BIOTECHNOLOGY - ENGINEERING PLANTATION CROPS FOR FUTURE

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INTRODUCTION

Kerala is one of the few States in the country which cultivates all the plantation crops. These crops play and important role in the economy of the state. Coffee, Tea, Cocoa, Rubber, Spices, Medicinal plants, Coconut, Arecanut and Oil palm are the crops included under the plantation crops. Considering their importance, lot of research is being carried out in various spheres of crop improvement and production. The frontier science of biotechnology offers great scope for improvement of these crops. The tools available to biotechnologists are tissue culture, recombinant DNA technique and molecular markers besides bioprocess engineering. The various areas, where biotechnology could be used in plantation crops are as follows (Iyer, 1995).

1. Rapid clonal propagation of elite, disease-free selections, through cell, tissue and organ cultures, especially via somatic embryogenesis.
2. Conservation of native and exotic genetic resources, through *in vitro* gene banks, under both short-term limited growth conditions as well as long-term cryogenic storage.
3. Use of molecular markers to characterize cultivars, hybrids and elite materials, and estimate genetic distances within and between populations.
4. To identify molecular marker loci linked to desirable traits such as disease/drought resistance/tolerance for effecting marker assisted selections and subsequent gene cloning and transfer.

5. Use of molecular markers to construct saturated molecular linkage maps and identify genomic regions for desirable traits.

6. Production of haploids and isogenic (inbred) lines through anther/pollen culture for use in heterosis breeding and linkage analysis.

PALMS

Although breaking the yield barrier still remains our major objective in coconut, arecanut and oil palm, the problem of disease and pest resistance/tolerance should now receive top priority attention in all these palms (Iyer and Dhamodaran, 1994). The recent finding that the causal agent (MLO) which incites root (wilt) disease (RWD) in coconut, yellow leaf disease (YLD) in arecanut, and spear-rot disease in oil palm can cross-infect each other, makes the problem more complicated and a cause for alarm (Anon, 1995). Our internal quarantine being what it is, with little or no restriction on movement of planting materials between States, utmost caution is called for in detecting one or the other disease in areas where oil palm has been introduced amidst coconut or arecanut plantations. Vector control being a very tedious, costly and impracticable operation, constant vigil is needed to eradicate palms where spear-rot, YLD or RWD is detected in any one of them, in order to reduce the inoculum level and prevent further spread of the disease. Resistance breeding work also assumes prime importance, and the sooner we develop DNA probes and RFLP/RAPD/AFLP markers for these three diseases of the three palms, the better it would be for planning future strategies.

The heartening feature in our efforts since the seventies (Iyer *et al.*, 1979) to locate palms showing resistance/field tolerance in the 'hot spots' for the dreaded RWD, is the indication of field resistance/tolerance of the Chowghat Green Dwarf (CGD) parent and its F1 hybrid with disease-free West Coast Tall cultivar located in the 'hot spots' of Kerala where the disease is endemic (Anon, 1994). If the F1 is showing field tolerance, its genetic implications need to be understood in terms of the number and nature of the gene(s) involved. Being a long-duration perennial, it is not conceivable to attribute single gene control for continued resistance/tolerance to MLO infection, since the microbial pathogen will most certainly have a faster rate of mutation for its survival in the host which lives in the farm for several decades (50-80 years for Talls and 40-50 years for Dwarfs). Only the polygenic control of this character can afford the internal buffering in the host against the onslaught of a rapidly mutating MLO. Molecular markers constitute an excellent tool for characterizing such polygenic traits, since only a single population is sufficient for identifying different genomic regions imparting disease resistance/tolerance. The strainal similarity of the MLOs causing the above three disease symptoms as revealed on cross-inoculation between the three host-palms, makes the situation alarming. Here is a strong case for constructing RFLP/RAPD/AFLP markers for these three disease-causing MLO's and their resistant/tolerant hosts.

Meanwhile, progress made on the tissue culture front is also encouraging, protocols for oil palam and coconut being worked out, and the IRHO having commercialized oil palm clones derived from leaf/inflorescence culture. Success in coconut is limited to a few clones obtained at CPCRI from seedling leaf tissues (Raju *et. al.*, 1984), and inflorescence tissue at Wye College, U.K. (Blake, 1991). Coconut tissue culture work done at none different centres in India has been summarized by Iyer (1993). All centres except CPCRI have since discontinued this work. Both IRHO (Verdeil *et. al.* 1989, 1992, 1993, 1994) and Philippine scientists (Rillo and Ebert, 1993) are closing in towards achieving success, and by the turn of this century it is hoped that coconut clones will also become a routine possibility, with advancement in our knowledge on basic aspects of this recalcitrant palm.

