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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Studies on organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* cultured *in vitro* （*In vitro* におけるデンドロビウム・キンギアナムのプロトコーム様球体(PLBs)の器官形成に関する研究）

学位論文要約：

Dissertation Summary

The orchids are one of the most beautiful flowering plants in the world. There are many factors like fragrance, color, pattern of blooms are responsible for the popularity of any flower. The propagation of orchids is almost difficult. Thus, organogenesis of orchids *in vitro* is a more controllable and reliable process. There are over 1,000 types of *Dendrobium* orchids and hybrids. They vary in size, bloom color, appearance, and growing requirements. For this reason, it is important that you get detailed care information for your certain type of *Dendrobium* because the care can greatly vary depending on the type you have. Studies on organogenesis of PLBs on two *Dendrobium* cultivar describe some new organogenesis methods of *Dendrobium* tissue culture. Based on to increase the efficiency of *in vitro* techniques, environmental factors in particular medium composition, temperature and light are considerable important for modification and innovation of cultivation technologies for orchid plants.

Chapter I: The effect of bio polysaccharides of different molecular weights hyaluronic acid (HA9, HA12 and HA20) and sodium alginate added to modified Murashige and Skoog (MS) media on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. The use of polysaccharides as a plant growth regulator for orchid plants has attracted considerable interest in recent years (Kaewjampa et al.2010; Nahar et.al. 2011; 2012) because of; it's a widely available and generally viewed as a safe material for humans and the environment. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. Poly-or oligosaccharides are signaling molecules with elicitation pathways that have been intensively studied because these compounds can substitute for fungal elicitors during a pathogen attack (Zhao et al., 2005). So it can be an important role for tissue culture. In addition to being a structural component of homogeneous polysaccharides like chitin, hyaluronic acid (HA)

is a linear hetero-polysaccharide that is composed of repeating D-glucuronic acid and *N*-acetyl-glucosamine (GlcNAc) residues. HA has been used in the applications in cosmetic, food, healthcare, and pharmaceutical fields (Laurent et al., 1996; Morra, 2005). 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 (Scott, 1992). Recently, HA has been reported in various agricultural applications; Park el al. (2008) tested systemic resistancy induction by HA to cucumber, tomato and pepper. In orchid tissue culture, Nahar et al. (2011) firstly demonstrated that HA in culture media have also shown to improve orchid organogenesis *in vitro*. The objectives of this section were investigated the effect of various type and concentration of hyaluronic acid on the organogenesis of *Dendrobium kingianum* 'Hallelujah' *in vitro* culture.

Plant material and explants source prtocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah' were used for explants. After PLBs were excised individually, each PLB was used as an explant. Modified Murashige & Skoog medium (Shimasaki et al., 1990) supplemented with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20 g/L sucrose and 2.2 g/L Phytagel (Sigma-Aldrich) were used as a culture medium. HA9, HA12 and HA20 (Shiseido) were added separately at concentrations of 0, 0.01, 0.1, 1, 10 mg/L were added to culture media before sterilization. Jars of 250 ml (UM culture bottle, Asone, 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℃ for 15 min. Five explants cultured in one vessel and three vessels were used for each treatment. Cultures were maintained at 25 $\pm$1℃ under white flourescent light (45 μmol/m^2s^1) during 24 h photoperiods for 42 days.

Experimental data were collected by counting the number of PLBs; number of shoots, roots 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 ($P \leq 0.05$).

