Production of coconut planting material through plumule culture

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Coconut is the most important palm species cultivated in more than ninety countries in the tropics, especially on small and marginal holdings. The coconut industry at present is burdened with a number of problems such as prevalence of various pest, diseases and senility of existing plantations. Crop improvement in coconut is a difficult and time consuming programme due to its long pre-bearing age, high heterozygosity, long interval between generations and exclusively seed propagated nature. The predominantly cross-fertilized nature of coconut results in enormous variability in the seedling progenies. Though India was the first country in the world to evolve a commercial hybrid of coconut and the country has since released 12 high yielding hybrids and varieties, current production of quality planting materials meet only about 20 percent of the annual requirement for replacement of senile and disease ravaged plantations.

In vitro vegetative multiplication through somatic embryogenesis of high performance individuals offers hope for the production of homogenous planting materials and for substantial improvement in plantation productivity. Standardization of a viable protocol for clonal propagation would open up tremendous possibilities of meeting the requirement for the quality, uniform, disease resistant/ tolerant planting material and of breaking down productivity barriers.

Coconut is a highly recalcitrant species with respect to tissue culture. Over the past two decades, many researchers have directed their efforts towards developing a method for clonal propagation of coconut. Despite these concerted efforts, success in the area has been limited and only a few clonal plants have been established in the field. Various problems encountered during *in vitro* propagation of coconut are intensive tissue browning (due to oxidation of polyphenols), slow *in vitro* response, low rate of somatic embryogenesis and variation in tissue response due to heterogeneity of explants taken from different individuals. A variety of protocols have been developed using a range of explants, immature inflorescence (Blake, 1991), immature and mature zygotic embryos, young tender leaflets, leaf basis from unopened spindle and plumular tissue (Iyer 1982; 1993; Nair *et al.*, 1999; Iyer and Parthasarathy, 2000), but the protocols lack repeatability.

Plumular tissues have been found to be the most responsive explants till date for clonal propagation of coconut. Plumule culture of coconut has been successfully applied for enhancing multiplication rate of known hybrid and genetic materials that are observed to be resistant to diseases, production of homogeneous breeding materials, multiplication of cryopreserved coconut germplasm and basic studies on somatic embryogenesis.

Coconut somatic embryogenesis is possible through a callus phase; therefore, the initial calli and subsequent embryogenic calli are prerequisite for successful plantlet regeneration. Among the auxins, the 2,4-D was more responsive and gradual reduction of the concentration of 2,4 –D was necessary for induction of embryogenesis. It was reported that the regeneration protocol for plumule through organogenesis and somatic embryogenesis utilizing plumular slices from *in vitro* germinated embryos. Embryogenic callus induction as well as plantlet regeneration was achieved in media supplemented with 2, 4- D and polyamines. By using this protocol a maximum of 13 plantlets/ plumule was achieved. The varying response of individual embryos to *in vitro* regeneration has remained a major bottleneck for large scale multiplication.

With the initial success achieved in regeneration of plants from plumule explants of coconut, a new project entitled "*Scaling up the production of planting material from released varieties of coconut palms through plumule culture*" was initiated at CPCRI, Kasaragod with financial assistance from Coconut Development Board during 2009. The major objectives of the project includes refinement

Fig. 1. Collection of embryo, extraction and inoculation of plumular explants of coconut

of existing plumule culture protocol for higher multiplication rate, seasonal variation for plantlet recovery, enhancing production of coconut plantlets from released varieties *viz.*, COD, MGD, PHOT, WCT and Chandra Sankara (COD X WCT) through plumule culture and clonal fidelity studies of *in vitro* retrieved plantlets using molecular markers.

