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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 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）

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Light is one of the basic factors needed by plant for its development. For horticultural researchers and crop producers to benefit from LEDs (light emitting diodes) use, a variety of preliminary findings should be considered. The increased use of LEDs in environmentally controlled closed-type plant production systems allows crop production throughout the year, regardless of external weather conditions. *Cymbidiums* have been arguably the most important genus of orchid in horticulture. The majority of *Cymbidium* spp. are commercially produced using tissue culture methods. 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 environment. 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. 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. 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. 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*. At these study, trehalose used as a carbon sources with modified MS medium and new formation occurred within short duration of culture periods.

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*. 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. 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.

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*. 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.