Table 1. Effect of HA9, HA12 and HA20 on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'

Concentration		Average no of PLBs/ explant	Average no of shoots/ explant	Average no of roots/ explant	Fresh Weight (g)
HA9(mg/L)	0	6.06±0.18c	0.06±0.02c	0.13±0.05a	0.1±0.005c
	0.01	16.9±0.5a	0	0	0.3±0.009a
	0.1	11.53±0.3b	0.07±0.02c	0.13±0.03a	0.2±0.004b
	1	3.5±0.2c	0	0	0.07±0.003c
	10	4.0±0.3c	0	0	0.3±0.007a
HA12(mg/L)	0.01	8.7±0.4bc	0	0	0.5±0.021a
	0.1	10.1±0.3b	0	0	0.4±0.011a
	1	7.9±0.3bc	1.0±0.1a	0.4±0.05a	0.4±0.01a
	10	6.5±0.1c	0.3±0.05b	0	0.2±0.004b
HA20(mg/L)	0.01	9.4±0.4b	0.13±0.3c	0.06±0.01b	0.2±0.009b
	0.1	8.7±0.4bc	0.3±0.07b	0.04±0.05b	0.3±0.015a
	1	10.9±0.05b	0.7±0.1a	0	0.2±0.013b
	10	8.1±0.2bc	0	0	0.2±0.004b

Value represents means ± SE followed by the different letters in the same column indicate significant differences using Tukey HSD tests at the 5% level.

Average number = Number of cultured explants with new PLBs or shoots/ total number of cultured explants

This result indicates that low concentration of HA9 in 0.01 mg/L is found highest number of PLBs to compare with other concentrations of HA12 and HA20 (Table 1). HA is a polysaccharide, an abiotic elicitor that enhances secondary metabolite production in plat tissue culture (Zhou and Wu, 2006). Hyaluronic acid shortens the adaption period of cell on the materials surface, and then cells enter the normal cell cycle quickly (Mao Jinshu et al. 2003). PLBs and shoots were successfully regenerated on modified MS medium supplemented with hyaluronic acid (HA9) (Nahar, 2012). When we found the best result in 0.01 mg/L of HA9 to compare with HA12 and

HA20 and that's why we use this same concentration under different LED to find out the best combination with LED and HA on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. In this study, HA9 were used at 0.01 mg/L under different LEDs. 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 ℃ for 15 min. Jars of 250 ml (UM culture bottle, Asone, Japan) with plastic caps containing 30ml of medium were used for culture vessels. Five explants were put in each culture vessel and three culture vessels were used for each treatment. To determine the effective light sources for PLB propagation of the *Dendrobium* cultivars *in vitro*, PLBs were cultured under different light conditions with a photon flux density (PFD) of (45 µmol/m^2s^1). Five light sources were used in this study: white fluorescent lamp (18W 1XFL20SS/18 as control; National), red LEDs (LT20R 9W 1449; Beamtech), blue LEDs (LT20B 9W 1447; Beamtech), green LEDs (LT20GS 9W 1524; Beamtech), and white LEDs (LTL T20KY 9W 1532; Beamtech). All cultured were maintained at 25±1^0 C and 24 h photoperiods for six weeks. In the experiment, addition of HA9 (0.01 mg/L) under different LED we found the highest number of PLBs (10.33/ explant) under white LED and the PLB formation rate 100% found under all LED lights. The maximum roots and shoots (0.013/explant) are found in under blue LED light and the highest fresh weight recorded in (0.36g) under blue LED and red LED. HA9 is the product name of sodium bio hyaluronic acid having the molecular weight0.8-1.17x106da. HA in culture media have also shown to improve *in vitro* organogenesis of (Nahar et al., 2011; Kaewjampa et al., 2012; Teixeira da Silva et al., 2013). LED lights are a fundamental environmental cue in the life of plants, playing crucial roles directly and indirectly in the regulation of plant growth and development (Sultana et al., 2014). The highest number of PLBs and the PLB formation rate at 0.1 mg/L HA9 under white LED (Sultana et al., 2014). In this study shown that use of low concentration of HA9 (0.01 mg/L) manipulated MS medium produce no significant different. Although the maximum average number of PLBs found in white LED to compare with others LED on *D. kingianum* 'Hallelujah' .The Effect of sodium alginate on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah' were used in this experiment. The objective of this section to find the effect of different concentration of sodium alginate on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. Protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah' were used for explant. After PLBs were excised individually, each PLB was used as an explant. Modified Murashige & Skoog medium (Shimasaki et al., 1990) supplemented with 412.5 mg/L ammonium nitrate, 950 mg/L potassium nitrate, 20 g/L sucrose and 2.2 g/L Phytagel (Sigma-Aldrich) were used as a culture medium. Sodium alginate were added separately at concentrations of 0, 0.01, 0.1, 1, 10 mg/L was added to culture media before sterilization. The highest number of PLB (17.2/explant) we found in 0.01 mg/L in alginate). Maximum number of shoots (0.1/explant) found in 0.1 mg/L and roots (0.13/explant) found in 0, that's mean control. The highest fresh weight recorded (0.3

