DEVELOPMENT OF A CHARCOAL-FREE PROTOCOL FOR CALLOGENESIS AND SOMATIC EMBRYOGENESIS IN IMMATURE EMBRYO EXPLANTS OF COCONUT *(Cocos nucifera L.)*

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Activated charcoal is often used in coconut tissue culture media for its beneficial effects. However, the use of activated charcoal leads to variable results as it adsorbs growth regulators from the culture medium. Therefore the feasibility of developing a charcoal-free protocol for immature embryo culture of coconut was studied. Four antioxidants namely, ascorbic acid, polyvinylpyrrolidone (PVP), citric acid and L-cysteine-HCl were tested for their ability to replace charcoal in the callus induction medium of immature embryo culture.

Various treatments comprising of different concentrations of the 4 antioxidants in combination with different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) were tested against 0.25% activated charcoal with 24 μM 2,4-D (control). After culturing into different treatments the intensity of browning, precentage callus production and the size of callus formed in immature embryo explants were recorded.

The results indicated that activated charcoal was better for reducing browning and inducing callus in immature embryo explants than the four antioxidants tested. All 4 antioxidants effectively reduced browning in immature embryo explants. Ascorbic acid (filter-sterilized) and PVP were more effective in inducing callus in immature embryos than L-cysteine-HCl and citric acid. Apart from the control, the most effective treatments for callus

induction were PVP at 20,000 mg/l and ascorbic acid at 1100 μM in combination with 0.5 μM 2,4-D. Soaking the explants in ascorbic acid prior to culturing reduced callus production.

The feasibility of inducing somatic embryogenesis in immature embryo-derived callus in a charcoal-free medium was also tested. About 40% of the callus cultures containing 20,000 mg/l PVP (with 0.25-0.1 μM 2,4-D) produced somatic embryos. A few of these somatic embryos germinated and produced shoots when 6-Benzylaminopurine (BAP) or zeatin (5 μM) was incorporated in the medium. The results of this preliminary investigation indicated that the development of a charcoal-free protocol for immature embryo culture is feasible.