Success has been achieved on the routine use of embryo culture for field collection. Kumaran *et al.*, 1998 have successfully collected 1342 embryos of Tall and Dwarf accessions from Indian Ocean Islands, in sterile distilled water and nearly 90% of these could be retrieved and stored (Anitha Karun and Sajini, 1994, Batugal and Engelmann 1998, Anitha Karun *et al.*, 1998) and retrieval of coconut (Assy-bah *et al.,* 1989, 1991) and oil palm germplasm. The future priority will be to standardize cryo-storage methods using liquid nitrogen, so that *in vitro* gene banks will become a distinct possibility. All the above effort, particularly RFLP/RAPD/AFLP mapping is going to cost a lot of money and questions of economy will be raised. However, considering the enormous losses of over 960 million nuts incurred from RWD alone, the above lines of work would be worthwhile and cost effective. Sources of finance will not be wanting, since organizations like Govt. of India's Dept. of Biotechnology are eager to support such inter-disciplinary and even inter-institutional research, across all bureaucratic barriers.

Our future research on *in vitro* culture of palms should be directed towards finding answers for (1) reduced response of adult palms vs. juvenile seedling tissues, (2) trigger for callus-mediated vs. direct somatic embryogenesis, from leaf/inflorescence tissues, (3) role of antioxidants and activated charcoal (AC) in prevention of browning vs. absorption of growth factors and inhibitory chemicals like ethylene, or 5-hydroxymethyl furfural produced by sucrose dehydration, and of growth promoting auxins like 2,4-D. There is evidence from work done in Philippines that AC adsorbs 99.5% of the 14C labeled 2,4-D by 4th day, leaving only 0.5% available for uptake by the explant (Ebert and Taylor, 1990), especially in liquid as against semi-solid media. Higher temperatures (20-30°C) and low pH of medium accelerated adsorption of 2,4-D by AC. Similar studies are needed to track the fate of other growth factors like cytokinins and vitamins to ascertain if they become growth limiting due to adsorption by AC.

Another aspect of *in vitro* culture that deserves our intensive attention is the production of haploids through anther/pollen culture, where preliminary success was reported both in India (Iyer, 1982), the Philippines (Thanh Tuyen and Guzman, 1983; Thanh-Tuyen, 1985), and in France (Monfort, 1985), upto the pollen embryoid stage. The production of haploids and isogenic diploids in coconut and other palms will greatly accelerate the pace of our heterosis breeding and genetic engineering programmes to evolve rare recombinants.

Oil quality

So far this aspect has not received the attention it deserves from coconut breeders, but in *tenera* oil palm hybrids, variation has been recorded in the ratio of saturated vs. unsaturated fatty acids. In the annual oilseeds like rape (*Brassica rapa)* and *Arabidopsis,* possibilities are indicated, of evolving “designer oilseed crops” through the induction of useful oil-quality mutations in *Arabidopsis thaliana* and their subsequent transfer into rape species by a process known as ‘shuttle mutagenesis’ (Robbelen, 1991; Murphy, 1991; Ashri, 1992; 1993). Mutants for fatty acid composition have already been induced in *Arabidopsis* (Browse, 1989). Another possible way of integrating mutation breeding and genetic engineering is being considered in *Sesamum,* whose oil contains a natural phenolic anti-oxidant, ‘sesamol’ derived from sesamolin, which is being considered for reducing oil oxidation and prolonging shelf life of all vegetable oils. If genes controlling sesamol biosynthesis in *Sesamum* could be identified through RFLP/RAPD/AFLP analysis, transferred to other oil plants and expressed, their oil stability can be greatly improved. Willmitzer and Toepfer (1992) have reported that several DNA sequences coding for enzymes of plant lipid metabolism have already been cloned, and most of the enzymes of plant lipid metabolism are expected to be mapped in the next couple of years. Several commercial Biotech. companies have major programmes in this area (Cubitt, 1991). Genetic engineering in rape (*Brassica napus* and *B. rapa)* leading to a modified fatty acid composition has also been achieved, besides transferring anti-sense genes from rat into rape for medium chain hydrolase.

