学位論文要旨 Dissertation Abstract

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学位論文題目: Studies on organogenesis of *Cymbidium* cultured *in vitro* Title of Dissertation (*In vitro* におけるシンビジウムの器官形成に関する研究)

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Different environmental factors affecting in vitro organogenesis in rhizome-forming type Cymbidium (Cymbidium goeringii 'Getsurin', Cymbidium encifolium 'Saga' and Cymbidium kanran 'Murotonishiki'), and in PLB (protocorm-like body)-forming type Cymbidium (Cymbidium Sweet Waffle 'Tarte' and Cymbidium floribundum) were studied in 4 chapters. Chapter 1. Effect of different plant growth regulators (PGRs) and elicitors in modified MS media on the organogenesis in rhizome culture of Cymbidium species was studied. The addition of 30 mg/l and 0.1 mg/l adenine sulphate (AdS) to culture media enhanced the regeneration of rhizome and shoot in Cymbidium kanran 'Murotonishiki' and Cymbidium goeringii 'Getsurin', respectively. Application of thidiazuron (TDZ) to culture media was found to reduce the organogenesis by developing protocorm-like shoots (PLS) in both Cymbidium species. The addition of 0.01 mg/l naphthaleneacetic acid potassium salt (NAA-K) with 0.1 mg/l AdS to the culture media, and addition of sodium hyaluronic acid (0.1 mg/l FCH and 0.01 mg/l FCH-SU; Kikkoman) to PGR free culture media was effective in inducing plantlet in Cymbidium goeringii 'Getsurin'.

Chapter 2. Effects of various light sources and intensities on the organogenesis of different *Cymbidium* species were studied. At 40 µmol/m²/s, red LED enhanced the regeneration of rhizomes and shoot in *Cymbidium kanran* 'Murotonishiki', and green LED enhanced PLB formation and red LED increased regeneration of shoots and roots in *Cymbidium* Sweet Waffle 'Tarte'. Light sources with 100 µmol/m²/s intensity were found inhibitory for organogenesis in both species. Alternate green and red LED enhanced PLB proliferation, shoot formation and fresh weight in *Cymbidium* Sweet

Waffle 'Tarte'.

Chapter 3. The effects aluminum chloride (AlCl₃) under different light sources, polyethelene glycol (PEG), and combined effect of AlCl₃ and PGRs in modified MS media on the organogenesis of different *Cymbidium* species cultured *in vitro* were studied. Culture media supplemented with 1.0 mg/l AlCl₃ enhanced PLB formation under green LED, and shoot formation from PLBs and root formation from shoot under red LED in *Cymbidium* Sweet Waffle 'Tarte'. It also induced shoots from rhizome and developed roots from shoot under red LED in *Cymbidium kanran* 'Murotonishiki. 10 mg/l AlCl₃ in culture media was found inhibitory for the organogenesis because it resulted PLS in both *Cymbidium* species. Addition of PEG to culture media at lower concentrations (< 0.1 mg/l) was not effective but higher concentrations (1.0 - 10 mg/l) acted as an inhibitor for organogenesis in rhizome culture of *Cymbidium encifolium* 'Saga'. Addition of AlCl₃ to the basal media was found to suppress the activity of 6-Benzylaminopurine (BAP), TDZ and Gibberellic acid (GA₃). Addition of auxin to culture media supplemented with AlCl₃ inhibits the effect of AlCl₃ in rhizome culture of *Cymbidium*.

Chapter 4. Effects of citric acid (CA), and iron salt in modified MS media on the organogenesis of different *Cymbidium* species were studied. Addition of 0.1 g/l CA in culture media reduced the browning intensity in the cultured PLBs of *Cymbidium floribundum*, which significantly increased PLB formation, shoot formation from the PLBs, and root formation from the shoots. Addition of CA in culture media was found ineffective in rhizome culture of *Cymbidium encifolium* 'Saga' and *Cymbidium kanran* 'Murotonishiki'. Reducing iron salt concentrations (Fe-EDTA) at quarter in modified MS medium enhanced the regeneration of shoots and roots, and using CA as an additive increased PLB proliferation in *Cymbidium floribundum*. In *Cymbidium encifolium* 'Saga', Fe-EDTA at full concentration in modified MS media enhanced the rhizome branch formation, increased the regeneration of shoots and roots, and developed no protocorm-like rhizome (PLRh).

This study first time reported that AlCl₃ can enhance organogenesis of *Cymbidium in vitro* in PGR free culture media, and can inhibit activity of PGRs on *in vitro* growth of rhizomes. It also confirmed that the responses of organogenetic pathways of PLBs and rhizome culture of *Cymbidium in vitro* to various factors are different. Alternative red and green LED, reduced concentration of iron salt in modified MS media and applying culture media additive like AlCl₃, CA at lower concentration are suitable for improved PLB proliferation and shoot organogenesis in PLB culture of *Cymbidium*. Red LED, full concentration of iron salt in modified MS media and applying culture media additive like AlCl₃, sodium hyaluronate at lower concentration are suitable for improved rhizome formation and shoot organogenesis in rhizome culture of *Cymbidium*.