STUDIES ON OVARY, OVULE AND ANTHER CULTURE OF *Cocos nucifera* L. (COCONUT)

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STUDIES ON OVARY, OVULE AND ANTHER CULTURE OF COCONUT *(Cocos nucifera* L.)

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Breeding in coconut is hampered by the lack of homozygous lines for hybrid production and a clonal propagation method. Thus the present study was undertaken to develop suitable techniques for production of dihaploids (DH) *via* anther and ovule culture. Further, the unfertilized ovary was tested as a novel explant for clonal propagation through somatic embryogenesis. The suitable developmental stages of explants were determined by a detailed histological study on inflorescence development.

Consistent callogenesis was observed when unfertilised ovaries at -4 stage were cultured in CRI 72 medium containing 100 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1% activated charcoal. Callusing was improved by application of Thidiazuron (TDZ). Embryogenic calli were sub-cultured into somatic embryogenesis induction medium containing 66 µM 2,4-D followed by maturation medium devoid of any hormones. Germination of somatic embryos and shoot formation was induced by addition of 6-benzyl aminopurine (BAP) and Gibberellic acid (GA_3) while 2-isopentyl adenine (2iP) increased the frequency of plant regeneration. Histological studies illustrated the sequence of events during somatic embryogenesis while genetic stability of calli and somatic embryos was confirmed by ploidy analysis. *In situ* hybridization indicated that the cell cycle controlling gene, Rb is a potential marker to assess regeneration potential of cultures.

Induction of embryogenesis in ovules failed while anther culture gave promising results. Embryogenic calli / embryoids were produced when anthers at 3 WBS stage, pre-treated at 38 °C for 6 days, were cultured in Eeuwens Y_3 liquid medium containing 9% sucrose, 100 µM 2,4-D and 0.1% activated charcoal. Androgenic response was enhanced by combining 2,4-D with 1- naphthalene acetic acid (NAA) and cytokinins (2iP and kinetin). Use of phytagel-solidified medium promoted plant regeneration. Anther orientation of 'abaxial surface up' promoted formation and germination of embryoids. Histological studies revealed that the origin of calli/embryoids were from pollen. Ploidy analysis revealed that some of these structures were haploid whereas the others were diploid. Microsatellite marker analysis revealed that all the tested samples containing diploid chromosome complement were DHs. To the best of our knowledge, this was the first successful attempt of obtaining haploid calli/embryoids and DH plants *via* anther culture in coconut.