Callus cultures of oil palm have been used to study the formation of a storage lipid whose biosynthesis was monitored by incubating the cells with 14C - acetate and determining radioactivity in the triacyl glycerol fraction of labeled lipids formed (Turnham and Northcote, 1984). Sharp increase in radioactive triacyl-glycerol 5 weeks after culture, indicated the production of embryoids. Studies on developing somatic and pollen-derived embryos of *Brassica napus* and other oil crops show that embryo formation in culture parallels embryogenesis in developing seeds, e.g., in storage lipid assembly and fatty acid elongation. Specific activities of the Kennedy pathway (whose enzymes assemble the triacyl glycerols in the endoplasmic reticulum,) are found to be much higher in cultured somatic and microspore (haploid) embryos than in zygotic embryos. Such embryos could hence be used as models to help studies on assembly of storage lipids and regulation of corresponding genes in oil seeds. Thus, genetically modified embryos in culture may be useful for the prognosis of alteration in fatty acid composition of seed oils at a very early stage (Weber and Taylor, 1990).

Thus, it will become possible to develop within a few years, genotypes that will produce 'tailor made' oils of specific fatty acid ratios to meet the needs of the edible oil (for human consumption), and of the industrial consumers for soap manufacture and other chemical industries. Let us hope that in coconut and oil palm too we will witness a shift from "petrochemistry" to "botanochemistry" as predicted by Pryde and Rothfus (1989), so that both developed and developing countries will stand to benefit from such a shift.

Breeding for tolerance to stress: Biotic and abiotic

Among the biotic stress factors, MLO incited diseases of palms, such as lethal yellowing (LY) in Jamaica, root (wilt) disease (RWD) in Kerala, spear rot in oil palm, yellow leaf disease in arecanut (YLD), and Tatipaka disease of coconut in Andhra Pradesh, are the major diseases to engage our concerted effort to evolve strategies to contain the spread of these diseases, screen cultivars, hybrids and natural populations for possible sources of field resistance/tolerance, and finally, to develop DNA probes as diagnostic tools as also for genetic engineering studies.

The disturbing factor is the wide host range of the lethal yellowing (LY) MLO which not only affects coconut but several other palm genera - thus threatening the valuable collection of palms at the Fairchild Botanic Gardens in Florida. Thus, in a study conducted in Mexico, MLOs were detected in coconut palms of Yucatan Province, using radiolabelled cloned DNA probes. It was found that two probes LYI-43 and LYD-9 containing DNA segments from a Florida LY MLO, hybridized at moderate frequency with DNA extracted from 6 Yucatan LY-diseased but not healthy palms, thus suggesting similarities between LY MLO from Yucatan, Mexico, and LY MLO from Florida, USA. The MAYPAN hybrids replanted in LY-ravaged gardens of Jamaica are now said to be in full bearing and show no significant losses (Harries, 1991). Commercially, losses in both Malayan Yellow Dwarf (MYD) and its hybrids are reported to be less than one percent.

In India as stated earlier, the MLO's of coconut RWD, arecanut YLD and oil palm spear-rot appear to be similar as they can cross-infect all the three hosts (Anon., 1995). This makes it all the more difficult to keep constant vigil of mixed plantations, or contiguous plantations of coconut, arecanut and oil palm, to detect any possible disease appearing. Only through RFLP/RAPD/AFLP analyses one can unravel the real identity of the three MLO's and the nature of homology/dissimilarities among them. This would also help in identifying the nature of field tolerance/resistance of the CGD and its D x T hybrid with field tolerant/resistant WCT palms located in hot spots of RWD (Nair *et al.* 1996, Jacob *et al.* 1998).

