REVIEW OF LITERATURE
Over the millennia, forests had always played a pivotal role in shaping the life of human beings particularly in India. It was essentially Ararya Samskriti or forest culture in India where, the cultural diversity of the people was intimately interdispersed with forest habitats and resources. Needless to say, ancient Indians worshipped nature and elements comprising it. Even today, relics of the once virgin forests, in parts of India are preserved with religious reverence and worshipped out of faith or fear of associated deities or favour of receiving direct or indirect benefits from an otherwise wood land. Harbouring extensive repository of useful materials, the forests that occur in vast stretches along the mountain chains or as congregated and isolated sholas in the slopes and valleys or as protected sacred groves even in the plains, have been meeting the ever changing needs and aspirations of the local people since ancient times with an approximate 3500 and an amazingly large number and variety of serviceable sylvan items (Rao and Rangaswamy 1971). In today’s scientific parlance, as against the impending international regimes like World Trade Organization (WTO) and Intellectual property Rights (IPR), and as an invaluable source of unique genes and gene products, the plant resources of the forests represent a treasure trove to be conserved, prospected and harnessed with efficient use of science and technology including sunrise technologies like biotechnology and information technology for the economic wellbeing of mankind.

Often forests are mistaken for trees which medicinal or otherwise, have towered above their fellow land plants for 200 million years. They are 16 times more ancient than grasses and were already a venerable group when the first dinosaurs roamed on the earth.
They are the fundamental and prominent components of many ecosystems and human economics. Ecologically, forest is said to be a plant community dominated by trees and other woody vegetation with a closed canopy. In India, such was the veneration for trees that Vrikshayurveda states: “Das Koop Samavapi, Das vapi Samahad, Dashad Samaputro, Dasputra Samataru which means that ten wells are equal to a pond, ten ponds are equal to a lake, ten lakes are equal to a son and ten sons are equal to a tree”. Undoubtedly trees, medicinal and non-medicinal serve man in more ways than any other living group of objects by providing a variety of products in addition to major ecosystem services of protection of water sheds and top soil and stabilization of climatic conditions. Air, water and fertile earth are the least applicable contributions that trees make to life on this planet.

India’s biological diversity is estimated to be over 47,000 plant species excluding aquatic forms and 81,000 animal species, representing about 7% of the world’s flora and 6.5% of world’s fauna respectively (Govt. of India 1999-2000). According to Natesh (1999) the country harbours about 45,000 plant species excluding aquatic forms, of which 20,000 are higher plants. The floristic exploration of Gamble (1921) recorded that, of the 4500 species of flowering plants known from south India, 20% are trees, 16% shrubs, 11% climbers and the remaining 52% herbs. As elsewhere in the world the impact of anthropogenic pressure and consequent degradation of the natural plant wealth has become an issue of serious concern in India (Frankel 1995). This situation has arisen not because forests have just started disappearing or that mankind had all along been oblivious of the importance and significance of the tree resource. It has arisen
because trees have for long been considered as that undepletable resource which can be exploited continuously by man, as the bottomless pit kind of resource and are replenished through the natural processes of regeneration and reforestation (Singh 1996). The database of "World Conservation Monitoring Centre" (WCMC) in 1995 contained records of slightly fewer than 600 tree species as globally threatened using the old IUCN categories of threat. One year later the database contained around 14,000 tree species of which 3200 were recorded as globally threatened (Eksplorasi 1997).

