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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 environmental control of organogenesis in orchids
（ランの器官形成における環境制御に関する研究）

学位論文要約：
Dissertation Summary

Orchids are one of the most popular ornamental crops worldwide. Demands and consumption of orchids in Asian countries around Japan have been steadily increasing. The increase in orchid production is leading to higher public demand for technical assistance and information in order to improve orchid growth, development, production and quality. The development of more efficient micropropagation methods will be advantageous for orchid industry. In orchid, the growth and development *in vitro* are affected by host of factors as well as environmental factors (plant growth regulators, light quality, light intensity, temperature, photoperiod, humidity etc.) during the culture period. Among the various environmental factors, plant growth regulators and light are the most important variables affecting growth and development of tissue cultures on many plant species. In chapter I, 5-aminolevulinic acid (5-ALA), lysozyme and Me-JA (methyl jasmonate) were used as elicitors on organogenesis through PLBs culture *in vitro* of *Cymbidium* spp. In *C. insigne*, a 100% PLB formation and a high number of PLBs (7.9 PLBs/explant) were observed in medium containing 1 mg/l ALA. The maximum percentage of shoot formation (66.7%) was obtained with 0.1 mg/l ALA and was significantly different compared to the control treatment. Similarly, in *C. finlaysonianum*, 0.01 mg/l and 0.1 mg/l ALA had significant effects on growth of new PLBs and shoot formation. In *C. dayanum*1 mg/l ALA had significant effects on growth of new PLBs, shoot and root formation. Other new elicitor lysozyme, successfully worked as a plant growth regulator in PLBs culture of *C. insigne*. Dipping (PLBs) into 30 minutes at lysozyme aqueous solution, 0.1 mg/l lysozyme significantly increased the number of PLBs, shoot and root formation. Dipping (PLBs) into 60 minutes at lysozyme aqueous solution, 1 mg/l lysozyme increased average number PLBs and formation rate of PLB was high. Me-JA is a catabolite of JA and both activate genes controlling the secondary metabolic pathways and are highly active elicitor compounds inducing similar responses in several plant species. PLBs of *C. insigne* were dipping into different concentrations of Me-JA aquoues solution into 30 minutes and 60 minutes; than PLBs were proliferated into modified MS medium for 6 weeks under white fluorescent tube. Results of this study revealed that 0.1 mg/l Me-JA had effective for PLB and shoot formation. Maximum PLB and shoot formation were observed which PLBs were dipping into 30 minutes under Me-JA aqueous solution. Hyaluronic acid (HA) and *N*-acetylglucosamine (NAG) are of elicitors, which became worldwide used as plant growth regulators in orchid propagation. In chapter II, combination effect of this two (HA and NAG) polysaccharides were conducted. Results of this present study indicated that in *C. insigne*, the combination treatment of HA and NAG was more effective than single addition. In C. insigne, 0.1 mg/l HA + 0.1 mg/l NAG was effective for the highest percentage of PLB and shoot formation. In *C. finlaysonianum*, 0.1 mg/l HA+ 10 mg/l NAG was more effective for the highest percentage of PLB formation. Single addition of NAG with culture medium was effective for promoting shoot formation. 20% root formation found single addition of NAG (0.1 and 10 mg/l) HA (0.1 mg/l) treatment respectfully. In *C. dayanum*, 0.1 mg/l HA + 1 mg/l NAG was effective for the highest percentage of PLB and shoot whereas lowest formation rate was found at single addition of NAG (40% and 20%) treatment. Chondroitin sulfate is polysaccharide elicitor, which was firstly used in *Cymbidium* tissue culture. Application of chondroitin sulfate in growing media, new PLB, shoot and root were successfully regenerated within

