THEME 3

Expanding Frontiers in Forestry Sciences

Expanding Frontiers of Forestry Science: Meeting the Future Challenges of Forestry in India[1]

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"The rapid growth of cross-disciplinary and interdisciplinary work would indeed argue that new knowledge is no longer obtained from within the disciplines around which teaching, learning and research have been organized in the nineteenth and twentieth centuries" **(Drucker 1989)**

INTRODUCTION

All over the world forests and forestry are undergoing major changes in response to the larger societal changes. Several factors – demographic changes (including population growth, urbanization, ageing and migration), growth and distribution of income, globalization, political and institutional changes, local, national and global environmental concerns, developments in science and technology, etc. – are collectively impacting society, changing the demand for goods and services from forests. As already evident, the transformation of India from a largely agrarian society to an industrial and post industrial society is accentuating resource use conflicts stemming from divergent needs of different segments in the society and the differing pace of societal transition. Persistent inequalities in wealth and income in society have added to the severity of resource use conflicts.

With 1.21 billion people in 2011 accounting for about 17% of the world's population in an area about 2.3% of the world's land area, resource use conflicts in India are already severe and are bound to escalate in the context of continued population growth. India has one of the highest population densities in the world (382 people/km^2 as per the 2011 census) and consequently all critical resources, including arable land, water, forests, etc. are in short supply. India's per capita forest area is only 0.06 ha (about a third of Asia-Pacific region and one-tenth of the world), making forests one of the most contested resource in the context of increasing demand for food, fibre, fodder, fuel and raw materials. Climate change related events could further accentuate the problems. Mitigation and adaptation to climate change will require concerted efforts to sequester carbon and to reduce carbon footprints.

Advancements in science and technology are critical in addressing the above challenges. Broadly forestry science will have to deal with three kinds of problems: those that have been with us for a long time and continue to defy any solutions; the more predictable problems characteristic of the changing society – nature relationship; and those which are unpredictable and in the realm of uncertainty, but need to be addressed as they unfold. This paper provides an overview of the challenges facing forestry science and technology in meeting the emerging challenges of sustainable forest management in India. While outlining science and technology developments in key areas, it also gives an indication of some of the developments in frontier areas of science and the issues in their wider application in Indian forestry.

FORESTRY SCIENCE AND TECHNOLOGY: GENERAL TRENDS

An overview of the forest science and technology system

Technological improvements take place through a series of interlinked steps, involving the unraveling of the science underlying natural and human-made processes (upstream basic and strategic research), the use of that knowledge to improve existing technologies or to develop new technologies (applied and adaptive research) and the application of technologies to achieve the desired outputs/ outcomes. The overall structure of science and technology in the forest sector is depicted in Figure 1.

As in other sectors, technology development and application in the forest sector have focused on one or more of the following depending on the objectives of resource managers/ owners and the larger economic and social context.

- ***Cost reducing and productivity enhancing technologies***: The main thrust is on improving output: input ratios, mainly focused on wood and other products.
- ***Environment conserving/improvement technologies***: These aims to improve the flow of environmental services, including conservation of biodiversity, improved stream flow through better watershed management, reduction *in effluent loads, increased carbon sequestration, etc.*
- ***Energy saving/conserving technologies***: Higher costs of non-renewable energy and climate change policies have led

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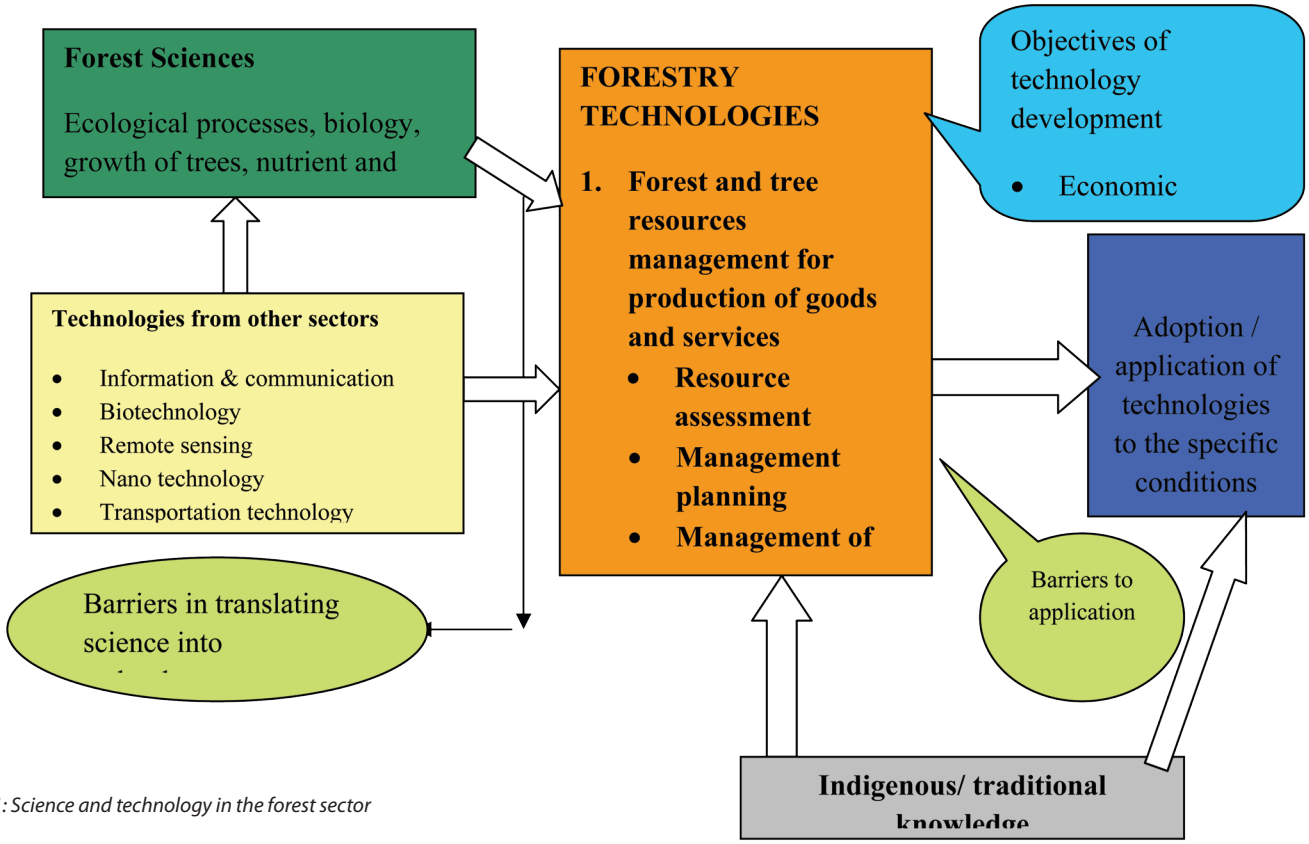

Fig 1: Science and technology in the forest sector

to investments in science and technology to improve energy efficiency and to improve carbon balance.

- ***Socially relevant technologies***: Fulfillment of critical social objectives – like poverty alleviation, uplifting socially and economically weak sections in society – is the main objective of these technologies.

Broadly three streams of knowledge have contributed to the development of forestry as indicated below:

1. Developments in forest science, especially to understand ecosystems and ecological processes, including growth of trees, water and nutrient cycling, forest pests and diseases, carbon fluxes, etc. form the core of silviculture and forest management.

2. Science and technology developments in other sectors: These include information and communication technologies, remote sensing, transportation technologies, biotechnology and, more recently, nano-technology and a host of developments in the field of industrial processing. Often the application of technological developments taking place outside the forest sector impact forestry more profoundly than developments in forestry science *per se.* One such example is the developments in information and communication technologies. Another is plant genetics and crop breeding, which initially revolutionized agriculture, found application in forestry significantly enhancing productivity of plantations.

3. The third stream of knowhow is traditional knowledge, acquired through long practical experience and field observations by local communities, especially farmers. This forms the basis of a large segment of informal sector activities. Indigenous knowledge about the use of several plant and animal species belongs to this category and there is increasing effort to strengthen the scientific basis of this through the use of modern science and related analytical tools.

Though considerable work has been done in enhancing basic knowledge (especially in taxonomy, ecology, wood science, chemistry of forest products, etc.), application of science has largely been focused on wood production with the following getting most attention:

- Thrust on industrial wood production (which includes a whole range of activities including inventory of resources, harvesting technologies, regeneration (both natural and artificial) of commercially important species;
- Emphasis on large scale production, catering to large markets; and
- Simplification of complex ecosystems, focusing on intensively managed forest plantations to enhance wood production.

KEY DEVELOPMENTS IN FOREST SCIENCE

Science and technology developments in forestry have largely focused on the following:

- Resource assessment to give a better picture of key forest characteristics (area, density, forest type, growing stock, increment, biodiversity, carbon stock, etc.), the flow of goods and services and how the stock and flow are changing over time;

- The science underlying human interventions, enabling the alteration of the flow of goods and services; and
- Innovation on processing of wood and non-wood forest products, including production of new products and services or improvements in production processes.

Forest resource assessment

Assessing the status of forest resources has been one of the thrust areas of forest science since the beginning of forest management. Resource assessment in the early years has largely focused on the estimation of the area under forests and the volume of commercial timber. Technological developments in resource assessment have aimed at:

1. Improvements in the technologies for assessing the extent of forests including differentiation according to density. Satellite based remote sensing technologies (or "eyes in the sky") have brought about major improvements in the speed and accuracy of resource assessment; ; yet challenges persist in providing an accurate picture of the extent of forests and the changes over time, for example forest degradation.
2. Forest inventory techniques have largely focused on providing estimates of growing stock and increment of timber, especially of commercially valuable species to facilitate the determination of annual allowable cuts. Technological progress has improved the assessment of products and services including non-wood forest products, biodiversity and several of the environmental services. In the context of climate change mitigation and adaptation, measuring and monitoring changes in forest carbon stocks have become important and substantial efforts are underway to develop systems for monitoring, reporting and verification of carbon stock in the context of the REDD+ initiatives.
3. Improvements in remote sensing technologies and information and communication technologies are paving the way for real time assessment of changes in forest conditions, enabling tracking of forest clearance and incidence of forest fires and pest and disease outbreaks so that preventive/ remedial measures can be initiated without losing time. Satellite based systems are able to locate outbreaks of fire, provide details of location and direction of spread and alert fire fighting teams to take timely action.
4. Much of the traditional resource assessment technologies have focused on two dimensions, largely providing the area of forests and to a limited extent the density of forests. Volume estimation has largely been made through sample measurements and extrapolation. LiDAR technologies are however revolutionizing forest resource assessment through providing information on the third dimension and the scope for its application is extremely wide (Box 1).

Forest and tree resources management

Developments in several fronts of science and technology have influenced forest management, including the process of preparation of management plans and actual management of forest and tree resources. Broadly management of forests can be grouped into (a) management of natural forests and (b) management of plantations. With the increase in farm tree planting under agroforestry increasing attention is also being paid to understand the problems as regards tree growing in mixed farming systems recently attention is being given to managing trees in mixed farming systems, especially to optimize the flow of different products and services.

Box 1: **LiDAR technology application in forestry**

Light Detection and Ranging (LiDAR) remote sensing is a major breakthrough in forest resource assessment as it provides three-dimensional data as opposed to the two-dimensional resource assessment of traditional technologies. The technology facilitates high precision assessment of stand/tree height, helpful in estimating growing stock/ biomass and the development of digital terrain model and digital stand model. 3D models built with LIDAR have been successfully applied to ecological studies, especially to assess overall ecosystem health. LiDAR technologies have significantly improved the precision in estimating key forest characteristics and are more cost effective, especially in high value management. Wider adoption however depends on the relative costs and the intensity of management.

Resource management planning

Forest management planning has undergone revolutionary changes with the advent of Geographic Information System (GIS). *"GIS is a system of hardware and software designed to store, retrieve and analyze spatial and temporal data, whether generated using remote sensing or other means and to generate the required information in desired forms for management purposes"* (Kushwaha, 2011). It is an integrating technology helping to combine different information enabling the analysis of the linkages between different components. Ability to rapidly collect a wide array of information – on topography, soil, climate, vegetation, volume of different products, environmental services, access to markets, availability of inputs, potential impact of different treatments, etc. – and to integrate them to assess the different options and their economic, social and environmental implications has brought about major changes in forest management planning. Although use of GIS in forest management planning has been in vogue for quite some time, it is yet to be used on a wider scale and most efforts to use GIS remains fragmented.

Certainly GIS technologies will continue to improve, especially in the context of improvements in remote sensing technologies, sensor technologies and new software that permits integration of different information. The main challenge relates to the actual application of GIS in management decision making. As a tool there is considerable awareness of its relevance, but still forest departments are far from fully taking advantage of its potentials.

Science and technology for management of natural forests

Before the advent of plantation forestry most of the timber was being sourced from natural forests. Consequently much of forestry research was focused on developing practices intended to manage natural forests sustainably and this resulted in

the development of a wide range of silvicultural systems. In fact India's rich forest science tradition largely stems from the enormous efforts in the first half of the 20th century to develop scientific practices to manage its natural forests. Substantial work was done on forest ecology and to use such knowledge to develop various silvicultural practices. Considerable emphasis was given to regulate harvesting to provide conditions favourable for regeneration of valuable species and to nurture them to ensure sustained yields.

While there was considerable potential to develop the knowledge stream on natural forest management, the declining importance of natural forests in wood production led to a near total neglect of further efforts in this direction. Widespread bans on logging of natural forests and the setting aside of large tracts of forests as protected areas led to the neglect of further development of the science of managing natural forests. Increasing dependence on plantations and liberalized wood imports also contributed to the neglect of management of natural forests for wood production. There is however some signs of growing interest in refining management of natural forests to fulfill multiple objectives, giving thrust to an ecosystem approach (Box 2) and development of precision forestry integrating different streams of information.

Box 2: **Ecosystem approach to forest management**

In many countries the traditional nature dependent low intensity management (with most of the focus on protecting forests from adverse human interventions) based on limited understanding of natural processes has largely been replaced with intensive management focusing on wood production through forest plantations. In a way this implies a near total change in the ecosystem. Increasing concern about environmental services provided by forest ecosystems is encouraging the pursuit of the ecosystem approach to the management of natural forests. This means that (a) the management maintains or improves the ecosystem and (b) the ecosystem is managed in a way that provides not only timber but a wide range of goods and services now and in future. Ecosystems are hierarchical and decision making in management should proceed in hierarchical steps. Owing to the great number of factors involved, usually a generic approach that aims to minimize disturbances are adopted. There is considerable scope for wider application of ecosystem approach to managing natural forests to produce very high quality timber for specific niche markets. While both of SFM and EA are based on the concept of sustainability, the former is an out-come based approach, while EA gives greater emphasis to holistic management with greater stress on conservation of the ecosystem integrity. Ecosystem approach forms the basis of "close to nature" silviculture. Understanding the complexity of forests remains the major challenge in implementing an ecosystem approach to forest management.

Precision forestry is an outcome of efforts to develop fine tuned management integrating a wide array of developments including on ecosystem research, information and communication technologies, sensor technologies, etc. Traditionally forest management has neglected micro-scale conditions and most practices have been developed on large scale models, aimed to overcome the complexities of natural ecosystems. Precision forestry is a highly knowledge intensive approach giving considerable attention to the space-scale-time dimensions and in-depth assessment of the totality of changes arising from natural processes and human interventions (Box 3).

Box 3: **Precision Forestry**

Science is reaching the stage at which it could enable intensive management of complex forest systems. Computer simulations could make it possible to manage forests for an optimal mix of products and services. Precision forestry uses high technology sensing and analytical tools to support site-specific, economic, environmental and sustainable decision making for the forestry sector supporting the forestry value chain from land to the user of products and services from forests. New sensor technologies are improving the ability to determine with greater precision such things as what do we do in seeding to the customer forestry supply chain. A whole array of applications have emerged to provide a much better understanding of the natural and human driven processes so that they all are fully integrated to provide optimal solutions. Precision forestry enables planning and conducting site-specific forest management activities and operations to improve wood product quality and utilization, reduce waste, and increase profits, and maintain the quality of the environment.

Three broad categories of precision forestry can be identified:

1. Using geospatial-information to assist forest management and planning;
2. Site-specific silvicultural operations; and
3. Advanced site-specific technology to meet market demands for higher valued products.

The common thread in all three of these areas of precision forestry is the emphasis on site-specific practices and geospatial technologies. Rather than conducting operations at the stand level, operations and activities are conducted in smaller management.

Source: Ackerman et al 2010

The science of forest plantation management:

As plantations are highly modified systems, focused on obtaining high productivity, they obviously require substantial science and technology inputs. Understandably a major chunk of investments in forestry science is centred on forest plantations. Site species matching, enhancing productivity through tree improvement and mass multiplication, management of pests and diseases, and site management to maintain and improve productivity have been the thrust areas of forest plantation

research. Especially in the case of afforestation/ reforestation of difficult sites, considerable attention has been given to identify suitable species and appropriate practices to ensure regeneration and subsequent performance.

Undoubtedly tree improvement has been one of the core areas of forestry science, and a wide array of techniques have been developed to enhance productivity through faster growth rates, to improve wood quality, to withstand adverse environmental conditions, and to overcome pest and disease infestation. Productivity increase has been particularly phenomenal in the case of fast growing short rotation species like Eucalyptus, tropical pines, and poplars. Through a combination of careful selection, mass multiplication and intensive site management, there are examples of attaining very high levels of productivity (in some cases a mean annual increment of 50m3 per hectare) although concern still remains on sustainability of such productivity levels. Important features of these technologies are:

•As in the case of the "green-revolution technologies" in agriculture, productivity increase is largely due to the cumulative impact of various components (the right planting material, improved site management, effective control of pests and diseases, etc.). Isolated use of any one of the components is unlikely to have much impact.

•The focus on short-rotation fast growing species, especially Eucalyptus, tropical pines and poplars is, directly related to the demand from the processing industry like pulp and paper and reconstituted fibre boards. Such backward linkage from the processing sector is one of the main drivers of innovation in plantation technologies.

Private sector is in the forefront of development and application of productivity enhancing technologies (as in the case of ITC Bhadrachalam). While public sector research institutions have pioneered the science of tree improvement, adoption of technologies by forestry departments, who manage most of the plantations, severely lag behind. Tree improvement research aims to "fast-track" the development of progenies with desirable characteristics and their mass multiplication. Molecular markers have helped in the identification of the loci of desirable characteristics. Advancements in forest tree genome mapping will further strengthen the efforts in tree improvement. While traditional improvement techniques relied on natural variations, genetic modification aims to implant desirable characteristics selectively manipulating the genetic makeup. As in any innovation, the pros and cons of genetic modification are under intense discussion (Box 4).

Box 4: **Genetically modified trees: Potentials and challenges**

Increasing demand for wood has drawn attention to the potential of genetically engineered or "designer" trees. Advancements in gene transfer technologies and tree genomics are providing new avenues for genetic improvement of trees. Genetic composition is changed to produce trees with characteristics that are economically important, but could not be produced by conventional techniques within a reasonable period of time. Traits considered for genetic modification include herbicide tolerance, reduced flowering or sterility, insect resistance, wood chemistry (especially lower lignin content) and fibre quality. Developing planting materials to withstand adverse environmental conditions – for example high levels of acidity and alkalinity (or other toxicities) – will be a thrust area for genetic modification, enabling the use of vast stretches of degraded lands. Further, in the context of climate change thrust will be on identifying traits that could help in the adaptability of species will be a thrust area. Genetic modification is also getting increasing attention in the context of cellulosic biofuel production.

Production and deployment of GM trees however remain a contentious issue. Genetic modification is not merely a technical and economic issue. Concerns have been raised on their impact on ecosystems, especially invasiveness, impacts on biodiversity and the transfer of genes to other organisms. Since expression of a desired trait is an outcome of several factors, long term effectiveness of genetic modification also needs careful consideration. Society is also concerned that many of the trials of GM trees are spearheaded by large biotech companies, largely focused on immediate profits potentially underplaying their long term adverse social and environmental impacts. Reliable and tested protocols for assessing risks associated with GM trees are necessary and in most cases such capacity is non-existent. Considerable uncertainty exists on the use of GM trees and substantial work needs to be done to assess their long term potential impacts. The extent of research on potential impacts has been extremely limited in comparison with the efforts to develop GM trees.

Source: El Lakany 2004, UN, 2008

Forest protection–emerging trends

Managing forest fires – to assess vulnerability, identifying hotspots, locating fire outbreaks and taking timely action – requires deployment of a wide array of technologies. With satellite based infra-red detection devices, it is possible to locate fire outbreaks, assess the intensity and direction of their spread and alert fire-fighting crews for taking appropriate counter measures.

Another major concern, especially with large scale plantations is the outbreak of pests and diseases, as has been the case of the sal borer and teak defoliator. Much of the thrust has been to study the life cycle of pests and the factors contributing to outbreak and spread of infestation. Climate change related infestation largely attributed to changes in temperature could spur pest outbreaks. Among the various measures to control outbreaks of pests and diseases have been developed, much of the thrust had been on chemical pesticides. Increasing awareness of their adverse impacts on human health and damage to ecosystems has led to a shift in favour of adopting bio-control measures (for example pheromone trapping), including the use of bio-pesticides (Box 5). Some work has also been done as regards sequencing the genome of the sal borer symbiont (a fungus) that undermines the tree defenses.

Box 5: **Bio-pesticide application to manage teak defoliator**

Substantial efforts have been made to understand the ecology and management of teak defoliator based on which the baculovirus bio-pesticide has been developed, field tested and the technology for its mass production standardised. Hybcheck is an HpNPV formulation which holds the promise to contain teak defoliator populations below economic threshold levels. Yet several constraints exist in its wider adoption, which could significantly increase productivity of teak plantations. As such routine teak defoliator management is not taking place anywhere in the country. Quite different from the agricultural scenario, the major share of teak plantations is with governments. In general the silvicultural operations in teak plantations are limited only for a short span of about 3-4 years. Beyond this period, plantations are left to nature. Undertaking pest management operations thus depends on the policy decision on this matter. Once such a positive decision is taken, it will be necessary to build up necessary infrastructure for mass production of the bio pesticide. Along with this, suitable training on pest monitoring, decision making on pest control, biocide application, etc. is to be imparted to the field staff.

Source: Sajeev et al 2008

THE SCIENCE OF AGROFORESTRY

Agroforestry comprises of a broad group of land uses where crops, livestock and trees are integrally managed. Most of the research in agroforestry aims to identify appropriate crop and tree combinations to enhance the economic, environmental and social benefits. Agroforestry practices attempt to optimize the different components taking full advantage of the micro variation in soils, topography and moisture availability. Some important characteristics of agroforestry technologies include:

- ecological and cultural specificity;
- integral part of the traditional knowledge system developed through hands-on experience and transmitted between generations;
- holistic nature of knowledge in that economic and other benefits are assessed in their totality considering the strong linkages between the different components; and
- local communities and land holders form the most important innovators and adaptors.

There are several successful agroforestry systems and practices – including alley cropping, silvopasture, wind breaks, hedgerow intercropping, parklands, home gardens and relay cropping (FAO 2009). Again, as is with the shift from natural forests to plantations, a significant share of research efforts are focused on simplifying management with particular focus on a limited number of species in mixtures. This to some extend permits easy transfer of traditional plantation technologies to farm forestry and suits well in the context of increasing demand for industrial roundwood for large scale processing.

However, in the context of climate change adaptation, increasing emphasis will be to enhance the resilience and to reduce the vulnerability of existing monocrop farming systems. There are several examples of multi-functional agroforestry systems in India appropriate to the divergent ecological, economic and social conditions (Singh and Pandey 2011). Mixed crop farms are reported to be more efficient in sequestering and storing carbon and in maintaining key economical functions, especially water and nutrient cycling. Singh and Pandey have listed a number of agroforestry issues to be resolved through research and innovation (Table 1)

Table1: **Unresolved challenges for future agroforestry research and innovations in India**

Issue	Background
Crop yields: Increase or decrease?	Although some traditional agroforestry systems do increase crops yields near trees, there are instances where fast growing trees have reduced crop yields in the short term. Context-specific long-term studies are required to resolve this issue.
Nutrients: additional supply or redistribution?	Mature and scattered agroforestry trees are associated with improved soil nutrient supply in traditional agroforestry systems, it is not known if trees additionally supply nutrients by increasing the total quantum of nutrients in agroecosystems or just redistribute the available quantity horizontally and vertically
Water-Tree interaction: high water uptake or no change?	High water use by fast-growing species and therefore alleged groundwater depletion is a common concern in dry regions that remains unresolved. Do trees actually extract more groundwater or use the residual water available either through irrigation, or use the rainwater when crops have been harvested? It may be possible that rather than letting the rains be lost as runoff, agroforestry may increase the utilization of rainwater by extending the growing season. Furthermore, it is not clearly understood if trees harvest and accumulate water from surrounding area and release it during the soil-moisture stress. If this is so then, agroforestry as an adaptation to monsoon variability may actually benefit the crops.
Climate change mitigation and adaptation	Studies on the carbon sequestration potential are limited both by their location specificity as well as uncertainty related to sequestration in biomass and soils. Often, the rate of carbon sequestration is derived from the growth of above ground biomass. In addition, role of agroforestry in as an adaptation to climate change needs to be explored further.
Soil amelioration and conservation	Agroforestry systems with mature trees capable of yielding enough litter are known to conserve soils and ameliorate soil nutrient status, but knowledge on the full range of species and their attributes useful for all the agro-climatic regions and problem-soils in India are required.

Genetically improved trees	Genetically improved trees may provide more biomass and other products valued by the society, but presently research results in this field mostly remain in the laboratory. A full mechanism starting from developing and registration of clones, decentralized certification, and mass multiplication of suitable stock to ensure availability to farmers is required.
Multiple-use species adapted to multiple agro-climatic conditions	Multiple-use species with a wide range of geographic and climatic adaptation can enhance the success and spread of agroforestry. This is a crucial area of research involving multi-location research in all the climatic regions in India.
Domestication of useful species	Many wild populations of species that yield commercially-valued products are getting depleted, research efforts are required to domesticate these species and integrate with the agroforestry systems in India.
Policies to promote linkages between markets and tree growing in agro ecosystems	On the one hand smallholder systems in India supply about 50% of wood andfuelwood demand, on the other here are still many restrictive regulations that potentially deter farmers from growing trees in agroecosystems and selling these in markets. This issue needs to be addressed.
Value addition innovations	NTFPs have the potential to improve livelihoods of poor farmers, but vigorousefforts are needed to provide knowledge on the on-farm value addition innovation.

Source: Singh and Pandey, 2011

Advances in processing technologies

Wood industries

Technological developments in wood processing have taken place a much faster pace largely on account of the compulsion to respond to the changing consumer demands, competition between producers to bring out new or improved products and the combined impact of innovation in a wide array of areas. While traditional use of timber focused on inherent structural properties (especially strength, durability, working quality and to some extent appearance), wood processing technologies have advanced to such an extent that these have become less important, as almost any wood can be transformed into almost any form with desirable properties. Technological developments in wood processing have:

- enabled the use species that were once considered less useful – a typical example being the use of rubberwood for a wide range of products including as furniture and for medium density fibre board,
- enhanced the rate of recovery of raw material,
- increased the use of small dimension logs,
- improved the extent of recycling, and
- reduced the extent of pollution and provided new technologies for treatment of effluents.

Development of composite wood, with desirable properties by incorporating other materials, has changed the pattern of wood use. Some of the major developments in recent years include:

1. use of microorganisms to bleach pulp and treat effluents in the paper industry, reducing costs and adverse environmental impacts;
2. total use of wood and other biomass through biorefineries producing a range of biomaterials and energy (Box 6).

Development of composite materials has changed the world of wood processing and as such it just doesn't matter what kind of wood is grown. New processing technologies have made it possible to produce a wide range of products from diverse biomass materials.

Box 6: **Biorefineries**

Biorefineries are integrated bio-based processing units, using a variety of technologies to produce chemicals, biofuels, food and feed ingredients, biomaterials (including fibres) and power from biomass raw materials. They are similar to petroleum refineries. Biomass is fractionated to give purified cellulose, hemicelluloses and lignin which form the feed stock for a wide range of products. There are several research and development initiatives, including pilot/ demonstration plants. India's first biorefinery – Godavary Biorefineries Ltd. - has been set up at the Godavari Sugar Mills Ltd, converting bagasse into a number of products. The pulp and paper industry is in some sense a biorefinery and in several countries pulp and paper units are being transformed into full-fledged biorefineries, extracting the entire range of potential products. With the increasing emphasis on pursuing a green path to development, improving energy and material efficiency, there will be increasing thrust on biorefineries and all the indications are of continued efforts to extract the full range of products from biomass in the most energy efficient and environment friendly manner.

Sources: Mamta Kumari and Sudhir Kumar 2010, van Ree R &B. Annevelink 2007 http://www.biorefinery.nl/fileadmin/biorefinery/docs/publications/StatusDocumentBiorefinery2007final211107.pdf,

Among the various emerging technologies, nanotechnology has the potential to revolutionise the wood processing sector. Nanotechnology is defined as the manipulation of materials measuring less than 100 nanometres (one nanometre is one-billionth of a metre) and substantial research is underway in its application to forestry. Potential uses of nanotechnology include:

- Production of lighter-weight but stronger products developed from nano-fibres;
- Coatings to improve surface qualities;
- "Intelligent" products with nanocensors for measuring forces, loads, moisture levels, temperature, etc. (FAO, 2009) (Box 7)

Innovations in wood energy

Wood along with other biomass, especially agricultural residues, is expected to remain the most important source of domestic energy, especially for cooking and space heating in

Box 7: **Nanotechnology applications in forestry: Opportunities and challenges**

Nanotechnology could bring about fundamental changes in the manufacturing of all products and forestry and forest industry will also be impacted. One area that is attracting particular interest within the paper industry is the modification of natural fibres at the nano level, for very thin opaque paper coatings that allow more brilliant colour to set. The cellulose nanofibrils found in wood and other natural materials are also strikingly similar to carbon nanotubes and could be used to strengthen composites for manufacturing, offering a new value stream from forest resources. The nanofibrils are about as strong and stiff as carbon versions and should be cheaper to produce. Nanotechnology has the potential to produce wood products that are water, fire and pest resistant. In paper industry one of the thrust areas of nanotechnology application is in the production of e-paper, which has a wide array of applications. All the indications are that nanotechnology is opening up opportunities for developing new products and processes on an unprecedented scale.

As in the case of all technologies, nanotechnology has to address a lot of challenges. At the nano scale the properties tend to be very different and this is raising concerns about human and animal health issues and a host of environmental problems. As in the case of GM crops, there is a need to undertake systematic assessment of the environmental and human health aspects, requiring substantial public sector research and studies. In fact there is a need to make systematic independent assessment of the environmental and social impacts of nanotechnology, as

Sources: McCrank 2009, R.Atalla et al 2004

colder environment. Although there has been some fuel-switching as households move up the energy ladder in response to increased income and improved availability of commercial fuels like liquefied petroleum gas (DeFries and Pandey, 2010), there are doubts about sustained energy switching in the context of increasing fossil fuel prices and policies that encourage the use of renewable fuels in the context of climate change concerns. Biomass energy is thus being "rediscovered" with particular attention being paid to improve energy efficiency of devices making it a more acceptable and attractive option, especially through reducing in-door pollution and to enhance the convenience in use. Two thrust areas of development in wood biomass use are:

- Design and development of biomass gasifiers for small scale use, especially households;
- Developments in cellulosic biofuel technologies.

Considerable progress has been made in the above two areas. Wider application of these innovations largely depends on their competitiveness with other energy technologies and more importantly public policies, especially in the context of combating climate change. If the current high energy prices persist, cellulosic biofuel could become an important source of commercial energy. Cellulosic biofuels have the advantage of potentially higher overall production volumes, and an improved net carbon emissions balance. However, currently the process of converting cellulosic biomass to sugar and then to biofuel is more expensive than for example grains, largely due to the complexity of processing requiring expensive enzymes to facilitate biomass decomposition. Economic viability will depend on a substantial reduction in enzyme costs. Research is underway to reduce these costs, especially through synthetic biology, to produce engineered enzymes or microbes that break down cellulose replacing the current costly techniques.

Non wood forest products

NWFPs are diverse, and thus many different technologies are used in their production and processing. Although most NWFPs are subsistence products, collected from the wild and consumed locally with minimal processing, some have been domesticated and are cultivated and processed using sophisticated technologies to meet the demand from global markets. Science and technology development for these products has largely focused on more organized systems of production, while subsistence production has almost entirely relied on traditional/ local knowledge.

Natural resource degradation coupled with increasing demand has been the main driver of organized cultivation of many NWFP-yielding species – much as wood production has shifted from natural to planted forests. Research on domestication and cultivation has also been encouraged by the complexity and uncertainty of managing production in the wild. For many products, such as rubber, rattan, bamboo and some medicinal and aromatic plants, organized production and chemical substitution of natural components have virtually replaced collection from the wild, except for products intended for niche markets paying a high premium.

Scientific research has focused on:

- understanding the composition, properties and potential uses of different products;
- technologies for extraction and isolation of marketable components and for addition of desirable characteristics, e.g. to facilitate storage and transportation;
- improvements in processing technologies and development of new products, e.g. new plant-based pharmaceuticals and health and beauty products (the area where most technological advancements are taking place).

Technological developments, for example in biotechnology, have opened up new opportunities and challenges for many NWFPs. While new uses and markets have emerged, so have substitute products which undermine existing markets. Petrochemicals and new technologies for processing glass and metals have significantly changed the markets for a number of plant-based products. NWFPs with limited end uses are particularly vulnerable to such developments. In contrast, Bamboo, a widely cultivated species, has been developed for diverse end uses and has thus become a widely distributed material and an important source of income (FAO, 2007).

In view of their importance in the livelihood of local communities, a significant share of research has been focused on the social, economic and cultural aspects of non-wood forest products. Much less has however been done to develop and adopt value addition technologies that could significantly improve the livelihood of local communities.

Environmental services

Provision of environmental services – in particular water conservation, carbon sequestration and storage, conservation of biodiversity, arresting land degradation and desertification and rehabilitation of degraded lands, amenity values especially recreation and improvement in environmental quality especially in urban areas – has become a major thrust of forest management. Yet our understanding of the ecosystem services and how they are impacted by human interventions remain extremely limited. Myths, misconceptions and misinformation are widespread. In most cases the studies are not comprehensive and there is a strong tendency to extrapolate, exaggerate and sensationalize the issues. Even reputed scientific bodies are not free from such problems, as evident from the discussion on the rate of shrinkage of the Himalayan glaciers on account of global warming.

Research relating to environmental services has largely focused on quantification of environmental services, assessment of the impact of various interventions and valuation of environmental services. A large share of such studies is taken up in the context of diversion of forest land for alternative uses. Putting a price tag has been a major thrust of environmental economics, especially in the context of using market mechanisms, in particular payment for environmental services. Emergence of carbon markets, both regulated under the clean development mechanism and the less regulated voluntary markets, has led to several studies on quantification and valuation. Yet concerns remain whether market mechanisms, in particular payment for environmental services, will be able to significantly reduce environmental degradation. Notwithstanding all the efforts during the last two decades, payment for environmental services is yet to make any dent on the problem. For every successful reported examples of payment for environmental services, there could be many unreported instances of failures.

Most research on forest related environmental services remain fragmented and there is a strong tendency to extrapolate results from limited spatial and temporal studies resulting in incorrect conclusions. In general the approach to provision of environmental services is to minimize human interferences enabling nature to take its course. However with the increasing demand for a wide array of environmental services and the need to establish acceptable tradeoffs between competing needs, the need to fine tune management of forests for provision of environmental services will become very critical.

Three areas that will require particular attention as regards the science of provision of environmental services are:

- Restoration/ rehabilitation of degraded ecosystems;
- Improved management of water and carbon; and
- Better conservation and management of biodiversity.

Especially in the context of the adverse impacts of climate change and the increasing demand for food, fibre and fuel, much more systematic work will be required in these areas to provide better clarity as regards public decision making and to establish appropriate tradeoffs between competing objectives.

CHALLENGES FOR INDIAN FORESTRY SCIENCE

While considerable progress has been made in improving the science and technology base of Indian forestry, many challenges persist. The world in 2011 is very different from what it was a decade or two earlier and so will be 2020 in comparison with what it is now. Despite the various uncertainties, it is possible to visualize how the society in 2020 and beyond will look like and to identify the challenges forests and forestry are likely to face in the context of larger changes. Key issues that require particular attention include the following:

Understanding the complexity of forests

Forest is a complex system, extremely challenging to understand the interaction between the different components and the processes over time and space. We still haven't really done adequate work to fully understand the system as much of the focus has been on simplifying it for the convenience of single purpose management (mainly wood production) the system. Although earlier work has provided some basic knowledge, this has not been taken forward and developed benefitting from the various advances in methodologies and research tools. As provision of environmental services becoming a major thrust of forest management, multiple use management based on a better understanding of ecosystem processes will become unavoidable.

Need to bring science to the people

Most of the science and technology developments in forestry have focused on industrial development and that too on large scale production and processing, catering to large markets with substantial scale economies. Largely this has been the outcome of the predominance of public ownership of forests and the thrust given to meet the large scale demand for industrial round wood. The needs of small producers and traditional communities have seldom received much attention, Although this situation has changed somewhat, largely on account of the emergence of farm forestry as a source of wood supply and the increasing attention that government and industries are paying, still a very large share of production and processing takes place in the informal sector mostly by small and medium enterprises. A typical example is that of woodfuel, which accounts for about 90% of the total wood production most of it being produced and traded in the informal sector. Vast majority of wood industries also operate in the informal domain with very limited access to technology. Low levels of technology imply reduced efficiency (in particular high levels of wastage and low raw material recovery), poor sustainability and adverse environmental impacts. Any improvement in the forest situation is dependent on remedying the significant technology divide that currently exists within the forest sector.

Institutional challenges

Probably the greatest challenge facing forestry science in India relates to the institutional arrangements. Although some effort was made to improve the situation through the establishment

of the Indian Council for Forestry Research and Education, the situation remains extremely worrying. In particular the institutional problems manifests in the following ways:

• **Mediocre output quality and fragmentation of research***:* A considerable share of research continues to remain highly fragmented and in many cases there is very little collaboration between individuals and institutions. Duplication and overlap are rampant and notwithstanding the enormous scope for collaborative research, the attempt in that direction is very limited. In fact the unhealthy competition is having a negative impact and many of the outputs are at best mediocre, largely intended to increase the number of papers published. Shifting the focus to more demand driven research, linking resource allocation to clearly defined outputs and outcomes involving user agencies in determining priorities will to a great extent address this problem.

• **Science management**: Of course poor quality of research is largely a reflection of severe deficiencies in science management. There is over-bureaucratisation of Indian forestry research – whether it is national or state level institutions, including universities – and in many cases those who have no research background are heading key institutions. There is an urgent need to improve management of forest science, especially in building up competent leadership.

• **Strengthening public sector research:** Environmental and social issues will continue to become more pronounced in an era of economic liberalization and globalization. Resource conflicts will continue to escalate and resolution of these will require strong science based policy and market interventions, which will be extremely difficult in the absence of a vibrant public sector research system.

There is an urgent need to undertake a very thorough social audit of forestry R&D system and to take suitable corrective action to make the system more vibrant, responsive and accountable, especially in the context of the rapid changes taking place in the social and economic conditions. This is all the more so in the context of increasing emphasis on pursuing a green development path. Although forestry is one of the greenest of the economic activity, still there is a need for major changes in the direction of development with the concomitant shifts in science and technology development.

CONCLUSION

Innovation remains the cornerstone of meeting future challenges, especially in a severely resource constrained situation like India. There are a number of key developments in science and technology, including information and communication technologies, biotechnology and ongoing efforts in nanotechnology and bio-refineries. While a whole spectrum of technologies exists and more are being developed, there are several challenges in their application, including their relevance and appropriateness to the social, economic and environmental context. Currently the technology divide within the forestry sector is extremely wide and a large segment of forestry remains outside the purview of any science, an important cause of unsustainability. Particular thrust needs to be given to:

- Enhancing clarity of purpose and defining a long term vision on what will be the role of forests and forestry in a rapidly evolving diverse social, economic and ecological mosaic in India and how innovation has to be steered to address the emerging challenges, particularly benefitting people who remain marginalised and whose current access to innovation is limited.
- Ensuring a right balance between public and private sector research so that the totality of scientific developments in forestry is more balanced taking into account the economic, social and environmental dimensions.
- Addressing the barriers in the application of innovations. As such diffusion and application of innovation is extremely slow due to a wide array of problems, in particular deficiencies in the institutional arrangements. Accountability of R&D institutions should be defined better and there is a need to create a more congenial environment for innovation to flourish. Striking the right balance as regards scientific freedom and social accountability will be a major challenge.
- Reinventing the institutional system to create vibrancy in the research system rewarding competence and productivity.

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Geomatics: Applications and Opportunities

S.P.S. Kushwaha *

GEOMATICS

Geomatics (also known as geospatial technology or geomatic engineering) is the discipline of gathering, storing, processing, and delivering geographic or spatially referenced information. The term Geomatics was coined in 1969 by Dubuisson by combining the terms geodesy and geoinformatics. It includes the tools and techniques used in land surveying, geography, remote sensing, cartography, geographic information systems (GIS), global navigation satellite systems (GPS, GLONASS, Galileo, Compass), photogrammetry, and related forms of earth mapping. A geographic information system (GIS), geographical information system, or geospatial information system is a system designed to capture, store, manipulate, analyze, manage, and present all types of geographically referenced data. Remote sensing is the technology of gathering information about objects or phenomena without being in physical contact with them. For this, different wavelength regions of the electromagnetic spectrum are utilized. Electromagnetic radiation is comprised of a large spectrum of wavelengths right from very short wavelength cosmic rays to long radio waves. In remote sensing, the most useful regions are the visible (0.4-0.7 μ), reflective infrared (0.7-3.0 μ), thermal infrared (3.0 to 5.0 μ and 8.0 to 14.0 μ) and the microwaves (1mm to 1m). Maximum radiation occurs around 0.55μ, which is in the visible region. The solar radiation reaching to the earth surface is modified by the atmospheric gases, aerosols and the water vapors by so called the "path radiance". Hence, it is useful to apply atmospheric corrections to satellite imagery to obtain natural contrast among various features. The atmosphere, including haze and clouds, is much more transparent to microwaves than to visible and infrared waves. Hence, remote sensing using active microwave sensors has all weather capability.

All remote sensing systems, capture radiation in different wavelengths reflected/emitted by the earth surface features and record it either directly on the film as in case of aerial photography or on a digital medium for generating the image. As no two objects in nature are theoretically ditto, their signatures are most likely to be unique. This property of the objects is exploited in remote sensing to differentiate the objects from one another. The reflectance from vegetation is controlled by leaf pigments, cell structure and leaf water content. The radiation absorbed in red region is primarily used for photosynthesis. In healthy vegetation, both absorption and reflectance are more pronounced. Diseased and senescent vegetation show lesser absorption as well as reflection. These spectral properties of vegetation are exploited to detect their type, density and condition through image interpretation. Forests are one of the most conspicuous terrestrial features on the surface of the earth. Hence, identification and mapping of different types of forests in time and space using remote sensing techniques is relatively easy and accurate. Aerospace sensing could be divided into two types i.e. aerial remote sensing and satellite remote sensing depending upon whether sensing is done from aerial or space platforms. Table 1 gives details of various satellites and sensors.

Table 1: **Remote sensing satellites and sensors**

Satellites	Sensors	No. of Bands	Wavelength (µm)	Spatial Resolution (m)	Swath (km)
Landsat 1-5 (USA)	MSS	4	05 – 0.6 0.6 – 0.7 0.7 – 0.8 0.8 – 1.1	80	185
Landsat 4/5	TM	7	0.45 – 0.52 0.52 – 0.60 0.60 – 0.69 0.76 – 0.90 1.55 – 1.75 2.08 – 2.35 10.4 – 12.5	30 120	185
SPOT-1 (France) SPOT-2/3	XS PAN XS PAN	3 1 1 3	0.50 – 0.59 0.61 – 0.68 0.79 – 0.89 0.51 – 0.73 -do- -do-	20 10 20 10	117
IRS-1A/1B (India)	LISS-I & II	4	0.45-0.52 0.52-0.59 0.62-0.68 0.77 – 0.86	72.5 (LISS-I) 36.25 (LISS-II)	148 (LISS-I) 2x74 (LISS-II)
IRS-1C (India)	LISS-III PAN WiFS	4 1 2	0.52-0.59 0.62-0.68 0.77-0.86 1.55-1.70 0.5 – 0.75 0.62-0.68 0.77-0.86	23.5 5.8 188	141 70.5 810
IRS-1D (India)	LISS-III PAN & WiFS	4 1 2	0.62-0.68 0.77-0.86 1.55-1.700.5 – 0.75-0.62-0.68 0.77-0.86	23.5 5.2-5.8 3.6-70.5 169-188	141 133-148 63-70.5 728-812

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IRS P6 (India)	LISS-III	4	0.52-0.59 0.62-0.68 0.77-0.86 1.55-1.70	23.5	141
	LISS-IV	Mono	0.62-0.68	5.8	23
		MX	0.52-0.59 0.62-0.68 0.77-0.86	5.8	23
	AWiFS	4	0.52-0.59 0.62-0.68 0.77-0.86 1.55-1.70	56	56
ERS-1(EU)	SAR Image Mode	1	C (5.3 GHz)	30	100
ERS-2 (EU)		2	C (5.3 GHz)	30	100
JERS-1 (Japan)	SAR	1	L (1.275 GHz)	18	75
RADARSAT-1 (Canada)	SAR	1	C (5.3 GHz)	10x50	100 165 150 45
IKONOS (USA)	PAN MX	1 4	0.45-0.90 0.45-0.52 0.52-0.60 0.63-0.69 0.76-0.90	1 4	11

The GIS has assumed very high significance with the passage of time. It allows one to analyze and integrate a variety of information layers either individually or in different combinations to arrive at suitable and scientific solutions. It helps planners and managers to generate different alternative planning and management scenarios before choosing the one best suited for a particular area. GIS facilitates in cutting across the sectoral style of planning, hitherto practiced by various line departments and government agencies. Many consider remote sensing, GIS, and GPS (Global Positioning System) as three completely different yet amazingly complimentary technologies essential for any development planning. While remote sensing applications in India are nearly fifty years old, the GIS and GPS applications are of relatively recent origin. Last two decades have witnessed the unprecedented development in remote sensing, GIS and GPS technologies globally, including hardware and software.

The GPS is a device, which provides information on the geographic coordinates, altitude, time, direction of movement, and the distance covered. A GPS, also called NAVSTAR (Navigational System with Time and Ranging), consists of a constellation of radio-navigational satellites, a ground control segment, which manages satellite operation and users, who use specialized GPS receivers to receive a wide range of data. The system was established by the Department of Defense (DOD) of United States of America primarily to fulfill the defense needs and, as a by-product, to serve the civilian community. The NAVSTAR satellite constellation consists of 21 satellites and three spare satellites positioned 20,000 km (about three times the earth's radius) above the earth. Each satellite receives and stores information from the control segment, maintains very accurate time through onboard precise atomic clocks, and transmits signals to the earth. Table 2 shows the characteristics of GPS satellites. The satellites are distributed in a manner, which ensures that at least four satellites are visible anywhere in the world at any time. Some other countries like Russia and Japan have their own GPS satellites. India's own GAGAN GPS satellite system is also on anvil.

Table 2: **Characteristics of GPS satellites**

Orbital height	20,200 km
Period	12 hr
Frequency	1575 MHz
Navigational data	1228 MHz
Availability	Continuous
Accuracy	15m in point positioning (P-Code)
Satellite constellation	24
Geometry	Repeating
Satellite clocks	Rubidium, Cesium (Atomic)

GPS has been found very useful in gathering all-weather locale-specific information. A geodetic GPS provides sub-metre positional accuracy while differential GPS provides accuracy of up to 1 m. The use of global positioning system has added much desired precision to surveying and mapping tasks including ground truth collection and mapping. Good quality GPS receivers provide data even under the forest canopy. In forestry, the GPS has been found useful for the determination of geo-coordinates of various forest features and for forest area mapping.

APPLICATIONS AND OPPORTUNITIES

The sustained supply of forest resources for the present day needs and future requirements of mankind have made the forest managers conscious about the compelling need for rational utilization of forest resources. The conservation and utilization of natural resources requires detailed knowledge of the resource with respect to their quality and distribution so as to strike a balance between utilization and regeneration as also to ensure the environmental quality. The conventional methods for assessment and monitoring of the forest resources are not only uneconomical but also time consuming. Many a times they do not match with the forest dynamism and hence, become obsolete by the time the results are available. Moreover, the reliability of the results obtained through such methods is considerably less. The advent of the technology of remote sensing has revolutionized the entire process of forest resource assessment and monitoring. Some of the advantages of remote sensing technology are: large area coverage, high frequency of observations and real time images available on multiple scales. It is perhaps the only technology, which allows retrospective assessment of the forest.

Aerial Remote Sensing of Forests

The aircraft mounted cameras are used to photograph the forests and other land covers/land uses in different wavelengths. Photographs are generally acquired using visible, photographic infrared (also called near infrared) and far-infrared regions of the electromagnetic spectrum. When they are acquired in black and white, using only visible portion of the spectrum, they are generally referred to as panchromatic (or just PAN). They can also be acquired in true colour or infrared false colour depending upon the requirement. Infrared false colour photos are normally preferred over true colour ones as they provide much needed clarity and contrast for interpretation. Besides wavelength and the colour for acquiring air photos, the scale of the photograph is another important consideration, for scale determines whether or not an object or feature such as a small patch of forest could be resolved on the air photo. Aerial photographs provided much needed high spatial resolution until high resolution satellite imagery became available for planning and management in forestry sector. They are amenable to visual monoscopic or stereoscopic interpretation. Latter provides three-dimensional view of the terrain and the forests thus enabling the user to estimate the heights of objects like trees and buildings. Photo elements like tone, texture, shape, size, shadow, pattern, association and location help greatly in identification of the features.

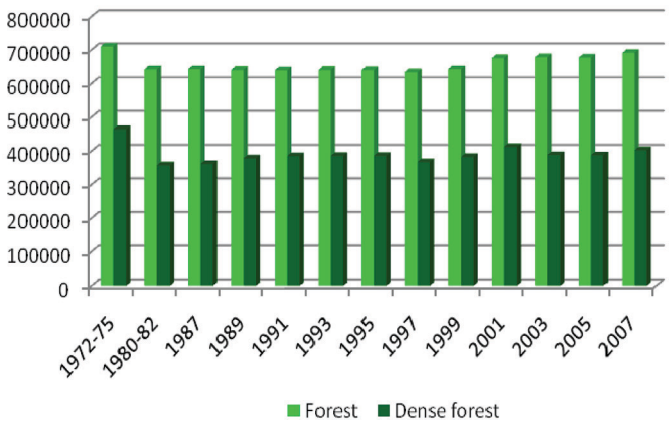
Different parts of the world have different types of the forests. Accordingly, the classification schemes and the interpretation keys vary from region to region. The identification of forest types and tree species depends upon the scale, season of photography and the photo quality. The scale of photograph is chosen depending upon the details of information required, level of analysis, time in hand and the budgetary constraints. On a 1:10,000 scale photograph not only different forest features but also trees and their density could be identified. The finer drainage patterns and even narrow paths could be distinguished. On small-scale photographs, the ease and accuracy of interpretation and quantification of minor features becomes difficult. The small-scale photographs, on the other hand, cover larger area and can give extent of broad forest types and some idea about their density. The colour photographs help in identification of different forests while false colour photographs yield information about different tree species, their physical and physiological conditions and provide good contrast between the vegetation and the soil underneath. Compared to true colour, the false colour photographs are more advantageous in forestry as latter registers invisible (to human eye) differences also within the vegetation cover (Kushwaha and Madhavan Unni, 1987).

The Forest Survey of India and State Forest Departments have used aerial photo-interpretation techniques for forest type mapping and monitoring since long time. NRSC and IIRS have so far carried out a number of studies on forest stratification and mapping. In one such study (Madhavan Unni, 1990) in Andhra Pradesh, it was possible to delineate pure teak, mixed teak, *Hardwickia binata*, mixed *Anogeissus*, degraded forests and forests blanks. The forest types were further divided on basis of crop height and terrain conditions. Tree height, crown diameter, crown cover and number of trees can be estimated using photo-interpretation techniques. The information so obtained is correlated to the actual on ground using two-stage inventory design. A regression model is developed for stratification of timber volume on air photos. Standard aerial volume tables are available for many species for quite some time. For large areas, it is advisable to go far multi-phase sampling techniques. A two-phase sampling design involving photo interpretation and field inventory does not suffice the purpose if the study area is very large. In multi-phase design, visually or digitally classified imagery makes the first stage followed by photo-interpretation and ground measurements. Köhl and Kushwaha (1994) used a multi-phase sampling design for estimation of timber volume of a 12,000 km^2 area of Western Ghats in southern Karnataka. The multi-phase sampling reduced cost by a factor of three and a half and time by a factor of six.

