

Optimum Density of Callus Inoculum and Sub-Culturing Interval in Callus Culture of *Gyrinops walla*

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Gyrinops walla is an endemic and endangered species of Sri Lanka, produces precious fragrant resin infused wood called, agarwood. *In vitro* callus culture of *G. walla* is the best and viable alternate to produce fragrant compounds commercially, while conserving the existing species. The present study was aimed to select optimum inoculum density and subculture interval in callus culture, which would be the foremost and determining step of successful callus culture and product synthesis. In the present study, five different densities of callus inoculums (100, 150, 200, 250 and 300 mg on fresh weight basis) were examined to choose the best inoculum density from the growth curves. Callus specific growth rate (mg/week) were calculated by regressing natural logarithms of callus dry weight overtime and doubling time were calculated by dividing the growth rate by 0.693. Highest cell biomass was assessed from the point where exponential growth declined. Sub-culturing frequency was determined as at which point the stationary phase commenced in the growth curve. Among the treatments, 200 mg fresh weight of callus was chosen as the optimum initial inoculum density with highest specific growth rate (398.3 mg/week on dry weight basis), fastest cell doubling time (1.74 weeks) and maximum cell biomass (128 mg dry weight). Sub-culturing interval was decided as end of six weeks of inoculation. Present study concluded that 200 mg fresh weight of initial inoculum density and six weeks of sub-culturing interval should be considered during the periodic sub-culturing of callus to maintain the healthy and alive culture with the ultimate aim of producing fragrant compounds through *in vitro* cultures of *G. walla*.

Keywords: Callus, Cell-doubling time, Inoculum density, Sub-culturing frequency, Specific growth rate