Indian Journal of Biotechnology
Vol. 3, April 2004, pp. 159-170

Micropropagation for quality propagule production in plantation forestry

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Plantation forestry is the major source of raw material for industrial and domestic wood products and perpetually provides renewable energy, fiber and timber. Successful plantation forestry is dependent on effective research and development leading to technological advances. In hard wood plantation establishment, micropropagation is preferred for the production of quality planting stock because of its very high multiplication rate with instant silvicultural gains. Integration of micropropagation in tree improvement is essential for the replication of improved genetic material and rapid release of quality propagules. Further, *in vitro* propagation is also required for rejuvenating productive clones, which are difficult to root; rescuing of important mature individuals and natural hybrids; and producing stock plants for clone bank establishment and conserving the important germplasm. Thus, the approaches for micropropagation should be species oriented to fit into the existing strategies of tree improvement. In this paper, the recent advances made towards the production of quality plantlets through micropropagation of hardwood tree species, such as eucalypts, acacia, teak and bamboos, are presented. Further, the strategies for the multiplication of elite genotypes and the use of microproagules as planting stock in plantation forestry are discussed.

Keywords: micropropagation, tree improvement, plantation forestry, eucalypts, acacia, teak, bamboos

IPC Code: Int. Cl.[7] A 01 H 4/00, 5/00; C 12 N 15/00

Introduction

Plantation forestry is recognized as a raw-material base for industrial and domestic wood products, which provides perpetually renewable energy, fiber and timber. The economic benefits of planted forests have led to their widespread adoption throughout the world. Globally, 48% of the forest plantation is established for industrial use, 26% for non-industrial use (fuel wood, soil and water conservation) and the remaining 26% is not specified. The current extent of world's plantation forest area is about 187 million hectares (m ha) with the annual planting of 4.5 m ha[1]. India is one of the largest hardwood plantation resources comprising about 32.5 m ha with eucalypts, acacia and teak as major species[1]; whereas, bamboo species occupy 8.96 m ha. The annual planting target of India is about 3 m ha, which requires roughly 6100 m seedlings for 10 major species, viz. *Eucalyptus*, Bamboos, Acacias, Albizias, *Prosopis cineraria*, *P. juliflora*, Casuarinas, Dalbergias, Conifers and Teak[2].

The National Forestry Action Programme (NFAP) and National Forest Policy of India have identified the expansion of area under forest plantations with increased productivity as one of the important thrust areas in forestry. Significance of plantation establishment and the use of improved planting stock have also been emphasized in a number of recent reviews[3-5]. Although, the planting of genetically superior stock was started in 1970 but the planting of genetically modified trees has just begun in parts of the subtropics, New Zealand and South America[6]. Till date a large portion of the planting stock originates from unknown seed origin, when it is no longer considered valid for the establishment of end use oriented plantations. In the past decade, however, intensive work on forest tree breeding combined with biotechnological interventions has allowed the choice of species and planting materials, and has also provided a vehicle for the introduction of improved propagules. With increasing yields and shortened rotations, the planted forests have increasingly become attractive as an investment for producing future industrial wood[7].

Role of Micropropagation in Plantation Forestry

Plantation forestry, either of introduced or indigenous species, involves the use of genetically improved planting materials derived from tree genetic improvement programmes. Traditional breeding approaches are a long-term process to produce propagules of high quality; however, they are

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considered prerequisite for the effective utilization of biotechnological techniques, such as micropropagation and genetic engineering. The major challenge for the tree breeder is to quickly transfer the ever-improving material from the breeding programmes into plantations. The basic method of achieving such transfers is the use of seeds from orchards or clonal propagation. In view of the fact that open pollinated orchards are not an option for capturing high genetic gains, clonal propagation of selected genotypes through micropropagation is commonly used for acquiring genetic gain in a short time. Advantages of cloning in plantation establishment have been well discussed[8-10].

Micropropagation systems, such as axillary and adventitious shoot multiplication and somatic embryogenesis, employed in plantation forestry is regarded as an imperative strategy to achieve rapid genetic gain[9,11,12]. These techniques are currently in use for the large-scale multiplication of important tree species[13]. Further, micropropagation is the only choice for the multiplication of difficult-to root but economically or industrially important genotypes. Micropropagation has been applied to many tree species for rejuvenation and mass multiplication[14,15]. The potential impact of cloning through micropropagation methods on planted forests[16], forest industry and global timber supplies[6] is now well recognized. The role of tissue culture for the production of quality propagules in tree species has, recently, been also emphasized[17].

Micropropagation of Hard Wood Tree Species

Micropropagation through proliferation of axillary buds is a common technique in *in vitro* multiplication of forest trees. In the last decade, it has been applied extensively to important hardwood plantations of forest tree species[15,18]. Micropropagation exploits the regeneration capacity of the selected tissue; however, it is difficult to achieve in materials beyond the juvenile stage. As a result, most of the investigations, on the development of protocols for clonal propagation, are concentrated on juvenile materials. On the other hand, micropropagation of mature trees is only possible through manipulation of phase change and application of stress under *ex vitro* or *in vitro* condition that has the potential to overcome developmentally related restrictions[19].

Among various Asian countries, the success in micropropagation of tree species, including bamboos, differs and depends on their breeding programmes. For example, Malaysia has done extensive work in micropropagation of timber species, such as Acacias; the protocols for tissue culture were developed and the plantlets were tested in the field[20]. In India, the major accomplishment has been made in the *in vitro* propagation of various plant species, including forest trees like teak, eucalypts, bamboos and sandal[21]. To some extent, industries have also contributed in the field of tissue culture. The main focus of the industry has been on high value crops with tremendous export potential including few forestry species, such as teak, bamboos and eucalypts. However, the involvement of industry is considered essential to commercialize forestry propagules, cost-effective mass multiplication and supply of high quality products at competitive prices in the market. Indo American hybrid seeds (IAHS) and Southern Petrochemical Industries Corporation (SPIC) Agro Biotech Ltd., which have large micropropagation units in Asia, have successfully adopted the micropropagation technique for mass multiplication of teak, bamboo and eucalypts. The Micropropagation Technology Park at Tata Energy Research Institute, New Delhi, produced about 10 lakh plants of various forest tree species and planted them in 12 different states over an area of 2500 ha. Similarly, National Chemical Laboratory, Pune, produced about 10 lakh plants of *Eucalyptus*, teak and bamboo and established trials at 100 locations over 1000 ha[22]. Commercial multiplication of *Eucalyptus* was successful in cooperative venture between the Agroforestry Foundation and EPC Irrigation, a private company, and over 60,000 plants of *E. camaldulensis* were supplied to farmers of Maharashtra, Gujarat and Madhya Pradesh[23]. Technology for rapid mass clonal multiplication of the most promising tree of *Populus deltoides* was developed by National Botanical Research Institute, Lucknow and has been transferred to the Tata Energy Research Institute, New Delhi,for up scaling and mass production of clonal plants for afforestation. The technology has also beentaken up for commercial launching by Cadila Pharmaceuticals Ltd., Ahmedabad (http://www.nbri-lko.org/mraksharma.htm).

In spite of these efforts, the continual use of *in vitro* produced planting stock for the large-scale operations is seldom practiced because the significance of integrating tree improvement with micropropagation techniques in producing genetically improved planting stock is still not fully recognized. Moreover, for the *in vitro* clonal programme to be successful in plantation forestry, the gain achieved must be much higher. Thus, *in vitro* propagation should be practiced in combination with tree genetic improvement activities for the rapid release of quality planting material. However, most of the reports available on tissue culture of forest trees give emphasis on the techniques followed to produce the plantlets rather than the circumstances and type of material used. The approach for micropropagation should be species oriented to fit into the existing strategies of tree improvement. The present article reviews the recent advances in the production of quality planting material for hard wood plantation species, such as *Eucalyptus*, Acacias, teak and bamboos.