SATELLITE REMOTE SENSING OF FORESTS

The first civilian remote sensing satellite, Landsat-1 was launched by USA in 1972 for natural resources survey and monitoring. The low resolution (57m x 79m) satellite data provided by Landsat 1, 2 and 3 satellites facilitated in identification and mapping of broad forest types and density. Landsat-4 and -5 provided low as well as medium resolution data (30m x 30m), which significantly improved forest resources assessment. The availability of 20 m and 10 m resolution imagery from French satellite SPOT from 1986 onwards significantly advanced remote sensing applications in forestry. NRSC (1983) used Landsat MSS false colour imagery for the periods 1972-75 and 1980-82 on 1:1 m scale and mapped and monitored India's forests for the first time. The forests were classified into closed forests, open/degraded forests, mangroves and non-forest based on the crown density/cover/closure and location. This resulted in the first-ever forest cover map of the entire country for two periods of time. Fig. 1 shows the forest cover situation in the country between 1972-75 and 2007. As evident from the figure, there had been an overall depletion of the forests to the tune of 2.74% between 1972-75 and 1980-82. During this period, dense forest area decreased by 3.24%. Between 1980-83 and 1997-99, forest cover did not increase or decrease to any significant extent. A marginal increase in forest cover, however, has been reported by FSI after 2001.

Fig. 1: **Forest cover in India between 1972-75 and 2007**



The launch of first Indian Remote Sensing Satellite (IRS-1A) in March 1988 dawned a new era in the history of Indian Satellite Remote Sensing Programme. Both IRS-1A and -1B carried 72.5 m and 36.25 m spatial resolution sensors on board and thus provided not only the continuity of satellite data from American programme to indigenous one but also an opportunity to Indian scientific community to test their data for natural resources inventory and monitoring. The IRS-1C, IRS-1D satellites provided further improvised sensor data of 23m and 5.8m resolutions along with a new Wide Field Sensor (WiFS) data having 188 m spatial resolution. The launch of Resourcesat-1 in October 2003 provided further opportunities to increase the mapping/monitoring precision using high resolution LISS-IV data. The 10-bit Advanced Wide Field Sensor (AWiFS) data available from Resourcesat-1 has found global acceptability for natural resources monitoring. The availability of still higher resolution data from IKONOS (1m), QuickBird (61cm), WorldView (50cm) has tremendously helped the remote sensing community to bring in precision in mapping and monitoring.

Table 3: **Appropriate season for aerial/satellite data acquisition in forestry (Roy, 1986).**

No.	Vegetation Type/Forest Region	Proper Season
1.	Humid/moist evergreen and semi-evergreen forests of western and eastern ghats	January-February
2.	Humid and moist evergreen and semi-evergreen forests of north-east India and Andaman and Nicobar Islands	February-March
3.	Tropical moist deciduous forests of northern and central India	December-January
4.	Temperate evergreen forests of western Himalayas	March-May
5.	Temperate, sub-alpine, alpine evergreen, deciduous forests of Jammu and Kashmir	September-October
6.	Arid and semi-arid dry deciduous and scrub forest	October-December
7.	Mangrove forests	February-March, low tide period

It is now well established that the present day satellite data combined with GIS has a lot of potential in the field of preparation and/or revision of forest working plans. Porwal *et al.* (1994) has demonstrated on the use of satellite data for working plan preparation and growing stock assessment. Dutt *et al.* (1996) helped Karnataka Forest Department in working plan preparation for Karwar Forest Division. The terrestrial aboveground biomass in Madhav National Park, Shivpuri was studied using spectral response modeling (Roy and Ravan, 1996). Tiwari (1994) estimated biomass in Rajaji National Park using IRS 1A LISS-II data and digital processing. Kushwaha and Jyotishi (1996) studied browse biomass for Asian elephant using a combination of satellite remote sensing and field sampling in Chilla Sanctuary of Rajaji National Park. The land use/land cover maps generated using remote sensing make good base for landscape analysis.

WILDLIFE HABITAT MODELLING

The application of remote sensing to wildlife habitat analysis is relatively recent. Food, water, cover and terrain are four major parameters of any wildlife habitat, which could be assessed using remote sensing. Remote sensing thus provides nearly complete information needed for wildlife habitat evaluation and monitoring. This information when integrated with other parameters like proximity from potential sources of disturbances, grazing, etc., in a GIS environment can help in scientific evaluation of any habitat in general or for a particular wild animal. Kushwaha *et al.* (2000) used geospatial modelling to evaluate the Kaziranga habitat for great Indian one-horned rhino (*Rhinoceros unicornis*). They found 73% of the national park suitable for rhino. The study also revealed a reduction in the overall Kaziranga landmass by 20.8 km^2 between 1967-68 and 1997. Kushwaha *et al.* (2001) studied habitat suitability for goral (*Nemorhaedus goral*) in Chilla Sanctuary of Rajaji National Park in Uttaranchal using habitat parameters like forest/habitat type, forest density, waterholes, slope, proximity to roads and settlements. Alfred *et al.* (2001) used remote sensing and GIS-based model to find out the habitat suitability for chinkara (*Gazella bennetti*) Rajasthan state. Kushwaha and Roy (2002) have reviewed the contemporary studies on wildlife habitat evaluation. Imam *et al.* (2010) used multiple logistic regression model to work out the potential wildlife habitat for tiger in Chandoli National Park. Singh and Kushwaha (2010) have used a modified form of logistic regression for mountain goral habitat modeling in Binsar Wildlife Sanctuary. A web-enabled wildlife information system (WILIS) is needed for enabling the people interested in similar studies as well as for providing information on wildlife related database. Time is now ripe to initiate action towards realization of such an information-cum-decision support system.

BIODIVERSITY CHARACTERIZATION AT LANDSCAPE LEVEL

Remote sensing can also play a very useful role in assessment of biologically rich areas, which could then be related to actual values of biodiversity, got through ground methods (Roy and Tomar, 2000). This can be done through landscape characterization wherein a digitally or visually classified image is taken as direct input. Many landscape parameters *viz*., porosity, patch size and shape, interspersion and juxtaposition etc. have been said to have a direct relationship with a variety of vegetation feature like biodiversity, physiognomy, composition and other stand parameters (Behera *et al*., 2005). More than 37 work centers spread across the country were involved in the project. A software, SPLAM was developed for this purpose. The work envisaged preparing maps on 1:50,000 scale showing biological (plant) richness in the natural vegetation regions. By now entire country has been covered. The project reports numbering nine have been published. This first of its kind spatial and not-

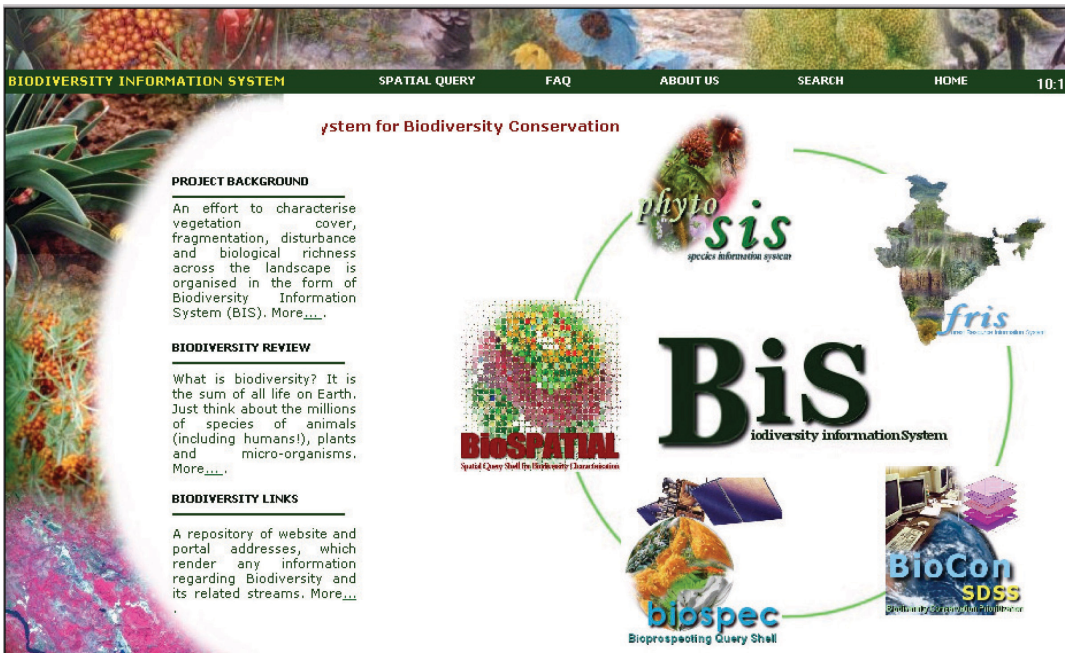


Fig. 2: **The biodiversity information system**

spatial database has been shared with government departments, universities and the research institutions. Data is being put to a variety of uses by users. A biodiversity portal, www.bisindia.org (Fig. 2), has been created for effective data dissemination.

REVISION AND UPDATION OF FOREST WORKING PLANS

The information about the size of forest areas and the growing stock are two most important outputs of inventory and mapping. The inventory, therefore, makes an attempt to describe the quality and quantity of the forests. It is undertaken at national or state level for knowing the location, extent, condition and productive capacity (timber production) of the forests. The outputs are used for formulating the national level policies and monitoring purposes. At Division level, the most important use of inventory and mapping is in the preparation of working plan or management plan, which is a comprehensive document, dealing with all aspects of forest management at the divisional level. Its preparation if based on stock mapping mainly through ground surveys. Needless to mention, it takes lot of time and labour to prepare revised stock maps through ground surveys. It is now well established that the present day satellite data combined with computer technology has a lot of potential in the field of preparation and/or revision of forest working plans. Porwal *et al.* (1994) has demonstrated on the use of satellite data for working plan preparation and growing stock assessment. Dutt *et al.* (1996) used remote sensing and GIS in working plan preparation of Karwar Forest Division, Karnataka.

FOREST BIOMASS AND PRODUCTIVITY ASSESSMENT

SAR (synthetic aperture radar) data are of high interest for forest biomass mapping (Santoro *et al*., 2002a, b; Thiel *et al*., 2006) especially for reasons of their relative weather independence. There are numerous publications on the use of radar data for forest cover and biomass assessment. For forest cover mapping and biomass estimations, three basic approaches based on SAR data are used: backscatter, coherence and phase-based approaches (Koch, 2010). Only SAR interferometric techniques such as repeat–pass or single pass interferometry (InSAR) and polarimetric interferometry (PolInSAR) can provide information on tree or canopy height. A comprehensive overview of the phase-based approaches is provided by Baltzer *et al*. (2007). Most of the recent investigations on phase-based extraction of forest height used experimental airborne data to avoid problems with temporal and atmospheric decorrelation (Balzter, 2001; Askne *et al*., 2003; Askne and Santoro, 2005). In international scientific community, the study of global environmental change has been put on highest research agenda.

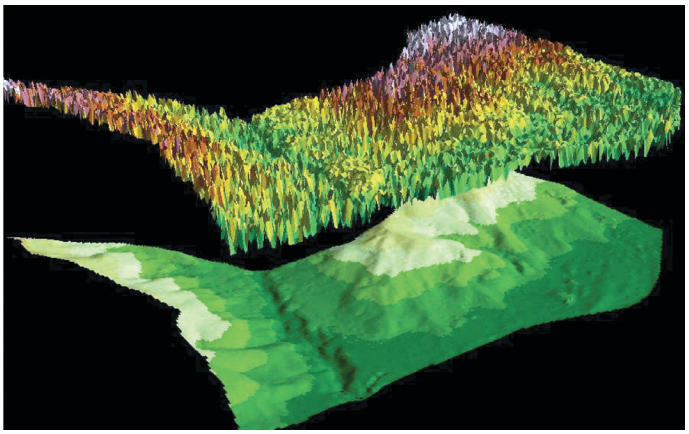


Fig. 3: **DTM and DSM of a forest area obtained from LiDAR**

The main research in forestry in the last decade has focused on the use of small footprint airborne laser scanning systems, polarimetric synthetic radar interferometry and hyperspectral data (Koch, 2010). Fig. 3 illustrates the digital terrain model (DTM) and digital surface model (DSM) obtained from LiDAR data for a black forest area in Germany. The IceSat/GLAS (Ice, Cloud and Land Elevation satellite/Geoscience Laser Altimeter System) system has proven its suitability for forestry applications (Lefsky *et al*., 2005; Rosette *et al*., 2010). The space-borne full wave laser system ICESat/GLAS has also been used to derive biomass parameters and canopy height for large areas (Pang *et al*., 2008; Wu *et al*., 2009). The Ground Penetrating Radar (GPR) has made belowground biomass (root biomass) assessment simpler. An ISRO Geosphere-Biosphere Programme-funded project on soil, vegetation-atmosphere carbon flux monitoring using tall tower and eddy covariance technique was undertaken in 2009. The project aimed to cover all natural biomes with installation of 15 towers in forest and agriculture areas. Five towers have been installed already at Haldwani, Barkot forests in Uttarakhand, Modipuram farm of S.P. University of Agriculture and Technology in U.P., Tropical Dry Teak Forest at Betul in Madhya Pradesh, and Sunderban Mangrove Forest in West Bengal. The eddy covariance technique is being used for this purpose as is the case globally. The eddy covariance method measures ecosystem level carbon exchange between atmosphere and vegetation canopy at 10 GHz i.e. ten observations per second. Remotely sensed data is planned to be used to upscale the results to biome level (IIRS, 2010).

FUTURE SCENARIO

With launch of high-resolution satellites, the scope of the applications of remote sensing and GIS in forestry has increased tremendously. The high-resolution satellite imagery is going to fill up the gap between aerial photographs and satellite imagery. The availability of high-resolution multispectral images is expected to provide the unprecedented opportunities for tree species identification, association level mapping and assessment of forest Type Groups and Sub-Groups as well as density classification. Kushwaha and Roy (2002) have attempted trees and other vegetation identification on IKONOS XS and PAN merged image. They reported significant improvements in terms of vegetation type and density mapping but poor differences among various tree types and hence, difficulty in tree type mapping. They attributed poor tree type differentiation to broad bands used for sensing and suggested on the scope of improvements in spectral resolution of the sensor. The hyperspectral remote sensing, with its capability to look at any ground feature using 240 narrow spectral bands has helped in the species level discrimination and identification. Several studies have reported use of hyperspectral data for lignin, cellulose and other materials sensing (Huber *et al*., 2008). Table 4 gives details of some latest high spatial resolution satellites and sensors.

The use of SAR interferometry and polarimetry in forestry is gaining importance with passage of time. It has been already used for forest stand height, basal cover and growing stock/biomass assessment. LiDAR (**Li**ght **D**etection **A**nd **R**anging) remote sensing is a major breakthrough for forest resources inventory. LiDAR data needs no geo-rectification since sensors have in-built GPS system. The data facilitates high precision assessment of stand/tree height, helpful in growing stock and biomass estimation. LiDAR and to a lesser extent radar facilitate in generation of digital terrain model (DTM) and digital surface model (DSM). For both economic and environmental reasons, it is critical to measure and understand the spatial organization of nature. Remote sensing images allow analysis of various attributes of environment, but are limited in their ability to represent spatial patterns only in two-dimensional space. The advantage of using LiDAR remote sensing is that it provides three-dimensional data through direct and indirect retrievals. If cautiously planned, LiDAR can form one of the most scientific and accurate means of environmental management in the country.

The measurement of forest parameters is either direct or indirect. For direct measurement, a characteristic such as height is estimated by first minus last return of the raw data alone or by applying a linear transformation to the raw data. Indirect estimates are most often based on first estimating a fundamental parameter such as height, which is then fed into a predictive model for biomass and volume estimation. Laser technique may prove most useful to detect changes in the aboveground carbon storage in the tropics, where the most rapid and significant climate and vegetation changes are expected over the next few decades. Such measurements will improve our understanding of the effects of these factors on land degradation and the hydrological and biological systems. The combination of LiDAR data and satellite remote sensing data could also be very useful for describing biodiversity and monitoring changes in biodiversity.

The vertical component (z-axis) measurement capability of LiDAR technology is exploited in a very straightforward way for tree height estimation in comparison to the ground. Tree canopy height is obtained by subtracting the elevations of the first and last returns. Vegetation height is a function of species composition, climate and site quality, and can be used for land cover classification. If coupled with species composition and site quality information, height serves as an estimate of stand age or successional stages. Like simple height estimate, the vertical distribution of laser returns provides basis to classify vegetation, and to estimate other important canopy characters *i.e.,* canopy cover, crown volume (foliage, trunk, twigs, branches *etc*.). LiDAR can accurately estimate the rate of photosynthetically active radiation (PAR) absorption and define the location and depth of the zone, where the maximum rate of PAR absorption occurs. LiDAR data provides input for modeling of biophysical parameters to estimate aboveground biomass with reasonably high accuracy. Vertical foliage diversity can be derived from LiDAR data using extinction co-efficient. But the problem in deriving vertical foliage diversity is light obscuration through dense forest; shaded sparse layers become close to the detection limits of sensors. The lack of a completely quantitative relationship between LiDAR waveforms and foliar profiles does not preclude developing empirical relationships useful in particular regions and stand types, thus offering a challenge for more and more works in varied conditions (Kushwaha and Behera, 2002).

There is a high potential for savings, if laser data and image data could be collected simultaneously and stand delineation and characteristics usable for stratification could be derived from existing auxiliary data and automated methods. It may be

Table 4: **High resolution earth observation satellites**

Organisation	Digital Globe (EarthWatch) USA	ORBIMAGE, USA	Space Imaging, USA	Imagesat International (West Indian Space), Israel	ISRO, India	CNES, France
System	QuickBird 1&2	OrbView 3&4	IKONOS 1&2	EROS-A&B	Cartosat 1&2	SPOT 5A
On-Orbit Date	QB 1-Nov 2000 QB 2-Oct 2001	OV 3-End 1999 OV 4-Sep 2000	I-1-Apr 1999 I-2-Sep 2000	A:#1-Dec 2000-#2-Sep 2000 B:#3-#8 Dec'02-Dec'04	#1-2003-04 #2-2004	May 2002
Spatial Resolution (m)	0.61 PAN 2.50 MS	OV-3&4 1.0 PAN OV-3&4 4.0 MS OV-4 8.0 HS	1.0 PAN 2.0 4.0 MS	#A 1.8 PAN #B 0.82 PAN 3.28 MS	#C1 – 2.50 PAN #C2-<1.0 PAN	2.5 PAN 10 HRG 20 SWIR
Revisit Interval	1-3.5 days	Less than 3 days	1-3 days	3 days	#1-5 days #2-4 days	5 days
Altitude (km)	600	470	680	480 & 600	#2 – 600	824
Spectral Band Width (μ)	0.45 – 0.90 PAN 0.45-0.52 0.52-0.60 0.63- 0.69 0.76- 0.89	0.45- 0.90 0.45- 0.90 200 bands 0.45- 2.50 80 bands 3.0-5.0	0.45- 0.90 0.45-0.52 0.52-0.60 0.63-0.69 0.76-0.89 MS	A & B PAN 0.5-0.9 #B-MS 3 bands or more	PAN 0.5-0.7	PAN 0.51-0.730
Imaging System	Pushbroom	Pushbroom	Pushbroom	Pushbroom	CCD	NA
Swath Width (km)	16.5	8.0 PAN 5.0 HS	11-11.0 12-12.6	EROS A-12.6 EROS B-16.4	#C1-30 #C2-10	60
System Life	5 yrs	5 yrs	7 yrs	#A-4 yrs #B-6 yrs	5 yrs	5 yrs
Stereo	Along & Across : $\pm 38^0$ & $\pm 30^0$	Along & Across : $\pm 45^0$ & $\pm 45^0$	Along & Across : $\pm 45^0$ & $\pm 45^0$	Along : $\pm 45^0$	#1-Along: $+26^0$ & -5^0 #2-along & Across: ± 45	Across : $\pm 26^0$

useful in detecting habitat features associated with particular species, including those of rare and endangered. Different indices of structural complexity could be useful to identify areas of probable high biodiversity, thus providing inputs to GAP analysis programmes. By identifying various classes of forest structures and canopy gaps, associated with varying fire behaviour, it may help in fire prevention. In future, it would boost the study of canopy science and physical attributes of vegetation canopy structure. The presence of specific organisms and the richness of wildlife communities can be highly dependent on the three-dimensional spatial pattern of vegetation, especially in systems where biomass accumulation is significant. Individual animal and bird species are invariably associated with specific three-dimensional features in forests. Other functional aspects such as forest productivity may be related to forest canopy structure, which is measured precisely from LiDAR data.

Multi-frequency GPR will perform sub-surface profiling at different depths to map subsurface features and characterize dielectric property and electrical conductivity of the subsurface materials. Each of different frequency ranges (e.g., 12.5MHz, 25MHz, 50MHz, 100MHz, 250MHz, 500MHz and 1000MHz) are capable of profiling different depth and precision for addressing applications: (1) detection and mapping of underground mining cavities/galleries, subsurface coal fire voids, (2) profiling sub-surface rock formations for current tectonics study, (3) detection and identification of subsurface archaeological objects and evidences, (4) detection and mapping of buried palaeo-channels, (5) snow cover depth, snow water content, (6) estimation of below ground biomass (BGB), and (7) soil moisture.

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Cost Effective Microsatellite Genotyping of *Eucalyptus* Mapping Population

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INTRODUCTION

DNA sequences contain valuable information about the evolutionary history of the particular region of the genome or species studied. The analysis of DNA polymorphism between and among the species is of major importance in genetic studies. Genetic variations occurring among the individuals are best studied using molecular markers. A variety of molecular markers have been used for genetic analysis including restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs). Among these, SSR markers are widely used for a variety of applications in plant genetics and breeding. Comparatively SSRs are preferred over other type of marker systems since they are highly reproducible, multiallelic in nature, codominantly inherited, relatively abundant and have good genome coverage (Varshney et al. 2005).

Simple sequence repeats (SSRs) are a group of repetitive DNA sequences that represent a significant portion of eukaryote genomes. Microsatellites thus find use in detecting polymorphism of the human and mammalian genome and found to play an important role in genetic improvement of plants (Powell et al. 1996). Microsatellites genotyping was performed by polymerase chain reaction (PCR) with specific forward and reverse oligonucleotide primers. Resolving of the amplified fragments were routinely carried out through poly acrylamide gel electrophoresis (PAGE) and stained using silver which gives higher resolution than the high performance agarose gel electrophoresis. This type of analysing the fragments is labour intensive, time consuming and prone to scoring errors. However, capillary electrophoresis based automated genotyping methods are prevalent in plant genome analysis. In the automated high-throughput genotyping system, the microsatellite primer is labelled fluorescently and used for PCR amplification and detection of polymorphism. Such genotyping method is beneficial when few primers are used routinely for genotyping. However, in genetic analysis of tree species the number of SSR loci analysed are high in number particularly during the development of dense genetic linkage maps and all these loci are not targeted always for QTL mapping or any other routine analysis. Hence development of fluorescently labelled SSR primers is not favourable and cost-effective particularly for tree species, where microsatellite genotyping is not a routine tool for genetic analysis. Few alternate genotyping methods to reduce the cost were proposed by Schuelke (2000) and Arruda et al (2010). These fluorescent labelling protocols include a third universal primer to detect the amplified PCR products. To date several reports were published on the use of three primer strategy for cost-effective genotyping of crop plants and tree species (Homolka et al. 2010; Chapmana et al. 2008; Oblessuc et al. 2009). However, in eucalypts the strategy was applied only for initial screening for SSR polymorphism and genotyping was carried out by individual primer labelling (Ottewell et al 2005). In this study a high throughput method for amplification and detection of fluorescent-labelled SSR markers was optimised for genotyping of the mapping population of *E. camaldulensis* x *tereticornis* and *E.tereticornis* X *grandis* to use in generation of genetic linkage map.

MATERIALS AND METHODS

Plant material

Juvenile leaves were collected from the parent trees of *E. camaldulensis*, *E. tereticornis, E. grandis* and from their F1 inter-specific hybrids (*E. camaldulensis* x *tereticornis* and *E.tereticornis* X *grandis)*. Genomic DNA was extracted from 100 mg of the plant sample using Qiagen DNeasy Plant Mini Kit. The extracted DNA quantity was assessed using agarose gel electrophoresis with ethidium bromide staining and the quantity of DNA was confirmed using Picodrop spectrophotometer (Picodrop microlitre spectrophotometer version 3.01,UK). DNA samples were diluted to get the final concentration of 10ng/μl using sterile water.

SSR primer synthesis, amplification and genotyping

The locus-specific forward, reverse primer and a 5' FAM-labelled M13universal primer (5' TGT AAA ACG ACG GCC AGT-3') were synthesized from Integrated DNA Technologies, USA. The forward primer of the SSR loci was modified to accommodate the M13 universal sequence (5'-TGT AAA ACG ACG GCC AGT-3') in its 5' end. In this study the SSRs were cross amplified from other species of *Eucalyptus* like *E. camaldulensis*, *E. grandis*, *E. nitens*, *E. tereticornis* and from *Corymbia*. Hence, to identify the polymorphic SSRs between the parents, PCR was carried out

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without the addition of third universal primer. One hundred and forty five SSR loci were analysed for the parents. The PCR reagents and conditions are given below. The PCR amplification was carried out in 10 μl volume with 10X buffer containing 100 mM Tris–HCl pH 8.3, 500 mM KCl and 15mM MgCl2, 0.4 pmol of each primer, 1 unit of Taq DNA polymerase, and 100 μM of each dNTPs and 20 ng of template DNA. The PCR amplification was carried out for 5 min at 94°C, 30 cycles of 1 min at 94°C, 60 or 30 sec at the primer specific annealing temperature, 2 min at 72°C, and 7 min at 72°C for final extension. Annealing temperatures varied from 48°C to 60°C, to amplify specific microsatellite marker. PCR products were size-separated using an 5% denaturing polyacrylamide (PAGE) gels of size 21 cm X 50 cm (Sequi-Gen GT System, BIO-RAD, USA) containing 7 M urea and 1 X TBE buffer, and visualized by silver staining.

Once the SSR loci showing polymorphism were identified using PAGE, the mapping population was genotyped in Genetic Analyzer (ABI 3500, USA) using the following PCR reagents and conditions. PCR amplification was carried out in 10 μl volume with 10X buffer containing 100 mM Tris–HCl pH 8.3, 500 mM KCl and 15mM MgCl2, 125μM dNTP mix, 0.1pmol of forward primer with M13 tail at 5' end, 0.4pmol of reverse primer and 0.2 pmol of fluorescently labelled universal M13 primer, 10ng of template and 1 U of Taq DNA polymerase. The PCR for fluorescence based genotyping was carried out in two sequences. The first sequence involved initial denaturation at 94°C for 5 min, then 30 cycles of 94°C for 45sec, annealing temperature varying from 48°C to 60°C for 30 sec and 72°C for 1 min and an elongation step at 72°C for 15 min. The second sequence consisted of 20 cycles with 94°C for 30sec, annealing of labelled M13 at 50°C for 45sec and 72°C for 45sec, and final extension of 72°C for 30 min. PCR enhances such as Dimethyl Sulphoxide (DMSO) and betaine in concentration of 5% and 2M respectively were used in PCR reactions to address the problems of no amplification or stutter peaks.

An aliquot of 1 μl of fluorescently labelled PCR product was mixed with 0.2μl of GeneScan 600 Liz size standard and 8.8 μl of Hi- Di formamide (ABI). The mixture was electroinjected in a 8 capillary ABI 3500 genetic analyzer having POP7 polymer . The data was collected under dye set Ds33 spectral calibration using Genemapper software. Data obtained after 45 minutes of injection was analyzed with Genemapper software (Version 4.1) and used for further analysis. Multiloading of PCR products by combining upto three loci in one injection was performed. While loading more than one product the concentration of the each product was standardised (0.5 or 1.0 μl) because over loading of a products may result in off-scale peaks.

RESULTS AND DISCUSSION

The results obtained from genotyping of the parents through silver staining showed 126 SSR loci polymorphic between the parents. Among these 126 loci, 38 loci showing perfect polymorphic alleles were selected initially for genotyping of F1 hybrids and their parents using the Genetic Analyser. Results showed that 3 SSR loci produced monomorphic alleles when genotyped with fluorescence detection. This observation could be because of the poor resolution obtained in PAGE. Optimization of exact product size with no non-specific amplification in PAGE was one of the practical approaches to obtain better results in Genetic analyser. The alleles with the 1 base pair difference were well detected and clear peaks were observed. Problems like stutter peaks, split peaks and no amplification were noticed. Stutter peaks were reduced by increasing the annealing temperature of the locus specific primers. However, the addition of PCR enhancers like DMSO and betaine, which reduce the secondary structure formation in GC rich primers, did not produce positive results. Few of the SSR loci could amplify when the annealing temperature of labelled M13 primer was reduced to 50°C.

High throughput analysis was performed by multiloading the SSR loci in single injection. SSR loci having amplification range difference of 25 bp were used for multiloading and upto three SSR loci were combined. Two loci multiloading was found to be optimal as three loci produced off-scale readings. The Genemapper software could resolve the peaks according to the allele call for each of the loci. Further inclusion of additional labelling dyes such as VIC (green), PET (RED) and NED (Yellow) allow detection of at least 8 loci in a single injection thus reducing the cost effectively.

Labelling cost of individual primer is very expensive and also labelling more than hundred primers for genetic mapping studies of single species is still more expensive. Further, locus specific primers can be used only for the particular species. In this case three primer strategy with multiloading will be cost effective since only the M13 universal primer alone need to be labelled and can be used along with the other species specific primers for genetic studies..

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National Bureau of Forest Genetic Resources for Economic and Ecological Security

N. Krishna Kumar * and R. Anandalakshmi **

INTRODUCTION

Wide variability within species marks the richness and multi-faceted potential of the forest resources. Forest Genetic Resources (FGR) represents the genetic variation in trees of potential or present benefit to humans. Also, the term FGR is used variedly and encloses a range of components from intraspecific diversity to inter-specific genetic diversity among a set of taxonomically and/or ecologically related species, to the entire range of forest species that are economically important and/or potentially useful (Young *et al.* 1999). Many reports indicate that FGR is in imminent danger due to adverse abiotic and biotic stresses resulting from urban expansion, infrastructural development, agriculture and global warming (Bawa and Dayanandan 1998; Brown and Pearce 1994; Stedman-Edwards 1998). It has been reported that in India there are about 6270 economically important species (ICE, 1994). According to Ahmed (1997), the total annual value of India's forest products is estimated to be Rs 300 000 million (about US$ 6662 million) compared to the meagre investments of Rs 8000 million (US$ 176 million) in this sector. Therefore, in today's era of climate change, FGR is one of the most important components to be conserved and managed through networking and involvement of stakeholders. In other words, forest gene conservation through a network approach, the Forest Genetic Resources Management Network (FGRMN) is crucial in ensuring that the rural population and the national economy benefit from the sustainable use of tree species, whether in meeting commercial or livelihood objectives. The use of quality germplasm is fundamental for successful tree breeding and planting and is directly linked to the production of high quality trees and tree products, which attract higher market prices. It also ensures that seed and planting materials of different populations of desired tree species will be available in addition to long term conservation of these valuable resources in the form of field and seed gene banks. As a supplement, non income generating values of forest gene conservation which are also equally important in meeting the long term interests of the country and its future generations such as environmental protection is also taken care.

The Ministry of Environment and Forests, Govt. of India, considering the importance of forest genetic resources has initiated various programmes for FGR conservation. One such recent programme is the establishment of the Forest Genetic Resources Management Network (FGRMN) under the umbrella of Indian Council of Forestry Research and Education (ICFRE) with the FGRMN Chair at the Institute of Forest Genetics and Tree Breeding (IFGTB). The first meeting of FGRMN, 'The Consultative Workshop on Strategies for Formulation of Forest Genetic Resources Management Network' was held at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore during 9th and 10th March 2011, with the participation of all the stakeholders concerned with FGR. This Network discussed mainly on economically important tree species that have significant present and potential use for people. The two day workshop through extensive deliberations identified about 30 important tree species that need immediate attention under FGR research and conservation. The roles, responsibilities of various stakeholders, the research and extension needs and policy initiatives required for effective management through networking were also discussed. Today the onus of initiating the FGRMN activities lies with IFGTB, the State Forest Departments and other stakeholders. The present paper details about FGRMN, its future plan and responsibilities of the stakeholders.

FGRMN AND ITS PROGRAMMES

The FGRMN has the mandate to act as nodal agency at national level for acquisition and management of indigenous and exotic forest genetic resources for their exploration, documentation, conservation and their sustainable utilization. The network shall function with the following objectives (Krishna Kumar and Anandalakshmi, 2011),

- To plan, prioritize, organize, conduct and coordinate exploration, collection and documentation of indigenous and exotic forest genetic resources to strengthen *in situ* and *ex situ* conservation.
- To undertake introduction, exchange and quarantine of genetic resources of forest origin.
- To characterize, evaluate and conserve forest genetic resources and their sustainable management in collaboration with state forest departments, ICFRE institutes, other national organizations, research institutes, universities, industries and NGOs.
- To develop and maintain a national information network on

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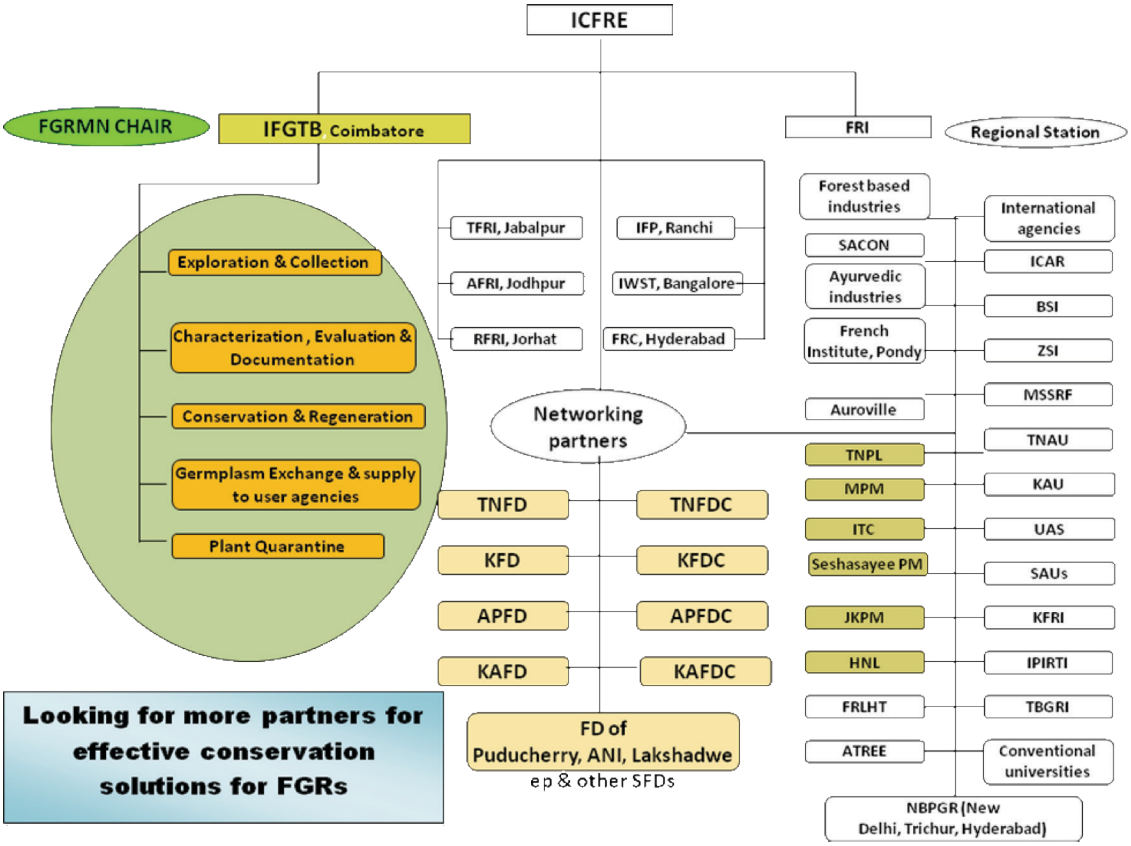
The Forest Genetic Resources Management Network



FGRs

- To develop genomic tools, techniques and approaches to characterize and validate the germplasm
- To conduct research, undertake teaching and generate public awareness on FGRs through trainings, teaching, seminars etc.

Its following components/programmes would constitute its activities,

- Exploration and Collection
- Characterization, Evaluation and Documentation
- Conservation and Regeneration
- Germplasm Exchange and Supply to user agencies
- Plant Quarantine

The above programmes will be backed up by capacity building and public awareness related to FGR management and conservation in the long run.

Species prioritized for FGRMN with identified partners

No.	Prioritized Species	Networking partner for species
Phase I		
1	*Tectona grandis*	IFGTB, IWST, TFRI, AFRI, TNFD, KFD, APFD, KAFD, MFD, KFRI, KAU, FCRI, ASPEE, CTCRI, CARI, DBSKKV
2	*Gmelina arborea*	IFGTB, IWST, TFRI, RFRI, TNFD, KFD, APFD, KAFD, MFD, DBSKKV, ASPEE, TNPL, TBGRI, KFRI
3	*Melia dubia*	IFGTB, TNFD, KFD, APFD, KAFD, MFD TNPL, FCRI
4	*Casuarina equisetifolia*	IFGTB, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, DBSKKV, ASPEE, TNPL, CTCRI, TAFCORN
5	*Eucalyptus camaldulensis*	IFGTB, AFRI, IWST, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, ANGRAU, TNPL, TAFCORN, MPM, WCPM
6	*Ailanthus excelsa*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, ASPEE, FCRI, TBGRI
7	*Eucalyptus tereticornis*	IFGTB, AFRI, IWST, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, TNPL, TAFCORN, MPM, WCPM
8	*Anthocephalus cadamba*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, FCRI, TBGRI, KFRI
9	*Pterocarpus santalinus*	IFGTB, IWST, TNFD, KFD, APFD, APFDC, KAFD, CTCRI, NBPGR (Thrissur), FCRI
10	*Acacia mangium*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KAU, KFRI, MPM
11	*Acacia auriculiformis*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KAU, KFRI, MPM
12	*Casuarina junghuhniana*	IFGTB, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, ASPEE, TNPL, TAFCORN

13	*Calophyllum inophyllum*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, DBSKKV, NBPGR (Thrissur), TBGRI
14	*Sapindus emarginatus*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI
15	*Azadirachta indica*	IFGTB, IWST, AFRI, TFRI, TNFD, KFD, APFD, KAFD, MFD, CTCRI, ANGRAU, FCRI, MFD
Phase II		
16	*Tamarindus indica*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, CARI, FCRI
17	*Dalbergia latifolia*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, KFRI
18	*Dalbergia sissoo*	IFGTB, AFRI, TNFD, KFD, APFD, KAFD, MFD, CTCRI, FCRI, TNPL
19	*Artocarpus heterophyllus*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, ASPEE, CTCRI, NBPGR (Thrissur), TBGRI
20	*Santalum album*	IFGTB, IWST, TNFD, KFD, APFD, KAFD, KAFDC, MFD, ASPEE, CTCRI, FCRI
21	*Pongamia pinnata*	IFGTB, TFRI, TNFD, KFD, APFD, KAFD, MFD, FCRI, KFRI, DBSKKV, CARI
22	*Aegle marmelos*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, TBGRI, KFRI
23	*Pterocarpus marsupium*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI
24	*Ailanthus triphysa*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI, FCRI, CTCRI
25	*Terminalia chebula*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CSGRC, ASPEE, CTCRI, KFRI
26	*Albizia lebbeck*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI, FCRI
27	*Leucaena leucocephala*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, FCRI, WCPM, CARI
28	*Thespesia populnea*	IFGTB, TNFD, KFD, APFD, KAFD, MFD
29	*Bombax ceiba*	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CARI
30	*Bamboos* (13 economically important bamboo species identified by NMBA)	IFGTB, IWST, RFRI, TNFD, KFD, APFD, KAFD, MFD, TNPL, KFRI, CARI, FCRI, TBGRI

** IFGTB- Institute of Forest Genetics and Tree Breeding (Coimbatore), IWST- Institute of Wood Science and Technology (Bangalore), AFRI- Arid Forest Research Institute (Jodhpur), TFRI- Tropical Forest Research Institute (Jabalpur), RFRI- Rain Forest Research Institute (Jorhat), TNFD- Tamil Nadu Forest Dept., KFD- Kerala Forest Dept., APFD- Andhra Pradesh Forest Dept., KAFD- Karnataka Forest Dept., MFD- Maharashtra Forest Dept., APFDC- Andhra Pradesh Forest Development Corporation, DBSKKV- Dr. Balasaheb Sawant Konkan Krishi Vidypeeth (Dapoli, Maharashtra), ASPEE- Aspee College of Horticulture & Forestry (Navsari, Gujarat), TNPL-Tamil Nadu Newsprint and Papers Ltd.(Karur, Tamil Nadu), TBGRI- Tropical Botanic Garden and Research Institute (Palode, Kerala), KFRI- Kerala Forest Research Institute (Peechi, Kerala), NBPGR- National Bureau of Plant Genetic Resources (Thrissur, Kerala), FCRI- Forest College and Research Institute (Mettupalayam, Tamil Nadu), KAU- Kerala Agricultural University (Thrissur), CARI- Central Agricultural Research Institute (Port Blair, Andamans), CTCRI- Central Tuber Crops Research Institute (Trivandrum, Kerala), WCPM- West Coast Paper Mills Ltd. (Dandeli, Karnataka), MPM- Mysore Paper Mills Ltd. (Shimoga, Karnataka), CSGRC - Central Sericultural Germplasm Resources Centre (Hosur, Tamil Nadu).*

RECOMMENDATIONS OF THE CONSULTATIVE WORKSHOP

With the guidance of the Former Chairman – Protection of Plant Varieties and Farmer's Rights Authority (PPV & FRA), Dr. S. Nagarjan, who chaired the workshop, and with the thoughts and experiences of all the participants of the workshop, the following recommendations (Krishna Kumar *et al.*, 2011) emerged.

1. Co-operation and support of State Forest Departments should be ensured for effective functioning of the Network.
2. FGR of both indigenous and exotic species, including their landraces are recommended for conservation and preservation.
3. The network would be handling many species of forest origin, which must be allocated to respective network institutions for comprehensive database development, genetic improvement and sustainable utilization.
4. FGRMN starts with a focused list of species which have present and future importance. Species of indigenous and exotic tree species of economic importance need prioritization to begin with Forest Genetic Resources Management. Species prioritization needs to be done both at country and regional level. Tree species for wood should be taken up as a priority in FGRMN and other species like lac could be taken up in the second phase. There is a need to spell out the forest genetic resources that are to be covered in the first phase of the Network.
5. Species on which genetics and improvement work has done should not be missed out.
6. Species other than given in the indicative list need to be explored at regional level for next stage of conservation. Species having conservation value can be added to the prioritized indicative list.
7. The potential of each partner in the species prioritized have to be enlisted to strengthen FGRMN.
8. Nodal centres are to be identified for various species for development and maintenance of database containing all relevant information, including GPS coordinates, soil parameters and variability range of economic traits for stakeholders.
9. Taxonomical research has to be promoted in nodal centres and network institutions / organizations to deal with inventorisation, characterization and documentation of FGR.
10. Capacity building for frontline officials, researchers, forest field staff, community and students is very essential for effectively managing FGR.
11. Forest dwelling communities / adjoining rural communities and self help groups need to be involved as stakeholders and their support and co-operation in this venture to be ensured.

The knowledge of farmers and tribals to be documented.

12. Division for Forest Genetic Resource may have to be created in the Forest Departments and other partners for smooth functioning of FGRMN.
13. Stakeholders should submit a note containing the following information,
    - Name of the Institution / Organisation
    - Name of the designated Nodal Officer
    - Name and short details of the species with which they are working
    - Level of variability existing within the species with the institution
    - Scientific and other strengths of the Institution to work with the species
    - The requirements of the Institutions for developing the database and linkage with Nodal Centre.
    - Financial requirements from the Network
14. A task force may be constituted to finalise the Network on the basis of notes submitted by the stakeholders on the guidelines provided. The Task force shall decide upon the priority species fund allocation for each species and each stakeholder in the Network.
15. A subsidiary Network of all botanical gardens, bambusetums, arboreta, palmetums etc. can be made as a part of the main Network to enlist the database of all species and variability conserved in them.
16. A nodal and single window system for allotting “Identification Numbers” to the accessions in the Network need to be developed. These numbers should be unique and only these should be used by the members of the Network.
17. A digital and GPS record of the mother plants needs to be collected during explorations along with minimum passport data and indigenous knowledge if any, associated with it.
18. Due recognition should be given to the stakeholders who are providing germplasm to the Network. A database may be created for the exploration (with GPS records) being taken up by the stakeholders to ascertain the availability of the species in the natural conditions.
19. The database should be comprehensive with access being provided to them in the form of digital library, publications etc.
20. Economic value and utility aspects of genetic resources from Tropical Dry Evergreen forests need to be comprehended.
21. Documentation on globally threatened mangrove taxa in different parts of the country to be attended.
22. FGR selections in industrial forestry R&Ds need to be included in the Network.
23. Bureau of Forest Genetic Resources should be a central institute. Regional institutes, State Agricultural Universities and private institutions may have access to germplasm and their usages.
24. Exploration, Collection, Conservation, Evaluation, Characterization, Documentation and Exchange of FGR are the functions of the Bureau.
25. “Bureau of Forest Genetic Resources " should be a committed Institute, with committed people and it should have a committed funding and regional centers.
26. Initially, to begin with it can be started as a Division for forest Genetic Resources at the Institute of Forest Genetics and Tree Breeding. Later, in subsequent years, it can be developed into a full fledged independent Institute, and tree breeding should be separated from FGR conservation.
27. The forest genetic resources include very large number of trees, shrubs, lianas, algae, Fungi, pteridophytes, lichens, microbes and medicinal plants. Initially it may not be possible to cover all the species. To begin with, few tree species of importance may be taken and later other species including medicinal plants can also be covered. It should be called "Bureau of Forest Tree Genetic Resources ". Later, as Institute grows separate Divisions/ Institutes –like "Forest Medicinal Plant Resources" or "Forest Animal Genetic Resources" may be bifurcated.
28. The varieties for forest trees should also be registered within one year of release as in case of Agricultural crop varieties.
29. Already existing varieties, provenances, clones etc., of Forestry Species should be brought to "Common Knowledge" through publications to avoid bio- piracy. A National Gene Bank may be established at IFGTB.
30. Under equitable sharing of benefits, benefits sharing from final product –out of Biological material taken by access should flow back to the area from where the biological material was drawn for conservation of species and development of the area.
31. Identify people who conserve land races and recognize them to know more about land races available with farmers.
32. CAMP workshops have to be organized for different species or a group of related species to understand the level of diversity, utilization and conservation needs for prioritizing species.
33. Different units of NBPGR have already either prioritized or started accumulating accessions of tree species in their respective regions. Such initiatives have to be compiled by consulting these NBPGR stations for allocation of species to different partners.
34. Sustained interaction between organizations concerned with genetic resources of horticulture trees and forestry institutions to learn from the strategies adopted for tree crops and modify them for forestry tree species. In particular the long-term pollen storage techniques available for horticulture species may be useful for forestry species.
35. The extent of variation required to be maintained for exotic germplasm may be determined after characterizing the germplasm currently available.
36. IFGTB/FGRMN and State Forest Departments should be equal partners in FGR related activities. A National Coordinator in the FGRMN Secretariat and State Coordinators for participating States will be necessary for effective networking.
37. Molecular characterization of assemblages required for conservation and management of FGR. Advanced molecular tools like SNP genotyping may be considered during characterization.
38. The Bureau of Forest Genetic Resources should have a plant protection and quarantine system. Molecular characterization could be taken up in phase two while documentation and publication be taken up immediately.
39. Different agro climatic zones may have zonal offices, and zonal level co-ordination is important. Major FGR are available with Forest Departments and they have an important role in

the FGRM network.

40. An exclusive website for FGRMN should be created for effective information exchange.

ROLE AND CHALLENGES OF FOREST DEPARTMENTS

The departments handling the fragile forest resources have to play a significant role in conservation of the forest genetic resources. When we witness rapid conversion of forests into agricultural lands, over exploitation, pollution and phenomenon of climate change coupled with invasive species causing great pressure on forest ecosystem, the following points need to be taken care.

- It is increasingly essential to increase the area of protected and well managed forests.
- Incorporate management of trees and forest patches into agricultural landscapes and promote agroforestry systems
- Create an environment for increased communication among stakeholders and take lead in inter-sectoral co-ordination
- Plan for increased research with institutes, universities to better understand the interactions within and among the ecosystems
- Strengthening local community institutions for FGR conservation through participatory approaches
- Improving investment for tree planting and forest conservation
- Improved monitoring of FGR at planning, input, outcome, and impact level
- Commissioning Research in support of the Mission aim
- Making the Mission a people's programme
- Forest species needs priority setting for conservation based on economic and ecological significance.
- Programmes to support strong mechanism for science and technology findings in FGR conservation and exchanges at all levels on annual basis
- Setting genetic diversity indicators for conservation gains.
- Tree breeding programmes covering many species for abundant yield of genetically improved seeds for production of quality planting stock need to be implemented.
- Increase in area under orchards for various species as seed orchards to assure supply of quality seeds from authentic sources is essential
- Provenance and progeny test need to be conducted for major timber species and should be maintained as *ex-situ* conservation stand.
- Better understanding of FGR for decision support systems (DSS) is essential for forest management applications and reintroduction of species in areas where population have depleted or diversity has diminished.
- The management plans of the divisions should necessarily provide details on FGR resources while also underlining conservation measures.
- Establishment of conservation banks, e.g., Medicinal Plants Conservation Area (MPCA), Permanent Preservation Plots (PPP) to protect genetic diversity, in order to employ these resources as breeding parental sources in restoration strategies and sustainable utilization.
- Conserve the available fragmented forests and take efforts to establish corridors to enable gene flow.
- Renewed thrust on control of genetic erosion by regulating the drivers of change namely over exploitation, invasive species and thereby provide scope for ecosystem recovery in human induced ecosystems.
- Responsible forest management also includes looking beyond forest landscapes like agricultural landscapes where agro-biodiversity has to be protected along forest edges.
- Mapping of distribution of priority species through GIS and regular updation
- Designate a nodal officer at the department headquarters to co-ordinate the network activities with IFGTB
- Active participation of the officer and his team (working groups) in the errand of exploration, collection, multiplication, characterization, evaluation and documentation of germplasm.

EXPECTED OUTCOME OF FGRMN

- Conservation of Forest Genetic Resources
- Establishment of National germplasm bank in the form of field and seed gene banks of economically important tree species for their sustainable utilization
- Validated and characterized forest genetic resources in the form of genetic stocks, provenances, seed source, land races, improved planting materials, clones and hybrids will be available for productivity enhancement and forestry research.
- Database on Forest genetic resources in India
- Exchange of germplasm within and outside the country
- Establishing National Bureau of Forest (Tree) Genetic Resources.

CONCLUSION

In the process for establishing a National Bureau for Forest (Tree) Genetic Resources (NBFGR) it is increasingly important to realize and recognize the role and responsibilities of each stakeholder handling forest genetic resources and also draw examples from the experiences of NBPGR in India and other international PGR authorities. It is noteworthy to emphasize that FGR management is a challenging task that demands the fullest involvement and support from all stakeholders, especially the forest departments who are the core guardians of this valuable resource.

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Present Status of Indian Species of *Trichogramma* and their Application in Biological Control of Forest Insect Pests

Mohd. Yousuf *

INTRODUCTION

Genus *Trichogramma* belongs to order Hymenoptera and these wasps are close to Honey bees in appearance with small size ranging from 0.4-0.6 mm. The genus was erected by Westwood in 1833, with the type species *Trichogramma evanescens*. *Trichogramma* spp. are utilized in biological control of insect pests all over the world, and are familiar not only among entomologists, but also field workers are well known. *Trichogramma* spp. control the population of harmful insects at egg stage and the releases of *Trichogramma* spp., have been made extensively all over the world for biological control of lepidopterous pests (Debach and Rosen, 1991). These parasitoids have been utilized against the pests of agricultural crops, commercial cash crops, orchards and forest insect pests as well. In India, record on release of *Trichogramma chilonis* in forestry goes as early as 1937 (Beeson, 1941; Nair *et al*., 1995). Several new species of *Trichogramma* have been described from India. These include *T. flandersi*, *T. chilotraeae*, *T. achaeae* (Nagaraja and Nagarkatti, 1969); *T. agriae, T. hesperidis, T. pallidiventris, T. plasseyensis, T. poliae, T. raoi* (Nagaraja, 1973); *T. brevifringiata* (Yousuf and Shafee, 1987), *T. convolvuli, T. cuttackensis, T. danausicida, T. hebbalensis, T. sankarani* (Nagaraja, 1996); *T. manii* (Nagaraja and Gupta, 2007), *T. kashmirica* (Nagaraja *et al*., 2007), *T. breviciliata* (Yousuf and Hassan, 2007), *T. latipennis, T. kankerensis* (Yousuf and Hassan, 2008a, b); *T. danaidiphaga, T. giriensis, T. pieridis* and *T. rabindrai* (Nagaraja and Mohanraj 2010a, b).

INDIAN SPECIES OF *TRICHOGRAMMA*

Doutt and Viggiani (1968) recognized 64 genera and 344 species under Trichogrammatidae. Yousuf and Shafee (1986a, b) have catalogued 71 genera and 549 species under Trichogrammatidae. Further Yousuf and Shafee (1987) have published an account of Indian Trichogrammatidae with 15 species of *Trichogramma*. Till date twenty eight species of *Trichogramma* have been recorded from India, of which twenty four species have been originally described from India which are: *Trichogramma achaeae*, *T. agriae*, *T. breviciliata*, *T. brevifringiata*, *T. chilotraeae*, *T. convolvuli*, *T. cuttackensis*, *T. danausicida*, *T. danaidiphaga, T. flandersi, T. giriensis, T. hebbalensis, T. hesperidis, T. kankerensis*, *T. kashmirica*, *T. latipennis*, *T. manii*, *T. pallidiventris, T. pieridis, T. plasseyensis, T. poliae*, *T. rabindrai, T. raoi* and*, T. sankarani*. Other four species *T. chilonis* Ishii, *T. japonicum* Ashmead, *T. semblidis* (Aurivillius) and *T. thalense* Pinto and Oatman have also been recorded from India with their natural distribution.

