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Effect of hyaluronic acid on the organogenesis of an *Oncidium* cultivar

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ABSTRACT

Protocorm-like bodies (PLBs) multiplication is one of the most preferable in vitro methods to increase the number of orchids in a short time. The current investigation was conducted to understand the impact of two types of hyaluronic acid (HA12 and HA20) using various concentrations on the regeneration of Oncidium Aloha 'Iwanaga'. The highest number of PLBs were found at 0.1mg/l of HA12 (20.7/explant) and HA20 (23.1/explant) respectively. The highest number of shoots were found at 0.1mg/l of HA12 (2.1/explant) and HA20 (3.5/explant) respectively. The highest formation rate of PLBs and shoots was found in both hyaluronic acids at 0.1mg/l. No root formation was observed. The highest fresh weight was obtained with 0.1mg/l HA12 (195.5mg) and 0.1mg/l HA20 (212.3mg) respectively. So use of hyaluronic acid could be a potential source of PLB proliferation within short time in Oncidium Aloha 'Iwanaga'.

Key Words*: Orchid, Protocorm-like bodies (PLBs), In vitro micropropagation and Organogenesis*

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I. Introduction

Orchids have a huge commercial appreciation and grown worldwide as cut flower and indoor potted plants with an 8% share in floriculture trade. It is considered outstanding as ornamentals due to its diverse colors, shapes, forms and long lasting blooms (Toukuhara et al., 2001). Among various orchid species, *Oncidium* orchids are economically important for the flower market, unlike other orchid hybrid groups. It represents one of the genera with most ornamental applications and used in floriculture for pot and cut flower production (Faria et al., 2015). Propagation by the conventional method of orchid is extremely slow as its seeds lack endosperm and need fungal stimulants for germination in nature (Islam et al., 1982). The complicated propagation nature of orchid lead some of

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the indigenous species to extinction (Basker et al., 2006). So, *In vitro* cultural techniques are now adopted for quick propagation of commercially important orchid species (Alam et al., 2020). Several reports were available demonstrating the micropropagation of orchid using different explants (leaf, roots tips, lateral buds from young flower stalks) but none of those methods proved effective in producing huge number of PLBs in a short time (Tanaka, 1987, Kobayashi et al., 1991, Ichihashi, 1992, Tokuhara et al., 1993). Use of plant growth regulators into culture media can play a vital role to increase the number of PLBs (Nahar et al., 2011). Hyaluronic acid (HA) is a polymer of disaccharides; composed of D-glucuronic acid and N-acetyl-D-glucosamine. Due to having growth regulative properties, HA is used as an additive in plant tissue culture (Kaewjampa et al., 2012). In recent times the beneficial effect of HA has been demonstrated in the rapid proliferation of PLBs. Sultana et al. (2015) showed that the addition of HA9 (Hyaluronic Acid with molecular weight 0.8～1.17×10^6 Da) or HA12 (Hyaluronic Acid with molecular weight 1.1～1.6×10^6 Da) in culture media increased PLBs of *Phalaenopsis* at a certain concentration. Based on that it was hypothesized that the application of HA12 and HA20 (Hyaluronic Acid with molecular weight 1.9～2.7×10^6 Da) could also promote the organogenesis of *Oncidium* Aloha 'Iwanaga'. However, there was a lack of detailed information about the promotive effect of HA12 and HA20 on the organogenesis of *Oncidium.* Regarding the Hypothesis, the current investigation was done to understand the effect of HA12 and HA20 using at various concentrations on the organogenesis of *Oncidium* Aloha 'Iwanaga'.

II. Materials and Methods

Protocorm-like bodies (PLBs) of *Oncidium* Aloha 'Iwanaga' were proliferated to the modified Murashige and Skoog (MS) medium (Shimasaki et al., 1990) at the Lab of Vegetable and Floricultural Science, Faculty of Agriculture and Marine Science, Kochi University, Japan on February 2020. After excising PLBs into individuals, each was used as explant. MS medium containing 412.5 mg/l ammonium nitrate, 950 mg/l potassium nitrate, 20 g/l sucrose and 2 g/l Phytagel (Sigma) were used as culture medium. HA12 and HA20 (Shiseido, Japan) were added separately at various concentrations such as control(0), 0.01, 0.1, 1.0 and 10mg/l to the culture medium before sterilization. Jars (250 ml UM culture bottle; As one, Japan) with plastic caps containing 30 ml of medium were used as 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^{0}C for 15 min. Five explants were put in each culture vessel. Three culture vessels were used for each treatment. All cultures were maintained under white LED(NNLK41509) (Panasonic) at 25±1^0 C. Experimental data were collected after 6 weeks of culture by counting the number of PLBs, the number of shoots and measuring the fresh weight (mg). The data were analyzed using one-way analysis variance (ANOVA) and differences between means were tested using Tukey's HSD test ($p \leq 0.05$).

