

Patcharawadee Wattanawikkit 2014: Development of *In Vitro* and Cryogenic Protocols for *Ex Situ* Conservation of Orchids. Doctor of Philosophy (Botany), Major Field: Botany, Department of Botany, Thesis Advisor: Associate Professor Sureeya Tantiwiwat, Ph.D. 123 pages.

This study has derived a novel *in vitro* method for asymbiotic clonal propagation of *Caladenia latifolia* R.Br. (an endemic terrestrial orchid species from Western Australia) for horticulture and breeding from seedling explants. The highest induction of protocorm rate and size were achieved in MS medium plus 10 μM 2,4-D for a 90 day incubation period. The most effective medium for protocorm proliferation was $\frac{1}{2}$ MS plus 10 μM BAP. The best shoot induction and protocorm size were obtained on PGR-free Thomale GD medium. The development of a cryopreservation protocol for orchid protocorms of *C. latifolia* was showed that large cryopreserved protocorms had the highest viability and potential survival rate. There were no significant differences in potential survival among desiccation medias, cryoprotectant solutions, and incubation time at 0 °C. Potential protocorm survival was also not significantly different among recovery media treatments.

Development of *in vitro* propagated-protocorms of *Paphiopedalum insigne* (Wall. ex Lindl.) Pfitzer (endangered Thai slipper orchid) was also investigated. The result revealed that $\frac{1}{2}$ MS medium containing 10 and 40 μM BAP induced the greatest multiple shoot numbers. Nevertheless, both BAP and TDZ had no significant effect on the shoot length. Although the $\frac{1}{2}$ MS plus 5 μM TDZ or 20 μM BAP gave the maximum root length, both BAP and TDZ inhibited root formation at above 10 μM . *In vitro*-propagated protocorms cultured on $\frac{1}{2}$ MS medium plus 0.8 M glycerol showed the highest potential survival of both non- and encapsulated protocorms following cryostorage. Protocorm exposure to PVS2 solution \geq 20 minute is sufficient for a great survival rate of non-encapsulation, the 90 minute air-drying time gave the best maximum survival rate of encapsulated protocorms.

Student's signature

Thesis Advisor's signature