Though some exotic species including *Trichogramma brasiliensis, T. chilonis (=T. confusum)*, *T. evanescens, T. exiguum, T. perkinsi, T. pkcal* (hybrid) and *T. pretiosum* were reared in India in different laboratories and work has also been carried out on their laboratory and field efficacy against agricultural pests and also by few workers against forestry insect pests (Patil and Thontadarya 1983 & 1984; Ahmad 1990 & 1992; Yousuf 2005 and Joshi *et al*. 2007). These exotic species of *Trichogramma* were released against various insect pests, which were established temporarily, and no evidence is available on their permanent establishment.

HOST-RANGE OF INDIAN SPECIES OF *TRICHOGRAMMA*

Complete host-range has been up-dated after consulting the World literature on the subject, for Indian species of *Trichogramma* (Table 1):

Table 1: **Host -range of Indian species of *Trichogramma*:**

S. N.	*Trichogramma* species	Host-range (Host-insects)
1.	*Trichogramma achaeae* Nagaraja and Nagarkatti	*Achaea janata, Agrius convolvuli, Catopsilia pyranthe, Clostera cupreata, Corcyra cephalonica, Earias insulana, Earias vitella, Ergolis merione, Helicoverpa armigera, Pectinophora gossypiella, Spodoptera litura* and *Tiracola plagiata.*
2.	*Trichogramma agriae* Nagaraja	*Agrius convolvuli* and *Corcyra cephalonica.*

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3.	*Trichogramma breviciliata* Yousuf and Hassan	*Corcyra cephalonica, Eutectona machaeralis, Hyblaea puera* and *Hasora alexis*
4.	*Trichogramma brevifringiata* Yousuf and Shafee	*Chilo infuscatellus*
5.	Trichogramma chilonis *Ishii*	*Achaea janata, Acherontia styx, Acigona steniellus, Acrobasis caryae, Aglossa dimidiata, Agraulis vanillae, Agrius cingulata, Agrius convolvuli, A. juglandis, Ampillia dioscoridea, Anomis flava, Arctia coerulea, Argyroploce schistaceana, Ascotis selenaria dianerla, Atherigona soccata, Bactra sp., Barathra brassicae, Cerura vinula, Chilo indicus, Chilo infuscatellus, Chilo partellus, Chilo sacchariphagus, Chilo suppressalis, Chilo venosatus, Clanis bilineata, Clostera anachoreta, Cnephalocrocis medinalis, Cocytodes coerulea, Corcyra cephalonica, Creatonotus transiens, Crocidolomia binotatus, Danaus plexippus, Deilephila nerii, Diatraea saccharalis, Earias insulana, Earias vitella, Emmalocera depressella, Ephestia cautella, Ergolis merione, Etiella zinekenella, Eucosma schistaceana, Euproctis flavinata, Eutectona machaeralis, Gastropacha populifolia, Grapholitha glycinivorella, Helicoverpa armigera, Heliothis assulta, Heliothis zea, Hemerophila atrilieneata, Herse convolvuli, Homona coffearia, Hyblaea puera, Hymenia recurvalis, Jaspida distinguenda, Laspeyresia caryana, Macroglossum pyrrhosticum, Mycalesis gotama, Naranga aenescens, Oebia undalis, Olethreutes schistaceana, Ostrinia furnalis, Ostrinia nubilalis, Papilio xuthus, Parasa consocia, Parnara guttata, Pelopidas mathias, Philosamia cynthia ricini, Pieris rapae, Plutella xylostella, Procera sacchariphagus, Procera venosatus, Prodenia litura, Prodesaia kurosawai, Psara* spp.*, Samia cynthia, Scirpophaga exceptalis, Scirpophaga incertulas, Scirpophaga innotata, Scirpophaga nivella, Scirpophaga* sp.*, Sesamia inferens, Sitotroga cerealella, Spilarctis obliqua, Spodoptera litura, Spodoptera mauritiana, Tiracola plagiata, Trichoplusia ni, Tryporyza inceretulas,* and Unidentified Lycaenid, Noctuid, Pyralid and Sphingid eggs.
6.	*Trichogramma chilotraeae* Nagaraja and Nagarkatti	*Agrius convolvuli, Bactra sp., Chilo infuscatellus, Chilo partellus, Chilo suppressalis, Corcyra cephalonica, Helicoverpa armigera, Pelopidas mathias, Ostrinia furnacalis* and *Trichoplusia ni*
7.	*Trichogramma convolvuli* Nagaraja	*Agrius convolvuli* and *Corcyra cephalonica*
8.	*Trichogramma cuttackensis* Nagaraja	*Psalis* sp.
9.	*Trichogramma danausicida* Nagaraja	*Corcyra cephalonica* and *Danaus chrysippus*
10.	*Trichogramma danaidiphaga* Nagaraja and Mohanraj	*Danaus chrysippus*
11.	*Trichogramma flandersi* Nagaraja and Nagarkatti	*Agrius convolvuli, Chilo infuscatellus* and *Corcyra cephalonica*
12.	*Trichogramma giriensis* Nagaraja and Mohanraj	Undetermined lepidopterous eggs
13.	*Trichogramma hebbalensis* Nagaraja	*Corcyra cephalonica*
14.	*Trichogramma hesperidis* Nagaraja	*Corcyra cephalonica, Pelopidas mathias* and Hesperiid eggs
15.	Trichogramma japonicum Ashmead	*Aglossa dimidiata, Agrotis ypsilon, Anchonoma xeraula, Anomis flava, Aphomia gullaris, Ascotis dianeria, Ascotis selenaria, Biston margina, Cataoela adjurella, Chilo suppressalis, Chilo* spp.*, Chilortaea auricilia, Chilotraea polychrysa, Cnaphalocronis medinalis, Corcyra cephalonica, Cocyttodes coerulea, Creatonotus transiens, Dendrolimus punctatus, Dendrolimus spectibilis, Ephestia cautella, Ephestia kuehniella, Eutectona machaeralis, Hyblaea puera, Jaspida ditinguenda, Lampides boeticus, Leucania seperata, Melanitis leda, Naranga aenescens, Notiphila dorsopunctata, Notiphila similis, Notiphila spinosa, Ostrinia furnalis, Ostrinia nubilalis, Pelopidas mathias, Parnara guttata, Plutella xylostella, Prodenia litura, Pyralis farinalis, Scirpophaga excerptalis, Scirpophaga incertulas, Scirpophaga nivella, Semia cynthia, Sesamia inferens, Sepedon aenescens, Sepedon plumbellus, Sepedon sauteri, Sepedon sphegens, Sepedon violaceua, Sitotroga cerealella, Spodoptera mauritiana, Spilarctia obliqua, Susumia exigua, Trichoplusia ni, Tryporyza incertulas, Tryporyza innotata* and *Tryporyza nivella*

16.	*Trichogramma kankerensis* Yousuf and Hassn	*Corcyra cephalonica*
17.	*Trichogramma kashmirica* Nagaraja *et.al.*	Eggs of unidentified Sciomyzid
18.	*Trichogramma latipennis* Yousuf and Hassan	*Corcyra cephalonica*
19.	*Trichogramma manii* Nagaraja and Gupta	*Deudorix isocrates*
20.	*Trichogramma pallidiventris* Nagaraja	*Corcyra cephalonica* and *Scirpophaga incertulas*
21	*Trichogramma pieridis* Nagaraja and Mohanraj	*Catopsilia pyranthe*
22.	Trichogramma plasseyensis Nagaraja	*Chilo auricilius, Chilo infuscatellus, Chilo terrenellus, Chilo tumidicostalis, Corcyra cephalonica, Eutectona machaeralis* and *Hyblaea puera*
23.	Trichogramma poliae Nagaraja	*Chilo auricilius, Chilo infuscatellus, Chilo tumidicostalis, Clostera cupreata, C. Fulgurita* and *Corcyra cephalonica*
24.	Trichogramma rabindrai *Nagaraja and Mohanraj*	Unidentified eggs of sphingid
25.	*Trichogramma raoi* Nagaraja	*Achaea janata, Corcyra cephalonica, Eutectona machaeralis, Hyblaea puera* and *Naranga aenescens*
26.	*Trichogramma sankarani* Nagaraja	*Agrius convolvuli* and *Corcyra cephalonica*
27.	*Trichogramma semblidis* (Aurivillius)	*Acantholyda pinnivora, Achaea janata, Cactoblastis cactorum, Calopodes ethlius, Chilo infuscatellus, Chrysops sp., Colias eurytheme, Conchylis ambiguella, Corcyra cephalonica, Diatraea saccharalis, Eupoecilia ambiguella, Helicoverpa armigera, Hylesinus crenatus, Lesperisinus fraxini, Lespericinus orni, Lobesia botrana, Mamestra brassicae, Meliana albilinea, Ostrinia nubilalis, Papilio pergamea, Phthorimaea operculella, Pieris rapae, Plathnota stullana, Polychrosis botrana, Rhychites botrana, Rhychites auratus, Sialis californica, Sialis flavilaterata, Sialis ifiumata, Sialis lutaria, Sialis rotunda* and *Tabanus macer*
28.	*Trichogramma thalense* Pinto and Oatman	*Diatraea grandiosella, Heliothis zea, Trichoplusia ni, Venessa sp.* and Noctuid eggs

APPLICATION OF *TRICHOGRAMMA* SPP. IN BIOLOGICAL CONTROL OF FOREST INSECT PESTS

Few attempts have been made in India for controlling forest insect pests by *Trichogramma* spp.. Record on release of *Trichogramma* in forests goes as early as 1937, with the release of *T. chilonis* (=*T. minutum*) at Nilambur, where 9, 250 parasitoids were released in 1937 against *Hyblaea puera* in teak forest of Kerala (Beeson, 1941). Patil & Thontadarya (1983) carried out laboratory efficacy of nine exotic *Trichogramma* species against teak skeletonizer, *Eutectona machaeralis;* which gave good parasitisation with higher parasitisation by three species *viz. T. evanescens, T. brasiliensis* and *T. pkcal* (hybrid). They have also carried out field efficacy of *Trichogramma evanescens, T. brasiliensis* and *T. pkcal* (hybrid) by releasing 5000 parasitoids of each species in 5 hectare of three years old teak plantation (Patil and Thontadarya 1984). The rate of field parasitisation was less, 22.44% by *T. pkcal*, 19.73% by *T. evanescens* and 19.46% by *T. brasiliensis*. Further they had observed that *Trichogramma evanescens* survived for 60 days, *T. brasiliensis* for 90 days and *T. pkcal* (hybrid) for 105 days at release sites.

Ahmad (1990) tested laboratory efficacy of *T. japonicum*, *T. confusum* and *T. brasiliensis* against teak defoliator, *Hyblaea puera* and teak skeletonizer, *E. machaeralis*. In *H. puera* by five pairs of parasitoids in 100 eggs, parasitisation was recorded as high as 87.40%, 94.60% and 94.80% while in case of *E. machaeralis* parasitisation was 89.40%, 98.00% and 95.60% respectively. Further Ahmad (1992) carried out the laboratory testing of seven *Trichogramma* spp:, showing parasitisation by *T. achaeae*-64.40%, *T. confusum*-3.20%, *T. chilonis* 58.40%, *T. exiguum*-52.40%, *T. japonicum 0.0*, *T. perkinsi* 64.80% and *T. pretiosum*-54.20% against poplar defoliator, *Clostera cupreata*.

Ramachandra *et. al.* (2001) had also recorded the field efficacy of *Trichogramma* spp. against teak leaf skeletonizer, *E. machaeralis*. By releasing the parasitoids @ 1 lakh / ha. in 1987 at Haliyal division, Karnataka maximum percentage of parasitisation was recorded as 50%. Further they had released *Trichogramma* spp. @ 4 lakh / ha. in 1989 in teak plantation of Karka in Haliyal range and the maximum percentage of parasitisation was recorded 42.80%. Yousuf (2005) had carried out the laboratory testing of four exotic species of *Trichogramma* (*T. brasiliensis*, *T. chilonis*, *T. japonicum* and *T. pretiosum*) and one indigenous species *T. raoi* against *E. machaeralis* and *H. puera,* and recorded parasitisation in case of *E. machaeralis* by five pairs of parasitoids in 100 eggs as much as 95%, 78%, 81%, 72% and 89%; while in case of *H. puera,* parasitisation was 90%, 85%, 83%, 74% and 87%, respectively. Further, Yousuf (2005) also carried out the field efficacy of these species against teak leaf skeletonizer, *Eutectona machaeralis* and concluded that *T. chilonis* and *T. raoi* controlled up to 50% skeletonization by releasing @ 1.5 lakh parasitoids per hectare. Out of these two species; being indigenous species, *T. raoi* persists

for a longer period in the field. Joshi *et al.* (2007) had recorded laboratory efficacy of four exotic *Trichogramma* spp. (*T. chilonis*, *T. brasiliensis*, *T. japonicum* and *T. pretiosum* against *E. machaeralis* and recorded the parasitisation in three days 48%, 61%, 46% and 42% respectively. They had also carried out the field efficacy of *Trichogramma brasiliensis* against *E. machaeralis* by releasing parasitoids @ 0.25 lakhs/ ha.; 0.50 lakhs/ ha.; 0.75 lakhs/ ha.; 1.00 lakhs/ ha.; 1.25 lakhs/ ha.; 1.50 lakhs/ ha.; 2.00 lakhs/ ha. and 3.00 lakhs/ ha; and concluded that the lowest effective quantity of *T. brasiliensis* is @ 1.25 lakhs/ ha for controlling the teak leaf skeletonizer. Yousuf (2008) carried out laboratory efficacy of three indigenous species: *Trichogramma raoi*, *T. plasseyensis* and *T. breviciliata* against the eggs of teak defoliator, *H. puera*, teak leaf skeletonizer, *E. machaeralis* and *T. breviciliata* against defoliator of *Holoptelia integrifolia* (*Hasora alexis*). By five pairs of parasitoids, maximum parasitisation was recorded by *T. raoi,* 81% in *H. puera* and 89.70% in *E. machaeralis* while in case of *T. plasseyensis,* it was 80% in *H. puera* and 80.30% in *E. machaeralis.* Similarly *T. breviciliata* gave parasitisation 71.10% in *H. puera* and 72.90% in *E. machaeralis* while in case of *Hasora alexis* it was 62.30%, respectively.

FUTURE PROSPECTS OF APPLICATION OF *TRICHOGRAMMA* IN FORESTS

There are several lepidopterous insect pests, causing serious defoliation, skeletonization and damage to the forest tree species. Some of these are: teak defoliators, *Hyblaea puera*; teak skeletonizer, *Eutectona machaeralis*; Poplar defoliator, *Clostera cupreata, C. fulgurita*; Shisham defoliators, *Plecoptera reflexa, Leucoptera sphenograpta*; defoliator of Kadam, *Arthroschista hilaris*; Deodar defoliator, *Ectropis deodara*; Sal defoliators, *Ascotis imparata, Lymantria* spp., *Achaea janata*; Bamboo leaf roller, *Crypsiptya coclesalis*; Toon feeder, *Diacrisia oblique*; Arjun defoliator, *Lymantria* spp.*;* Lagerstroemia defoliator, *Achaea janata*; Cassia defoliator*, Catopsilia crocale* etc. For controlling these key insect pests in large forestry and agro-forestry areas; application of chemical pesticides is not only expensive but also environmentally unsafe and in many cases, especially in hilly areas pesticide application is not practically feasible. In such situations, biological control plays the key role for controlling the key forest insect pests. It is environmentally safe, reached in difficult situations and also self sustainable.

Discussion: *Trichogramma* has an important place among all biological control agents, as the species belonging to this genus, check the population of insect pests at egg level. India has very rich trichogrammatid fauna (Yousuf and Shafee 1987). Very little work has been carried out on Indian species of *Trichogramma.* In last two decades, work on identification increased (Nagaraja 1996, Nagaraja and Gupta 2007, Nagaraja *et al*. 2007, Yousuf and Hassan 2007, 2008a and 2008b, Nagaraja and Mohanraj 2010a and 2010b). In most of the biological control laboratories and field releases in India, several exotic species of *Trichgramma* were utilized, which established temporarily at release sites (Nagarkatti and Singh 1985). Updated, host list of Indian *Trichogramma* species is clearly showing that indigenous species can be utilized for controlling several key pests of agricultural crops, commercial cash crops, insect pests of orchards and forest tree species. Data on laboratory and field efficacy clearly indicate that the *Trichogramma* spp. can give the promising results for controlling the serious key insect pests like teak skeletonizer, *Eutectona machaeralis,* poplar defoliator, *Clostera cupreata,* etc. Hence in future more emphasis should be given on exploration of *Trichogramma* species, their cultures maintenance, laboratory multiplication and also on their laboratory and field efficacy against important key insect pests of Forestry, so that by applying *Trichogramma* species, strong biological control package can be developed.

CONCLUSION

Till date twenty eight species of ***Trichogramma*** have been recorded from India, which are: ***T. achaeae***, ***T. agriae***, ***T. breviciliata***, ***T. brevifringiata***, ***T. chilonis***, ***T. chilotraeae***, ***T. convolvuli***, ***T. cuttackensis***, ***T. danausicida***, ***T. danaidiphaga, T. flandersi, T. giriensis, T. hebbalensis, T. hesperidis, T. japonicum***, ***T. kankerensis***, ***T. kashmirica***, ***T. latipennis***, ***T. manii***, ***T. pallidiventris, T. pieridis, T. plasseyensis, T. poliae***, ***T. rabindrai, T. raoi, T. sankarani, T. semblidis*** and ***T. thalense***. These ***Trichogramma*** species have considerably good range of theirs hosts, including forest insect pests. Several species of ***Trichogramma*** have been tested in laboratory and fields/ forests for controlling the forest insect pests. These egg parasitoids can be utilized, as biological control agents for controlling the key insect pests of Forests.

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Forests Seed Certification: Problems, Limitations and Needs

Nawa Bahar and V.R.R. Singh *

INTRODUCTION

Seed certification is a legally sanctioned system designed to control and maintain high- purity seed and for propagating material of genetically distinct crop varieties. It allows one to check on the origin of seed and trueness to its cultivar purity, to evaluate the growing crop and supervise the preharvest, harvest and postharvest operations during seed production and processing, as well as conduct sample inspection (laboratory test), bulk inspection for homogeneity, and controlled plot testing.

SEED CERTIFICATION

Unlike several attributes of seed such as purity, germination capacity, moisture content, health etc., which can be assessed in the laboratory, one attribute of prime importance- varietal purity- cannot be assessed in the laboratory. The principal characters differentiating one variety from another are visible not in the seed, but in the plant. Therefore it is not sufficient only to examine the seed offered to the farmer/ forester, as the case may be, but examination of the mother plant, from which the seed was harvested, is equally important. This involves not only the organization of a field inspection service, but also schemes to control the identity of the seed from harvest to final processing and packaging in a merchant's ware- house. Control of varietal purity, or seed source identity, as it may apply to forestry, therefore, involves three examinations: in the field, in the plot and in the laboratory, though the relative importance of each is not the same in all the crops.

A large proportion of the seed used in forestry in India, at present, is obtained from unspecified sources, from stands, natural or planted, that are neither classified nor managed specifically for seed production. Now, with the growing knowledge of forest tree genetics, the benefits that can be reaped through the application of this science in forestry are being realized. With this realization, there is now a general awareness of the need to formulate, and adopt, certification of forestry seeds, in order to ensure the use of quality seeds for raising plantations in India.

DEFINITION OF SEED CERTIFICATION

According to Baldwin (1942) "Certification of tree seed, like guarantees on manufactured articles, is affirmed to be of the quality and come from the source stated. Any attribute of the seed may be so certified. The guarantee is only as good as the person or agency, which thus pledges their honour; hence the greater confidence bestowed on governmental certification, on that of disinterested public agencies and testing associations. Guarantees imply knowledge of the qualities guaranteed, and nothing can be stated as true which has not been ascertained by testing or certification of records".

Some foresters have proposed the following definition. "**Seed certification is the guarantee of seed character and quality by an officially recognized organization usually evidenced by a certificate, which includes such information as certification category, genuineness of species and variety, year of collection, origin, purity, soundness, and germinative capacity" (Rudolf *et al*., 1963b).**

MINIMUM STANDARDS

The following minimum certification standards for forest tree seeds have been provided by the AOSCA(Association of Official Seed Certifying Agencies) to aid local seed certifying agencies in developing standards.

I) Application and amplification of general certification standards:

a) The general seed certification standards, as adopted by the Association of Official Seed Certifying Agencies, are basic and together with the following specific standards constitute the standards for forest tree seed.

b) The general standards are amplified as follows:

Eligibility requirements for certification

Forest trees include all species normally used in forestry including specialized products or uses such as agroforesty species.

Classes and sources of certified seed

Certified seed

Certified seed shall be seed from trees of proven genetic superiority, as defined by the certifying agency, produced so as to assure genetic identify (Seeds from inter specific hybrids of forest trees may be included). In addition the following subclasses may be accepted for certification.

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Selected seed

Selected seed shall be seed from untested parentage of rigidly selected trees or stands that have promise but not proof of genetic superiority.

Source - identified seed

Source-identified seed shall be seed from (1) natural stands with the geographic origin known and (2) from plantation of known provenance, as specified in the standards of the various certifying agencies.

For all classes of forest tree seed, the exact geographic source of the parent trees and the stand history must be known. Location of the source of certified seed and selected seed shall be designated by section or comparable land survey unit. Location of source-identified seed shall be defined by means of administrative and geographic boundaries and, where applicable, by altitudinal and other appropriate boundaries judged to be significant by the certifying agency. In all cases where seed or other propagating materials are produced from planted or otherwise artificially established trees, the origin of the parent material must be known. Exception may be made by the certifying agency in the plantations or trees outside the natural range of a species.

Limitations of Generations

Limitation of generations for forest tree seed shall be in terms of a specified period of time as determined for each species by the certifying agency.

Field Inspection

Inspectors for forest tree seed shall be professional foresters or persons trained specifically for the job by such foresters. For certified and selected seed at least one field inspection shall be made prior to pollination. At this time compliance in regard to rouging and isolation as covered by the applicable agency standards will be checked. For all classes of seed, an inspection will be made prior to seed maturity and the size of the crop will be estimated.

Unit of Certification

An individual tree, clone, or stand of trees may be certified in producing certified, selected, or source-identified seed.

Sampling and Testing

For seed of species not covered by the rules for testing seeds of the Association of Official Seed Analysts, the analyses and testing shall be in accordance with the rules of the International Seed Testing Association or appropriate State or Governmental Laboratories as determined by the certifying agency.

Labelling and Sealing

The following tag colours shall apply:

Certified Tree Seed - Blue Label

Selected Tree Seed - Green Label

Source Identified Seed – Yellow Label

Labels shall be affixed to the containers and the containers sealed to the satisfaction of the certifying agency.

II) Land Requirements

Elevation of the original geographic source and average height and age of the trees from which collected shall be shown on the tag for all forest tree seed. If available, site index (the Capability of a given site to produce trees as measured by the height of the trees at a specified age) may be recorded instead of tree height and age.

III) Field Standards

For certified or selected seed, an adequate isolation zone shall be maintained free of off-type plants and other species that might cross-pollinate producing trees. The isolation distance and specifications for off-type plants shall be set for each variety of species by the certifying agency. There shall be no requirement for source-identified seed. All clones used in seed orchards shall be tested in accordance with the requirements of the certifying agency.

Certification procedure

The certification process for the seed producer begins when he files an application with the certifying agency. The application should include information on the identity of the seeds and on the zone, locality, seed-production area, or seed orchard involved. An inspector from the agency (usually a forester or a man trained by foresters) checks the information on the ground. He also checks to see that seed-production areas and seed orchards are sufficiently isolated from other trees or stands that might contribute to the pollination of the trees on the designated area. Preferably he should check the areas both at the time of flowering and near the time of seed harvesting. (For pines species this requires a check for each seed crop in two successive years). Inspection of seed-production areas and seed orchards is comparable to that for agricultural field crops. The identification of the exact origin of seeds collected from wild stands, however, may be more difficult and more expensive. The certifying agency normally charges a fee to cover the costs of inspection. The primary factor influencing the cost of seed-source identification would be the exactness of identification needed. Any sound programme would contain these four elements:

a) The inspector must determine that a crop is available. To do this he might estimate size of crop, mark specific seed trees, or delimit the seed-collection area.
b) The inspector must observe the collectors at work. He must be assured that the proper fruits are being harvested. If warranted, he might seal or mark bags to prevent contamination or adulteration.
c) The inspector must ascertain that the identity and purity of the crop is safeguarded during collection, fruit shipment, seed extraction and storage.
d) The inspector must affix evidence or identification to the seed before shipment and document his action (Rudolf at al. 1963a and Matthews, 1964).

Other factors also will influence the cost of seed identification. These include size of collection, kinds of seed trees, remoteness of seed collection areas and precision of their demarcations, methods of collection, and the many facets of separate handling of fruits and seeds to maintain identity and purity. Large collection from poorly defined areas and seed trees should cost less per kg than small collections from selected trees in well-defined

but remote localities (Rudolf et al., 1963a). Certified tree seed may cost from a few to several Rupees more per kg than ordinary seed. Source-identified (origin certified) seed might cost from other seed (Hopkins, 1968). This added cost s may seem high, but when they are spread over an acre of plantation they become modest.

Justification

Whether or not certification is economically justifiable depends on the improved productivity of the stands grown from certified seed. Calculations based on logical assumptions as to costs and returns have been made for the pines by several investigations. Perry and Wang (1956) concluded that genetic improvements of 1 or 2% would more than justify the extra cost involved in establishing seed orchards or harvesting from seed- production areas. Bergman (1968) reported that increases in commercial wood production of between 2 and 6% would justify the production costs of seed in loblolly pine seed orchards. The general conclusion was that investment in seed orchards was economically feasible from the stumpage producer's view (Davis, 1967). We can infer, therefore, that the tree grower could justify the cost of certified seed.

Role of seed testing laboratory

The essence of good seed testing is the application of reliable standard methods of examination of seed to ensure that uniform and reproducible results are obtained.

The Seed Testing Laboratory in the Silviculture Division of the Forest Research Institute was established in the year 1962. In the year 1966, the laboratory was granted the status of an accredited station for tree seeds in India by the International Seed Testing Association (ISTA). The ISTA accreditation authorized this laboratory to issue International Seed Analysis Certificates after examination of seed samples in accordance with the International Rules for Seed Testing, but has remained mainly a research laboratory devoted to all aspects of forest tree seed problems, viz. seed collection, processing, germination, dormancy, viability and storage.

The task before the Forest Tree Seed Laboratory at present is to take up research on tree seed which may form the basis for compilation of a set of rules for the Indian tree species for use in tree seed laboratories and eventual inclusion in the ISTA (1971) rules.

Seeds are evaluated for germination, viability and vigour whenever samples are received. The laboratory has facilities for viability tests through germination tests or rapid tests of viability using Triphenyl Tetrazolium Chloride (TTZ) or conductivity tests.

The laboratory has in its research programme, technology for proper seed collection, development of indices of fruit and seed maturation, pre-harvest surveys of seed crops; development of procedures for seed extraction and processing; morphological studies on seed for identification; seed germination physiology, dormancy with emphasis on variation due to seed source or provenance and development of suitable methods of pretreatments; indirect methods of viability and vigour testing; screening of seeds for recalcitrant and intermediate storage physiology and development of protocols for the storage of orthodox seeds.

The laboratory has also shared knowledge with other institutions with similar interests and has been largely responsible for creating infrastructure (seed testing laboratories) for tree seed research and testing in the Indian Council of Forestry Research and Education (ICFRE) Institutes, State Forest departments and Agricultural Universities (under the Research Grant Fund (RGF)). Seed Laboratories of ICFRE Institutes can similarly conduct testing of forestry seeds in accordance with the ISTA guidelines

A brochure highlighting the multifarious activities of the Forest Tree Seed Laboratory have been published which may be useful to individuals or organizations interested in the production, maintenance and distribution of quality seeds of forest tree species.

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Stem and Branch Wood Volume Equations and Variable Density Yield Model for *Dalbergia sissoo* Plantations in IGNP Area of Rajasthan

V.P. Tewari *

INTRODUCTION

Forest yield tables and growth models are an essential source of information to forest management and forest planning. Their predictions have made it possible to develop the idea of a sustainable yield and to optimize silvicultural management. Today, there are numerous yield tables and growth models used all over the world. However, in certain regions and for some species, growth and yield estimates are obsolete or even not available. This is only partly due to the fact that there are no basic inventory data sets accessible (Nagel and Kehr, 1997).

Estimates of total volume and product yields are an important part of a stand model. Such estimates are indispensable when silvicultural decisions are based on economic criteria. It is impossible to measure the stand volume directly in the field. Therefore, this quantity must be calculated from other variables, such as basal area and dominant height, and sometimes also stand age. Thus, although sometimes estimated directly, stand volume is often a derived quantity, a *by-product* of modelling (Gadow and Hui, 1999).

Yield in economic terms is defined by the dimensions and quality attributes of the harvestable logs, and estimating timber products is a central issue of production-oriented growth and yield research. The term yield is used in forestry with a number of qualifiers e.g. annual, intermediate, final, sustained, and financial. Each has a special connotation for management.

Regional yield forecasts are based on aggregate area information, typically in the form of age-class distributions, involving a considerable lumping of area detail with an associated uncertainty. General yield models, covering all possible growing sites and silvicultural regimes, are simply not available.

Volume equations

Estimation of stem and branch wood volume in a tree with greater accuracy has always been a matter of interest for forest managers. Volume equations play a very crucial role in forest management. The importance of volume equations is well indicated by the existence of numerous such equations and the constant search for their improvement. The objective of any volume equation is to provide accurate estimates with acceptable levels of local bias over the entire diameter range in the data. Equations that provide accurate predictions of volume without local bias over the entire range of diameter are one of the basic building blocks of a forest growth and yield simulation system (Tewari and Kumar, 2003).

Yield tables

A yield table is essentially a tool of long term planning. It is a type of growth or 'experience' table which lists expected productivity/volumetric yield for a given age, site or crop quality and sometimes other indices such as density. Thus, yield tables usually refer only to even-aged stands. Data to prepare such tables may be obtained from permanent sample plots, temporary sample plots or stem analysis. Permanent sample plot information is by far the most satisfactory on which to base yield tables.

The main purpose of yield tables is to provide estimates of present yield and future increment and yield. The tables may be presented in tabular or graphical form or in the form of a regression equation relating yield to age, site and stand density. There are three main types of yield table, viz. normal, empirical and variable density.

A normal yield table is based on two independent variables, age and site (species constant), and applies to fully stocked (or normal) stands. It depicts relationships between volume/unit area together with other stand parameters and the independent variables. 'Normal' is an unfortunate term as fully stocked stands are rather unusual.

Since only two independent variables are involved, normal yield tables are conveniently constructed by graphical means. The density variable is held constant by attempting to select sample plots of a certain fixed density assessed as full (or normal) stocking. The data presented in normal yield tables are averages derived from many stands considered to be fully stocked at the time they were sampled.

In contrast to normal yield tables, empirical yield tables are based on average rather than fully stocked stands. This simplifies the selection of stands for sampling. The resulting yield tables describe stand characteristics for the average stand density encountered during the collection of field data.

Normal and empirical yield tables essentially have the same

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limitations, namely: the difficulty of locating fully stocked stands or representative average stocked stands from which to collect the basic data; stocking may not have always been 'fully stocked' or 'average'; the problem of selecting correction factors to apply to stands of density other than normal or average.

The limitations of normal and empirical yield tables led to the development of techniques for compiling tables with three independent variables, stand density being included as the third variable: hence the term variable density yield tables. Basal area/ unit area, mean diameter or stand density indices are used to define the density classes. Such yield tables are particularly useful for abnormal stands e.g. abnormal due to early establishment problems, insect and fungal attack, drought, fire, fluctuating demands for produce, etc.

Mathematical models for estimating timber yields are usually developed by fitting a suitable equation to observed data. The model is then used to predict yields for conditions resembling those of the original data set. It may be accurate for the specific conditions, but of unproven accuracy or even entirely useless in other circumstances. Thus, empirical yield models tend to be specific rather than general.

Yield tables that represent the *normal* development of a forest are a frequently used data base for regional timber resource forecasting. A yield table estimates the production potential for a discrete number of site quality classes, assuming average silvicultural treatment. The volume of the remaining stand may be modelled as a function of site class and age (Smaltschinski, 1997, p. 132). An empirical yield function can be used for regional resource forecasting (Shvidenko *et al*., 1995).

To improve agriculture and living conditions of the people in the drought prone arid parts of Rajasthan State in India, the Indira Gandhi Canal Project (IGNP) was developed. To combat the desertification, the State Forest Department has taken up massive afforestation activities along the main canal and its distributaries by planting various tree species like *Acacia nilotica*, *Dalbergia sissoo*, and *Eucalyptus camaldulensis*. The plantations were raised throughout the area at different sites with varying stand density.

The objective of the present study was to develop stem/ branch wood equation and variable density yield model for *D. sissoo* that can be used by the forest managers for the productivity/ yield estimation and management of plantations of this species in the study area.

MATERIALS AND METHODS

Study area

The IGNP area is characterized by large variation in the diurnal and seasonal temperatures. The maximum daily summer temperature often exceeds 46-48°C; the night temperature occasionally touches 0°C owing to cold waves associated with the western disturbance, which may cause frost conditions. The mean monthly maximum temperature varies between 39.5°C and 42.5°C while mean monthly minimum temperatures vary between 14-16°C. Soil temperature in desert sandy tracts often reaches 62°C during May and June and they remain higher than the air temperature at least by 10°C. The mean annual rainfall in the area varies from 120 mm to 300 mm. The major quantity of rainfall is received during the southwest monsoon season (July-September). The number of rainy days varies from 8 to 17 days in the area. The mean monthly relative humidity in the IGNP area fluctuates largely during the year from 15 to 80%. The mean evaporation in the area varies from 2.7 to 4.7 mm per day in winter and 13.2 to 15.3 mm in the summer with the annual mean being of the order of 7.3 to 8.5 mm per day. A strong wind is the characteristic feature of the summer and monsoon season in the area, which often causes soil erosion. Wind speeds as high as 130 km per hour have been experienced during the summer months. Dust storms are also common in the region (3-17 days per year). Droughts are a recurring feature of the area and often persist for 2-3 years. The terrain of the area is very undulating consisting of moving sand dunes, dry undulating plains of hard sand and gravelly soil and rolling plains of loose sand. The soil is rich in potash but poor in nitrogen and low in organic matter with very low productivity. There is presence of semi-consolidated lime concretionary or gypsum strata in many places. The soils are coarsely textured with a low water retention capacity (Tewari and Kumar, 2005).

Data and field procedure

A total of 30 ample plots of *D. sissoo* were laid out at various locations throughout the IGNP area, covering the available age groups, stand densities and sites, using stratified multistage sampling. Each plot was representative of the growing conditions in the stand. For identification and demarcation, trenches were dug at the four corners of the plot and the trees inside the plot were numbered and enumerated. The check trees (closest trees bordering the sides of the plot) surrounding the plots were marked with rings. The study was started in 1995 and measurements were taken in the sample plots annually for five years. Trees, representing different diameter classes in the plots, were felled from the surround of the plots for volume estimation. A total of 90 trees were felled from the plantations. For the computation of total volume, stem and branch wood with a minimum diameter of 5 cm was considered. The volume was then calculated by dividing the stem and branches into logs of 3m length, measuring the mid-diameters and applying Huber's formula to estimate individual log volumes. Equations showing the relationship between volumes and D^2H were derived for volume prediction. The volume equation developed was applied on the trees within the plots to estimate wood volume per hectare. The plot data included records of age, dominant height, average height, number of trees per hectare, top diameter, basal area per hectare, quadratic mean diameter and timber and wood volume (over bark and under bark) per hectare and are summarized in Table 1.

Table 1: **Summary statistics for the pooled data of the thirty plots of *D. sissoo***

Attribute	Minimum	Maximum	Mean	Standard deviation
Age (years)	3.20	33.40	12.30	6.57
Dominant height (m)	8.71	22.78	14.40	3.22
Stems per hectare	342	2632	1465	553.36
Quadratic mean diameter (cm)	5.76	29.83	13.29	5.45

Basal area (m^2/ha)	4.82	32.80	17.61	5.64
Site index (m)	8.65	18.68	14.46	2.77

Site index, the dominant height of the trees in a stand at a given reference age, has been the most widely used means for estimating potential forest site productivity (Payandeh and Wang, 1994). The dominant height is practically independent from the stand density (Kramer, 1988) and thus is used as an indicator of the site productivity. For estimating site index, the base-age was selected as 15 years. Accordingly five site classes/qualities were defined at 2m intervals.

The combined variable equation [$V = a + b\ D^2H$ where, V=volume (m^3), D=dbh (cm), H=height (m)] has been well recognised in volume predictions of many tree species with R^2 usually above 95% (Avery and Burkhart, 1994) and, therefore, was used to generate stem and branch wood volume equation.

The following equation (modified from Nagel and Kehr, 1997) was found best among all other equations tested and, hence, was used to develop variable density yield model for *D. sissoo* plantations in IGNP area of Rajasthan using the data from the 30 sample plots measured annually for five years:

$$V = \text{Exp}\ [a + b*\ln(TH/A) + c*\ln(N) + d*\ln(BA)] \quad (1)$$

where, V=volume/ha (m^3), TH=dominant height of the trees in the stands (m), A=age of the stand (years), N=stems/ha, BA=basal area/ha (m^2).

RESULTS AND DISCUSSION

The data collected from the 90 sample trees felled from the surround of sample plots were used to develop stem and branch wood volume equations. The parameter coefficients, coefficient of determination and root mean squared error obtained by fitting the equation to the data set are given in Table 2.

Table 2: **Parameter coefficients and fit statistics for stem and wood volume equation**

	a	b	R^2	RMSE (m^3)
Stem	-0.001337	3.399E-05	0.991	0.00012
Branch	-0.000373	2.459E-06	0.938	0.00015

The corresponding stem and branch wood volume tables are given in Tables 3 and 4.

Table 3: **Stem wood volume table for *D. sissoo* (volume in m^3)**

Diameter (cm)	Height (m)					
	5	10	15	20	25	30
5	0.002912	0.007161	0.011409	0.015658	0.019907	0.024156
10	0.015658	0.032653	0.049648	0.066643	0.083638	0.100633
15	0.036902	0.075141	0.113379	0.151618	0.189857	0.228096
20	0.066643	0.134623	0.202603	0.270583	0.338563	0.406543
25	0.104882	0.211101	0.317319	0.423538	0.529757	0.635976
30	0.151618	0.304573	0.457528	0.610483	0.763438	0.916393
35	0.206852	0.415041	0.623229	0.831418	1.039607	1.247796
40	0.270583	0.007161	0.814423	1.086343	1.358263	1.630183

Table 4: **Branch wood volume table for *D. sissoo* (volume in m^3)**

Diameter (cm)	Height (m)					
	5	10	15	20	25	30
5	0	0.000242	0.000549	0.000857	0.001164	0.001471
10	0.00086	0.002086	0.003316	0.004545	0.005775	0.007004
15	0.00239	0.005160	0.007926	0.010693	0.013459	0.016225
20	0.00455	0.009463	0.014381	0.019299	0.024217	0.029135
25	0.00731	0.014996	0.022680	0.030365	0.038049	0.045733
30	0.01069	0.021758	0.032824	0.043889	0.054955	0.066020
35	0.01469	0.029750	0.044811	0.059873	0.074934	0.089995
40	0.01930	0.038971	0.058643	0.078315	0.097987	0.117659

Variable density yield model was developed by fitting Equation 1 to the 5 year dataset of 30 sample plots taking wood volume (over bark and under bark) per hectare as dependent variable and age, dominant height, number of trees per hectare, and basal area per hectare, as independent variables. The parameter coefficients, coefficient of determination and mean squared error obtained by fitting the equation to the data set are given in Table 5.

Table 5: **Parameter coefficients and fit statistics for variable density yield model**

	a	b	c	d	R^2	RMSE (m^3)
Value	2.0593	0.12150	-0.24769	1.48353	0.949	13.06308
Std. error	0.31013	0.04905	0.03127	0.05240		

The standard errors show that all the partial regression coefficients were statistically significant. The R^2 value is generally high while RMSE values are very low. This shows the high accuracy and precision of the equation used in making yield predictions in *D. sissoo* plantations in the study area under varying age, density and site conditions. The provisional yield tables for the five site classes, generated based on the field data collected for five years from the 30 sample plots, are given in Tables 6-10 as an Appendix.

A regional yield model is a useful tool for evaluating the effects of different harvest levels on a given age-class distribution and a simple age-class simulation is often the only feasible way to predict the dynamic development of a forest resource on a regional basis. The method involves, however, considerable aggregation over growing sites, forest types and management regimes.

More refined methods of simulation need to be applied in regions where intensive production forestry is practiced, and the first step towards refinement should be involving a method for considering the effects of different levels of stand density. The density of a forest may be expressed using the variable *degree of stocking*, which implies the observed stocking (basal area or volume per ha) as a proportion of some normal yield table stocking.

Projections based on yield tables need to be adjusted for variable density. This may be done using tables of reduction factors (Kramer and Akca, 1987) or specific growth reduction functions for adjusting current increment in accordance with the actual degree of stocking (Smaltschinski, 1997, p. 155).

Variable density yield model too have some limitations (which apply also to normal and empirical tables), namely:

- no confidence limits are attached to trends;
- extrapolations are made outside and beyond thinning regimes and ages sampled;
- volume functions used are mostly two-dimensional and of regional application;
- volumes are computed for normal trees only and no account is taken of malformation and other such factors affecting recoverability;
- usually, no account is taken of the pruned component of a stand.

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APPENDIX

Table 6: **Provisional yield tables for *D. sissoo*, Site class I (top height 17-19 m)**

Age (years)	Stems/ha (No.)	Vol/ha (m^3)	Top ht (m)	BA/ha (cm^2)	Crop dia (cm)	Crop ht (m)	FF	MAI (m^3)
6.5	1684	118.17	16.34	194169.8	12.13	12.22	0.497	18.180
8.0	1684	129.14	16.71	20493.3	12.44	12.64	0.499	16.140
8.0	1636	89.75	13.24	172655.8	11.59	11.29	0.461	11.220
8.5	1599	136.33	16.83	223141.6	13.33	12.10	0.505	16.040
8.5	1583	129.60	16.75	203668.1	12.79	12.84	0.497	15.250
8.5	1438	84.00	14.25	169582.2	12.25	9.89	0.501	9.882
9.5	1398	91.41	14.38	164473.4	12.23	12.11	0.460	9.622
9.5	1356	99.90	14.72	184977.9	13.18	11.09	0.487	10.520
9.5	1332	133.69	16.78	201178.3	13.86	13.91	0.478	14.070
10.0	2537	93.65	14.54	198532.8	9.98	9.71	0.486	9.365
10.0	1902	100.11	14.34	192820.0	11.36	11.01	0.472	10.010
10.0	1316	96.07	14.86	183094.9	13.30	10.47	0.502	9.607
10.0	1249	87.41	14.78	150868.7	12.39	12.70	0.457	8.741
10.5	1316	107.19	15.35	155187.7	13.74	10.92	0.503	10.210
11.0	1891	111.14	14.96	202865.0	11.68	11.45	0.479	10.100

11.0	1356	116.66	16.09	199520.8	13.68	11.82	0.495	10.610
11.0	657	109.61	18.48	141057.2	16.52	15.78	0.494	9.965
11.5	2502	111.96	15.62	215108.1	10.46	10.83	0.481	9.736
11.5	1356	118.84	16.16	200920.2	13.76	12.01	0.493	10.330
11.5	1292	120.22	16.49	204663.6	14.20	11.55	0.509	10.450
12.5	1149	125.63	17.1	195575.4	14.72	13.15	0.489	10.050

Table 7: **Provisional yield tables for *D. sissoo*, Site class II (top height 15-17 m)**

Age (years)	Stems/ha (No.)	Vol/ha (m^3)	Top ht (m)	BA/ha (cm^2)	Crop dia (cm)	Crop ht (m)	FF	MAI (m^3)
7.0	1884	93.26	12.49	170242.6	10.72	9.87	0.556	13.32
8.5	1807	100.65	13.46	175209.2	11.11	10.43	0.551	11.84
8.5	1592	107.33	13.60	207883.7	12.89	10.72	0.482	12.63
9.0	1768	103.99	13.98	176454.2	11.27	10.71	0.551	11.55
9.5	1651	120.58	16.09	201912.5	12.48	11.93	0.501	12.69
10.0	1426	111.01	13.56	210876.7	13.72	10.99	0.479	11.10
10.5	1296	114.63	13.90	211268.7	14.40	11.50	0.472	10.92
11.0	1606	123.65	16.36	205959.1	12.77	12.18	0.494	11.24
11.5	1271	99.94	15.95	167445.2	12.94	12.21	0.490	8.69
11.5	1111	115.23	14.94	197429.4	15.04	12.25	0.477	10.02
12.5	937	82.73	15.99	135856.7	13.58	12.46	0.489	6.62
19.0	861	155.63	18.39	202725.1	17.31	14.57	0.527	8.19
20.0	778	158.96	19.99	203095.4	18.23	14.91	0.525	7.95
21.0	694	164.17	19.72	199851.1	19.13	16.06	0.513	7.82

Table 8: **Provisional yield tables for *D. sissoo*, Site class III (top height 13-15 m)**

Age (years)	Stems/ha (No.)	Vol/ha (m^3)	Top ht (m)	BA/ha (cm^2)	Crop dia (cm)	Crop ht (m)	FF	MAI (m^3)
7.5	1972	53.14	11.47	138399.3	9.45	8.47	0.454	7.085
8.0	2500	73.22	12.30	162828.0	9.10	9.62	0.468	9.153
8.0	2289	49.00	11.40	122356.4	8.25	8.73	0.459	6.125
8.5	1567	42.23	11.24	107945.2	9.36	8.90	0.440	4.968
9.0	2385	75.33	12.57	164062.3	9.36	9.83	0.467	8.370
9.0	1972	58.34	11.85	144976.9	9.67	8.86	0.455	6.482
9.5	2289	63.16	13.19	140244.7	8.83	9.49	0.475	6.648
9.5	1972	61.37	12.14	147647.9	9.76	9.12	0.456	6.460
10.0	1962	67.05	12.63	141271.6	9.57	10.20	0.466	6.705
10.0	1484	44.57	11.79	108445.4	9.64	9.22	0.447	4.457
10.5	1945	67.29	13.01	151392.8	9.95	9.63	0.462	6.409
10.5	1381	45.18	12.16	105602.9	9.86	9.47	0.453	4.303
11.0	2102	64.74	13.35	138828.9	9.17	9.80	0.476	5.885

11.0	905	53.88	13.68	99547.8	9.07	10.96	0.841	4.898
11.5	1361	48.60	12.24	110028.5	10.14	9.82	0.451	4.226
12.0	1929	65.51	13.55	136777.6	9.50	10.11	0.474	5.459
13.5	1622	95.15	13.51	182648.1	11.97	11.26	0.463	7.048
15.0	1622	99.58	13.75	187707.1	12.14	11.44	0.464	6.639
18.0	832	78.71	14.40	142022.8	14.93	11.81	0.47	4.373
19.0	796	80.04	14.43	143153.0	15.12	12.02	0.466	4.213

Table 9: Provisional yield tables for *D. sissoo*, Site class IV (top height 11-13 m)

Age (years)	Stems/ha (No.)	Vol/ha (m^3)	Top ht (m)	BA/ha (cm^2)	Crop dia (cm)	Crop ht (m)	FF	MAI (m^3)
7.0	1616	57.60	11.62	138284.5	10.44	9.79	0.426	8.889
8.5	2046	67.06	11.43	164503.2	10.11	8.76	0.466	7.886
8.5	1599	68.21	11.91	158114.8	11.22	10.06	0.429	8.025
10.0	2021	68.28	11.58	165485.7	10.21	8.86	0.466	6.828
10.0	1582	71.88	12.17	162066.5	11.42	10.33	0.430	7.188
10.5	1228	51.33	11.62	118590.7	11.09	9.39	0.461	4.889
11.0	1548	73.70	12.61	162449.7	11.56	10.48	0.433	6.700
24.0	476	236.99	20.08	291593.7	27.90	16.60	0.491	9.875
25.0	476	270.07	21.26	314748.2	28.99	17.49	0.492	10.800
27.0	476	289.71	22.22	325158.8	29.47	18.05	0.495	10.730
28.0	476	299.50	22.78	327961.4	29.47	18.49	0.499	10.700

Table 10: **Provisional yield tables for *D. sissoo*, Site class V (top height 9-11 m)**

Age (years)	Stems/ha (No.)	Vol/ha (m^3)	Top ht (m)	BA/ha (cm^2)	Crop dia (cm)	Crop ht (m)	FF	MAI (m^3)
3.0	1850	11.46	9.14	48200.75	5.76	6.97	0.341	3.820
4.5	1775	26.07	9.33	79193.77	7.53	8.51	0.388	5.793
6.0	1750	30.60	9.69	88084.96	8.00	8.73	0.399	5.100
7.0	1650	32.56	9.90	89333.74	8.30	9.08	0.402	4.656
7.5	2632	20.28	8.71	86666.80	6.47	6.35	0.369	2.704
9.0	2179	19.38	9.00	76463.46	6.68	6.62	0.384	2.153
9.0	1560	31.86	9.78	102277.90	9.13	7.88	0.396	3.540
10.5	1170	25.40	9.41	80854.83	9.38	7.01	0.392	2.419
11.0	910	23.78	9.94	17650.42	9.94	8.64	0.390	2.162
30.5	356	117.70	18.52	232219.70	28.80	15.79	0.322	3.859
32.0	342	182.94	19.02	230990.70	29.30	16.38	0.485	5.717
32.5	342	189.02	19.24	236062.70	29.62	16.51	0.486	5.816
33.5	342	193.37	19.35	239612.20	29.84	16.65	0.486	5.772

Expanding Frontiers in Forest Genetics and Biotechnology

Dr. N. Krishna Kumar *

The divergent requirements of conservation and production forestry, in the current context of limited land availability for trees outside forests and climate change, have resulted in the opening up of a number of avenues for scientific intervention for addressing challenges faced by the forestry sector. Modern tools of genetics, breeding, and biotechnology developed for agricultural crop species are already being applied in forestry. There has been a growing realization that the goal of the National Forestry Policy for increasing green cover in India requires community participation. A number of institutes and forest departments are therefore, selecting and breeding for superior clones and varieties, so that farmers are benefited through tree plantations / agroforestry. Tree breeding programmes largely rely on open–pollinated breeding populations established using diverse seed sources, in combination with clonal propagation of desired genotypes for planting stock production, and for establishment of seed orchards. Tree improvement programme for species like Teak, Eucalyptus, Poplars, Albizias, Acacias, Neem, Dalbergia, Pines, Casuarina, Bamboos and Gmelina are already in place. The present status and advances being made in the Southern states of India in the field of forest genetics and biotechnology are enunciated below

TREE IMPROVEMENT: PRESENT STATUS AND FUTURE DIRECTIONS

The Tree Improvement activities in India got an impetus during the 1960s under the leadership of Dr. S. Kedarnath, the Forest Geneticist of FRI. Plus trees of teak were identified in the States of Tamil Nadu, Kerala, Andhra Pradesh and Karnataka. Clonal Seed orchards were established in these States by deploying the selections through grafts. Later in the 1970s the "Indo Danish Project on Seed Procurement and Tree Improvement" came into existence with its base in Hyderabad and Centres at Dehradun and Coimbatore. Under this project emphasis was laid on the improvement of valuable species like teak, rosewood, *Gmelina* and *Bombax*. Apart from identifying plus trees and establishment of orchards, Seed Production Areas were established in many States. The project also laid emphasis on "Certification of Forest Reproductive Material" following the OECD Scheme practiced in the Scandinavian countries and some of the countries in the Europe. The third Phase in Tree Improvement started during 1990s with the implementation of World Bank aided "Forestry Research, Education and Extension Project" (FREEP) by ICFRE. Under this project different Institutes established Seed Production Areas and, Seed Orchards for various species in collaboration with the State Forest Departments.

There were three plan projects under the erstwhile FRI and Colleges which contributed significantly in the field of Tree Improvement. The "Sandal Research Centre" at Bangalore strived to eliminate the sandal spike disease. Plus trees of sandal were selected based on the heartwood and sapwood ratio and oil content. These plus trees were assembled and seed orchards were established. The "Tropical Pine Centre' at Kodaikanal was involved in the introduction and evaluation of provenances of *Pinus carribea, Pinus keysia, Pinus oocarpa* particularly in the hills of Southern States. The "Eucalyptus Research Centre" at Ooty coordinated the introduction and evaluation of new species and provenances of Eucalyptus. Provenance trails of Eucalyptus species were established in places like Warangal (AP), Pudukottai (TN) and Kodaiakanal (TN). These trials formed the base for large planting of Eucalyptus in Southern States and also they were the source for many clones which are commercially planted in the recent times. India also participated in the International Provenance Trial of *Gmelina* and Teak. Provenance trails of these species have been established in many countries in 1981-83. One of the International provenance trail of teak is still existing in Maredumilli in Andhra Pradesh.