6/8 weeks of culture. In *C. insigne* 0.1 mg/l chondroitin sulfate was effective; in *C. finlaysonianum*, 1 mg/l chondroitin sulfate and in *C. dayanum*, 10 mg/l chondroitin sulfate was more effective for PLB and shoot formation after 6/8 weeks of culture. In chapter III, comparative study was conducted with two elicitors (Chitosan H and HA9) under different sources of lights. Green LED promoted on the proliferation of protocorm-like bodies (PLBs) of *C. insigne* and *C. finlaysonianum* and blue LED promoted on the PLBs of *C. dayanum in vitro*. It should therefore be possible to improve the induction of PLBs, shoot and root formation by using chitosan and hyaluronic acid under different light on *Cymbidium* spp. *in vitro*. Effects of chondroitin sulfate under different sources of lights were conducted. These experiments indicated that if chondroitin sulfate added to culture media acts as plant growth stimulator to induce PLB and shoot formation of *Cymbidium* spp. *T*here was no malformation observed in regenerated shoots. Based on this experiment results, it is hypothesis that effect of chondroitin sulfate on orchid tissue culture partly depend on the action of elicitor. The present study demonstrated the potential of another polysaccharides, chondroitin sulfate could induce PLB and shoot formation in *Cymbidium* orchid plants. In *C. finlaysonianum*, 10 mg/l chondroitin sulfate was effective under green LED, 0.1 mg/l chondroitin sulfate was effective under blue LED and 0.1 mg/l and 1 mg/l chondroitin sulfate was effective under red LED for highest PLB formation. In *C. insigne*, 0.1 mg/l chondroitin sulfate under red LED was effective for highest formation of PLB and shoot. Every concentration of chondroitin sulfate was induced 100% PLB under green LED. In *C. dayanum* 1 mg/l chondroitin sulfate was effective under blue LED, 0.1 mg/l chondroitin sulfate was effective under green LED and 1mg/l and 10 mg/l chondroitin sulfate was effective for highest formation of PLB and shoot. In chapter IV, disaccharide (trehalose) were used as an alternative of sucrose on modified MS medium than added HA9 at different concentrations. Results of the study revealed that trahelose and HA successfully regenerated new PLB, shoot and root in *C. insigne* and *C. dayanum*. 0.1 mg/l HA9 and 1 mg/l HA9 with trehalose induced maximum PLB formation and 10 mg/l HA9 with trehalose induced maximum shoot formation after 5 weeks of culture. In C. dayanum, 0.01 mg/l HA9 with trehalose induced maximum PLB and shoot formation after 3 weeks of culture. PLBs of *C. insigne*, were cultured on modified MS medium under four sources (white fluorescent tube, green LED, red LED and blue LED) of lights. Disaccharide (trehalose) was used as an alternative of sucrose. Results of this study revealed that compare with four sources of lights, green LED with trehelose had good effect for PLB (100%) formation and blue LED and trehalose increases maximum shoot (68%) formation after 6 weeks of culture. Overall this study results suggest that trehelose can be used as an alternative of sucrose with modified MS medium. In chapter V, different sources of lights were used for regeneration of *Anoectochilus formosanaus in vitro*. Compare with five different sources of lights (white fluorescent tube, green LED, blue LED, red LED, red FEL), green LED was best light source for organogenesis of *Anoectochilus formosanaus* plantlets *in vitro*. Shoot tips of *A. formosanaus*, dipping into MeJA and HA9 aqueous solution at three hours, 0.1 mg/l MeJA was effective for maximum fresh weight, highest leaf number and highest rhizome number. 100 mg/l HA9 was effective for maximum fresh weight. The results indicated that *A. formosanaus* shoot tips were successfully increased fresh weight, leaf number, root number and also elongated leaf size. Reports of this study suggest an easy, fast and reliable *in vitro* regeneration system for the propagation system of *A. formosanaus*.

Phytohormones, both from natural substance, such as coconut water, which contain cytokinin and synthetic ones, such as 6-benzyl amino purine (BAP), thidiazuron (TDZ), naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA), have been carefully applied to stimulate PLB and plantlet development. The disadvantages of using synthetic hormones are the additional costs, the inhibitory effect on shoot regeneration and the increased mutation rate amongst the plantlets produced. The use of elicitors/polysaccharides elicitor as a plant growth regulator for orchid plants has attracted considerable interest in recent years because of; it is a widely available and generally viewed as a safe material for humans and the environment.

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