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ACKNOWLEDGEMENTS. We thank UKIERI–UGC collaborative grant to Oxford University and NCBS (UKIERI UGC Thematic Partnership UGC-2013-14/056), for this conference and workshop.

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MEETING REPORT

Somatic embryogenesis and bioreactors*

Production of homogeneous quality planting material to the farmers is one of the major constraints in coconut productivity. The present annual production of coconut seedlings, through conventional techniques, is unable to meet the annual requirement of quality planting materials. Rapid multiplication of coconut through *in vitro* techniques, therefore, is of paramount importance. However, coconut is highly recalcitrant to *in vitro* culture.

Tissue culture of plantation crops was initiated at the Indian Council of Agricultural Research-Central Plantation Crops Research Institute (ICAR-CPCRI), Kasaragod three decades back and repeatable *in vitro* protocols have been developed for oil palm (seedling meristematic column) and arecanut (inflorescence culture). Efforts for developing a protocol for *in vitro* regeneration of coconut using plumular explants were initiated in 2000. Even though plantlets have been regenerated and successfully established in the field, a commercial-scale protocol has not been achieved and conversion of somatic embryos into plantlets has remained one of the major bottlenecks.

Recognizing its importance, ICAR has incorporated development of a commercially viable coconut tissue culture protocol as one of the flagship programmes of ICAR-CPCRI in the XII Plan period. In this connection, a brainstorming session was organized at ICAR-CPCRI.

In his presidential address, George V. Thomas (ICAR-CPCRI) stressed upon the role of biotechnology as a solution to problems confronting conventional methods. He gave a brief history of tissue culture work taken up at the Institute since 1970s and emphasized the importance of having a commercially viable tissue culture protocol in coconut. He hoped that the brainstorming session would be purely business-like, where innovative ideas could be discussed in detail.

In the inaugural address, T. Janakiram (ICAR) lauded the efforts made by the Institute for developing coconut embryo culture technique, which has received international acclaim from Bioversity International. He also highlighted the point that a repeatable tissue culture protocol would be the only solution for production of homogenous planting material on a large scale. He also suggested exploring the possibility of a public–private partnership to commercialize the coconut tissue culture protocol after the technique had been perfected.

Sugatha Ghosh (Coconut Development Board) emphasized that the current multiplication rate of 1 : 1 (one seed to one palm) in coconut should be modified to at least 1 : 20 using rapid multiplication protocols without compromising on quality, to meet the demand of 10 million quality planting materials of coconut per year, of which only 3–3.5 million could be met by conventional techniques. He suggested formation of a network approach, through collaborative efforts, to achieve success in the development of a tissue culture protocol in coconut, with a dedicated team led by CPCRI, and with possible funding from ICAR, CDB, DBT and BIRAC.

Anitha Karun (ICAR-CPCRI) spoke about the current status of plumule culture in coconut. Achievements made in this area, including procedure for excision of shoot meristem directly from the zygotic embryo, hastening *in vitro* culture period and identification of best performing palms and seasons for collection of explants were also discussed. Presence of genotypic differences in response to *in vitro* culture, low rate of somatic embryo formation, conversion of somatic embryos into plantlets, and formation of abnormal somatic embryos were the major constraints.

M. K. Rajesh (ICAR-CPCRI) gave a talk on deciphering somatic embryogenesis in coconut through molecular approaches. He gave a concise picture on the progress made in understanding somatic embryogenesis pathway in coconut through bioinformatics and transcriptomic approaches.

Working on *Santalum album* (sandal wood) and ghanera (*Nothapodytes foetida*), Devanand P. Fulzele (BARC, Mumbai) observed that conversion of somatic embryo into plantlets was always a challenge in recalcitrant species.

*A report on the Brainstorming session on 'Somatic Embryogenesis and Use of Bioreactors' organized by ICAR-Central Plantation Crops Research Institute, Kasaragod on 2 August 2014.

Manipulation in plant growth regulators (2,4-D, indole acetic acid) helped in improvement of callus formation and the somatic embryogenesis process could be scaled up manyfold using bioreactors. He highlighted the significance of size of inoculum, and other physical factors such as size of the container, rotation per minute, air flow volume, etc. for success in somatic embryogenesis using bioreactors.

R. R. Nair (ICAR-Indian Institute of Spices Research, Kozhikode) gave a talk on his experience with black pepper and long pepper. Direct somatic embryogenesis from micropylar region was achieved in hormone-free Schenk and Hidebrandt (SH) medium, signifying the importance of chemical composition in the basic explants. Maturation and germination of somatic embryos could be achieved in the same medium. Cyclic secondary somatic embryogenesis was obtained from radicle end of primary embryos in liquid suspension cultures. Somatic embryogenesis was genotype dependent in black pepper. The talk highlighted the use of additives such as abscisic acid for better proliferation of embryogenic callus. He also drew attention to the importance of assessing the total available nitrogen and ammonium to nitrate ratio in the culture medium.

The talk by Pious Thomas (ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru) opened up avenues for a new area of research on biofortification of *in vitro* culture with endophytic bacteria. His presentation highlighted the point that tissue culture samples are not fully sterile, but have an intense and inseparable association with endophytic bacteria. Sharing his experience with papaya tissue culture, Thomas observed an *in cyto* ecosystem in *in vitro* cultures. Endophytic association of bacteria could enhance or retard the response to *in vitro* culture.

Sharing his experience with somatic embryogenesis in mango, papaya and guava, Maneesh Misra (ICAR-Central Institute of Subtropical Horticulture (CISH), Lucknow) discussed about developing successful protocols for somatic embryogenesis in guava (mesocarp) and genotype-independent protocol for papaya (using immature zygotic embryos), but the failure in acclimatization of *in vitro*-derived mango plantlets through *in vitro* nucellar embryogenesis. Induction of globular somatic embryos in mango could be enhanced by supplementation of spermidine (a polyamine), malt extract in the medium and also shock provided by low temperature (15°C) for maturation of globular somatic embryos into cotyledonary embryos and PEG for conversion of embryos into plantlets. For guava, a unique combination of basal media (B5 major and MS minor), supplemented with ascorbic acid and glutamine, was necessary for maturation of somatic embryos.

S. Uma (ICAR-National Research Centre for Banana, Tiruchirapalli) had utilized immature male flowers as explants; for the development of a robust technique for suspension culture and somatic embryogenesis in banana; however, the protocol was not genotype-independent. Critical factors which could enhance somatic embryogenesis include quantity of initiation medium, intervals of sub-culture, removal of self-destructing cells, frequent monitoring of cell viability, heat stress, starvation, anaerobic stress and mechanical wounding. Proteomic and transcriptome work has also been undertaken to obtain deeper insights into somatic embryogenesis pathway in banana.

E. Muralidharan (Kerala Forest Research Institute, Thrissur) gave a talk on the achievements made in somatic embryogenesis of bamboo, eucalyptus, teak and rattans. Ashalatha Nair (Kerala University, Thiruvananthapuram) presented her work on somatic embryogenesis on diploid cultivars (AA, AB) of banana utilizing male inflorescence, bract meristem and leaf bases and shoot apices *in vitro* shoots. Supplementing biotin in the medium could enhance somatic embryogenesis in banana. She also presented work done on epigenetic basis of embryogenesis through DNA methylation studies.

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