（様式５）(Style5)

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

氏名：　Shahrin Sharmin
Name

学位論文題目：
Title of Dissertation　Studies on *in vitro* organogenesis of an *Oncidium* cultivar (オンシジウムの*in vitro*における器官形成に関する研究)

学位論文要約：
Dissertation Summary

Background of the study

Orchid is an important group of ornamental plants comprising of several thousand species and hybrid. Among this huge number of species *Oncidium sp.* (Commonly known as dancing lady) is a tropical epiphytic orchid comprising over 750 species. They can last several weeks thereby providing a marketing potential worldwide as cut flowers and potted plants. Generally, *Oncidium* is propagated by simple division of pseudobulbs vegetatively but it is time-consuming and expensive. On the other hand, propagation from seeds is highly undesirable due to high heterozygosity. Micropropagation provided an important breakthrough for mass propagation of orchid species which have high heterozygosity and very slow sexual reproduction capability (Kanjilal et al., 1999).

The success of plant tissue culture is highly influenced by the nutritions supplied in the media, carbohydrate and growth regulators. The media used for tissue culture of orchids is mainly high in salt, minerals, vitamins, growth regulators and water (Uddain et al., 2015). Carbohydrate (CHO) is an essential component for plant tissue culture as it supplies energy to the plants particularly during the early stage of tissue culture when plantlets are not able to photosynthesize their own food (Al-Khateeb, 2008). CHOs can be added in the form of simple or complex sugars (Akter et al., 2007). Among CHOs, sucrose has generally been used as the most suitable source of CHO (Mehraj et al., 2019). Another source of CHO, Trehalose, a nonreducing disaccharide, is found in some organisms including several plants in which it serves as an osmo protectant. It stabilizes proteins and biological membranes under a variety of stress conditions including hydrostatic pressure and osmotic stress (Benaroudi et al., 2001). It has been in many reports that various environmental factors affected on the growth and development of plants in both acclimatization and in vitro culture.

Among the environmental factors, light is one of the most important abiotic factor affecting the growth and development of plant species (Sivakumar et al., 2006). Light act as an energy source that regulates the photosynthesis process allows the plant to capture the light energy and turn in to chemical energy in the form of carbohydrate. Over the years in *in vitro* culture, fluorescent lights (FLs) are a common source of light but FLs have some disadvantages such as high electricity consumption with low output efficiency ultimately resulted in high production cost and it produces an unnecessary wide range of wavelengths (350–750 nm). To solve this in recent years, with the technological advancement of light emitting diodes (LEDs), numerous studies have been carried out on a variety of plant species to investigate the effects of LEDs as an alternative light source for *in vitro* culture. Different light colors such as blue, red, far-red, green and yellow are commonly used *in vitro* system.

LED as a new light source have many advantages 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). Red and blue LEDs alone or in combination have been used for studies in photosynthesis, chlorophyll synthesis and morphogenesis in many plants and orchid (Lee et al., 2007; However, few studies have been carried out on the effect of green LED lighting on plants growth and morphogenesis of *in vitro* culture.

Plant hormones, also called phytohormones, are organic compounds other than nutrients that are naturally produced by plant tissues in response to specific stimuli. In orchid micro propagation, 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). Auxins (NAA-K) and cytokinin (TDZ) were found to be most effective in regenerating plantlets in a number of orchids (Nayak et al., 1997).

In vitro condition polyethylene glycol (PEG) can induce water stress in plants (Ruf et al., 1967; Kaufman et al., 1971). PEG is a non-penetrating inert osmoticum that lowers the osmotic potential of nutrient solutions, but it is not taken and is not phytotoxic (Lawlor, 1970). Mode of action of inducing water stress in the cultured plant cells as same as the cells of intact plants. Cross tolerance refers to the exposure of tissue to a moderate stress that induces resistance to another stress (Genoud et al., 1999); however, very little is known about how different stresses interact with each other. Alginate is a newly emerging 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.