Eucalyptus

Eucalyptus plantations contribute 25% of the total amount of wood consumed in the developed countries, where the primary uses are as an energy source (charcoal) and industrial round wood for pulp and paper production. In fact, short rotation intensive culture (SRIC) practice and experience of clonal propagation at Aracruz, Brazil have paved the way for the establishment of eucalypt plantations in many countries. In Brazil, improvement from 33 to 70 m^3/ha/yr over the past ten years was reported for *E. grandis* and *E. urophylla*, and up to 100 m^3/ha/yr was estimated for hybrid *Eucalyptus* growing under research conditions[24]. The propagules for these plantations were primarily obtained through clonal propagation. Various species of eucalypts are planted in different locations, for example, *E. globulus, E. grandis* and *E. nitens* are preferred in temperate regions; whereas, *E. camaldulensis*, *E. tereticornis* and *E. viminalis* are capable of withstanding under irrigation and very hot summer temperatures. The species are also selected for their end uses, such as suitability for the manufacturing of quality paper and charcoal. For instance, Andhra Pradesh Forest Development Corporation has raised clonal plantations of *E. tereticornis* over an area of 8795 ha for pulp wood production (http://apfdc.apts.gov.in/Vision2020.htm).

The importance of *in vitro* clonal propagation of *Eucalyptus* is evident from the amount of work available on the subject[13,25-29]. *In vitro* propagation in *Eucalyptus* is highly demanded in difficult-to-root situations, where productive clones need to be rejuvenated or mass multiplied, to rescue important mature individuals and natural hybrids to produce stock plants for clone bank establishment and also to conserve the important germplasm. From 20-year-old trees of *E. citriodora*, plantlets were obtained in culture and it was estimated that 1,00,000 plants could be produced from one bud in one year[30]. In Brazil, about 2,50,000 micropropagated plants of 12 superior clones, identified from provenances, were taken to the field[31]. About 170 superior trees of *E. grandis*, selected from 5-year-old progeny test, were micropropagated by organ cultures for breeding orchard establishment[32]. *In vitro* propagated clones were also used to establish stock plants for grafting of *E. camaldulensis*[33], and for rooting of cuttings of *E. tereticornis*[34], *E. grandis*[29] and *E. globulus*[35].

Epicormic shoots in eucalypts have been considered the best source of juvenile[33,35-37]. In *E. camaldulensis*, explants collected from epicormic shoots, which sprouted from branch segments of 6 to 7-year-old fast-growing phenotypes, showed greater response than buds collected directly from the tree[38]. Single and successive graftings have been employed in *Eucalyptus*, whereby mature scions are grafted to juvenile rootstock and explants from these materials are used for shoot induction[33,39]. Shoots from clonal hedges[28] and shoots produced after girdling[40] have also been used as a source of juvenile explant. Plantlets were produced from macropropagated ramets of 4-year-old ortets of *E. pellita* and *E. grandis* × *E. urophylla*. Rate of shoot multiplication and plantlet development were always better in coppice shoots of mature *E. tereticornis*[41-44], *E. camaldulensis*[44], *E. grandis*[45] and *E. citriodora*[46]. In contrast, higher rate of shoot multiplication was reported in mature explants than in coppice shoots of *E. radiata* ssp. *radiata*[47]. Axillary buds obtained directly from mature trees of *E. tereticornis*[48], *E. deglupta* and *E. camaldulensis*[49] were also successfully multiplied. In mature trees, *ex vitro* manipulation of explant source coupled with *in vitro* propagation increased the production of plantlets[50].

The production of inter-specific F1 hybrids in eucalypts has received a lot of attention since the outstanding success of the *E. urophylla* × *E. grandis* hybrid clonal propagation programme in Brazil. There is a lot of evidence that hybrids could also extend commercial plantations to marginal lands. Many hybrids are already in use in Morroco, China, Colombia and South Africa. Axillary or adventitious shoot bud multiplication was found successful in mature hybrid clones of *E. grandis* × *E. urophylla*[51-53], *E. camaldulensis* × *E. tereticornis*[52,54] and hybrids of

E. grandis[40]. It was reported that hybrid vigour remained unaffected in axillary shoot bud multiplication of mature hybrid trees of *E. torrelliana* × *E. citriodora*[55], *E. camaldulensis* × *E. tereticornis*[56], *E. grandis* × *E. urophylla* and *E. pellita* × *E. tereticornis*[57]. Seedlings obtained from F1 hybrids of *E. tereticornis* × *E. camaldulensis*[58,59], *E. camaldulensis* × *E. tereticornis*[53] and *E. macarthurii* × *E. grandis*[28] were also mass multiplied *in vitro*.

Root induction in *in vitro* produced shoots varies with species[44], genotype[38,51], explant source[33], growth regulator used in multiplication stage[60] and number of passage[40]. Generally, shoots obtained from seedling explants root easily[61]. Culture of explants, obtained from coppice shoots[43], grafts and epicormic shoots[38], showed better rooting than the explants collected directly from mature trees. Repeated subculturing of shoot tips produced *in vitro* rejuvenation with increased root induction in *E. camaldulensis*[33], *E. globules*[35] and *E. tereticornis*[34]. Further, *in vitro* rejuvenation achieved up to 80% rooting in the hybrid clones of cold-tolerant *E. nitens* and *E. grandis*, where the poor rooting ability of cuttings hampered plantation establishment[62]. Technically viable and economical *ex vitro* rooting methods have also been developed for the hybrid clones of *E. grandis* × *E. urophylla*[53].

The type of propagules produced has a general effect on their performance in the field. Moreover, to evaluate the different kind of propagation systems, field demonstration trials need to be established with appropriate controls[11]. Differences in field performance between micropropagated plants, rooted cuttings and zygotic seedlings were documented for *E. grandis*[63]. Tissue cultured plants performed better than cutting derived plants in *E. camaldulensis*[33] and *E. tereticornis*[41]; however, there was no significant difference except for flowering in *E. grandis* × *E. urophylla*[64]. Results from field trial of 4-year-old *Eucalyptus* clones[52] indicated that growth rate and uniformity and quality of micropropagated plants were superior to that exhibited by the individuals of the same genotype produced by rooted cuttings[29]. Under field performance, the tissue-cultured plantations of *E. tereticornis* were capable of producing 152% more yield than seedling plantations[65]. Uniform growth and self-pruning was observed in the 5-year-old micropropagated plants of *E. camaldulensis* and *E. tereticornis* but the growth rate was not better than the seedling raised plants[66]. Further, the inoculation of root symbiotic organisms was found to augment successful establishment of micropropagated plants in the field. *In vitro* produced plants of *E. tereticornis*, when grown with *Pisolithus tinctorius*, showed significant increase in growth[67].

Acacias

Acacias are one of the short-rotation and fast-growing tree species. Australian acacias are grown in over 70 countries and cover approximately 1,750,000 ha in plantations[68]. India has the largest plantation area under Acacia species[69], comprising 3 m ha with *Acacia nilotica*, *A. tortilis*, *A. catechu* and *A. leucophlolea* plantations. Species, such as *A. auriculiformis*, *A. mangium* and *A. crassicarpa*, are grown for timber and pulp. The bark of *A. mearnsii* has high content of tannins. *A. senegal* is grown primarily for gum and also plays a role in agricultural systems by restoring soil fertility and providing fuel and fodder. Hybrid combinations of *A. auriculiformis* and *A. mangium* have higher pulp productivity than either parent[70] and widely planted in Vietnam, Malaysia, Papua New Guinea and India. South Indian paper and pulp industries are also producing hybrid-planting material on large-scale for their plantations. Outstanding hybrid clones have been identified, tested and mass propagated for operational clonal forestry[70]; however, the propagation of mature trees is still a problem[71].

