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Callus Development Potential of *Gymnema sylvestre* (Masbadda) in Organ Culture using Coconut Water in Media

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Gymnema sylvestre (Masbadda) is a valuable medicinal plant and a vulnerable species in Sri Lanka. This plant is widely used for its antidiabetic and pharmacological action because it contains "Gymnemic acid". However, seed germination and vegetative growth are suppressed in its propagation through conventional methods. Therefore, the present study was conducted to establish an efficient *in-vitro* callus induction. This study was intended to assess the influence of coconut water on callus development, and to determine the most suitable 2,4-D (2,4-dichlorophenoxyacetic acid) concentration for initiation and callus proliferation. Leaf explants were sterilized using 70% ethanol for 30 sec, then 0.5% NaOCl for 7 min, and 50 mg/L stock solution of streptomycin sulphate for 15 min, cultured on MS medium with varied coconut water levels (2.5%, 5%, 7.5% and 10%) and 2,4-D (2.0-2.5 mg/L). Reactions for growth in fresh weight, dry weight, callus length, texture, and color variation were recorded after inoculation for five weeks (35 days) from inoculation. Data were analyzed for treatment effects using non-parametric tests by using the Kruskal-Wallis rank sum test and the Chi-square test. Throughout the five weeks, color reduction rate was highly significant, and callus fresh weight, dry weight, callus length, and callus texture; all parameters were highly significant ($P < 0.05$). Results revealed that 7.5% coconut water and 2.5 mg/L 2,4-D containing medium produced maximum callus induction frequency with yellowish-cream friable callus for leaf explant with high significance. A watery or compact callus was obtained with increased concentration of coconut water due to hormonal or osmotic imbalance. It can be emphasized that the synergistic combination of optimized sterilization, 2,4-D levels, and intermediate coconut water had significantly enhanced *G. sylvestre* callus proliferation. This finding provides a foundation for subsequent studies on organogenesis, metabolite yield, and mass propagation of the medicinal crop in Sri Lanka.

Keywords: Callus induction; coconut water; 2,4-D; friable callus; gymnemic acid; *Gymnema sylvestre*