Among different plant growth regulators, 5-ALA is a linear five carbon compound thought to act as a plant growth regulator (Nahar et al., 2014). 5-ALA is synthesized from glutamate in a reaction involving a glutamyl-tRNA intermediate and requires ATP and NADPH as cofactors (Wettstein et al., 1995). It has been reported in several studies that 5-ALA was involved in the regulation of plant growth, development and stimulates physiological activity (Bindu et al., 1998, Akram et al., 2013). Recently, ALA has been demonstrated to be involved in the PLB culture of *Cymbidium insigne* and *Cymbidium finlaysonianum* (Nahar et al., 2014), *Dendrobium kingianum* (Habiba et al., 2014) and *Phalaenopsis* 'Fmk02010 (Sultana et al., 2015). The effect of different LED using a certain concentration of 5-ALA was first reported in this study. Prior to the present study, the effect of fluorescent lamp *in vitro* propagation of *Cymbidium* has been reported by Nahar et al. (2015) using 5-ALA but no research had been reported using LEDs. NAG (N-acetyl-D-glucosamine) is a monosaccharide derivative of glucose. An amide between glucosamine and acetic acid play significant role in several biological functions (Nahar et al., 2011). Low concentration of NAG played a significant role in PLB and shoot proliferation of *Dendrobium kingianum* (Habiba et al., 2014). Similar results were found in *Epidendrum* 'Rouge Star No.8', *Cymbidium dayanum* and *Phalanopsis* 'Fmk02010 (Kaewjampa et al., 2010, Nahar et al., 2011, Sultana et al., 2015). Very few reports were avavible describing the effect of LED on the organogenesis of *Oncidium* Aloha 'Iwanaga' using NAG. Kamal et al. (2014) investigated the effect of LED on the organogenesis of *Cymbidium* hybrids using various concentrations of NAG. However, it has not been so far studies in *Oncidium* Aloha 'Iwanaga' *in vitro* yet.

Hyaluronic acid (HA) is a polymer of disaccharides; composed of D-glucuronic acid and N-acetyl-D-glucosamine. Hyaluronic acid (HA) is a linear heteropolysaccharide that is composed of repeating D- glucuronic acid and N-acetyl-glucosamine (GlcNAc) residues. Due to having growth regulative properties, HA is used as an additive in plant tissue culture (Kaewjampa et al., 2012). 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). 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 0.8～1.17×106 Da) or HA12 (Hyaluronic Acid with molecular weight 1.1～1.6×106 Da) in culture media increased PLBs of *Phalaenopsis* at a certain concentration. However, there was a lack of detailed information about the promotive effect of HA12 and HA20 on the organogenesis of *Oncidium*. Several reports showed the effect of HA9 on the organogenesis of several orchid species like *Phalaenopsis* 'Fmk02010' (Sultana et al., 2015, Mehraj et al., 2017), *Cymbidium* (Kaewjampa et al. 2012), *Dendrobium kingianum* (Habiba et al., 2014). Very few reports were available where effect of LEDs was demonstrated in the case of orchids specially in *Oncidium* Aloha 'Iwanaga'. Habiba et al. (2014) reported that effect of 6-Benzylaminopurine (BA) and Hyaluronic Acid (HA) under white LED on organogenesis PLBs of *Dendrobium kingianum*.

Therefore, considering these aspects our investigation was conducted in order to explore new methodologies of organogenesis by observing the The effect of environmental factors, plant hormones, biopolysacharides, elicitors and different LEDs on the organogenesis of *Oncidium* Aloha 'Iwanaga'.

Experiment 1
Effect of hyaluronic acid on the organogenesis of an *Oncidium* cultivar

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 0.8～1.17×106 Da) or HA12 (Hyaluronic Acid with molecular weight 1.1～1.6×106 Da) in culture media increased PLBs of *Phalaenopsis* at a certain concentration. 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'.

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 1210C 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).

Statistical Analysis

The data were analyzed using one-way analysis variance (ANOVA) and differences between means were tested using Tukey's HSD test ($p \leq 0.05$).

Results and summery

The effect of HA12 and HA20 on the organogenesis of *Oncidium* Aloha 'Iwanaga' using different concentrations after six weeks of culture. 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 HA12 and 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 (195.5mg) 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. The 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.

In sum, our findings indicated 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'.