In fact, Harrison *et. al.* (1992) have cloned and identified 5 Eco RI restriction fragments comprising chromosomal DNA of MLO associated with lethal yellow (LY) disease of Manila Palm (*Veitchia merrillii)* occurring in Florida. When used individually as (32P)d ATP - labeled probes in dot and Southern hybridizations at high stringence, four or five probes consistently hybridized to DNA extracts derived from LY-affected palms only. However, at moderate stringency, all probes hybridized with DNA of other MLO's that occur in Florida, and 3 probes also hybridized to DNA of several *Acholeplasmas* and/or *Spiroplasma* species. In addition to Manila palms, the probes also detected the presence of LY MLO DNA in DNA samples extracted from heart tissues of LY affected true date (*Phoenix dactylifera),* cliff date *(P. nupicola),* chinese fan palm *(Livistona chinensis),* and five coconut palm cultivars. Probes also hybridized to DNA from symptomatic *Caryota rumphiana* and *Livistona rotundifolia,* two palm species previously not known to be affected by LY mycoplasma. This extends the known host range of LY MLO and increases our understanding of epidemiology of the disease. These probes may be useful in identification of primary and alternate plant hosts and insect vector(s) as both the probes and the diseases are further characterized.

Similarly, molecular diagnosis has been developed for the viroid agent of Cadang-Cadang disease (CCCVD) of coconut in southern Luzon of Philippines (Hanold and Randles, 1991 a,b; Maramorosch, 1993). This disease develops very slowly and cannot be unequivocally identified on the basis of visual symptoms in a single observation. Another disease of Guam coconuts called "tinangaja" is also caused by a related viroid (CTiVD). Oil palm also develops bright orange leafspots under natural infection or artificial inoculation, resulting in loss of nuts and the palm. Other palms inoculated successfully with CCCVD are arecanut, golden palm *(Chrysalidocarpus herbaceous lutescens),* date palm, royal palm *(Roystonea regia)* and Manila palm. Several other herbaceous monocots growing near coconut also contained viroid-like molecules with similar DNA sequence as CCCVD. These authors have cautioned against unrestricted movement of germplasm following their recent identification of CCCVD-like sequence in the Pacific region, far away from Cadang Cadang area. Even embryo/tissue cultures of coconut and oil palm should be derived only from material tested for freedom from viroid. In fact, Rillo *et al.* (1988) have used *in vitro* cultured embryos to screen coconut populations for resistance to Cadang-cadang.

In Ivory Coast, Franqueville and colleagues (1991) have located resistance/tolerance to the bud rot pathogen, *Phytophthora heveae.* Whereas, the West African Tall (WAT) was sensitive to bud rot, it was tolerant to immature nut fall. Within PB-121 hybrid (MYD x WAT) MAWA, there was considerable variability, some trees showing vertical resistance. MYD was tolerant to this disease. Other hybrids like Cameroon Red Dwarf (CRD) x Rennel Tall (RLT), MYD x RLT and D x T of Vanuatu showed tolerance to both bud rot and immature nut fall. These findings need to be confirmed in India and other countries where bud rot is prevalent due to *Phytophthora palmivora.*

Somerville (1994) at the Carnegie Institution of Washington has proposed a gene-for-gene hypothesis for molecular mechanism of disease resistance in barley against powdery mildew. The resistance genes encode receptor proteins that intercept a pathogen signal/and activate defensive responses.

Breeding for drought tolerance in coconut

The abiotic stress that adversely affects palms is drought that occurs frequently in many areas in the country. Although there is no authentic data on the extent of production losses caused by drought in coconut and arecanut, it was the unprecedented drought of 1982-83, particularly in North Kerala that led to a systematic approach to screen germplasm collections for drought-tolerance in coconut. Having identified the coconut cultivars and hybrids possessing desirable traits to withstand drought, like stomatal regulation (Rajagopal *et al.*, 1990), biochemical characterization of enzymes (Shivashankar *et al.*, 1991), and lipid peroxidation (Chempakam *et al.*, 1993); the next logical step would be to understand the genetic basis of tolerance to drought (Iyer and Dhamodaran, 1994). Application of modern techniques like RFLP/RAPD/AFLP for identification of genes controlling either stomatal regulation, epicuticular wax deposition, or specific enzyme markers, would help in opening up new vistas in drought research in coconut. For instance, the location of genes controlling the production of wax components like hydrocarbons or b-diketones, as also the synthesis or activation of enzymes like Superoxide Dismutase, in response to field stress might prove useful. Transfer of such genes to less tolerant or susceptible palms could result in evolving transgenic plants in coconut for drought prone areas. Karunaratne *et al.* (1991) have also developed an *in vitro* assay for drought tolerant coconut germplasm.