g) in 0.01 mg/L of sodium alginate. The highest formation rate of PLBs (100%) was obtained in 1 mg/L of alginate. In plants, Sodium alginate used as a soil fertilizer. Sodium alginate is the sodium salt form of alginic acid and gum mainly extracted from the cell walls of brown algae, with chelating activity. Upon oral administration, sodium alginate binds to and blocks the absorption of various radioactive isotopes. Sodium alginate absorbs water quickly which makes it useful as an additive in dehydrated cells and tissues in plants. In present experiment, we found very good impacts of sodium alginate on PLBs in *Dendrobium kingianum* 'Hallelujah' because our study showed that very low concentration (0.01 mg/L) of sodium alginate with modified MS medium produced maximum number of PLBs within short period.

Chapter II. Effect of environmental factors and carbohydrate sources on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium* cultivar. Among various physical environmental factors applied to plants, the temperature stress is most relevant to plant growth and development in both the natural environment and *in vitro* culture. Micropropagation provided an important breakthrough for mass propagation of orchid species which have high heterozygosity and slow sexual reproduction capability (Kanjilal et al., 1999). The success of plant tissue culture is highly influenced by the nutrition supplied in the media, carbohydrate, growth regulators and environmental factors. In recent year, LED emerged as a new light source has many advantages. Advanced features of LEDs compared over the conventional one, such as longer life, wavelength specificity and narrow band width, less heat radiation and low power consumption (Kaewjampa et al. 2012). Optimization of medium compositions was an important approach to fasten the micropropagation process and the improve the quality of regenerated plantlets through culturing callus, adventitious shoots, or protocorm-like bodies (PLBs) (Ichihashi, 1992; Chen et al.,2000; Park et al., 2002). The effect of LEDs on plantlets cultured *in vitro* were reported in plants such as potato (Miyashita et al., 1995), *Cymbidium* (Nahar et al., 2012, 2013), *Dendrobium* (Sultana et al., 2014) and strawberry (Nhut et al.,2003). The Effects of sucrose and trehalose on *Dendrobium kingianum* 'Hallelujah' under different light conditions. A light-emitting diodes (LEDs) is a semiconductor device that emits visible light when an electric current passes through it. The light is not particularly bright but, in most LEDs, it is monochromatic, occurring at a single wavelength. It is represented a fundamentally different technology from the gaseous discharge-type lamps currently used in horticulture. To elucidate the effect of different light conditions on the in vitro PLBs growth of *Dendrobium kingianum* **'**Hallelujah**'**, the cultures were established and grown under different light conditions of photon flux density (PFD) of (45 µmol/m^2/s).

Table 2. Effects of sucrose and trehalose on *Dendrobium kingianum* 'Hallelujah' under different light conditions