Experiment 2

***In vitro* regeneration of an *Oncidium* cultivar using different concentrations of BAP under different LEDs**

Orchidaceae family is one of the largest and most diverse plant family producing beautiful flowers and exhibiting more than 25000 species and 700-800 genera (Samarfard et al., 1995). Among this huge number of species, *Oncidium* is one of the commercially important orchids. It produces brightly attractive flowers and highly adaptable to culture under a wide range of climatic conditions (Kalimuthu et al., 2007). Several factors work in conjunction which facilitates the reproduction of orchid in nature (Mondal et al., 2013). However, regulation of these factors is quite difficult which leads to inadequate production of orchid propagules through conventional way. On the contrary, through tissue culture method, a large number of propagules of desired quality can be produced within least amount of time (Alam et al., 2020). Several studies reported the *in vitro* culture of *Oncidium* using multiple parts of plant as the explants such as root tips (Kerbauy, 1984), leaf tissue (Chen et al., 1999), flower stalk internodes (Chen et al., 2000), leaf tip segments (Chen et al., 2003) and young leaves (Wu et al., 2004). Teixeira da Silva et al. (2005) stated protocorm-like bodies (PLBs) can be a good source of explant in the proliferation of orchids as these immature formations are meristematic and have strong potential for totipotency thus resulted in fast proliferation. Regarding growth promotors used in tissue culture system, cytokinin acts as the promoter of cell division and development of meristematic centers leading to the formation of organs, mainly shoots.

Among different types of cytokinins, 6-benzylaminopurine (BAP) is a first generation synthetic cytokinin that elicits plant growth and development by stimulating cell division (Siddiqui et al., 2011). Several reports are available showing the impact of BAP on organogenesis of orchid genera like *Oncidium* (Rahman et al., 2005), *Cymbidium* (Shimasaki et al., 1990) and *Dendrobium* (Habiba et al., 2014). On the other hand, importance of light among the other environmental cues is undeniable as it is a fundamental necessity in each stage of a plant's life cycle (Sivakumar et al., 2006). Plants under natural illumination conduct multiple physiochemical activities in its leaf to store the energy in the form of carbohydrate, a process termed as photosynthesis. As such, the quality of the light source is of prime importance in regulating plant physiology and morphology. Over the year's fluorescent lamps (FLs) were used a common source of light but FLs have some disadvantages such as high electricity consumption with low output efficiency ultimately resulted in high production cost and it produces an unnecessarily wide range of wavelengths (350–750 nm). Recently light emitting diodes (LEDs) emerged as a new alternative light source with many advantages over the conventional one, such as longer life, wavelength specificity and narrow bandwidth, less heat radiation and low power consumption (Kaewjampa et al., 2012). The effects of BAP under different LEDs have been demonstrated in *Vanilla* and *Dendrobium* (Bello-Bello et al., 2016 and Habiba et al., 2014). However, till now no such research is done regarding the organogenesis of *Oncidium* Aloha 'Iwanaga'. In light of this, the present study was conducted to determine the appropriate concentration of BAP for the organogenesis of *Oncidium* and the impact of subsequent LED spectrum treatment on it.

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 December 2019. After excising PLBs into singles, 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) was adjusted to pH 5.5-5.8 before autoclaving. BAP at various concentrations such as control (0), 0.01, 0.1, 1 and 10 mg/l were added to culture media. Jars (250 ml UM culture bottle; Asone, Japan) with plastic caps containing 30 ml of medium were used as culture vessels. Five explants were put in each culture vessel. Three culture vessels were used under each treatment. All cultures were maintained under white LED (NNLK41509, Panasonic, Japan) at 25±1^0 C for continuous photoperiod. Based on the results of the initial experiment culture media was prepared using 0.1mg/l BAP and each jar contained 30ml of medium. Five explants were put in each culture vessel and three culture vessels were used under each treatment. Jars were placed under different light sources e.g. green LED (Jefcom, LT20-G, peak wavelength: 517 nm), red LED (Jefcom, LT20-R, peak wavelength: 631 nm), blue LED (Jefcom, LT20-B, peak wavelength: 460 nm), white LED and white fluorescent lamp (FL20SS, Toshiba). Experimental data were collected after 6 weeks of culture by counting the number of PLBs, number of shoots and measuring the fresh weight (mg).

Statistical Analysis

The data were analyzed using one-way analysis variance (ANOVA) and differences between means were tested using Tukey's HSD test ($p \leq 0.05$).

Results and summery

In the experiment, addition of BAP in media stimulated both PLB and shoot proliferation (Table 01). Maximum numbers of PLBs (28.1/explant) were found at 0.1mg/l and minimum (9.6/explant) was

obtained with 0mg/l which was statistically similar to other concentrations. The highest formation rate of PLB (93.3%) was obtained with 0.1mg/l and the lowest (80.0%) was obtained with 0mg/l. No significant variation was found with the number of shoots and fresh weight. The highest formation rate of shoot (86.6%) and the lowest (66.6%) was obtained with 0mg/l. Regarding light spectrum evaluation significant variation was found under different light conditions. The highest number of PLBs (28.1/explant) was obtained with white LED to which blue LED showed statistical similarity whereas the lowest was obtained in a white fluorescent lamp. The maximum formation rate of PLB (100%) was obtained with white and blue LED whereas the lowest (60.0%) was obtained with green LED. The highest number of shoots (2.0/explant) was obtained with white LED and the lowest (0.2/explant) was obtained with statistically similar red and blue LED. The highest fresh weight (260.0 mg) was obtained with white LED and the lowest was observed in green LED.

