AsOne, JAPAN) with plastic caps were used, each bottle receiving 30 ml of medium.

Effect of Hyaluronic acid (HA) on Organogenesis: To study the effects of Hyaluronic acid (HA) on organogenesis of *Phalaenopsis sp.*, single protocorm-like bodies (PLBs) were cultured in modified MS medium supplemented with two types to HA which are Hyaluronic acid 9 (HA9) and Hyaluronic acid 12 (HA12) with a difference molecular weight of $1.08x10^6$ Da and $1.2x10^6$ Da, respectively (Shiseido, Japan) at difference concentrations at 0,0.01, 0.1, 1.0 and 10.0 mg/L. Five PLBs explants were put in each culture vessel and three culture vessels were used for each treatment.

Data Collection: The numbers of PLBs, the numbers of shoots, the percentage of PLBs, the percentage of shoot and fresh weight of PLBs were recorded after 42 days of culture. The experiment was a completely randomized design with 3 replications and each replicate contained 5 PLBs. Data were statistically analyzed by calculating standard error of the means (means ± SE)

RESULTS AND DISCUSSION

Effect Hyaluronic acid (HA) on the growth and development in PLBs of *Phalaenopsis* 'Fmk02010': In the present study, we have tested two types and concentrations of HA in order to find the highest growth and development of PLB after six weeks of culture. This is the first report demonstration two types of HA including HA9 and HA12, act as a plant growth regulators in *Phalaenopsis* (Table 1). The experiment revealed that there was greatly affected on the number of PLBs per explant in *Phalaenopsis*. In all medium tested, the earliest morphological sign of PLB formation appeared as swelling on the part of explants within 10 days of culture and then PLBs grew into small round bodies with smooth surfaces and greenish in color. The best response for enhanced the percentage of PLBs formation and producing the highest number of PLBs per explant was recorded for the medium containing 0.1 mg /L HA12, on which 100% of the explant developed on average 23.3 PLBs per explant within six weeks. Concentration of HA9 at 1.0 mg/L inhibited PLBs formation. Highest percentage of shoot formation and number of shoot per explants were also recorded in the medium with 0.1 mg/L HA12, on which 93.3% with an average of 15.1 shoots per explant within six weeks of culture. The maximum of fresh weight of PLBs also was highest in medium containing 0.1 mg/L HA12 (0.596 gm FW). This study indicated that lower concentration of HA12 was most effective to PLBs induction for shoot development and fresh weight. HA12 was most effective for PLBs and shoot development in comparison to HA9, the medium without any plant growth regulator.

Table 1: Effect of HA on growth and development in PLBs of *Phalaenopsis* 'Fmk02010'

HA types	Conc.(mg/L)	PLB number	Shoot number	Fresh weight (g)
Control	0	12.9±1.8	8.6±0.7	0.198±0.05
HA9	0.01	11.7±3.4	14.8±1.2	0.236±0.08
	0.1	18.2±2.1	9.3±0.4	0.291±0.03
	1	12.6±2.6	12.1±1.1	0.238±0.04
	10	14.8±2.1	8.4±0.5	0.199±0.03
HA12	0.01	13.4±3.4	8.1±1.2	0.333±0.08
	0.1	23.3±2.1	15.1±0.4	0.596±0.03
	1	7.96±2.6	12.1±1.1	0.233±0.04
	10	15.5±2.1	8.2±0.5	0.257±0.03

Data were statistically analyzed by calculating standard error of the means (means ± SE)

Average number = Number of cultured explants with new PLBs or shoot / Total number of cultured explants

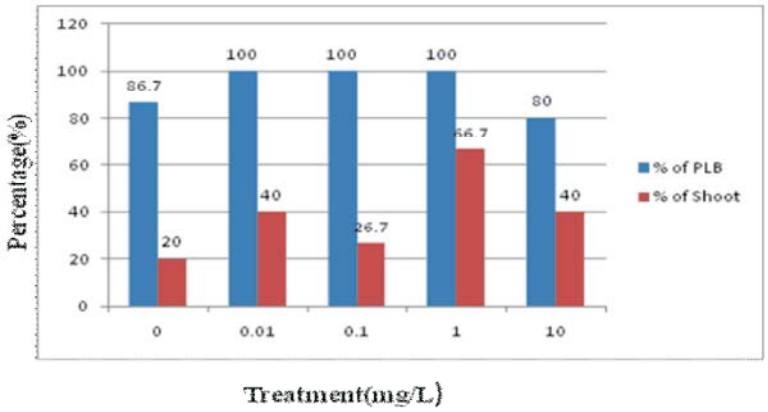

Fig. 1: Effects of HA9 on percentage of PLBs and Shoots formation rate in *Phalaenopsis* 'Fmk02010'

