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# Molecular Tools for Improvement of Forest Trees

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**Abstract:** During the 21st century, humankind will have confrontation with an extraordinary set of challenges. Hunger and poverty around the globe must be addressed, while the life-support systems provided by the world's natural environment are maintained. Meeting these challenges will require new knowledge generated by continued scientific advances, development of appropriate new technologies and a broad dissemination of appropriate information, knowledge and technology. It will also require that wise policies be implemented through informed decision making on the part of national, state and local governments in each nation. A new set of tools has become available in the past twenty years that combined with traditional plant breeding-will allow people to generate products, the genetically improved varieties of the future. This set of tools, which comes under the general title of 'Biotechnology'. Biotechnology-derived crops promote the adoption of conservation tillage and forest saving practices, resulting in substantial environmental gains. These include preservation of 37 million tons of top-soil, 85 % reduction in green house gas emission from farm operations, 70% reduction in herbicide run-off, 90% decrease in soil erosion and saving 4-7 gallons of fuel oil per acre.

**Key words:** Clonal forestry · micropropagation · somatic embryogenesis · microsatellites

## INTRODUCTION

We are inseparable from trees probably because we had an arboreal ancestry. Reposing under a tree is like being cuddled by a mother. As principal components of forests that form the green mantle over the earth or as individuals, trees play multiple roles in nature. They are unmatched as providers and protectors. They are the largest and the longest-lived organisms and offer an amazing diversity of form. Trees have come to symbolize benevolence, fertility and nobility. Considering the productive, protective, ecological, conservational, educational and recreational roles of forests, the number of trained scientists and the extent of research and developmental activities in forestry area are dismally low. For too long forestry was excluded from the mainstream of Indian education, in contrast to the situation with agriculture [1]. Economic, political, demographic and social trends are shaping the management of forests and influencing national forest policy formulation and institutional arrangements. Demographic changes, the growing size of population of the world and increasing urbanization have had and will continue to have, major impact on forest cover and condition, demand for wood and non-wood forest products and the ability of forests to fulfill essential environmental functions. Political and economic trends affecting the forestry sector are decentralization, privatization, trade liberalization and globalization of world economy; and overall economic growth coupled with a widening gap between rich and poor in many countries.

Deforestation and forest degradation are occurring in dry lands and upland areas. These already have limited forest cover and are fragile environments susceptible to soil erosion and other forms of degradation. In these areas poor communities are highly dependent on forests for food, fuel and income. Causes of forest degradation include excessive collection of fuel wood, overgrazing, over-harvesting of timber and poor harvesting practices.

While the world's forest area has been steadily decreasing, there has been a continued increase in demand for wood products. Wood is the most important natural and endlessly renewable source of energy and therefore has major future role as an environmentally cost-effective alternative to burning fossil fuels. Wood is the fifth most important product of the world trade. The complex chemical make up of wood also makes it an ideal raw material for what could be a future "lingo-chemical" industry that could replace petrochemical industry, in providing not only plastic and all kind of chemical

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products, but also food and textile products [2]. Trees are the defining feature of forest and woodlands, the most biologically diverse terrestrial ecosystems which occupy the world's ice-free land surface. These long-lived woody plants have been little domesticated relative to the crop plants and animals on which agricultural systems are based and for which genetic knowledge and manipulations are most advanced.

The size and importance of agricultural economy and the fundamental role of improved crops and animals in agricultural production system have fostered high levels of activity in crop and animal breeding. Tree breeding programmes remain modest by comparisons partly because products harvested from natural forests have been dominant in world market and partly as a consequence of historical perception that trees played relatively minor role in sustaining rural livelihood. Demand for fuel wood, which is the main or sole source of domestic energy for two-fifth of the world's population, continues to grow by 1.2 percent per year. About 90% of the world's fuel wood is produced and used in developing countries. By contrast developed countries account for more than 70% of the total world production and consumption of industrial wood products. While the rate of consumption in developed countries has leveled off, it continues to rise in developing countries. Many countries are relying more on plantations and in some places, on farm forestry and agro-forestry to supply their wood needs.

## CONVENTIONAL METHODS OF TREE BREEDING

Conventional breeding technology for forest involves well-known quantitative genetic techniques such as progeny testing and efficient selection for utilizing existing genetic resources and also selection of superior families of clones within particular species. Traditional genetic improvement programs have been utilized to produce very large genetic gains across the world.