With the establishment of IFGTB in 1988, systematic long term breeding programmes for Casuarinas (*C. equisetifolia* and *C. junghuhniana*) and Eucalypts (*E. camaldulensis* and *E. tereticornis*) were initiated. Systematic genetic improvement programme for Eucalypts were initiated in the year 1995 with a written breeding plan (Doran *et al*. 1995). Seeds were obtained from the Australian Tree Seed Centre, CSIRO, Australia. To achieve short term deliverables in the form of quality seeds, unpedigreed seedling seed orchards were established with bulked seeds of about 500 trees of natural provenances of *E. camaldulensis* and *E. tereticornis* at two locations namely Panampally (Kerala) and Pudukkottai (Tamil Nadu). As a long-term strategy, first generation progeny trials were established at 3 locations– Panampally (Kerala), Pudukkottai (Tamil Nadu) and Sathyavedu (Andhra Pradesh) using open-pollinated families from natural provenances in Australia and plus trees selected from India. After early evaluation for growth performance, inferior trees were culled out and the trials were converted into seedling seed orchards. Quality seeds are being collected from the seed orchards since 2002 and supplied to Forest Departments, Industries and Farmers. The seeds collected from these SSOs yielded 17% more than the local seed-

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lots in genetic gain trials established using the SSO seeds. Based on growth and tree form 126 superior trees were identified from pedigreed and un pedigreed orchards, cloned and evaluated in three clonal trials located at Karunya (Tamil Nadu), Sathyavedu (Andhra Pradesh) and Kulathupuzha (Kerala). These tests were evaluated, 110 clones short-listed and finally 30 outstanding clones were earmarked for large-scale propagation. These 30 clones were tested for resistance to Eucalyptus gall and 4 clones have been released for raising commercial plantations. The Institute has also initiated an exhaustive hybridization programme to enhance productivity of Eucalyptus plantations. The experiments envisage combining the desirable economic traits especially pulping traits of different species. Hybrids of *E. tereticornis x E. camaldulensis, E. grandis, E. pellita, E. urophylla* and *E. alba* have been developed and deployed in field tests in coastal and inland agro-climatic conditions**.** Collaborative projects with TNPL and ITC have been initiated for creating new generation hybrids to meet the challenges of gall infestation, moisture limitation and increased pulp yield and quality.

Genetic improvement of *Casuarina equisetifolia* was taken up as per a written breeding programme (Pinyopusarerk, 1996) since 1996. In casuarinas, provenance tests were conducted by IFGTB with the support of Australian Tree Seed Centre (ATSC) of CSIRO, Australia to identify fast growing natural and introduced populations for further improvement. Provenance - progeny trials were established with open-pollinated families at 7 locations (Nellore, Balukhanda, Rajahmundry, Neyveli, Coimbatore and Pondicherry) in 3 States and 1 UT as breeding populations during 1996-98. Nearly 40 seedlots from the entire natural distribution and cultivation range of *C. equisetifolia* were tested in these trials. Natural provenances from South East Asia (Thailand and Malaysia) and Pacific (PNG and Solomon Islands) showed faster growth than Indian seedlots in all locations and landrace seedlots from Kenya showed superior growth in inland locations. These trials were evaluated, thinned and converted to Seedling Seed Orchards in 2003. Seeds collected from these orchards are being supplied to the farmers, forest departments and other planting agencies. SSO seeds produced 13% more wood in rainfed conditions and 28% more in irrigated conditions compared to the local source. 100 outstanding individuals from the SSOs were selected and planted in Hoskote (Karnataka) and Karunya (TN) for the evaluation of their performance. From these clones, 40 outstanding clones in terms of growth and form have been selected for seed orchard establishment and breeding. Four top-ranking clones have been released for raising commercial clonal plantations. Inter and intra-specific hybrid clones are under testing at multi-locations.

New germplasm of *C. junghuhniana* was introduced in the form of provenances from Indonesia and East Timor and landraces from Kenya and Tanzania. Two provenance trials were established at Pondicherry and Panampalli (Kerala) in 1996 and 6 superior provenances were selected for further improvement. The provenance tests were thinned to retain only the best trees of selected provenances and converted into seedling seed orchards. *C. junghuhniana* has coppicing ability unlike *C. equisetifolia*. This species is also drought and disease tolerant. Hybrids between different provenances of *C. equisetifolia* and *C. junghuhniana* have been developed to obtain optimum wood density and coppicing ability. Eight inter-provenance hybrid families of *C. equisetifolia* and 24 inter-specific hybrid families were generated and their progenies were established in the nursery for field testing in 2007.

Seed orchards were also developed for phyllodinous Acacias (*A. auriculiformis* and *A. mangium*) and Neem. In all 216 clones of *Casuarina equisetifolia*, 351 clones of Eucalypts, 129 clones of *Tectona grandis*, 59 clones of *Tamarindus indica* and 11 clones of *Emblica officinalis* have been selected and assembled in clone banks. Seed orchards to the extent of 28 ha for Eucalypts, 26 ha for *C. equisetifolia*, 4.5 ha for *A. auriculiformis*, 4 ha for *A. mangium* and 7 ha for *T. grandis* have been established. These orchards produce more than 100 kg of seeds annually and these seeds are supplied to different tree growers like farmers, wood-based industries and forest departments. Under the Planting Stock Improvement Programme (PSIP) of the Forestry Research, Education and Extension Project (FREEP) funded by the World Bank, seed production areas (SPAs) were established for the priority species by converting the existing plantations of the State Forest Departments to meet the immediate demand of quality seeds. A total area of 256 ha of SPAs were established in Tamilnadu (104 ha), Kerala (122 ha) and Andaman and Nicobar Islands (30 ha) for *T. grandis* (134 ha), *E. grandis* (21 ha), *E. globulus* (9 ha), *A. auriculiformis* (5 ha), *A. mangium* (10 ha), *Artocarpus hirsuta* (7 ha), Thorny Acacias (40 ha) and *Pterocarpus dalbergioides* (30 ha).

BREEDING PRIORITIES FOR IMMEDIATE FUTURE

Breeding objectives have so far been mostly focused on increasing growth and form in species for which active breeding programmes are being implemented. Wood properties have to be included as priority objectives while advancing the ongoing breeding programmes to the next generation. Absence of adverse correlation between most of the wood and growth traits provides opportunity for simultaneously improving both the traits. In the case of farmers who are not sure till harvest to what end use their plantations will be used it is desirable to have superiority for both wood volume and quality in the varieties / clones developed through breeding. Breeding for drought, disease and insect attack assume greater significance than before in the light of climate change. Selections have to be made to cope-up with changing temperature regimes, rainfall pattern and virulence and epidemics of pests. Hybrid breeding in particular helps in pushing the boundaries of a species' tolerance to the biotic and abiotic stresses by introgressing new genes from related species. Molecular markers-assisted breeding will support in early and efficient selections for various end use and adaptation traits leading to shorter breeding cycles and greater genetic and economic gains than before.

EXPANDING THE REACH OF IMPROVED PLANTING STOCK TO TREE GROWERS

The immediate benefit from the breeding programme is revenue earned through sale of seeds and clones to different user groups. Despite the cost of seed orchard derived seeds being many times costlier than locally available unimproved seeds, there has been a heavy demand for the improved seeds as its potential has been clearly demonstrated. During the last five years more than 250

kg of seeds worth Rs. 10 lakhs have been supplied to farmers, wood-based industries and forest departments. The long term gain is the linking of breeding programme to plantation development programme connects the research institution with the end users. The economic gain from the use of genetically improved seeds from seed orchards has been realized by the farmers as the plantations raised with improved seed come to harvest. A mere 10% increase in wood production results in an additional income of Rs. 12,000 to 20,000 per ha for most of the short-rotation species. Assuming this minimum gain from all the 9000 ha of plantations raised during the last five years with seed orchard seeds, an additional income of around Rs.14 crores will be realized. If the average gain of 20% is taken into consideration, this amount will be doubled.

Following the production of genetically improved seeds and clones, the focus is now shifted to making them available to the tree growers at affordable cost. Since research institutions will not be in a position to produce and supply all the planting stock needed, involvement of other stakeholders and decentralizing the seed production are necessary to bridge the gap between demand and supply. The wood-based industries, in particular the paper mills have been encouraged to establish their own orchards with technical support from IFGTB through Consultancy / Collaborative Projects. The Tamil Nadu Newsprint and Papers Limited and The Andhra Pradesh Paper Mills are in possession of productive orchards established with IFGTB's technical inputs.

In order to increase the accessibility and affordability of genetically improved planting stock to smallholding farmers, IFGTB initiated a project with funding support from the Public Sector Linkages programme of AusAID and technical collaboration of CSIRO. A new concept of "Community Seed Orchards' has been developed through production of high quality seed from orchard established in community/government lands is undertaken to meet the seed demand locally. The planting stock needed for the establishment of the orchard (clones/seeds) is provided by IFGTB which also bears the cost of initial planting and maintenance for two years. All works are carried out by farmers or traditional nursery operators under the supervision of the Scientists of IFGTB. After two years the orchard will be maintained by the community itself through the revenue generated from the sale of seeds. Wood-based industries also expressed their willingness to procure the surplus seeds from community orchards after meeting the local need. So far three model community orchards have been established in Tamil Nadu and Puducherry involving local farmers and nursery growers in their establishment and maintenance.

It is the long term goal of IFGTB to use community seed orchards and the genetic gain from their seed output as a means of enhancing livelihood opportunities for traditional nursery operators. At present the traditional low-cost nurseries are operated by landless agricultural labourers who hail from socially deprived communities with minimal livelihood security. Since they are not trained in modern nursery techniques and lack the means to provide additional inputs to their nursery operations, the planting stock is priced low and the per capita income is meager. IFGTB is working with this highly skillful group of nursery operators to impart training in latest nursery techniques. One of the community seed orchards of Casuarina is established adjacent to the traditional nurseries in the Union Territory of Puducherry. Once the orchard starts yielding seeds, they will be deployed in the nursery under the supervision of Scientists from IFGTB and the seedlings will be sold at a premium price compared to the other seedlings raised from unimproved seed sources. If this model of decentralized seed production and enhancing genetic quality of planting stock from traditional nurseries leading to livelihood enhancement to landless agricultural labourers is successful, it will be extended to other tree species and locations

GERMPLASM CONSERVATION AND CHARACTERIZATION FOR TREE IMPROVEMENT

The recent gall outbreak in Eucalyptus has, emphasized the importance of plantations to be deployed with diverse clones with different desirable traits. With breeding programmes increasingly moving from selection for enhanced biomass to breeding for desired traits like better wood properties for pulping, veneers and biofuels, and enhanced pest, disease, drought, flooding, salt and CO_2 tolerance, availability of germplasm resources characterized for these traits, therefore, becomes important. In this direction, ICFRE has embarked on a Forest Genetic Resource Management Network, which would help in germplasm collection, characterization and conservation. To facilitate such a work of this magnitude, through a workshop at the Institute of Forest Genetics and Tree Breeding, Coimbatore, species of focus was prioritized for conservation in partnership with different stakeholders (Table 1). Maintenance of Germplasm collections are subject to inadvertent costly errors including mislabeling and maintenance of redundant germplasm. DNA markers have been applied for maintaining the identity of germplasm collections and identification of redundants/ duplicates in germplasm collections.

Tree breeding is multivariate in nature and aims at simultaneously improving several traits. In recent years, phenotypic characterization has taken new dimension with help of digital computer technology. Images of plant parts from whole tree to leaf, flower, fruits, seeds, pollen, wood fibre, etc., can be captured using digital camera and processed in computer platform in extracting information related to the two and three dimensional data. Tree volume and form can be measured and made essentially a part of the plus tree selection. Estimation of volume and form traits helps in identification of trees with high biomass. The digital technology also allows in storage and retrieval of the pedigree details at later date. Large samples can be studied in short period of time.

The most commonly targeted trait in tree breeding programmes is wood property traits. For several decades, wood basic density was considered as the canonical trait in wood quality research due to ease of assessment, high genetic control and high degree of variation. However, with the increase in understanding of the wood quality parameters and their effect on pulp yield, mere estimation of wood basic density may not predict the overall wood quality. Hence, non-destructive sampling methods to evaluate pulp yield using near infrared reflectance analysis, Raman spectroscopy, estimation of chemical wood components including hot water extractives content and cellulose/lignin/pentosan content are considered during intensive selections in breeding programmes. Further, microstructure analysis like microfibril angle

and fibre characters are also targeted during selection. Recently, use of acoustic tools which are based on sound wave, ultrasound velocity or sonic resonance were demonstrated for easy estimation of module of elasticity and used for log segregation and tree selection (Lindstrom et al., 2002).

APPLICATION OF TISSUE CULTURE IN PLANTATION FORESTRY

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. Integration of clonal propagation in tree improvement is essential for the replication of improved genetic material and rapid release of quality propagules. Currently, the area of clonal propagation is gaining momentum for some of the industrial species like Eucalypts and acacia. The Two Micropropagation Technology Parks (MTPs) established by DBT at NCL, Pune and TERI, New Delhi, provide an effective platform for interaction between the Academics and the Industry. The MTPs have produced forest trees through tissue culture, and research efforts have concentrated on development of tissue culture protocols for economically important species that are either difficult to propagate or show marked variability. At the MTP – NCL, Pune, Teak, Eucalyptus and Bamboo were produced from material collected from the elite clones identified by the Forest Department. Field trials have been conducted, 10 lakh plants have been field planted at 100 locations over 1000 ha. At MTP – TERI, New Delhi, large scale production of *Anogeissus* sp, *Dendrocalamus strictus*, *Eucalyptus tereticornis, Populus deltoides, Paulownia* sp, in addition to other agricultural and floricultural species are being carried out. A total of 10 lakh plants were produced and distributed for field trials during the year. The forest trees are being evaluated in mainly 12 different states over an area of 2500 ha. Field evaluation results indicate that the tissue culture raised plants have a better root system and taller than the conventionally raised plants. The plants are also being used extensively as agroforestry species intercropped with other crops.

Among the vital factors determining the success of clonal plantations, rooting competence of the cuttings ultimately influences the genetic diversity of the clones in plantations. Despite rejuvenating techniques like coppicing and efficient nursery techniques practiced in clonal planting stock production, the rooting responsiveness of the coppice shoots to auxins is not equal. Due to the variations existing in rooting potential among the ortets finally only few clones enter into the operational plantations and attracting problems like pests and diseases. As rooting is a physiological process, several parameters determine the root formation in cuttings. Thus, by improving the rootability of less responsive clones through various approaches including tissue culture, the possibilities of introducing more number of clones can be enhanced. Evidences are available on phase change and rejuvenation during tissue culture process favouring root formation. Several superior phenotypes of *E. camaldulensis* and *E.tereticornis* have been identified through the tree improvement programmes. The rooting competence of these clones has been assessed through rooting of cuttings and found that tremendous variation exists among clonal individuals. Micropropagation strategy for rejuvenating productive clones, which are difficult to root for improved rooting success in *E. camaldulensis* and *E. tereticornis* mature clones is being practiced (Yasodha et al., 1997; Sharma et al., 2009). Further, *in vitro* propagation is required for 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. Advances were made towards the production of quality plantlets through micropropagation of species, such as eucalypts, acacia, teak and bamboos (Yasodha et al., 1997, 2003; Arya et al., 2009). Techniques of somatic embryogenesis developed in different species allow for reprogramming of juvenile phase in addition to fixing the desirable traits (Muralidharan, and Mascarenhas, 1987; Muralidharan et al., 1989; Sumathi et al., 2003; Arya et al., 2008). Somatic embryogenesis protocols are amenable for development of genetic transformation procedures.

APPLICATION OF MOLECULAR MARKERS IN TREE IMPROVEMENT AND PLANTATION FORESTRY

Tissue culture tools allow scalability for the propagules of identified clones. However, the techniques some time throw up altered phenotypes when they go through the process of repeated subculture. Molecular markers have been used for quality control of tissue cultured propagules and clonal fidelity testing (Tripathi et al., 2006). Tree breeding is aimed at increasing the frequency of superior phenotypes in plantations. The phenotype of an individual in a segregating population is the result of genotype of the individual and its surrounding environmental condition and developmental history. In trees, most phenotypic traits of interest are characterized by continuous variation. Hence, in tree breeding there has to be a clear distinction between traits targeted for breeding referred as “objective traits” and characters assessed during progeny trials referred as “selection criteria” (Apiolaza and Greaves, 2001). Reducing time in breeding for clones/ varieties is crucial, and therefore markers for early selection of these desired traits are being developed. Species/ clone diagnostic markers have been integrated into hybridization programmes at IFGTB (Balasaravanan et al., 2006). Markers have also been used for understanding the existing diversity of natural and breeding populations (Balasaravanan et al., 2005; Kamalakannan et al., 2006), determining mating patterns within seed orchards (estimating out crossing and selfing rates), and quantifying genetic drift in seed orchards. IFGTB is now developing markers for quantitative traits like wood property traits, adventitious rooting traits, and salinity and biotic tolerance using mapping populations developed for this purpose. In the recent years, Genomic selection methods have been shown to be more powerful than QTL mapping and candidate gene based association mapping in animal breeding (Meuwissen et al., 2001), and are being increasingly developed for application in tree breeding as well (Kirst et al., 2011; Grattapaglia et al., 2011).

APPLICATION OF GENOMICS AND TRANSGENIC TOOLS FOR TREE IMPROVEMENT

Development of reliable markers for these traits requires understanding the genes contributing to these traits. A number of genes governing desired traits are being identified as in the case of the WALLDOF gene, which could be used for wood density improvements (Gerhardt et al., 2011). With the availability of Eucalyptus Genome sequence, comparative genomic approaches with the poplar can provide valuable information regarding the function of the genes (Myburg et al., 2011). Gene homologues corresponding to two pathogen defense- related genes (class I chitinase, *Cechi1* and glucanase, *Ceglu1)* have been isolated, cloned and characterized from *Casuarina equisetifolia*. Isolation of secondary xylem specific cellulose synthase (*CesA*) genes, from *Eucalyptus tereticornis* is in progress. Transgenic methods for functional characterization genes *in planta* have been developed (Gherbi, et al., 2008; Creux, et al., 2011; Balasubramanian et al., 2011). System biology approach of studying integrated diverse networks is being pursued for understanding the phenotypic manifestation of traits like high and low lignin (Chiang et al., 2011). Transgenic methods provide an opportunity for integrating desired traits in high biomass selections, thereby complementing breeding efforts. Worldwide, a number of field trials of transgenic trees have shown promise (Strauss and Viswanath, 2011, Seguin, 2011, Lu and Hu, 2011). Field trials of trees with altered fertility, reduced lignin, enhanced insect, freezing, drought, and salt tolerance, and properties for bioremediation of mercury contaminated soils are underway. Leading private companies including Arborgen, USA, Shell Research Ltd, UK., and Monsanto do Brasil Ltd in collaboration with public research institutions are pioneering to alter the plantation forestry scenario towards transgenics. The perennial nature of trees species may also be an advantage for molecular pharming of drugs and vaccines. Advances have been made for bioproduction of vaccines like Chikunguna. Crucial bottlenecks for transformation have been the development of suitable tissue culture regeneration protocols like somatic embryogenesis for the desired genotypes amenable for transformation. Understanding the molecular basis of regeneration may be important for making regeneration possible from recalcitrant tissues and trees. Recently, it has been shown that there may be an association between telomeric repeats, and aging and regeneration ability in gymnosperm tissue culture (Aronen, 2011). Challenges like development of clone specific tissue culture regeneration protocols, knowledge on physiology / biochemistry of traits in tree species, long generation time, assessment of ecological risks and stability of transgenes, and public concern for biosafety remain to be addressed for tapping this powerful technology for the benefit of forestry.

APPLICATION OF BIOTECHNOLOGICAL TOOLS FOR CONSERVATION

Tissue culture tools provide non-destructive options for rescuing endangered plants. Molecular markers are being applied for obtaining information on diversity hot spots, the most diverse individuals and populations, and the trees contributing to the gene flow in natural forests, so that the extant diversity is captured in these conservation programmes (Drummond *et al*., 2000). DNA markers have been used to compare and assess the extant diversity of mangroves. RAPD and RFLP markers have been used to understand the intra population and inter population genetic variations in the mangrove species, *Excoecaria agallocha*. It was shown that populations from the West and East Coasts of India grouped into separate clusters (Lakshmi *et al*., 2000). Microsatellite analysis of the genetic structure in another mangrove species, *Avicennia marina,* revealed that most of the variation was between populations (41-71%), and within individuals in the total population (31-49%). There was little variation among individuals within populations (0-10%). Significant levels of inbreeding were also noticed in some populations (Maguire *et al.,* 2000).

DNA barcoding uses unique DNA sequences like the mitochondrial cytochrome c oxidase subunit I (COI) gene or the chloroplast genes, rbcL and matK, for identification of many species. These tools have also been developed for application in the field of forest forensics for detection of crimes related to overexploited trees and wild life, like enable verification of the country of origin of the CITES listed species. Bioprospecting for genes and novel molecules with pharmacological and pesticidal properties from our forest genetic resources is another area of intense research. Metabolic engineering for large scale non-destructive production of phytochemicals like taxol, from transgenic plant cell and hairy root cultures are being developed.

CONCLUSIONS

With the achievement of substantial genetic improvement of widely planted tree crops as evidenced by the increase in returns from plantations, the focus now should shift to making the benefits accessible and affordable to all those involved in plantation development. The demand for genetically improved planting stock is so huge that no single organization can fulfill it on its own. Hence networking among all stakeholders and decentralizing the planting stock production are prerequisites for making the research output useful to tree growers. IFGTB has introduced a new concept of developing "Community Seed Orchards" through which farmers and self-help group members are encouraged to establish their own seed orchard in government / village land with inputs from IFGTB in the form of planting material and technical support. The cost of maintaining the orchards for two years is also taken care of by the Institute in addition to capacity building of farmers in orchard management. The seeds produced will be used by the farmers themselves and any surplus will be sold to others like wood-based industries with quality assurance from IFGTB. The model orchards are already established in Tamil Nadu and Puducherry and several more are proposed to be established in the near future. The learning's made by working with exotics now need to be applied for the improvement of the thus far neglected indigenous species (Table 1)

To achieve the national goal of achieving a growth of 3 to 4 cubic meters of biomass per hectare per year, there is a need for enhanced scientific intervention using available genetic material and biotechnologies. The enhanced capacity of industries for utilization of pulp wood, in the context of limited land availability, demands that the productivity per unit area be significantly increased. In this context as well as considering scenarios of cli-

mate change, investments on breeding for traits like adaptability to flooding, drought, high salt and CO_2 environs need to be made. High-end molecular breeding tools like genomic selection and transgenics need to be developed and applied for addressing the most pressing issues of the rural society. Solutions would emanate from long-term mission mode approaches that involve investment in germplasm conservation and characterization, development of tools that facilitate quicker breeding, development of new breeding methods, and understanding fundamental biochemical pathways and gene networks governing desired traits. Comparative biology between Eucalyptus and poplars, functional gene testing and systems biology approaches should make possible the dissection of individual pathways for enabling targeted modification. Genetic engineering has been shown to be a powerful tool for breeding of plants with desired traits. Understanding gene flow, understanding public concerns and addressing them and development of novel trees that address issues that affect rural livelihood like enhanced tolerance to drought, salinity or flooding may eventually help in the improving public perceptions on transgenics. Ongoing linkages between forest departments, academia, and industries like ITC and members of Indian Plywood Manufacturing Association, need to be strengthened through funding concerted cross-institutional research that breaks transnational boundaries for bringing together domain experts so that solutions emerge for the challenges faced in forestry. To quote the Nobel Laureate, Richard Feyman, "We are at the very beginning of time of the human race. It is not unreasonable that we grapple with problems. But there are tens of thousands of years in the future. Our responsibility is to do what we can, learn what we can, improve the solutions and pass them on"

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Table 1: **The list of species prioritized for Forest Genetic Resource Management Network is given below**

S.N.	Prioritized Species		Networking partner for species
Phase I			
1	Tectona grandis	Indigenous	IFGTB, IWST, TFRI, AFRI, TNFD, KFD, APFD, KAFD, MFD, KFRI, KAU, FCRI, ASPEE, CTCRI, CARI, DBSKKV
2	Gmelina arborea	Indigenous	IFGTB, IWST, TFRI, RFRI, TNFD, KFD, APFD, KAFD, MFD, DBSKKV, ASPEE, TNPL, TBGRI, KFRI
3	Melia dubia	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD TNPL, FCRI
4	Casuarina equisetifolia	Exotic	IFGTB, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, DBSKKV, ASPEE, TNPL, CTCRI, TAFCORN
5	Eucalyptus camaldulensis	Exotic	IFGTB, AFRI, IWST, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, ANGRAU, TNPL, TAFCORN, MPM, WCPM
6	Ailanthus excelsa	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, ASPEE, FCRI, TBGRI
7	Eucalyptus tereticornis	Exotic	IFGTB, AFRI, IWST, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, TNPL, TAFCORN, MPM, WCPM
8	Anthocephalus cadamba	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, FCRI, TBGRI, KFRI
9	Pterocarpus santalinus	Indigenous	IFGTB, IWST, TNFD, KFD, APFD, APFDC, KAFD, CTCRI, NBPGR (Thrissur), FCRI
10	Acacia mangium	Exotic	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KAU, KFRI, MPM
11	Acacia auriculiformis	Exotic	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KAU, KFRI, MPM
12	Casuarina junghuhniana	Exotic	IFGTB, TNFD, KFD, APFD, APFDC, KAFD, MFD, FCRI, ASPEE, TNPL, TAFCORN
13	Calophyllum inophyllum	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, DBSKKV, NBPGR (Thrissur), TBGRI
14	Sapindus emarginatus	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI
15	Azadirachta indica	Indigenous	IFGTB, IWST, AFRI, TFRI, TNFD, KFD, APFD, KAFD, MFD, CTCRI, ANGRAU, FCRI, MFD
Phase II			
16	Tamarindus indica	Exotic	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, CARI, FCRI
17	Dalbergia latifolia	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CTCRI, KFRI

18	Dalbergia sissoo	Indigenous	IFGTB, AFRI, TNFD, KFD, APFD, KAFD, MFD, CTCRI, FCRI, TNPL
19	Artocarpus heterophyllus	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, ASPEE, CTCRI, NBPGR (Thrissur), TBGRI
20	Santalum album	Indigenous	IFGTB, IWST, TNFD, KFD, APFD, KAFD, KAFDC, MFD, ASPEE, CTCRI, FCRI
21	Pongamia pinnata	Indigenous	IFGTB, TFRI, TNFD, KFD, APFD, KAFD, MFD, FCRI, KFRI, DBSKKV, CARI
22	Aegle marmelos	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, TBGRI, KFRI
23	Pterocarpus marsupium	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI
24	Ailanthus triphysa	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI, FCRI, CTCRI
25	Terminalia chebula	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CSGRC, ASPEE, CTCRI, KFRI
26	Albizia lebbeck	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, KFRI, FCRI
27	Leucaena leucocephala	Exotic	IFGTB, TNFD, KFD, APFD, KAFD, MFD, FCRI, WCPM, CARI
28	Thespesia populnea	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD
29	Bombax ceiba	Indigenous	IFGTB, TNFD, KFD, APFD, KAFD, MFD, CARI
30	Bamboos (13 economically important bamboo species identified by NMBA)	Indigenous	IFGTB, IWST, RFRI, TNFD, KFD, APFD, KAFD, MFD, TNPL, KFRI, CARI, FCRI, TBGRI

Forest Genetic Resource Conservation and Improvement: Aspects and Prospects

Dr. H.S. Ginwal *

BACKGROUND

Prof. Champion, who realized the importance of geographical variations and application of the knowledge of forest genetics, started tree improvement work in India during 1930. He established a provenance trial of chirpine at New Forest, Dehradun and found that spiral gains in chirpine are inherited. Laurie during the same period established an all-India Teak provenance trial, which remained the most important one in Asia. Isolated efforts were made for improving forest trees by selection and breeding, but what has been lacking were a systematic co-ordination and a good action programme. Realizing the importance of this subject Forest Research Institute, Dehradun opened a Forest Genetics section during 1959-60 attached to Botany Branch under the then Directorate of Biological Research at this institute. In the year 1961 Prof. J.D.Mathews an expert from F.A.O. prepared guidelines for tree improvement programme of the country and work initiated on some priority species viz. *Tectona grandis, Bombax ceiba, Pinus spp. Dalbergia sissoo, Santalum album, Morus alba* and *Pterocarpus santalinus*.

Formation of Indian Council of Forestry Research and Education and thereafter reorganization of Forest Research Institute lead to the creation of Division of Genetics and Tree Propagation, during 1988. Similarly a full-fledged national institute i.e. the Institute of Forest Genetics and Tree Breeding (IFGTB) was established in April 1988 at Coimbatore. Consequently during 1994 World Bank funded project (FREEP) gave a significant boost to the field of forest genetics and forest biotechnology in the country.

Developing appropriate strategies for tree and integrating it with the tree improvement activities of the State Forest Departments is crucial. In this approach, the emphasis is on species oriented tree improvement programme in collaboration with State Forest Departments. The approach is in terms of development of Seed Production Areas (SPA), Bambusetum, Canatum, Clonal Seed Orchards (CSO), Seedling Seed Orchards (SSO), select germplasm for progenitors and clonal accessions of high value. Several hundred trees have also been identified as plus trees, propagated vegetatively and established as germplasm banks. Forest Research Institute, Dehra Dun, TFRI, Jabalpur, AFRI, Jodhpur, RFRI, Jorhat and IFGTB, Coimbatore has assembled different populations of various species. In addition, international provenances of Neem, Casuarina, Eucalyptus and Acacias have also been assembled. National level provenances for various species like; *Dalbergia sissoo*, Pines, acacias have also been assembled. Improved seeds from Clonal Seed Orchards and Seedling Seed Orchards of Casuarina, Dalbergia, Eucalyptus, Acacias and Neem are already made available for planting to user agencies. Forest Departments of different States and many Universities have also selected and developed their own genetic resources in consultation with ICFRE institutes in the form of seed production areas, seed orchards, plus trees and vegetative multiplication areas. The species in focus in various regions of the country by different ICFRE institutes is depicted in table 1.

Table 1: **Species in focus by different ICFRE institutes**

Region	Institute	Species focus
Northern	Forest Research Institute (FRI), Dehradun	*Eucalyptus* spp., *Shorea robusta, Dalbergia sissoo, Acacia* sp., poplars, *Leucaena leucocephala,* Himalayan pines, tropical pines etc.
	Himalayan Forest Research Institute (HFRI), Shimla.	*Pinus roxburghii, Picea smithiana, Abies pindrow, Dalbergia sissoo, Salix* sp., *Populus* sp., *Cedrus deodara* etc.
Western	Arid Forest Research Institute (AFRI), Jodhpur	*Tecomella undulata, Salvadora spp, Prosopis cineraria, Dalbergia sissoo, Tectona grandis*, *Acacia* sp., *Azadirachta indica*, *Ailanthus excelsa*, *Capparis decidua, Commiphora wightii, Eucalyptus* spp. etc.
North eastern	Rain Forest Research Institute (RFRI), Jorhat	*Gmelina arborea*, *Tectona grandis*, Bamboos, *Acacia* spp., *Dipterocarpus* sp. etc
Central	Tropical Forest Research Institute (TFRI), Jabalpur	*Tectona grandis*, Bamboos, *Acacia nilotica*, *Dalbergia sissoo*, *Albizia procera*, *Azadirachta indica*, *Gmelina arborea* etc.
	Institute of Forest Productivity (IFP), Ranchi	Eucalyptus, *Acacia catechu*, *Adina cordifolia*, tropical pines, *Leucaena leucocephala*, *Gmelina arborea* etc.

Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun

Southern	Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore	Eucalypts, *Casuarina* sp., *Tectona grandis, Acacia* spp., Bamboos, *Azadirachta indica, Tamarindus indica, Ailanthus excelsa* etc.
	Institute of Wood Science and Technology (IWST), Bangalore	*Santalum album, Pterocarpus santalinus*, Eucalypts, *Casuarina* sp., *Tectona grandis*, Bamboos, *Dalbergia latifolia* etc.

FOREST GENETICS AND TREE IMPROVEMENT IN NORTHERN STATES

During Vth plan period Forest Genetics and Tree Improvement got a significant boost when two central sector schemes were initiated. The first one in collaboration with DANIDA as " Indo-Danish on Seed Procurement and Tree Improvement" under which tree improvement was carried out on *Pinus roxburghii, P.wallichiana, Cedrus deodara, Dalbergia sissoo, D.latifolia, Albizia lebbek, Tectona grandis, Bombax ceiba, Gmelina arborea* etc. The second was "Creation of Radio Isotope Laboratory Facilities" to work on physical and chemical mutagenesis of forestry species, calculate the LD-50 dose and screen out variants for future tree breeding work. Two PL. 480 projects viz. "Techniques for inducing mutations and polyploidy in some hardwood and conifer species of importance in forestry (Project No. A7-F5-35) and other on "studies on induced poyploidy and mutagenesis in some hardwood tree species (Project No. A7-F5-73) were successfully completed in 1970 and 1981 respectively. The results of these studies indicate that mutation breeding and polyploidy breeding could be useful adjunct to conventional breeding methods in the species studied and on the related species. Improvement through hybridization, induced mutation and polyploidy of multipurpose tree species like Eucalyptus, Populus, Siris and Bamboos were other areas of research in which significant progress was made. To improve the quality of trees, intensive surveys were made throughout the country for selection of superior trees and collection of area specific seed material. This was intended to establish progeny trials to know the genetic worth of the selected superior trees, establish their orchards and clonal gardens for the production of quality seeds and vegetative material for raising improved plantations. In the process of genetic improvement, promising hybrids were produced, superior trees were selected, seed orchards, vegetative multiplication gardens and breeding populations of different species like Teak, Chir pine, Semul, Eucalyptus, Sandal, Red sander, Babul, Poplars, Neem, Rose wood and Bamboos etc. across the country were established. Propagation techniques for mass multiplication of improved tree species were developed. Germplasm of poplars, tropical pines, eucalyptus and Paulonia were introduced in different parts of India. Some of the species and their varieties are grown on commercial scale and are playing a vital role in economy of the country.

Beside FRI, University of Horticulture and Forestry, Solan; Punjab Agriculture University, Ludhiana; G.B.Pant Agriculture University, Pantnagar; JNKVV Jabalpur, Himalayan Forest Research Institute (HFRI), Shimla and State Forest Departments are also involved in tree improvement programmes of important tree species. The various activities of research-involved development of new clones and hybrids, establish seed production areas, plus tree selection and provenance delineation and establishment of seed orchards. The species in their priority were teak (*Tectona grandis*), chir pine (*Pinus roxburghii*), deodar (*Cedrus deodara*), bhimal (*Grewia optiva*) and shisham (*Dalbergia sissoo)*. The state agriculture universities were more focused on improvement of agroforestry trees species that can be grown under agroforestry system like Eucalyptus, Acacia, Poplars, Grewia, Shisham etc. G.B. Pant Agriculture University, Pantnagar has developed new promising clones of *Dalbergia sissoo* and is being tested under agroforestry systems. Punjab Agriculture University, Ludhiana has also been working on Poplars and Eucalyptus.

A brief summary of major tree improvement programmes initiated in past have been given below:

1. Eucalyptus

The genetic improvement for higher productivity of Eucalyptus through breeding approaches was initiated during 60's and has been continuing with strength till now. At Forest Research Institute, Dehradun promising inter specific hybrids in Eucalyptus popularly known as FRI 4, FRI 5 to FRI 15 have been developed (Venkatesh and Sharma, 1977 a, 1977 b, 1978, 1979, 1980**;** Kapoor and Sharma, 1984, 1984a, 1984b). Some of the species hybrid combinations have displayed sustained and pronounced degree of hybrid vigour (heterosis) both in respect of height and diameter and produced 3 to 5 folds more volume of wood than the parental species (Venkatesh and Sharma, 1977a, 1977 b, 1979, Kapoor and Sharma,1984). Wood properties of some of the hybrids possess better pulping quality. Some of the hybrids have been clonally multiplied through tissue culture and deployed in the field under different eco-climatic conditions. The superior recombinant of FRI-14, FRI-15 and FRI-5 were multiplied through tissue culture technique and their trials/demo plots established at multiple locations at Hoshiarpur, Hissar, Bithmeda, Pantnagar, Haldwani, Dehradun, Meerut and Jodhpur for evaluation of their performance.

- For improvement of seed quality and planting stock in various states, seedling seed orchards and clonal seed orchards of Eucalyptus were established at Seothi and Bithmeda (Haryana) (approx. 20 ha). The research wing of Haryana is able to harvest approx. 100 kg seeds per year of Eucalyptus from these orchards for their plantation programmes. Seed production area of *E.tereticornis* established in Uttarakhand (11.80 ha) and Punjab (1.0 ha).
- In order to broaden genetic base and productivity of Eucalyptus plantations in India, collaboration was made with Australian Seed Centre, CSIRO Australia and open-pollinated seeds of 91 seedlots (families) of *E. tereticornis* belonging to 13 provenances of Papua New Guinea and Australian origin were obtained. Seed source evaluation trials were established at different locations viz. FRI campus, Manakpur (Haryana) and Midnapur (West Bengal).
- Evaluation of different sources and families of *Eucalyptus tereticornis* revealed better field performance of North Queensland provenances and in particular two provenances

viz. Walsh River and Burdekin ranked the best in comparison to others. These two sources are recommended for their use in seed raised plantation programmes.

- Twenty-four new clones developed from the Australian germplasm and established in VMG at FRI. A clonal trial with 13 new clones established at Manakpur (Haryana).
- Multi-location evaluation of sources of *Eucalyptus camaldulensis* of Australian origin revealed two seed sources viz. Emu Creek Petford and Laura river, from Queensland as best growth performing source, and recommended for plantation programme in semi arid region of Haryana and Punjab.
- Provenances of *Eucalyptus tereticornis* of Australian origin were characterized with DNA marker technique for genetic diversity and genetic relatedness of populations.
- Established breeding arboretum of Eucalyptus by assembling genetically divergent genotypes from different sources to facilitate controlled pollination studies and obtain hybrids.
- Clonal multiplication areas (VMGs) for production of juvenile cuttings for raising clonal plants of Eucalyptus established in Haryana (8.5 ha with 42 clones).
- Besides above, it is to mention here that FRI was instrumental in introduction of germplasm of Eucalyptus in various parts of the country, which is being used by forest department, progressive farmers and industries. Many private companies have used this germplasm for selection and development of clones being marketed in their own name.

2. Poplars

Populus deltoides, an exotic poplar, is the most important poplar species in India's forestry horizon. It is intensively planted in agroforestry plantations in north Indian region of Punjab, Haryana, western Uttar Pradesh, plains/ outer valleys of Uttarakhand, Himachal Pradesh and Jammu. It grows very fast and gives a mean annual increment (MAI) of 20 to 25 m^3/ha/yr in irrigated fields of farmers.

- Exotic poplars were first introduced in India during 1950; their plantation in hills gave encouraging results. Systematic research on exotic poplars began in 1958. Till 1983, more than 440 clones of various species of *Populus* were introduced from Europe, North America and Australia.
- From field trials, *Populus deltoides* emerged as one of the most promising species of this genus in India. Superior clones of this species were identified and ranked on the basis of field trials. Germplasm that originated from the southern part of USA usually showed the best growth rate.
- To further increase the productivity of poplar in India, FRI Dehradun introduced germplasm of poplar, in form of seeds, from southern and south-eastern region of the USA in 1996.
- Cloning of the best seedling progeny and field trial of the clones, that concluded in 2008, resulted in identification of some (26) highly promising clones : FRI-AM-58 recorded mean annual increment (MAI) of 43.25 m^3/ha/yr in comparison with 28.75 m^3/ha/yr recorded by G48, the most popular clone in agroforestry plantations. As many as 26 clones have shown greater growth rate than G48.
- Hybridisation has also been carried out among the best clones of previous introductions. Hybridisation has also been achieved between *P. deltoides* and *P. euphratica*. The new hybrid clones have been assembled in germplasm bank for further testing.
- Poplar has been introduced by ICFRE in Bihar state so as to extend area under its cultivation. A field trial of FRI's promising clones has also been established in district Vaishali.
- Clone G48, presently the most popular clone of *P. deltoides* in Indian agroforestry, was introduced by FRI Dehradun. It constitutes about 55% of the entire poplar plantations in the northern India, i.e. about 275 lakh trees of this clone are standing in agroforestry plantations in India . Clone D121, S7C8, 82-35-4, S7C4, S7C15, etc. introduced by FRI make up about 75 lakh trees. Clone G3, another clone introduced by FRI in 1960s is not much planted now, except in relatively drier sites, due to its increasing susceptibility to diseases. New clones produced from progenies of G48 by private companies and Uttarakhand State Forest Department make another 130 lakh trees in agro forestry.

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- Various species and clones of poplars were introduced from Germany, UK, USA, Netherlands, Japan, China, and Australia.
- Clonal selection of hybrids of *P. cliliata* x *maximowiczii* (UCM-3287, UCM-3296, UCM-113) and a three way hybrid of *P.deltoides* (UD-Solan-1, UD-Hyb-U *)* have also been found to be promising under field demonstration trials.
- The *Populus ciliata* and its hybrid clones recommended for higher productivity include UFC-1000, UFC-1900, UFC-2200, UFC-010, UFC-6403, IL-3B for areas above 1,500 m; and HybridsUCM-3287, UCM-3220, UCM-3296, UCM-2801, UCM-113 for areas between 1,000 to 1,500 m.
- The recommended clones and hybrids for different areas are UD-5503, UD-6502, UD-10007, UD-3210, UD-3296, UD-8800 and Hybrids Solan-1, Hyb-U for areas between 1,000 to 1,500 m; UD-0102, UD-0700, UD-6500, UD-4400, UD-7007, UD-8800, IC, 200/86, 52/86 and P1/92 for areas between 300 to 1,000 m; and UD-5501, UD-5512, UD-6501, UD-6502, UD-1007, UD-63N, G-3, D-121 and S_7C_{16} for areas less than 300 m.
- The clones namely UD-63N, UD-8800, UD-5501 and UD-6502 along with hybrids Solan-1, UCM-3287 and UCM-3296 are recommended for plantation in middle Himalayan zone.
- Clonal archieve (gene bank) of about 200 poplar clones are being maintained at germplasm bank of the university for future breeding trials, as well as a source of clonal material for stool-bed and for reference identification.

3. Willow (*Salix* species)

- About 250 clones of different willows (*Salix* species) have been procured form 20 different countries covering 40 species for testing and selection in Himachal Pradesh. Amongst these 22 clones have been found to be promising in the nursery trials. However, out of these 15 were superior in first stage field trial. Some of these clones have also been planted on farmers fields as multilocation trials in Himachal Pradesh.
- Two in *situ* conservation plots cum demonstration trials have been raised at RHFRS, Bhota and RHRS, Bajaura for testing, multiplication and distribution of planting material to the

farmers and plantation agencies etc.
- Clonal material of promising clones of willows has been supplied to H.P. Forest Department, HFRI, Shimla, Punjab Agricultural University, Ludhiana, Haryana Agricultural University, Hissar, North Bengal Agricultural. University, Coochbehar, GBP University, Pantnagar and FRI Dehradun for further testing.
- DNA finger printing of different clones have been done.
- Control breeding (hybridization) work and development of hybrids are in progress.

4. Teak (*Tectona grandis*)

- Work on the selection of Teak plus trees was started in the year 1960 and to date about 700 plus trees are available for establishing clonal seed orchards.
- The first experimental seed orchard, was established at New Forest, Dehra Dun using 20 clones with 20 ramets replicated two times, and this orchard has provided very useful information on initiation of first flowering, variation in flowering of different clones, seed production and their germination.
- To meet the interim need of seeds for planting about 10,000 hectare seed production areas have been created in different states by treating good stands.
- To meet the future demand of quality teak seeds nearly 900.00 hectare of seed orchard has been created in different states of the country.
- Identified 3 genotypes for superior growth, frost resistance, and wavy grain timber characteristics. Established one biclonal, clonal seed orchard to produce hybrid seed combining two traits.
- Interspecific hybridization work has been carried out between *T. hamiltoniana* and *T. grandis* but no seed could be obtained due to abortive embryo.
- In a study of 80 batches of teak trees, selection differential and predicted genetic gain values were estimated. This involved individual character variation analysis, estimation of heritability and computation of predicted gain values. Predicted gain values were estimated for individual batches alongwith overall estimates.

5. Pine improvement

- In the provenance trial of *Pinus roxburghii* at Dehra Dun considerable variation in oleoresin yield was found among the trees in the different provenances, in some provenances there were high-yielders of 8 to 9 kg. A number of plus trees specifically for the trait of high oleoresin yield and growth were selected in the State of Uttar Pradesh and Himachal Pradesh. National Provenance trials of this species has been established at a number of locations and data so far collected and analysed from 4 locations has revealed the superiority of two provenances in all the four locations. Precocious flowering for 7 provenances at the age of 5.5 years was also recorded.
- Using a half-sib progeny trial, studied genetic variation in morphological growth and wood characters and obtained heritability estimates for different traits, phenotypic and genotypic correlations among various wood characters were also studied.

6. Semul (*Bombex ceiba*)

- Studies have been conducted on the reproductive biology and breeding behaviour of this species. The objective of tree improvement work in this species was to evolve varieties, which will be fast growing with good stem form, narrow crown and without buttresses. Since each fruit contains 200-300 seeds and the percentage of germination of seed is very high, it has been estimated that half a hectare of seed orchard can yield enough seeds to plant up to 500 hectares. Seed orchards of the species were established in U.P. and are in production.

7. Santalum album

- Sandal is a highly variable polymorphic species, six types namely, ovate, lanceolate, elliptic, linear, big and small leaves have been identified To maintain the variability in this species both in situ and ex situ conservation work has been carried out.
- Clonal propagation by top cleft grafting method carried out in Sandal Research Centre, Bangalore, gave 80% success and three clonal seed orchards were established.
- Clonal multiplication through surface root bits was developed at Forest Research Institute. Polyploidy has been induced with colchicine treatment and tetraploid have been produced.

8. Shisham (Dalbergia sissoo)

The Forest Research Institute, Dehradun has been working on the genetic improvement programme of *Dalbergia sissoo* since 1990. Though this species has a number of promising attributes, it exhibits poor stem form (crooked stem), forking, ramicorn branching and susceptibility to the dieback. In genetic improvement programme of the species, a number of plus trees from various locations have been selected and assembled in the gene/clone bank. Initially the selection of promising trees was carried out in the states of undivided UP, Rajasthan, Bihar, Nepal and other shisham growing reasons, the genetic worth of these genotypes *per se* needs to be tested in the filed. Keeping these points in view, different programmes with following objectives are being carried out;

- To produce quality seed through establishment of seed orchards (both seedling and clonal seed orchards)
- To identify most promising genotypes through multi-locational clonal trials and estimate genetic parameters and genetic correlations
- To assess adaptability, stability and genetic resemblance among the clones
- To characterize and develop genetic relationship in selected genotypes for physiological and wood traits

The quality seed being produced in the seed orchard of the species established in the states of Punjab, Haryana, Uttarakhand, Himachal Pradesh and Uttar Pradesh. The seed used for raising the plantations of the species in different states and majority of new plantations of the species are being raised using the improved seeds. Though the second-generation seed orchards have also been established recently, the production of seeds for the same would still take 4 to 5 years.

- From the various clones developed, four clones of *Dalbergia sissoo* viz. 66, 218, 237, 12 have been found most productive and adaptable. These clones are being multiplied since last 3

years and used by the SFDs of Haryana and Punjab and also being used by farmers and industries.

- Seed orchards of *D.sissoo* established at Hoshiarpur, Ludhiana, Patiala and Bithmeda are producing quality seeds and these improved seeds are being used by SFDs for plantation programmes. (During the year i.e. 2010, Haryana Forest Department has harvested 2500 kg. pods of *D.sissoo* from the seed orchard established by FRI)
- An advanced generation seed orchard established at Pindori Mindo Mind (Punjab) for production of quality seeds (producing 10 Kg seed per year).

Further, the clonal banks and the multiplications gardens have also been established in above-mentioned states to encourage the deployment of superior clones so that productivity of the plantations could be improved. On the other hand the clones, which are showing the resistance to substantial degree, are being multiplied and planted. Training the officials of the state forest departments in the clonal technology has done this and the infrastructure has also been established in the respective states.

9. Neoza pine (Pinus gerardiana)

- Twenty-five plus trees has been selected in Kinnaur districts.
- Variation studies revealed significant differences among and within stands for height, diameter, number of cones, cone size, number of seed per cone, seed size kernel/nut coat ratio etc.
- If all biotic interference is excluded, complete closure of the area was observed to encourage natural regeneration.
- Cleft grafting techniques at Sharbo and Kalpa during February on two to three years old seedlings with partial shade in the species gave 70-80% success.

Achievements in other species

- Many exotic tree species viz. *Pinus radiata, Pinus pseudostrobus, Pinus systeris, Platanus occidentalis, Eucalyptus glauca, Acacia mollissima, Robinia pseudoacacia* were introduced by UHF Solan.
- The plus trees have been selected in sal, chir-pine, deodar, chilgoza, wild pomegranate, Himalayan poplar, white poplar, toona, alder, khair etc
- Genetic diversity of *Taxus wallichiana* based upon morphological and molecular observations was estimated.
- High yielding trees of Harar (*Terminalia chebula*) have been identified and grafting technique standardized and the genotypically superior grafted plants are being made available to the farmers.
- Reproductive biology of *Bombax ceiba*, *Populus ciliata, Bauhinia variegate*, *Salix* species and *Grewia optiva* has been studied for seed orchard development and hybridization.
- Multilocation trials on promising clones of willow are being laid out within Himachal Pradesh and other parts of the country (Punjab, Haryana, U.P, J&K, Gujarat, Maharashtra, Rajasthan, Tamil Nadu, Kerala, Assam and Uttarakhand).
- The variation in wood basic density and fibre length was assessed in twenty-five promising willow clones collected from five different countries.
- Propagation technology of seabuckthorn has been developed. Studies on variation, pollination, propagation, plantation, fruit harvesting and seed pre- treatment, physical and chemical evaluation of seed and pulp oil and ecological studies of the seabuckthorn have been done.
- Progeny trial-cum-seedling seed orchard of chir pine, beul and stackling orchard of poplar species has been established.
- Protocols for multiplication of *Robinia pseudoacacia*, *Morus alba, Grewia optiva*, C*eltis australis* and *Bauhinia variegata in vitro* conditions have been developed.
- Mutation studies through physical and chemical mutagens revealed that lower doses of continuous and fractionated gamma irradiation and hydrazine hydrate stimulated the germination and survival of seedlings. The treatment of dormant shoot cuttings treated with lower doses of gamma radiation above (1to2 KR) lead to the reduction of thorn size.
- Large variation among different seed sources was assessed with respect to seed, physical characters and seedling growth characters. Seed sources of *Berberis lycium* viz; Dharampur, Rajgarh and Palampur were found to be the best for growth and biomass related characters. Berberene content was found highest in Rajgarh and Palampur seed sources.
- Clonal evaluation of mulberry carried out and the superior clones being recommended for raising in farm forestry and Agroforestry. The prelimnary observation revealed clones; China white, S-30, S-799 and S-1531 are quite promising.

2. Forest Genetics Tree Improvement in Northeast

Rain Forest Research Institute, Jorhat carried out genetic improvement and conservation work in six species of bamboo namely *Bambusa balcooa, B. pallida, Bambusa nutans, B. bamboos, Dendrocalamus hamiltonnii* and *Bambusa tulda* and more than 300 plus clumps has been selected in different ecological regions of north eastern states namely Assam, Nagaland, Mizoram, Tripura, Arunachal Pradesh, Meghalaya and Manipur. The important characters considered for selection were internodal length, culm diameter, fiber and pulping characteristics. Propagation protocols have been developed for these species and propagation work is in progress. Gene bank of selected bamboo germplasm is established at RFRI Jorhat and Aizwal. A multilocational trial of six species and twelve clones of each species is going on at six places in four states namely Assam, Mizoram, Nagaland and Tripura.

Species wise field assets established by RFRI are listed below. These assets are being utilized for raising stock for plantations and various training programmes as well as for on field demonstrations.

Gmelina arborea

- Clonal seed orchard, Seedling seed orchard–cum-progeny trial, Vegetative multiplication garden at Deovan campus, Nahorani, Jeypore (Assam), Agartala (Tripura) and Imphal (Manipur)

Tectona grandis

- Vegetative multiplication garden established at Deovan; CSO and SSO at Nahorani (Assam); CSO at Teliamura (Tripura); CSO and SSO at Imphal (Manipur)

Dipterocarpus retusus

- SSO-cum-*progeny* trial established at Deovan; SSO-cum-progeny trial at Nahorani; SSO at Jeypore (Dibrugarh) (Assam)

Acacia mangium

- SSO-cum-progeny trial established at at Deovan (Assam)

3. Forest Genetics Tree Improvement in Western States

The establishment of Arid Forest Research Institute, Jodhpur has added the much-needed impetus to the forest genetics and tree Improvement research endeavours of Western part of the country on initiation and implementation of planting stock improvement programme. After WB-FREEP the activities related to tree improvement were continued under various research projects viz. UNDP, NOVOD etc. During all these project emphasis was given to provenance testing, selection of phenotypically superior individuals their cloning and genetic testing. During last one and half decade AFRI has established many provenance trials and seed production populations, and vegetative propagules production populations. Besides CAZRI Jodhpur and universities has also contributed significantly in planting stock improvement programme of western states.

The brief account of the Tree Improvement activities pursued by AFRI is as follows.