III. Results and Discussion

The effect of HA12 and HA20 on the organogenesis of *Oncidium* Aloha 'Iwanaga' using different concentrations after six weeks were shown in (Table 01 and Table 02). Each concentration of HA12 and HA20 stimulated both PLB and shoot proliferation compared to 0mg/l. In the case of HA12, the highest number of PLBs (20.7/explant) was obtained with 0.1mg/l whereas the lowest (5.8/explant) was obtained with 0mg/l. The highest formation rate of PLBs (100%) was obtained with 0.1mg/l HA12and the minimum was obtained with 0mg/l (73.3%). In the case of shoots, the highest number of shoots (2.1/explant) was obtained with 0.1mg/l and the lowest (1.0/explant) was obtained with 0mg/l. The highest formation rate of shoot (93.3%) was obtained with 0.1mg/l compared to other concentrations. In this experiment, no root formation was observed. The highest fresh weight (195.5 mg) was obtained with 0.1mg/l of HA12 treatment while the lowest (86.0mg) was at 0mg/l. The highest number of PLBs (23.1/explant) and the highest formation rate of PLB (100%) were obtained while the PLBs were cultured with 0.1mg/l HA20 compared with other concentrations. Results in the case of shoots showed that the highest number of shoots (3.5/explant) was obtained with 0.1mg/l HA20. The highest shoot formation rate (93.3%) was obtained with 0.1mg/l compared to others. The highest fresh weight (212.3mg) was obtained with 0.1mg/l HA20 whereas the lowest (98.1mg) was with 0mg/l.

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Table 01. Effect of HA12 on the organogenesis of *Oncidium* Aloha 'Iwanaga'

HA12 (mg/l)	PLB		Shoot		Fresh weight (mg)
	No./explant	Formation rate (%)	No./explant	Formation rate (%)	
0	5.8 ± 1.0 b	73.3	1.0 ± 0.3 b	53.3	86.0 ± 9.2 b
0.01	11.4 ± 1.7 a	93.3	1.1 ± 0.3 ab	60	144.6 ± 13.2 ab
0.1	20.7 ± 1.9 a	100	2.1 ± 0.5 a	93.3	195.5 ± 20.4 a
1	9.4 ± 1.3 ab	93.3	1.1 ± 0.4 ab	53.3	115.8 ± 23.8 ab
10	10.8 ± 1.7 ab	86.6	1.1 ± 0.4 ab	53.3	116.5 ± 24.9 ab

Values represent mean±SE followed by different letters which indicating significant differences by the Tukey HSD test (p≤ 0.05).

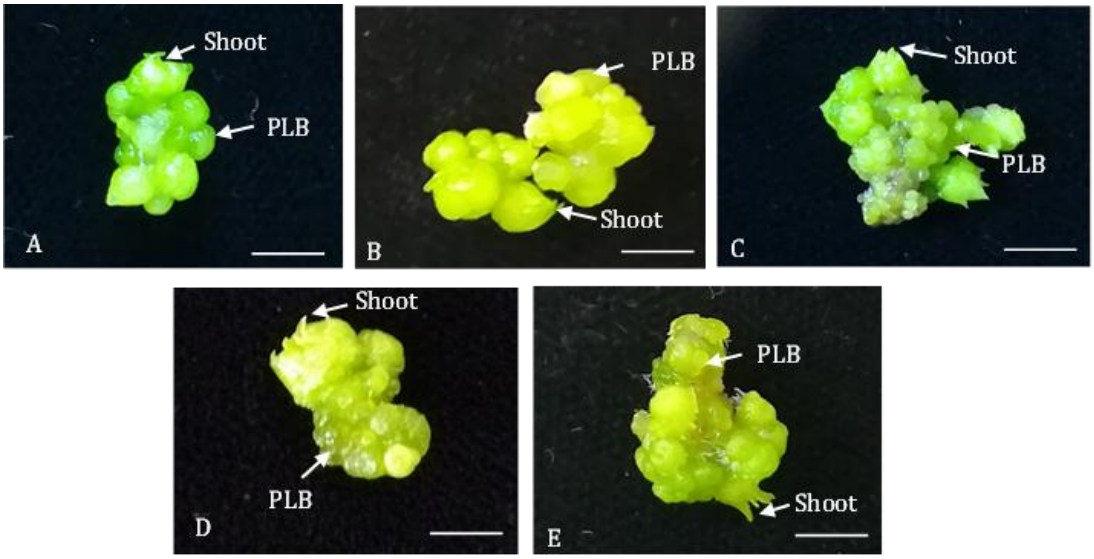

Figure 01. Effect of HA12 on the organogenesis of *Oncidium* Aloha 'Iwanaga'; A: 0mg/l; B: 0.01mg/l; C: 0.1mg/l; D: 1.0mg/l and E: 10mg/l HA12; Bar=1cm.

