

## Biotechnological achievements in plantation crops

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The conventional breeding techniques in improving plantation crops are slow and time-consuming, because of their long juvenile phase, heterozygous outcrossing nature, and large area needed for their experimentation. Biotechnology can only be considered as a supplementary new tool to solve specific problems. Generally the plantation crops are beset with a narrow genetic base, lack of an inbred population, adequate land for field testing the selections and hybrids for yield and quality characters. The most popular and widely commercialized global application of biotechnology has been in the sphere of plant tissue culture—micropropagation. In India, only micropropagation in cardamom has been successfully commercialized among plantation crops.

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RECOMBINANT DNA technology has provided a great opportunity for direct gene manipulation, gene function and expression for production of transgenic plants with desirable chimeric genes for commercialization of economically important crops.

### Palms

The planting material requirement in commercially cultivated palms coconut, oil palm and arecanut is met from seedling progenies. This situation has led to the indiscriminate planting of seedlings of unknown pedigree without any quality control, resulting in low productivity. This underlines the need for a commercially viable protocol in coconut and oil palm tissue culture.

The first attempt in the country to culture immature rachillae explants excised from the leaf axil was made at the Central Plantation Crops Research Institute, Kasaragod, during 1980, which failed to regenerate any viable plantlets.

After that a successful protocol was made for regeneration of leaf explants of WCT seedlings and field planting of 4 plantlets. Work on coconut tissue culture has also been conducted at the Jawaharlal Nehru University, New Delhi, the National Chemical Laboratories, Pune, and Hindustan Levers Ltd, Bombay. However a repeatable and commercial protocol is yet to be achieved.

The clonal propagation of a few genotypes may lead to narrow genetic base. The genetic variability in

the population then has to be increased by introduction of new germplasm and evolving new hybrids. Because of large nut size, short duration of dormancy and phytosanitary regulations, the movement of coconut germplasm has been restricted. An alternative method for collection of coconut germplasm has been developed at the CPCRI, Kasaragod. Now it is possible to collect coconut embryos aseptically from the field in the form of embryo cultures. The embryos from 8–12 months old nuts can be extracted and retrieved *in vitro*. Significant correlation has been observed between initial root volume (at the time of transplanting to the pots) and vigour of the plantlets in the pots. Vigorous root growth has been achieved by supplementing the

medium with IBA 5 mg/litre and NAA 1 mg/litre.

Since immediate transport of the field collected embryos may not be possible from distant collection sites, *in-vitro* active conservation (short-term storage) of coconut zygotic embryos become necessary. Now coconut embryos can be stored successfully for 2 months in sterile water with very low contamination.

Other biotechnological programmes undertaken at the CPCRI, Kasaragod, include attempt to develop a protocol of micropropagation from somatic tissues and DNA fingerprinting of coconut germplasm using RFLP/RAPD markers. Experiments are also in progress in the field of DNA fingerprinting and character tagging in coconut germplasm, molecular diagnosis of different pathogens, MLO for root (wilt); and molecular indexing of virulency of different strains of pathogens (*T. paradoxa*) which are causing stem bleeding in coconut.

The success in vegetative propagation of oil palm through tissue culture have been reported from France (IRHO) UK (Wye College, University of London); unifiield TC Unilever/Harrison and Crossfield, Malaysia (Palm Oil Research Institute of Malaysia or PORIM); Indonesia (Marihat Reserarch Station); Nigeria (Nigerian Institute of Oil Palm Research or NIFOR).

At the CPCRI, Kasaragod, somatic embryos have been produced from leaf explants of seedlings which were hardened and transplanted in the field during 1989. The performance of these seedlings is being monitored at the CPCRI Regional Centre, Palode. Recently plantlet regeneration was achieved from leaf explants

of 18-month-old *dura* and 6-month-old *tenera* oil palm seedlings. Plantlet development from calli has been achieved through both somatic embryogenesis and organogenesis. Experiments on regeneration protocol for adult palm, embryo rescue in *pisifera*, multiple shoot induction in *tenera* sprouts, and protoplast culture are in progress at the CPCRI, Kasaragod. Histological studies of somatic embryogenesis and organogenesis have also been studied. Studies on protoplast culture and regeneration, rDNA techniques for manipulation of desirable traits including oil quality, biocontrol of Rhinoceros beetle, rapid screenign techinques for disease tolerance are the major areas of need to be focussed.