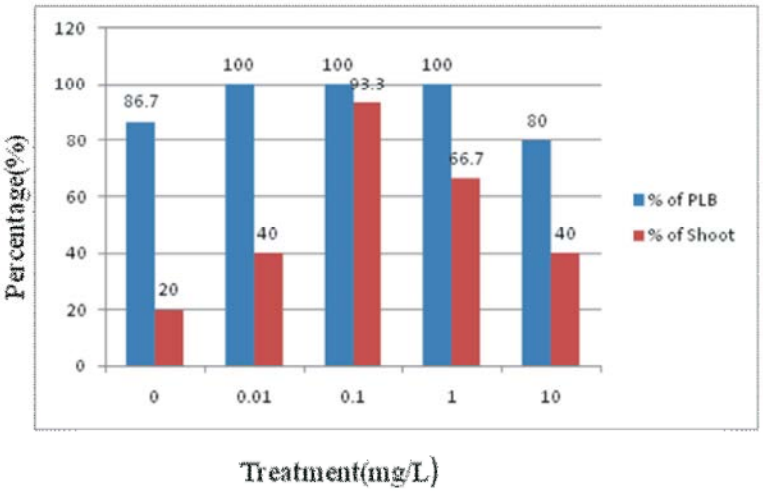

Fig. 2: Effects of HA12 on PLBs and Shoot formation rate (%) in *Phalaenopsis* 'Fmk02010'

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

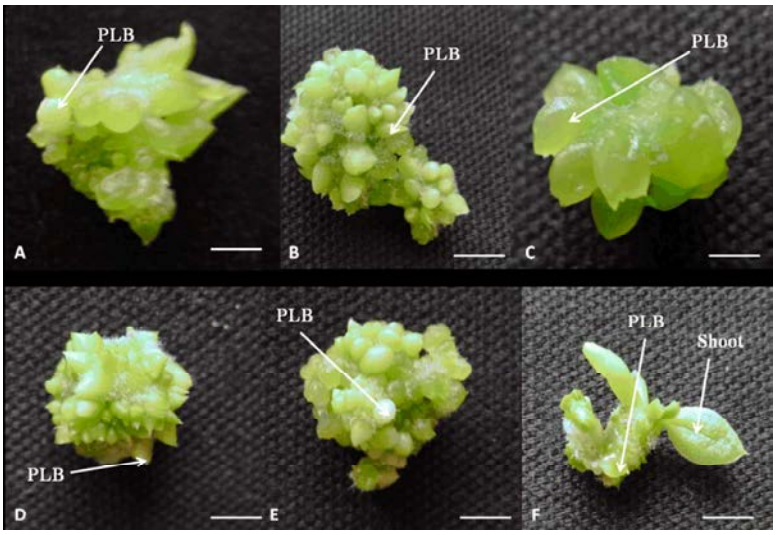

Fig. 3: Effects of HA9 and HA12 on PLBs and Shoot formation rate (%) in *Phalaenopsis* 'Fmk02010' A:Control; B: 0.1mg/L HA9, C: 10mg/L HA9; D: 0.01mg/L HA12 ; E: 0.1mg/L HA12 and F: 10mg/L HA12; Bars: 1cm.

Hyaluronic acid (HA) recently used as aplant growth regulators in orchid propagation. Bacterial fermentation methods for large scale economic production of HA have been well standardized as HA is of used in medical and cosmetic industry extensively [14, 15]. Hyaluronic acid shortens the adaptation period of cells on the material surface and then cells enter the normal cell cycle quickly [16]. In the present study, low concentration of Hyaluronic acid induced PLBs formation very rapidly in *Phalaenopsis.* HA are kind of biotic elicitors, has been one of the most effective for enhancing secondary metabolite production in plant tissue culture [17] and elicits systemic resistance in cucumber, tomato and pepper [11]. In orchid, Nahar *et al*. [12] who reported that the optimal concentration of HA9 for promoting the highest PLB formation rate of *Cymbidium dayanum* tissue was 1 mg/L HA9, whereas the highest shoot formation rate was found in medium supplemented with 1 and 0.001 mg l^{-1} HA9. In contrast to what we found here with HA9, the optimal amount of 0.1 mg l^{-1} of HA 12 supplementation for the highest PLBs and to promoting shoot formation. Thus, a function of Hyaluronic acid is quite different from orchid species to species. During the culture period there was no malformation observed in regenerated shoots. Therefore, much more work is still needed on hyaluronic acid for *Phalaenopsis* micropropagation.

CONCLUSION

Based on this research and discussion, it can concluded that low concentrations of HA9 and HA12 promotes organogenesis of PLBs in very speedily within short period of time and act as growth regulator in *Phalaenopsis* 'Fmk02010'.

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