Provenance Trials: Provenance trials of Neem **(***Azadirachta indica),* Babool *(Acacia nilotica),* Marwar Teak *(Tecomella undulata)* and Sisham *(Dalbergia sissoo)* were established at AFRI and a brief summary of results is given in following table:

Species	Year	Provenances	Best Performer
Acacia nilotica	1991	28	Shivpuri
Tecomella undulata	1991	13	Sikar
Azadirachta indica	1992, 1995	39 (Ind), 18 (Int.)	Palanpur , Sunyani
Dalbergia sissoo	1995	13	Pilibhit

Selection of Phenotypically Superior Trees:

Candidate plus trees of priority tree species namely *Acacia nilotica* (80), *Eucalyptus camaldulensis* (30*), Dalbergia sissoo* (50), *Tectona grandis* (60), *Tecomella undulata* (30) and most importantly *Azadirachta indica* (400) were selected.

Establishment of Production Populations:

Seed Production Area (SPA): The Institute has established 200 ha. SPA of four priority trees species *viz. Acacia nilotica* (75 ha), *Tectona grandis* (85 ha), *E. camaldulensis* (10 ha) and *Dalbergia sissoo* (30 ha).

Seedling Seed Orchards (SSO): *Acacia nilotica* (20 ha), *E. camaldulensis* (16 ha) and *Dalbergia sissoo* (19 ha).

Clonal Seed Orchard (CSO): *D. sissoo* (15 ha) and *E. camaldulensis (*14 ha).

Vegetative Multiplication Garden (VMG): *D. sissoo (*72 clones) and *E. camaldulensis (*26 clones).

Genetic Test Trials

Progeny trials: Progeny trials of following three species are going on under different projects.

A. *Tecomella undulata* (40 CPTs)
B. *Azadirachta indica* (30 CPTs)
C. *Jatropha curcas* (116 CPPs)

Clonal trials: Clonal trials of following four species are going on under different projects.

A. *Dalbergia sissoo* (30)
B. *Eucalyptus camaldulensis* (35)
C. *Jatropha curcas* (185)
D. *Commiphora wightii* (22)

In addition this, the Institute had also developed macro and micro propagation techniques for seven and four species namely *Ailanthus excelsa, Tecomella undulata, Azadirachta indica, Acacia nilotica, Dalbergia sissoo , Eucalyptus camaldulensis* and *Ailanthus excelsa:* and *Acacia nilotica, Azadirachta indica* and *Balanites aegyptiaca,* respectively.

Hence, a tremendous effort has been put in and the populations are on the ground. It needs networking, integration and development. Many of these collections can be of use as base population for tree improvement programme and production of high yielding varieties or clones. More importantly, the high yielding clones can form the basic material for development of regeneration protocols and for direct introduction of required genes through transformation approaches. In view of the fact that the trees have long rotation period, the clonal material can be effectively used for introgression of genes for different traits. In fact, biotechnology will play much greater role in tree improvement in future. "Tree Improvement " to "trait improvement" for "product" is the approach. It can provide trees tailored for cellulose, lignin, sodic and saline soils, even ideotypes for agroforestry. Support is required to inventorise, evaluate and characterise the germplasm and network it as Tree Genetic resource for which a massive effort is needed. Converting the germplasm into tree genetic resource is a requirement for production forest

CONSERVATION AND MANAGEMENT OF FOREST GENETIC RESOURCES

The growing attention to conservation reflects the increasing concern about alterations in the forests and the long-term maintenance of the health and overall productivity of forests and forest ecosystems. Considerations related to FGR in India have been integrated within broad frameworks, such as national forest programmes and biodiversity action plans (the Biodiversity Bill 2002). Both *in situ* and *ex situ* conservation strategies are followed for the conservation of forest genetic resources The management of an appropriate combination of genetic resources in various locations under diverse environmental and silvicultural practices, such as provenance trails and progeny trials, is considered to be the most efficient way to conserve various levels of genetic variation to increase the productivity. The *in situ* conservation measures include establishment of preservation plots, seed stands, seed production areas, biosphere reserves, national parks and sanctuaries, protected areas and tiger reserves in ev-

ery state. There are 14 biosphere reserves, 97 national parks and 508 wildlife sanctuaries, which include natural habitats of bamboo and rattan as well. Biodiversity on these plots is periodically monitored. The local people in sacred groves also protect these species. *Ex situ* conservation virtually safeguards and provides a required supply of germplasm for research and breeding. The various possible approaches in *ex situ* conservation of FGR are establishment of botanical gardens, arboreta, herbal gardens, clonal repositories, herbaria, provenance trials, seed orchards and cryogene banks. A list of seed production areas and seed orchards in different states is given in Table 2 and Table 3 respectively.

APFORGEN

The Asia Pacific Forest Genetic Resources (APFORGEN) Programme was initiated in 2003. APFORGEN is a regional programme with a holistic approach to conservation and management of forest genetic resources. Its aim is to enhance technical and scientific cooperation, training and information exchange among countries in the region. It is managed by the Asia Pacific Association of Forestry Research Institutions (APAFRI) with technical support from Bioversity International (Bioversity). Target beneficiaries of this programme include forest research institutions, policymakers, local communities, government forestry departments, NGOs and private forestry companies. Other international and regional organizations such as FAO are also participating in the development of the programme and its activities. The objective of APFORGEN is to manage tropical forest genetic diversity more equitably, productively and sustainably in the participating countries, specifically the programme aims to:

- Strengthen national programmes on forest genetic diversity
- Enhance regional networking and collaboration
- Facilitate to locate and conserve genetic diversity of selected priority forest species
- Increase sustainable use of genetic diversity in natural and man-made forests

APFORGEN currently has fourteen participating country organizations from India (Indian Council for Forestry Research and Education), Bangladesh (Bangladesh Forest Research Institute), Nepal (Department of Forest Research and Survey), Pakistan (Pakistan Forest Institute), Sri Lanka (Forest Department), Cambodia (Department of Forestry and Wildlife), China (Research Institute of Forestry, Chinese Academy of Forestry), Indonesia (Centre for Plantation Research and Development, Bogor), Lao PDR (Forest Research Centre), Malaysia (Forest Research Institute Malaysia), Myanmar (Forest Research Institute, Yezin), Philippines (College of Forestry and Natural Resources, University of Philippines Los Banos), Thailand (Royal Forest Department/National Park, Wildlife and Plant Conservation Department) and Viet Nam (Forest Science Institute of Viet Nam).

ICFRE has been regularly participating in the meetings and programmes of APFORGEN in past. A 'National workshop to Identify Stakeholders and Capacity Building Needs in Forest Genetic Resource Conservation' was organized at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore on 11 July 2007. As a result of various national and international workshops under APFORGEN the following task have been targeted by various member countries under APFORGEN:

- Review of the national *in-situ* and *ex-situ* FGR conservation and development programmes of various countries, including the priority tree species.
- Identification of capacity-building needs of the stakeholders in support of the national FGR programmes.
- Identifying the R & D needs necessary to support the FGR conservation and development programmes.
- Proposal for national FGR strategies and programmes.
- Commitments and support from institutions and individuals to be part of regional and National Coordinating Committee for FGR. Updated information on FGR conservation and use (including *in situ* and *ex situ* FGR conservation programmes)

Forest Genetic Resource Management Network (FGRMN)

The Forest Genetic Resources are provider of products and services for economic development. There is huge wealth of unexplored potential of genetic resources, which are very vital, more so in view of impending threats of climate change looming large for extinction of threatened/vulnerable species. Undocumented and uncontrolled movement of germplasm is also a major cause of concern of the day. Hence, there is an emerging need for conservation and preservation of forest genetic resources for biodiversity/ecological security and for posterity. Recent international developments have led to sharp focus on management and conservation of forest genetic resources including their access and benefit sharing. In the agriculture sector, Indian Council of Agriculture Research has initiated major steps for conservation of the genetic resources of agriculture importance through five dedicated national bureaus at various parts of the country for various organisms (National Bureau of Plant Genetic Resources, National Bureau of Fisheries Genetic Resources, National Bureau of Agriculturally Important Microorganism, National Bureau of Animal Genetic Resources, National Bureau of Agriculturally Important Insects). In the forestry sector most of the genetic resources are scattered and there was no dedicated institutional mechanism in the country for collection, reposition and preservation of forest genetic resources at central point.

Keeping in view, the proposal for creation of National Bureau of Forest Genetic Resources (NBFGR) was deliberated in, MoEF, Govt. of India. It was decided that it would be appropriate to create and support a Forest Genetic Resource Management Network (FGRMN) which will help in the establishment of NBFGR in future. It was decided that in the first phase the ongoing activities towards activities related to Forest Genetic Resource Management in two institutes at FRI Dehradun and IFGTB Coimbatore may be institutionalized in the form of a network. Hence, a Forest Genetic Resource Management Network (FGRMN) has been initiated at FRI Dehradun and IFGTB Coimbatore by utilizing a part of one time special grant of Rs. 100 crore.

Mechanism

- Collection, documentation, characterization of forest genetic resources and their conservation in collaboration with state forest departments, ICFRE institutes and other national organizations/institutions.
- Establish ex-situ germplasm banks/repositories for conserva-

tion of FGR using appropriate tools technologies and dissemination of material and information.

INTERVENTION THROUGH FOREST BIOTECHNOLOGY

The increasing demand for wood and wood products, has recently led to the introduction of several molecular and biotechnological tools into forest-tree improvement and breeding. Forest tree biotechnology emerged during the 1980s and encompasses a developing collection of tools for modifying tree physiology and genetics to aid breeding, propagation, and research (Burdon and Libby 2006). Biotechnology is not a single approach, but instead encompasses tissue culture, micropropagation, genetic engineering, and genetic markers. A combination of biotechnological tools and classical breeding techniques may benefit to the improvement of forest-tree species. The potential of biotechnology for accelerating forest tree breeding programmes can be realized at several levels:

1. Clonal propagation of superior genotypes using tissue-culture techniques;
2. Somatic hybridization using protoplasts, haploidization and exploitation of somaclonal variations;
3. The use of induced mutations;
4. Molecular breeding to complement classical breeding (e.g. the use of marker-assisted selection); and
5. Direct rapid introduction of specific traits via genetic engineering of forest-tree species.

Techniques for the *in vitro* propagation of several forest species, both angiosperms and gymnosperms, have been extensively researched and developed that include axillary-shoot multiplication, organogenesis and somatic embryogenesis. Somatic-cell techniques have already been developed for several forest-tree species. With the advances made in establishing genetic maps for some forest-tree species, notably *Populus* and many conifers, the use of DNA-marker-assisted selection should help in assessing the products of tree breeding and identifying superior offspring. Molecular markers can assist in the identification of diverse populations to begin with breeding programme and isolation of tree-specific genes from selected species. These genes can then be introduced through transformation methods into selected tree species and the transgenic trees cloned and propagated in tissue culture. When these goals are realized, future forest trees will be more tolerant to abiotic and biotic stresses, express genes for accelerated growth rate and have a modified wood structure. Such trees will bring forestry into a new era of productivity and quality.

In India, forest biologists are applying biotechnology in forest trees because these methods can help save time, reduce costs, or accomplish new goals. For example, genetic markers are beginning to be integrated into traditional breeding programmes to enhance genetic diversity, speed the notoriously slow rate of progress over generations, and to reduce the costs of selection. Other approaches, such as embryogenesis as a means of multiplication and amplification of the best performing clones, is seeing increasing use in conifer forestry. For the forestry industry, intervention of biotechnology could be more important. Identifying links between specific gene markers and desirable traits makes it possible to breed genetically superior trees for specific traits, higher yields and new end uses for specific species.

Overall understanding of tree molecular biology has advanced dramatically in recent years. The completion of genome sequences for model plants such as *Arabidopsis* and especially *Populus*, along with development of high throughput genomics tools for gene discovery and functional assignment have set the stage for continued rapid progress. This includes our understanding of the genetic characterization of complex traits such as growth, cold and drought tolerance, insect and disease resistance, and so forth.

The following are the main tools and techniques of biotechnology being largely used in forestry sector:

Molecular markers: The use of molecular markers has greatly enhanced our understanding of the genome structure of forest trees. Development and optimization of specific markers such as microsatellites and SNPs for tree species are currently very active in India. Studies on population genetics, genetic structure, genetic diversity in natural and planted forests and gene flow are based on the use of molecular markers. Many tree species of temperate and tropical origin viz. Pines, Cedrus, Poplars, Eucalypts, Dalbergia, Bamboo, Teak, Acacias, Casuarina, Gmelina and Dipterocarps have been well investigated using DNA marker techniques. Markers will probably continue to enjoy increased application in forest genetic studies (diversity and conservation, phytogeography, mating systems) and tree improvement (fingerprinting, paternity analysis, breeding and testing, QTL mapping, MAS, association genetics), though most effort is likely to be concentrated on a few highly valued species. These applications are becoming increasingly commercial in scope. There is need to determine the extent of genetic variation for species native to our country viz. teak, *Pinus roxburgii*, *Dalbergia sissoo* etc to devise *in situ* conservation strategies

Genetic maps, Marker assisted selection and Genomics: Marker-assisted selection (MAS) has given further impetus to tree breeding and selection. Molecular markers are genetically linked to a given allele on a given locus and can therefore be used to predict the presence of the allele with great accuracy. Genetic maps can be used to assess the degree of evolutionary relatedness of a number of species and identify the zones where gene variations are statistically linked to the variability of quantitative traits. These zones are called QTLs (quantitative trait loci). QTL research focuses on the genetic architecture of traits of interest. Proteomic analysis offers an important field of basic research that many research teams are willing to develop. Proteomic studies can find applications in such fields as biotic and abiotic stress response, effects of genetic mutations, gene expression regulation, QTL validation and genetic variability (Plomion 2000; Pilate *et al*. 2002).

Bioinformatics: Biotechnology requires the handling of massive catalogues of genes, transcripts and proteins hence investments in the field of bioinformatics are particularly valuable for the country.

Micropropagation: The development of large-scale vegetative propagation techniques, essentially based on micropropa-

gation or somatic embryogenesis, makes it theoretically possible to deploy superior planting materials rapidly and effectively. This is already the case for some coniferous and broad-leaved tree species including *Eucalyptus, Dalbergia, Pines, Bamboos, Azadirachta indica Acacia nilotica Ailanthus excelsa* and teak (*Tectona grandis*). Several Indian laboratories have developed protocols for micropropagation of woody forest plants. Several funding agencies including ICFRE, DBT, NMPB and DST have supported research activities throughout the country. Two micropropagation technology parks are established at TERI (New Delhi) and NCL (Pune). Extensive work on micropropagation of tree species have been carried out in last two decades but still there is a need to establish linkages between institutions and user agencies

Genetic modification: Genetic modification has been applied to forest trees mainly during the last two decades, at the experimental stage in the laboratory. However, globally, genetic modification research seems to diminishing, and some scientists have expressed mixed feelings about future applications of the technology. Many private forestry products companies are reluctant to engage in research on genetic modification, because of several factors: consumer unease, public relations risks for companies engaged in the research, unpredictable and costly government regulations, government bans against genetic modification, limitations due to fragmented patent estates and strict intellectual property rights, etc.

The Energy and Resources Institute (TERI)

TERI has set up an MTP (Micropropagation Technology Park) in its sprawling 36-hectare campus at Gual Pahari, Gurgaon, having an annual capacity of producing 2 million tissue cultured plants, with the financial support of Department of Biotechnology, Government of India. Micropropagation protocols for over 70 plant species including forest species, fruit crops, medicinal and aromatic plants, and ornamentals (both foliage and flowering) have been developed. TERI has been working in the areas of crop improvement and molecular characterization in various plant species for identification of varieties/clones. The resulting information helps the breeders to select the best parental plants for generating hybrids/improved varieties. With the help of DNA profiling, the genetic diversity of tea, neem, poplar, and some medicinal plants has been estimated. Besides putting applications 'into practice' the Group at TERI is engaged in gene cloning, DNA sequencing, molecular mapping, and tagging of genes and DNA fingerprinting.

Prospects and Priorities in Forest Genetics and Biotechnology

The productivity of the plantation can be increased sustainably by promoting technology based plantations. Of late in India, clonal forestry is gaining acceptance among the progressive Foresters and Plantation Managers. The ultimate objective of tree improvement is also to produce quality seeds that are the carriers of entire improvement made in one generation to the next generation. Keeping in view the improved superior seeds are the major source for propagation. Selection and testing the best clones is a prerequisite for clonal forestry. For that purpose, newer and further improved sexually produced material through seeds needs to be developed which will displace the material, which is currently in use. This calls for the continuity of seed and tree improvement programmes in the country. Priorities on Forest genetics and Biotechnology are summarized below:

- Developing a regional and national action plan for priority species
- Developing institutional capacity for characterization and management of FGR
- Developing guidelines and strategy for prioritized species and common strategies for tree breeding and FGR conservation
- Assessing diversity of species and variations within species. Wide genetic diversity is a prerequisite for healthy forests
- Exchange of genetic material between countries should be encouraged
- Modern biotechnology may have potential for more productive forestry.
- Development of the comprehensive national FGR strategies and action plans
- Evaluation, characterization, documentation and sharing the information of FGR
- Strengthening national programmes on forest genetic resources and enhancement of the linkage between conservation of forest genetic resources and national forest programmes
- Participation of local communities and the role of livelihood in conservation and sustainable use of forest genetic resources

Challenges

- Financial sustainability to long term breeding programmes
- Networking and communication system within the region and globally
- Utilization of the improved germplasm must be enhanced so that it can adequately contribute to improvement of the productivity in the region
- Demand for improved planting stock by the stake holder
- Exchange of genetic material within the region
- Promotion and strengthening conservation of wild crop relatives, medicinal, fodder and wild ornamentals still a challenge.
- Need for training facilities within the region.
- Capacity building and training

International Collaboration

- Awareness and exchange of germplasm being increasingly appreciated in light of threats posed by climate change
- Possibility of forging strategic partnerships in FGR conservation and improvement
- Local/regional training in FGR conservation
- Harnessing of modern technologies such as molecular characterization, cryopreservation and tissue culture for vegetatively propagated material
- Formation of the sub–regional groups
- Expansion of scope of ICFRE to work includes forest genetic resources at regional level.

Future Line of Action

- Development of inter/intra specific hybrids for specific traits (viz. resistance, pulp quality, adaptability) to exploit high heterosis and other intrinsic properties of mandate species.
- Development of advance generation seed orchards, production populations for the improvement of quality of seed & other planting material
- Development of site matched clones with traits of economic importance
- Cautious evolvement of transgenic approaches in forestry species for genetic modification of desired traits and functional analysis of genes
- QTL (Quantitative Trait Loci) mapping for economically important traits and initiate work on functional genomics
- Develop greater understanding of the genomes of economically important tree species and establish collaboration with international research groups
- Understanding biotic and abiotic stress responses of trees for forest conservation and reclamation of sites.
- Study of reproductive systems and inheritance patterns at genotype level for important species
- Application of molecular techniques in timber forensics and molecular taxonomy
- Study the population genetics of natural forests for their management and conservation employing DNA markers

CONCLUSION

The coordinated and well implemented forest genetics and tree improvement programme will have a multiplier effect in terms of production of propagules of desired quality and adequate quantity. Adding quality to volume in trees is a necessity not only to provide high value products but also for conservation programme both in *in situ*, *ex situ*. The advancement of biotechnology need to be involved for tree improvement through non conventional approaches, like engineered plants for drought tolerance, salt tolerance, pest and disease resistance in addition to scaling up the hybrids and superior genotypes. Such cooperative approach would lay foundation for developing infrastructure and human resource and rapidly transfer quality products at user level, like forest departments and farmers.

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Table 2: **Seed stands and seed production areas**

State	Species (area in ha)
Arunachal Pradesh	*Bombax ceiba* (1.0), *Dalbergia sissoo* (974.9), *Duabanga grandiflora* –(1.0), *Terminalia myriocarpa* (1.0)
Andhra Pradesh	Eucalyptus hybrid (30), *Tectona grandis* (6406)
Chhattisgarh	Bamboo (35), *Emblica officinalis* (10.0), *Eucalyptus* (5.5), *Gmelina arborea* (18.0), *Tectona grandis* (301), *Terminalia tomentosa* (100.0)
Gujarat	58 different species (4967.00)
Haryana	*Eucalyptus* (2.0)
Himachal Pradesh	*Abies pindrow* (3615.50), *Cedrus deodara* (1379.94), *Pinus roxburghii* (2834.20), *P. wallichiana* (968.68)
Jammu and Kashmir	*Pinus wallichiana* and *Abies pindrow* (total 238.6), *Dalbergia sissoo*, *Gmelina arborea*, *Tectona grandis* and Bamboo (250.0)
Karnataka	*Acacia amara* (10.0), *A. catechu* (10.0), *Casuarina equisetifolia* (10.0), *Grevillea robusta* (10), *Hardwickia pinnata* (10.0), *Santalum album* (7.0), *Sterculia campanulata* (10.0), *Swietenia mahagony* (1.0), *Tectona grandis* (316.6), *Terminalia tomentosa* (1.0), *Trewia nudiflora* (1.0), *Xylia xylocarpa* (14.0)
Kerala	*Acacia auriculiformis* (2.3), *Bombax ceiba* (12.5), *Casurina equisetifolia* (4.5), *Dalbergia latifolia* (45.8), *Eucalyptus grandis* (6.84), *Eucalyptus* hybrid (4.9), *Gmelina arborea* (4.0), *Tectona grandis* (1100), *Santalum album* (22.7)
Madhya Pradesh	*Dalbergia latifolia* (5.0), *Hardwickia binata* (5.0), *Tectona grandis* (400),
Maharashtra	*Tectona grandis* (677.36)
Manipur	*Tectona grandis* (1.0)

State	Species (area in ha)
Orissa	*Casuarina equisetifolia* and *Tectona grandis* (total 500)
Rajasthan	*Bombax ceiba* (8.0) *Dalbergia sissoo* (58.0), *Tectona grandis* (15)
Tamil Nadu	*Acacia ferruginea* (3.1), *A. mearnsii* (9.5), *A. melanoxylon* (0.5), *A. nilotica* (1.63), *Albizia falcataria* (7.22), *Ancardium occidental* (21), *Eucalyptus citriodora* (0.5). *E. globulus* (10.0), *E. grandis* (29.5), *E. tereticornis* (12.7), *Hardwickia binata* (5), *Pinus patula* (8.9), *Pterocarpus santalinus* (20.75), *Tectona grandis* (92.8)
Uttarakhand	Different species (total 6024)
Uttar Pradesh	*Acacia catechu* (146), *Adina cordifolia* (98), *Albizia lebbek* (4.0), *Bombax ceiba* (56), *Cedrus deodara* (20), *Dalbergia sissoo* (146), *Eucalyptus* hybrid (3), *Haplophragma adenophyllum* (1.0), *Jacaranda ovalifolia* (1.0), *Kydia calycina* (9.6), *Lagerstroemia flos-regine* (3.0), *Morus alba* (56), *Parkinsonia aculeata* (0.5), *Pinus roxburghii* (100.00), *P. wallichiana* (15), *Poinciana regia* (4.0), *Prosopis juliflora* (1.0), *Tectona grandis* (240), *Terminalia arjuna* (3.0), *Toona ciliata* (9.1)

Table 3: **Seed orchards developed in different states (ICFRE 2003)**

State	Species (area in ha)
Arunachal Pradesh	*Bombax ceiba* (4), *Chukrasia tabularis* (0.75), *Duabanga grandiflora* (1), *Gmelina arborea* (3), *Michelia champaca* (1), *Phoebe goalparensis* (1), *Tectona grandis* (17), *Terminalia myriocarpa* (2)
Bihar	*Dalbergia sissoo* (1.65), *Tectona grandis* (134.43)
Chhattisgarh	*Emblica officinalis* (20.0), *Eucalyptus* (15.0), *Gmelina arborea* (39.0), *Tectona grandis* (98.0)
Haryana	*Dalbergia sissoo, Tectona grandis, Azadirachta indica, Ficus benghalensis, F. religiosa, Eucalyptus* spp., *Populus deltoids, Acacia nilotica* and *Melia azedarach* (total area 43), *E. tereticornis* (12)
Jharkhand	*Acacia catechu, Cassia siamea, Tectona grandis* and *Dalbergia sissoo* (total 60.00)
Karnataka	*Eucalyptus* (18.0), *Tectona grandis* (109.8)
Kerala	*Tectona grandis* (50.65)
Madhya Pradesh	*Tectona grandis* (113)
Maharashtra	*Dalbergia sissoo* (1.11), *Tectona grandis* (234.65)
Manipur	*Pinus kesiya* (0.5), *Tectona grandis* (0.3)
Orissa	*Tectona grandis* (12.37)
Punjab	*Dalbergia sissoo* (4)
Tamil Nadu	*Anacardium occidentale* (12), *Casuarina equisetifolia* (5), *Eucalyptus tereticornis* (1.5), *Pterocarpus marsupium* (2.0), *Santalum album* (2.4), *Tectona grandis* (18.7), *Terminalia sp.* (6.00)
Tripura	*Gmelina arborea* (5), *Tectona grandis* (5),
Uttarakhand	*Cedrus deodara, Pinus roxburghii, P. wallichiana, Ficus micrantha, Juglans regia* and *Abies pindrow* (total 216)
Uttar Pradesh	*Acacia nilotica* (6.0), *Bombax ceiba* (7), *D. sissoo* (95), *Tectona grandis* (3)
West Bengal	Various species, total area 54.0 ha

Efficacy of IDS Technique on Improving the Quality of *Jatropha curcas* Seedlot

***R. Anandalakshmi, V. Sivakumar, B. Gurudev Singh and Rekha R. Warrier**

INTRODUCTION

Jatropha curcas seeds encounter problems of insect attack mainly during storage resulting in substantial damage to seedlots. Due to the insect attack the seeds though appear intact lose the seed kernel partially or fully resulting in ill-filled or empty seeds. Presence of insect attacked/ill-filled seeds reduces the germinability of the seedlot categorizing it as poor quality. Furthermore, the quantity and quality of oil from ill-filled seeds is very low. The oil extracted from the fraction of poor quality insect attacked seeds is high in free fatty acids and has undesirable odour. Therefore the poor quality oil contaminates the oil extracted from the particular seedlot in total by accelerating rancidification. Hence it becomes economically important to separate out the infested seeds from a seedlot to upgrade its quality.

The present study was carried out to identify a quick, feasible and cost-effective method to upgrade the quality of an insect attacked seedlot of *J. curcas*. Various grading methods such as seed blowing using Dakota blower and grading using specific gravity separator failed to successfully separate the insect attacked seeds. Therefore the IDS (Incubation, Drying and Separation) technique which was developed in Sweden to upgrade conifer seed lots was applied on *Jatopha curcas*. This technique is based on the principle that water imbibed by live seeds is lost at a slower rate than water imbibed by dead seeds when both are subjected to uniform drying conditions. The seeds can then be separated in liquid medium into a floating and sunken fraction based on the incurred density difference (Simak, 1983).

MATERIALS AND METHODS

A one year old seedlot of Sathyamangalam source which was found to be attacked by insects was taken up for germination up-gradation study. Four samples were drawn randomly from the seed lot each containing about 25 seeds. X-ray images of the sampled seeds were taken using a Faxitron model X-ray unit to understand the seed filling pattern. X-ray imaging for *Jatropha* seeds was standardized at 19 kvp (kilovolt potential) with an exposure time for 2 minutes. As a quick test of viability, the seeds were subjected to Tetrazolium (TZ) test. The seed kernels were extracted carefully by cracking the shells, pre-moistened and soaked in 1% solution of 2,3,5 triphenyl tetrazolium chloride and allowed to stand at 30° C for 24 hours in dark, which were then assessed for viability based on the staining pattern (Willan, 1985). Seeds with embryos and cotyledons stained red were counted as viable seeds. The results revealed that the seed lot had viability of only 58%.

The above seedlot was apportioned into four sublots with 800 seeds per sublot. Each sublot was subjected to different regimes of IDS treatments namely,

T1- Control- 24 hrs. soaking in water + 0 hr. drying
T2- 24 hrs. soaking in water + 1 hr. drying
T3- 24 hrs. soaking in water + 2 hrs. drying
T4- 24 hrs. soaking in water +3 hrs. drying

The seeds were soaked in water for 24 hours to allow imbibition and then drying was carried out for different durations under ambient conditions (30 ± 1 ºC and RH 65 ± 2%). Following drying, the seeds were separated into floaters and sinkers using water as separation medium. The number of floaters and sinkers in each treatment (four replications per treatment) was counted and the values were expressed as percentage. The individual identities of the separated seeds, in each of the floater and sinker fractions, were marked and then subjected to X-radiography. The marked seeds were then tested for germination in sand medium in the nursery. The quality of a seed lot can be checked radiographically before and after IDS treatment (Kamra, 1976).

Germination test

Germination study was conducted on sand medium in the nursery (32 ± 2°C; RH: 65 ±2%) (ISTA, 1993). The test was carried out in 4 replications for floater and sinker fractions under each treatment. The final count of germination test was taken after 40 days of sowing. The germination was expressed in percentage.

Statistical analysis

The experiments were carried out in Completely Randomized Design. One-way ANOVA was applied to test the effect of IDS on improving germination percentage of the seed lot. The means were tested for significance at 5% level of confidence ($\alpha = 0.05$) (Panse and Sukhatme, 1995).

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RESULTS

The results of separation of seed lot into floaters and sinkers and their respective germination percentage have been furnished in Table 1. The actual germination percentage of the seedlot was found to be 54% (average germination of floaters and sinkers in T1-control). In T1, the seeds separated into 67.25% floaters and 32.75% sinkers. Whereas, in T2, T3 and T4 the separation followed the order of 61.5:38.5, 56.5:43.5 and 46.38:53.63 for floaters and sinkers respectively. T4 gave the highest germination in sinker fraction showing 84.61%. It was found that least number of viable seeds were found as floaters only in T4 (20.91%). Therefore the maximum recovery of viable sinkers was found in T4.

Table 1: **Effect of IDS grading of *Jatropha* seeds on germination percentage**

IDS treatment	Recovery %		Germination %	
	Floaters	Sinkers	Floaters	Sinkers
T1- Control - 24 hrs. soaking in water + 0 hr. drying	67.25 (55.11)	32.75 (34.91)	58.59 (49.96)	48.92 (44.39)
T2- 24 hrs. soaking in water + 1 hr. drying	61.50 (51.66)	38.50(38.36)	59.13 (50.27)	53.28 (46.89)
T3- 24 hrs. soaking in water + 2 hr. drying	56.50 (48.74)	43.50(41.27)	44.87 (42.05)	70.67 (57.26)
T4- 24 hrs. soaking in water +3 hr. drying	46.38 (42.92)	53.63 (47.09)	20.91 (27.19)	84.61 (66.97)
S.e.d.	**0.860**	**0.859**	**1.356**	**1.468**
C.D.	**1.873**	**1.871**	**2.955**	**3.199**

**The values in parentheses are arc sine values*

DISCUSSION

The sound *Jatropha curcas* seeds were able to imbibe water like insect attacked seeds but were very slow in losing the gained moisture when subjected to drying. In the case of insect attacked seeds, the water was drawn into the seeds due to increased porosity in the shell as a result of infestation. The water accumulates in the hollow cavity between the shell and the kernel and was lost at a faster rate when these seeds were allowed to dry unlike the sound seeds. As a result difference in density was created and when allowed to float in water medium, the lesser dense insect infested seeds separated out as floaters while the viable intact seeds remained as sinkers. The appropriate drying time was crucial in creating the significant density difference between the sound and insect attacked seeds so that marked separation of sound and insect attacked seeds as floaters and sinkers respectively could happen. This was successfully achieved by T4 were the drying duration was 3 hours, where maximum number of viable seeds were found in the sinker fraction as shown by very high germination and minimum number in floater fraction recording very low germination. In addition, it is evident that both 1 and 2 hours of drying (T2 and T3 respectively) were insufficient to bring in clear density difference between the two categories of the seed as these recorded substantial germination percentages for the floater fractions compared to sinker fractions. The x- ray images of the seeds supported in non-destructive identification of insect-attacked seeds in the each sublot which was confirmed by germination test. X-radiography was first applied to tree seeds by Simak in Sweden to discriminate between viable and nonviable seeds with some species namely, Lodgepole pine (Simak, 1983), Scots pine and Norway spruce (Bergsten, 1987) and *Pinus roxburghii* seeds (Singh and Vozzo, 1990). The X-ray analysis of *Casuarina equisetifolia* (Sivakumar *et al*., 2007) revealed a large quantity of shrivelled, empty and insect-damaged seeds, altogether accounting 50%, which were the causes of low percentage germination of un-graded seed lots (48%).

For broad-leaved species, Falleri and Pacella (1997) and Demelash *et al*. (2003) reported 12.5% and 22.5% improvement in germination of London plane (*Platanus* X *acerifolia* (Ait.) Willd) and pepper tree (*Schinus molle* L.) seed lots, respectively after specific density separation. The technique has also worked well for *Acacia leucophloea* Willd (Raunja) seed lots using water as flotation medium (Mani *et al*. 2002). Grading of neem (*Azadirachta indica*) seeds by the floatation method gave 18% floaters which weighed comparatively less than the sinkers, possessed a higher endocarp content and recorded low viability in terms of maximum germinability and vigour. The sinkers in contrast recorded an increase of 35% in viability and 92% in vigour index (Ponnuswamy *et al*., 1990). Seed quality of *Simarouba glauca* seedlots can be upgraded by separation of full and empty seeds by means of specific gravity differences induced in liquid flotation techniques (Sekar, 2003). Srimathi *et al.* (2007) revealed that simple density grading of aged *Syzigium cuminii* seeds using water could select good seeds from dead / low vigour seeds. Density grading of *Sapindus emarginatus* seeds by floatation technique, separated sinkers which recorded 75% germination and floaters with minimum germination of 57% (Srimathi *et al*., 1994). It should be noted that the separation efficiency not only depends on seed size variability within a seed lot (e.g., *Cupressus lusitanica* Mill.) (Bergsten and Sundberg 1990), but also on inadequacy of the specific density gradient between germinable and nongerminable seeds (e.g., *Albizia schimperiana* Oliv.) (Tigabu and Oden, 2003).

CONCLUSION

The experiment revealed that soaking *Jatropha* seeds for 24 hours in water followed by 3 hours drying helps in recovery of sound seeds and therefore helped improving the germination percentage of a poor quality seed lot from 54% to 84.61%, almost two-third increase from initial germination capacity. Thus IDS has been found suitable for upgradation of *J. curcas* seedlot and would help better utilization of resources with minimal wastage.

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Characterization of Different Species of Bamboo Through ISSR Molecular Marker

Santan Barthwal*, Anup Chandra, Anita Rawat, Shweta Saini and H.S. Ginwal

INTRODUCTION

Bamboos are vital to many Asian economies, having important uses ranging from domestic items to rural housing and raw materials for industry (Dransfield and Widjaja, 1995). The total annual bamboo demand in India has been estimated to be approx. 5 million tonnes, of which about 3.5 million tonnes are required by the paper and pulp industry alone (Sharma, 1987). Basic knowledge of the biology and genetics of bamboo is severely lacking therefore relationships among a lot of bamboo species remain controversial and unclear. This is a direct result of the unusual life cycle of bamboo. Among bamboo species, the vegetative growth phase varies from 1 year to 120 years, moreover some species have never been known to flower (Janzen, 1976). So it is very difficult to get genetic information from the traditional means. Due to the unusually long sexual cycle and unavailability of any other diagnostic tool, identification of bamboo is mainly dependent on vegetative descriptors such as culm morphology, and the morphology of the culm-sheath including ligule and auricle (Ohrnberger and Goerrings, 1986).

Nowadays, greater attention is needed in the classification and identification of bamboos (Hui and Yang, 1998). Identification of sterile plants is therefore problematic as taxonomic studies of bamboos have traditionally depended heavily on inflorescence and floral morphology because: (1) vegetative characters are often environmentally influenced, which makes them less constant for systematic purposes (Wu, 1962); (2) characters that delimit species may be more subtle and not available for study; and (3) bamboo clones found in Asia are selected for economic value and are widely distributed without proper identification at the species level. The application of modern molecular techniques is therefore of great assistance in species identification. PCR-based genetic markers are now well documented for species/cultivar identification (Khasa and Dancik, 1996; Samec and Nasinec, 1996; Raina et al., 2001; Johnson et al., 2003). Studies include the use of restriction fragment length poly-morphisms (RFLP) in Phyllostachys (Friar and Kochert, 1991, 1994), isozyme analysis of a limited selection of bamboos from five genera (Heng et al., 1996), chloroplast DNA phylogeny of Asian bamboos (Watanabe, Ito and Kurita, 1994) and world bamboos (Kobayashi, 1997), and the use of chloroplast rpl16 intron sequences in determining phylogenetic relationships within the genus Chusquea (Kelchner and Clark, 1997). In this investigation, we used ISSR markers to study the genetic variation among different species and to determine the genetic similarities between the species.

MATERIALS AND METHODS

Young leaves of three different species of the genus *Bambusa viz. Bambusa bambos, Bambusa balcooa* and *Bambusa vulgaris* were collected and stored at -80ºC in deep refrigerator.

DNA EXTRACTION AND QUANTIFICATION

DNA was extracted from young leaves using CTAB method described by Doyle and Doyle (1990). 0.5 g of bamboo leaves were ground to a fine powder in liquid nitrogen and extracted for 30 min. at 60^0C in an extraction buffer (100 mM Tris pH=8.0, 20mM EDTA, 1.42M NaCl, 2% (w/v) CTAB and 2% (w/v) PVP 40) and 0.3% β-mercaptoethanol. The supernatant was extracted with chloroform-isoamyl alcohol (24:1). Equal amount of chilled isopropanol was added, stored at -20 ºC overnight and centrifuged at 13,000 rpm for 5 min. The pellet was washed with 76% and 70% alcohol and finally dissolved in 100 μl of TE buffer (10mM Tris pH-8.0 and 1 mM EDTA). The concentration of DNA samples was determined using Biophotometer (Eppendorf) and the DNA samples were diluted to 50 ng/μl concentration for polymerase chain reaction (PCR) amplification.

ISSR ANALYSIS

Seventeen ISSR primers (University of British Columbia (UBC), Biotechnology laboratory, Vancouver, BC, Canada) were initially screened for the molecular characterization of three different species of bamboo. The PCR amplifications were carried out in a 25 μl reaction mixture containing 50 ng of template DNA, 0.4 μM of ISSR primer, 1X PCR buffer containing 15 mM $MgCl_2$, 0.05 mM dNTPs and 0.6 unit of Taq DNA polymerase (Bangalore Genei, India).

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Amplifications were carried out in a thermal cycler (BIO-RAD, My Cycler) programmed for a preliminary 5 min. denaturation step at 94ºC, followed by 40 cycles of denaturation at 94ºC for 30 sec, annealing at 40ºC-50ºC (as per the primer) for 30 sec and extension at 72°C for 1 min and a final extension at 72°C for 5 min. Amplification products were separated alongside a molecular weight marker (100 bp GeneRuler™ ladder) by electrophoresis on 1.5% agarose gels run in 1X TBE (Tris Borate EDTA) buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc System (GelDoc-It System, UVP Ltd.)

GEL SCORING AND DATA ANALYSIS

The PCR-amplified fragments were scored as present (1) or absent (0) and data were entered in a binary data matrix as discrete variables. Jaccard's similarity coefficient was calculated to develop a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA). The computer package NTSYS-pc version 2.20e (Rohlf 1998) was used for cluster analysis and matrix correlation.

Genotyping data obtained for the ISSR markers was used to assess the discriminatory power of the markers by evaluating three parameters: Polymorphism Information Content (PIC), Marker Index (MI) and Resolving Power (RP).

The PIC values measure the informativeness of a given DNA marker and these were calculated for each SSR marker based on Anderson *et al.* (1993) as follows:

$$PIC = 1 - \sum_{i=1}^{k} P_i^2$$

where k is the total number of alleles detected for a given marker locus and P*i* is the frequency of the i^{th} allele in the set of genotypes investigated.

The average number of DNA fragments amplified/detected per genotype using a marker system is considered as multiplex ratio (n). The number of loci polymorphic in the germplasm set of interest, analyzed per experiment, called effective multiplex ratio (E) is estimated as:

$E = n\beta$

where β is the fraction of polymorphic markers and is estimated after considering the polymorphic loci (n_p) and non-polymorphic loci (n_{np}) as $\beta = n_p/(n_p + n_{np})$.

The utility of a given marker system is a balance between the level of polymorphism detected and the extent to which an assay can identify multiple polymorphisms. A product of information content, as measured by PIC, and effective multiplex ratio, called as marker index may provide a convenient estimate of marker utility Powell *et al.* (1996)

$MI = PIC \times E$

Or

$MI = n \times \beta \times PIC$

Pairwise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard 1908) using the SIMQUAL format of NTSYS-pc 2.20e (Rohlf 2000). The dendrogram was constructed using the UPGMA with the SAHN module of NTSYS-pc 2.20e to show a representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal 1973).

RESULTS

Of the 17 ISSR primers screened, 5 primers produced distinct, reproducible, polymorphic profiles among the 3 bamboo species surveyed. The approximate size range of ISSR products was 300 bp to 1 kb. A total of 71 fragments were amplified, of which 68 fragments were polymorphic achieving 97.27% polymorphism.

The Polymorphism Information Content (PIC) values for these markers in the examined genotypes ranged from 0.414 to 0.283 with a mean of 0.342 per primer. Marker Index (MI) for each marker ranged from 2.883 to 6.031 with an average of 4.537 per primer. Resolving power (RP) of the markers ranged from 12.664 to 4.11 with a mean of 7.510 per primer. Average effective multiplex ratio per primer was found to be 13.08 (Table 3).

UPGMA based dendrogram grouped the different species into different clusters to a great extent. The Jaccard's similarity coefficient values ranged from 0.1268 to 0.9437 (Table 4). Cluster analysis indicates that the three species of bamboo formed two major clusters (Fig. 2) based on similarity indices, cluster I and cluster II. Cluster I comprises *B. bambos, B. balcooa and B. vulgaris*. The second major cluster was further divided into three minor clusters A, B and C which clearly differentiated the three species of bamboo. The minor cluster A comprised of *B. bambos*, second minor cluster B comprised of *B. balcooa* and the third minor cluster C comprised of *B. vulgaris*. As per the cluster analysis, *B. bambos* was more closely related to *B. balcooa* than *B. vulgaris*.

DISCUSSION

Taxonomists have been relying solely on vegetative characters for the identification of all the bamboo species in the absence of reproductive characters. Bamboos flower once in a lifetime and then die: the vegetative phase lasts from 1–120 years depending on the species. Usually, genera are identified on the basis of culm, rhizome and branching characters, while species characterization is mainly dependent on culm sheath, ligule and auricle characters (Ohrnberger and Goerrings, 1986). Species identification based only on morphological characters may be misleading as vegetative characters are often influenced by environmental factors (Wu, 1962). In spite of the serious, outstanding need to develop a method to identify bamboo species at the molecular level, attention has been lacking.

The traditional method of identifying species by morphological characters is now gradually being replaced by protein that is more reliable or DNA profiling largely because of several limitations of morphological data. In recent years, DNA profiling through RAPD technique has been used for the analysis of diversity and identification of duplicates within the large germplasm populations (Virk et al. 1995), phylogenetic relationship (Millan et al. 1996), rational designing of breeding programmes (Powell et al. 1996), and management of genetic resources (Bretting and Widrelechner 1995).

The results from this study indicate that ISSR markers are a useful tool for the identification of germplasm and analysis of genetic relationships between and within the bamboo species. Such an analysis, together with data from other classical methods could thus be used to make a more accurate reconstruction of bamboo evolution. Furthermore, such an approach might be help-

ful in identifying taxa of potential value in genetic improvement programmes.

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Table1: **List of samples used in the study**

S.N.	Species	Code	Location
1	Bambusa bambos	Bb1	Old Agroforestry site, Pantnagar
2	Bambusa bambos	Bb2	Pantnagar, TERI
3	Bambusa bambos	Bb3	TERI
4	Bambusa bambos	Bb4	Pantnagar, Itanagar, Arunachal Pradesh
5	Bambusa bambos	Bb5	Ambiwala, Dehradun
6	Bambusa bambos	Bb6	Navada, Dehradun
7	Bambusa balcooa	Bba1	Pantnagar, Kishanpur, Kichha (U.K)
8	Bambusa balcooa	Bba2	Pantnagar, Nagao Assam, Grownurse Biotech House
9	Bambusa balcooa	Bba3	Gangapur, U.K.
10	Bambusa balcooa	Bba4	Pantnagar, Uday nagar
11	Bambusa balcooa	Bba5	Pantnagar, Gadapur, Ward No.5
12	Bambusa balcooa	Bba6	Lalkuan, Pantnagar
13	Bambusa balcooa	Bba7	Pantnagar, Kalinagar, U.K.
14	Bambusa vulgaris	Bv1	Lalkuan, FRI
15	Bambusa vulgaris	Bv2	Seetpur, Dineshpur
16	Bambusa vulgaris	Bv3	Pantnagar, Chandan nagar, Dineshpur
17	Bambusa vulgaris	Bv4	FRI
18	Bambusa vulgaris	Bv5	Old Agroforestry site, Pantnagar

Table 2: **List of ISSR primers used in the study**

S.N.	Primer	Primer	Ta	Primer sequence(5'-3')
1	ISSR-3	UBC811	50	GAGAGAGAGAGAGAGAC
2	ISSR-4	UBC812	53.3	GAGAGAGAGAGAGAGAA
3	ISSR-8	UBC855	53.3	ACACACACACACACACYT
4	ISSR-9	UBC856	53.3	ACACACACACACACACYA
5	ISSR-11	UBC858	53.3	TGTGTGTGTGTGTGTGRT

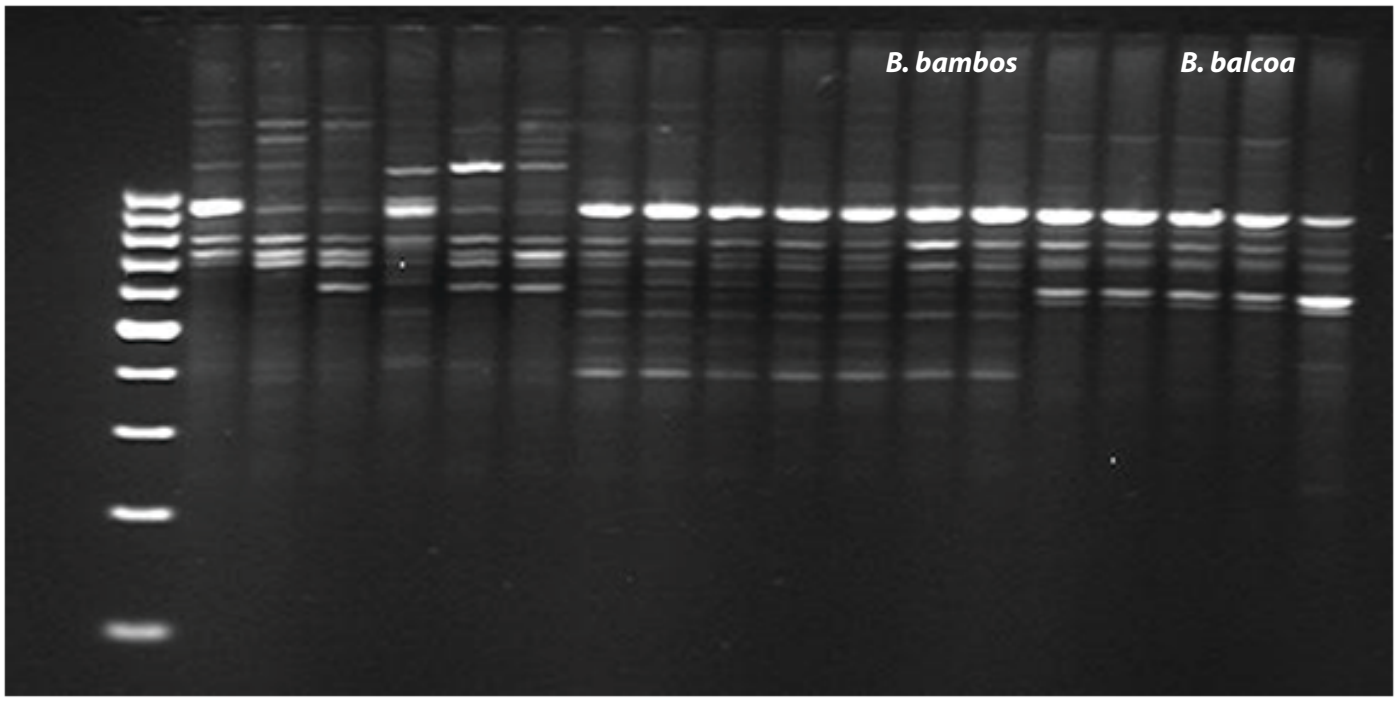
Table 3: **Total number of bands, polymorphic bands, polymorphism, Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Marker Index (MI), and Resolving Power (RP) obtained from 5 ISSR markers.**

S.N.	Primer	Total no. of bands	Poly-morphic bands	% polymor-phism	PIC	EMR	MI	RP
1	UBC811	**22**	**19**	86.36	0.368	**16.409**	**6.031**	**12.664**
2	UBC812	16	16	100	0.356	16	5.691	8.89
3	UBC855	11	11	100	**0.283**	11	3.117	4.334
4	UBC856	12	12	100	**0.414**	12	4.963	7.552
5	UBC858	**10**	**10**	100	0.288	**10**	**2.883**	**4.11**
Mean		14.2	13.6	97.272	0.342	13.082	4.537	7.510
Total		71	68					

Table 4: **Jaccard's similarity matrix table based on cluster analysis of three species of bamboo.**

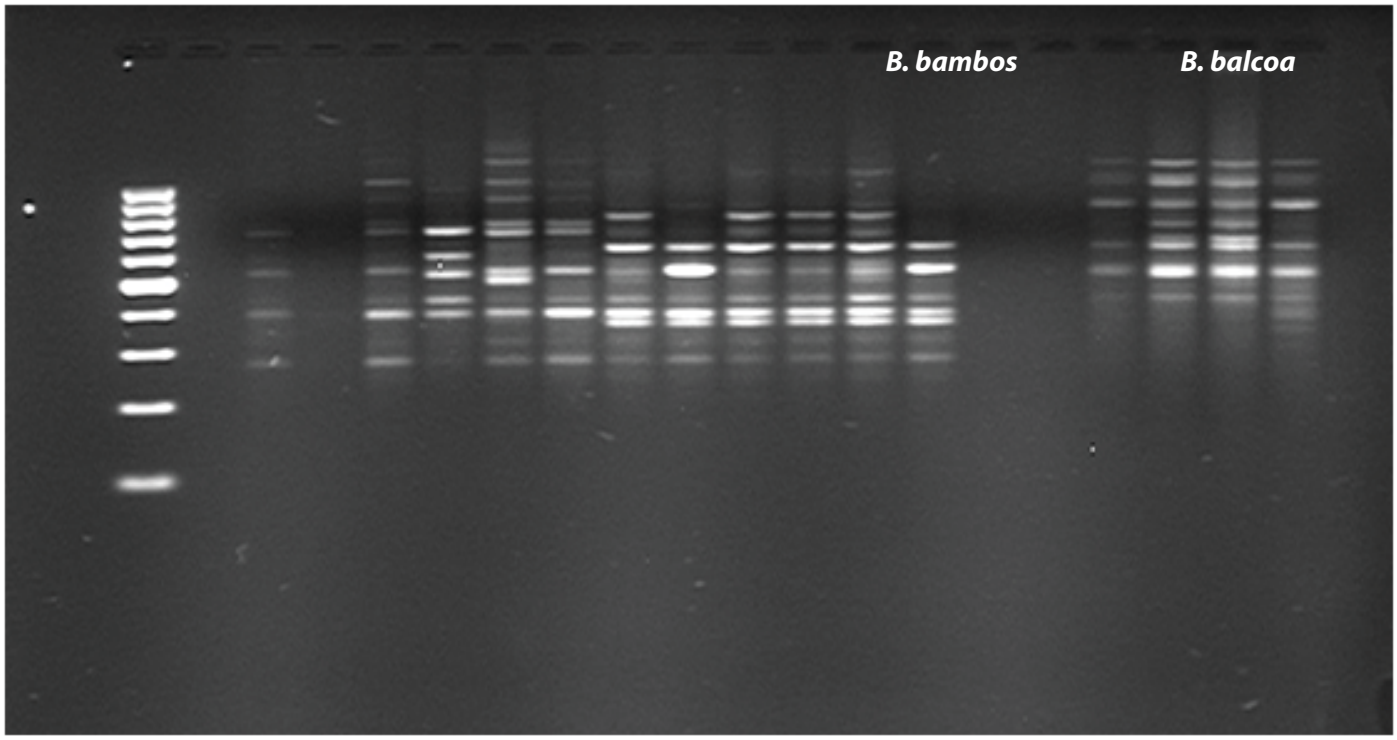
	Bb1	Bb2	Bb3	Bb4	Bb5	Bb6	Bba1	Bba2	Bba3	Bba4	Bba5	Bba6	Bba7	Bv1	Bv2	Bv3	Bv4	Bv5
Bb1	1.00																	
Bb2	0.75	1.00																
Bb3	0.56	0.37	1.00															
Bb4	0.35	0.17	0.55	1.00														
Bb5	0.41	0.24	0.56	0.63	1.00													
Bb6	0.44	0.27	0.63	0.63	0.77	1.00												
Bba1	0.32	0.15	0.44	0.62	0.59	0.68	1.00											
Bba2	0.32	0.15	0.49	0.56	0.54	0.59	0.80	1.00										
Bba3	0.45	0.27	0.68	0.54	0.46	0.51	0.70	0.62	1.00									
Bba4	0.31	0.13	0.51	0.55	0.49	0.55	0.82	0.85	0.72	1.00								
Bba5	0.28	**0.13**	0.48	0.55	0.49	0.55	0.79	0.82	0.69	**0.94**	1.00							
Bba6	0.34	0.14	0.45	0.63	0.58	0.63	0.79	0.87	0.66	0.83	0.83	1.00						
Bba7	0.63	0.83	0.28	0.20	0.15	0.13	0.27	0.24	0.44	0.27	0.27	0.28	1.00					
Bv1	0.69	0.89	0.34	0.17	0.21	0.18	0.18	0.18	0.27	0.15	0.15	0.17	0.83	1.00				
Bv2	0.51	0.35	0.70	0.48	0.52	0.48	0.42	0.45	0.58	0.41	0.41	0.46	0.30	0.44	1.00			
Bv3	0.44	0.34	0.61	0.46	0.48	0.49	0.41	0.46	0.51	0.37	0.39	0.45	0.25	0.42	0.87	1.00		
Bv4	0.28	0.20	0.46	0.54	0.56	0.54	0.44	0.46	0.39	0.37	0.39	0.51	0.14	0.25	0.70	0.80	1.00	
Bv5	0.31	0.17	0.51	0.54	0.54	0.54	0.52	0.55	0.46	0.48	0.51	0.62	0.20	0.25	0.72	0.73	0.83	1.00

Fig. 1: **Gel photographs of the ISSR primers UBC 811 and UBC 812 run on 1.5% agarose.**

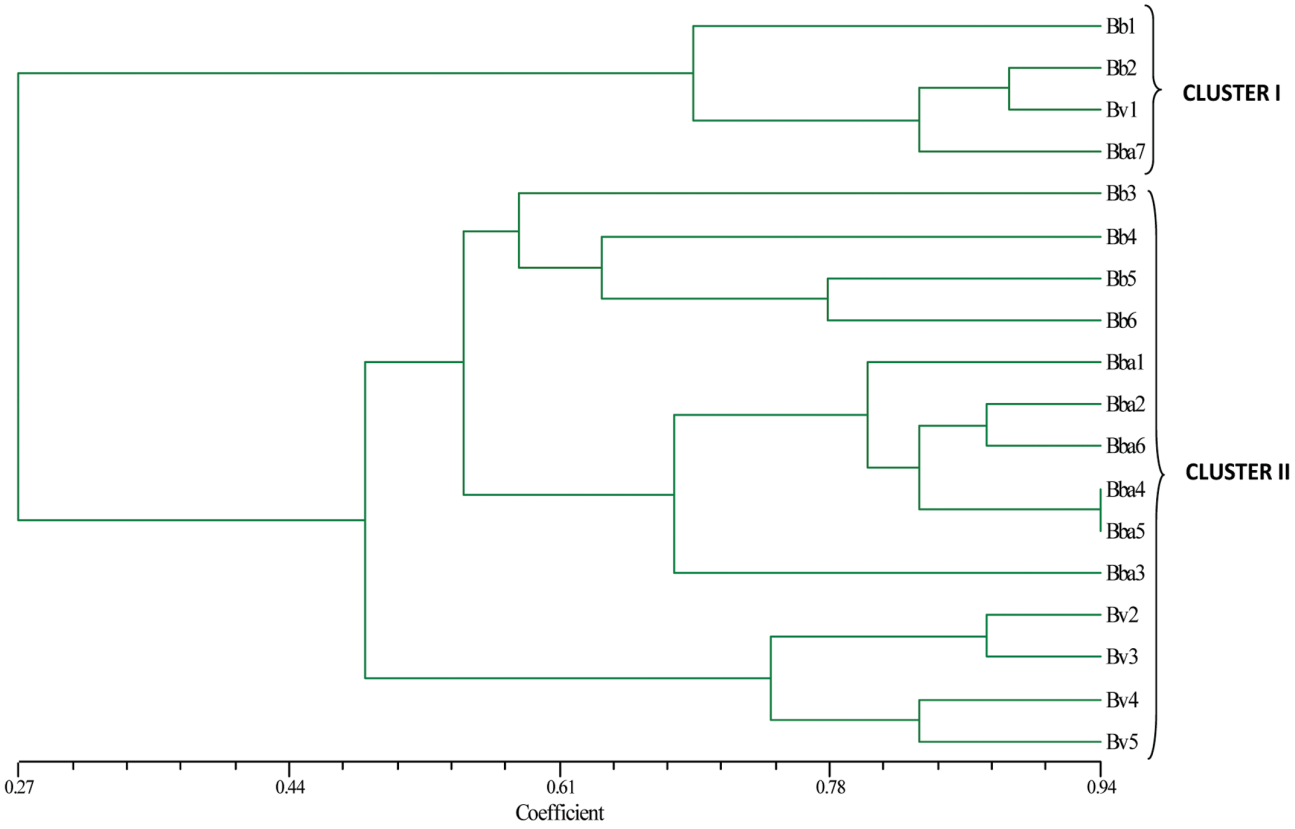


ISSR primer-UBC811

Fig.2: **UPGMA dendrogram of cluster analysis of RAPD markers illustrating the genetic relationships among the three (3) species of bamboo.**



ISSR primer-UBC812


Bb1
Bb2
Bv1
Bba7
CLUSTER I
Bb3
Bb4
Bb5
Bb6
Bba1
Bba2
Bba6
Bba4
Bba5
Bba3
CLUSTER II
Bv2
Bv3
Bv4
Bv5
0.27
0.44
0.61
0.78
0.94
Coefficient

Molecular Characterization of High and Low Resin Yielding Genotypes of *Pinus roxburghii* Sarg. Using Microsatellite Markers

Anita Rawat*, Santan Barthwal and H S Ginwal

INTRODUCTION

Diverse habitat of chir pine in different geographical regions of Himalayas and Shivalik range supports the existence of natural variation. This natural variation can be exploited for tree improvement programmes. Under various tree improvement programmes in the country attempts have been made to select plus trees for growth and high resin yield (Sharma et al., 2006). Gupta et al., (1970) reported 115 plus trees of *Pinus* for high resin yield. Dogra (1985) selected 58 plus trees for growth and 10 for resin yield. Forty nine plus trees form the distributional range of chir pine falling in different forest divisions of Himachal Pradesh, India were selected for growth form and high resin yield (Chauhan and Kanwar 2001).