The application of restriction fragment length polymorphism (RFLP) for genetic fingerprinting of 111 elite oil palms has been studied at Cambridge, UK, using a highly variable RFLP probes for palm identification.

In order to achieve genetic improvement of oil yield and quality, foreign gene (s) may be introduced into the genome of the oil palm. Microprojectile bombardment method (Biolistics) hve been proven to be a versatile technique for monocotyledon plants. Evaluation of 5 promoters for use in transformation of oil palm was undertaken at PORIM, Malysia. Results suggested that *Ubil* and *Emu* were the best promoters to begin developing a genetic transformation system for oil palm.

### Spices

Spices are generally vegetatively propagated and those having heterozygous recalcitrant seeds. Methods have been developed for the storage of culture of black pepper, car-

damom, ginger, turmeric, kasturi turmeric, mango ginger, kaempferias, vanilla and some seed and herbal spices. The experiments on *in-vitro* methods of cryopreservation for long-term storage of genetic resources are also in progress at the Indian Institute of Spices Research, Calicut. A number of successful exploitation of biotechnological tools are reported in spices crops, which includes rapid multiplication of elite, disease-free planting material, embryo rescue, generation of somaclonal varition, *in-vitro* screening for resistance/tolerance to pathogens, *in-vitro* germplasm conservation and exchange, and *in-vitro* production of active components of flavours and essences.

Protocols for micropropagation are now available in black pepper, long pepper, *Piper colubrinum*, *P. barberi*, betel, cardamom, ginger, turmeric, mango, cinnamon, dill, fennel, thyme, lavender, peppermint, sage, oregano, celery, camphor, vanilla, anise, savory, marjoram, parsley and ocimum. The tissue cultured plantlets are being evaluated for field performance. Field evaluation of micropropagated cardamom has demonstrated their superiority in yield and better tiller production.

Tree spices have proved more recalcitrant in tissue culture, particularly the explants from mature trees. Micropropagation of clove from seedling explants have been achieved. Scientists at the IISR, Calicut, have successfully multiplied the mace itssue of nutmeg, obtaining a 10-fold increase in fresh weight in 2 weeks and retaining deep red colour and its characteristic flavour intact. This can be a commerical proposition for *in-vitro* production of myristicin and myristic acid.

Somaclones have been developed in black pepper, ginger, turmeric, cinamom, vanilla, anise, savory and fennel. Somaclonal variation is being exploited especially in ginger, where conventional breeding programmes are ineffective due to lack of seed set in vanilla; the available genetic variability is also very narrow.

These regeneration systems have been used in studies on *Agrobacterium* mediated transformation for transfer of resistance (to *Phytophthora capsici* causing foot-rot and *Radopholus similis* nematode causing slow wilt) from *Piper colubrinum* of S. America to *P. barberi* at the IISR, Calicut.

Somatic embryos/shoot buds have been encapsulated in calcium alginate beads to develop synthetic seeds in black pepper, ginger, turmeric, cardamom, cinnamon, camphor, vanilla and sage to evaluate them as possible material for *in-vitro* conservation and exchange of germplasm. Cell suspension cultures have been established to exploit possible production of secondary metabolites *in vitro*. Protoplast isolation has been achieved in black pepper, cardamom and vanilla, which is a prerequisite for genetic manipulation programme.

At the IISR, Calicut, conservation of spices germplasm *in-vitro* repositories, as an alternative to field gene bank, is also in progress and over 300 accessions are in *in-vitro* gene bank.

### Tea

The domestic demand for tea is rapidly increasing, and by the turn of the century, about 1,000 million kilos of tea would have to be produced. A protocol for mass multiplication of planting material through tissue culture has been standardized. Haploids have been developed and initial success have been obtained in genetic engineering through protoplast iso-

lation and protoplast fusion. Somatic cell hybridization using protoplast fusion has been tried to transfer the Darjeeling tea flavour of 'China' clones to the Assam cultivars possessing strong and brisk liquors.

*In-vitro* approaches for evolving drought tolerant teas are being followed at the UPASI Tea Research Institute, where the protocols for rapid clonal multiplication have been developed in 4 cultivars. Drought tolerant somaclones through irradiation and chemical mutagens are in progress at the UPASI Tea Research Institute, using UPASI 3 clone whose *in-vitro* response is good and productive.