Although the importance of germplasm conservation and its impacts has long been discussed and greatly acknowledged in global and even local level, the depreciation of forest cover is ever increasing. The perusal of ancient Indian literature (Puranas) revealed that since 2\textsuperscript{nd} century A.D. Indians had paid attention in organized manner, on conservation and perpetuation of plants. The ancient Indian sages had a clear understanding about the importance of trees and their role in nature. They prevented wanton destruction of forests by attaching religious faith. Sacred groves, pieces of land with undisturbed natural flora found in the North-East and in the Western Ghats, are the best example wherein many threatened tree species in the IUCN list for example, *Syzygium travancoricum, Vateria macrocarpa, Myristica malabarica* are well protected. Vayupurana (200-600 AD) states that various types of natural calamities occur whenever man start felling trees on a massive scale. Matsyapurana cautions the public to the extent that, felling of trees without permission is a penal offence.
Although ancient Indians were aware of disasters of tree destruction and showed keen interest in tree conservation, it could not stand the rapid growth of human population, and its increasing material requirements. Many present day authors also emphasize the consequences caused by felling of trees such as scarcity of food, fodder, energy, raw materials for various purposes, wood demand and natural calamities (Pushpaghadan 1992, Khoshoo 1996). It is also a serious concern of other scientists that tree felling would lead to the extinction of many organisms or biodiversity because tree provides microclimate and habitat for many other organisms (Mohan Ram 1983, Raven 1999). Several international (FAO, IPGRI, PROSEA), Governmental Forest Departments, Botanic Gardens and NGOs (Chipko, FRLHT) have been encouraging the cultivation of trees. However, in most cases, industrially useful clones especially wood, fodder and fruit yielding crops are preferred. Cultivation of woody species used as medicines have been greatly ignored (Singh and Kumar 1988). This tendency has led to serious erosion of diversity among the natural population of trees (Wilkins 1991). Moreover, phytochemical screening of tree species by the application of modern tools and technologies reveals that most species have immense potential as medicine and in most cases this potential is yet to be tapped. While employing IUCN criteria for determining the status of selected species of medicinal plants of southern India, the FRLHT based at Bangalore, stated that out of 144 species evaluated, 52 are trees of which 3 are included in the list of critically endangered, 11 are endangered and 25 are vulnerable. The number would increase on further evaluation (FRLHT 1997).
The recent revival of interest in "green drugs" and consequent exploitation of woody medicinal plants for factory scale preparations of even traditional medicine without any effort to augment the slow-growing perennials indicate the emerging importance of woody plant conservation. Conservation of trees especially medicinal and fruit trees, not only contribute to protection of other wild fauna and flora but should also help to improve the soil and environmental health. Besides, the rural and tribal communities, which depend on them for fruits, dwelling and medicine, also get their share of benefits. Unfortunately compared to other plant taxa, conservation of woody medicinal species is greatly ignored because of certain inherent problems related to their propagation and maintenance (Bonga 1981, Pushpaghadan 1992).

It is often stressed that conservation is not merely preservation and the conserved species especially medicinal plants should be utilized sustainably through cultivation and domestication of wild plants. The larger objectives of a true conservation programme with trees or for that matter other taxa will not be achieved unless the conserved taxa are also used by the communities around. According to Raven (1999), about 30 percent of the world's 300,000 plant species are in cultivation now and BGCI reports that nearly 35,000 species of higher plants belong to RET category.

The Western Ghats region had most luxurious and extensive flora in the past. The tree wealth of the region remains unrivalled in its diversity and of utility only in certain pockets today. The region as a whole has registered considerable environmental degradation in recent years. Although, Tamil Nadu part of the Ghats has the wetland,
moist tropical and most biologically diverse forests, it is also one of the worst sufferers of
large scale deforestation primarily due to burgeoning population increase, human
settlements, various developmental projects, irrigation and electricity and extensive
cultivation of cash crops like tea, rubber, coffee, cardamom, pepper, etc right in the hot
spots of biodiversity in the region.

Of the total 16,000 sq.km area in the Western Ghats, forests now constitute less
than one-fourth. Ruthless and illicit cuttings of vast woodlands for timber and stripping
away the trees for fuel, fodder and medicine continue unabated across the entire stretch of
Agasthiamalai mountain.

Against the backdrop of continued degradation or loss of pristine forest habitats
and looming exturrpation and extinction of precious germplasm, non-conventional
approaches are needed for the conservation of medicinal trees. Biological, ecological or
anthropogenic, whichever be the cause, certain prioritization is needed for selection of
conservation and their use. Since species of prospective value are accorded special
consideration in conservation oriented programme, the most pragmatic and practical
approach would be to select rare and economically prospective medicinal trees. A
combination of strategies and ingenious use of both ex situ and in situ methods have been
advocated to achieve conservation of rare and endangered plants (Wyse Jackson and
Sutherland 2000). It is also known that for a thickly populated developing country like
India, there are ever so many pressing needs on the socio-economic front and therefore, it
is not practical to extend the in situ protection cover beyond the present 4.2% of the total
geographic area of the country.
Although tropical trees are characteristically narrow in their ecological preferences, they too have a variety of breeding options. However, vegetative propagation methods are not prominently displayed in trees as in their herbaceous counterparts. In such cases, the application of tissue culture technology to salvage and multiply selected species as part of an ex situ conservation strategy to solve the problems of species depletion is recommended (Mohan Ram 1983).