In vitro propagation methods have been reported for many species of acacia[72-77]. However, only few species, such as *A. senegal*, *A. seyal*, *A. nilotica, A. mangium*, *A. auriculiformis* and *A. melanoxylon*, were identified for advanced breeding programme. *In vitro* clonal propagation has also been practiced in large scale for the hybrid clones of *A. auriculiformis* × *A. mangium*[77,78].

To overcome the maturation effect on propagation, coppice shoots were used in *A. auriculiformis* × *A. mangium*[78] and *A. mearnsii*[79,80]; whereas, sprouting buds from stem cuttings were used in *A. auriculiformis*. From 4-year-old hybrid (*A. auriculiformis* × *A. mangium*) ortets maintained as hedge, axillary buds were collected from 1-year-old ramets and rejuvenated *in vitro*, where cultures restored the seedling traits. The culture-derived shoots were found morphologically identical to the seedling-derived acacia plants with bipinnate leaves[81]. The *in vitro* micrografting technique has also been a useful tool for vegetative, non-destructive propagation of mature selected *A. mangium* ortets[82,83]. Apical meristem was found more susceptible to rejuvenation during certain stages of development[84]. Apical meristems obtained from micropropagated plantlets and mature trees were used as source material for *A. mearnsii*[74] and complete plantlets were produced. The explants obtained from mature trees of *A. mangium*[85,86], *A. auriculiformis*[86,87], *A. catechu*[88] and *A. Senegal*[89] were also micropropagated successfully.

Seedlings of hybrid acacia[75], *A. mangium*[90], *A. stenophylla*[91], *A. seyal*[92], *A. mearnsii*[80,93], *A. catechu*[94] and *A. auriculiformis*[95,96] were multiplied *in vitro* through axillary bud proliferation. Cotyledonary nodes of *A. mangium*[97] and hypocotyls of *A. sinuate*[98] were also used to regenerate adventitious shoots. In *ex vitro* rooting, shoots more than 0.5 cm long gave high rooting success. In the case of *A. auriculiformis* × *A. mangium* hybrids, the rooting ability declined with the increase in number of passages[75,90]. Further, the growth of micropropagated *A. mangium* plants was found better than seedling raised plants[99]. The field performance of tissue cultured hybrid acacia was also found better than the seedlings and rooted cuttings[75].

Teak

Tectona grandis (teak) is one of the valuable timber species in tropics and can easily be established in plantations. The total area under teak plantations is about 2.2 m ha[100]. In India, apart from state forest departments, many private companies and farmers are also involved in teak planting and the projected annual planting of teak is about 0.12 m ha. Presently, planting stock production is mostly through seed, although it has certain disadvantages, like low fruit production, emptiness of fruits and low germination rate. In Thailand, only 5 seedlings of plantable size were obtained from 100 seeds sown[101]. As a part of teak improvement programme in India, establishment of clonal seed orchards started since 1960 and so far about 800 ha are under them. However, only 15 per cent of the planting requirement could be met through clonal seeds because of the very low seed production. Various vegetative propagation methods, like grafting, budding and rooting of cuttings, were followed for teak multiplication[102,103]. However, the quantity of production was limited because of the poor rooting of cuttings collected from mature trees, limited availability of stocks and intensive maintenance of stock plants. Only cuttings produced from juvenile seedlings and tissue-cultured plantlets gave high rooting percentage. At present, therefore, it is not considered to be economically feasible to produce cuttings from older trees; although, good results were obtained from 15-year-old trees[104]. Teak genetic improvement programmes have been initiated in several countries, such as Thailand, India, Indonesia, Myanmar, Bangladesh, Sri Lanka, and China[101]. One of the short-term objectives of teak improvement programme is to quickly make available the large number of planting stock with better genetic quality. However, the maximum possible genetic gain in forest tree improvement can only be achieved through both sexual reproduction and vegetative multiplication[105].

In vitro propagation, using shoot tips and axillary buds, has been a reliable method of mass clonal propagation of teak[106-109]. In this technique, nodes or shoot apices were induced to produce multiple shoots, followed by root induction either under *in vitro* or *ex vitro* conditions. Cultures were most readily obtained with juvenile explants derived from seedlings, or shoots arising at the base of trees or shoots produced by repeated grafting of buds from mature trees onto seedlings. Buds obtained from grafts of elite teak trees were used for culture establishment[108]. Girdling at the base of teak trunk promotes the growth of epicormic shoots. It was reported that teak meristems obtained during a very short period of bud break were more amenable to rejuvenation[83].

Tissue culture technique has successfully been applied in Thailand for commercial production of improved teak planting stock. The cost of production of tissue-cultured plants was of the same level as the cost of production of seeds from clonal seed orchard[110]. Micropropagation, using improved seed and aseptically grown seedlings as explants, enhanced the quantity of genetically improved planting stock[111]. *In vitro* multiplication was found to be very useful in teak for rapidly increasing the number of individuals from seeds of genetic value but their availability is in restricted number or with low germination capacity[109]. Tissue culture conditions also improved the germination percentage of teak seeds. Bulk propagation of seeds without maintaining any individual identity helps to introduce many genotypes in propagation cycle. However, the risks of narrowing the genetic base over a period of time in bulk propagation may be overcome by maintaining the individual identity of *in vitro* germinated seedlings[112].

Mature plants of teak, selected for their superior characteristics, have been micropropagated using single nodal and terminal shoot cuttings, and apical shoot meristems. Single nodal and terminal cuttings were collected preferably from herbaceous or semi-hard wood shoots with a diameter of less than 1.5 cm. These shoots were produced from epicormic shoots of mature teak trees, which were girdled and mound layered[104]. A micropropagation protocol, suitable for intensive production of superior quality teak planting stock, has been developed[109]. The protocol was optimized in as simple a manner as possible in order to cope with the constraints of large-scale application, i.e. low costs and a high productivity rate. Nodal segments with 0.5 to 1.5 cm diameter were divided longitudinally into two primary explants, each bearing one axillary bud. Then, inoculated on the medium individually into two separate tubes to avoid the loss of two potential sources of new shoots in case of explant contamination. Apical meristems of size 0.3 mm were also used for the production of microshoots. The *ex vitro* rooting-cum-acclimatization phase was achieved in nursery conditions under a mist system with 90% success. More than 50,000 micro-shoots have been produced following this approach. Thus, the plants are developed rapidly into vigorous and true-to-type vegetative offspring of superior quality. This technology enables the mass micropropagation of any genotype with exponential multiplication rates from three to four new shoots every two months. The combination of tissue culture with subsequent rooting of cuttings from tissue culture plantlets could be both technically and economically feasible for large-scale production of clonal planting material[101].

Bamboos

The importance of bamboos in paper and pulp industries and its domestic utilization is well-known. It has been realized long back that *in vitro* propagation is essential to meet the ever-increasing demand of planting stock of bamboos[113]. The International Network on Bamboo and Rattan (INBAR) has also identified the availability of planting material as the major constraint in bamboo production.