The earlier protocol using plumuler slices obtained from *in vitro* germinated embryos was further modified by using plumules from freshly excised embryos . The percentage of embryogenic calli and its multiplication was enhanced using this method. It was observed that the individual response of embryos for plantlet formation depended on genotypic differences in individual nuts from the same palm as well as from different palms from the same variety. The explants were initially inoculated in Y3 medium supplemented with 2,4-dichloro phenoxy acetic acid (16.5mg/L) with its gradual reduction in the later stages. Polyamines [spermine, 50μM/L] and cytokinin [Thidiazuron,1mg/L] were also incorporated in the initial

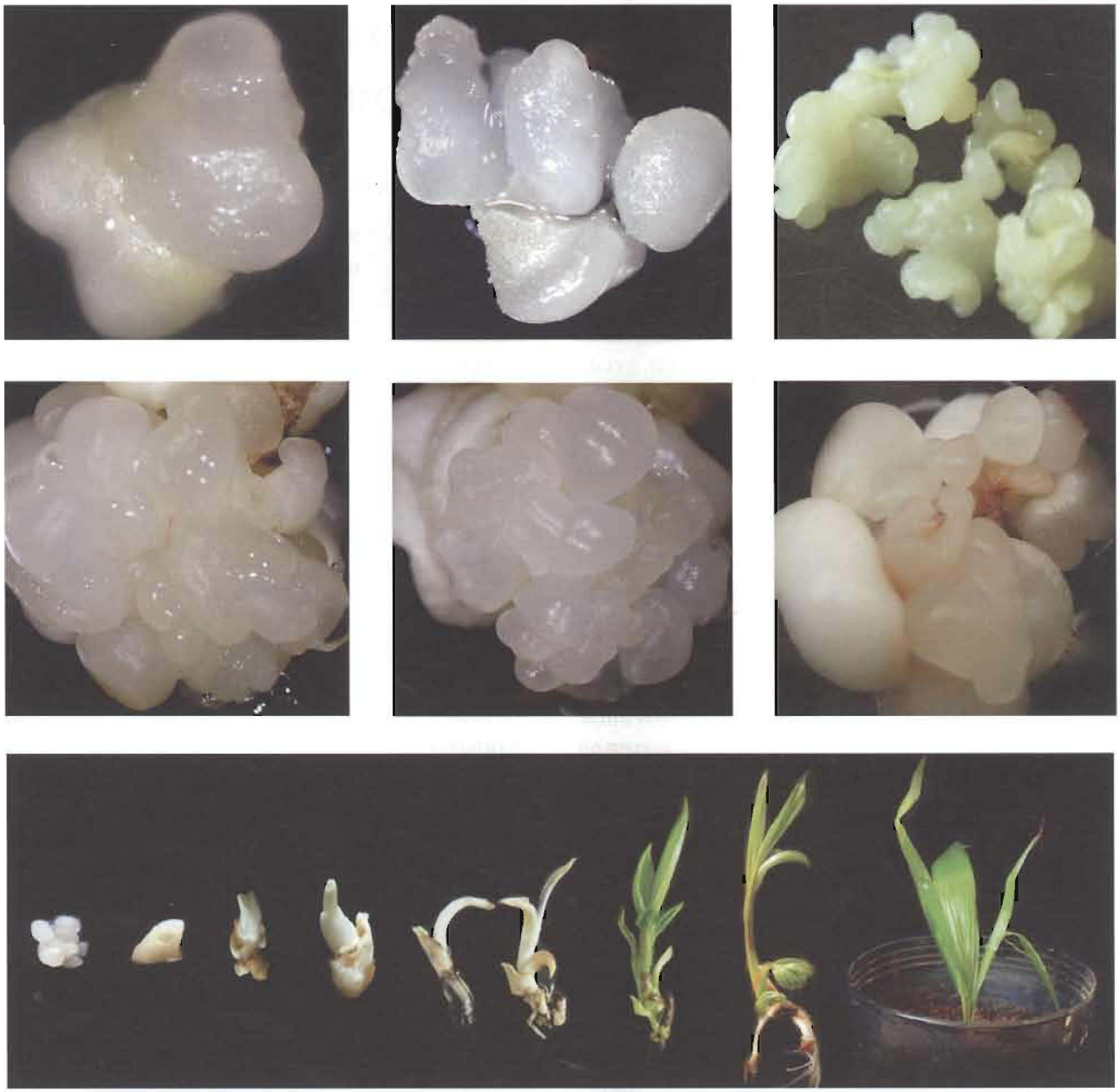

Fig. 2. Callus initiation, somatic embryogenesis and regeneration of planlets from plumular explants of coconut

medium for better callus multiplication. This protocol was executed in selected tall varieties (WCT and PHOT) and dwarf varieties (MGD, COD, CGD and GBGD).