Pines have long generation periods with vegetative phase extending up to several years and because of which multiple generations are not readily obtained and traditional approaches of tree improvement involving the identification of mature trees with desirable phenotypes, followed by their incorporation into breeding programmes are rather a slow process. The length of time needed for trees to reach reproductive maturity before controlled crosses is the limiting factor for tree improvement (Martin-Trillo and Martinez Zapater, 2002). Resin yield is a phenotypic trait and it can be scored in fully grown trees. The conservationists are more concerned on the damaging of pine forests due to large scale tapping of resins. However, if it is possible to identify the high resin yielding genotypes at the nursery stage, than we can establish plantations that are raised solely for the purpose of resin tapping. This will reduce the harm to the naturally occurring forests of chir pine.

The objective of the present research was to characterize the different genotypes of Pinus roxburghii varying in resin yield through DNA based molecular markers.

MATERIALS AND METHODS

Plant material

Chir pine (*Pinus roxburghii* Sarg.) from a natural population in Tiunee range, Chakrata division, Uttarakhand had been evaluated for resin yield in a study being conducted by Dr. S. S. Negi and Mr. Shashi Malik, Forest Research Institute, Dehradun. Based on the resin yield, 53 genotypes were selected for the present study (Table 1). The selected genotypes belonged to different sites and were representative of the variability in resin yield in the study area. Fresh needles or sapwood (in case needles were not available due to extreme height of trees) samples were collected and frozen at -80ºC.

DNA extraction and quantification

DNA was extracted from fresh needles or sapwood using the method described by Stange et al. (1998) and Doyle and Doyle (1990) with some modifications. The concentration of DNA was determined using Biophotometer (Eppendorf) and the DNA samples were diluted to15 ng/μl for polymerase chain reaction (PCR) amplification.

SSR Analysis

A total of 80 microsatellite markers were screened for amplification. 20 SSR markers were from P. thunbergii (Vendramin et al., 1996); 13 SSRs were from P. sylvestris (Provan et al., 1998); 3 SSRs were from P. resinosa (Boys et al., 2005); 32 SSRs were from P. taeda (Zhou et al., 2002; Chagne et al., 2004; Elsik et al., 2000); 5 SSRs were from P. merkussi (Nurtjahjaningsih et al., 2005) and 7 SSRs were from P. densiflora (Watanabe et al., 2006). Based on polymorphism, finally 19 microsatellites were used in the study (Table 2). PCR was performed in a 15 μl reaction volume containing 15 ng of template DNA, 1X Taq buffer, 3.0 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each primer and 0.06U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). All PCR reactions were performed in a thermal cycler (BIO-RAD, My Cycler) as follows: 5 min. at 95ºC followed by 30 cycles of 1 min. at 94ºC, 1 min. at 55ºC to 60ºC (as per the annealing temperature of the primer) and 1 min. at 72ºC and a final extension of 8 min. at 72ºC. Amplified products were electrophoresed on 3% (w/v) metaphor agarose: agarose (3:1) gel with 1X TBE buffer and stained with ethidium bromide (0.5μg/ml). Fermentas O'GeneRulerTM Ultra low range DNA ladder and GeneRulerTM 100 bp ladder were used as a size standard to ascertain that the amplified products were of the desired product size. DNA fragments were visualized under UV light and documented with the gel documentation imaging system (GelDoc-It System, UVP Ltd.).The primers which were not resolved on metaphor-agarose gel were then separated on 8% (w/v) polyacrylamide gel casted in 'MEGA-GEL High Throughput Vertical Unit' (model C-DASG-400-50) marketed by C.B.S Scientific Co. (Del Mar, CA, USA) with 1X TBE buffer and stained with ethidium bromide (0.5 μg/ml). DNA fragments were visualized under UV light (ULTRA Lum Inc) and photographed with digital camera (Cannon, EOS 400D).

Gel scoring and Data Analysis

The PCR-amplified fragments were scored as present (1) or absent (0) and data were entered in a binary data matrix as

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discrete variables. Jaccard's similarity coefficient was calculated to develop a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA). The computer package NTSYS-pc version 2.20e (Rohlf 1998) was used for cluster analysis and matrix correlation.

Genotyping data obtained for the microsatellite markers was used to assess the discriminatory power of the markers by evaluating three parameters: Polymorphism Information Content (PIC), Marker Index (MI) and Resolving Power (RP).

The PIC values measure the informativeness of a given DNA marker and these were calculated for each SSR marker based on Anderson et al. (1993) as follows:

$PIC = 1 - \sum Pi2$

where k is the total number of alleles detected for a given marker locus and Pi is the frequency of the ith allele in the set of genotypes investigated.

The average number of DNA fragments amplified/detected per genotype using a marker system is considered as multiplex ratio (n). The number of loci polymorphic in the germplasm set of interest, analyzed per experiment, called effective multiplex ratio (E) is estimated as:

$E = n\beta$

where β is the fraction of polymorphic markers and is estimated after considering the polymorphic loci (np) and non-polymorphic loci (nnp) as $\beta = np/(np + nnp)$.

The utility of a given marker system is a balance between the level of polymorphism detected and the extent to which an assay can identify multiple polymorphisms. A product of information content, as measured by PIC, and effective multiplex ratio, called as marker index may provide a convenient estimate of marker utility Powell et al . (1996)

$MI = PIC \times E$

Or

$MI = n \times \beta \times PIC$

Pairwise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard 1908) using the SIMQUAL format of NTSYS-pc 2.20e (Rohlf 2000). The dendrogram was constructed using the UPGMA with the SAHN module of NTSYS-pc 2.20e to show a representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal 1973).

RESULTS

The 19 SSR markers detected 2 to 5 alleles with an average of 2.42 alleles per marker in all 53 genotypes examined. In total, there were 46 alleles, of which 43 were polymorphic achieving 93.68% polymorphism. The three marker attributes, PIC, MI and RP (Table 3) all displayed discriminatory power of the SSR markers.

The PIC values for these markers in the examined genotypes ranged from 0.115 (pdms 221) to 0.499 (Pt 71936). Average PIC value per primer was 0.339. MI for each marker ranged from 0.057 to 1.168 with an average of 0.757. MI value was maximum for the SSR marker Pt 30204 and minimum for the SSR marker pdms 221. The primer RP test 9 showed the highest resolving power (3.698) whereas Pt 79951 showed the minimum (1.509) with an average of 2.282 per primer.

The Jaccard's similarity coefficient ranged from 0.409 to 0.955 suggesting wide genetic variability among the genotypes. The UPGMA-based dendrogram (Fig.1) clustered the genotypes clearly into two distinct clusters on the basis of resin yield. Most of the genotypes with low resin yield (0.25-2.5 kg) were grouped in cluster I (B-14, D-30, D-7, A-1, D-14, C-12, C-10, C-4, D-8, B-8, A-3, C-3, D-31, A-6, B-18, D-24, D-26, B-13, D-29, A-19, B-4, C-20, D-37, B-19, B-26, C-15 and A-24) and the genotypes with high resin yield (3-8 kg) were grouped in cluster II (D-27, D-38, C-9, A-10, A-13, C-8, D-33, B-24, A-9, D-39, A-28, A-2, B-25, A-12, B-10, A-25, A-7, D-11, B-2, C-1, b-7, C-7, B-6, B-9, B-3and B-12). There was no evidence of separate groupings by geographic locations from where samples were collected.

DISCUSSION

For any genetic improvement work, knowledge of genetic variability present in available germplasm, the type of genetic association between various attributes and the Genotype × Environment interactions are essential. Theses help in choosing the appropriate plant material for selection. In several reports, attempts have been made to develop and identify microsatellite markers in different Pinus species and cross-species amplification of these microsatellites in other species of Pines. These microsatellite markers can be used as valuable tools for the genetic diversity studies in Pine species.

In the present study, 19 SSR markers were used for the characterization of 53 genotypes of P. roxburghii varying in resin yield. The number of alleles per locus ranged from 2 to 5 with an average of 2.4 alleles per locus which was quite close to 3 to 6 alleles per locus obtained in Pinus merkusii (Nurtjahaningsih et al., 2005) and 1 to 9 alleles per locus in Picea abies (Scotti et al., 2002). The genetic divergence in terms of percent polymorphism was 50 to 100%. The PIC values for the microsatellite markers in P. roxburghii ranged from 0.115 to 0.499 with an average of 0.339 per primer. Earlier PIC values for microsatellites ranging from 0.1283 to 0.9529 were reported in P. taeda (Elsik et al., 2000) and 0.306 to 0 .929 in P. densiflora (Watanabe et al., 2006). The MI which is considered to be an overall measure of the efficiency to detect polymorphism ranged from 0.057 to 1.168 with an average of 0.757 per primer. The resolving power (Rp) ranged from 1.509 to 3.698 with an average of 2.282 per primer. Since, SSR markers assay only a few genetic loci per primer pair so the effective multiplex ratio was low ranging from 1-4 with an average of 2.263 per primer pair. Among the 19 microsatellite markers used in the study, RP test 9, Pt 71936 and Pt 30204 had the greater capacity to distinguish different genotypes of P. roxburghii as they have comparatively higher PIC, MI and RP values. The Jaccard's similarity coefficient values ranged from 0.409 to 0.955 suggesting wide genetic variability among the genotypes.

The dendrogram based on UPGMA analysis clustered all the genotypes in two major groups (clusters) and clearly separated most of the genotypes with low resin yield (0.25-2.5 kg) in cluster I and the genotypes with high resin yield (3-8 kg) in cluster II. Thus, the characterization through SSR markers showed that the two predefined groups (low and high resin yielding) were also genetically different.

Thus, this study clearly reveals the genetic basis of the variation in resin yield in P. roxburghii. Methods to improve the ac-

curacy of early selection at the individual level would be of considerable value for increasing the genetic gain per time unit. To achieve this objective, Marker assisted selection (MAS) is the promising method which results in indirect and nondestructive identification of genotypes, using a diagnostic system based on molecular markers which cosegregate with the trait of interest. Selection based on molecular markers will have an enormous impact on cost reduction in breeding programmes and will be of prime importance for forest product based industries, by guaranteeing quality forest products (Markussen et al., 2005).

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Table 1: **Location and resin yield of 53 genotypes of Pinus roxburghii used in the study**

S.No.	Sample	Location	Yield (gm)	Longitude (^{o}E)	Latitude(^{o}N)	Altitude (m asl)
1	A-1	Aspect 01, Site Quality 01; Chatra	2.2	77056' 16.9"E	30058' 02.3"N	1301
2	A-10	Aspect 01, Site Quality 01; Chatra	3.3	77056' 18.4"E	30058' 02.2"N	1298
3	A-12	Aspect 01, Site Quality 01; Chatra	8	77056' 42.9"E	30056' 54.9"N	1437
4	A-13	Aspect 01, Site Quality 01; Chatra	3.35	7709' 58.0"E	30051' 26.2"N	1203
5	A-19	Aspect 01, Site Quality 01; Chatra	5.2	77056' 17.4"E	30058' 02.7"N	1307
6	A-2	Aspect 01, Site Quality 01; Chatra	6.5	77056' 42.9"E	30056' 53.3"N	1423
7	A-24	Aspect 01, Site Quality 01; Chatra	2.8	77056' 20.3"E	30058' 01.0"N	1292
8	A-25	Aspect 01, Site Quality 01; Chatra	2.9	77056' 20.3"E	30058' 1.6"N	1292

S.No.	Sample	Location	Yield (gm)	Longitude (°E)	Latitude(°N)	Altitude (m asl)
9	A-28	Aspect 01, Site Quality 01; Chatra	6.2	77056' 43.3"E	30056' 52.9"N	1421
10	A-3	Aspect 01, Site Quality 01; Chatra	0.9	77056' 43.0"E	30056' 53.3"N	1423
11	A-6	Aspect 01, Site Quality 01; Chatra	1.4	77056' 43.2"E	30056' 53.6"N	1425
12	A-7	Aspect 01, Site Quality 01; Chatra	4.1	77056' 18.0"E	30068' 03.3"N	1293
13	A-9	Aspect 01, Site Quality 01; Chatra	5.8	77056' 42.5"E	30056' 54.4"N	1432
14	B-10	Aspect 01, Site Quality 02; Chatra	2	77056' 47.4"E	30057' 48.2"N	1454
15	B-12	Aspect 01, Site Quality 02; Chatra	4.6	77056' 48.4"E	30056' 49.3"N	1451
16	B-13	Aspect 01, Site Quality 02; Chatra	2.1	NA	NA	NA
17	B-14	Aspect 01, Site Quality 02; Chatra	0.25	770 56' 47.6"E	30056' 49.3"N	1442
18	B-18	Aspect 01, Site Quality 02; Chatra	1.2	NA	NA	NA
19	B-19	Aspect 01, Site Quality 02; Chatra	2.7	77056' 22.1"E	30057' 57.6"N	1315
20	B-2	Aspect 01, Site Quality 02; Chatra	2.5	77056' 46.4"E	30057' 47.5"N	1450
21	B-24	Aspect 01, Site Quality 02; Chatra	5.7	77056' 46.6"E	30056' 47.1"N	1438
22	B-25	Aspect 01, Site Quality 02; Chatra	6.4	77056' 46.8"E	30056' 42.2"N	1437
23	B-26	Aspect 01, Site Quality 02; Chatra	2.8	77056' 21.7"E	30057' 58.3"N	1306
24	B-3	Aspect 01, Site Quality 02; Chatra	4.9	77056' 46.7"E	30056' 47.4"N	1450
25	B-4	Aspect 01, Site Quality 02; Chatra	0.8	77056' 22.7"E	30057' 58.0"N	1316
26	B-6	Aspect 01, Site Quality 02; Chatra	4.5	77056' 47.0"E	30056' 47.3"N	1452
27	B-7	Aspect 01, Site Quality 02; Chatra	4.3	77056' 21.6"E	30057' 58.0"N	1324
28	B-8	Aspect 01, Site Quality 02; Chatra	2.25	77056' 47.3"E	30057' 48.5"N	1462
29	B-9	Aspect 01, Site Quality 02; Chatra	4.7	77056' 47.0"E	30056' 48.3"N	1454
30	C-1	Aspect 02, Site Quality 01; Chatra	4.2	77056' 36.5"E	30057' 9.8"N	1379
31	C-10	Aspect 02, Site Quality 01; Chatra	2.25	77056' 48.6"E	30056' 47.9"N	1406
32	C-12	Aspect 02, Site Quality 01; Chatra	2.25	77056' 37.2"E	30057' 10.1"N	1364
33	C-15	Aspect 02, Site Quality 01; Chatra	2.9	77056' 47.7"E	30056' 49.3"N	1404
34	C-20	Aspect 02, Site Quality 01; Chatra	2.9	77056' 46.6"E	30056' 50.3"N	1401
35	C-3	Aspect 02, Site Quality 01; Chatra	5	77056' 47.8"E	30056' 46.1"N	1404
36	C-4	Aspect 02, Site Quality 01; Chatra	2.7	77056' 37.2"E	30057' 9.8"N	1379
37	C-7	Aspect 02, Site Quality 01; Chatra	4.5	77056' 48.4"E	30056' 47.7"N	1404
38	C-8	Aspect 02, Site Quality 01; Chatra	4	77056' 38.8"E	30057' 11.2"N	1360
39	C-9	Aspect 02, Site Quality 01; Chatra	5.6	77056' 49.5"E	30056' 47.5"N	1405
40	D-11	Aspect 02, Site Quality 02; Chatra	2.6	77056' 30.4"E	30058' 4.9"N	1171
41	D-14	Aspect 02, Site Quality 02; Chatra	2.3	77056' 31.0"E	30058' 5.1"N	1171
42	D-24	Aspect 02, Site Quality 02; Chatra	1.75	NA	NA	NA

S.No.	Sample	Location	Yield (gm)	Longitude (^{0}E)	Latitude(^{0}N)	Altitude (m asl)
43	D-26	Aspect 02, Site Quality 02; Chatra	1.7	NA	NA	NA
44	D-27	Aspect 02, Site Quality 02; Chatra	0.9	77056' 38.2"E	30057' 4.4"N	1360
45	D-29	Aspect 02, Site Quality 02; Chatra	2.1	77056' 38.0"E	30057' 6.2"N	1353
46	D-30	Aspect 02, Site Quality 02; Chatra	1.6	77056' 38.3"E	30057' 6.5"N	1355
47	D-31	Aspect 02, Site Quality 02; Chatra	4.2	77056' 43.7"E	30056' 53.9"N	1396
48	D-33	Aspect 02, Site Quality 02; Chatra	5	77056' 42.4"E	30056' 53.5"N	1393
49	D-37	Aspect 02, Site Quality 02; Chatra	4.5	77056' 42.4"E	30056' 54.1"N	1432
50	D-38	Aspect 02, Site Quality 02; Chatra	4	77056' 43.2"E	30056' 53.4"N	1423
51	D-39	Aspect 02, Site Quality 02; Chatra	5.6	77056' 42.2"E	30056' 54.3"N	1421
52	D-7	Aspect 02, Site Quality 02; Chatra	1.3	77056' 38.8"E	30057' 4.5"N	1370
53	D-8	Aspect 02, Site Quality 02; Chatra	1.1	NA	NA	NA

Table 2: **Details of SSR primer pairs used for the study**

S.N.	Locus	Primer Sequence (5'-3')		Ta (^{0}C)	Size range (bp)	Repeat motif
1	pdms 011	Forward	TGTCAACTATATTGCTACCAAAC	55	141-147	$(GT)_{16}$
		Reverse	CGTCAATGATTCAAAATTCC			
2	pdms 221	Forward	GAGAGTTGTATGACGGAAATAC	55	151-201	$(GA)_9G_3(GA)_5$
		Reverse	CCCACACAAAAGTGTACTTC			
3	pm 05	Forward	GAGTCTAATTGCAAACCCCA	60	98-120	$(TG)_9$
		Reverse	TGGAGATCTACCACTTTTTC			
4	pm 07	Forward	GAATCTAAGCATATGAAATGAG	55	190-230	$(AC)_8(AT)_4$
		Reverse	CTTGTTAATGCTACTAGTTATG			
5	PtTX 3025	Forward	CACGCTGTATAATAACAATCTA	55	273-290	$(CAA)_{10}$
		Reverse	TTCTATATTCGCTTTTAGTTTC			
6	RPTest6	Forward	AGGATTCCAACAGCATCACC	60	73-77	TGC
		Reverse	CTGAACATGAAGCGCAGTGT			
7	RPTest9	Forward	CCAGACAACCCAAATGAAGG	60	314-357	AGC
		Reverse	GCCTGCTATCGAATCCAGAA			
8	Pt1254	Forward	CAATTGGAATGAGAACAGATAGG	57.8	146-161	$(T)_{17}$
		Reverse	TGCGTTGCACTTCGTTATAG			
9	Pt71936	Forward	TTCATTGGAAATACACTAGCCC	55	141-143	$(T)_{16}$
		Reverse	AAAACCGTACATGAGATTCCC			
10	Pt87268	Forward	GCCAGGGAAAATCGTAGG	55	138-182	$(T)_{14}$
		Reverse	AGACGATTAGACATCCAACCC			

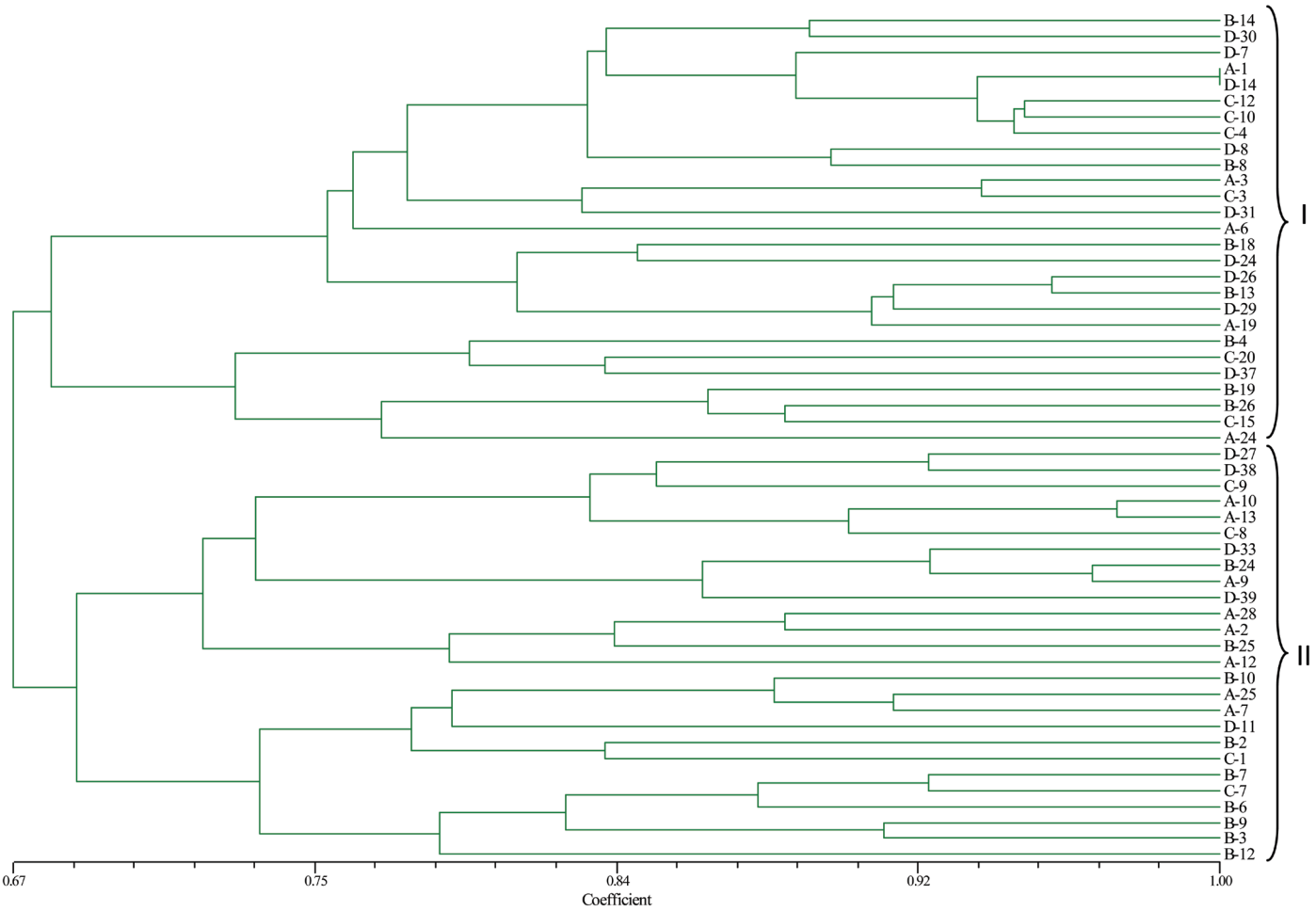
11	pm 09a	Forward	CCTTCTCATTTCGATATGCAC	58.5	108-115	$(AT)_5(GT)_{18}(AT)_2$
		Reverse	ATTAAAGGTTATATGGGGCT			
12	PCP26106	Forward	AATCCGACAAAAAAGATTCGG	58.5	144-155	$(A)_{14}$
		Reverse	GCTCCATTTCACGTGGTTG			
13	PCP30277	Forward	TGTTGATGTCGTAGCGGAAG	58.5	124-182	$(A)_{12}(G)_{10}$
		Reverse	ATGAAATGAATCACTTCCCCC			
14	Pt30204	Forward	TCATAGCGGAAGATCCTCTTT	56	143-145	$(A)_{12}(G)_{10}$
		Reverse	CGGATTGATCCTAACCATACC			
15	Pt45002	Forward	AAGTTGGATTTTACCCAGGTG	58	154-165	$(T)_{15}$
		Reverse	GAACAAGAGGATTTTTTCTCATACA			
16	Pt79951	Forward	CTTTTGTTTTTCAACAATTGCA	58.5	124-126	$(T)_{12}$
		Reverse	ACATCTATCTCCCATATCGGC			
17	PCP9434	Forward	AAACTGACGTAGATGCCATGG	58.5	114-117	$(A)_{10}$
		Reverse	GCGGTATGAGGGAAGAAGC			
18	Pt36480	Forward	TTTTGGCTTACAAAATAAAAGAGG	52	137-145	$(T)_{11}$
		Reverse	AAATTCCTAAAGAAGGAAGAGCA			
19	PCP41131	Forward	AAAGCATTTCCAGTTGGGG	58.5	137-142	$(T)_{11}$
		Reverse	GGTCAGGATTCATGTTCTTCC			

Table 3: **Total number of alleles, polymorphic alleles, polymorphism, polymorphic information content (PIC), marker index (MI), effective multiplex ratio (EMR) and resolving power (RP) obtained from 19 SSR markers.**

S. No.	Primer	No. of alleles	Polymorphic alleles	Polymorphism (%)	PIC	EMR	MI	Rp
1	pdms 011	2	2	100	0.253	2	0.507	3.396
2	pdms 221	2	1	50	0.115	0.5	0.057	2.264
3	pm 05	2	2	100	0.376	2	0.752	2.415
4	pm 07	2	2	100	0.311	2	0.622	3.170
5	Pt TX 3025	2	2	100	0.497	2	0.994	2.151
6	RP test 6	2	1	50	0.164	0.5	0.082	3.585
7	RP test 9	2	2	100	0.137	2	0.273	**3.698**
8	Pt 1254	2	2	100	0.414	2	0.827	2.038
9	Pt 71936	2	2	100	**0.499**	2	0.998	1.962
10	Pt 87268	3	3	100	0.324	3	0.971	1.887
11	pm 09a	3	3	100	0.339	3	1.018	1.962
12	PCP 26106	3	3	100	0.348	3	1.045	2.000
13	PCP 30277	2	2	100	0.391	2	0.782	2.679

14	Pt 30204	4	4	100	0.292	**4**	**1.168**	1.887
15	Pt 45002	5	4	80	0.262	3.2	0.838	1.887
16	Pt 79951	2	2	100	0.459	2	0.917	1.509
17	PCP 41131	2	2	100	0.486	2	0.971	1.660
18	Pt 36480	2	2	100	0.303	2	0.607	1.660
19	PCP 9434	2	2	100	0.473	2	0.946	1.547
	Total	46	43					
	Minimum	2	1	50	0.115	0.5	0.057	1.509
	Maximum	5	4	100	0.499	4	1.168	3.698
	Average	2.421	2.263	93.680	0.339	2.168	0.757	2.282

Fig. 1: **Dendrogram of 53 *Pinus roxburghii* genotypes based on Jaccard's similarity coefficient. Codes of the genotypes follow those given in table 1.**



Establishment of Nodulation and Nitrogen Fixation in *Casuarina junghuhniana* Miq. Rooted Stem Cuttings with *Frankia* under Aseptic Conditions

A. Karthikeyan*, K. Chandrasekar and M. Geetha

INTRODUCTION

Actinorhizal plants usually form root nodules in association with the nitrogen fixing actionmycete *Frankia* that helps them to survive even in nutrient poor soils by Nitrogen (N) fixation. *Frankia* is a filamentous soil bacteria, which interacts with the roots of appropriate host plants to form nitrogen fixing nodules also called actinorhizae (Benson and Silvester, 1993). Actinorhizal plants include Casuarinaceae which is a major family of trees that have been disseminated throughout the tropics owing to their ability to grow in adverse conditions (Echhab *et al.* 2007). *Casuarina junghuhniana* Miq. belongs to Casuarinaceae and due to to its high economic value farmers are interested in planting this tree as an agroforestry crop in the states of Tamilnadu and Pondicherry. It is useful as wind break, an ornamental plant, soil improvement, live fencing and building material (Jayaraj, 2010). *Frankia* associated with *C. junghuhniana* for N fixation and it has been estimated that *Frankia* fixes atmospheric nitrogen up to 362 kg N/ha/yr, which is an essential nutrient for all plant metabolic activities and growth (Shantharam and Mattoo, 1997).

To inoculate *Frankia* generally farmers used to collect the root nodules from mature trees of Casuarina trees and then crush and add at the time of planting in new sites .along with seedlings/cuttings of *Casuarinas spp.* (Karthikeyan *et al.* 2010). This practice is often unsuccessful if the crushed root nodules contain dead or inactive *Frankia*. Further, for pulp and paper production high yielding genetically superior trees of *C. junghuhniana* are selected and multiplied by rooted stem cuttings through Farmers of Tamilnadu and Pondicherry. But the rooted stem cuttings are being propagated in an inert material (vermiculite) so that there is no chance for *Frankia* association. Though inoculation of *Frankia* is essential in rooted stem cuttings of *C. junghuhniana* there is no report found on nodulation of rooted stem cuttings in *C. junghuhniana.* However, Echhab *et al.* (2007) found *Frankia* nodulation in *C. cunninghamiana* under axenic conditions. Hence, there is an urgent need to find an alternate solution for use of these chemical fertilizers for the rooted stem cuttings of *C. junghuhniana* during plantation. We attempted to study the effect of inoculation of cultured *Frankia* strain in rooted stem cuttings of *C. junghuhniana* on growth, biomass and nodulation which could reduce the use of chemical fertilizers. Further, it is intended to decide the effect of *Frankia* strain on the efficiency of N uptake and N use efficiency of *C. junghuhniana* rooted stem cuttings.

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MATERIALS AND METHODS

Location of the study

The study was conducted at the Model Nursery of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore (11° 01`N and 96°93`E; altitude 410 m a.s.l) Tamil Nadu, India. The climate is monsoonal with an annual precipitation of 640 mm and a dry season between January and April. The maximum and minimum monthly temperatures are 31 and 21°C respectively.

Isolation and multiplication of Frankia

The *Frankia* used in this study was isolated from *C. junghuhniana* root nodules collected from coastal area and the location and characteristics of collected nodules were shown in the Table.1 as presented below.

Table.1: **Source of *Frankia***

Place	Soil type	Source of Nodules	Nodules Colour	Nodules diameter
Cuddalore (T.N) Coastal zone	Sandy clayloam	Coastal plantations of *Casuarina junghuhniana*	Brown	1-1.5 cm

The nodules were collected in ice box and stored in frozen condition at - 4 °C. Afterwards the nodules were surface sterilized with 30% H_2 O_2 and kept in shaker for 30-40 minutes. Under aseptic conditions the nodules were rinsed in sterile water and 0.2 gram of nodule was ground manually in sterile mortar and pestle. Then the nodule solutions were centrifuged at 1000 rpm for 20 minutes and the supernatant was filtered through Whatman No.1 filter paper. The suspension was then plated in P media and incubated at 25°C for 3- 4 weeks. One litre of P medium was prepared as follows (Shipton and Burgraff, 1983) : 10g

$CaCl_2$,2H_2O, 20g $MgSO_4$, 0.46g Propionic acid, 0.15g H_3BO_3, 0.15g $ZnSO_4$.7H2O, 0.45g $MnSO_4.H_2O$, 0.004g $CuSO_4.5H_2O$, 0.028g $Na_2MoO_4.2H_2O$, 0.009g $CaCl_2.6H_2O$, 0.04g Biotin, 100g K_2HPO_4, 67g $NaH_2PO.2H_2O$, 0.1g FeNa EDTA, and 8.g agar. The pH of the medium was adjusted to 6.8. After 21- 30 days of incubation the *Frankia* growth was observed as fluffy white colonies on P media plates. These colonies were transferred in to P media broth for mass multiplication.

Analysis of Nitrogenase activity

The Nitrogenase activity of *Frankia* was determined in 15-21 days old culture in N free P media broth by using the acetylene reduction technique (Hardy *et al*. 1968) to confirm the presence of Nitrogenase in the *Frankia* strain which is essential to break down the triple bond of Nitrogen. 30 ml of culture placed in 130 ml capacity of sterilized vials and sealed with rubber stoppers. About 10% of the air space in each vial was replaced by pure acetylene and allowed to stand for one hour incubation at room temperature. About 0.5 ml of the gas was removed from each vial and injected in to a gas chromatography (GC: Model: Nucon 910980) equipped with a flame ionization detector and a 2m x 2.1 mm stainless steel column packed with Porapak Q on 80-100 mesh. The oven temperature was adjusted to 70° C; injector temperature 50°C; detector temperature 120°C. The Nitrogen carrier gas flow rate was adjusted to 30 ml/min) to measure ethylene production. Blanks comprised air from bottles to which no acetylene was added. Peaks of ethylene were compared with ethylene standard (purity 99.9%) injected in to the GC to calculate concentrations. The nanomoles of ethylene produced per time unit were standardized to total cell protein. The protein concentration of the cells was determined as described by Lowry *et al* (1951) with bovine serum albumin as the standard. The specific activity of nitrogenase was expressed as nanomoles of ethylene produced per mg of protein per hour. The rate of Nitrogen fixation was calculated using the formula:

$$\text{Nitrogenase activity} = \frac{\text{Peak area count} \times 0.0006 \times \text{volume of gas injected in to vial}}{\text{Incubation time} \times \text{volume of gas injected in to GC} \times \text{total mg of protein in the sample}}$$

Collection and propagation of *C. junghuhniana* stem cuttings

The stem cuttings were collected from the clones Cj 18 at Model Nursery, IFGTB and treated with 0.1% carbendazim fungicide and 2000 ppm of Indole Butyric Acid (IBA). After the treatment with IBA the cuttings were placed in 100 cc root trainers contains the inert media vermiculite. The rooted cuttings were thereafter placed in polytunnels made of poythene sheets (32-35°C and 60-65% RH) for 30days. After 25 days the cuttings showed initiation of 2-3 lateral roots with 1 cm -1.5 cm length. In this stage the rooted stem cuttings were transferred to shade house and watered regularly.

Inoculation of *Frankia* in *C. junghuniana* rooted stem cuttings

The cultured *Frankia* strain P media broth was inoculated in the root zone of rooted stem cuttings of *C. junghuhniana* clones Nos. Cj 18 at the rate of 5 ml^{-1} cutting and maintained 15 replicates. Root trainers containing inoculated cuttings and uninoculated controls were placed in the shade house and watered regularly. The initiation of nodules and nodule numbers in each rooted stem cuttings were assessed. These planting stocks were maintained for 3 months in the model nursery of IFGTB and harvested for analysis of growth and biomass. The dry weights of *Frankia* inoculated these planting stocks were determined after oven drying at 50^0C to a constant weight.

Analyses of growth, biomass and tissue Nitrogen (N) content

The growth of *Frankia* inoculated rooted stem cuttings and un-inoculated cuttings were analyzed in terms of shoot length, root length, number of lateral roots, collar diameter, dry weights of shoot, root, number of nodules and nodule bio mass. The dry weights were determined after oven drying at 50^0C to a constant weight. The total N content was estimated in root and shoot sample using KELPLUS auto analyzer to determine the N fixation by inoculation of *Frankia* in the rooted stem cuttings of *C. junghuhniana.* The dried plant sample (0.25gm) was digested with 3gm of Catalyst Mixture: (Potassium Sulphate and Cupric Sulphate in ratio of 5:1) and 10ml of H_2SO_4 in Kjeldhal digestion system (KELFLOW) at 420^0C for 1 hour. Then the digested sample was diluted with 10ml of distilled water before distillation. After distillation, the collected distillate was titrated against 0.1N Hydrochloric acid.

Statistical analysis

Each measured variable in the nursery experiment were subjected to analysis of variance and means were separated using Duncan's multiple range test (SPSS ver. 10).

RESULTS

Morphological Characteristics of *Frankia* Isolate

Under optimal conditions (28 – 32° C) the growth of the isolate formed white fluffy colonies on the P media plates were examined under light microscope. It showed branched and septate hyphae and round vesicles. The morphometrics of *Frankia* are shown in Table.3.

Nitrogenase activity

The nitrogenase activity of *Frankia* was measured at various intervals. The experiment was repeated three times and the mean value of the isolate was calculated. The observation of ability to reduce acetylene *in vitro*, support the fact that actinomycete bacteria isolated in this experiment able to fix nitrogen in the *Frankia* inoculated rooted stem cuttings. The *Frankia* showed the nitrogenase activity at 21 days old liquid culture that results an amount of 158.23 nano moles of ethylene produced per mg of protein per hour (Fig.1).

Growth and Biomass of *C. junghuhniana* rooted stem cuttings

Nodulation of *Frankia* was observed at 25-30 days after inoculation in the rooted stem cuttings of *C. junghuhniama*. The ini-

tial infection at 25 days showed clubbed roots in the rooted stem cuttings and the nodule development occurred at 30 days. The rooted stem cuttings of the inoculated with *Frankia* strain showed significantly increased growth in shoot length, root length, collar diameter and bio mass than the un-inoculated control seedlings. The rooted stem cuttings showed higher nodule biomass than the un-inoculated control. *Frankia* inoculated cuttings showed dense root nodules in the root region whereas the uninoculated cuttings showed absence of nodules. The root nodules developed in the rooted stem cutting weighed up to 43mg and number root nodules were also obtained 12 per cuttings in the rooted stem cuttings of the clone 73. The R/S ratio was showed significantly lower in *Frankia* inoculated rooted stem cuttings than the un-inoculated control (Table. 3). The new finding in the present study is successful nodulation establishment in the *C. junghuniana* rooted stem cuttings in inert media without using soil.

Tissue N content

Significant differences in total nitrogen content in comparison with uninoculated controls were observed. The total N content was found 5.64 mg/g dry weight in the rooted stem cuttings whereas the un-inoculated control rooted stem cuttings showed a mean value of 0.41 mg/g dry weight. (Fig 2).

DISCUSSION

The results of this study have clearly showed that *Frankia* can improve the plant growth through increased uptake of N. *Frankia* results in positive effect on the rooted stem cuttings of *C. junghuhniana* growth through improvement of growth and bio mass. In earlier studies were also reported that the increase in growth and biomass of casuarinas due to inoculation of *Frankia* might be strongly correlated with improved accumulation of nitrogen due to *Frankia* (Reddell *et al*. 1988). This study further supports the positive response of *C. junghuhniana* rooted stem cuttings in the nursery to *Frankia* application and strengthens the *Frankia* dependency of *C. junghuhniana* in low fertility. Similar results were reported for *Frankia* (nodule suspension) inoculation employed in *C. equisetifolia* seedlings (Muthukumar and Udaiyan, 2010). In several studies (Lesueur and Duponnois 2005; Yamnaka *et al.* 2005) the *Frankia* effects on the plant growth promotion has been demonstrated in sterile soil substrates. However the growth promoting effect of *Frankia* on *C. junghuhniana* rooted stem cuttings in inert media has not been reported. It has been repeatedly reported that spontaneous nodulation of the genera Casuarina is unlikely out side their natural habitat. This may be attributed to the fact that *Frankia* is not possible to transmit with the seed either within or on its surface (Torrey, 1983). Inoculation experiments of this kind in nursery conditions is essential for *C. junghuhniana* rooted stem cuttings which bring together the root system and nodulation as they propagated in inert media. In this study nodulation occurs in 30 days in the rooted stem cuttings of *C. junghuhniana* however Vergnaud *et al.* (1983) obtained axenic nodulation in *Alunus glutinosa* within 10 days. This also showed that there is a difference in nodulation behaviour between *Alnus* species and *C. junghuhniana*. In this study nodulation biomass and nodule number were increased the rooted stem cuttings of *C. junghuhniana* raised in inert media. This reflects the symbiotic nitrogen fixation is dependent on host photosynthesis (Arnone and Gordon 1990) where the ATP supplemented to *Frankia*. The increased biomass in the rooted stem cuttings of both the clones could be the result of increased nutrient inflow rates through *Frankia*. The nitrogenase activity of *Frankia* in this study showed that the *Frankia* culture contains more vesicles. Because vesicles of *Frankia* have been considered as the sites of nitrogenase for many years (Fontaine *et al*. 1984; Gauthier *et al.* 1981). The nitrogenase activity of *Frankia* also corroborates supply of Mg-ATP from the *Frankia* inoculated *C. junghuhniana* cuttings that gives energy for N fixation (Huss- Dannel and Hablin 1988; Huss–Dannel and Selsted 1985; Sundstrom and Huss–Dannel 1987). The increased tissue N content of *Frankia* inoculated rooted stem cuttings of *C. junghuhniana* raised in inert media than the un inoculated control plants showed that the influence of *Frankia* in N fixation.

CONCLUSION

The results from this study support the inoculation of cultured *Frankia* to the rooted stem cuttings of *C. junghuhniana* for enhancement of growth, biomass and nutrient uptake. It is essential to introduce potential *Frankia* in the rooted stem cuttings of *C. junghuhniana* as they propagated in inert media (vermiculite). This method of inoculation of *Frankia* in the rooted stem cuttings of C. *junghuhniana* will be beneficial for early establishment in the field without additional chemical fertilizers even in low fertile lands.

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Table 2: **Morphometrics of *Frankia***

Hyphal width	Vesicle dimension	Sporangia	No. of days grown
(in µm @ 40x)	**(in µm @ 40x)**	**shape**	**in media**
1- 1.5	2 - 3	Circular	25 days

Table 3: **Growth and bio mass of *C. junghuhniana* rooted stem cuttings to *Frankia* inoculation at 90 days under nursery conditions.**

myria	Treatments	Collar Diameter (cm Plant -1)	Shoot length (cm Plant -1)	Root length (cm Plant -1)	No. of lateral roots /plant	Shoot dry weight (mg Plant-1)	Root dry weight (mg Plant -1)	R/S ratio	Nodulation time	No. of nodules	Nodule biomass (mg-1 nodule)
Cj 18	*Frankia*	1.871 b	18.89 b	14.3 b	15.1 b	0.905 b	0.557 b	0.615 b	30 days	12.12	43
	Control	0.542 a	5.9 a	4.8 a	1.8 a	0.288 a	0.199 a	0.690 a	-----	----	-----

Data was Mean of 15 replicates; Means followed by same letters are not significantly different at p<0.05 according to Duncan's Multiple Range Test.

Fig.1: **GC analysis for Nitrogenase activity of *Frankia***

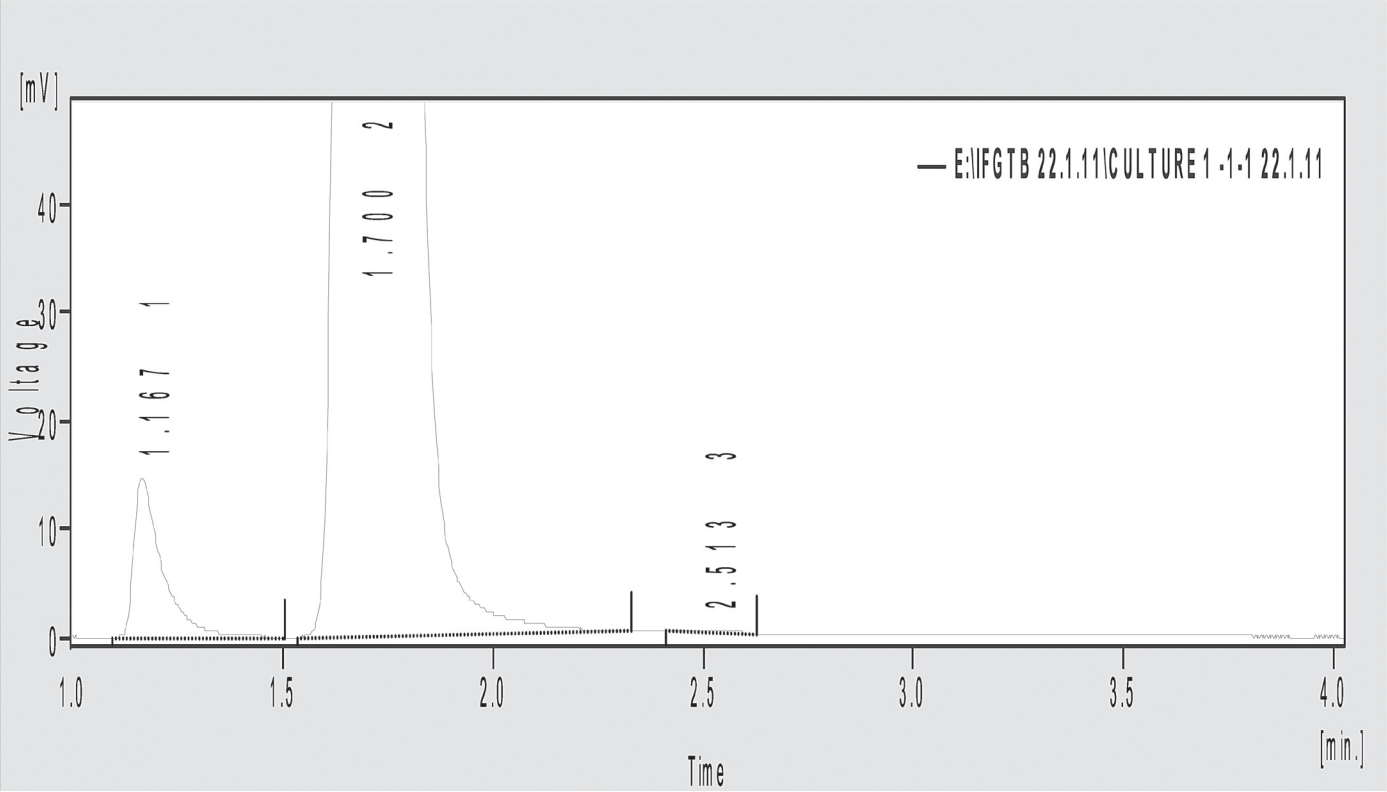
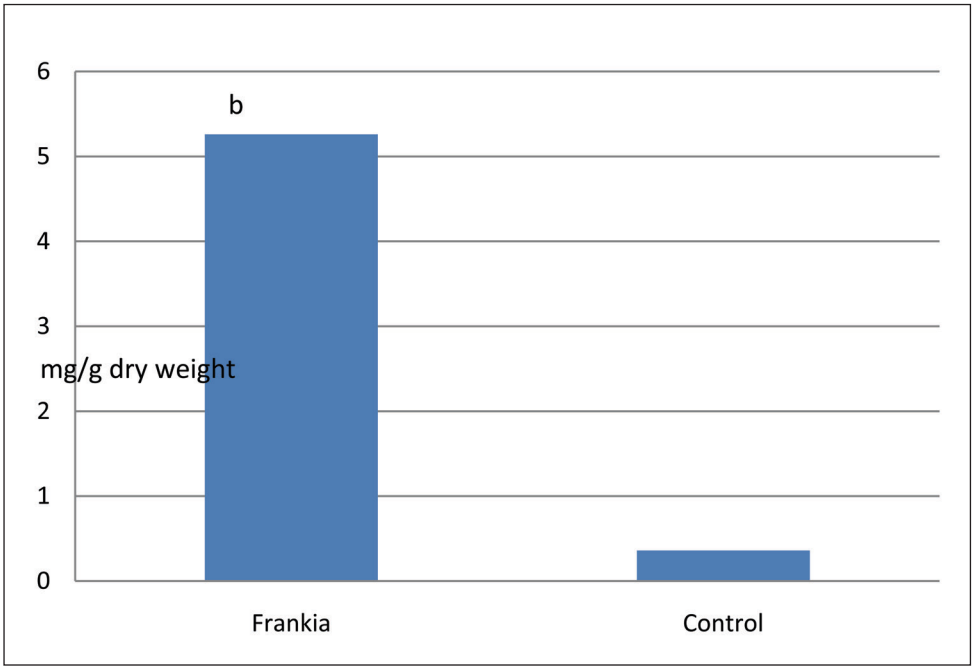


Fig. 2: **Total N content in the rooted stem cuttings of *C. junghuhniana* inoculated with Frankia**



Means followed by same letters are not significantly different at p<0.05 according to Duncan's Multiple Range Test.

Utilization of Tissue Culture Technique for Propagation of *Melia dubia* Cav.