Genetic improvement programmes commonly practiced for eucalypts, casuarina, teak and acacias cannot be followed for bamboo. The unique flowering nature of bamboo is the major limiting factor in improvement programmes in addition to their being polyploids. Simple procedures, such as selection, cannot be carried out in bamboo due to its peculiar architecture. Thus, there are no fixed selection criteria. The parameters that can be considered are number of culms per clump produced annually, internodal length, culm height and fiber length[114]. Even these parameters can be influenced by soil, water availability, temperature and humidity. However, vigour and growth habit of seedlings can be considered as effective criteria for early selection. Thus, selection strategy needs to be concentrated on characters, like seed quality and seedling vigour[115]. In case of *Bambusa arundinacea*, a direct correlation between the culm size and the seed weight was observed and vigorous clumps were found producing abundant flowers and seeds[116].

Extensive research on micropropagation of bamboo species has been carried out using seed or seedlings derived materials and mature tissues[114,117,118]. Procedures based on seeds/seedlings have the advantage of having greater number of genotypes in culture, from where propagation may proceed ensuring greater diversity of the species. While, procedures using tissue of mature clumps have the advantage of propagation of identified superior genotypes. However, seed/seedling tissue is preferred in bamboos because of their monocarpic nature and non-availability of any genetic improvement method.

In many economically important bamboo species, the majority of research to date has been aimed at the development of shoot multiplication systems[119-122]. Comprehensive reviews on micropropagation of various bamboo species are available, providing details on the media composition and rooting procedures[118,123,124], and on *in vitro* flowering aspects[120]. Tissue culture strategies are also advocated for genetic improvement of bamboo species[125,126]. Successful mass multiplication of *Dendrocalamus strictus* and *B. arundinacea* has been achieved in the authors' laboratory, where the physically good quality seeds were used for *in vitro* culture establishment. Seedling explants have also been used for multiple shoot induction in *D. strictus*[117,127,128], *B. nutans* and *D. membranaceous*[129], and *Thamnocalamus spathiflorus*[118] and *D. hamiltonii*[130].

Tissue culture protocols for multiplication from mature tissues of bamboo have been limited to only few species[131-133] because of the difficulties in continuous multiple shoot production and rooting. Multiple shoots were produced from mature nodes of *D. strictus, B. vulgaris* and *B. arundinacea* but the shoots could not be rooted except for *D. strictus*, where 20% rooting occurred[121]. Along with regeneration of axillary shoots, 30% rooting was obtained when nodal segments were cultured on a medium containing phloroglucinol[131]. In *B. glaucescens*, the axillary buds produced multiple shoots during first subculture but further multiplications failed[134]. Similarly, *B. tulda* produced multiple shoots only upto four subcultures[133]. Nodal segments were induced to produce multiple shoots in *D. strictus* but rooting was not reported[135]. Explants from small branches of *Thyrsostachys oliveri, D. asper* and *B. nana* produced multiple shoots but the cultures did not multiply after 2 months and none of the shoots could be rooted[136]. Four-year-old plants of *D. longispathus* were successfully multiplied and 70% rooting was obtained but vitrification of cultures was noticed[137]. Axillary shoot cultures from *D. strictus, B. arundinacea* and *T. siamensis* were initiated but no information on age of the tissue, shoot multiplication and root development was provided, and problems like high contamination rate and poor bud breaking were noticed[138]. From single node segments of 5-year-old plants, plantlets of *D. giganteus* were produced with 77% rooting. In the process, cultures were supported with the fibrous material obtained from luffa (*Luffa acutangula*) fruits to avoid vitrification[132]. Micropropagation technique has also been developed for a non-seeding bamboo, *Oxytenanthera stocksii* using the nodal segments from mature trees (Patented technique of the Institute of Forest Genetics and Tree Breeding, Coimbatore). Further, rapid clonal propagation has been achieved for ornamental bamboo, *B. vulgaris* using nodal cuttings as explants[139]. Commercially feasible micropropagation techniques have also been reported for large-scale propagation of 60 different temperate taxa of *Arundinaria, Chimonobambusa, Fargesia, Phyllostachys, Pleioblastus, Sasa, Sasaella, Semiarundinaria, Shibataea* and *Yushania*, and tropical taxa of *Bambusa, Dendrocalamus, Dinochloa, Oxytenanthera* and *Thyrsostachys*[140]. Recently, axillary shoot proliferation and *in vitro* flowering was also reported in an adult giant bamboo, *D. giganteus*[141].

Conclusion and Future Perspectives

The beneficial impact of using biotechnology in forestry would be greater than for most agronomic or horticultural species. The long breeding cycles and prolonged progeny analyses have greatly limited the potential of conventional breeding programmes for producing improved planting stocks. However, research on micropropagation, over a reasonable period, has enhanced the realization of the benefits of tree improvement. Improved ability to propagate vegetatively the selected material, to regulate flowering and breeding, and to regulate growth rates and wood quality would be of great benefit to tree improvement programmes. The most promising research areas in this context could be those concerned with rejuvenation, somatic embryogenesis and genetic transformation. These research areas are strategic in underlying future applications, and should be pursued with model species such as eucalypts. In recent years, liquid culture systems based on somatic embryos have become an option of interest to commercial micropropagations. Somatic embryogenesis has a vast potential to produce plants in millions, and now has become a routine practice in few gymnosperms. However, its use in hard wood plants is not yet to be realized because of several limitations, such as low number of somatic embryo germination, genotype influence and limited number of explants inducing somatic embryogenesis. Further, the availability of embryogenic system is now considered as an important prerequisite for genetic transformation studies. In short rotation hard wood trees, such as eucalypts, genetic transformation with pedigreed clones can be used to improve desired traits. The Council for Scientific and Industrial Research (CSIR), South Africa has selected a first generation commercial clone of *E. grandis* × *E. camaldulensis* with high *in vitro* culture potential, which is being licensed to Biotechnology laboratories across the world for genetic transformation studies.

In India, the tissue culture of trees has become commercially important and also feasible. Even, if the production cost of the tissue cultured plants are slightly higher than that of seeds, the expected increase in production makes the operation a profitable venture. Further, the technology delivery with effective dissemination channels has to play a major role in the commercial production of micropropagated plants in India. In this context, the technology adoption constraints need to be identified and addressed to introduce tissue-cultured plants in plantation or farm forestry. Furthermore, technology has always to be understood in a dynamic way. For instance, adoption of tissue culture technology will have to facilitate the use of genetically engineered plants as soon as they become available in near future. About 75 commercial tissue culture units, situated all over India, are operating in collaboration with overseas companies, with buy-back arrangements, having varying capacity of 1 million to 25 million. The spare capacity of these units may be used effectively for micropropagating tree species and for enhancing improved planting stocks to meet the immediate requirements. Commercial micropropagation is market driven and demand based. If enough demand is created, the cost of the planting stock can be cut down by 50%. When costs are reduced, the use of micropropagules as planting stock in industrial plantation forestry will broaden dramatically. In this approach, not only the improved planting stocks are produced on a large-scale but also the propagules are made available to planters in large volume year after year without the dependence on seed availability. At present, Indian tissue culture units are mainly involved in propagation of fruit crops and ornamentals for export market. However, the production for domestic market has been increasing from 10 to 40% in recent years.

To make tissue culture cost-effective and accepted practice in plantation forestry, it should be practiced for high value, highly productive and intensively managed systems. Thus, it requires upstream technology for production of appropriate plant material, developed through tree improvement and hybridization programmes, and downstream technology in terms of appropriate silvicultural practices, biofertilizer application, planting and post-planting care. The tree tissue culture/micropropagation will only be highly successful, where breeding and propagation programme for the defined species are in place and the produce is in high demand with good market value. The reported criticism that micropropagated plants of tree species do not perform well is because of the non-availability of improved planting stocks as base material. Furthermore, the acclimatization and hardening are important aspects for micropropagated tree species. It requires different types of greenhouses for hardening. In certain instances good shading, misting/fogging etc. are also necessary to achieve appropriate condition for optimizing the quality of the planting stock.

