Tissue culture: a strategy to produce quality planting materials in coconut

Renuka R, Chandrasekar A, Gnanam R and Mohanalakshmi. M*

Coconut Development Board under its Technology Mission on Coconut is investigating possibilities of developing rapid multiplication techniques in coconut to address the shortage of disease free quality planting materials through collaborative research projects. Presently Tamilnadu Agricultural University is conducting a research project called 'Standardization of *in vitro* culture techniniques through somatic embryo genesis for propagation of elite coconut cultivars'

Coconut is a tropical palm grown in more than 90 countries worldwide. Area under coconut plantaions is stagnating and the production has also declined in recent times, as majority of palms are old, senile and are affected by a number of devastating pests and diseases. However, the demand of coconut is expected to be 21,795 million nuts, while the supply is projected to be 15,734 million nuts by 2025, with short fall of 6,061 million nuts. Eventhough, India has attained the foremost position globally in terms of production and productivity of coconut it needs to be improved further to meet the increasing demand.

Improving productivity of coconut palm is a herculean task as it is an out-breeding perennial, normally propagated by seed, due to lack of vegetative propagation methodology for the production of elite planting materials. However, vegetative propagation of superior palms is the promising way for producing quality seedlings which is absoultely necessary for the establishment of new orchards and the rejuventaion of traditional coconut-based farming system. Plant tissue culture is one of the feasible alternatives for the rapid production of true to type planting materials.

Over past few decades, vegetative cloning of coconut through plant tissue culture has been addressed by many researchers around the globe. The studies revealed the use of various explants *viz.*, immature inflorescence, tender leaf, mature embryos, immature zygotic embryo, plumules and unfertilized ovaries for coconut *in vitro* studies. The response of plumule explants to *in vitro* culture conditions was found to be better than that of other explants in terms of callus formation, regeneration and embryogenic capacity. The findings of these studies have resulted in the limited success in mass multiplication of coconut

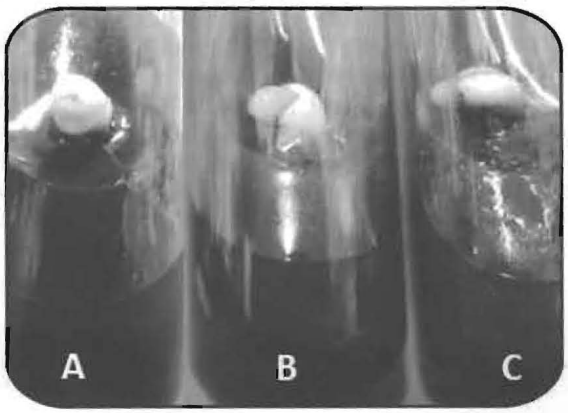

Plate 1: Mature embryos of COD inoculated in different orientations (45 days after inoculation) – (A): Inverted upright position (Plumule facing upwards), (B): Upright (Plumule facing downwards) and (C): Horizontal (Plumule facing sidewards)

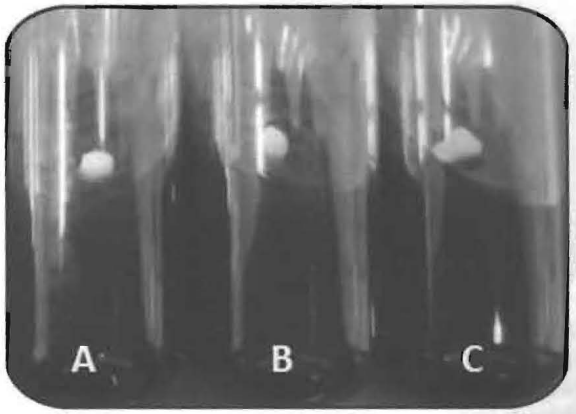

Plate 2: Mature embryos of COD inoculated in different orientations (A): Inverted upright position (Plumule facing upwards), (B): Upright (Plumule facing downwards) and (C): Horizontal (plumule facing sidewards)