With regard to arecanut palm, in view of the self-sufficiency in production achieved through conventional breeding and selection, there has not been any attention towards biotech research. However, the use of dwarfing genes for evolving hybrids with improved talls such as Sumangala, Srimangala and Mohitnagar, is considered promising, for ease of harvesting and resistance to YLD (Ananda, 1998). Use of molecular markers for genotype characterization and DNA fingerprinting, especially of the MLO causing YLD, would be rewarding.

CASHEW

Cashew (*Anacardium occidentale* L.), a native of Brazil, has acclimatized well in India and it is one of the important plantation crops of Kerala. Biotechnological approaches in cashew have not advanced much. The areas where biotechnology would be of importance in cashew are:

1. Intensification of research on somatic embryogenesis and plantlet regeneration which could subsequently be useful for genetic transformation to introduce genes for resistance to (a) tea mosquito, and (b) cashew stem and root borers.
2. Standardization of micrografting techniques for building mother blocks of elite orchards for supply of scion materials.
3. Molecular characterisation of existing genetic diversity.

One of the major objectives in cashew improvement is to achieve stability of performance in diverse environments. Being a highly heterogeneous, outcrossing population, the selections and hybrids need to be tested for particular locations in order to evolve region-specific cultivars and hybrids, since their performance over locations is highly inconsistent. Nevertheless, we have exceptionally adapted varieties like M44/3 which has performed consistently well in all locations. However, it is not possible to pinpoint the specific gene combination or complex which has contributed to the consistently stable performance of this variety over diverse locations. The use of modern tools of biotechnology such as RFLP/RAPD/AFLP markers can certainly help in identifying specific Quantitative Trait Loci (QTLs) which determine the high performance of varieties. Also, the RFLP mapping of varieties will clarify many doubts regarding correct identity of germplasm collections and duplicates, in molecular terms. Selection of parents for breeding programme will acquire a new dimension of precision, so that predictable hybrids with heterotic expression of characters of qualitative and quantitative inheritance can be realized (Iyer, 1995). The work in this direction has been initiated for characterising Tanzanian cashew germplasm using RAPDs (Mneney *et. al,* 1997). The molecular markers available at present are many and suitable markers have to be identified for characterizing and tagging genes controlling kernel quality, and resistance to pests and diseases.

Micropropagation of cashew using shoot tips of seedlings has been reported recently by Hegde *et al.* (1998). Thimmappaiah and Shirly (1998) have reported limited success with mature-tree explants.

Use of tissue, cell and anther culture in cashew, should not be looked upon merely for generating clones of elite trees, both as rootstock as well as scion material. Efforts should also be directed towards developing a cell-suspension system, for induction and multiplication of somaticembryos. The problem of browning, particularly of adult tree tissues can be overcome to a great extent, by the use of liquid cultures under dark incubation, and use of chemicals like phenyl-3 methyl-5 pyrazolone, ammonium chloride, urea and sugar to remove inhibitors secreted by explants, which on reaching a certain threshold value inhibit the growth of tissues, bud-break and elongation. Successful *in vitro* micrografting and subsequent transfer of grafts to soil has been reported by Kesavachandran *et al.* (1998).

Multiple shoot formation

Bud culture in cashew has given only a limited number of shoots, and increase in dose of cytokinin often resulted in fasciated micro-shoots. However, lowering BA levels and increase of sucrose, has induced elongation of microshoots (D'Souza *et al.*, 1994). Recently, Cardoza and D'Souza (1998) have studied salt tolerance of multiple shoots..

Immature cotyledon segments have given direct somatic embryogenesis without any intervening callus, or a very incipient callusing. This system if augmented and made regular can be used for basic studies on somatic embryogenesis, as also in encapsulation studies for developing protocols for germplasm storage both under limited growth conditions as well as cryostorage. Thimmappaiah and Shirly (1998) have reported 2-6 buds/explant of nodal segments excised from mature trees, on media containing Thiadiazuron or BAP + GA + IBA.

