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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 of *Cymbidium in vitro* under controlled environments**

（制御環境下における*in vitro*でのシンビジウムの器官形成に関する研究）

学位論文要約：
Dissertation Summary

Introduction

Orchids belonging to the family Orchidaceae, are one of the largest and most evolved flowering plants. To meet the demand of orchids in the future, as well to conserve their biodiversity, the development and deployment of new technologies is very important for improving flower quality, resistance to biotic and abiotic stresses, and to rapidly mass propagation members of this phylum. The successful application of new approaches will help to further improve orchids and orchid products. *Cymbidiums* have been arguably the most important genus of orchid in horticulture. In this study four *Cymbidium* spp were used (*C. devonianum*, *C. pumilium*, *C. insigne* and *C. finlaysonianum*). The objectives of this study was to develop some new procedures of rapid organogenesis methods of *Cymbidium* orchid which become environmental friendly and safe; to identify the most effective light source for the rapid propagation of *Cymbidium* plantlets *in vitro*; to identify the suitable concentration of trehalose and also to determining the appropriate concentration of the application of plant growth regulators under controlled environments. To fulfill the objectives, four sources of lights were used such as white fluorescent tube, green LED, red LED and blue LED; different concentrations of trehalose used as a carbon sources with modified MS medium without use any plant growth regulators (chapter 2). To use plant growth regulators (PGR) with media is a traditional methods. PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements. The functions of the exogenous PGRs are quite different from orchid species to species. In this study, combination of cytokinin (6-Benzylaminopurine or BA) and elicitors (Hyaluronic acid or HA) were examined. BA is a first-generation synthetic cytokinin that elicits plant growth and development responses. HA is well known elicitors has been used in the applications in cosmetic, food, healthcare, and pharmaceutical fields (chapter 3). Jasmonates (jasmonic acid and its derivatives) are plant growth regulators that appear to be common in higher plants. Application of jasmonates and analysis of mutants indicate that jasmonates also have a significant role in various developmental processes of plants. In these studies combinations of two elicitors (hyaluronic acid and methyl jasmonic acid) of Cymbidium PLBs under white fluorescent tube were examined (chapter 4).

Materials and Methods

Modified MS medium were used for culture; MS medium with 412.5 mg/l ammonium nitrate, 950 mg/l potassium nitrate, 15, 20, 25 and 30 g/l trehalose, and 2.2 g/l Phytagel (Sigma) were used. BA , HA and Me-JA at concentrations of 0, 0.1, 1 and 10 mg/l, were added to culture media. The pH of the medium was adjusted 5.5- 5.8 using 0.1mM 2-(N-morpholino) ethanesulfonic acid sodium salt (MES-Na) before autoclaved at

121^0 C for 15 min. Jars of 250 ml (UM culture bottle, As one, Japan) with plastic caps were used, each bottle receiving 30 ml of medium. Five explants were placed in each culture vessel and 3/4 culture vessels were used for each treatments.

The cultures of *in vitro* plantlets were illuminated using different lights. Four sources of light were used: white fluorescent tube (National FL20SS), blue LED (Jefcom, P18W-E1701-B, peak wavelength: 450 nm), red LED (Jefcom, P18W-E1701-R, peak wavelength: 640 nm) and green LED (Jefcom, P18W-E1701-G, peak wavelength: 510 nm). The plantlets were exposed to a 16 h photoperiod for 3/4/6 weeks.

Results and Discussions

In chapter 2, the activity of trehalose with modified MS medium under different sources of lights on organogenesis of *Cymbidium* PLBs was demonstrated. Trehalose (a-D-glucopyranosyl a-D-glucopyranoside) is a disaccharide composed of two molecules of glucose. In spite of the fact, that its biosynthesis is similar to that of sucrose. The observation that trehalose can be used to preserve biological structures has been obtained from *in vitro* studies. And light emitting diodes (LEDs) have been proposed as a primary light source for space-based plant research chambers or bioregenerative life-support systems, and a potential alternative light source for *in vitro* plant growth and development. The increased use of LEDs in environmentally controlled closed-type plant production systems allows crop production throughout the year, regardless of external weather conditions.