WOODY PLANTS

Wood is a highly versatile renewable material and has been closely associated with man's basic survival and development and in the process of growing trees fixes atmosphere carbon and thus helps ameliorate the environment. India as one of the major users of wood in the Asia-Pacific region country had, till recently, the privilege of having abundant supply of wood from several tropical hardwood species including Shorea, Gluta, Teak, Rose Wood, etc. Utilization of these species for different end was, by and large, based on practical experience rather than scientifical validation, product development and application technologies (Arun Bansal 2004). Rational use of wood and wood products in manufacturing maximum value added marketable products is one of the determinant factors to the socio-economic benefits finally derived from the forestry sector. In India, wood products research hitherto has been material oriented and to some extent limited to primary processing aspects. There has been no emphasis on downstream processing of these primary products - sawn wood and panel materials into end products like joinery and furniture. Research and development in the following areas is vital to the effective utilization of plantation timbers (Schari-Rad and Welling 2002).
The challenge before industry is to enhance capability to process available wood raw materials into quality products through implementation of innovative and adaptive technologies developed at R & D institution. Necessary enabling environment needs to be created by appropriate institutional, legal and policy initiatives. One can be optimistic about the future of wood products in India provided responsible agencies like foresters, processing industries, develop technologies and consumer organizations realize their respective roles in view of the changing scenario of raw material supplies, and industrial and economic reforms presently unfolding the Indian Industrial Sector.

Cellulose is the major constituent of wood comprising nearly 50 percent of wood substances by weight. The intimate association of cellulose with lignin and hemicelluloses imparts to wood its useful physical properties. Cellulose is the basic raw material for paper, rayon, films, lacquers and explosives. Lignin comprises 23 to 33 percent of softwoods, but only 16 to 25 percent of hardwood. As a chemical lignin is an intractable, insolvable material. To remove it from the wood on a commercial scale requires vigorous reagents, high temperature and high pressures. One sizable commercial use for lignin is in the formulation of drilling muds, used in the drilling of oil wells, where it’s dispersant and metal combing properties are valuable. It has found use also in rubber compounding and as an air-ent -aining agent in concrete mixes. Lesser amounts are processed to yield vanillin and solvents such as dimethyl sulphate and dimethyl sulphoxide.
Hardwoods contain an average of 20-30 percent hemicelluloses with xylose as the major sugar. Softwoods contain an average of 15 to 20 percent hemicelluloses, with mannose as the main sugar unit. The hemicelluloses play an important role in fiber-to-fiber bonding in the paper making process. Therefore, the properties of wood make the trees unique and incomparable in terms of utilization to shrubs and herbs which together constitute a community in the natural forests. Besides, as already stated other species, as many as 30, including fungi, mosses, epiphytes, birds and ants depend on trees for their very survival.

MICROPROPAGATION

Tissue culture (in vitro) technology is now successfully developed and applied for large scale multiplication of many important species including medicinal plants. This technology is widely used for the vegetative propagation of selected plant in agriculture and horticulture and, to a lesser extent in forestry. The major objective is to produce large number of plants with uniform quality at a definite space. Historically, commercial applications of this technology were restricted to herbaceous plants (Hussay 1975, Hussay and Stacey 1981). However, for the last two decades, considerable success has been obtained in woody plants (Zimmerman 1986 and Thorpe et al., 1991). These includes both gymnosperms and angiosperms, i.e., softwoods and hard woods, and both trees and shrubs. Economically, the trees are extremely important for wood products, including lumber, pulp and paper, forestry plantations and reforestation. The Wisconsin Paper Mill, for example, is run mostly with the trees raised through tissue culture. Large-scale clonal systems can be an asset for selected high performance trees, and reliable
protocols are necessary for further genetic manipulation. However, a major problem in the propagation of woody plants is that most success is achieved with juvenile tissue and not from proven mature trees in 1990’s (Harry and Thorpe 1990, Dunstan and Thorpe 1986). According to Abbott (1997), woody plants were assumed to be intractable in culture and research workers therefore paid only scant attention to them. Needless to say, many woody plants need to be thoroughly and urgently investigated especially those connected with food, oil, spices and medicinal products.