In sum the experiment was conducted to determine the effective concentration plant hormone increased the generation of orchids. From the data of our experiment we found 0.1mg/l BAP effective compared to other concentrations and white LED accelerate PLB formation using that certain concentration.

References:

Akram, N. A. and Ashraf, M. (2013). Regulation in plant stress tolerance by a potential plant growth regulator, 5-aminolevulinic acid. Journal of Plant Growth Regulation, 32(3), 663–679.

Akter, S., Nasiruddin, K. M. and Khaldun, A. B. M. (2007). Organogenesis of *Dendrobium* orchid using traditional media and organic extracts. Journal of Agriculture and Rural Development, 5, 30-35.

Alam, M. M., Shimasaki, K., Habiba, S. U. and Jahan, N. (2020). Effect of alginate on protocorm like bodies (PLBs) formation of *Dendrobium kingianum* cultivar. Asian Journal of Crop, Soil Science and Plant Nutrition, 2(2), 62-67.

Al-khateeb, A. A. (2008). Regulation of *in vitro* bud formation of date palm (*Phoenix dactylifera* L.) cv. Khanezi by different carbon sources. Bioresource Technology, 99(14), 6550-6555.
Arditti, J. R. (1993). Micropropagation of Orchids. John Wiley and Sons, New York. USA.

Bello-Bello, J. J., Martínez-Estrada, E., Caamal-Velázquez, J. H. and Morales-Ramos, V. (2016). Effect of LED light quality on *in vitro* shoot proliferation and growth of vanilla (*Vanilla planifolia* Andrews). African Journal of Biotechnology, 15(8), 272-277.

Benaroudi, N., Lee, D. H. and Goldberg, A. L. (2001). Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. Journal of Biological Chemistry, 276(26), 24261-24267.

Bindu, R. C. and Vivekanandan, M. (1998). Hormonal activities of 5-aminolevulinic acid in callus induction and micropropagation. Plant Growth Regulation, 26(1), 15–18.

Chen, J. T. and Chang, W. C. (2000). Plant regeneration via embryo and shoot bud formation from flower-stalk explants of *Oncidium* Sweet Sugar. Plant Cell, Tissue and Organ Culture, 62(2), 95-100.

Chen, J. T. and Chang, W. C. (2003). Effect of GA3, ancymidol, cycocel and paclobutrazol on direct somatic embryogenesis of *Oncidium in vitro*. Plant Cell, Tissue and Organ Culture, 72(1), 105-108.

Chen, J. T., Chang, C. and Chang, W. C. (1999). Direct somatic embryogenesis on leaf explants of *Oncidium* Gower Ramsey and subsequent plant regeneration. Plant Cell Reports, 19(2), 143-149.

Genoud, T. and Metraux, J. P. (1999). Crosstalk in plant cell signaling: structure and function of the genetic network. Trends in Plant Science, 4, 503-507.

Habiba, S. U., Shimasaki, K., Ahasan, M. M. and Alam, M. M. (2014). Effect of benzylaminopurine (BA) and hyaluronic acid (HA) under white light emitting diode (LED) on organogenesis in protocorm-like bodies (PLBs) of *Dendrobium kingianum*. American-Eurasian Journal of Agricultural Environment Sciences, 14, 605–609.

Kaewjampa, N. and Shimasaki, K. (2012a). Effects of green LED lighting on organogenesis and superoxide dismutase (SOD) activities in protocorm-like bodies (PLBs) of *Cymbidium* cultured *in vitro*. Environmental Control in Biology, 50(3), 247-254.

Kaewjampa, N., Shimasaki, K. and Nahar, S. J. (2012b). Hyaluronic acid can be a new plant growth regulator for hybrid Cymbidium micropropagation. Plant Tissue culture and Biotechnology, 22(1), 59-64.

Kalimuthu, K., Senthilkumar, R. and Vijaya kumar, S. (2007). *In vitro* micropropagation, *Oncidium* sp. (Dancing Dolls). African Journal of Biotechnology, 6(10), 1171-1174.