Table 1. Effect of trehalose (15, 20. 25 & 30 g/l) with modified MS medium) under different sources of lights on organogenesis of *C. devonianum in vitro*

Trehalose (g/l)	Fresh weight (mg)				Average no. of PLBs				Formation rate of PLB (%)			
	WFT	GL	RL	BL	WFT	GL	RL	BL	WFT	GL	RL	BL
15	21.6	43.1	32.1	37.7	5.6	6.1	4.8	6.0	85	80	90	100
20	45.7	72.7	62.8	65.6	5.7	7.9	7.1	7.5	100	95	95	100
25	47.8	76.0	65.4	58.7	8.8	12.5	10.7	9.4	100	95	100	100
30	25.8	66.7	51.0	45.0	6.9	11.9	10.1	8.0	90	95	95	100

Each value represents means (n=20). PLB is calculated only growing of green PLB. At table, WFT= White fluorescent tube; GL= Green LED; RL= Red LED; BL= Blue LED

According to this study reports suggested that in *C. devonianum* 20 & 25 g/l trehalose induced maximum formation rate of PLB under white fluorescent tube; under green LED, blue LED and red LED 25 g/l trehalose induced maximum PLB formation within 3 weeks of culture (Table 1). In *C. pumilium* 25 g/l trehalose induced maximum PLB and shoot formation under white fluorescent tube, green LED and blue LED; under red LED 20 g/l trehalose induced best formation of PLB and shoot within 4 weeks of culture (Table 2).

Table 2. Effect of trehalose (15, 20. 25 & 30 g/l with modified MS medium) under different sources of lights on organogenesis of *C. pumilium in vitro*

Trehalose (g/l)	Fresh weight (mg)				Formation rate of PLB (%)				Formation rate of Shoot (%)			
	WFT	GL	RL	BL	WFT	GL	RL	BL	WFT	GL	RL	BL
15	39.7	58.2	32.5	46.9	75	80	75	70	0	20	0	0
20	53.5	68.7	66.3	45.7	90	100	100	100	30	50	40	40
25	48.7	82.8	47.2	87.4	85	100	85	100	50	70	15	55
30	32.4	69.8	39.8	41.9	80	80	75	80	20	25	0	20

Each value represents means (n=20). PLB and shoot is calculated only growing of green PLB. At table, WFT= White fluorescent tube; GL= Green LED; RL= Red LED; BL= Blue LED

In *C. insigne* 15 g/l trehalose induced new PLBs, shoot and roots after 6 weeks of culture very successfully and green LED is a suitable light source for *C. insigne*. 100% PLB formation observed under green LED and red LED. Maximum shoot formation 80% observed under green LED but root formation found green and blue LED. At this study, trehalose used as a carbon sources with modified MS medium and new formation occurred within short duration of culture periods (Table 3).

Table 3. Effect of trehalose (15 g/l with modified MS medium) under different sources of lights on organogenesis of *C. insigne in vitro*

Light Treatments	Fresh weight (mg)	Formation rate of PLB (%)	Formation rate of Shoot (%)	Formation rate of Root (%)
White fluo. tube	31.8	65	15	0
Green LED	56.0	100	80	20
Red LED	52.1	100	55	0
Blue LED	40.4	95	65	25

Each value represents means (n=20). PLB and shoot is calculated only growing of green PLB. FW= Fresh weight; White fluo. Tube= White Fluorescent Tube.

In chapter 3 combination treatments of cytokinin (BA) and elicitors (HA) were conducted of *C. insigne* and *C. finlaysonianum* under white fluorescent tube. The combinations, concentration and the ratio of plant growth regulators are critically important for *in vitro* culture techniques. Plant growth regulators such as 6-benzylaminopurin (BA) is a first-generation synthetic cytokinin that elicits plant growth and development responses; hyaluronic acid (HA) has been reported to act as a plant growth regulator and considered to elicit the induction of plant defense mechanisms in many plants. Results of this study revealed that, among all the concentrations and combinations, 0.1 mg/l BA+0.1 mg/l HA were found to be the most effective combination concentration for PLB, shoot formation and maximum fresh weight of PLBs of *C. insigne*. Figure 1 shown the effect of 6-benzylaminopurine (BA) and hyaluronic acid (HA9) on formation rate of PLB and shoot of *C. insigne*.