Carbon source 20g/L	Light conditions	No. of PLBs	Formation rate of PLBs	No. of shoots	No. of roots	Fresh weight (g)
Sucrose	White fluorescent lamp	6.1±0.45b	93	1.27±0.12a	-------	0.21±0.01ab
	White LED	9.8±0.26a	100	0.4±0.06ab	-------	0.35±0.01a
	Blue LED	9.3±0.22a	100	-------	-------	0.35±0.01a
	Red LED	11.0±0.27a	100	-------	-------	0.44±0.01a
	Green LED	11.1±0.30a	100	-------	-------	0.45±0.02a
Trehalose	White fluorescent lamp	7.4±4.06b	93	0.86±1.4a	0.53±1.1a	0.22±0.09c
	White LED	10.3±4.1ab	100	-------	-------	0.37±0.12a
	Blue LED	11.6±3.3a	100	-------	-------	0.35±0.11a
	Red LED	11.8±3.8a	100	-------	-------	0.27±0.11b
	Green LED	13.8±4.5a	100	-------	-------	0.42±0.16a

Value represents means ±SE followed by the different letters in the same column indicate significant differences using Tukey HSD tests at the 5% level.

Average number = Number of cultured explants with new PLBs or shoots/ total number of cultured explants

The *in vitro* sucrose and trehalose supplemented culture media under green LED stimulated the PLBs organogenesis of *D. kingianum* 'Hallelujah'. The average number of PLBs and the amount of fresh weight are very important for successful and healthy PLB regeneration *in vitro*. LEDs are an effective light source for plants (Manivannan et al. 2015; Lin, et al. 2011) and play an important role in plant growth and development under *in vitro* conditions, depending on irradiation intensity (quantity of light), wavelength (quality), and light duration (photoperiod). Light is absorbed by chlorophyll (Topchiy et al. 2005). Carbon sources are another important factor in orchid PLB regeneration because it supplies energy to the plants especially when they are not ready to photosynthesize in the early stage of growth (Al-Khateeb, 2008). Moreover, trehalose itself can affect development by acting as a signal molecule in carbohydrate metabolism. The results of this study revealed that

green LEDs with trehalose produced the highest average number of PLBs, but the maximum fresh weight was obtained with same light using sucrose in the medium for the *Dendrobium kingianum* 'Hallelujah' cultivar. A similar result has also been found for the growth of young tea plants, for which green LED were effective for the growth of potted and rooted cuttings (Homma et al., 2009) and also for strawberry plants, for which the growth and enlargement of the strawberry fruit plants were promoted by green light irradiation (Kudo et al., 2009).Several studies have reported that orchid PLBs cultured under red LEDs showed the lowest differentiation rate, while using blue LED resulted in the highest differentiation rate in cultures of *Oncidium* and *Dendrobium officinale in vitro* (Xu et al., 2009 ;Lin et al.,2011). The results of this study revealed that green LEDs with trehalose produced the highest average number of PLBs (Table 2), although the maximum fresh weight was obtained with same light using sucrose in the medium for the *Dendrobium kingianum* 'Hallelujah'. The effect sucrose and trehalose on *Dendrobium kingianum* Jonathan's Glory 'Dark Joy' under different light conditions.

Table 3 . Effects of sucrose and trehalose on *Dendrobium kingianum* Jonathan's Glory 'Dark Joy' under different light conditions

Carbon source 20g/L	Light conditions	No. of PLBs/ explant	Formation rate of PLBs	No. of shoots/ expalnt	No. of roots/ explant	Fresh weight (g)
Sucrose	White fluorescent lamp	5.3±2.1b	100	1.9±2.3a	--------	0.17±0.1ab
	White LED	6.9±2.7ab	100	0.7±1.4a	0.1±0.4a	0.13±0.1bc
	Blue LED	6.1±2.7ab	86	1.8±2.2a	0.5±1.1a	0.18±0.1ab
	Red LED	5.4±1.3b	100	0.8±1.1a	0.1±0.3a	0.10±0.1c
	Green LED	8.0±2.9a	100	2.6±1.9a	0.1±0.3a	0.22±0.1a
Trehalose	White fluorescent lamp	6.9±3.1a	100	0.8±1.4a	---------	0.14±0.1ab
	White LED	8.1±2.9a	100	1.6±1.1a	0.1±0.5a	0.23±0.2a
	Blue LED	5.0±4.1ab	93	1.3±1.6a	0.8±1.2a	0.14±0.1ab
	Red LED	5.2±3.0ab	100	1.4±1.5a	0.5±0.8a	0.15±0.1ab
	Green LED	4.1±2.7b	87	1.3±1.2a	0.5±0.7ab	0.10±0.1b

Value represents means ±SE followed by the different letters in the same column indicate significant differences

using Tukey HSD tests at the 5% level.

