

Establishment of In Vitro Regeneration Protocol for Sabah's Jewel Orchid, *Macodes limii* J.J. Wood & A.L. Lamb

ABSTRACT

Habitat disturbance and excessive collection of wild orchids from their natural habitat have threatened many orchids species at risk of extinction. In this study, the in vitro regeneration protocol for *Macodes limii*, a jewel orchid endemic to Sabah was established. The effects of explant source and plant growth regulators (PGRs) including naphthaleneacetic acid, picloram, 2,4-dichlorophenoxyacetic acid, 6-benzylaminopurine, kinetin, and thidiazuron on the in vitro regeneration capacity of *M. limii* plantlets were examined. Both factors showed a significant interaction in promoting axillary shoot formation. Nodal explants from the third and fourth positions cultured with 1.0 mg/L TDZ, induced 95% of shoot regeneration, with an average of three shoots/explant (1.6–1.8 cm of shoot length) after 90 days of culture. The well-developed plantlets went through an acclimatization phase for 60 days with a 60% of survival rate. An inter simple sequence repeat (ISSR) marker analysis confirmed the genetic stability of the in vitro regenerated plants to the mother plant. The successfully acclimatized plantlets were finally transferred to Poring Orchid Conservation Centre for reintroduction. The established protocol provides the means for large-scale production of this endemic jewel orchid, as well as a basis for further research aimed at the conservation and genetic improvement of this plant.