Traditional methods for propagation include rooted cuttings, grafting and layering. For *in vitro* micropropagation, the methods generally used are first, the induction of adventitious buds or auxiliary bud breaking, both of which produce shoots that are subsequently elongated and rooted, and second, somatic embryogenesis mainly from juvenile tissue. Source of the trees successfully multiplied through adventitious bud / axillary buds are *Diospyros kaki* (Takuya et al., 1998), *Albizzia lebbeck* (Gharyal and Maheswari 1981), *Pterocarpus santalinus* (Arockiasamy et al., 2000), *Morus australis* (Pattnaik et al., 1996), *Pinus sylvestris* (Andersone and Levins 2000), *Dalbergia sissoo* (Singh et al., 2002), *Tectona grandis* (Daqueinta et al., 2001) etc. and others multiplied through somatic embryogenesis includes *Santalum album* (Ravisankar and Jen Mc Comb 2002), *Eucalyptus globules* (Greg Nugent et al., 2001), *Eucalyptus* (Muralidharan and Mascarenhas 1995), *Anacardium occidentale* (Shilpa and Rajani 2000), *Citrus reticulata* (Sheshu et al., 2002), *Panax ginseng* (Tang 2000), *Gossypium hirstum* (Ikram-ul-Haq and Yusuf Zafar 2004) etc.
Established tissue culture methods are appropriate for the mass propagation of some crop species, but for the others serious difficulties remain. In general work on economic and food crops has been rather limited when compared with model systems such as carrot, tobacco, and other members of Solanaceae. Woody plants also propagated using in vitro system belonging to the former category.

In fact a serious beginning has yet to be made in most of the tropical species. In the Indian context Muralidharan and Mascaranhas (1989) have reviewed the progress of the work done on Indian trees including the endangered species. Unfortunately, in many cases, the plants supposed to have been multiplied in vitro were not established in the field or the forest areas. It is particularly so in Indian tissue culture research where not many applications have been realized. Although, many Indian laboratories are involved in plant tissue culture research, commercial applications have always remained a distant reality.

Things have lot impaired now in Indian laboratories. The Plant Biotechnology Group at Tropical Botanic Garden and Research Institute has cloned such tree species or woody climbers as Aegle marmelos (Ajith Kumar and Seeni 1998), Crataeva magna (Ganga Prasad et al., 2005), Blepharistervira membranifolia and Celastrus paniculatus (Lakshmi Nair and Seeni 2001), Calophyllum apetalum (Lakshmi Nair and Seeni 2003), Celastrus paniculatus (Lakshmi Nair and Seeni 2002) and established all the species in the field or reintroduced them back into native with more than 65% establishment rates. Other woody tree species so far successfully multiplied through in vitro
methods and established in the field are *Eucalyptus camaldulensis* (Arezki *et al*., 2000), *Ginkgo biloba* (Gang Ping *et al*., 2000), *Acacia sinuta* (Vengadesan *et al*., 2003) and *Cinnamomum comphora* (Nirmal *et al*., 2003). Based on the indigenous knowledge’s developed, National Facility for Plant Tissue Culture (NFPTC) have been established at National Chemical Laboratories, Pune and Tata Energy Research Institute, Delhi for commercial production and supply of several woody taxa including Bamboos, Teak, *Populus*, *Shorea*, etc.

**CONSERVATION OF TREES**

A wide generalization about tropical tree species is that most of them occur at very low adult densities and are of relatively uniform dispersion, such that adult individuals of the tree species are thinly and evenly distributed in space. If true, this generalization has potentially profound consequences for the reproductive biology of tropical tree species. The slow growing and perennial nature of the trees in general together with the tendency to realize quick returns have largely dissuaded the public from taking up their illustration. Besides, unlike timber species, the general public is not familiar with the arborescent medicinal taxa and education and awareness programmes are most lacking in this area. In nature, with or without over harvesting, all sorts of stresses imposed on them in an otherwise shrinking ecosystem may be overwhelming and may push them into peril. Often natural regeneration of these species is poor, because of sporadic seed production and production of recalcitrant seeds that are intolerant to desiccation, so that their genetic diversity can not be preserved in conventional seed banks (Martinez *et al*., 1997).
The conservation of forest genetic resources in situ being ideal for the pollination and evolutionary processes to continue but impractical implies that complementary ex situ strategies must be implemented to increase the success of overall gene conservation, ex situ conservation measures include collection of seeds from seed stands, storage of seeds and developing an efficient germination system. The seedlings so raised from different populations of the same species, perhaps from different phytogeographical regions, may retain most, if not all the variability available within the species and can also be used for reintroduction (into the natural habitats of the species) or translocation (into alien forest sites having similar or identical agroclimatic conditions of the native habitat) for reestablishment. In such a case the seed propagation may be a far cheaper alternative to tissue culture through micropropagation for plant gene conservation and species enrichment. Unfortunately, seed propagation studies for ex situ conservation of truly endangered tree species in the Indian context are also scarce (Calophyllum austrindicum, Diospyros barberi, Garcinia imbertii, Poeciloneuron pauciflorum, Syzygium beddomei, Garcinia rubroechinata, Gluta travancorica, Pavetta travancorica etc.) and plant restoration through such a route is not attempted to any significant extent. Seed germination and storage studies carried out in certain endemic tree species of the Western Ghats point to their recalcitrance (Tectona grandis (Masilamani and Dharmalimgam 2001), Dalbergia retusa (Garcia and Stefano 2000), Mangifera indica (Girija and Srinivasan 2000), Peltophorum dubium (Perez et al., 2001), Asparagus recemosus (Gupta et al., 2002), Ilex aquifolium (Sagrario and Francisco 2004), Heracleum candidans (Jitendra et al., 2004). Unlike embryo culture and seed
propagation, vegetative propagation, seldom practiced in RET tree species results in the multiplication of the existing genotypes and as such, it is similar to in vitro clonal multiplication in principle (multiplication through sprouting of existing meristem) though it makes use of macro-cuttings and rate of multiplication is slow.