There are a number of constraints in the tree improvement process. The major constraint in the improvement of forest tree is time. Cheliak and Rogers [3] identified four major ways in which time influences the three phases of the tree improvement process- (i) evolutionary time, (ii) time of harvest (iii) time elapsed before age-stable performance is achieved and (iv) time to reproductive maturity. As such evolutionary time cannot be directly influenced by technology, but it can influence what technologies are appropriate. The remaining three time factors affect the rate at which progress can be achieved. The existence of long juvenile phases in fruit and forest trees has been one of limiting factors for there genetic improvement preventing full domestication of economically important woody species.

Tree improvement is a process of managing genetic resources. Through this process the inherent genetic variability in a particular population is managed by recurrent cycles of selection and breeding. Tree improvement involves three distinct, but related phases. Conservation, selection and breeding and propagation as we progress from one phase to the next, decreasing amounts of variations are being managed. Therefore, at any one time, more variation is being conserved or selected than is being deployed through propagation. Conservation is directed towards both wild and managed germplasm resources. Breeding and selection are the central activities of a tree improvement program and involve formation of mating designs, evaluation of resultant progeny and selection of desirable genotypes for propagation and further breeding for the next generation. Tree improvement programs are well under way for a wide variety of commercially valuable forest tree species throughout the world [4].

Breeding of forest plants/trees and clonal forestry are major activities that fast growing hardwood forest and forest products companies worldwide ate using to maximize productivity, quality and profitability. Clonal forestry is one of the most exciting and controversial issues facing the industry today. There is no doubt about clonal forestry not working; rather the issue is about a proper and responsible assessment. During the last three decades the world has witnessed the explosion of interest and research efforts in vegetative propagation of commercial timber species. We are in the midst of a technological revolution in forestry. Clonal forestry is component of the total system of genetic management of forestry which represents the ultimately refinement in tree breeding [5, 6].

## BIOTECHNOLOGY AND TREE IMPROVEMENT PROGRAMMES

The US Forest Service predicted in 1983-84 that demand for wood would double within the next 50 years. The largest demand should come from outside the US, from tree-poor countries like India [7]. It was postulated that biotechnology offers the potential for revolutionary changes in forest productivity to meet that demand. Forestry would probably realize the greatest improvement of any crop from biotechnology, one of the most important things that biotechnology could do for us in forestry was cut the long periods required for tree improvement using conventional technologies, it was predicted.

The advent of biotechnology does not means that conventional breeding will be swept away conventional genetic improvement programmes will still be the methods of choice for improving quantitative and multigenetic traits. Long-term multi-generation breeding program will still be necessary to obtain improvements in traits such as growth rate and overall environmental adaptation. Biotechnology is a collection of techniques that can be used for enhancing the impact of biological programs. The major emphasis in forest biotechnology has been on systems of tissue and protoplast culture.

**Selection and clonal propagation of trees:** It is expected that genetically improved forest trees will not be available for planting for some years even in the advanced countries. It will be necessary meanwhile to clone pre-existing, superior trees. An initial problem encountered involves the identification of those features of a superior adult phenotype that can be attributed to genotype and those that result from its environment. A possible route to cement this problem would be to use molecular probes to screen the genome [8-10] but such diagnostic tools are not yet available. In the meantime, the most realistic approach will be to select superior phenotypes and assume that, at worst, a tree with a superior phenotype is a genotype that responded well to good management practices, while, at best, in the absence of human inference, the genotype is totally responsible for its enhanced field performance. A further difficulty is encountered in collection of material from large and old trees for propagation. Since woody material obtained from adult trees is usually difficult or impossible to root, taking cutting from the parent tree would be wasteful procedure to produce nursery stock plants. A more effective method would be to graft buds onto suitable root-stocks as is traditionally practiced in the propagation of fruit trees and for range of ornamental trees. While clonal root-stocks would enhance the uniformity of the propagated stock such clones would not be available in early experiments with mast species and grafting anta a mixed seedling population would be mare realistic.