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INTRODUCTION

Melia dubia Cav. family Meliaceae is an indigenous species to India, Southeast Asia and Australia. It is a fast growing multipurpose tree species, which is in high demand as high quality termite and fungus resistant timber and has potential to use in biomass power plants (power generation), plywood industry, apart from the urban landscaping and in afforestation. Conventionally, *M. dubia* is propagated through seeds, which has short period of viability. The seed are very hard and hardly germinate without any treatment. Fruits generally, do not drop after ripening and cling to the branches for several months even after the leaves have fallen and often loose viability on the tree itself. Therefore, production of planting material of *M. dubia* has become a problem. Seed germination has been reported in *M. dubia* very poor 14% (Nair *et al*., 2005). There is only one report on vegetative propagation of *M. dubia* with success rate of 76-94% rooting from the coppice juvenile stem cuttings treated with 5% Indole-3-butyric acid (IBA) (Manjunatha, 2007).

It is very difficult to take up large scale plantation of *M. dubia* due to above cited problems. The use of modern *in vitro* techniques on woody trees has opened new possibilities for rapid and mass multiplication of plus trees/elite genotypes for improved productivity and *ex situ* conservation of germplasm (Husain *et al*., 2008).

There is no report on *in vitro* cloning through axillary shoot proliferation, adventitious regeneration and somatic embryogenesis in *M. dubia*. In the above background and importance of this species, to produce desired quality of identical saplings, investigations were carried out with the objective to find out suitable propagation techniques using *in vitro* culture methods.

MATERIAL AND METHODS

Collection, processing and surface sterilization of the explants

Newly grown shoot segments with 6-8 nodes were collected during morning hours from the experimental field station of Karnataka State Forest Department Nallal, Bangalore. After excision of leaves, shoots were cut into 2.5-3.0 cm nodal shoot segments. To remove dust particles from the explants surface, explants were dipped in Tween-80 (0.1% v/v) liquid detergent solution for 15 minutes, shaken periodically and washed 3-4 times with double distilled water to remove carryover effect of detergent. In order to minimize fungal contamination, explants were treated with 0.1% (w/v) solution of Bavistin (Carbendazim 50% WP- a systemic fungicide) for 10 min and washed 3-4 times with double distilled water. Later on, explants were surface sterilized with 70% (v/v) ethanol for 50 seconds and washed 3-4 times with sterile double distilled water, followed by the treatment with freshly prepared 0.1% (w/v) $HgCl_2$ for 8-10 minutes, based on type and nature of explants used and washed 6-7 times with sterile double distilled water.

Culture media and incubation conditions

Murashige and Skoog (1962) medium (MS) containing 3% sucrose and gelled with 0.7% agar (Hi-media, India) was used in all the experiments. After adjusting the pH to 5.8, using 0.5M NaOH or HCL 0.1N, about 20ml of medium was dispensed into each 150×25mm Borosil culture tubes. The culture tubes were plugged with non-absorbent cotton wrapped in cheesecloth and autoclaved at 1.06 kg cm^{-2} and 121°C temperature for 20 min. Surface sterilized explants were inoculated in the medium vertically, inserting 1/3 of the explant in contact of medium. All the cultures were maintained at 25±2°C temperature and 2500 lux intensity of light for 16h photoperiod.

Shoot initiation

Nodal shoot segment as explants was cultured on MS medium supplemented with different concentration of BAP (0.3, 0.5, 1.0, and 2.0) either alone or with combination of auxins; IAA or NAA (0.1-0.2mg/l). In order to obtain multiple shoots, explants along with *in vitro* differentiated shoots were subcultured on MS medium with NAA (0.1 mg/l) and BAP (0.5 mg/l) for 2-3 passage at the intervals of 2 weeks.

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Shoot multiplication

Healthy shoots (2-3 shoots/clump) of 2 to 3cm long obtained from the mother nodal explants were excised after four weeks and sub cultured on MS medium supplemented with various concentrations of BAP (0.1-2.5 mg/l) alone as well as with NAA or IAA (0.1-0.2 mg/l) for shoot multiplication.

In vitro rooting and hardening

Shoots of 3-4 cm in length were excised from the shoot multiplication cultures and cultured on MS/2(basal salts) medium supplemented with IBA (0.1 -0.5 mg/l).

The rooted plantlets after four weeks were washed with water to remove adhered agar-agar and transferred to potting mixture of, sand, soil and compost (1:2:1, v/v), and kept under polytunnel to maintain high humidity for four week in mist chamber at 28°C±2°C temperature and 85% relative humidity. The natural illumination was cut 50% by using a shade net. The hardened plants were then transferred to a net house under ambient humidity and temperatures and maintained for another 30 days for hardening.

Data Analysis

Experiments were laid in a completely randomized design with a minimum of 3 replicates per treatment and each replicate consisted of 7 explants. Experiment was repeated at least thrice. One way analysis of variance was carried out for all experiments.

RESULTS AND DISCUSSION

The effect of PGRs was found significant on frequency of shoot initiation, and shoot length. Among the various concentrations of BAP (0.3-2.0) and auxins; IAA and NAA (0.1-0.2mg/l) used in MS medium, BAP(0.5mg/l) + NAA (0.1 mg/l) in medium proved to be better for high frequency (90%) nodal shoot initiation with maximum shoot length 2.8±0.80 cm.(Table 1 and Fig.1A). Similarly, auxiliary effect of combined use of NAA (0.1mg/l) and BAP (1.0-2.0 mg/l) proved the most effective in shoot initiation in *Camptothec acuminate* (Liu and Li., 2001). Contrary to above, Arora *et al.* (2010) reported that combination of BAP (1.11 μM) + IAA (1.43μM) and adenine hemisulphate (81.43 μM) in the medium proved the most effective for inducing multiple shoots in *Azadirachta indica*.

Among the different concentrations of BAP (0.1-2.5 mg/l) either alone or with NAA/IAA (0.1-0.2 mg/l) tested in MS medium for shoot multiplication, medium consisted BAP (0.5mg/l) alone was found the best for multiple shoot induction (93%) with highest (9.83±0.29) shoot number per clump with maximum shoot length 3.73±0.12 (Table 2, Fig.1B). The use of auxins in the medium favoured callus induction from the shoot base. In *Azadirachta indica* (Venkateswarlu *et al*., 1998; Chaturvedi *et al*., 2004 and Quraishi *et al*., 2004) revealed that the use of BAP without auxins was the best cytokinins for *in vitro* shoot multiplication.

Among the different concentrations of IBA (0.1 -0.5 mg/l) used in MS/2 basal salt medium, IBA 0.5 mg/l in the medium favoured highest (98.00%) rooting with good root number and maximum root length (4.41, cm) within four weeks (Table 3 and Fig.1C). Similar to our findings, San *et al*. (2010) found that the half strength MS medium supplemented with IBA was most suitable for rooting in *Melia azedarach*. Generally, IBA has been observed to induce strong rooting response and extensively used in a wide range of plant and specifically tree species (Eeswara *et al*., 1998; Husain *et al*., 2008). Contrary, Venkateswarlu *et al*. (1998) revealed that IAA (2-3mg/l) in MS/2 medium was the best auxin for rooting of neem shoots. The micro propagated plantlets of *M. dubia* were successfully acclimatized with 90% survival rate. Protocol developed will be useful for the clonal propagation of *M. dubia*.

CONCLUSION

The result of present study shows that *M. dubia* can be successfully propagated by tissue culture form plus tree with more than Ninety percent shoot inductions in MS agar medium supplemented with NAA 0.1 mg/l + BAP 0.5 mg/l. High rate of shoot multiplication can be achieved on MS medium with BAP (0.5 mg/l). More than (>90%) rooting frequency can be achieved from *in vitro* shoot of 3 to 4 cm in length on MS/2 medium supplemented with IBA 0.5 mg/l in 4 weeks period with high rate of survival.

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Fig. 1: **Micropropagation of Melia dubia Cav.**

A-C, A. Shoot proliferation from nodal shoot segment in MS medium with NAA (0.1 mg/l) +BAP (0.5 mg/l), B. Multiplication of shoots in MS medium + BAP (0.5 mg/l) and C. Rooting of shoots in MS/2 medium + IBA (0.5mg/l).

Table1: **Effect of cytokinin and auxins in MS medium on shoot initiation from nodal shoot segments of *M. dubia* after four weeks.**

Cytokinin	Auxins		% of response on shoot initiation	Average shoot length (in cm)
BAP (in mg/l)	NAA (in mg/l)	IAA (in mg/l)		
0.3	-	-	67.60	1.00±0.20^{b}
0.5	**-**	**-**	**85.03**	**1.97±0.50^{a}**
1.0	-	-	77.21	1.37±0.40^{b}
2.0	-	-	55.30	0.30±0.20^{b}
	0.1	-	**90.00**	**2.80±0.80^{a}**
	0.2	-	87.34	1.10±0.10^{b}
		0.1	82.42	1.53±0.06^{b}
		0.2	75.43	1.20±0.20^{b}

Treatments followed by different letters are significantly different from each otherand treatments followed by the similar alphabet superscript are statistically on par with each other at α=0.05.
Numbers of replicates for each treatment were 3 and each replicate consisted of 7 explants.

Table2: **Effect of cytokinin (BAP) and auxins (NAA and IAA) on shoot multiplication of M. dubia on MS medium after three weeks**

Cytokinin	Auxins		% of multiple shoot production	Number of shoots/ clumps	Shoot length (in cm)	Remarks (callusing)
BAP (in mg/l)	NAA (in mg/l)	IAA (in mg/l)				
-	-	-	51.93	3.00±0.50^{d}	1.13±0.12^{b}	-
0.1	-	-	60.20	4.97±0.68^{c}	1.97±0.57^{b}	-
0.5	**-**	**-**	**93.00**	**9.83±0.29^{a}**	**3.73±0.12^{a}**	-
1.0	-	-	79.67	7.36±0.31^{b}	2.57±0.51^{b}	++
2.0	-	-	55.15	5.24±0.15^{c}	1.50±0.56^{b}	+++
0.5	0.1	-	70.27	7.00±0.30^{b}	1.40±0.10^{b}	+++
0.5	0.2	-	75.17	4.33±0.58^{c}	1.43±0.32^{b}	++++
0.5	-	0.1	90.00	8.49±0.25^{b}	3.63±0.51^{a}	+++
0.5	-	0.2	74.87	7.90±0.10^{b}	2.80±0.26^{b}	++++

Treatments followed by different letters are significantly different from each other and treatments followed by the similar alphabet superscript are statistically on par with each other at α=0.05.
Numbers of replicates for each treatment were 3 and each replicate consisted of 7 shoot clumps

Growth Performance of Industrially Important Bamboo Species in Two Different Agro Climatic Conditions

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INTRODUCTION

Bamboos are fast growing, short rotation, ecologically and economically important plant species. They contribute, to a great extent to the economy for rural communities in developing countries, particularly in the Asia-Pacific region. Out of 75 genera and 1250 species of bamboo recorded from all over the world (Soderstrom and Ellis, 1987). India constitute 125 species of bamboo in 23 genera including 10 exotics, which provide scope for selection of the fast growing, economically and industrially important bamboo species suitable for various agro climatic conditions for plantations. Bamboo species have proven potential to act as a substitute of timber in many ways including new generation products. Bamboo have a wide range of distribution and covering an estimated area of 8.96 million hectares, which constitutes 11.71% of the forest area in deciduous and semi evergreen regions of India (Rai and Chauhan, 1999). Demand of bamboo is estimated to 26.6 million tonnes/year, whereas supply is of 13.7 million tonnes/year (Anonymous, 2003). Due to its wide spread use and demand, bamboo is harvested mainly from the forests, which is depleting gradually and will not be sustainable for future. To meet the indigenous demand, plantation outside forest (agroforestry, farm forestry and social forestry) reduces pressure on existing forest.

A long flowering cycle and cumbersome in conventional propagation (rhizomatous, culm and branch cutting) methods, limits mass multiplication and improvement in bamboo. Biotechnological tools provide scope for rapid and mass production of high quality planting material of bamboos. Selection of superior genotypes based on morphological traits like; culm height, diameter, wall thickness and strength properties provide scope of increased productivity and uniformity of plantation. Tissue culture based biotechnology has the potential to overcome the problems encountered in traditional methods of propagation for commercially important bamboo species. *In vitro* propagation of bamboo through axillary shoot proliferation from nodal explants have been reported in *Dendrocalamus asper* (Arya *et al*., 2002), *Dendrocalamus hamiltonii* (Sood *et al*., 2002), *Pseudoxytenanthera* stocksii (Sanjaya *et al*., 2005, Somashaker *et al*., 2008), *Bambusa balcooa* (Das and Pal, 2005), *Guadua angustifolia* (Jimenez *et al*., 2006) and *B. nutans* (Yashoda *et al*., 2008).

Present study was taken up to evaluate the field performance in different agro climatic zones of six industrially important micropropagated bamboo species (*Bambusa balcooa, Bambusa nutans, Dendrocalamus asper, Dendrocalamus hamiltonii*, *Dendrocalamus stocksii* and *Guadua angustifolia*) at Chintalpudi, Andhra Pradesh and Shimoga, Karnataka.

MATERIALS AND METHODS

The *in vitro* cultures of *Bambusa nutans, Dendrocalamus asper, Dendrocalamus stocksii* and *Guadua angustifolia* were established at tissue culture lab, IWST through axillary shoot proliferation from mature culm nodal explants collected from Bamboo gremplasm bank, Gottipura, Bangalore. Whereas, *Bambusa balcooa* and *Dendrocalamus hamiltonii* were outsourced from Growmore Biotech, Hosur, Tamil Nadu and IHBT, Palampur respectively.

Field trials were conducted during onset of monsoon (2007-2008) at two different locations viz; Chintalpudi, Eluru, Andhra Pradesh and Navatoor, Shimoga, Karnataka. Six months old hardened plant with 25-35 cm in height and 2.0 to 3.0 tillered were used for the field trials. Planting was carried out in 1cu.m earthern pits at spacing of 5x5m. At the time planting 1kg FYM + 100g neem cake + 50g SSP were used in each pit. After planting, 0.1% (v/v) Chloropyrophos solution was applied in each pit as prophylactic measures. Weeding and soil work was done as and when required. Percentage survival, number of culms and culm height (in cm) were taken into consideration as parameters to evaluate growth performance of each bamboo species.

All the experiments were conducted in a randomized block design (RBD), the data was analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel version 5.0 and means were compared using Tukey's *t*-test at 5% level of significance (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSION

The initial (six months) survival rate of micropropagated plants

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was varied from 33.47-92.88% (Data not shown) and final survival (after 30 months) rate varied from 54.61-90.46% depending upon species and locations. In the present study results revealed that, *B. nutans* proved the best species for both the states. Maximum survival (90.86% and 89.44%) was observed in *B. nutans* at Navatoor site, Shimoga, Karnataka and in Chintalpudi, Eluru, Andhra Pradesh (Plate 1, A and B) respectively. This was followed by ***B.*** *balcooa*, *D. hamiltonii* and *D. stocksii* (Fig 1). However *D. asper* and *G. angustifolia* were survived only in Navatoor, Shimoga demo plot. Whereas, complete mortality was observed at Chintalpudi site over a period of time even casualty replacement were previously undertaken. This may be due to they are belongs to high rainfall area.

Subsequently, higher growth performance in terms of number of culms (12.69 and 6.67) and culm height (388.93 and 256.91 cm) was observed in *B. nutans* at both the respective planting sites of Andhra Pradesh and Karnataka (Fig 2 and 3), followed by ***B.*** *balcooa, D. hamiltonii* and *D. stocksii* in the order of sequence. However among exotics, *D. asper* was superior over *G. angustifolia* at Navatoor site which produced more culms (5.48) with culm height of 130.16 cm. Lowest survival of *D. asper* and *G. angustifolia* in Navatoor and Chintalpudi site, may be due to susceptibility to termite attack and may need intensive management for large scale adaptation.

CONCLUSION

To promote bamboo plantation at mass scale, mainly depends on quality planting stock, site of planting and climatic conditions. In the present study, survival percentage and growth performance of micropropagated plants that were assessed after 30 months that leads an information about suitability of a particular bamboo species at specified site for large scale plantation. Overall, *B. nutans* and *B. balcooa* proved the best species in both agro climatic conditions based on survival percentage, culm number and culm height fallowed by *D. hamiltonii* and *D. stocksii*. Whereas, both exotic species (*D. asper* and *G. angustifolia*) were not suitable for large scale plantation in both the locations.

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Clonal Propagation of an Economically Important Woody Tree of the Arid Zone-*Tecomella undulata* (Sm.) Seem.

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INTRODUCTION

Tecomella undulata is an important timber tree of Rajasthan and locally known as Marwar teak. Its distribution is restricted to the drier parts of the Arabia, southern Pakistan and northwest India. In India, it occurs naturally in Maharashtra, Gujarat, Rajasthan, Punjab and Haryana. The species is mainly found in western Rajasthan. Its wood is strong, durable and forms a great source of timber. It is also used widely in various Ayurvedic medicines and truly a multipurpose very important tree of western India. Overexploitation and poor regeneration are the main reason for its population decline and resulted as threatened species. Forest department of Rajasthan and Gujarat taken this species as one of priority species for their plantation schemes and tree improvement programme. Arid Forest Research Institute is also running tree improvement research projects. (Anonymous 2009). However the major hurdle in tree improvement programme is the lack of reliable clonal propagation technique.

Genetic variation reported among the trees population/sources of *T. undulata* was reported by Jindal et al., (1987), Bhau et al., (2007) and Negi et al., (2011). Thus there is a scope of tree improvement in this species for enhancing productivity. Vegetative propagation is an important aspect of the tree improvement programmes, to establish clonal seed orchards and clonal trials to screen superior genotypes. Clonal propagation has higher potential of genetic gain and genetic uniformity as compare to clonally raised plantations through seeds. Sexual method of propagation through seeds has limitation as often essential superior qualities or plus traits of a mother plant fails to get transmitted to the selected young ones and progeny also lack the uniformity and resemblance to mother tree (Kesari et al., 2009). Vegetative propagation of mature trees through conventional method is very difficult.

Micropropagation studies on *Tecomella undulata* has been done by Rathore et al., (1991), Bhansali (1993) and Robinson et al., (2005) and reported rooting percentage of 60%, 30% and 66% respectively. The drawback of these studies is that they lack statistical test and sound experiments. The major requirement was to develop macropropagation technique and to study the rooting procedure. Due to poor rooting and hardening success these methods are commercially unviable needs refinements. Macropropagation methods are still preferred because of low cost and less complexity over micropropagation techniques. Therefore, the current study was undertaken to examine the rooting ability of mature trees through macro and micropropagation. The aim was to develop a suitable clonal technique for the large-scale production of superior clonal planting stock.

MATERIAL AND METHODS

Experiments were conducted in tissue culture laboratory and poly mist house to standardize micro and macro propagation methods.

A. Micropropagation

Micropropagation techniques are developed by standardizing various steps, such as source of explant, sterilization, shoot multiplication, rooting and hardening.

a. Media Preparation

In the present work Murashige and Skoog (MS, 1962), Gamborg's (B_5, 1968), Lloyd, G. and McCown, B. (WPM, 1980), and Hogland's (1950) nutrient solution with agar were used during various experiments.3% sucrose and 0.8% (W/V) agar (Himedia) were used as carbohydrate source and gelling agent in media. Plant growth regulators were added according to the experimental requirement. The pH of media was adjusted to 5.8. The culture vessels containing media were autoclaved at 15 Ib/cm^2 and 121^{o}C for 20 minutes.

b. Explant Collection and Sterilization

Trees of *Tecomella undulata* 10-15 years old were selected from AFRI field and campus. Single Node explants (2 cm) long were excised from mature trees as well as from new sprouts of the stem cuttings raised in mist chamber. After removing leaves, the explants were thoroughly washed with running tap water. The clean cuttings were washed thoroughly with distilled water containing 2-3 drops of Tween-80 and followed by treating with the solution of Bavistin and streptomycin for 20 minutes. These were then surface sterilized with 5% NaOCl solution for 5 min followed by 3-4 washings in sterile distilled water. All the surface sterilization procedure was carried out in aseptic conditions in a laminar airflow cabinet. The explants were slightly trimmed at both ends

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to expose fresh tissue before planting them on Murashige and Skoog (MS) medium.

c. **Inoculation, culture condition and subculture**

The nodal shoot segments (2-3 cm long) were cut with sharp sterilized scalpel and inoculated by keeping them vertically or horizontally on MS medium in test tubes. After bud break and few subcultures, healthy shoot cultures were maintained by repeated subculturing of the stock after 3-4 weeks on fresh MS + 1.0 mg/l BAP medium for their multiplication. These individual excised shoots were used for the rooting experiments. Shoot multiplication experiments as well as maintenance of *in vitro* shoot stock experiments were conducted in 150 ml conical flasks. All rooting experiments with micro shoots were inoculated in test tubes.

d. **In vitro rooting**

Shoot segments of (3-5cm) in length were isolated from shoot multiplication cultures and used for *in vitro* rooting. Experiment for *in vitro* rooting was in a complete randomized design with a minimum of three replicates per treatment and each replicate comprised of 20 explants. Cultures were incubated in dark for 5 days, followed by incubating them at 2000 lux light intensity for 16h photoperiod, 60-65% relative humidity and 28±2 ºC temperature. To initiate rooting two step procedures was adopted. In the first step the microshoots were given short duration treatment of autoclaved IBA and NAA (100 mg/l) solution and then transferred to the hormone free medium. Observations were recorded after regular interval of 10 days up to 40 days and root length and root number were recorded.

B. Macropropagation

Macropropagation methods involves maintenance of mother trees, selection and preparation of cutting, auxin and fungicide treatment, raising them in root trainer inside polyhouse, hardening and field transplantation.

a. **Collection and Preparation of Cuttings**

All the stem cuttings were collected from 15 to 16 year old trees growing in AFRI's experimental area. These trees were coppiced, pollard and lopped, 5 trees for each group to produce different level of rejuvenated shoots. These managed trees were watered at 15 days interval to increase the number of new sprouts. Trees were also treated with fungicide and insecticide to prevent the attack of pathogens and insects. Stem cuttings were collected from coppiced, pollard and lopped trees. Fresh stem cuttings of 1.0-2.0 cm thick, unless otherwise mentioned in the experiment were collected from coppiced, pollard and lopped trees. The stem cuttings were harvested with the help of sharp secateurs and were kept in wet gunny bags to avoid desiccation during transportation from collection site to mist polyhouse. The branches were cut into approximately 8.0-12.0 cm long shoot segments with 3-5 nodes.

b. **Treatment**

The lower portions of vegetative cuttings were treated with auxins. Upper portions of cutting were covered with choupatia paste (petroleum jelly, red lead and copper carbonate) in the ratio 2:1:1, to prevent fungal attack. This paste also partially checks the desiccation from the upper cut end. Auxins (IBA, NAA and 2, 4-D) solutions were prepared by dissolving them in very small volume of absolute alcohol or 1N NaOH and finally obtained the desired concentration by adding Milli 'Q' water. Auxin treatment is either given for different duration or at different concentrations. The stem cuttings were planted in such a way so that, one bud remains inside the soil and upper portion bears 3 or 5 buds. The proximal/basal end of cuttings was firmly embedded in the soil.

c. **Potting Mixture**

A mixture of sand: compost (3:1) ratio was filled in root trainers Hyko trays. The soil mixture used was sterilized by formalin. Black colour 150cc root trainers were used. These root trainers (20 cells each) were placed in a raised platform inside polyhouse/mist chamber.

d. **Environmental Conditions**

A mist polyhouse is used for different rooting experiments in which high humidity and desired temperature is maintained. The temperature and relative humidity (RH) of polyhouse were controlled with the help of cooling and misting system, respectively. The temperature and RH of polyhouse were recorded at an interval of 15 minutes with help of Gemini data loggers. Water was provided through intermittent misting for 3 minutes after every 15 minutes interval. Average temperature and relative humidity maintained in polyhouse was 30±2 ºC and 70±5% respectively. To reduce sunlight polyhouse was covered with 25% shade agronet. The actual light intensity in the polyhouse ranged between 5500±100 lux in winter and 8700±100 lux in summers. The cuttings were maintained for 2 months in summer and 3 months in winter, during rooting experiments.

C. Hardening and Field Trial of micro and macropropagated plants

One and half month old rooted microshoots were removed gently from the vessels and washed to remove the adhered agar and traces of the medium to avoid microbial contamination and transferred to the mixture of vermiculite and wetted with ½ MS liquid solution for *in vitro* hardening for 4-5 weeks. Then the plants were transferred to plastic cups containing vermiculite and placed in mist chamber. Acclimatization was carried out in mist camber (30 sec misting at 15 minutes intervals to maintain relative humidity between 85-90%). Plants with well developed roots were then transferred to the earthen pots for further growth. Many such micropropagated plants are growing well in the field.

Six to seven months old macropropagated plants kept in polythene bags were transferred to the field by slit cutting the polythene and put into 45x45x45 cm^3 pit along with the earth ball attached to roots. The cuttings were transferred to field in the months of July and August. The cuttings when transferred were given water immediately and thereafter at an interval of seven days for 2 months (except in rainy day) and then at an interval of 15 days for 4 months and then 30 days for 6 months. Many such macropropagated plants are growing well in the field.

D. Statistical analysis

All the experiments for micro and macropropagation were conducted in CRD design. In both the cases of micro and macropropagation test of significance was carried out by one way ANO-

VA and data means were compared by Duncan Multiple Range Tests (DMRT) using the SPSS statistical package (Version 8.0).

RESULTS

Micropropagation

1. **Seasonal effect on in vitro shoot establishment in Tecomella undulata**

In the winter months of January to February (During flowering time of *Tecomella undulata*), 73% cultures showed axillary bud sprouting. In the months of November to December 63% cultures showed axillary bud sprouting. Season has been reported to be an important factor during *in vitro* establishment of cultures from mature tree-derived explants. During July to August (period corresponding to rainy season), the contamination rate was high and only 23% cultures were sprouted. The rate of contamination or bud-break was highly dependent on the season during which the material was collected. Therefore, November to February was the best period for culture initiation and *in vitro* establishment of *T. undulata* as shown in Table 1.

2. **Effect of BA on shoot multiplication**

The axillary shoots obtained on MS medium supplemented with (2 mg/l) BA+ (0.1 mg/l) NAA were excised and further subcultured on the shoot multiplication media MS, MS + (1 mg/l) BA & MS + (2 mg/l) BA. MS medium supplemented with (1mg/l) BA was found most suitable for shoot multiplication of *Tecomella undulata* with mean shoot number of 1.8, mean shoot length of 22 (mm) and moderate callus as heavy callusing is not desirable as shown in Table 2.

3. **Effect of different medium on in vitro rooting**

Many rooting experiments were carried out and it is found that rooting in *T. undulata* by one step procedure is very difficult. The main reason behind this was callus formation at the base of the plant which leads to shoot tip necrosis. After many different rooting experiments, two step procedure was found suitable for root initiation. The best medium for rooting is found to be Gamborg's (B5) medium. 43.4% rooting success was achieved by short duration treatment of autoclaved IBA & NAA (100 mg/l) solution for 15 minutes on ½ B5 medium as shown in Table 3. Photographs of various stages of micropropagation are shown in Figure 1 from (A-G).

Macropropagation

1. **Rooting response in different seasons**

Season play an important role in many plant physiological and development process. Therefore it was essential to find out the best period in a year for raising cuttings for optimal root induction. Experiment was conducted in different months and the highest rooting (15%) was obtained in the month of January-March as shown in Table 4.

2. **Stem cutting collected from different trees**

Experiment was also conducted with stem cutting collected from four different trees. Rooting response varied significantly for these four different genotypes as shown in Table 5.

3. **Stem cutting collected from different locations in a tree**

Branches collected from top, middle and bottom crown were divided in three portions of selected tree (tree no 9) i.e. upper, middle and lower part. Experiment revealed that stem cuttings of middle crown if analyzed collectively produced the best result (17.8% rooting) but stem cuttings belonging to upper portion of the middle crown branches rooted (33.3%) maximally as shown in Table 6.

Hardening and Field trial

About 35% of micropropagated rooted plants were survived in hardening procedure and 75% of macropropagated plants were survived and growing well in the field and they are growing well since last two years. Photographs of various stages of macropropagation are shown in Figure 2 from (A-F).

DISCUSSION

Season play an important role for factor during *in vitro* establishment of shoot cultures from mature tree-derived explants (Nadgauda et al., 1993). Explants collected during winter (November - December) gave the maximum response as measured by percentage of explants with axillary bud break *in vitro* (Bhatt and Dhar, 2004). Similarly maximum bud break was observed in winter season (November to January) by (Siril and Dhar, 1997). It also has marked effect on rooting of stem cuttings (Singh et al., 2004). The best period for establishment of shoot cultures have been standardized for various species. Before onset of leaf fall trees generally accumulate nutrients in the shoots which are subsequently utilized for emergence of new sprouts (Palanisamy et al. 1998). The formation of new sprouts leads to elevation of endogenous root forming substances including auxins (Went 1929; Bouillenne and Went 1933; Avery et al. 1937). In *Pongamia*, stem cuttings collected during January rooted better in terms of percentage response, average root number and average root length than those collected during October (Rangan et al., 2010). This variation in seasonal rooting response may be attributed to the physiological condition of the plant cuttings. Cellular activities during root initiation require availability of sugars which are synthesized due to activity of various hydrolytic enzymes (Nanda K.K. 1975). The activity of these enzymes might have been at the highest level during monsoon and post monsoon months. The failure of cuttings to produce good root system in non-monsoon months may be due to a high rate of metabolism and increased inhibitor–promoter ratio (Eganathan et al., 2000). In present studies time span from November-February is suitable for *in vitro* explant establishment and January –March period is best for rooting in stem cuttings of *T. undulata*.

In shoot multiplication BAP alone was found to be suitable for both multiple shoot bud induction and proliferation. Explants required a higher concentration of BAP (2.0 mg/l) at the initial stage of shoot bud regeneration, but further growth and proliferation of the shoot buds was observed only after subculture to fresh medium with lower level of BAP. The optimal BAP concentration for shoot elongation was 1.0 mg/l (Geetha et al., 1998).The main

objective in multiplication is to obtain shoots suitable for rooting. Rooting experiment showed the optimal medium for root induction was 1/2 B5 basal medium with 43.4%. 1/2 B5 medium was more effective than MS medium, also reported in *Papaver orientale* by (Zakaria et al., 2011).

It has been reported in many woody species that genetic make of individual genotype also influence rooting response through stem cuttings. Wide range of variation in rooting ability in different genotypes of *Eucalyptus* species is reported by (Verma et al., 1993). In *E. camandulansis* rooting response varied from 0-96% and in *E. teriticornis* it was from 0-75% through coppice shoots of different genotypes. Genotype effect has also been observed in *A. auriculiformis,* where some portion of field ortet genotype demonstrated rooting rates similar to young seedlings (Haines et al., 1992). Cuttings of *Salix planifolia* collected from five provenances (distinct geographical populations) exhibited different adventitious rhizogenesis distinct to each provenance (Houle and Babeux 1983). The rooting ability of *Pongamia* is linked to its genotype, in addition to the seasonal effects and other contributing factors (Rangan et al., 2010).

The effect of position of cuttings were also been reported in many species *Pinus radiata* (Libby & Hood, 1976), *Tectona grandis* (Nautiyal et al., 1992) and *Delbergia sissoo* (Ansari et al., 1995). Bonga (1982) suggested that this is because of some juvenile cells/tissue remain even in mature tissue. In case of tree species, the degree of juvenility is inversely proportional to the distance (along the trunk and branches) between the root shoot junctions and branches (Razdan, 1993). The endogenous auxin levels decreases as the distance from the apices of the branches within the same plant increases (Overbeek, 1938; Jacobs, 1979). The branch position on a hedged donor plant has an important effect on rooting and sprouting response of teak cuttings. Cuttings taken from the middle position had the best rooting percentage followed by apical and basal positions respectively (Husen and Pal, 2007). In most tree species rooting ability of cuttings has been reported to increase from apical to basal part of the crown/shoots which has been attributed to accumulation of carbohydrates at the base of shoot (Hartmann et al., 1997). Similar result was obtained in *Dalbergia sissoo* (Husen, 2004). Therefore, it is evident from these findings that optimal branch positions for the best rooting percentage vary with the plant species. The effect of position on rooting may be caused by variation in the physiological status of shoot/cutting tissues on stock plants resulting in occurrence of gradients along the stem axis in the cellular activity or in the level of assimilates or growth regulators or in the level of lignification etc. (Hartmann et al., 1997).

Further research work is required on micropropagation to improve rooting and hardening success as well as for macropropagation to enhance the rooting response by understanding more factors influencing these stages of micro and macropropagation.

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Table 1: **Effect of season on bud break of *Tecomella undulata* nodal segment on MS + 2mg/l BA + 0.1 mg/l NAA after 30 days.**

Months	Explant number	Responding explant % ± SE	Mean bud length ± SE
January-February	30	$75^{c} \pm 7.8$	$4.3^{d} \pm 0.4$
March-April	29	$40^{ab} \pm 9.3$	$2.0^{c} \pm 0.5$
May-June	27	$43^{ab} \pm 9.7$	$1.7^{bc} \pm 0.4$
July-August	31	$23^{a} \pm 7.6$	$0.5^{a} \pm 0.1$
September-October	28	$27^{a} \pm 8.7$	$0.6^{ab} \pm 0.2$
November-December	26	$63^{bc} \pm 9.8$	$3.4^{d} \pm 0.5$

Means bearing similar letters within a column are not significantly different at $P \leq 0.05$. The means separated using Duncan Multiple Range Test.

Table 2: **Effect of BA on shoot number and shoot length.**

Treatment	Explant number	Mean shoot Number ± SE	Mean shoot Length (mm) ± SE	Associated callus
MS	16	$1.4^{a} \pm 0.3$	$8^{a} \pm 1$	++
MS + BA (1 mg/l)	15	$1.8^{a} \pm 0.1$	$22^{b} \pm 2.1$	+++
MS + BA (2 mg/l)	17	$2.6^{b} \pm 0.3$	$15^{c} \pm 1.7$	++++

Means bearing similar letters within a column are not significantly different at $P \leq 0.05$. The means separated using Duncan Multiple Range Test.

'++' sign denotes less callusing

'+++' = moderate callusing

'++++' = heavy callusing

Table 3: **Rooting response of *Tecomella undulata* shoots on different media after short duration treatment of IBA + NAA solution (100 mg/l) for 15 minutes.**

Treatment	Explant number	Rooting%	Root length Mean ± SE	Root number Mean ± SE
½ MS	23	17.4^{a}	$7.0^{ab} \pm 3.3$	$0.6^{a} \pm 0.3$
½ B5	25	43.4^{b}	$12.3^{b} \pm 3.5$	$1.3^{b} \pm 0.3$
½ WPM	23	4.3^{a}	$0.2^{a} \pm 0.2$	$0.04^{a} \pm 0.04$
Hogland	23	4.3^{a}	$3.1^{a} \pm 2.1$	$0.1^{a} \pm 0.09$

Means bearing similar letters within a column are not significantly different at $P \leq 0.05$. The means separated using Duncan Multiple Range Test.

Table 4: **Effect of different season on sprouting and rooting response of stem cuttings.**

Months	Number of Cuttings	Sprouting% (15 days) ±SE	Primordia % (3 months) ±SE	Rooting % (3 months)
January –March	100	$95^{b} \pm 2.1$	$68^{c} \pm 4.6$	15^{b}
April – June	100	$86^{b} \pm 3.4$	$34^{b} \pm 4.7$	2^{a}
July – Sept	100	$82^{b} \pm 3.8$	$4^{a} \pm 1.9$	0^{a}
October – December	100	$29^{a} \pm 4.5$	0^{a}	0^{a}

Means bearing similar letters within a column are not significantly different at $P \leq 0.01$. The means separated using Duncan Multiple Range Test.

Table 5: **Effect of stem cutting collected from different trees on sprouting and rooting response.**

Treatments	Number of Cuttings	Sprouting% ±SE	Primordia% ±SE	Rooting%
Tree No 09	135	$99^{b} \pm 7.4$	$48.1^{b} \pm 4.3$	10.37^{b}
Tree No 12	135	$93^{a} \pm 2.2$	$7.4^{a} \pm 2.2$	0.74^{a}
Tree No 17	135	$92^{a} \pm 2.3$	$4.4^{a} \pm 1.8$	0.74^{a}
Tree No 21	135	$97^{ab} \pm 1.4$	$5.9^{a} \pm 2.0$	0.74^{a}

Means bearing similar letters within a column are not significantly different at $P \leq 0.01$. The means separated using Duncan Multiple Range Test.

Table 6: **Effect of stem cutting collected from different locations in the crown of a tree (No. 9) on rooting response.**

Crown Portion	Number of Cuttings	Rooting percentage ± SE			
		Part of the branch*			
		Upper	Middle	Lower	All
Top crown	45	$6.7^{a} \pm 6.7$	$13.3^{ab} \pm 9$	0^{a}	6.7
Middle crown	45	$33.3^{b} \pm 12$	$13.3^{ab} \pm 9$	$6.7^{a} \pm 6.7$	17.8
Bottom crown	45	0^{a}	$20^{ab} \pm 10$	0^{a}	6.7

*15 stem cuttings were raised for each part and a total 45 cutting from crown portion. Means bearing similar letters within a column are not significantly different at $P \leq 0.01$. The means separated using Duncan Multiple Range Test.

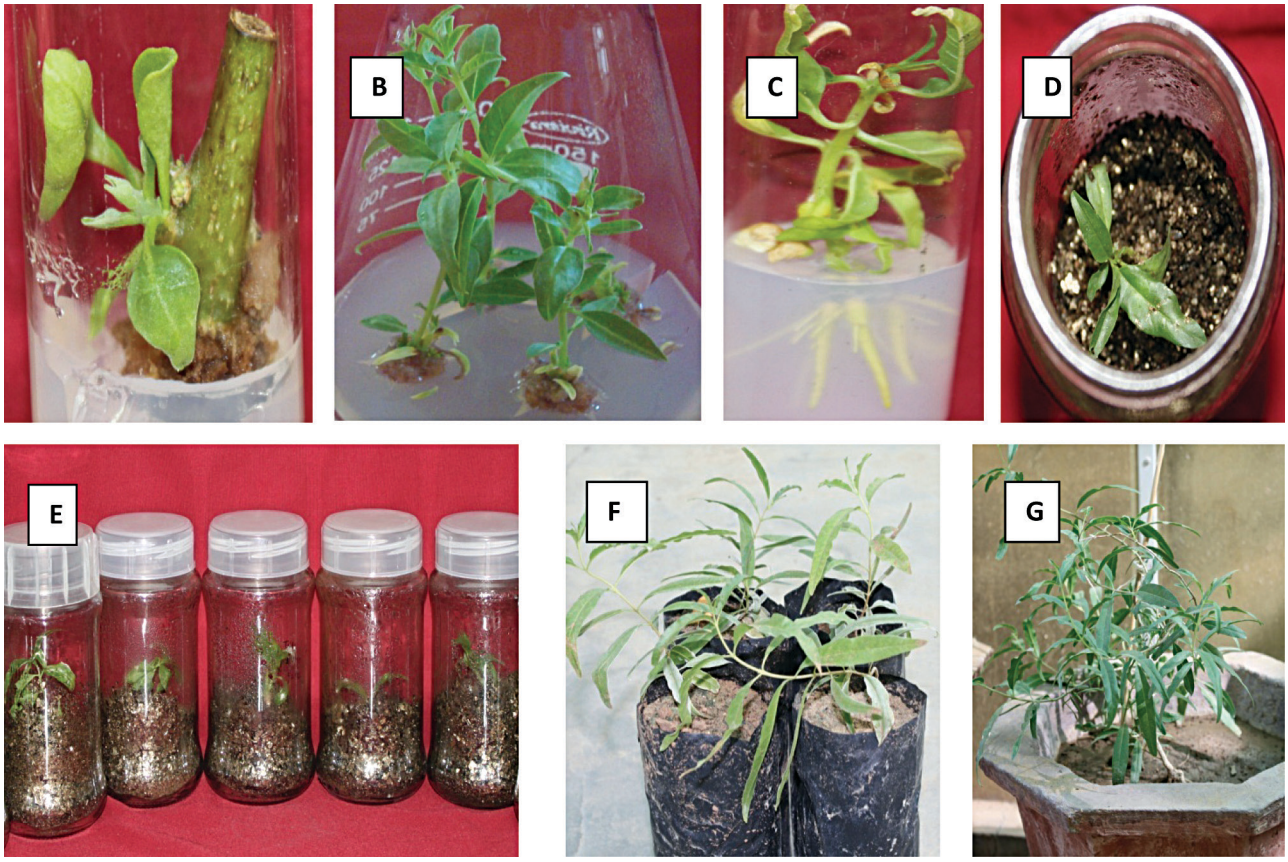

Figure 1: **Various micropropagation stages of *T. undulata*. (A). Shoot initiation from nodal part of *T.undulata* after 15-20 days of culture on MS + BA (2 mg/l) + NAA (0.1 mg/l). (B). Multiplication & elongation of shoots on MS + BA (1 mg/l). (C). Rooting of *in vitro* formed shoots on ½ B5 medium. (D & E). *In vitro* hardening of rooted shoots. (F). *Ex vitro* hardening. (G). 1 year old plant in pot.**

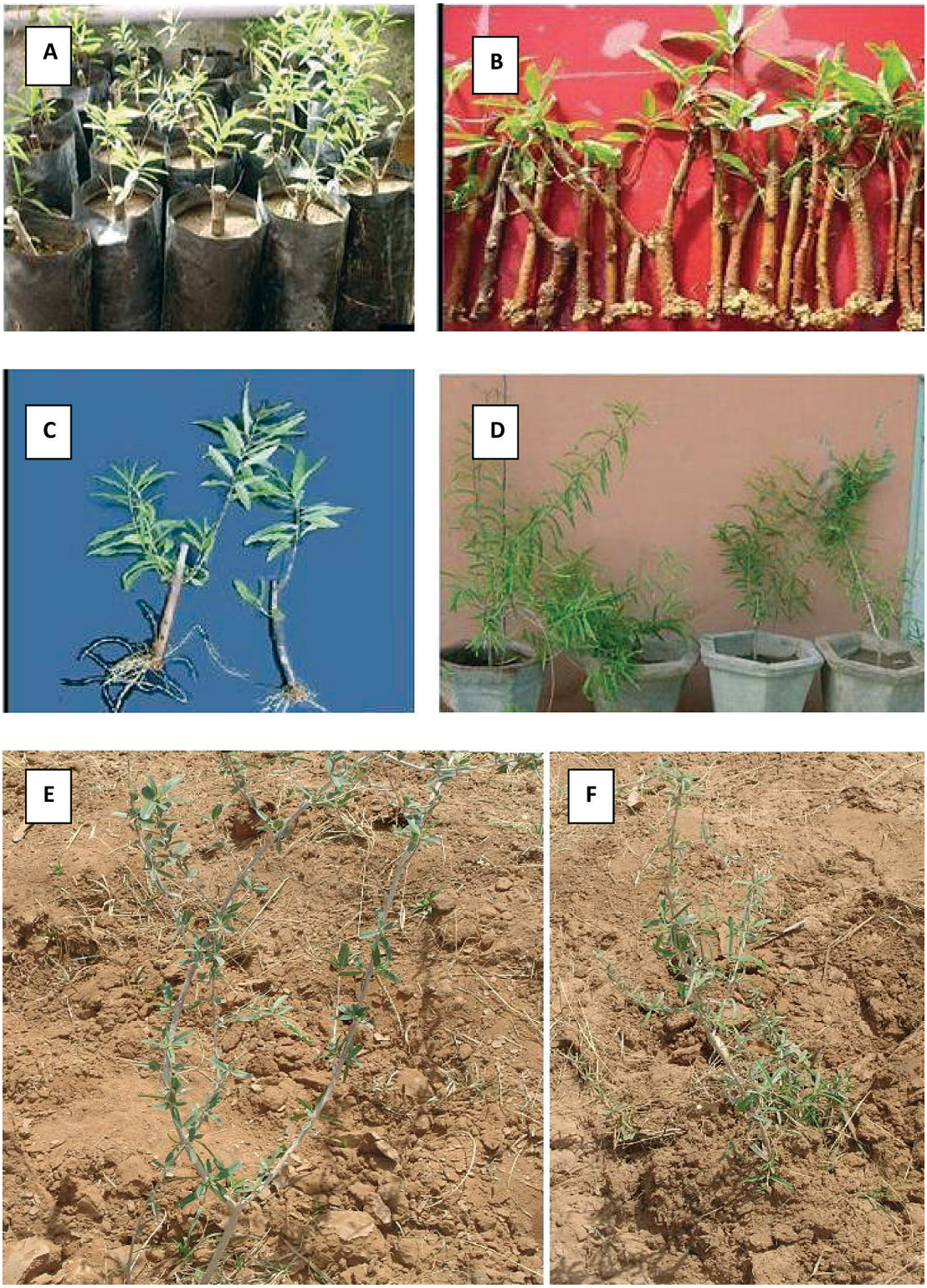

Figure 2: **Various macropropagation stages of *T. undulata*. (A). Sprouted cuttings of *T. undulata*. (B). Cuttings with developed root primordia. (C). Stem cuttings with well developed roots. (D). Well developed plants in pots. (E & F). Macropropagated plants of *T. undulata* in field.**

Tissue Culture Method for Multiplication of FRI Hybrids of Eucalyptus and their Field Trials

I.D. Arya, Sudhir Sharma, S.K. Chouhan, Barkha Kamal, and Sarita Arya *

INTRODUCTION

Men as per its natural habit to do something new, started to develop superior plant material through propagation. Plant tissue culture (Micropropagation) provides method to maintain the original genetic stock of the organism. The application of tissue culture technique for the regeneration and commercial propagation of whole plant is a more recent development in India. So far work has been done in horticulture and agricultural plants, while forest species were ignored because they were more difficult to propagate and were considered recalcitrant. *Eucalyptus* offers an ideal industrial field stock for pulp and paper and essential oil. *Eucalyptus* produces some of the heaviest, hardest and most durable wood, which makes this genus the most valuable source of hardwood in the world. To meet the increasing demand for timber in the future, fast growing *Eucalyptus* plantations are expected to make great contribution. In this direction, *Eucalyptus* hybrids have shown promising future with fast growth and development by giving 3-5 times more biomass than their parents. To meet the increasing demand for timber in the future, fast growing *Eucalyptus* plantations are preferred. *Eucalyptus* hybrids have been a widely planted species in India owing to its adaptability indifferent eco-climatic zones. Hybridization is a known process by which the desirable traits of the two parents may be combined in F1 offspring. It also offers a means to capture the benefits of hybrid vigour (heterosis), which is often manifested in certain specific parental species combinations in hybrids. Development of hybrids, their clonal multiplication is of utmost requirement as, when F2 population was raised through seeds a lot of segregation was observed which had reduced the average yield per unit area per unit time. At Forest Research Institute, Dehradun, work on hybridization in *Eucalyptus* was initiated during 1970s (Venkatesh and Sharma,1977). Based on cross - ability pattern, studies were initiated to produce controlled hybrids as well as natural hybrids from half-sib progenies raised from seeds collected from stands of two inter crossable species growing in vicinity to each other. The hybrids are artificially produced or spontaneous selected from New Forest area at Forest Research Institute campus, Dehradun. Out of these different interspecific hybrids developed, micropropagation work was initiated on five hybrids FRI-5 (*E. camaldulensis x E. terticornis*), FRI-10 (*E. grandis x E. terticornis*), FRI-13 (*E. camaldulensis x E. terticornis*) x (*E. grandis*) FRI-14 (*E. torelliana x E. citriodora*), and FRI-15 (*E. citriodora x E. torelliana*) in order to developed a protocol for true to type plant propagation. Two hybrids were selected for large scale T.C. plant production (FRI-5 and FRI-14) and for field trial at seven different agroclimatic conditions.

MATERIALS AND METHODS

Explant in the form of nodal segments bearing axillary buds were collected from the 30-32 years old mature trees of F1 hybrid of Eucalyptus growing at New Forest experimental field of Forest Research Institute, Dehradun. Nodal segments containing single axillary buds were used as source material for tissue culture. Nodal segments measuring 2.0 to 2.5 cm were cut and washed in liquid antiseptic detergent with antibiotics and Cetavelon solution. After washing, nodal explants were surface sterilized with various sterilizing agents like mercuric chloride, sodium hypochloride and hydrogen peroxide, followed by 3-4 washing with autoclaved distilled were to remove the traces of sterilent. The surface sterilized axillary buds were inoculated on semi solid and liquid MS (Murishige and Skoog 1962) medium supplemented with cytokinin (BAP/Kn). The pH of the medium was adjusted to 5.8 prior to autoclaving the medium at 121 0C for 15 minutes. Cultures were maintained at 25+2 0C temperature with 16 hours illumination.

In vitro multiplication of shoots

Once the culture conditions for optimum shoot induction from explants were established. Proliferated axillary shoots were excised and sub-cultured on fresh medium with suitable cytokinin for further in vitro shoot multiplication. These sub-cultured in vitro shoots were multiplied on suitable medium in every 4-5 weeks. Different sets of experiments were conducted to obtain maximum shoot multiplication rate. For this multiplied shoots were sub-cultured in propagules consisting of 1 to 7 shoots. Observations were recorded after an interval of 5 weeks. The number of propagule cultured and number of propagule derived at the end of subculture gave the multiplication rate.

Effect of BAP and Kn alone and in combination with auxin NAA were studied. Effectof myo-inositol at different concentrations in MS medium, effect of subculture duration was studied.

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Effect of sucrose (1-6%) and effect of different media (MS, B5, WPM and SH) was also tested for in vitro shoot multiplication.

In vitro rooting

The critical step in tissue culture plant production is *in vitro* rooting of *in vitro* developed shoots. Various concentration of auxins (IBA, NAA & IAA) in the medium were tested for rooting. Different types of media (MS, B5,WPM, and SH) and their strength (2x, 1x, 1/2x,1/4x) were tested for in vitro rooting. Rooting response was recorded in terms of rooting percentage number of roots produced and average root length.

Hardening and acclimatization

The tissue culture raised plants are heterotrophic in their mode of nutrition and cannot withstand the environmental conditions without proper hardening and acclimatization. In vitro raised plantlets need to be hardened and acclimatization before field transplantation. For this the *in vitro* rooted plantlets were taken out from the culture bottles and washed thoroughly with autoclaved water to remove adhered nutrient agar. These were carefully transferred to bottles containing autoclaved soilrite and moistened with 1/4 strength MS salts and covered with polycarbonate caps. These plantlets were kept for 2-3 weeks in mist chamber. Later they were transferred to polybags containing sand: soil: FYM in 1:1:1 ratio and placed in shade house conditions for acclimatization. During hardening the shoots elongated, leaves turned greener and expanded. In shade house the plants were further transferred to bigger polybags.

Field Transfer

Hardened and acclimatized plants were planted in field preferably during monsoon season July to October). Sivicultural practices were followed during plantation with 3m x 3m spacing with a pit size of 45 cm x45cm x 45cm. The field transferred plants were irrigated in 15 days for two months and were supplied with manure at the time of planting. Parameters like height, DBH, clear bole length and self pruning capability of both the hybrids were recorded up to three years to identify the suitability of these hybrids in different varied climatic zones.

RESULTS

Collection and Preparation of Explant

Nodal shoots segments containing axillary buds were harvested from the all the five F1 hybrid of Eucalyptus. The explants were harvested from newly developed fresh shoots during early in the morning and proved to be the best time for explant collection. It was found that the explants collected during January to February and August to September were the best for *in vitro* studies as they showed least phenolic exudation and gave 65 - 70% bud break response as compared to other months.

The nodal shoot segments containing axillary buds were collected from 30-32 years old mature trees of *Eucalyptus* hybrids growing at New Forest experimental field of Forest Research Institute, Dehradun. Axillary bud/nodal segments measuring 2-3 cm were cut. Explant were treated with Bavistin (1%) and antibiotics (Streptomycin and Chloramphenicol) for 3-5 minutes followed by surface sterilization with (0.15%) HgCl2 (12min for FRI-5,10 & 15 and 10 min for FRI-14 and 13) was found to be very effective in controlling 78-80% contamination with good survival rate of 65-75%.

Axillary bud break and Shoot Initiation

Four nutrient media viz. MS medium (Murashige and Skoog, 1962), Woody Plant Medium (Lloyd and McCown, 1980), B5 medium (Gamborg et al., 1968) and SH medium (Schenk and Hildebrandt, 1972) were tested for the establishment of aseptic cultures from axillary buds and for shoot multiplication. All the media containing 2% sucrose solidified (in case of FRI-5) and 3% (in case of FRI-10, 13, 14 and 15) with 0.6% bacteriological agar and supplemented with different concentrations of BAP individually and along with NAA was used for the establishment of bud induction and bud break. Bud break started without an intervening callus phase within 3 weeks from the date of inoculation of the nodal segments. A cluster of 3-5 shoots normally proliferated from the axillary bud in five weeks. These *in vitro* proliferated shoots from the axillary buds were excised in a group of shoots cluster (propagule) and subcultured on MS medium supplemented with 0.1- 3.0 mg/l BAP for shoot multiplication. It was found that MS+1.0mg/l BAP medium was optimal medium for shoot multiplicationin general for all the hybrids. A regular subculture of shoots (in a propagule of 5-6 shoots) on fresh MS medium (1.0 mg/l BAP) for five weeks gave a 6-12 folds average shoot multiplication rate in these hybrids. Among the four media, MS medium was found to be very effective for axillary bud induction and shoot initiation for all the hybrids. Nodal segments when cultured on MS medium supplemented with 1.0 -3.0 mg/l BAP, gave 80-90% bud break.