Each option has inherent biological problems requiring research depending on the nature of the material and the specific type and scope of activities (Isajev 1997). Seed storage is a relatively quick inexpensive and effective conservation measure for species which has orthodox seeds. However, long-term storage of seeds needs to be carefully planned and may present considerable problems due to intrinsic characteristics and added constrain by difficult seed handling and storage methods for such species. Under such conditions even small improvements in the maintenance of viability could mean a significant step forward for the bulk production and supply of seeds for use in large scale planting; well planned plantations, in turn could potentially make a significant contribution to more effective general conservation. Unfortunately, knowledge on seed collecting and seed physiology of these tree species is generally poor or even non existent, resulting in a very variable quality and poor availability of seed for planting (IPGRI 1996). Therefore, research is needed to develop cost-effective techniques for seed handling (eg. collecting, storage, germination testing) for a number of economically valuable trees species, particularly tropical forest tree and agro foresting species. In vitro conservation techniques have the potential to resolve ex situ conservation problems of species with recalcitrant seeds and those that can not propagate vegetatively. Studies on seed, vegetative and in vitro propagation of endemic RET species will be greatly
strengthened if the details of distribution, population and associated reproduction biology of the species are also worked out. Therefore, as part of the conservation work on the trees species selected for the present investigation, aspects of distribution in Agasthiyamalai region, associated species, phenology, reproduction biology, extent of variation within the populations, seed germination, embryo culture and tissue culture were investigated to assess the conservation options available.

**TREE TISSUE CULTURE**

The history of tissue culture in tree propagation and its importance in reforestation, conservation of genetic resource, forest products and clonal improvement has been dealt in a number of publications. In the pioneering work of Gautheret (1938), the cambial tissues of tree species were found to be highly responsive, giving rise to callus. Fully organized plantlets of tree species propagated *in vitro* and capable of subsequent transfer to soil were first obtained in *Populus tremuloides* and among gymnosperms, in Pinus. Thus there was a gap of almost 30–40 years between initial attempts and the achievement of propagating plantlets suitable for cultivation. As the practical application of tissue culture studies became more obvious, many workers were fortunately attracted to this subject. So far, more than 180 species of woody plants have been studied *in vitro* with variable success in the productive of shoots, roots, embryos and whole plantlets.

Though the potential application of micropropagation for woody plants, particularly tree species, has been well reviewed, the application of micropropagation
system to woody plants has a rather late beginning, and many problems remain to be solved. Basically, there are two main objectives for developing clonal techniques for forest trees. Firstly, viable large-scale clonal systems can be an asset for selected trees and secondly reliable protocols are necessary for genetic manipulation. To be able to use micropropagated plants for reforestation, it would be necessary that large scale production systems are simple, reproducible, cost-effective and adaptable to nursery practices (Ahuja 1987, Nandi et al., 2002, Yasodha et al., 2004). As already stated to date large number of protocols has been developed for micropropagation of woody trees, but only a few have been demonstrated at field level. Progress of micropropagation involves several stages, each of which may be critical to decide its success for a particular plant species. The success in micropropagation of woody trees is limited by many problems. Most important is inability to manipulate easily in vitro explants taken from nature trees. Woody plants often secret substances into the medium in response to wounding or excision often inhibiting the growth and development of explants in vitro (Pierik 1987).