Figure 1

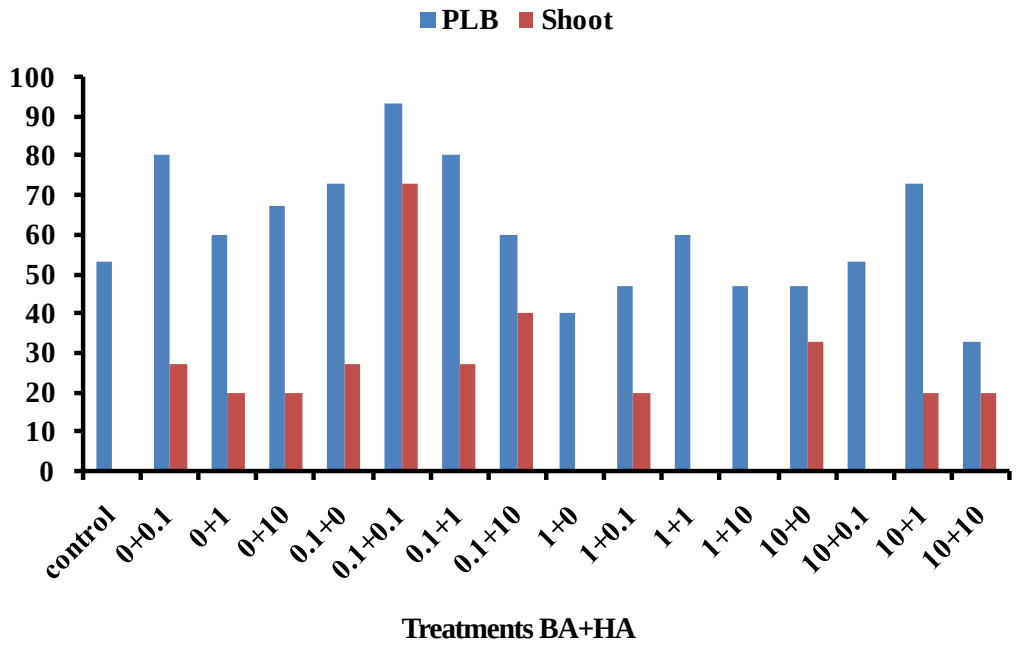
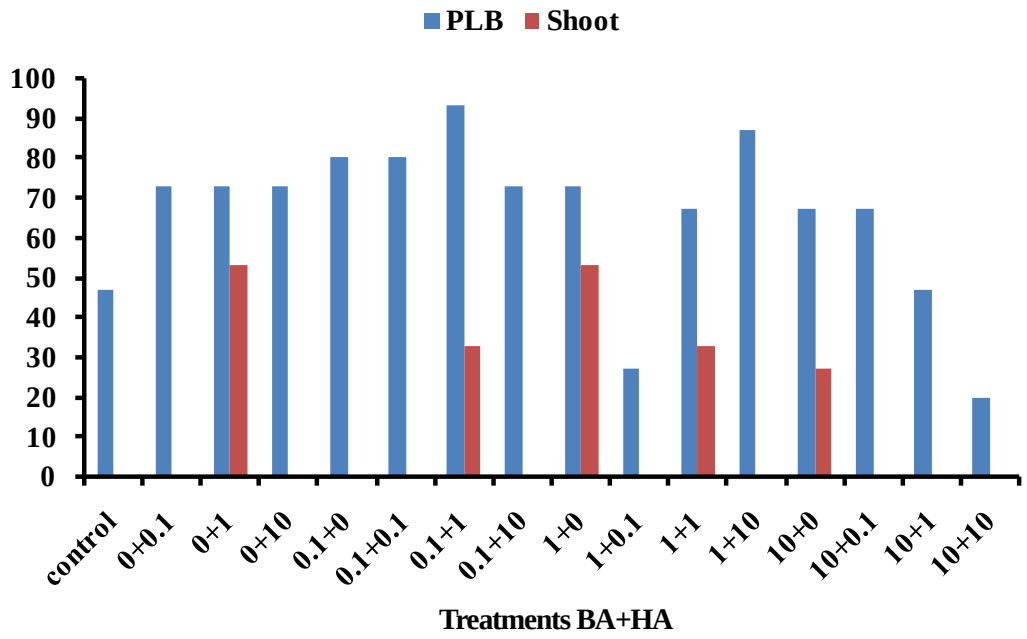


Figure 2 shown the effect of 6-benzylaminopurine (BA) and hyaluronic acid (HA9) on formation rate of PLB and shoot of *C. finlaysonianum.*

