STUDIES ON *IN VITRO* SOMATIC EMBRYOGENESIS OF *Cocos nucifera* L. (COCONUT)

By

W N I Shyama Celin Fernando

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Sri Lanka

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ABSTRACT

In vitro somatic embryogenesis is the most important means of mass propagating high quality-planting materials in *Cocos nucifera* L. (coconut). Despite long-term research, a reliable cloning technique has not yet been developed. The present study was undertaken to develop suitable culture conditions for *in vitro* somatic embryogenesis and plant regeneration from a local variety (Sri Lanka Tall) of coconut. It also included studies on markers for early detection of tissues with embryogenic potential, comparative histological and biochemical studies on zygotic and *in vitro* somatic embryos at different developmental stages.

During the study, embryogenic callus was initiated from plumule explants excised from pre-cultured mature zygotic embryos. The basal culture media, 72, Y_3 and MS were found to be suitable for callogenesis when supplemented with appropriate concentration of 2,4-D (24 μM). However, the adsorption capacity of activated charcoal present in the medium determines the level of freely available 2,4-D in the medium and therefore it plays a crucial role in callogenesis. Under the most suitable combination of charcoal and 2,4-D (0.25 % Pharmacos charcoal with 24 μM 2,4-D and 0.1 % BDH, acid-washed charcoal with 100 μM 2,4-D), about 50 % plumule explants produced embryogenic callus. The callusing frequency in charcoal-free media was found to be lower (about 29 %) than that of charcoal-containing media.

Attempts were made to produce friable callus for the establishment of cell suspensions. However, it was not possible to obtain friable callus during the course of the study and thus fine suspension cultures could not be established.

Several plant regeneration protocols were tested for *in vitro* somatic embryogenesis from plumule-derived callus. Application of 5.0 μM ABA for 5 weeks resulted in consistent plant regeneration at a low frequency (4.4 %). The effect of high agar-induced moisture stress, different cytokinins and $AgNO_3$ (at different concentrations) in combination with ABA on somatic embryogenesis and plant regeneration was assessed. Application of water stress induced by high agar concentration significantly increased the frequency of somatic embryogenesis (62.1 %) and plant regeneration (9.0 %). The effect of cytokinins varied with the type and concentration whereas the effect of $AgNO_3$ depended on the concentration. Application of cytokinin in combination with ABA showed multiple shoot regeneration.

Histological studies were conducted to identify the mode of plant regeneration and possible causes for low plant regeneration frequency. The results of the histological studies revealed that application of ABA leads to plant regeneration through somatic embryogenesis. It also revealed that a mature zygotic embryo and a complete (bipolar) somatic embryo process common features. The occurrence of incomplete somatic embryos (which lack shoot meristems) at a high frequency may be the main reason for low plant regeneration frequency.

During the present study, clonal plants were regenerated from plumule-derived callus and several of them were established in the field to evaluate their performance under field conditions. Analysis of ploidy level confirmed that the regenerated plants were diploid.

Content of proline, total sugars, starch, endogenous ABA and protein profiles of zygotic and *in vitro* somatic embryos at different developmental stages were analyzed. The results revealed that accumulation of proline and total sugar in the two types of tissues follows a similar trend.

Analysis of protein profiles in zygotic and *in vitro* somatic embryos revealed the occurrence of similar polypeptides in the two types of embryos. No embryogenic-specific proteins were detected. Generally, *in vitro* cultures of coconut are highly heterogeneous and this might have hindered the detection of specific polypeptides in embryogenic cultures.