Anther culture

There is a long standing need in cashew for developing haploids and isogenic lines to facilitate heterosis breeding as well as for gene transfer experiments. The major limitation in achieving *in vitro* androgenesis is the availability of only a single fertile anther whose size is quite small at the uninucleate microspore stage of the buds. Tea mosquito infestation compounds the problem further resulting in severe browning and contamination. Screening of cultivars and hybrids against tea mosquitoincited toxin, and further multiplication of the resistant selections is a priority area where tissue and cell culture combined with molecular techniques will pay dividents. The greatest advantage is the natural propensity for obtaining cashew grafts in large numbers, for which bio-technology approaches would help to supplement the additional requirement of scion shoots as well as root stock materials generated *in vitro* (Thimmappaiah, *et al.*, 1999). Recently, *in vitro* micrografting has been standardized by (Kesavachandran *et al.* 1998).

BEVERAGE CROPS

Tea, coffee and cocoa as a group form ideal model systems for combining conventional breeding with modern tools of biotechnology and genetic engineering for crop improvement, since the scientists working in these crops have nearly perfected the protocols for *in vitro* multiplication of elite materials. Both tea and coffee research institutions have developed necessary infrastructure for this work, and hence are well poised to enter the new era of Bio-tech breeding.

TEA

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important plantation crop. India is the leader in tea production. The Tea Research Institutes at Tocklai

(Assam), UPASI (Tamil Nadu), and CSIR Complex (Palampur) are carrying out research on tea biotechnology. The major areas where biotechnology would be useful in tea improvement are as follows:

i. Micropropagation for mass multiplication of elite tea clones.

ii. Application of molecular markers for characterizing tea clones as well as QTL.

iii. Genetic engineering for developing resistance to tea blight (*Exobasidium vexans).*

iv. Identification, characterization and gene transfer for low caffeine tea.

Protocols have been developed for micropropagation, somatic embryogenesis and their encapsulation to produce synthetic artificial 'seeds' of tea (Sood *et. al.*, 1993; Manivel., 1993; Rajasekharan and Mohankumar, 1992; Rajkumar and Ayyappan, 1992; Rajkumar and Marimuthu, 1998; Haridas *et al.,* 1998). Tea plantlets have been successfully induced from cotyledon callus (Wu, 1976; Wu *et. al.*, 1982) and from stem callus (Kato, 1985). Production of shoots and buds has been achieved using nodal explants (Phukan and Mitra, 1984 Thimmappiah and Shirly, 1998; and Thimmappiah *et al.* 1999). Tea being self-incompatible, it would be worthwhile producing haploids and homozygous diploids using anther/pollen culture technique, and preliminary success was reported upto the multicelled pollen stage and haploid callus formation (Raina and Iyer, 1974, 1983, Chen and Liao, 1987).

Low caffeine tea

Evolving low caffeine tea with improved solubility, which involves the applicationn of both conventional breeding as well as molecular tools like RFLP/RAPD, to unravel the molecular biology of cell wall polymers and architecture. Possibility of transferring low caffeine genes from other *Camellia* sp. should be explored both through conventional hybridization and transgenic approaches to the modification of the biosynthetic pathway for caffeine synthesis, using antisense RNA technology to selectivity swtich off individual genes and block biochemical pathways. The other approach is through accelerated particle gene delivery (using 'gene gun') which has brought many recalcitrant crops within range for genetic manipulation.

In vitro approaches for evolving drought tolerant teas are being followed at the UPASI Tea Research Institute, where, protocols for rapid clonal multiplication have been developed in four cultivars (Manivel, 1993). Induction of variation for selecting drought tolerant somaclones through irradiation or chemical mutagens, has been in force at the UPASI Tea Research Institute using UPASI-3 clone whose *in vitro* response was good and productive.

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, (Banerjee, 1986, Balasubramanian *et al.*, 1998).

Tea being a dicot species, it would be worthwhile pursuing *Agrobacterium* mediated transformation as the choice gene delivery system. Tea regeneration from both shoot tips and cotyledonary callus-derived somatic embryos has been reported from Japan (Nakamura, 1987). Recently Haridas *et al.* (1998) have reported 14% frequency of somatic embryogenesis produced directly or via callus on a nutrient medium containing BAP and NAA. Breeding for resistance/tolerance to blister blight fungus *(Exobasidium vexans)* would be facilitated if the *in vitro* system is employed for evaluating and screening fungicides, as also the resistance of the tissues to various races of the pathogen (Agnihothrudu and Chandra Mouli, 1991). Raj Kumar and Marimuthu (1998) have reported that Tissue Culture plants were more vigorous than vegetatively propagated tea plants, and produced higher number of laterals in response to centering and tipping.