Another important aspect, which is often not practiced in silviculture, is the plant quality standards. It is necessary to define the plant quality standard for each species in terms of length of the shoot, shoot-root ratio, number of leaves, collar diameter, the bark production at the upper region of the shoot, straightness, colour of leaf and resistance to disease and pests. Development of operational methods to characterize the *in vitro* plant quality and to estimate the establishment of plants in an accurate and economic way are essential. Moreover, a suitable quality control strategy is necessary to ensure the performance of plants and the genetic fidelity of clonal planting stocks. The Polymerase Chain Reaction (PCR) based techniques, such as RAPD, ISSR and AFLP, have to be integrated in mass (industrial) tissue culture systems to assess the genetic fidelity of micropropagated plants.

References

1 FRA, *Global forest resources assessment (2000)* (FAO, Rome) 2001, www.fao.org/forestry/fo/fra/index.jsp.

2 Gurumurthi K, Forest tree improvement in India─Baseline strategy, in *UNDP/FAO project on improved productivity of man made projects through application of technological advance in tree breeding and propagation* (FAO, Rome) 1994, 1-41.

3 Evans J, The sustainability of wood production in plantation forestry, *Unasylva*, 49 (1998) 47-52.

4 Kanowski P J, Plantation forestry for the 21st Century, *XIth World For Congr*, held on 13-22 Oct, 1997 (Antalya, Turkey) 1997, 23-34.

5 Pandey D & Ball J, The role of industrial plantations in future global fibre supplies, *Unasylva*, 49 (1998) 37-43.

6 Sedjo R A, Potential of high-yield plantation forestry for meeting timber needs, in *Special issue on planted forests: Contribution to the quest for sustainable societies,* edited by J R Boyle (Kluwer Academic Publishers, Dordrecht), *New For*, 17 (1999) 339-359.

7 Campbell M M, Brunner A M, Jones H M & Strauss Steve H, Forestry's fertile crescent: The application of biotechnology to forest trees, *Plant Biotechnol J*, 1 (2003) 141-154.

8 Zobel B, Vegetative propagation in production forestry, *J For*, 90 (1993) 29-34.

9 Ritchie G A, Operational use of vegetative propagation in forestry: World overview of cloning and bulking, in *Natl Proc For Conserv Nursery Assoc*, Portland, USA, 1996, 192-197.

10 Ahuja M R & Libby W J, Clonal forestry conservation and application, in *Clonal forestry I: Genetics and biotechnology*, vol 2, edited by M R Ahuja & W J Libby (Springer, New York) 1993, 277.

11 Libby W J & Ahuja M R, Micropropagation and clonal options in forestry, in *Micropropagation of woody plants*, edited by M R Ahuja (Kluwer Academic Publishers, Dordrecht) 1993, 425-442.

12 Haines R & Martin B E, Biotechnology and sustainable production of tropical timber, *For Genet Resour Inf*, 25 (1997) 52-58.

13 Sita G L & Swamy B V R, Application of biotechnology in forest trees–Clonal multiplication of sandalwood, rosewood, eucalypts, teak and bamboos by tissue culture in India, in *Tree improvement: Applied research and technology transfer*, edited by S Puri (Science Publishers, Enfield, USA) 1998, 233-248.

14 Merkle S A & Trigiano R N, *In vitro* propagation of hardwoods, (in *Applications of vegetative propagation in forestry*) *Proc 1992 SRIEG Biennial Symp For Genets*, held on 8-10, July (USDA, New Orleans, LA) 1992, 23-37.

15 Bonga J M & von Aderkas P, *In vitro* culture of trees, in *Forestry sciences*, vol. 38. (Kluwer Academic Publishers, Dordrecht) 1992, 236.

16 Yanchuk A D, The role and implications of biotechnological tools in forestry, *Unasylva,* 52 (2001) 53-61.

17 Jain S M & Ishii K, *Micropropagation of woody trees and fruits*, (Agritech Publications) 2003, 840.

18 Ahuja M R, *Micropropagation of woody plants* (Kluwer Academic Publishers, Dordrecht) 1993, 507.

19 von Aderkas P & Bonga J M, Influencing micropropagation and somatic embryogenesis by manipulation of phase change and culture environment, *Tree Physiol*. 20 (2000) 921-928.

20 Darus H A, *Forest tree improvement in Malaysia: A baseline study*, (UNDP/FAORAS/91/004) 1994.

21 Sharma M, Agricultural biotechnology, in *Country case studies*, edited by G J Persley & L R Maclntyre (CAB International) 2002, 51-60.

22 Anonymous. *Annual Report 2001-2002* (Department of Biotechnology, Government of India) 200, 169-170.

23 Sreedhar D, Rao M M, Ramesh C & Lawrence L, Commercial scale micropropagation of *Eucalyptus camaldulensis*, *Indian For*, 124 (1998) 217-224.

24 WFI**,** *Brazil: Forestry and the wood products industry* (World Forestry Institute, Oregon, USA) 1995, 50.

25 Castro R, Dagoberto & Gonzalez O J, Eucalyptus (*Eucalyptus grandis* Hill ex Maiden) micropropagation in a temporary immersion system, *Agric Tec*, 62 (2002) 68-78.

26 Gupta P K & Mascarenhas A F, Eucalyptus, in *Cell and tissue culture in forestry*, vol 3, edited by J M Bonga & D J Durjan, (Martinus Nijhoff, Dordrecht) 1987, 385-399.

27 Jones N B & Van Staden J, Micropropagation of *Eucalyptus*, in *Biotechnology in agriculture and forestry*, vol. V (39) (*High-tech and micropropagation*), edited by Y P S Bajaj (Springer-Verlag, Berlin) 1997, 286-327.

28 Le Roux J J & Van Staden J, Micropropagation of *Eucalyptus* species, *Hortic Sci*, 26 (1991) 199-200.

29 Watt M P, Blakeway F C, Herman B & Devison N, 1997. Developments in the use of biotechnology in commercial forestry tree improvement programmes in South Africa, vol 2, theme 8, *XIth World Fory Congrs*, held on 13-22 Oct, 1997 (Antalya, Turkey) 1997.

30 Gupta P K, Mascarenhas A F & Jagannathan V, Tissue culture of forest trees–Clonal propagation of mature trees of *Eucalyptus citriodora* Hook by tissue culture, *Plant Sci Lett,* 20 (1981) 195-201.

31 Grattapaglia D, Caldas L S, Machando M A & Assis T F, Large scale micropropagation of *Eucalyptus* species and hybrids, in *Abstract VII th Int Congr on Plant Tissue Cell Cult* (IAPTC, Amsterdam) 1990, 113 (A3-123).

32 Ikemori Y K**,** Penchel R M & Bertolucci F L G, Integration of biotechnology into *Eucalyptus* breeding, in *Int Wood Biotechnol Symp*, Tokyo, 1994, 77-84.

33 Yang J C, Ho C K, Chen Z Z & Chen C F, Comparison of different methods of vegetative propagation of *Eucalyptus camaldulensis* plus trees, *Taiwan J For Sci*, 11 (1996) 433-443.

34 Sumathi R, Yasodha R & Gurumurthi K. Rescuing of a mature candidate plus tree of *Eucalyptus tereticornis.* in *IUFRO Int Symp on developing the Eucalypt of the future*, held on 10-15 Sept, 2001 (Valdivia, Chile) 2001, 116.

35 Trindade H & Pais M S, *In vitro* studies on *Eucalyptus globules* rooting ability, *In Vitro Cell Dev Biol Plant*, 33 (1997) 1-5.

36 Da Silva L L & Teixeira S L, *In vitro* clonal propagation of *Eucalyptus grandis* Hill ex Maiden from nodal segments and epicormic shoots, *Rev Ceres*, 40 (1993) 390-396.