Average number = Number of cultured explants with new PLBs or shoots/ total number of cultured explants

The *In vitro* PLBs organogenesis of *D. kingianum* Jonathan's Glory 'Dark Joy' was stimulated by the sucrose supplemented media under green LEDs. Whereas the organogenesis was stimulated by the trehalose supplemented medium under white LEDs. The results of the present study revealed that green LEDs and sucrose also increased the number of PLBs and amount of fresh weight of *Dendrobium* Jonathan's Glory 'Dark Joy', but trehalose decreased the number of PLBs and the amount of fresh weight under different light emitting diodes for the same cultivar. Hew and Mah (1989) reported that carbohydrate hydrolysis by extracellular hydrolytic enzymes is possible, as demonstrated with the PLBs of *Dendrobium*. Carbohydrate is an important factor *in vitro* culture media and the appropriate amount is also important for the successful regeneration of PLBs. A deficient supply of carbohydrate for *in vitro* orchids can be detrimental to the cell growth rate. Sucrose is specifically needed in the plant embryo to increase cell division by encouraging cell expansion and reserve accumulation (Borisjuk et al., 2002). However, increasing sucrose over the threshold concentration could lead to excessive carbohydrate accumulation and hinder photosynthesis which eventually impairs the cell growth of rose plants (Capellades et al., 1991). The amount of carbohydrate is also responsible for good quality PLB formation. Sucrose (10 g/L) was found to be very inefficient in producing PLBs of *Dendrobium huoshanense* compared with 35 g/L sucrose (Zha et al., 2007). Pulse treatments using trehalose and sucrose were considered to be useful for plantlet production in *Cymbidium* spp. (Shimasaki et al., 2003). Both carbohydrates and light are responsible for good quality PLB formation. The results of the present study revealed that green LEDs and sucrose increased the number of PLBs and amount of fresh weight (Table 3) but trehalose and white LED produced the maximum number of PLBs (8.1/explant) and the amount of fresh weight on *D. kingianum* Jonathan's Glory 'Dark Joy'. The effect of different temperature on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. PLBs of *Dendrobium kingianum* 'Hallelujah' were incubated in five conditions. Different temperatures (15, 20, 25, 30, 35 ℃) were in 24 hours for six weeks period. All treatments were also incubated the same with continuous Toshiba fluorescent lamps (45 μmol/m^2 s^1) and other methods and materials almost same. In this experiment the growth and development of PLBs in *Dendrobium kingianum* 'Hallelujah' were significantly affected by different temperature treatment *in vitro*. The highest average number of PLB (15.5/explant) we found in 25 ℃ for 24 hours. There is no shoots found in this experiment and the roots (0.13/ explant) are found in 20 ℃ and 25 ℃. The fresh weight (.206 g) recorded also highest in 25 ℃ for 24 hours. When we set it under 35 ℃ we see that all the explants are burned. The highest formation rate of PLBs we found in 100% both 20 ℃ and 25 ℃. The temperature treatment is one of the most important factors

regulating plant development through photosynthesis in term of rate of carbon assimilation. In orchid, high temperature produce a growth of protocorms of *Dendrobium purpurella* was obtained at 23 ℃ (Harvais and Hadley, 1967). In our study we found highest number of PLBs (15.5) in 25 ℃ with shoots, roots and also fresh weight in same temperature. It was response to flower initiation of *Phalaenopsis* and Zygopetalum Redvale 'Fire Kiss' under 20-25 ℃ and 25.4-28.6 ℃, respectively (Su et al., 2001; Kataoka et al., 2004; Blanchard and Runkle, 2006; Lee et al., 2007; Chen et al., 2008; Lopez and Runkle, 2005).