COFFEE

There are about 70 species under the genus *Coffea* out of which *C. arabica* and *C.canephora* are the most important. India contributes to merely a 4% of the global export. Nevertheless, it is an important beverage crop of the country.The major constraints to coffee production and quality where biotechnological tools can offer solutions are as follows:

1. Development of resistance through genetic engineering techniques for fungal diseases particularly *Hemileia vastatrix* (leaf rust).

2. Introduction of Bt genes for control of coffee berry borer *Hypothenemes hampei* Ferrari, and stem borer *Xylotrechus quadripes.*

3. Use of embryo rescue techniques for interspecific crosses from resistant species like *C. stenophylla* for leaf miner (Medina *et al.,* 1984) and some species resistant to nematodes (Medina *et. al.,* 1984).

4. Development of tools for quality improvement for uniform maturity, short-maturation cycles, high soluble solids, large bean size and increased bean density and texture (Sondahl and Loh,1988), better aroma and low caffeine content.

Biotechnological research carried out in coffee has resulted in development of protocols for somatic embryogenesis, micropropagation, molecular markers and genetic transformation. Sondahl and Lauritis (1992) have presented a comprehensive review on coffee biotechnology. Since the successful induction of somatic embryogenesis in *C. canephora* by Staritsky (1970) numerous reports have appeared on the successful regeneration of plantlets from somatic embryos. Sondahl and Sharp (1977) developed a two step method for somatic embryogenesis from leaf explants. Later it was modified by Neuenschwander and Baumann (1992) as Self Controlled Somatic Embryogenesis (SCSE). In India Sreenath and his group (Sreenath *et al.,* 1995) obtained somatic embryogenesis from integument tissue of *C.canephora.* Staritsky and van Hasselt (1980) suggested a modified process for conversion of somatic embryos in liquid cultures. A later development was that of temporary immersion which is suggested for improvement of micropropagation through microcuttings as well as somatic embryoids (Berthouly *et al.,* 1995).

For micropropagation nodal orthotropic explants of *C. arabica* (Custers *et al.,* 1980) and apical meristems (Zok, 1985) have been used. Berthouly *et al.,* (1987) developed a long term programme for nodal culture of coffee. Use of haploidy has also been exploited in coffee breeding (Neuenschwander *et. al.,* 1993).

Synthetic seed technology for encapsulating embryos in sodium alginate has been developed. Anther culture technique has been successfully employed for callus induction and plantlet regeneration in C x R inter-specific hybrid between *C. congensis* and *C. canephora*, for rapid fixation of heterosis (Sreenath, 1998). This programme was carried out in collaboration with SPIC Science Foundation, Madras, where protocols for isolation of embryogenic cultures, using leaf and nodal explants were developed. Work has been initiated to develop the *Agrobacterium*-mediated transformation system using a leaf-disc procedure, and on protoplast isolation and culture from embryogenic calli of *C. arabica* and C x R hybrid (Mamatha and Sreenath, 1998).

Coffee micropropagation via somatic embryogenesis promises to be an efficient method of propagating elite individual trees from a segregating population, which will be competitive in time and cost.

The successful recovery of plants via somatic embryogenesis in several wild coffee species, five *C. arabica* cultivars and two interspecific hybrids demonstrates the potential of using *in vitro* methods for coffee improvement (Sondahl and Lauritis, 1992). Muniswamy *et al.*, (1998) have reported high frequency somatic embryogenesis in Cauvery on 1/2 MS medium with 4% sucrose.

Somaclonal variation in coffee would be an excellent way of shortening breeding programmes, since it provides access to genetic variability within existing cultivars, carrying few alterations, while preserving the genetic integrity of commercial varieties. This variation is either naturally occurring or induced *in vitro* during plant regeneration from callus, due to chromosome breaks, translocations, deletions, aneuploidy, polyploidy, gene amplification, transposons, somatic crossing over, or point mutations (Evans and Sharp, 1983).

In *C. arabica*, some 40 mutants have been identified which are controlled by single genes with expression of dominance, recessiveness, or partial dominance, over the alleles of *typica* variety. Appearance of mutants with monogenic inheritance indicates the partially diploidized nature of arabica coffee (Carvalho et al., 1969). Santa Ram and Sreenath (1998) have used DNA fingerprinting to locate coffee rust resistance genes.