37 Koriesh E M, Abd El-Fattah Y M, El-Dayem M A & El-Etriby M A, Micropropagation of juvenile *Eucalyptus citriodora*, *Acta Hortic (The Hague),* 625 (2003) 283-288.

38 Yang J C, Ho C K, Chang S H & Chen Z Z, Micropropagation of mature trees of *Eucalyptus camaldulensis* with fast-growing phenotype, *Taiwan J For Sci*, 11 (1996) 421-431.

39 Lubrano L & Loreti F, Micropropagation of *Eucalyptus trabutii*, *E. viminalis* and *E. gunnii*, in *Intl symp on vegetative propagation of woody species*, *Acta Hort (The Hague)*, 227 (1988) 428-429.

40 Warrag E I, Lesney M S & Rockwood D L, Micropropagation of field-tested superior *Eucalyptus grandis* hybrids, *New For*, 4 (1990) 67-79.

41 Gill R I S & Gosal S S, Micropropagation of economically important tropical forest trees, in *Tree improvement for sustainable tropical forestry, QFRI-IUFRO Conf*, held on 27 Oct -1 Nov, 1996 (Caloundra, Queensland, Australia), 1 (1996) 230-233.

42 Jambhale N D & Patil S C, Micropropagation of elite *Eucalyptus* types through shoot tip culture, *Indian For*, 122 (1996) 61-64.

43 Patil V & Kuruvinashetti M S, Micropropagation of *Eucalyptus teriticornis* using explants from mature and coppice shoots, *Phyton*, 63 (1998) 129-132.

44 Yasodha R, Sumathi R & Gurumurthi K, 1997. Micropropagation of difficult to propagate clones of eucalypts, in *Proc IUFRO Conf on silviculture and improvement of Eucalypts*, held on 24-29 Aug, 1997 (Brazil) 1997, 123-128.

45 Wachira F, *In vitro* shoot multiplication of *Eucalyptus grandis*, *Afr Crop Sci J*, 5 (1997) 239-251.

46 Sapulete E & Izudin E, Vegetative propagation of *Eucalyptus citriodora* Secara *in vitro*, *Bul Penelitian Kehutanan Pematang Siantar*, 15 (1999) 243-251.

47 Chang S H, Donald D G M & Jacobs G, Micropropagation of *Eucalyptus radiata* ssp. *radiata* using explants from mature and coppice material, *South Afr For J*, 162 (1992) 43-47.

48 Sharma S K & Ramamurthy V, Micropropagation of 4-year-old elite *Eucalyptus tereticornis* trees, *Plant Cell Rep*, 19 (2000) 511-518.

49 Capuli F A & Calinawan N M, Micropropagation of *Eucalyptus* species: Research success in a private laboratory, *Canopy Int*, 25 (1999) 10-11.

50 Bolstad P V & Libby W J, Comparison of radiata pine cuttings of hedge and tree-form origin after seven growing seasons, *Silvae Genet*, 31 (1982) 9-13.

51 Jones N B & Van Staden J, Micropropagation and establishment of *Eucalyptus grandis* hybrids, *South Afr J Bot*, 60 (1994) 122-126.

52 Watt M P, Duncan E A, Ing M, Blakeway F C & Herman B, Field performance of micropropagated and macropropagated *Eucalyptus* hybrids, *South Afr For J*, 173 (1995) 17-21.

53 Xavier A & Comerio J, '*Ex vitro*' rooting of '*in vitro*' multiplied and grown shoots of *Eucalyptus* spp., *Sci For*, 51 (1997) 29-36.

54 Bisht P, Kapoor M L, Sharma S K & Chauhan J M S, *In vitro* clonal propagation of juvenile *Eucalyptus* hybrid (*E. camaldulensis* Dehn.x *E. tereticornis* Sm.), *Indian J For*, 20 (1997) 93-97.

55 Yasodha R, Sumathi R & Gurumurthi K, Branch segments of mature Eucalypts—As a source of explant for micropropagation, in *Proc IUFRO Int Symp on developing the Eucalypts of the future*, held on 10-15, Sept, 2001 (Valdivia, Chile) 2001, 117.

56 Bisht P, Joshi I, Chauhan J M S & Sharma V K, *In vitro* clonal propagation of mature *Eucalyptus* F1 hybrid FRI-5 (*E. camaldulensis* Dehn. x *E. tereticornis* Sm.), *Indian J For*, 23 (2000) 28-32.

57 Wendling I, Xavier A, Gomes J M, Pires I E & Andrade H B, Effect of the growth regulator IBA on propagation of *Eucalyptus* spp. clones by microcutting. *Rev Arvore*, 24 (2000) 187-192.

58 Bisht P, Sharma V K, Joshi I & Kapoor M L, Micropropagation of newly produced F1 hybrid of *Eucalyptus* (*E. tereticornis* Sm. x *E. camaldulensis* Dehn. southern form), *Silvae Genet*, 48 (1999) 104-108.

59 Chauhan J M S, Bisht P, Kapoor M L & Rawat M S, *In vitro* clonal propagation of *Eucalyptus* hybrid FRI-4 (*E. tereticornis* Sm. x *E. camaldulensis* Dehn.), *Ann For*, 4 (1996) 186-191.

60 Bennett I J, McComb J A, Tonkin C M & McDavid D A J, Alternating cytokinins in multiplication media stimulates *in vitro* shoot growth and rooting of *Eucalyptus globulus* Labill, *Ann Bot (Lond)*, 74 (1994) 53-58.

61 Lubrano L, Micropropagation of *Eucalyptus*, *Cellul Carta*, 43 (1992) 27-33.

62 Mokotedi M E O, 1999. Studies on the *in vitro* rooting ability of cold-tolerant hybrids of *Eucalyptus grandis* x *nitens* and assessment of root quality, in *For Biotechnol 1999 Conf*, held on 11-16 July, 1999 (University of Oxford) 1999.

63 Rockwood D L & Warrag E I, Field performance of micropropagated, macropropagated, and seed-derived propagules of three *Eucalyptus grandis* ortets, *Plant Cell Rep*, 13 (1994) 628-631.

64 Sekhar C, Panda R C & Rai R S V, Comparative evaluation of tissue cultured plantation in Tamil Nadu. *J Trop For Prod*, 6 (2000) 127-133.

65 Yang J C, Chung J D & Chen Z Z, Vegetative propagation of adult *Eucalyptus grandis* x *urophylla* and comparison of growth between micropropagated plantlets and root cuttings, *Plant Cell Rep*, 15 (1995) 170-173.

66 Biswas R N, Karunakaran M & Moon A R, Field trials on tissue culture in *Eucalyptus*, *Indian For*, 125 (1999) 264-271.

67 Reddy M S & Satyanarayana T, Inoculation of micropropagated plantlets of *Eucalyptus tereticornis* with ectomycorrhizal fungi, *New For*, 16 (1998) 273-279.

68 Maslin, B R & McDonald M W, *A key to useful Australian acacias for the seasonally dry tropics* (CSIRO Forestry & Forest Products, Canberra, Australia) 1996.

69 Pandey D, *Forest resources assessment 1990—Tropical forest plantation resources*, (FAO Forestry Paper 128) 1995, 81.

70 Kha L D, Studies on natural hybrids of *Acacia mangium* and *A. auriculiformis* in Vietnam, in *Tree improvement for sustainable tropical forestry, QFRI-IUFRO Conf*, held on 27 Oct-1 Nov, 1996 (Caloundra, Queensland, Australia), 2 (1996) 328-332.