Chapter III: Effect of elicitors on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. The use of biotic elicitors to stimulate product formation has become an important progress strategy and has been very useful in reducing the process time required to attain high product concentrations and increased volumetric productivity. Plant cell culture has recently received a lot of attention as an effective technology for the production of valuable secondary metabolites. The effect of 5-aminolevulinic acid (5-ALA) on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. In this experiment I used four different concentration of 5-ALA and other methods and materials are same as HA treatment. In this experiment, the most effective concentration in terms of average number of PLBs in 1 mg/L to compare with control. The maximum number of PLBs (10.3/ explant) was found in 1 mg/L of 5-ALA. The highest formation rate of PLBs we found in this experiment 100% in 0.1and 1mg/L of 5-ALA and there are no roots and shoots we found in this treatment. The highest fresh weight (0.14) recorded in 0.1 mg/L of 5-ALA. 5-ALA is known to play a vital role in improving plant growth regulator in Cymbidium spp. 5-ALA improves the growth and yield of several plants by enhancing the chlorophyll content and the rate of photosynthesis, including spirulina, date palm and Arabidopsis (Sasaki et al., 1995; Al-Khateeb et al., 2006, Awad 2008; Maruyama-Nakashita et al., 2010). It is reported that the positive effect of 5-ALA on PLB and shoot proliferation of *Dendrobium kingianum*. In this study we can demonstrated that low concentration of 5-ALA significantly enhanced the formation of PLBs in *Dendrobium kingianum* 'Hallelujah'. Effect of N-acetylglucosamine (NAG) on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. N-acetylglucosamine (NAG) is the monomeric unit of the polymer chitin, which forms the outer coverings of insects and crustaceans. It is the main component of the cell walls fungi, the radulas of mollusks, and the breaks of cephalopods. Recently, Kyrychenko et al., (2007) reported the stimulatory effects of N-acetylglucosamine (NAG) on the plants. NAG has been reported to act as a plant growth regulator and considered to elicit the induction of PLBs in tissue culture on *Epidendrum* 'Rouge Star No.8' (Kaewjampa et al., 2010) and also in *Cymbidium* dayanum and *Cymbidium* insigne (Nahar et al., 2011 & 2012). The objective of this study to effect of different concentration of NAG on organogenesis of *Dendrobium kingianum* 'Hallelujah'.

Materials and methods, Data collection is same like other experiments. The effect of NAG at low concentration enhanced the growth of PLBs formation to compare with control. The maximum average number of PLBs (10.06/ explant), we found in 0.1 mg/L on NAG. There is no shoots found in this treatment and very little average number of roots (0.2/explant) is recorded in 0.1mg/L of NAG. The maximum fresh weight found in (0.10g) in 0.1mg/L of NAG. The maximum formation rate of PLBs (100%) we found in 0.1 and 10mg/L of NAG in this treatment. Low concentration of NAG (0.1 mg/L) supplementation in culture media enhance the maximum formation of PLBs of *Dendrobium kingianum* 'Hallelujah' and almost similar results found by the application of NAG at low concentration the growth of PLBs in *Epidendrum* 'Rough Star No.8' , *Cymbidium* dayanum and *Dendrobium kingianum in vitro* (Kaewjampa 2010, Nahar S.J. 2011 and Sultana 2016). In very recent, the effect of NAG on the organogenesis of *Oncidium* Aloha 'Iwanaga' is also reported that low concentration of NAG produced the maximum average number of PLB/ explant. In my studies also shown the similar results that low concentration produce highest number of PLBs and very few numbers of roots.