**Micropropagation as method of cloning:** An alternative approach to cloning is provided by micropropagation. This process is based upon the principle that a bud taken from a given specimen and cultured on a nutrient medium containing growth regulators, usually at least one cytokinin, develops into a shoot and its axillary buds grow out to form branches. Shimazo-Sato and Mori [11] have thoroughly discussed (i) development and potential of axillary meristems, (ii) biological functions of dormancy and apical dominance and (iii) physiological, genetic and molecular biological approaches that control outgrowth and dormancy in axillary buds of plants. These side branches can be rooted or transferred to fresh multiplication medium in which axillary buds again grow out. This process can be carried out indefinitely to produce a limitless number of shoots. Following success with large numbers of herbaceous species, Information is now slowly being gathered on the micropropagation of an increasing range of fruit and timber trees. For forest trees, most success has been reported in conifers. Based an published data, explants from mature trees are likely to be more difficult to establish *in vitro* owing to excessive polyphenol oxidation and rapid necrosis of the explant, but in same species this problem has been overcame. Few laboratories have yet reported on the field performance of micropropagated trees. Saxena and Dhawan [12] have reported on production of *Anogeissus pendula* and *A. latifolia* by micropropagation.

Micropropagation as related to forest trees has been reviewed by Bonga and Von Aderkas [13]. Several Indian laboratories have developed protocols for micropropagation of woody/forest plants. The DBT has supported R&D activities by establishing activities at various geographical regions of the country. Two Micropropagation Technology Parks are established at TERI (New Delhi) and NCL (Pune). There is need to establish linkages between the institutions (which are developing micropropagation technologies) and user agencies. Plant micropropagation has an outstanding place in biotechnology industry. Department of Biotechnology has published the achievements of R&D activities in tissue culture and micropropagation of plants of our country. The DBT has provided extensive support for technology development needed far forest trees of India. Plant production through this technique can benefit from the utilization of mycorrhiza, the naturalistic association between plant root and fungi [14].

**Somatic embryogenesis:** Since 1985, researchers have made a great deal of progress in the development of somatic embryogenesis for conifer reforestation [15]. It has been predicted that the biotechnology of somatic embryogenesis will be technology of 21$^{st}$ century in forestry. Bioprocessing for tree production in the forest industry was reviewed by Timmis [16] of Weyerhaeuser Company of USA. In India research on induction of somatic embryogenesis has been done by several laboratories including, Bhabha Atomic Research Center (BARC), Mumbai; NBRI, Lucknow; BHU, Varanasi and Department of Botany, JNV University, Jodhpur.

**Genetic transformation and genetically modified trees:** It was in the last decade of 20$^{th}$ century that research had been conducted on the characterization of DNA from trees and isolation and characterization of specific genes. Strauss *et al.* [17] reviewed and discussed genetic engineering of reproductive sterility in forest trees. Serious experiments in genetic engineering with tree species were attempted in the developed countries [17-22, 24-26]. Genetic transformations have been reported in number of tree species including hybrid poplar, loblolly pine, aspen, *Citrus* spp. apple [18]. Rugh *et al.* [27] developed transgenic yellow poplar for mercury phytoremediation. Phytoremediation of trichloroethylene with genetically engineered poplar has been discussed by Holton [28]. Hu *et al.* [29] produced transgenic aspen trees in which expression of lignin biosynthetic pathway gene PtCL 1 encoding coumarate: coenzyme A ligase (4CL) has been down regulated by anti-sense inhibition. Boudet and Grima-Pettenati [30] reviewed lignin genetic engineering. Eriksson *et al.* [31] reported on over-expression of a key regulatory gene in biosynthesis of plant hormone gibberellin in hybrid aspen. Pena *et al.* [24] transformed juvenile *Citrus* seedlings to constitutively express the *Arabidopsis,* LEAFY OR APETALAI genes, which promote flower initiation. The transgenic *citrus* produced fertile flowers and fruits as early as the first year. The issues related to genetically modified forest plants [32] and related controversies [33] have been discussed. Ghorbel *et al.* [23] constructed supervirulence vector A281 that provides super-transformation ability to *Agrobacterium tumefaciens* in *Citrus.*

## GENETIC MAPPING OF WOODY PLANTS - EXPRESSED SEQUENCE TAGS (ESTs)

There have been major changes during the last 20 years in plant biology. The genome sequencing of *Arabidopsis* has been completed. This is rightly heralded as a landmark event [34]. As plant biologists enter a new era in which genomics promises to address fundamental questions in botany. In, the post-genomic era, rice and *Arabidopsis* are often presented as examples of a "typical" monocot and a "typical" dicot respectively. It is important to realize that the intensive studies of these model systems also have their limitations. Developmental regulation of trees in general and leguminous and gymnospermous trees in particular, offers different area of research. Han *et al.* [35] identified 3000 ESTs in Black Locust.