Low caffeine tea with improved solubility from other *Camellia* sp. should be explored through transgenic approaches leading to modification of the biosynthetic pathway for caffeine synthesis, using anti sense RNA technology to selectively switch off individual genes and block biochemical pathways. The other approach is through accelerated particle gene delivery which has brought many recalcitrant crops range for genetic manipulation.

Recalcitrant seeds from tropical-subtropical spices are difficult to cryopreserve, primarily due to high moisture level and sensitivity to desiccation. Embryonic axes are highly tolerant for desiccation and cryoexposure.

### Coffee

Protocols have been developed for obtaining direct as well as indirect somatic embryogenesis at the Coffee Research Institute, Balehonnur. Coffee is considered as a model *in-vitro* system for crop improvement via tissue culture and genetic transformation. Immature embryo cultures have been established for plantlet regeneration through embryo rescue in hybrids. Synthetic seed technology for

encapsulating embryos in sodium alginate has been developed. Another culture technique has been successfully employed for callus induction and plantlet regeneration in CXR interspecific hybrid, for rapid fixation of heterosis. Work has been initiated to develop the *Agrobacterium* mediated transformation system using a leaf-disc procedure. High frequency somatic embryogenesis through bioreactor scale of micropropagation using embryogenic cell suspensions of Robusta coffee in flask cultures, estimated to yield 4,60,000 somatic embryos after 7 weeks of culture. For a 3-litre capacity bioreactor charged with 3 g fresh weight of embryogenic cells, approximately, 6,00,000 somatic embryos could be realized every 2 months of culture.

The work on the development of a coffee variety with low caffeine and pest and disease resistant varieties through the application of genetic engineering techniques are need to be focused.

### Rubber

Consumption of natural rubber in India is increasing year-after-year. The *Hevea brasiliensis* is a perennial crop with a long gestation period and economic life of 32-35 years. All the trees in South-East Asia covering millions of hectares are set to have been derived only from a few trees, thus showing a narrow genetic base. Of many high-yielding clones evolved at the Rubber Research Institute of India, 'RRII 105' is most popular, yielding 250-400 kg more yield than other clones. The RRII, has produced tissue cultured plants from high-yielding clones and these are under field evaluation. Plantlets have also been obtained from various explants, namely inner integuments of immature seed and haploid culture.

## Cocoa

Although conventional methods of clonal propagation such as budding, side grafting and rooting of cuttings are possible in cocoa, they are relatively expensive and provide only a limited number of propagules. Hence there is a good scope for exploitation of tissue culture for rapid clonal propagation and crop improvement in cocoa, as a supplement to conventional propagation techniques.

At the CPCRI, Kasaragod, *in-vitro* induction and growth of somatic embryos have been obtained from immature cotyledon segments. At the KAU, Vellanikkara, multiple shoots from nodal explants of fully grown trees of cocoa have been obtained. Scientists at the Purdue University have found that a high CO<sub>2</sub> environment (470–28000 ppm) promoted shoot and root development in cotyledon derived somatic embryos.

At the CPCRI Research Station, Vittal, the molecular diagnosis have

been developed for *Phytophthora* causing black-pod disease in cocoa. The extent of interspecific DNA diversity was studied in isolates of *P. palmivora*, *P. capsici*, *P. citrophthora* causing black-pod disease in cocoa in India.

## Cashew

Micropropagation in cashew using tissue culture has some problems such as contamination of field collected material, leaching of phenolics and recalcitrant nature of mature tissues. The major problem of browning of adult tree tissues can be overcome to a great extent, by the use of liquid cultures under dark incubation, and use of chemicals phenyl 1-3 methyl 1-5 pyrazolone, ammonium chloride, urea, activated charcoal, ascorbic acid, to remove the inhibitors secreted by explants. The explants used for micropropagation includes shoot-tips, nodal explants of both seedlings and mature trees. At the NRC for Cashew, Puttur, several experiments

noticed that the responses of nodal explants are better than shoot-tips. Multiple shoot buds (1–13 buds/explant) could be induced from each nodal culture of seedling.

Kernel quality of cashew is very important in international markets, one must precisely identify desired geotypes possessing the premium quality of kernels, not only in terms of size and weight, but also in protein value and essential amino acids. The use of RFLP/RAPD can identify the specific Quantitative Trait Loci (QTLs) which determines the high performance of varieties. The RFLP mapping of varieties would correct the identity of germplasm in molecular terms.

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