Chapter IV: Effect of plant hormones on the organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum* 'Hallelujah'. In orchid micropropagation, plant hormones are very important for plant growth and development and frequently used in different orchids especially, auxins and cytokinins are most commonly used for nutrient media to increase the production of orchids (Arditti and Ernst, 1993). The effect of 6-benzylaminopurine (BAP) on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. Materials and methods, Data collection same as other experiments. In this treatment, we found the highest average number of PLBs in the high concentration of BAP that means to compare with control we can see that when we use high concentration 10 mg/L of BAP, we found highest number of PLBs (15.7/explant) to compare with control (0). In control, very low number of roots and shoots found in this treatment. In low concentration of BAP 0.01 mg/L found little shoot also but in high concentration of BAP haven't any shoot and root in this treatment. Without control the others concentration of BAP we found almost maximum percentage of PLBs in this treatment. The fresh weight (0.3g) obtained better in 0.01, 0.1 and 1 mg/L of BAP to compare with control and high concentration of BAP 10mg/L. 6-Benzylaminopurine (BAP) is a widely used for micropropogation of orchids because of it's ability to induce organogenesis. This cytokinin constitutes a major class of plant growth regulator that is involved in a wide range of physiological processes. It is reported that BAP is the most commonly used cytokinin in tissue culture. Low concentration of BAP is very affective to produce the maximum average number of PLBs (Habiba et al.,2014), but for the cultivar of *Dendrobium kingianum* 'Hallelujah' results shown that high concentration of BAP produced the maximum number of PLBs (15.7/explant) and size is also big to compare with other concentrations of BAP.

The effect of 1-Naphthaleneacetic acid (NAA) on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'. 1-Naphthaleneacetic acid (NAA) is a synthetic plant hormone in the auxin family, it is a rooting

agent and used for the vegetative propagation of plants. It is also use for plant tissue culture. It is an amide between glucosamine and acetic acid. The Effect of NAA on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah' with different concentrations.

Table 4. Effect of 1-Naphthaleneacetic acid (NAA) on the organogenesis in PLBs of *Dendrobium kingianum* 'Hallelujah'

Concentration		Avg. No. of PLBs	Avg. No. of shoots	Avg. No. of roots	Fresh weight (g)
NAA(mg/L)	0	6.1±0.2bc	0.07±0.02a	0.13±0.05a	0.1±0.005ab
	0.01	11.1±0.5ab	----	----	0.2±0.005a
	0.1	8.2±0.3bc	----	----	0.1±0.003ab
	1	14.9±0.3a	0.20±0.05a	0.33±0.07a	0.2±0.003a
	10	5.3±0.3c	----	----	0.09±0.004c

Value represents means ± SE followed by the different letters in the same column indicate significant differences using Tukey HSD tests at the 5% level.

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

In this study results showed that low concentrations of NAA have promotive effect on PLBs formation. Successful utilization of tissue culture for the improvement and groundnut will be made possible only with an efficient shoot proliferation, formation of well-developed root system in microshoots, successful acclimatization and final establishment in field (Abdulmalik et al., 2012). Our result also shown that in low concentration 1 mg/L of NAA the PLBs, shoots and roots are significant effect of this treatment. Successful utilization of tissue culture for the improvement and conservation of groundnut will be root system in micro shoots, successful acclimatization and final establishment in field. In our result also shown that 1 mg/L of NAA produce the maximum number of PLBs (14.9/explant) and also roots (0.33/explant) this treatment (Table 4). The maximization of orchid production is leading to higher people demand for technical assistance and information in order to develop orchid growth, development, production and quality with quantity. The culture media are supplemented with phytohormones for controlling the efficient proliferation of PLBs, shoots and roots in orchidaceae plants *in vitro*. The different sources of LEDs are used in this study and all are widely available and generally viewed as well safe materials for environment

and also for human. Present studies clearly demonstrated that application of HA, 5-ALA, NAG, NAA and sodium alginate at low concentration significantly enhanced the formation of PLBs and shoots for *Dendrobium kingianum* ‘Hallelujah’ and during the culture period. The role of light quality in controlling in vitro morphogenesis is still obscure. Based on our studies, green LEDs and white LEDs were the most effective light source in propagation of *Dendrobium kingianum* cultivars.