71 Turnbull J W, Midgley S J & Cossaltar C. Tropical acacias planted in Asia: An overview, in *Proc Int Workshop*, held on 27-30 Oct, 1997 at Vietnam (ACIAR, Canberra) 1998.

72 Barakat M N & El Lakany M H, Clonal propagation of *Acacia saligna* by shoot tip culture, *Euphytica,* 59 (1992) 103-107.

73 Beck S L & Dunlop R W, Micropropagation of the *Acacia* species–A review, *In Vitro Cell Dev Biol Plant*, 37 (2001) 531-538.

74 Beck S L, Dunlop R & van Staden J, Meristem culture of *Acacia mearnsii, Plant Growth Regul*, 32 (2000) 49-58.

75 Darus H A, Micropropagation techniques for *Acacia mangium* x *Acacia auriculiformis*, in *Proc Int Workshop on breeding technologies for tropical acacias*, held on 1-4 July 1991 (ACIAR Malaysia), 37 (1992) 119-121.

76 Gassama Y K, *In vitro* culture and improvement of symbiosis using mature *Acacia albida*, in *Proc Regional Semin on Trees for development in sub-Saharan Africa*, held on 20-25 Feb, 1989 (ICRAF House, Nairobi, Kenya) 1989, 286-290.

77 Yusoff A M, David M K, Fadillah Z, Halilah A K & Haliza I, Establishing a protocol for commercial micropropagation of *Acacia mangium* x *Acacia auriculiformis* hybrids, *J Trop For Sci*, 11 (1999) 148-156.

78 Banik R L & Islam S A M N, *In vitro* clonal propagation of hybrid acacia (*A. auriculiformis* x *A. mangium*), *Bangladesh J For Sci*, 25 (1996) 1-7.

79 Beck S L & Dunlop R, Vegetative propagation of the black wattle (*Acacia mearnsii* de Willd), *ICFR Bull*, 8 (1999) 15.

80 Beck S L, Dunlop R & van Staden J, Rejuvenation and micropropagation of adult *Acacia mearnsii* using coppice material, *Plant Growth Regul*, 26 (1998) 149-153.

81 Yasodha R, Sumathi R, Malliga P & Gurumurthi K, *In vitro* expression of juvenility in acacia hybrid, in *Natl Symp on emerging trends in modern biology*, held on 10-12 Jan, 2002, (Loyola College, Chennai, India) 2002, 24.

82 Monteuuis O, *In vivo* grafting and *in vitro* micrografting of *Acacia mangium*: Impact of ortet age, *Silvae Genet*, 44 (1995) 190-193.

83 Monteuuis O, *In vitro* shoot apex micrografting of mature *Acacia mangium*, *Agrofor Systems*, 34 (1996) 213-217.

84 Monteuuis O, 1988. Maturation concept and possible rejuvenation of arborescent species– Limits and promises of shoot apical meristems to ensure successful cloning, in *Proc IUFRO Conf* (Thailand) 1988, 106-118.

85 Bhaskar P & Subhash K, Micropropagation of *Acacia mangium* Wild. through nodal bud culture, *Indian J Exp Biol*, 34 (1996) 590-591.

86 Zhang H W, Huang X L, Fu J, Yang M Q & Chen C Q, Axillary bud culture and plantlet regeneration of *Acacia*

auriculiformis and *A. mangium*, *J Trop Subtrop Bot*, 3 (1995) 62-68.

87 Reddy P, Patil V, Prasad T G, Padma K & Udayakumar M, *In vitro* axillary bud break and multiple shoot production in *Acacia auriculiformis* by tissue culture technique, *Curr Sci*, 69 (1995) 495-496.

88 Kaur K, Gupta P, Verma J K & Kant U, *In vitro* propagation of *Acacia senegal* (L.) Willd. from mature nodal explants, *Adv Plant Sci*, 11 (1998) 229-233.

89 Badji S, Mairone Y, Ndiaye I, Merlin G, Danthu P *et al*, *In vitro* propagation of the gum arabic tree (*Acacia senegal* (L.) Willd.) 1. Developing a rapid method for producing plants, *Plant Cell Rep*, 12 (1993) 629-633.

90 Darus H A, Micropropagation of *Acacia mangium* from aseptically germinated seedlings, *J Trop For Sci*, 3 (1991) 204-208.

91 Crawford D F & Hartney V J, Micropropagation of *Acacia mangium* and *Acacia stenophylla*, in *Proc ICIAR Int Workshop on Australian acacias in developing countries*, (Australian Centre for International Agriculture Research, Gympie,Qld, Australia) 16 (1987) 64-65.

92 Wasel A S, Micropropagation of *Acacia seyal* Del. *in vitro*, *J Arid Environ*, 46 (2000) 425-431.

93 Marguerite Q, da Silva M C, Martins K G & de Oliveira D E, Multiplication of juvenile black wattle by microcuttings, *Plant Cell Tissue Organ Cult*, 66 (2001) 199-205.

94 Kaur K & Kant U, Clonal propagation of *Acacia catechu* Willd. by shoot tip culture, *Plant Growth Regul*, 31 (2000) 143-145.

95 Ide Y, Watanabe Y & Ikeda H, Tissue culture of *Acacia auriculiformis* using the aseptically germinated seedlings, *J JPN For Soc*, 76 (1994) 576-583.

96 Watanabe Y, Ide Y & Ikeda H, Plant regeneration from axillary bud culture of one-year-old seedling of *Acacia auriculiformis* grown in green house, *Bull Tokyo Univ For*, 92 (1994) 29-35.

97 Douglas G C & Mcnamara J, Shoot regeneration from seedling explants of *Acacia mangium* Willd. *In Vitro Cell Dev Biol Plant*, 36 (2000) 412-415.

98 Vengadesan G, Ganapathi A, Anand R P & Anbazhagan V R, *In vitro* organogenesis and plant formation in *Acacia sinuata*, *Plant Cell Tissue Organ Cult*, 61 (2000) 23-28.

99 Setiawan I, Umboh M I & Supriyanto, Growth and rooting system of *Acacia mangium* obtained by tissue culture, *Biotropia*, 4 (1991) 1-8.

100 FAO, *Forest Resource Assessment, 1990–Tropical forest plantation resources* (FAO Forestry, Rome) 1995.

101 Kaosa-Ard A, Suangtho V & Kjær E D, *Experience from 30 years of tree improvement of teak (Tectona grandis) in Thailand* (Technical Note 50, Danida Forest Seed Centre, Humlebæk, Denmark) 1998.

102 Goh D & Monteuuis O, Vegetative propagation of teak, *ITTO Newslett, Trop For Update*, 7 (1997) 13.

103 Rawat M S & Kedharnath S, Field grafting and budding in teak (*Tectona grandis* L), *Indian For*, 94 (1968) 260-261.

104 Monteuuis O, Wallauri D, Poupard C, Hazard L, Yusof Y *et al*, Propagation clonale de tecks matures par bouturage horticole. *Bois For Trop*, 243 (1995) 25-39.

105 Hasnain S & Cheliak W, Tissue culture in forestry: Economical and genetic potential. *For Chron*, 82 (1986) 219-225.

106 Apavatjrut P, Kaosa-Ard A & Paratasilpin T, Current research on Teak (*Tectona grandis* Linn. F.)-Tissue culture in Thailand, in *BIOTROP Spl Publ No.* 35 (Indonesia), 1988, 107-115.

107 Devi Y S, Mukherjee B B & Gupta S, Rapid cloning of elite teak (*Tectona grandis* Linn.) by *in vitro* multiple shoot production, *Ind J Exp Biol*, 32 (1994) 668-671.