Table 02. Effect of HA20 on the organogenesis of *Oncidium* Aloha 'Iwanaga'

HA20 (mg/l)	PLB		Shoot		Fresh weight (mg)
	No./explant	Formation rate (%)	No./explant	Formation rate (%)	
0	7.1 ± 1.1 b	86.6	1.0± 0.3 b	60	98.1± 10.6 b
0.01	20.9± 4.0 b	93.3	1.4 ± 0.4 ab	66.6	126.3 ± 17.5 ab
0.1	23.1 ± 3.4 a	100	3.5 ± 0.7 a	93.3	212.3 ± 31.1 a
1	12.7 ± 2.9 b	93.3	2.1 ± 0.7 ab	60	181.7 ± 20.3 ab
10	9.8 ± 1.9 b	93.3	1.9 ± 0.5 ab	60	134.7 ± 10.7 ab

Values represent mean±SE followed by different letters which indicating significant differences by the Tukey HSD test (p≤ 0.05).

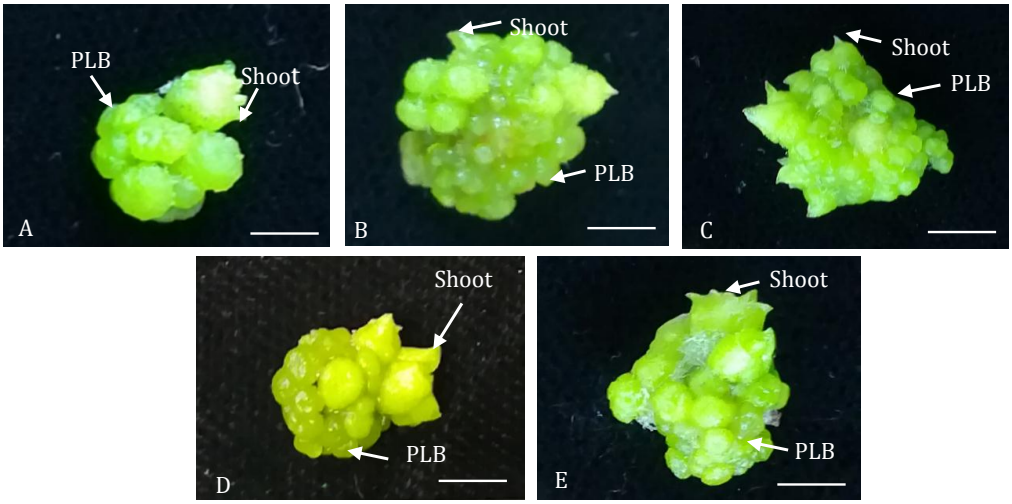

Figure 02. Effect of HA20 on the organogenesis of *Oncidium* Aloha 'Iwanaga'; A: 0mg/l; B: 0.01mg/l; C: 0.1mg/l; D: 1.0mg/l and E: 10mg/l HA20. Bar=1cm.

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Recently HA in culture media have also been shown to improve *in vitro* organogenesis of several orchid species such as *Cymbidium* (Kamal et al., 2014) and *Phalanopsis* 'Fmk02010' (Sultana et al., 2015). Literature regarding the effect of such elicitors on *Oncidium* was very less. Sultana et al. (2015) found the best results in terms of PLBs, shoots and fresh weight in comparatively less concentrations. HA is a polysaccharide, an abiotic elicitor that enhances secondary metabolite production in plant tissue culture (Zhou and Wu, 2006). HA has several biological functions including cell proliferation and differentiation and gene expression (Kogan et al., 2007). 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). Mehraj et al. (2017) confirmed that HA12 is better than not only HA9 but also the combinations of both HA9 and HA12 performed. From the results of our experiment, we found both HA12 and HA20 showed a promotive effect on the proliferation of PLBs, Shoots and increase their formation rate. The results of this study also indicated that HA20 showed better performance in the proliferation PLBs and shoots in comparison of HA12.

IV. Conclusion

Application of HA12 and HA20 at 0.1mg/l concentration in the culture media was found as the best and HA20 comparatively produced more PLBs than HA12 during the organogenesis of *Oncidium* Aloha 'Iwanaga'.

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Conflict of Interest

The author declared that there is no conflict of interest

V. References

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