Morgante and Olivieri [36] discussed PCR amplified microsatellites as markers in plant genetics. Pagalia and Morgante [37] utilized DNA finger printing techniques for analysis of conifer genome. Zyprian [9] reviewed genetic mapping in woody crops with focus on the major fruit-producing horticultural perennials and forest trees. In this paper the currently used types of molecular marker have been compiled. Lefort *et al.* [10] suggested microsatellite as new generation of molecular markers for-forest genetics. Bradshaw *et al.* [38] prepared a genetic linkage map of a hybrid poplar composed of RFLP, STS and RAPD markers. Chase *et al.* [8] used microsatellite markers for population- and conservation- genetics of tropical trees. Conservation of microsatellites among tropical leguminous trees was reported by Dayanandan *et al.* [39]. Hicks *et al.* [40] developed RAPD and microsatellite markers for lodge pole pine. Heinze [41] used PRC-based chloroplast DNA assays for the identification of *Populus.* Microsatellite analysis of *Quercus robur* was done by Lexer *et al.* [42]. Parasnis *et al.* [43] used microsatellite to reveal sex-specific differences in Papaya. In case of *Prunus cerasifera,* Lecouls *et al.* [44] identified root-not nematode resistance gene using RAPD and SCAR markers. Arcade *et al.* [45] applied AFLP, RAPD and ISSR markers for genetic mapping of larch. Using chloroplast DNA marker, Stoehr *et al.* [46] evaluated pollen competition in Douglas-Fir. Bukhari *et al.* [47] used chloroplast RFLP data for phylogenetic analysis of *Acacia.* Sosinki *et al.* [48] used microsatellite markers for characterization of peach. Butcher *et al.* [49] studied on microsatellite marker from *Acacia mangium.* Johnson *et al.* [50] analysed the financial feasibility of marker-aided selection in Douglas-fir. Van der Schoot *et al.* [51] developed microsatellite markers and characterized black poplar. Collevatti *et al.* [52] worked on high-resolution microsatellite based analysis of mating system that allowed the detection of significant biparental inbreeding in *Caryocar brasiliense*. Miller and Bayer [53] reported on molecular phylogenetics of *Acacia* based on the chloroplast DNA sequences. Sankar and Moore [54] evaluated ISSR analysis for making *Citrus* genetic linkage map. Yang *et al.* [55] optimized SSR-PCR system for *Panax ginseng* using orthogonal design. Plant height is an important trait for plant breeding because it is related to planting density and lodging resistance. It is influenced by many qualitative genes and quantitative trait loci (QTL). The genetic basis of plant height and its related traits can be dissected, using simple sequence repeat (SSR) markers within recombinant inbred lines (RIL) [56].

Genomics approaches and tools of molecular biology are revolutionizing the study of plants to discover the mechanisms, development and control of developmental processes. Among the tree legumes, the black locust *(Robinia pseudoaccacia)* has been investigated by the scientists of developed countries. These genomic studies may be valuable but in order to have better perspectives efforts should be made towards genomics of trees, which have wide ranges of environmental adaptations and have more economic and ecological values.

## TREES OF ARID AND SEMI-ARID ENVIRONMENTS

Several tree species contribute to production of biomass in arid and semi-arid regions in India. These include *Anogeissus* spp., *Acacia spp., Azadirachta indica, Aegle marmelos, Capparis* spp, *Boswellia serata, Calligonum polygonoides, Balanites aegyptiaca, Delbergia* spp., *Maytenus emerginata, Prosopis spp., Salvadora* spp., *Sterculia urens, Tecomella undulata, Terminalia* spp., *Zygyphus* spp and *Wrightia* spp. Considerable work has been done on tissue culture of some of the tree spp. However, there is meager knowledge about several important aspects of biology of even useful trees. It is suggested that research on tree genomics should be initiated in the country.

There is need for taking up projects on Expressed Sequence Tags (ESTs) and microsatellite as molecular markers for *Anogeissus* spp., *Capparis* spp., *Prosopis cineraria, Tecomella undulata* and *Terminalia* spp. These species bear important traits and genes that can be characterize and identified for future prospecting.

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