Figure 2

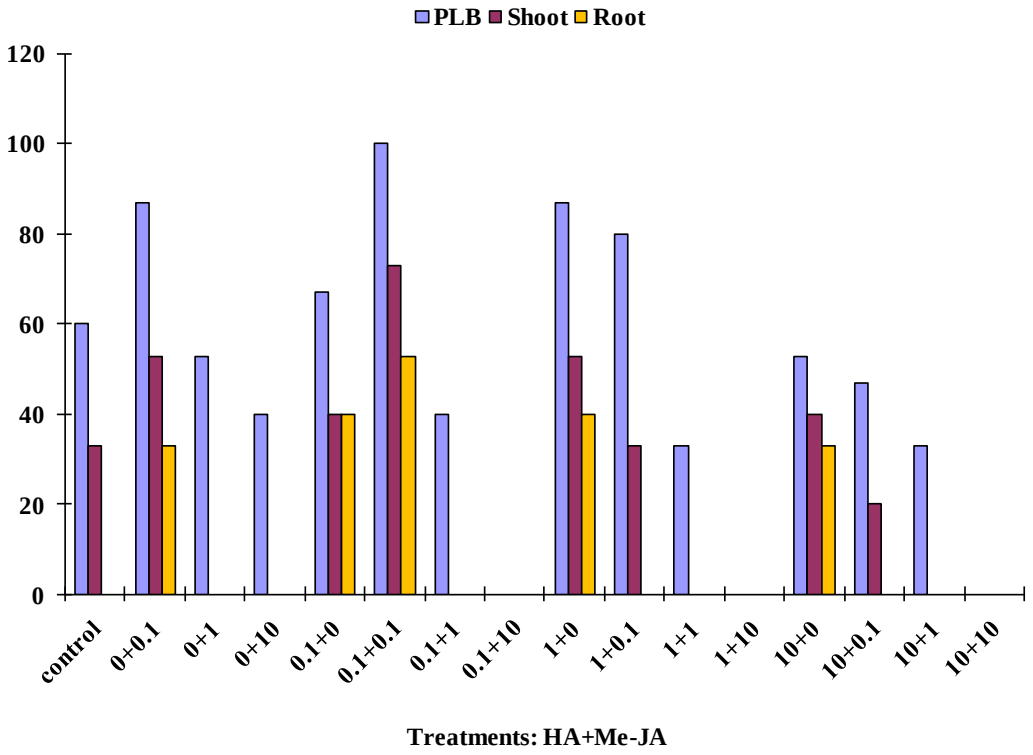


In *C. finlaysonianum* 0.1 mg/l BA + 1 mg/l HA was found to be effective for maximum number of PLBs, highest formation of PLB and shoot formation was effective for single addition of BA and HA treatment.

In chapter 4 combination treatments of methyl-jasmonate (Me-JA) and hyaluronic acid (HA) of *C. finlaysonianum* PLBs were conducted under white fluorescent tube. Elicitors are substances that induce protective responses in plants. Me-JA (methyl jasmonates) is well known elicitors and particularly interesting because of the myriad of plant responses associated with its synthesis and presence. Me-JA is emitted by wounded plants and therefore, may represent a means of communication between damaged plants. Figure 3 shown the effect of hyaluronic acid (HA) and methyl-jasmonate (Me-JA) on formation rate of PLB, shoot and root of *C. finlaysonianum.* According to this study, in *C. finlaysonianum* 100% PLBs, 73% shoot, 53% root formation observed on medium containing 0.1 mg/l HA + 0.1 mg/l Me-JA (when PLBs were dipping 30 minutes at Me-JA aqueous solution than cultured on modified MS media with HA) after 6 weeks of culture. The maximum average numbers of PLBs, shoots, roots and maximum fresh weight also found at same treatments. Findings of this study, 10 mg/l Me-JA did not induced any shoot and root within cultured period.

Elicitation is the induction of secondary metabolite production by either biotic or abiotic treatments. Nowadays, the use of pathogenic and non-pathogenic fungal preparations and chemicals as elicitors has become one of the most important and successful strategies to improve secondary metabolite production in plant cell culture. The use of elicitor as a plant growth stimulator is of interest as it is a widely available and generally viewed as a safe material for humans and the environment. *In vitro* culture plays important roles in the propagation of plants in large quantities with desired characters, and also has been used as a tool for the conservation and rapid propagation of plants. Based on present study, under different sources of lights-different concentrations of trehalose needs for rapid formation of PLB, shoot and root of *Cymbidium* spp. *in vitro.*

Figure 3



Plants treated with elicitors develop a general resistance. The use of phytohormones has a massive impact on the environment and could cause the formation of abnormal PLBs. This study reports establish a new protocol on organogenesis of *Cymbidium* spp. through the combination treatment of 6-benzylaminopurine (BA) and hyaluronic acid (HA) and also combination treatments of methyl jasmonate (Me-JA) and hyaluronic acid (HA) *in vitro*. There were no malformations observed on regenerated shoots within culture periods. As biological control becomes more prevalent, useful and important in horticultural crop production, targeted use of elicitors induced defenses may provide valuable augmentation of plant production. Finally it would be concluded that all of these (described) procedures were new, easy and safe for environment which would be fulfill our objectives successfully.