Clonal propagation is often practiced for the multiplication of important forest tree species and for bulking scarce or valuable forest tree seed. The transfer of non-additive characteristics is difficult through seed production approaches but is routinely possible through clonal propagation. Further, clonal options can be used to produce, maintain and multiply medicinal plant species. The macro and micropropagation approaches have the potential to meet the demands for good quality planting material. Vegetative propagation of Gmelina arborea has been successfully achieved through
budding and grafting and air layering, rooting of cuttings and bud sprouts (Arya and Haqua 1982, Rosales et al., 1992 and Tewari 1995). The existing tree cover is not sufficient to meet the requirement of major and minor forest products and for environmental conservation 33% area should be under forest cover. However, in practice only 19.47 percent of the total geographical area (329 million ha) of the country is now under vegetation cover as evidenced from visual and digital interpretation of satellite data in 1993. Out of this, dense forest with crown density 40 percent or more is only 11.73 percent and 7.61 percent is open forest with crown density 10 to less than 40 percent. The average productivity of forests in the country is only 0.7 m$^3$ ha$^{-1}$ (which compares poorly with the world average of 2.1 m$^3$) on one hand and on other hand, demand for forest product is tremendous. India is likely to touch 1,225 million human and 700 million livestock population by 2015 AD, necessitating 275 million tones food gains, 100 million tones fodder and 235 million m$^3$ fire wood, 27 million m$^3$ timber and 19 million m$^3$ industrial raw materials against our current supplying power of 40,12 and 4 million m$^3$, respectively of the latter forest products.

Latest information pertaining to the cultivation of trees and shrubs in agroforestry systems is available (Kritikar and Basu 1993, Anon 1994). Few authors have described the importance and cultivation of multipurpose tree species in agroforestry systems (Gill et al., 1998, Solanki and Shukla 1997, Bisaria and Tiwari 1997, Cook 1941, Tiwari et al., 1997). Recently, several investigators have well documented the use of higher plants as source of drugs on their use in medicines (Kamboj 2000; Phillipson 2001 and Atul et al., 2002).
Countries with sound scientific tree improvement programmes and large scale plantations will have definite advantage in the international markets for timber and wood products. Seven countries of the southern hemisphere i.e Brazil, Argentina, Indonesia, Chile, Newzeeland, Australia and South Africa will have nearly 9 million ha of *Eucalyptus* plantation by 2010. Other important countries which have intensively managed plantations include USA, Australia, Spam, Portugal, China and Korea.

The close relationship that exist between trees and man as well as other living organisms and the role of trees in maintaining the ecological balance of our planet, are too well known to be over emphasized. Among the higher plant species, trees have the distinction of being the largest and oldest living organism on earth with maximum life span. Some of the coniferous trees are supposed to be still growing well over 3000 years. Although the herbaceous habit has made unprecedented evolutionary gains since the middle and late coenozoic, trees still are the most conspicuous plants covering the habitat, the land surface of the earth (Zimmermann and Brown 1977). It was recorded that trees have been growing in our planet in various shapes and forms for the last 40 million years and life has evolved beneath their ever changing canopy. Due to the structural complexity and bounteous diversity of shape and forms, it became very difficult to propose a unique definition to tree species. Not surprisingly, the estimated number of tree flora in the world also varies according to the definition followed. The world tree flora is estimated to be around 100,000 species (Numbers vary according to the definition of tree followed). According to the most accepted definition "tree is a
woody plant growing on a single stem usually to a height of over two meters” and is adopted by the Temperate Broad leaved Tree Specialist Group of IUCN’s Species Survival Commission (SSC). Approximately 21,000 species of plant genera are predominantly woody and predominantly temperate in distribution (Eksplorasi 1997).

Micropropagation of various plant species, including many medicinal plants, has been reported. Propagation from existing meristems yields plants that are genetically identical with the donor plants. Plant regeneration from shoot and stem meristems has yielded encouraging results in medicinal plants like *Rauvolfia serpentina* (Roy et al., 1994), *Azadirachta indica* (Roy et al., 1996), *Isoplexis canariensis* (Perez–Bermudez et al., 2002), *Adhatoda vasica* (Azad et al., 2003), *Hemidesmus indicus* (Siddique et al., 2003), *Sterculia foetida* (Anitha and Pullaiah 2001). Numerous factors are reported to influence the success of *in vitro* propagation of different medicinal plants (Hu and Wang 1983, Hussey 1980).