The initial response of the explants for callus formation was found to be uniform in all tall and dwarf varieties upto the somatic embryo developmental stage. On an average of 12 somatic embryos/ plumule was obtained in all the types of plumules studied, with a maximum of 18 plantlets. Shoot induction from callus was observed after four months of culture initiation. Incorporation of BAP (1-5mg/L) along with GA_3 (0.1-1mg/L) and glutamine (1-5mg/L) in the regeneration liquid medium resulted in the development of shoots from somatic embryo. Gradual increase in the concentration of BAP (1-15 mg/L) along with NAA (5-10 mg/L) in the basal medium resulted in the recovery of normal plantlets.

Various abnormalities like compact calli, abnormal somatic embryoids, rudimentary shoots with multiplied roots and elongated scale leaves were also observed. In the case of dwarf varieties, root formation was found to be earlier

compared to shoot formation. Anatomical studies revealed the presence of intact cotyledonary leaves which seemed to inhibit the apical meristem development of somatic embryoids. It was also observed that the presence of vascular bundles in the early stages of callus formation may lead to the direct formation of meristemoids.

In case of dwarf cultivars, maintaining the cultures in high 2, 4-D medium for extended periods resulted in the formation of roots. Hence it is necessary to maintain the sub-culturing duration to a minimum of 20 days in each media. Multiple shoot formation was observed in some palms but its morphological uniformity remained too low.

Presently the modified CPCRI plumule culture protocol was found to be more suitable for WCT, PHOT and COD varieties, since more number of normal plantlets were achieved through somatic embryogenesis as well as meristemoid formation. But for dwarf embryos, plantlet recovery was hindered by the abnormal growth of callus into root formation, compact callus and abnormal somatic embryos. In case of MGD, root formation was more prevalent than shoot as a result plantlet recovery rate was too low. The clonal fidelity study was carried out using microsatellite markers. The banding pattern observed were uniform which showed the genetic uniformity of plantlets emerging from a single plumule.

Palms giving enhanced response for callus induction and somatic embryogenesis were identified at CPCRI, Kasaragod as well as CDB Farm at Neriamangalam. The effect of novel growth regulators on somatic embryogenesis and regeneration process is in progress to improve the protocol to get a uniform response from each plumular explant (Fayas *et al.*, 2012). Cell suspension cultures were initiated from embryogenic calli obtained from plumular explants of coconut. Cell multiplication was observed in suspension culture after two months of culture initiation. Refinement of media for regeneration from cell suspension is in progress.

The embryogenic potential of somatic cells is achieved by the reprogramming of gene expression patterns and other physiological changes. It involves different molecular events including differential gene expression and various signal transduction pathways for activating or repressing numerous genes sets. Several genes have been reported in connection with somatic embryo induction and formation. Somatic embryogenesis receptor-like kinase (*SERK*), is one of the important genes involved in somatic embryogenesis. Our future line of work include transcriptome and proteomic analysis of somatic and zygotic embryos in coconut, which would provide important clues in enhancing somatic embryogenesis of this recalcitrant species and validation of gene expression patterns using quantitative real-time PCR (qRT-PCR).

Coconut export crosses Rs.1000 crores

First time in the history of India, the export of coconut products excluding coir and coir products crossed Rs.1000 crores. Despite a decline in the total export in the country, the coconut sector has witnessed a growth rate of 26% in value during the year 2012-13 and reached at Rs.1050 crores. During this period 32% increase was registered in the volume of export of coconut products. Remarkable achievement is made in the export of activated carbon made out of coconut shell charcoal during 2012-13. A total of 58,000MT of activated carbon valued Rs.550 crores has been exported from the country during 2012-13 as against 38,500MT valued Rs.347.6 crores in 2011-12. A steady increase in coconut product export is observed since the Board is designated as an Export Promotion Council in 2009-10. Since then, export of coconut products register an annual average growth rate of 35% in value. Export of coconut products valued Rs.5,000 crores (1 billion US$) is targeted by the Board during the terminal year of the 12th Five Plan Period (2017-18).