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学 位 論 文 要 旨
Dissertation Abstract

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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）

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Orchids are marketed both as plants and as cut flowers and their production has been increased in recent years. *In vitro* propagation of orchids as an option for rapid propagation of commercially valuable cultivars progressed well during the last decades. Plant organogenesis *in vitro* is a more controllable and reliable process. Studies on environmental control of organogenesis in orchids describe some new organogenesis methods of orchid tissue culture by using different types of plant growth regulators/elicitors under different qualities of LED lights.

Chapter I. The effects of elicitors (5-aminolevulinic acid and lysozyme) on organogenesis of *Cymbidium* spp. were conducted. 5-aminolevulinic acid (5-ALA) has been suggested as a new natural and environmentally friendly regulator, which can be widely used in agriculture. In this study, 5-ALA added with modified MS medium in PLBs culture of *Cymbidium* spp. under white fluorescent tube. In *C. finlaysonianum*, 0.01 and 0.1 mg/L ALA supplementation in culture medium enhanced the best formation of PLBs and shoots, in *C. dayanum*, 1 mg/L ALA induced highest formation of PLBs, shoots and roots and in *C. insigne* 1 mg/L ALA with modified MS medium increased maximum number of PLBs and maximum formation of PLBs. 0.1 mg/L induced highest number of shoots and highest formation rate of shoots. These study results suggest that low concentrations of ALA added with culture medium enhance PLBs, shoots formation compare with control treatment. In this study lysozyme was firstly used as a plant growth regulator in *Cymbidium* tissue culture. Lysozymes are enzyme, also known as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases. 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 PLB, shoot and root formation of *C. insigne*.

Chapter II. The combination of HA and NAG (N-acetyl-D-glucosamine) at different concentrations with modified MS medium under white fluorescent tube was studied. NAG is a monosaccharide derivative of glucose; it is an amide between glucosamine and acetic acid. In *C. finlaysonianum* highest PLBs formation were found at combination of HA 0.1 mg/L + 10 mg/L NAG, in *C. dayanum*, cultures the maximum formation rate of PLBs, shoots and roots were found in treatment of 0.1 mg/L HA + 1

mg/L NAG. In *C. insigne* cultures, thr highest formation rate of PLBs and shoots were found combination of 0.1 mg/L HA + 0.1 mg/L NAG with modified MS medium. These results suggest that combination of two polysaccharides had best effect in PLBs culture of *Cymbidium* spp. compare with single 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.

Chapter III, comparative study was conducted with two elicitors (Chitosan H and HA9) under different sources of lights. These study results suggested that green LED is best light source for *C. insigne* and *C. finlaysonianum* and blue LED is best light source for *C. dayanum* PLBs culture and it should therefore be possible to improve PLB and shoot formation by using HA and chitosan under different light in *Cymbidium* spp. *in vitro*. Effects of chondroitin sulfate under different sources of lights were conducted. 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.

Chapter IV, disaccharide (trehalose) was used as an alternative of sucrose on modified MS medium than added HA9 at different concentrations. Results of the study revealed that 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. insigne*. In *C. dayanum*, 0.01 mg/l HA9 with trehalose induced maximum PLB and shoot formation after 3 weeks of culture.

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*. In other experiment, 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*.