The micropropagation protocols of some woody tree species and their importance are narrated here. The cotyledonary nodes of *Ferronia limonia* were cultured on MS medium for multiple shoot formulation with varying concentrations and combinations of auxins and cytokinins. The phenolic exudation was controlled by using sterilized distilled water containing antioxidants like PVP 50 mg.l⁻¹, ascorbic acid 100 mg.l⁻¹ and citric acid 150 mg.l⁻¹ for 4-5 times. In another example, cotyledonary or epicotyledonary nodes and hypocotyls explants of *Wrightia tinctoria* obtained from three-week old *in vitro* growth seedlings were culture on MS basal medium supplemented with various
concentrations of cytokinins (0.5-8.0 mg.l⁻¹ BAP and Kinetin) individually and in combination also with different concentrations of NAA (0.1 – 1.0 mg.l⁻¹) for multiple shoot formulation. In vitro rooting experiments were conducted in two different ways. In one method rooting hormone was incorporated into the medium while in the other a dip in sterilized IBA solution (50-200 mg.l⁻¹) followed by implantation of the treated shoots into medium of different salt strength induced root formation from the basal end.

In Wrightia tomentosa, nodal segments were treated with 1% NaOCl solution containing few drops of Tween-80 for 2-3 min followed by a dip in 90% ethanol for 2 minutes. Finally the explants were treated with 0.1% mercuric chloride for 5 minutes and washed with autoclaved distilled water. Explants were positioned both horizontally and vertically on different media containing TDZ (Thidiazuron), phloroglucinol, coconut milk and ascorbic acid to raise cultures successfully (Purohit et al., 1994).

In Sterculia urens, the role of thidiazuron was also tested individually. Also, various auxins (NAA, IAA, 2,4-D) were combined with the optimum concentration of BAP. Explants were cultured in conical flasks (100 ml) covered with non - absorbent cotton plugs and kept under controlled conditions of temperature (28 ± 2°C), light (45 μM m⁻² s⁻¹) for 16 hrs and 60 - 70% relative humidity (Sunnichan et al., 1998).

Aquilaria agallocha can produce fragrant agar wood used for incense, traditional medicine and other products. An efficient plant regeneration system was established via organogenesis from shoots developed from seedlings of Aquilaria agallocha. Shoots generated many buds on MS medium supplemented with 1.3 μM.l⁻¹ BA.
(6-benzylaminopurine) in the first 7 weeks, and the buds elongated on MS medium with 1.3 \( \mu M L^{-1} \) NAA (naphthalene acetic acid) in another 7 weeks, 2-3 shoots 2cm in length per explant were obtained within 14 weeks. Plantlets were rooted on \( \frac{1}{2} \) MS medium after being immersed in 5 \( \mu M L^{-1} \) of NAA for 48 hrs and 96.7% of roots grew up two weeks later. All plantlets survived well in the pots (Hej 2005).

*Hypericum perforatum* is a traditional medicinal plant with wound healing and antidepressive properties. Among the secondary compounds of interest is hypericin, a naphtodianthrone that seems to participate in the medicinal effects of this species. Cultures were initiated from nodal segments of mature plants inoculated onto MS medium supplemented with 4.5 \( \mu M \) BA, kinetin, thidiazuron, individually or in combination with 0.05 \( \mu M \) NAA. Organogenic explants were observed on medium with either BA or kinetin alone or in combination of these with NAA. Subculture of organogenic explants onto the proliferation medium containing 4.5 \( \mu M \) BA promoted the organogenic response. The highest average of shoot production (52.6 shoots) was obtained on those explants induced in the presence of BA and NAA. Rooted plantlets were successfully acclimated. Analysis of hypericin contents showed that levels found in callus represented only 0.11 % of what was detected in adult plants, while shoots and eaves from *in vitro* plants showed similar hypericin levels to those found in the leaves of the field-grown plants, suggesting that the accumulation of this compound is related to leaf differentiation (Leena Tripathi and Jaindra 2003).

A method for adventitious shoot bud regeneration from leaves of *Potentilla ulgens* has been developed. Leaves were obtained from plants growing in the natural
Explant browning, a major hurdle in the establishment of cultures, was overcome by treating leaves with a combination of antioxidants (100 mg.l\(^{-1}\) ascorbic acid, 100 mg.l\(^{-1}\) citric acid and 20 mg.l\(^{-1}\) L-cysteine HCl). Influence of the growth regulators BAP (6-benzylaminopurine) and NAA (α-naphthalene acetic acid) on adventitious bud differentiation and shoot regeneration was observed on modified Murashige and Skoog (MS) agar medium. The most effective treatment was MMS with BAP 0.1 mg.l\(^{-1}\) and NAA 0.1 mg.l\(^{-1}\), which gave 80% bud induction frequency with 38.4 BFC (Bud Forming Capacity) index and 48 shoots per explant of 3.5 cm length. Rooting was induced on MS basal medium. The regenerated plants had 70% survival rate (Laskar et al., 2005).

