

“Discovery of biomarkers involved in high plasticity callus formation and regeneration in medical orchid *Dendrobium aurantiacum* via NMR metabolomics study”

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Orchids are highly traded for its medicinal and ornamental values; therefore it is prone to extinction due to overharvesting and many growth limiting factors including the ambiguous seed maturation period and specific dependent on the specific fungi for germination. Orchid as a sessile organism had demonstrated great adaptive mechanisms such as totipotency and high plasticity towards unfavourable and stressful environmental condition. Therefore, this study examines the potential of micropropagation technique by shoot culture in orchid, *Dendrobium aurantiacum* (F. Muell.) and to examine the metabolomic profiling and metabolomic changes at different callus stage and compared to the adult plant using Nuclear Magnetic Resonance and liquid chromatography mass spectroscopy approach. This study reported an effective aseptic technique to develop sterilized *D. aurantiacum* tissue *in vitro* and promote callus induction and regeneration from shoot explant with different plant growth regulators. Among the treatments, 20% sodium hypochlorite with 15 mins sterilization period showed the highest sterilization efficiency on explants with only $16.7 \pm 5.8\%$ of contamination occurred after two weeks and obtained the highest survival rate $73.3 \pm 5.8\%$ after one month. Callus formed in all combinations of plant hormone treatments. Media treated with 10 mg/L 2,4-D showed the highest callus induction rate, but browning conditions occur after 3 months of culture. Cell count on callus proliferation showed a significant difference ($p < 0.05$) between control and treatments. The callus induced from the plant tissue showed several similar metabolites as in the adult. There are more than 200 metabolites detected in all sample groups except white callus (WC) with only 126 metabolites and 155 metabolites in the root. There are various metabolites detected in *D.aurantiacum* including alcoholic, flavonoids, benzyl-derivatives, fatty acid, glucopyranoside, polysaccharides, phenolic, steroids and triterpenoids showed high medicinal and market values. It is hence can significantly be beneficial to the pharmaceutical industry as it indicates a shorter timeframe used to obtain the metabolites with the mass propagation of orchid callus. Metabolomics profiling at each development stage of callus formation and regeneration not only provide insight into the biological background of this process but also provide a range of possibilities for productivity and diversification toward sustainable agriculture.