*Department of Plant Biotechnology, Tamil Nadu Agricultural University Coimbatore - 641 003 Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore - 641 003

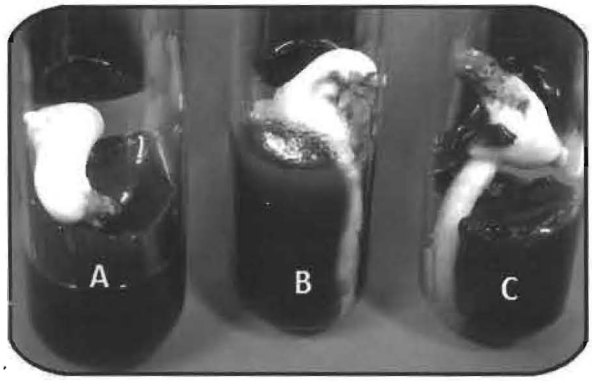

Plate 3: Mature embryos of COD inoculated in different orientations (75 days after inoculation) – (A): Inverted upright position (Plumule facing upwards), (B): Upright (Plumule facing downwards) and (C): Horizontal (Plumule facing sidewards)

through tissue culture, due to inconsistent results under *in vitro* culture and poor plant regeneration efficiency influenced by genotypic variations thus categorizing coconut as one of the most recalcitrant species for *in vitro* regeneration.

The influence of mature embryo orientation on regeneration potential of coconut was studied at the Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore. The fibrous mesocarp of nuts collected from 14 months old Chowghat

Plate 4: Mature embryos of COD inoculated in different orientations (105 days after inoculation) – (A): Inverted upright position (Plumule facing upwards), (B): Upright (Plumule facing downwards) and (C): Horizontal (Plumule facing sidewards)

Orange Dwarf (COD) palms, was removed and nuts were broken with machete. One half of the broken nut containing eyes was used for embryo isolation, where the embryo was removed along with endosperm (cylinder shaped) using a tender coconut opener and then stored in 0.6% sodium hypochlorite for transportation to the laboratory. Then, the endosperm cylinders were sterilised with 6% sodium hypochorite for 20 minutes followed by sterile distilled water rinses for three times. Mature embryos isolated from the endosperm cylinders were inoculated in three different orientations; horizontal (plumule facing sidewards), upright (plumule facing downwards) and inverted upright positions (plumule facing upwards) in modified Y3 medium (Eeuwens, 1976) fortified with 300 μM of Benzyl Amino Purine (BAP) (Plate 1) under 16 hours photoperiod at 24 ± 2 °C. The influence of mature embryo orientations on the regeneration ability was observed after 45, 75 and 105 days of inoculation (Plate 2, 3 and 4 respectively).

The results of this study showed that orientation of the mature embryos during inoculation had significant effect on plantlet production. Good quality plantlets were produced in mature embryos inoculated in upright position after 140 days of inoculation (Plate 5) while mature embryos inoculated in horizontal position produced stunted plantlets. In case of mature embryos inoculated in inverted upright position, enlargement of tissue was observed and organogenesis was not initiated even after 140 days of inoculation. This study demonstrated that coconut tissue culture is influenced not only by genotypic differences, culture conditions and explant variations but also by minor factors like orientation of explants, making coconut a recalcitrant crop to tissue culture. However, techniques like somatic embryogenesis and secondary somatic embryogenesis are explored by researchers to harness the potential of plant tissue culture in coconut.

Regeneration of coconut from plumule explants through somatic embryogenesis and the potential of secondary somatic embryogenesis in the production of somatic embryos and embryogenic calluses is reported in coconut. However, the information on reproducible production of embryogenic calluses and somatic embryos is lacking, although *in vitro* multiplication using secondary somatic embryogenesis is reported in many crops. In the Department of Plant Biotechnology, Tamil Nadu Agricultural University, Coimbatore, attempts are made to standardize reproducible protocol for generating somatic embryos from the callus.

Plate 5: Plantlet of COD produced through mature embryo culture (embryo inoculated in upright orientation: 140 days after inoculation)