Single nodes from young top shoots of *Decalepis arayalpathra*, a woody shrub were cultured on Murashige and Skoog (MS) agar medium supplemented with 0.1 to 5.0 mg.l\(^{-1}\) of BAP. All the concentrations of BAP induced single axillary shoot of varying length. However, MS medium supplemented with 1.0 mg.l\(^{-1}\) BAP supported rapid growth and produced the longest shoots (6.8 cm) in 60 days. For further multiplication, the nodes and shoot tips from *in vitro* derived shoots were recultured on MS medium fortified with 0.5 mg.l\(^{-1}\) of BAP, which produced 8 cm long shoots having 5-7 nodes in 30 days period. Further, the top microshoot cuttings (3-5 cm long) with 2-3 nodes, sub cultured onto MS medium supplemented with 1.5 mg.l\(^{-1}\) IAA, produced an average of 6.3 roots in 30 days period. The rooted plants after hardening were reintroduced into their natural habitat at Kallar reserve forest, Thiruvananthapuram. After two years, 84% survival of reintroduced plants was recorded (Gangaprasad et al., 2005).
An efficient protocol has been developed for plant regeneration from shoot tip and nodal explants of in vitro grown *Psoralea corylifolia*. Nodal segments were more morphogenic to shoot bud differentiation than shoot tips. Proliferation of shoots was achieved on Murashige and Skoog (MS) medium supplemented with various concentrations of BA, Kn, IAA, NAA either singly or in various combinations. The highest shoot regeneration frequency (90%) and number of shoots (12.0±0.57) were obtained from nodal segment on MS medium fortified with 5 \( \mu M \) BA and 0.5 \( \mu M \) NAA. Addition of CH (100 mg.l\(^{-1}\)) to the shoot induction medium enhanced the growth of regenerants. The regenerated shoots rooted best on MS medium containing 0.5 \( \mu M \) IBA. Regenerated plantlets with well-developed shoot and roots were hardened, successfully transferred to soil and maintained in greenhouse. The in vitro procedure so developed can be used in conservation and mass propagation of this endangered medicinal plant (Mohammad and Faisal 2005).

Survey of the petridophytes and angiosperms of Western Ghats especially Agasthiyamalai (Tirunelveli hills) were analyzed thoroughly by our team (Centre for Biodiversity and Biotechnology, St. Xavier's College Palayamkottai. Number of ferns and herbs and shrubs from the region were also micropropagated successfully by this group. Most of them were reintroduced successfully with 80-85% of survivability. Armed with these initial successes from this laboratory, the present author ventured to work on the selected tree *Calophyllum austroindicum* that is endemic and over exploited for various purposes. In addition to their taxonomic, conservation and medicinal importance, this species finds other economic uses as well, while taking up the study.
the author was always reminded of the dictum that cultivation and artificial propagation should be used as a last resort and as a crisis management tool with the purpose of reestablishing the species in natural habitat. It is hoped that success achieved in the *in vitro* multiplication of the woody taxa and reasonable establishment back in nature of the reintroduced micropropagated plants reported would help to demonstrate that all is not lost; where man can destroy, he can also reinstate the endangered species, with the ingenious intervention of appropriate technology. In the present investigation, the author micropropagated the selected tree through embryo and nodal culture.

**CLONAL UNIFORMITY**

The clonal uniformity of the natural and propagated plants is difficult to detect by visual inspection of the plants. Although isozyme markers may be used to detect variations / uniformity (Tobolsky and Kemery 1992), such markers are known to be affected by environmental conditions and different stages of plant development (Falkanhagen 1985) and do not always for discriminate between genotypes. In contrast, DNA based markers overcome these disadvantages and allow for the discrimination, on finger printing of individual genotypes.

At present, the most popular and the simplest of the DNA based marker systems is the PCR – based (Polymerase chain reaction – based) DNA technique referred to as RAPD (Randomly amplified polymorphic DNA) (William *et al.*, 1990). Apart from being inexpensive, this technique allows for linkage maps (Grattapaglia *et al.*, 1992).
RAPD assay is being used for discriminating *Eucalyptus* species and hybrids via a co-operative research programmes with researchers from the University of the Orange tree state (De Lange *et al.*, 1993) and Council for Scientific and Industrial Research (CSIR). The RAPD profiles are suitable for such applied techniques as discrimination between clones and verification of genotypes of *Eucalyptus* and *Pines*. Based on these earlier findings, the author concluded that the genetic uniformity of the propagated plants could be tested by using RAPD assay.