

Highly efficient proliferation and regeneration of protocorm-like bodies (PLBS) of the threatened orchid, *phalaenopsis bellina*

ABSTRACT

Phalaenopsis bellina is an important indigenous fragrant orchid threatened with extinction. In this study, we evaluated the effect of medium strength, sucrose, nitrogen (NH_4NO_3) and potato extract on proliferation of *P. bellina* protocorm-like bodies (PLBs) to improve micropropagation in this species. Optimal treatment for PLBs proliferation rate with an average fresh weight (FW) of 0.97 ± 0.16 g was obtained through culturing on half strength ($\frac{1}{2}$) MS medium containing 20 g/L sucrose, 15 mM NH_4NO_3 and 20% w/v potato extract supplemented with $0.8 \mu\text{M}$ 2,4 dichlorophenoxyacetic acid (2,4-D). The optimal treatment produced large, healthy and greenish PLBs with reduction in the occurrence of culture browning. In contrast, treatments with high potato extract ($>20\%$ w/v) or NH_4NO_3 (>30 mM) concentrations tend to have inhibitory effect and resulted in low PLBs proliferation rate, with an average FW of 0.77 ± 0.15 g and 0.69 ± 0.15 g, respectively. Plant regeneration of PLBs was achieved on plant growth regulator (PGR)-free $\frac{1}{2}$ MS medium. In total, 60 healthy PLBs from the optimal treatment were successfully regenerated, acclimatized with 100% survival percentage and grew well in a mixture of soil, sand and vermicompost (8:4:2 (w/w/w)). With the optimal treatment, PLBs proliferation rate was enhanced by 27.63%. Our findings offer an improved micropropagation protocol of the endangered *P. bellina* for conservation and commercial production.

Keyword: Indigenous orchid; *Phalaenopsis bellina*; Proliferation; Protocorm-like bodies; Regeneration