Kanjilal, B., De Sarker, D., Mitra, J. and Datta, K. B. (1999). Stem disk culture: development of rapid mass propagation of *Dendrobium moschatum* (Buch.-Ham.) Swartz-An endangered orchid. Current Science, 77, 497-300.

Kaufmann, M. R. and Eckard, A. N. (1971). Evaluation of water stress control with polyethylene glycols by analysis of guttation. Plant Physiology, 47, 453-456.

Kerbauy, G. B. (1984). Plant regeneration of *Oncidium varicosum* (Orchidaceae) by means of root tip culture. Plant Cell Reports, 3(1), 27-29.

Lawlor, D. W. (1970). Absorption of polyethylene glycols by plants and their effects on plant growth. New Phytologist, 69, 501-513.

Mehraj, H., Alam, M. M., Habiba, S. U. and Mehbub, H. (2019). LEDs combined with CHO sources and ccc priming PLB regeneration of *Phalaenopsis*. Horticulturae, 5(34), 1-12.

Mondal, T., Aditya, S. and Banerjee, N. (2013). *In vitro* axillary shoot regeneration and direct protocorm-like body induction from axenic shoot tips of *Doritis pulcherrima* Lindl. Plant Tissue Culture and Biotechnology, 23(2), 251-261.

Nahar, S. J. and Shimasaki, K. (2014). Application of 5-aminolevulinic Acid for the *in vitro* micropropagation of *Cymbidium* as a potential novel plant regulator. Environmental Control in Biology, 52 (3), 117-121.

Nahar, S. J., Shimasaki, K. and Haque, S. M. (2012). Effect of different light and two polysaccharides on the proliferation of protocrom-like bodies of *Cymbidium* cultured *in vitro*. ActaHorticulturae, 956, 307-311.

Nayak, N. R., Patnaik, S. and Rath, S. P. (1997). Direct shoot regeneration from foliar explants of an epiphytic orchid *Acampe praemorsa* (Roxb.) Blatter and McCann. Plant Cell Reports, 16, 583-586.

Rahman, S. M. M., Islam, M. S., Sen, K. P. and Begum, F. (2005). *In vitro* propagation of *Oncidium taka.* Biotechnology, 4(3), 225-229.

Ruf, R. H., Eckard, E. R. & Gifford, R. O. (1967). Compounds of osmotic adjustment of plants to rapid changes in root medium osmotic pressure. Soil Science, 104, 159-162.

Samarfard, S., Kadir, M. A., Kadzimin, S. B., Ravanfar, S. and Saud, H. M. (2013). Genetic stability of *in vitro* multiplied *Phalaenopsis gigantea* protocorm-like bodies as affected by chitosan. Notulae Botanicae Horti Agrobotanici, 41(1), 177-183.

Shimasaki, K. and Uemoto, S. (1990). Micropropagation of a terrestrial *Cymbidium* species using rhizomes developed from seeds and pseudobulbs. Plant Cell, Tissue and Organ Culture, 22, 237-244.

Siddiqui, M. W., Bhattacharjya, A., Chakraboty, I. and Dhua, R. S. (2011). 6-benzylaminopurine improves shelf life, organoleptic quality and health promoting compound of fresh cut broccoli florets. Journal of Scientific and Industrial Research, 70(6), 461-465.

Sivakumar, G., Heo, J. W., Kozai, T. and Peak, K. Y. (2006). Effect of continuous or intermittent radiation on sweet potato plantlets *in vitro*. The Journal of Horticultural Science and Biotechnology, 81(3), 546-548.

Teixeira da Silva, J. A., Yam, T., Fukai, S., Nayak, N. and Tanaka, M. (2005). Establishment of optimum nutrient media for *in vitro* propagation of *Cymbidium* Sw. (Orchidaceae) using protocorm-like body segments. Propagation of Ornamental Plants, 5(3), 129-136.

Uddain, J., Ganasekaran, P., Zakaria, L., Lynn, C. B. and Subramaniam, S. (2015). The effect of different growth media, carbon source and PGRs on *Dendrobium* Broga Giant orchid's protocorm-like bodies (PLBs) proliferation supported with sem and tem analysis. Pakistan Journal of Botany, 47(2), 587-593.

Wettstein, V. D., Gough, S. and Kannangara, C. G. (1995). Chlorophyll biosynthesis. Plant Cell, 7(7), 1039-1057.

（注） 要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。
(Note) The Summary should be about 10% of the entire dissertation and may include illustrations