108 Mascarenhas A F, Kundurkar S V & Khupse S S, Micropropagation of teak, in *Micropropagation of woody plants*, edited by M R Ahuja (Kluwer Academic Publishers, Dordrecht) 1993, 247-262.

109 Monteuuis O, Bon M C & Goh D K S, Teak propagation by *in vitro* culture. *Bois For des Trop,* 256 (1998) 1-11.

110 Kaosa-Ard A, *Teak (Tectona grandis Linn. f.) domestication and breeding* (UNDP/FAO, Los Baños, Philippines, RAS/91/004) 1996, 48.

111 Hutzell M J & Durzan D J, Improved aseptic germination and controlled growth for micropropagation of Douglas fir, in *Micropropagation of woody plants*, edited by M R Ahuja (Kluwer Academic Publishers, Dordrecht) 1993, 367-372.

112 Yasodha R, Sumathi R & Gurumurthi K, 1999. Enhanced production of genetically improved planting stock of teak through micropropagation, in *Intl Teak Conf on teak beyond year 2000*, held on 23-25 August, 1999 (Chiang Mai, Thailand) 1999.

113 Rao I V R & Rao I U, Tissue culture approaches to the mass–Propagation and genetic improvement of bamboos, in *Proc Int Bamboo Workshop* (Kerala Forest Research Institute, India & I.D.R.C, Canada) 1988, 151-158.

114 Banik R L, Selection criteria and population enhancement of priority bamboos, in *Genetic enhancement of bamboo and rattan*, edited by J T Williams, I V Ramanuja Rao & A N Rao, (Los Banos, Philippines) 1995, 99-110.

115 Gurumurthi K, Madhavi R & Verma R K, *Selection criteria and methodology for production of bamboo clones in genetic enhancement of bamboo and rattan*, edited by J T Williams, I V Ramanuja Rao & A N Rao (Los Banos, Philippines) 1995, 69-86.

116 Madhavi Rani A, Development of criteria for producing quality planting material in *Bambusa arundinacea.* Ph.D Thesis, Forest Research Institute (Deemed University), Dehra Dun, 1995.

117 Shirgurkar M V, Thengane S R, Poonawala I S, Jana M M, Nadgauda R S *et al*, A simple *in vitro* method of propagation and rhizome formation in *Dendrocalamus strictus* Nees, *Curr Sci*, 70 (1996) 940-944.

118 Zamora A B, 1994. Review of micropropagation research on bamboos, in *Constraints to production of bamboo and rattan* (INBAR Technical Report 5) 1994, 45-100.

119 Bag N, Chandra S, Palni L M S & Nandi S K, Micropropagation of Dev-tingal [*Thamnocalamus spathiflorus* (Trin.) Munro]–A temperate bamboo, and comparison between *in vitro* propagated plants and seedlings, *Plant Sci*, 156 (2000) 125-135.

120 Nadgauda R S, Parasharami V A & Mascarenhas A F, Precocious flowering and seeding behaviour in tissue cultured bamboos, *Nature (Lond)*, 344 **(**1990) 335-336.

121 Nadgir A L, Phadke C H, Gupta P K, Parsharami V A, Nair S *et al*, Rapid multiplication of bamboo by tissue culture, *Silvae Genet*, 33 (1984) 219-223.

122 Saxena S, *In vitro* propagation of (*Bambusa tulda* Roxb.) through shoot proliferation, *Plant Cell Rep*, 9 (1990) 431-434.
123 WuY M, Current research on bamboo tissue culture and plantlet regeneration, *J Bamboo Res*, 18 (1999) 32-37.
124 Zhang C X, Xie Y F, Zhang Y F, He D T, Chen T B *et al*, The current advances in bamboo tissue culture and its prospects, *J Bamboo Res*, 18 (1999) 46-49.
125 Gielis J, Peeters H, Gillis K, Oprins J & Debergh P C, Tissue culture strategies for genetic improvement of bamboo, *Acta Hortic (The Hague)*, 552 (2001) 195-203.
126 Huang L C & Huang B L, Tissue culture alternatives in bamboo improvement, *FORSPA Publ*, 6 (1994) 174-179.
127 Preetha N, Yasodha R, Sumathi R & Gurumurti K, Continuous shoot proliferation of *Dendrocalamus strictus* in stationary liquid cultures, *J Trop For*, 8 (1992) 105-108.
128 Singh M, Jaiswal Uma & Jaiswal V S, Thidiazuron-induced shoot multiplication and plant regeneration in bamboo (*Dendrocalamus strictus* Nees). *J Plant Biochem Biotechnol*, 10 (2001) 133-137.
129 Yasodha R, Sumathi R, Malliga P & Gurumurthi K, Genetic enhancement and mass production of quality propagules of *Bambusa nutans* and *Dendrocalamus membranaceous*. *Indian For*, 123 (1997) 303-306.
130 Sood A, Palni L M S, Sharma M & Sharma O P, Micropropagation of *Dendrocalamus hamiltonii* Munro using single node cuttings taken from elite seedling plants, in *Proc the 4th Int Bamboo Workshop on bamboo in Asia and the Pacific*, held on 27-30 Nov, 1991 (Chiangmai, Thailand), *FORSPA Publ*, 6 (1994) 165-168.
131 Chaturvedi H C, Sharma M & Sharma A K, *In vitro* regeneration of *Dendrocalamus strictus* Nees through nodal segments taken from field grown culms, *Plant Sci*, 91 (1993) 97-101.
132 Ramanayake S M S D & Yakandawala K, Micropropagation of the giant bamboo (*Dendrocalamus giganteus*. Munro) from nodal explants of field grown culms, *Plant Sci*, 129 (1997) 213-223.
133 Saxena S & Dhawan V, Micropropagation research in South Asia, in *Constraints to production of bamboo and rattan,* held on 9-13 May, 1994 at Bangalore, India, *INBAR Tech Rep*, 5 (1994) 101-142.
134 Banik R L, Techniques of bamboo propagation with special reference to pre-rooted and pre-rhizomed branch cuttings and tissue culture, in *Proc Int Bamboo Workshop on recent research on bamboos* (CAF China & IDRC, Canada) 1985, 160-169.
135 Mascarenhas A F, Nadgir A L, Thengane S R, Phadke C H, Khuspe S S *et al*, Potential application of tissue culture for propagation of *Dendrocalamus strictus*, in *Proc Int Bamboo Workshop on bamboo current research* (Kerala Forest Research Institute, India & IDRC, Canada) 1988, 159-166.
136 Vongvijitra R, Traditional vegetative propagation and tissue culture of some Thai bamboos, in *Proc Int Bamboo Workshop on bamboo current research* (Kerala Forest Research Institute, India & IDRC, Canada) 1988, 159-166.
137 Saxena S & Bhojwani S, *In vitro* clonal multiplication of 4-year-old plants of the bamboo, *Dendrocalamus longispathus* Kurz., *In Vitro Cell Dev Biol Plant*, 29 (1993) 135-142.
138 Kumar K B, Propagation of bamboo through tissue culture, *BIC Indian Bull,* 4 (1994) 20-24.
139 Das G & Samanta P K S, Rapid clonal propagation of an ornamental bamboo (*Bambusa vulgaris* L.), *Indian J Hortic*, 57 (2000) 268-272.
140 Gielis J & Oprins J, Micropropagation of tropical and temperate bamboos: From biotechnological dream to commercial reality, in *Bamboo for sustainable development* (VSP, The Netherlands) 2002.
141 Ramanayake S M S D, Wanniarachchi W A V R & Tennakoon T M A, Axillary shoot proliferation and *in vitro* flowering in an adult giant bamboo, *Dendrocalamus giganteus* Wall. ex Munro, *In Vitro Cell Dev Biol Plant*, 37 (2001) 667-671.