In vitro Shoot Multiplication

Axillary shoots were excised from the explant and were inoculated onto the MS media supplemented with different concentrations of BAP alone and in combination with NAA. Out of the different media combinations tested, best shoot multiplication occurred on MS + 1.0 mg/l BAP + 0.1 mg/l IBA in FRI-5 and on MS + 1.0mg/l BAP in case of other FRI hybrids. Multiple shoots were obtained after 5-6 weeks in all the hybrids. A regular sub-culturing was carried out every 5 weeks on fresh medium. Cultures were incubated at 25 ± 2°C 16 hours in light (illuminated by 40 watt fluorescent tubes,1200 lux) and for 8 hours in dark cycle irradiance by cool fluorescent tubes. The cultures were regularly transferred into fresh medium to check the browning of cultures. Maximum shoot multiplication rate (6-10 folds) was obtained on MS medium supplemented with 1.0 mg/l BAP + 0.1 mg/l IBA mg/l in case of FRI-5 and in case of other hybrids maximum shoot multiplication rate (6-12 folds) was obtained on MS medium supplemented with 1.0 mg/lBAP. Amongst all media, shoot multiplication was highest (found optimum) on MS medium with a average shoot multiplication rate of 6-12 folds. It was found that maximum shoot multiplication rate was obtained on full strength MS medium. *In vitro* shoot elongation was obtained on ½ MS medium without PGR.

Regeneration of roots

After *in vitro* shoot elongation individual shoots measuring 2.5 to 3.0 cm were inoculated on half strength MS medium sup-

plemented with IBA (0.1-2.0 mg/l) alone and in the combination with NAA (0.1-2.0 mg/l) for rooting. 87.50% rooting was achieved in FRI-5 on ½ MS supplemented with IBA (1.0 mg/l) and 91.66% rooting was achieved in FRI-14 on ½ MS supplemented with IBA (0.5 mg/l) without intervening callus phase.

Acclimatization and hardening and field transfer

Micropropagated rooted plantlets were hardened *in vitro* in liquid ¼ MS medium having 2% sucrose. Absorbent cotton soaked in this liquid medium was used for supporting root system of *in vitro* raised plantlets. Plantlets were maintained in this step for 2 weeks, transferred to mist chamber in polythene bags containing a mixture of soil, sand and manure (1:1:1) and covered with perforated polythene bags and then transferred to the net house. Holes were made in the polythene bags, which were withdrawn periodically, and the plantlets were finally transferred to the field. 85 - 95% success in field survival rate was observed in both the hybrids.

Field plantation

All the experimental sites were leveled and at places where irrigation facility was available for irrigation of the plantation was done every fourth night. The trial was laid out in block plantation along with the control local hybrid. Field study of tissue culture raised plants of both *Eucalyptus* hybrids FRI-5 and FRI-14 was done on following aspects viz survival rate and growth parameters like height, diameter and clear bole length. So far 6000 tissue culture raised plants each of FRI-5 and FRI-14 were planted in field during monsoon season of 2005 in different eco-climatic zones. Tissue culture raised plants of FRI-5 and FRI-14 were planted in three meter apart rows and the plant to plant distance was also 3 m (monsoon year of 2005). Each treatment had 20 plants in a row. The total rows were 10 in each location. The control plants raised by locally available seeds were also maintained at the same site. Casualty replacement was done during the second year. Other cultural operations such as weeding, fertilizing and mulching were done up to 3 years. The height, collar diameter and clear bole length were taken twice a year (June and December). The survival count was also noted during this period.

An average height of 7.5-9.5 m were observed with an average diameter of 6.5-8.5 cm and a survival rate of 90-92% at the sites of Dehradun, while at Pantnagar and Haldwani fields a survival rate of 86-90% was recorded after three years of plantation. Total height and collar diameter taken on breast height (~137.0 cm) were recorded after three years and variation was observed in height and collar diameter at different plantation sites. The growth performance and survival percentage of both *Eucalyptus* hybrids FRI-5 and FRI-14 showed their suitability in particular environmental conditions. The analysis of survival percentage showed that the hybrids as well as the localities also when compared do not differ significantly. It indicates that localities or hybrids have no effect on survivability of plants. Hence, it was considered to the best for commercial plantation at these three plantation sites. Both *Eucalyptus* hybrid FRI-5 and FRI-14 performed better for all these traits when compared with control. However, statistical analysis indicated non-significant differences among the hybrids and control for all the traits studied. The analysis of average height showed that the hybrids differ significantly but localities show no significant difference among them. The average height of the hybrids is significantly higher than the control. Likewise, the analysis of average collar diameter shows that the hybrids differ significantly but localities show no significant difference among them. The average collar diameter of the hybrids is significantly higher than the control. While the analysis of average clear bole length shows that the hybrids as well as the localities do not differ significantly. The uniformity obtained in the plantation with micropropagated plants confirms the feasibility of using this technique for commercial scale multiplication of both *Eucalyptus* hybrids (FRI-5 and FRI-14). The overall data collected after three years shows that both FRI-5 and FRI-14 performed well and are suited to the varied climatic conditions of Dehradun, Pantnagar, Haldwani and also showed good self pruning up to three years of age.

DISCUSSION

The present investigation demonstrates the successful multiplication by axillary meristem and field plantation at different eco-climatic zones. Systematic field evaluation data for tissue culture raised plants is not available. Although number of tissue culture raised plants have gone to the field but large-scale field trials is limited. Clonal plantations of *Eucalyptus* are being raised by conventional vegetative propagation method by private organizations having captive consumption. Plantation has displayed a very high degree of vigour (positive heterosis) both in height, diameter and wood quality. Data showed vigorous growth when compared with local *Eucalyptus* species in terms of total height, collar diameter and clear bole length. This may be attributed due to genetic constitution of the hybrid as well as environmental interaction constituting various physical factors like moisture content, soil pH, mean annual rainfall, mean temperature, etc. Preliminary investigation showed that vigoursity of the hybrids was transferred in clonal material fully and expressed even after the age of 36 months.

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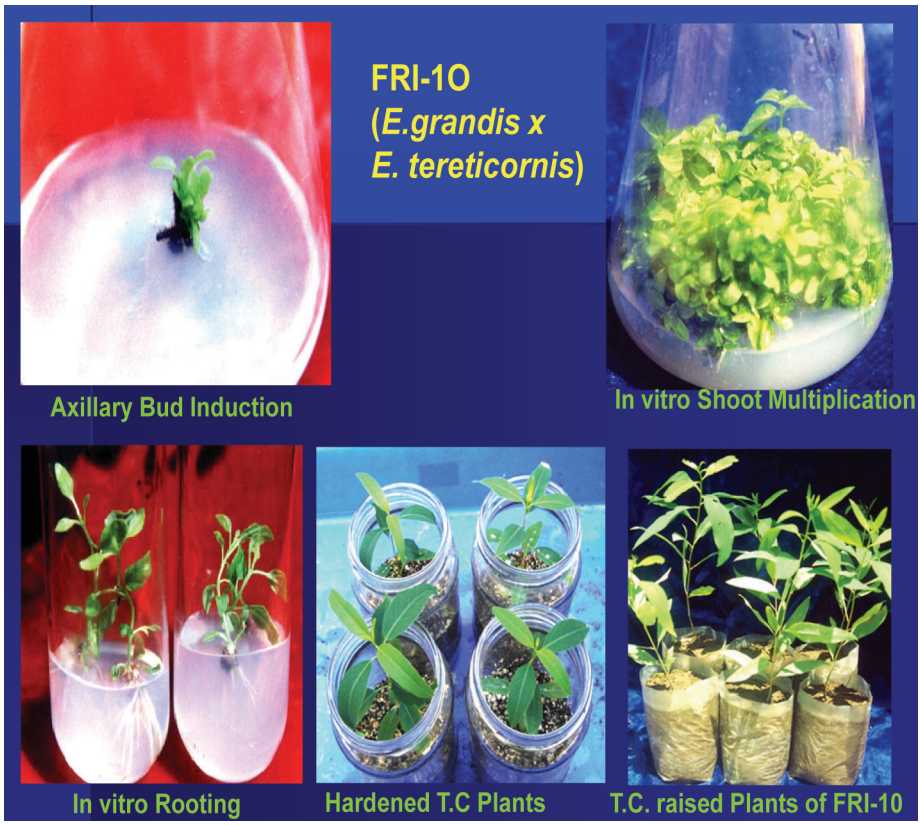

PROTOCOL FOR MICROPROPAGATION OR EUCALYPTUS

IN VITRO SHOOT CULTURES OF EUCALYPTUS

Stability Analysis in Clones of *Casuarina equisetifolia*

Kannan C.S. Warrier* and B. Gurudev Singh

INTRODUCTION

The phenotypic performance of a genotype is not necessarily the same under diverse environments. Some genotypes may fare well in some environments but not so well in others. Such G x E interaction have assumed greater importance in plant breeding as they reduce the stability of genotypic values under diverse environments (Dhillon *et al*., 1999). Several researchers have stressed on the importance to test trees at different growth phases or fertility regimes and in a range of environments and growing situations for selecting stable genotypes (Shelbourne and Campbell, 1976; Burdon, 1977; Zobel and Kellison, 1978; Noh and Lee, 1983; Ataga, 1993). Though stability studies have extensively been carried out in annual crops, limited studies only are available in tree species.

Casuarina equisetifolia L. is a multipurpose tree species extensively planted in the Indian sub continent, especially in the southern states. Its amenability to short rotation and a sustained market demand as scaffolding in building industry, cheap housing material, banana stakes and excellent fuelwood are keys for its success (Kondas, 1983). *C. equisetifolia* has proved to be an efficient soil reformer and finds its acceptability in many countries to stabilize and control sand dunes (Kumar and Gurumurthi, 2000). Its nitrogen fixing ability, desirable stem form, fast growth and light crown characteristics make it an ideal tree for agroforestry systems (Viswanath *et al*., 2001). *Casuarina* based farming is very popular in the coastal regions of peninsular India as it buffers the economy against crop failure and drought. It has also gained importance as a major pulpwood species (Amanulla *et al*., 2001; Jain and Mohan, 2001; Nicodemus, 2004). Its usefulness in environmental protection has been fully realized after the tsunami and is now a major component in any coastal afforestation programme in India (Nicodemus, 2007). Growing *Casuarina* is also steadily increasing in inland areas where it is not traditionally grown.

Realising the potential of clonal forestry for yield improvement of plantations, systematic studies have been in progress at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, India since 1996 and is a focal point for *Casuarina* research in India. A clone bank consisting of 275 accessions of *C. equisetifolia* selected from various parts of Tamil Nadu including Chidambaram, Chengalpet, Tiruchendur, Rameswaram, Pudupattinam, Kelampakkam, Vedapattinam, Agarammedu, Vedaranyam, Pichavaram, Pudukkottai and Cudallore is in place at present. The present study was mainly focused to understand the stability existing among the selected phenotypes of *Casuarina equisetifolia* assembled in the clone bank of IFGTB from Chidambaram, Chengalpet and Tiruchendur area of Tamil Nadu.

MATERIALS AND METHODS

The basic experimental materials comprised of clones of *Casuarina equisetifolia* selected and assembled in the clone bank of Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore (Latitude 11° 01' N, Longitude 77° 02' E) from Chidambaram (CH), Chengalpet (CP) and Tiruchendur (TCR) in Tamil Nadu, India. Thirty-three CH / CP clones and 43 TCR clones were used for the study.

Stability analysis was performed in both the groups of clones using the data on current annual increment (CAI) for total height (H), diameter at breast height (DBH), collar diameter (CDM), frustum volume (FV) and volume index (VI) obtained during age 4 to age 8. The analysis was carried out following the method suggested by Eberhart and Russel (1966). Considering Y_{ij} as the mean observation of i^{th} clone in j^{th} environment, the stability model described by them is as follows

$Y_{ij} = m + B_i I_j + \delta_{ij}$ (i = 1,2……t and j = 1,2, …..s)
(t = clones; s = growth periods)
where,
Y_{ij} = Mean of i^{th} clone in j^{th} growth period
m = Mean of all the varieties
b_i = Regression coefficient of the i^{th} clone on the environmental index which measures the response of the clone to varying growth periods
I_j = The environmental index which is defined as the deviation of the mean of all the clones in j^{th} growth period from the overall mean

$$= \frac{\sum_i Y_{ij}}{t} - \frac{\sum_i \sum_j Y_{ij}}{ts} \quad \text{with } \sum_j I_j = 0$$

and δ_{ij} = Deviation from regression of the i^{th} clone at j^{th} growth period

Two parameters of stability were calculated namely, (a) regression coefficient which is the regression of the performance

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of each clone under different growth periods on the growth period means and (b) mean square deviations from linear regression.

The regression coefficient is estimated as

$$bi = \sum_j Y_{ij} I_j / \sum_j I_j^2$$

where,

$\sum_j Y_{ij} I_j$ is the sum of products and

$\sum_j I_j^2$ is the sum of squares

Mean square deviations (Sd^{-2}) from linear regression is estimated as

$$- \frac{\sum_j \delta^2_{ij}}{(s-2)} \quad \frac{S^2_e}{r}$$

where,

$$\sum_j \delta^2_{ij} = \sum Y^2_{ij} - \frac{Y^2_i}{t} - \frac{(\sum_j Y_{ij} I_j)^2}{\sum_j I^2_j}$$

and S^2_e = Estimate of pooled error

RESULTS AND DISCUSSION

A special concern in tree improvement and genetic testing relates to genotype x environment interaction which means that the relative performance of clones, families, provenances or species differs when they are grown in different environments. Because of the possible presence of G x E interaction, it is always advisable that genetic tests be established in multiple environments (Zobel and Talbert, 1984). Environments may consist of different locations, different years or different site preparation or management treatments. In tree improvement, superior performing genotypes in a range of environments are highly desired (Hanson, 1970). The importance of selecting stable genotypes by testing the available selections at different growth phases, fertility regimes or locations have been highlighted by several researchers (Zobel and Kellison, 1978; Mullin, 1985; Ataga, 1993; Ades and Garnier-Gere, 1996; Song and Zhang, 1997; Larochelle *et al.*, 1998; Sonesson and Eriksson, 2000) as G x E interaction can reduce genetic gains or make a tree breeding programme more complex and expensive.

In the present study, stability parameters were estimated using the model proposed by Eberhart and Russel (1966). According to them, a high yielding genotype with unit regression coefficient (bi=1) and the deviation from regression not significantly different from zero ($s^{-2}di=0$) is considered as the stable one. Clones which possessed high mean (general mean + two SE) only were considered for classification and characterization for adaptability (Cavalcanti and Gurgel, 1973). The high yielding clones were further classified into four groups following the methodology suggested by Mehra and Ramanujam (1979) as under.

Group	Mean	Regression Coefficient 'bi'	Deviation from Regression 's^{-2}di'
I	High	Around unity	Around zero
II	High	Significantly deviating from unity	Around zero
III	High	Significantly deviating from unity	Significantly deviating from zero
IV	High	Around unity	Significantly deviating from zero

Clones in group I will be highly stable over the growth phases. An above or below average response could be expected from clones falling in group II and they will be suited for stress or favourable growth phases. Groups III and IV may be ignored as the behaviour of the clones falling in these groups will be unpredictable. When the current annual increment (CAI) for total height, DBH, CDM, frustum volume and volume index over five growth periods were subjected to stability analysis, the variance due to clone x growth period interaction was found significant for total height, frustum volume and volume index in case of CH / CP clones. Therefore, further analyses were carried out for these characters and stability parameters worked out (Table 1 and 2). Clones CP 4202, CH 3002, CH 2803 and CP 3903 were included in group I when stability parameters for height was considered and hence are the most stable clones with respect to total height (Table 3). Six clones though recorded high mean values, were found unpredictable over growth periods due to the significant deviation from regression. Clones CP 0207, CP 3903 and CH 0401 exhibited stability for both frustum volume and volume index. Clones CH 2703 and CH 2803 were found to be stable for frustum volume and volume index respectively. Even though, clones CH 3004 and CH 2703 exhibited superior growth, they could not register favourable values for stability parameters and proved their instability. No clones were found suitable for stress or favourable growth phases.

In TCR clones, clone x growth period interaction was significant for CAI of all the characters except volume index (Table 4 and 5). Among the 15 clones which recorded high mean values for total height, 10 were found to be highly stable over the growth periods (Table 6). Select clones were observed to be stable for DBH (7), CDM (9) and frustum volume (10) also. Clones TCR 060101, TCR 030202 and TCR 030101 exhibited high stability for all the four traits. Though showed instability for height, clones TCR 110202 and TCR 040103 were found to be stable for DBH, CDM and frustum volume. Clone TCR 040204 which registered superior growth characteristics was unpredictable across the environments due to the significant deviation from regression for characters, total height and CDM. However, it was found to be highly stable for DBH and frustum volume (indicated from the non-significant regression coefficient and least deviation from regression). The clone TCR 120102 which ranked first for most of the variables exhibited instability for DBH, CDM and frustum volume.

A considerable proportion among the superior clones in the TCR group exhibited instability for height (33%), DBH (46%), CDM (40%) and frustum volume (17%) when the CAI of the respective traits were subjected to stability analysis over five growth phases. In TCR clones also no genotype could be identified for stress or favourable environments. Kumaravelu and Paramathma (2001) studied the stability of 75 open pollinated families of *Casuarina equisetifolia* over 3 years and identified three stable performers in all the three growth phases.

Table 1: **Pooled analysis of variance for phenotypic stability in CH / CP clones**

Source	DF	Total Height	DBH	CDM	Frustum Volume	Volume Index
Clone	32	0.130*	0.135*	0.180*	1538285.515*	345740393.550*
Growth Period	4	11.021*	1.233*	1.282*	2872391.060*	160930309.285*
Clone x Growth Period	128	0.029*	0.014	0.017	75010.633*	17966799.371*
Growth Period + (Clone x Growth Period)	132	0.362*	-	-	159779.735*	22299026.784*
Growth Period (Linear)	1	44.083*	-	-	11489547.094*	643726380.000*
Clone x Growth Period (Linear)	32	0.044*	-	-	124835.935*	11982106.625*
Pooled Deviation	99	0.023*	-	-	56632.634*	19356748.938*
Pooled Error	825	0.012	0.013	0.015	121051.656	27050114.000

** Significant at 5% level*

Table 2: **Stability parameters in CH / CP clones**

Clone No.	Total height			Frustum Volume			Volume Index		
	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$
CH 3004	1.023	0.940	0.027*	2859.356	1.308	714746.750*	40515.543	3.519	3488389.120*
CP 4305	0.756	1.013	-0.008	250.225	0.385*	-118639.961*	2303.683	0.434*	-268728.540
CP 3703	0.881	1.062	0.033*	626.511	1.063	-111418.422	7308.503	0.936	-217952.840
CP 3702	0.836	1.265	0.000	520.273	1.112	-119402.805*	5683.975	1.049	-254711.780
CP 3901	0.796	1.049	0.016	402.083	0.804	-116027.453	3914.907	0.804	-268503.440
CP 1501	0.801	1.064	-0.003	639.355	0.887	-106370.117	7688.423	1.085	-264348.940
CP 1802	0.332	0.275*	-0.011	228.515	0.534	-116399.500	1006.549	0.130*	-269678.140*
CP 4403	0.703	0.781	0.017	614.653	0.598	-32909.219	5444.039	0.140	-229973.500
CP 4202	0.990	1.172	-0.005	605.921	1.325	-111365.758	5803.693	1.145	-264597.760
CP 0301	0.735	0.963	0.007	612.878	1.214	-114703.219	6565.782	1.303	-254597.300
CP 0110	0.750	1.144	0.006	399.938	0.806	-113711.664	3836.388	0.870	-264453.840
CP 0207	0.819	1.048	0.013	1156.934	2.068	61937.414	15879.232	0.503	-16269.269
CP 0108	0.797	1.021	0.026*	426.207	0.530	-114512.563	4344.923	0.505	-259317.560
CP 0203	0.830	1.011	0.006	661.720	1.302	-113990.852	8024.452	1.332	-215626.440
CH 2703	0.909	0.901	0.032*	2232.886	2.097	141477.656	30437.285	2.998	1001544.720*
CH 1802	0.626	0.802*	-0.011	283.564	0.522*	-119457.844*	2343.833	0.445	-267810.820*
CH 3002	0.902	1.019	0.011	454.263	1.221	-119517.242*	5130.304	1.011	-251542.560
CH 2604	0.783	1.258	0.002	374.386	1.148	-109204.789	5153.262	1.363	-174842.460

CH 2803	0.976	1.220	-0.005	778.892	1.452	-20278.039	11190.376	1.591	-68291.855
CP 3501	0.620	1.007	-0.005	522.593	0.772	-112931.914	4330.466	0.841	-269402.820*
CP 3101	0.580	0.795	-0.008	322.289	0.497	-113547.617	2309.091	0.307*	-269198.240*
CP 3903	0.974	1.177	-0.002	919.325	1.408	-77171.258	12246.478	2.066	-140139.770
CP 2401	0.596	0.982	-0.001	211.470	0.411*	-119027.156*	1768.347	0.302	-266903.900*
CP 4805	0.712	1.071	-0.007	543.574	0.876	-117079.422	6180.519	0.953	-258100.300
CH 2602	1.005	1.055	0.035*	488.906	0.750	-109403.289	5529.405	0.576	-249613.420
CH 2303	0.830	1.092	0.005	375.816	0.891	-118912.883*	3818.409	0.685	-267971.880*
CH 2002	0.709	0.873	0.025*	301.156	0.723	-119917.922*	3055.887	0.586	-255853.360
CH 0401	1.149	1.161	0.085*	1357.818	3.303	49353.246	20503.795	2.535	5512912.500
CH 0903	0.975	0.801	0.053*	816.590	0.362	-97966.563	7526.727	0.674	-238509.640
CH 1702	0.592	0.957	0.022*	271.387	0.524	-115119.898	1935.007	0.387*	-268333.000*
CH 0803	0.798	1.079	0.011	465.754	0.772	-103264.953	4483.463	0.737	-266838.760*
CH 1004	0.771	0.956	-0.005	770.759	0.714	-111561.047	7080.250	0.828	-255768.500
CH 1002	0.697	0.985	0.010	395.077	0.619*	-119529.430*	3051.514	0.360	-265992.800*
Grand Mean	0.796			663.366			7769.531		
SEM	0.028			96.555			1447.549		

** Significant at 5% level*

Continued

Table 2: **Stability parameters in CH / CP clones (continued)**

Clone No.	DBH			CDM		
	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$
CH 3004	0.971	-	-	1.173	-	-
CP 4305	0.249	-	-	0.312	-	-
CP 3703	0.421	-	-	0.461	-	-
CP 3702	0.340	-	-	0.366	-	-
CP 3901	0.317	-	-	0.428	-	-
CP 1501	0.415	-	-	0.454	-	-
CP 1802	0.220	-	-	0.252	-	-
CP 4403	0.425	-	-	0.461	-	-
CP 4202	0.368	-	-	0.470	-	-
CP 0301	0.403	-	-	0.458	-	-
CP 0110	0.333	-	-	0.377	-	-
CP 0207	0.568	-	-	0.577	-	-
CP 0108	0.325	-	-	0.409	-	-
CP 0203	0.412	-	-	0.451	-	-
CH 2703	0.911	-	-	0.976	-	-
CH 1802	0.326	-	-	0.315	-	-
CH 3002	0.307	-	-	0.398	-	-
CH 2604	0.272	-	-	0.289	-	-

Clone No.	DBH			CDM		
	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$
CH 2803	0.459	-	-	0.529	-	-
CP 3501	0.332	-	-	0.431	-	-
CP 3101	0.313	-	-	0.350	-	-
CP 3903	0.569	-	-	0.610	-	-
CP 2401	0.255	-	-	0.244	-	-
CP 4805	0.364	-	-	0.423	-	-
CH 2602	0.373	-	-	0.416	-	-
CH 2303	0.292	-	-	0.328	-	-
CH 2002	0.297	-	-	0.313	-	-
CH 0401	0.409	-	-	0.529	-	-
CH 0903	0.531	-	-	0.675	-	-
CH 1702	0.254	-	-	0.305	-	-
CH 0803	0.366	-	-	0.414	-	-
CH 1004	0.477	-	-	0.593	-	-
CH 1002	0.318	-	-	0.403	-	-
Grand Mean	0.400			0.460		
SEM	0.029			0.033		

Table 3: **Grouping of clones based on stability parameters in CH / CP clones**

Characters	Group I	Group II	Group III	Group IV
Total Height	CP 4202 CH 3002 CH 2803 CP 3903	Nil	Nil	CH 3004 CP 3703 CH 2703 CH 2602 CH 0401 CH 0903
Frustum Volume	CP 0207 CH 2703 CP 3903 CH 0401	Nil	Nil	CH 3004
Volume Index	CP 0207 CH 2803 CP 3903 CH 0401	Nil	Nil	CH 3004 CH 2703

Table 4: **Pooled analysis of variance for phenotypic stability in TCR clones**

Source	DF	Total Height	DBH	CDM	Frustum Volume	Volume Index
Clone	42	0.072*	0.008*	0.014*	119118.949*	46391495.148*
Growth Period	4	5.652*	0.276*	0.385*	521177.812*	114811851.476*
Clone x Growth Period	168	0.016*	0.004*	0.006*	15050.723*	2522096.744
Growth Period + (Clone x Growth Period)	172	0.147*	0.010*	0.015*	26821.120*	-
Growth Period (Linear)	1	22.610*	1.104*	1.541*	2084745.316*	-
Clone x Growth Period (Linear)	42	0.033*	0.005*	0.007	19759.308*	-
Pooled Deviation	129	0.011*	0.003*	0.006*	13167.414	-
Pooled Error	1075	0.007	0.002	0.003	10489.768	-

** Significant at 5% level*

Table 5: **Stability parameters in TCR clones**

Clone No.	Total height			DBH			CDM		
	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$
TCR 070201	0.568	0.874	0.005	0.161	0.394	0.001	0.195	0.357	-0.001
TCR 050203	0.638	1.040	0.002	0.187	0.792	0.000	0.274	0.102	0.019*
TCR 050202	0.546	0.875	0.025*	0.178	0.554	0.000	0.225	0.489	0.004
TCR 060202	0.562	0.754	-0.005	0.193	0.014	0.006*	0.236	0.485	0.004
TCR 020101	0.510	0.736	-0.002	0.178	0.527	0.000	0.189	0.364	-0.002
TCR 070102	0.481	0.648	-0.003	0.160	0.344*	-0.001	0.180	0.520	-0.002
TCR 080103	0.576	1.096	0.021*	0.173	0.717	-0.001	0.202	0.698	-0.002
TCR 060101	0.787	1.591	0.010	0.220	1.002	-0.001	0.288	0.978	-0.001

TCR 120102	0.895	1.429	0.000	0.374	0.331	0.040*	0.444	0.430	0.068*
TCR 080203	0.790	1.006	-0.001	0.240	0.858	0.009*	0.248	1.660	0.000
TCR 110202	0.893	1.331	0.014*	0.260	0.988	0.000	0.292	0.806	-0.001
TCR 080201	0.797	1.369	0.006	0.242	1.416	0.003*	0.290	1.430	0.009*
TCR 120203	0.665	0.890	0.023*	0.279	0.498	-0.001	0.394	0.741	0.005*
TCR 090102	0.730	1.196	-0.001	0.219	1.306	0.004	0.249	1.116	0.007*
TCR 030201	0.736	1.126	0.001	0.244	1.040	0.003*	0.261	1.289	0.004
TCR 020105	0.590	0.868	0.000	0.240	0.097	0.007*	0.331	0.734	0.005*
TCR 100104	0.521	0.782	-0.001	0.179	0.658	-0.001	0.231	1.035	0.000
TCR 100101	0.676	1.079	0.004	0.211	1.045	-0.001	0.270	0.861	-0.001
TCR 090201	0.543	0.785	-0.005	0.194	1.014	0.000	0.286	0.339	0.003
TCR 100203	0.579	1.054	-0.005	0.209	1.450	0.002	0.238	1.248	0.003
TCR 010103	0.592	0.928	-0.006	0.214	1.163	0.001	0.238	0.887	-0.002
TCR 010101	0.663	1.017	-0.004	0.215	1.238	0.000	0.273	1.088	-0.001
TCR 110101	0.614	0.863	-0.004	0.214	1.606*	-0.001	0.244	1.395	-0.002
TCR 110203	0.652	1.087	-0.005	0.210	1.310	-0.001	0.214	1.056	0.001
TCR 030203	0.635	0.924	0.001	0.199	1.255	-0.001	0.225	0.894	0.000
TCR 040104	0.573	0.892	0.001	0.175	1.017	0.000	0.191	0.904	0.000
TCR 090101	0.690	0.987	0.007	0.203	1.259	-0.001	0.244	0.973	0.000
TCR 020202	0.716	0.938	0.033*	0.195	1.054	-0.001	0.235	1.185	-0.003
TCR 100102	0.458	0.707	0.004	0.154	0.851	0.000	0.169	1.026	-0.002
TCR 090202	0.586	1.107	-0.004	0.169	1.250	0.000	0.210	1.424	0.000
TCR 030106	0.476	0.783	0.007	0.188	1.134	-0.001	0.215	1.013	-0.003
TCR 120202	0.391	0.447*	-0.003	0.152	0.469	0.000	0.213	0.841	0.001
TCR 020102	0.547	0.894	0.000	0.207	1.265	-0.001	0.234	1.532	-0.001
TCR 130202	0.550	0.747	-0.005	0.235	0.800	0.004*	0.268	1.048	-0.001
TCR 120204	0.554	0.799	-0.004	0.204	0.838	0.000	0.228	0.670	0.001
TCR 010102	0.469	0.853	0.004	0.168	1.080	-0.001	0.212	0.918	-0.001
TCR 110102	0.639	1.074	0.019*	0.201	1.200	0.000	0.260	1.311	-0.002
TCR 040103	0.769	1.516	0.028*	0.249	2.018	0.000	0.302	2.184	0.002
TCR 030202	0.695	1.220	0.006	0.256	1.340	0.001	0.279	1.675	0.000
TCR 080102	0.553	1.060	-0.003	0.182	0.997	0.000	0.212	0.925	-0.002
TCR 060204	0.496	0.851	-0.005	0.206	1.437	0.000	0.223	1.206	0.002
TCR 030101	0.727	1.237	0.000	0.229	1.567	-0.001	0.294	1.354	0.002
TCR 040204	0.861	1.543	0.015*	0.243	1.806	0.002	0.290	1.810	0.005*
Grand Mean	0.628			0.210			0.251		
SEM	0.018			0.006			0.008		

** Significant at 5% level*

Continued

Table 5: **Stability parameters in TCR clones (continued)**

Clone No.	Frustum Volume			Volume Index		
	Mean	bi	$S^{-2}di$	Mean	bi	$S^{-2}di$
TCR 070201	164.406	0.150	-8056.754	1755.311	-	-
TCR 050203	254.223	0.119	85.366	2725.929	-	-
TCR 050202	227.165	0.322	-4612.153	2139.269	-	-
TCR 060202	231.097	0.115	-745.515	2264.677	-	-
TCR 020101	192.598	0.249*	-9960.537	2071.922	-	-
TCR 070102	163.483	0.239*	-10169.874	1654.512	-	-
TCR 080103	207.334	0.499	-9484.104	2562.305	-	-
TCR 060101	382.847	0.975	-8576.845	5545.854	-	-
TCR 120102	845.493	0.285	263834.656*	14445.159	-	-
TCR 080203	309.425	1.232	-355.911	4419.963	-	-
TCR 110202	473.901	0.936	-4793.001	9351.834	-	-
TCR 080201	544.613	2.054	31923.736*	10079.689	-	-
TCR 120203	844.671	0.830	13432.391	12753.811	-	-
TCR 090102	357.259	1.302	7816.890	5433.511	-	-
TCR 030201	384.553	1.238	3500.701	5290.064	-	-
TCR 020105	460.115	0.452	13778.872	4904.879	-	-
TCR 100104	234.841	0.705	-8794.368	2253.415	-	-
TCR 100101	286.766	0.693	-8799.510	3344.570	-	-
TCR 090201	285.060	0.383	-7635.125	2341.650	-	-
TCR 100203	278.051	1.159	-843.307	2972.289	-	-
TCR 010103	284.187	0.811	-8156.358	3354.845	-	-
TCR 010101	366.946	1.177	-5796.290	4812.194	-	-
TCR 110101	303.360	1.323	-9507.216	3669.887	-	-
TCR 110203	279.283	1.109	-5992.268	3686.263	-	-
TCR 030203	273.583	0.957	-7684.989	3542.809	-	-
TCR 040104	201.369	0.744	-7699.845	2386.152	-	-
TCR 090101	376.316	1.277	-6105.212	5901.007	-	-
TCR 020202	341.786	1.321	-9971.626	5404.563	-	-
TCR 100102	134.875	0.573	-9988.503	1194.283	-	-
TCR 090202	272.635	1.447	-6539.129	3826.258	-	-
TCR 030106	274.084	1.051	-10185.143	2621.139	-	-
TCR 120202	190.162	0.475	-8226.604	1325.242	-	-
TCR 020102	285.665	1.326	-8578.102	2686.181	-	-
TCR 130202	386.765	1.059	-3035.719	3912.329	-	-
TCR 120204	261.112	0.625	-7677.159	2971.136	-	-
TCR 010102	231.570	0.861	-9095.445	1736.830	-	-
TCR 110102	357.352	1.479	-7135.428	4574.607	-	-
TCR 040103	538.713	3.074	5202.234	7457.349	-	-

TCR 030202	452.999	1.951	-6035.591	6717.007	-	-
TCR 080102	195.344	0.691	-9664.887	1800.473	-	-
TCR 060204	265.204	1.224	-4727.726	2822.638	-	-
TCR 030101	475.150	1.980	-4558.086	7152.130	-	-
TCR 040204	514.983	2.529	14752.276	10207.363	-	-
Grand Mean	334.682			4466.820		
SEM	23.538			464.515		

** Significant at 5% level*

Table 6: **Grouping of clones based on stability parameters in TCR clones**

Characters	Group I	Group II	Group III	Group IV
Total Height	TCR 060101 TCR 120102 TCR 080203 TCR 080201 TCR 090102 TCR 030201 TCR 100101 TCR 090101 TCR 030202 TCR 030101	Nil	Nil	TCR 110202 TCR 120203 TCR 020202 TCR 040103 TCR 040204
DBH	TCR 060101 TCR 110202 TCR 120203 TCR 040103 TCR 030202 TCR 030101 TCR 040204	Nil	Nil	TCR 120102 TCR 080203 TCR 080201 TCR 030201 TCR 020105 TCR 130202
CDM	TCR 060101 TCR 110202 TCR 100101 TCR 090201 TCR 010101 TCR 130202 TCR 040103 TCR 030202 TCR 030101	Nil	Nil	TCR 050203 TCR 120102 TCR 080201 TCR 120203 TCR 020105 TCR 040204
Frustum Volume	TCR 060101 TCR 110202 TCR 120203 TCR 030201 TCR 020105 TCR 130202 TCR 040103 TCR 030202 TCR 030101 TCR 040204	Nil	Nil	TCR 120102 TCR 080201

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Monitoring Genetic Fidelity of Somatic Embryo Regenerated Plants of *Bambusa bambos* by RAPD and ISSR markers

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INTRODUCTION

Bamboos are a group of plants that belong taxonomically to the subfamily of *Bambusoideae* under the family *Poacae*. Bamboo is known to be one of the fastest growing plants in the world, with a growth rate ranging from 30 to 100 cm per day in growing season (United Nations 1972). India is rich in genetic resource of bamboo with 125 species of bamboo in 23 genera spread (natural and planted) over an around 11.4 million ha in 25 states and union territories, which constitute 16.7% of the total forest area in the country (FSI, 2003).

Bambusa bambos is distributed throughout country and commonly found in homesteads of southern India (Tewari, 1992; Anonymous, 2003). It occupies 15% of the total bamboo area in India. The major uses of *B. bambos* are as a raw material for pulp, paper and panel products and other uses such as scaffoldings, rafters, thatching and roofing, basket making, bows and arrows, furniture, etc.,

Micropropagation is only methods for the production of large quantity of clonal planting material. The genetic stability of *in vitro* regenerated plants is an essential requisite for large scale clonal propagation and, somaclonal variation is of special relevance in perennial plants (Skirvin *et al*., 1994) and long generation forest trees since occasional mutations can sometimes only be noticed at very late developmental stages, or even in their off springs.

Genetic fidelity of the microproagated plants of *B. bambos* through somatic embryogenesis have been evaluated by using RAPD and ISSR markers at every six months interval up to two years.

MATERIALS AND METHODS

Nodal shoot segments of i*n vitro* grown axillary shoots were used as explants for callus induction. In order to identify the effect of auxins on callus initiation and multiplication, different auxins *viz;* IBA, NAA, 2,4-D and 2,4,5-T were tested in MS medium supplemented with additives (ascorbic acid 50mg/l, citric acid, 25 mg/l, cystein 25 mg/l, glutamine 100 mg/l) for callus induction and multiplication. To obtain homogenous callus multiplication of embryogenic callus, different combinations of carbohydrates (sucrose and glucose either alone or in combinations) was evaluated on MS medium supplemented with additives and BAP (2.0mg/l) + NAA (1.0mg/l). In order to obtain synchronized induction of somatic embryo, effect of Kinetin and BAP along with NAA 1.0mg/l in two different concentrations (1.0 and 2.0mg/l) in MS agar gelled medium with additives and another treatment with coconut water 10% (v/v) were tested in six different treatments. Optimization and synchronization of somatic embryo maturation and germination, experiments was carried out with various cytokinins *viz;* Kinetin, BAP, TDZ (1.0-2.5mg/l) and auxins (NAA, IBA, IAA and NOA) either alone or in combinations in MS agar gelled medium in twelve different treatments. MS medium with additives and 10% coconut water was also tested in other treatments. At every six months interval somatic embryo regenerated plantlets were transplanted in polybags consisted potting media; sand, soil and compost (5:1:4 v/v) enriched with neem cake 10kg/cum in mist chamber conditions for 4 weeks (30 $\pm$ 5° C temperature and 80 $\pm$ 5% relative humidity), followed by 2-3 weeks under 50% shade in agro shade net house. Regenerated plants acclimatized at nursery conditions were used for genetic fidelity studies.

Genomic DNA was extracted from the leaves of *in vitro* regenerated plants from randomly selected 10 plants from the nursery conditions by using modified Doyle and Doyle (1990) CTAB method at every six months interval and also from the mother plant. Genetic fidelity was evaluated using 20 primers of RAPD (8 OPD and 12 OPR primers) and 23 primers of ISSR (UBC 800 series) markers at every six months interval for two years. The PCR amplifications were carried out in 0.2ml PCR tube using gradient Mastercycler (Eppendorf, Germany) in 25μl reaction volume. The PCR reaction mixture containing 2.5μl of 30ng of genomic DNA as template DNA, 2.5μl of 1X PCR buffer, 2.5μl of $MgCl_2$ (2.75mM), 2.5μl of 10mM of primers, 1μl of 10mM dNTPs and 0.3μl of 1.2 unit Taq polymerase was performed with RAPD Operon markers and ISSR UBC markers.

RAPD amplification reactions were carried out with the following cycle profiles *viz;* initial denaturation at 94°C for 3 minutes, followed by repeated 40 cycles of denaturation at 94°C for 30 sec, annealing at 45°C for 30 sec, extension at 72°C for 30 sec and a final extension for 10 min at 72°C temperature.

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ISSR amplification reactions were carried out with cycle profile *viz;* initial denaturation at 94°C for 3 minutes, followed by repeated 35 cycles of denaturation at 94 °C for 30 sec, annealing at 50°C for 30 sec, extension at 72°C for 30 sec and a final extension of 10 min at 72°C temperature.

The RAPD and ISSR amplified products were electrophoresed on 2% agarose gel stained with ethidium bromide and visualized for RAPD and ISSR profile under UV in Herolab gel documentation system.

RESULTS AND DISCUSSION

Highest percentage of response (88.89%) on callus induction was on MS medium with additives and 2,4,5 - T (2.5 mg/l) this was followed by 2,4-D 2.5mg/l (Graph 1 and Fig. 1). The callus was whitish in colour, heterogeneous and compact in nature. Mucilaginous growth was also observed along with the embryogenic callus. Callus induction response was low in MS medium with NAA or IBA. Similarly, Jullien and Tran Thanh Van (1994) observed three types of callus from the leaf tissues on MS medium with 2, 4-D (18μM) in *B. glaucescens*. In accordance to our results, Hu *et al*. (2011) reported that among the two auxins (2,4 –D and 2,4,5 – T) and other PGRs; Kn, IAA and IBA, high callus induction frequency (96.0%), was observed in MS medium supplemented with 2,4,5 – T (2.0mg/l) in *D. farinosus,* a giant sympodial bamboo.

Similarly, various auxins (IBA, NAA, 2, 4-D, and 2,4,5-T) tested in MS medium for callus multiplication, 2,4,5-T (1.0 mg/l) not only favoured embryogenic callus multiplication (2.96g), but also reduced the amount of mucilaginous callus as compared with medium consisted other auxins (Graph 2 and Fig. 2). Increase in the concentration of 2,4,5-T (2.5mg/l) increased the intensity of callus multiplication, as evidenced by the fresh weight (3.20g) and the result was on par with 2,4,5-T 1.0mg/l (2.96g). It was observed that medium supplemented with 2,4-D favoured growth of mucilage callus along with embryogenic callus. Other auxins *viz;* IBA and NAA were not found suitable for embryogenic callus multiplication and also encountered problem of callus deterioration. In accordance to our findings, Hu *et al*. (2011) reported that MS medium with 2.0mg/l of 2,4,5-T resulted better multiplication of embryogenic callus in *D. farinosus*.

It was evident from the results that among the different type of carbohydrate tested (sucrose and glucose) either alone or in combinations in the MS medium + 2,4,5-T (1.0mg/l) resulted in proliferation of embryogenic compact callus. Medium with 3% sucrose alone proved better with 3.68g of fresh weight of callus, which was on par with combined use of glucose 1.5%+sucrose 3%, with fresh weight of 3.66g (Graph 3). Medium with sucrose produced more embryogenic callus as compared with glucose alone. Callus produced on the medium with glucose was relatively compact and also with mucilage**.** In accordance to your results, Komatsu *et al*. (2010) reported the best results in terms of inducing meristematic centers in callus cells on the medium with 4% glucose as compared to the medium with other carbohydrates in *P. bambusoides*. Yeh and Chang (1986) reported that higher concentration (6%) of sucrose favoured callus multiplication under dark conditions in *B. beecheyana*. In addition, sucrose at 6% favoured highest percentage of secondary embryogensis as compared with four different carbohydrates *viz*; sucrose, glucose, maltose and fructose (Agarwal *et al*., 2004).

Hormone free medium with additives (ascorbic acid 50mg/l + citric acid 25mg/l + cysteine 25mg/l + glutamine 100mg/l) + 10% coconut water favoured maximum (49.33%) somatic embryo induction (Graph 4). Whereas, among the cytokinins (Kinetine and BAP) in different concentrations (1.0 and 2.0mg/l) tested along with NAA 1.0mg/l in the medium, none of the combinations improved somatic embryo induction. Whereas, MS medium with additives + BAP 2.0 mg/l +NAA 1.0 mg/l responded second highest percentage response (46.0%) for production of somatic embryos like structures. In accordance to our findings, Godbole *et al.* (2002) observed elimination of 2,4-D and incorporation of 10% coconut water in MS medium was favourable for somatic embryo induction, and followed by incorporation of PGRs was necessary for embryo germination. Ramanayake and Wanniarachichi (2002) reported similar steps where, complete elimination of auxins resulted in 78% somatic embryo induction, which did not redifferentiate unless again exogenous supply of BAP concentration up to 2.5mg/l was used in *D. hamiltonii*, and in *D. giganteus*.

Effect of PGRs in MS medium was significant on somatic embryo maturation and germination. Among the various PGRs tested, highest (16.33%) percentage of somatic embryo germination into normal plant (root and shoot) 34.63% only shoots and 20.67% only roots was observed on MS medium with additives (ascorbic acid 50mg/l + citric acid 25mg/l + cysteine 25mg/l + glutamine 100mg/l) + BAP 2.0 mg/l + NAA 1.0 mg/l (Graph 5 and Fig. 3, 4). Increase in BAP concentration (2.5mg/l) did not have any significant difference with respect to somatic embryo germination. Low rate of germination was observed with TDZ and Kn combinations. Among the auxins (IAA, IBA, NAA and NOA) tested along with BAP (2.0mg/l) with MS medium, NAA, IBA and IAA (1.0mg/l) had an on par embryo germination response. Similarly, Arya *et al.* (2008) reported maximum somatic embryo germination on MS medium with 1.0 mg/l BAP + 1.0 mg/l GA_3 in *D. asper*. Woods *et al.* (1995) also reported the important role of BAP in promoting the production of somatic embryo and germination in Mexican weeping bamboo.

Plants regenerated by somatic embryogenesis were transferred in poly bags (600cc) consisted potting medium; sand, soil and compost (5:1:4, v/v) enriched with neem cake 10, kg/cum and fungicide (M-4.5, 0.4, kg/cum) + Phorate (0.4, kg/cum) as a prophylactic measures. Initially, plantlets were kept in a polytunnel for 4 weeks in green house (30 $\pm$ 5° C temperature and 80 $\pm$ 5% relative humidity), followed by 2-3 weeks under 50% shade in agroshade net house was found essential for high rate of survival. Plantlets showed new shoot and root growth within 2-3 weeks period in green house. At the end of 4 weeks in all the plants new shoot and root growth was visible. Plants transferred in agroshade net house were green and properly acclimatized in 2-3 weeks, before keeping them in open nursery

Before large scale plantations, genetic fidelity of the micropropagated plants is essential so as to maintain true to type characters of the mother plant. In the present study modified Doyle and Doyle (1990) CTAB method used for DNA extraction with 3% CTAB, 980 ng of DNA was isolated from 250 mg of fresh leaves extracted from both somatic embryo derived plants hardened at

nursery conditions and from leaves of mother plant. The purity of the DNA extracted was optimum with OD of 1.82 at A_{260}/A_{280} nm, which was pure enough for RAPD and ISSR studies.

Genetic fidelity of the plants raised through somatic embryogenesis from the embryogenic callus was ascertained in comparison with the mother plant every six months interval i.e., 6, 12, 18 and 24 months revealed that, genetic variations started to creep in the plants raised from 12th months old callus cultures with polymorphism of 1.77% (1329 bands) to 2.44% in the plants of 18th months old cultures (21 passages) and further increased to 3.03% in the plants raised at 24th months old callus culture (28th passage) (Fig. 5). Agnihotri *et al*. (2009) used six RAPD primers (OPA3, OPA4, OPA5, OP11, OPA19 and OPA15) which produced clear and scorable amplification products in all the samples for genetic fidelity studies in *D. hamiltonii* with no genetic variations from plants raised through axillary mode of regeneration. All the amplified 33 fragments ranged from 0.3 - 2.6kb and found to be monomorphic across the *in vitro* propagated plants and the corresponding mother plant up to 1.5 years.

Genetic stability was observed only in the plants regenerated from 6th month old callus cultures, followed by slight variations with 98.10%, 97.60% and 97.02% monomorphic bands in the genomic DNA of plants regenerated from 12th, 18th and 24th months old callus cultures, respectively using ISSR markers. A maximum percentage of polymorphic bands were observed in the genomic DNA of the regenerated plants from 28th passage (24 months) old callus cultures with 2.97% of polymorphism with 1793 monomorphic loci (Fig. 6). Similar to our findings, Mehta *et al*. (2010) reported a slight variation of 1.2% using AFLP from the plants regenerated through somatic embryogenesis in *B. nutans*.

Whereas, Negi and Saxena (2009) reported 100% genetic stability by using ISSR primers up to 33 passages in *B. balcooa* through axillary shoot proliferation. Again Negi and Saxena (2010) reported genetic stability for plantlets of 11th, 17th and 27th passages raised through axillary shoots proliferation in *B. nutans,* with a total of 1,581 bands produced by using 15 ISSR markers. Hwang and Tang (1996) reported in any micropropagation protocol the permissible limit of genetic variations is accepted up to 3-5%. Hence the variations observed in the present study are within the acceptable limits and the somatic embryogenesis mode of regeneration of *B. bambos* can be carried out for a period of two years.

CONCLUSION

This study describes an effective regeneration protocol of *B. bambos* by somatic embryogenesis through callus phase. This protocol is useful for production of quality planting material in large scale for afforestation programmes. Tissue culture methods allows the production of a large number of plantlets identical to mother plant by economical method, which offers distinct advantages over conventional methods for multiplication of elite bamboo clones. The assessment of genetic stability of the regenerated plants is essential to avoid greater losses due to long gestation periods of forestry species. The present, protocol of molecular markers can be utilized for other bamboo species to evaluate the genetic stability of the regenerated plants.

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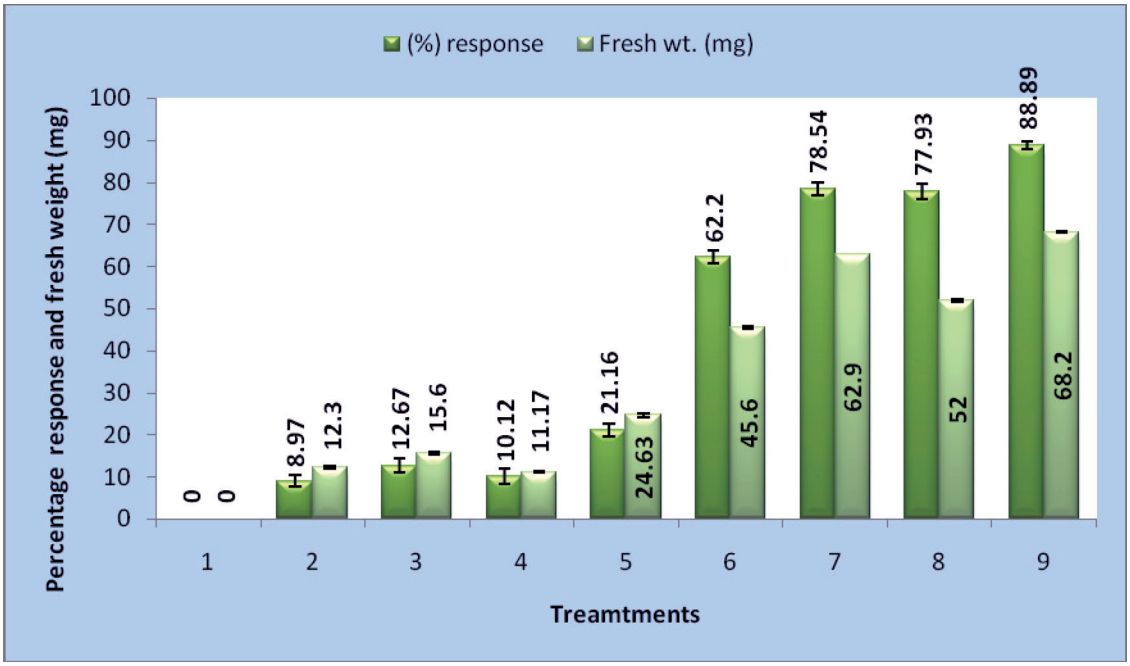
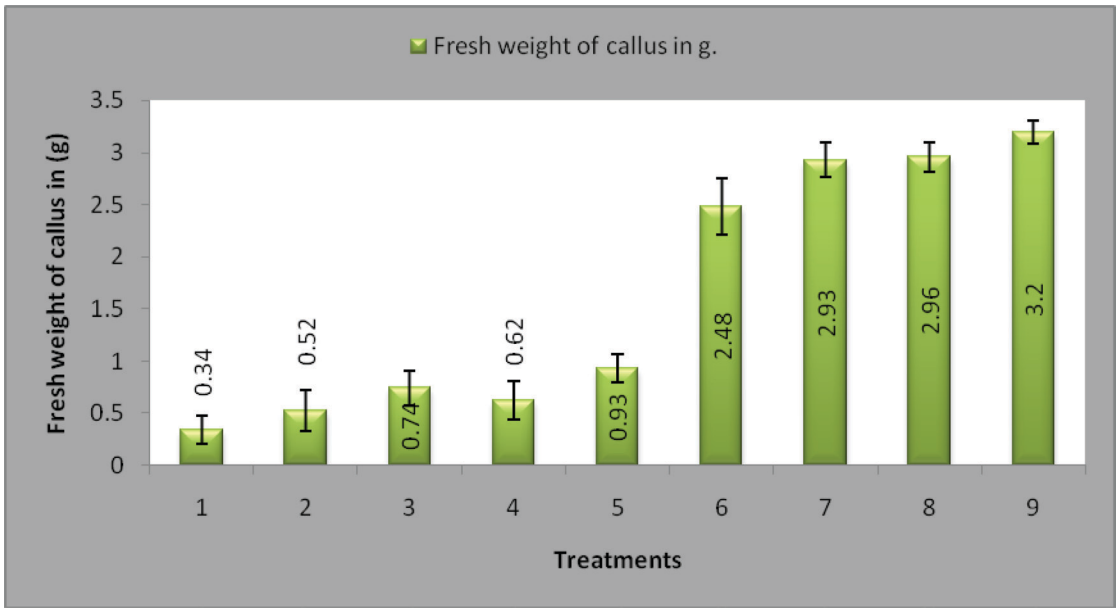
Graph 1: **Effect of various auxins and their different concentration on callus induction on MS medium with additives using nodal segments as explants from *in vitro* shoots**
Treatment details: T1-Control, T2-IBA 2.0, T3-IBA 4.0, T4-NAA 2.0, T5-NAA 4.0, T6-2,4-D 1.0, T7-2,4D 2.5, T8-2,4,5T 1.0, T9-2,4,5T 2.5

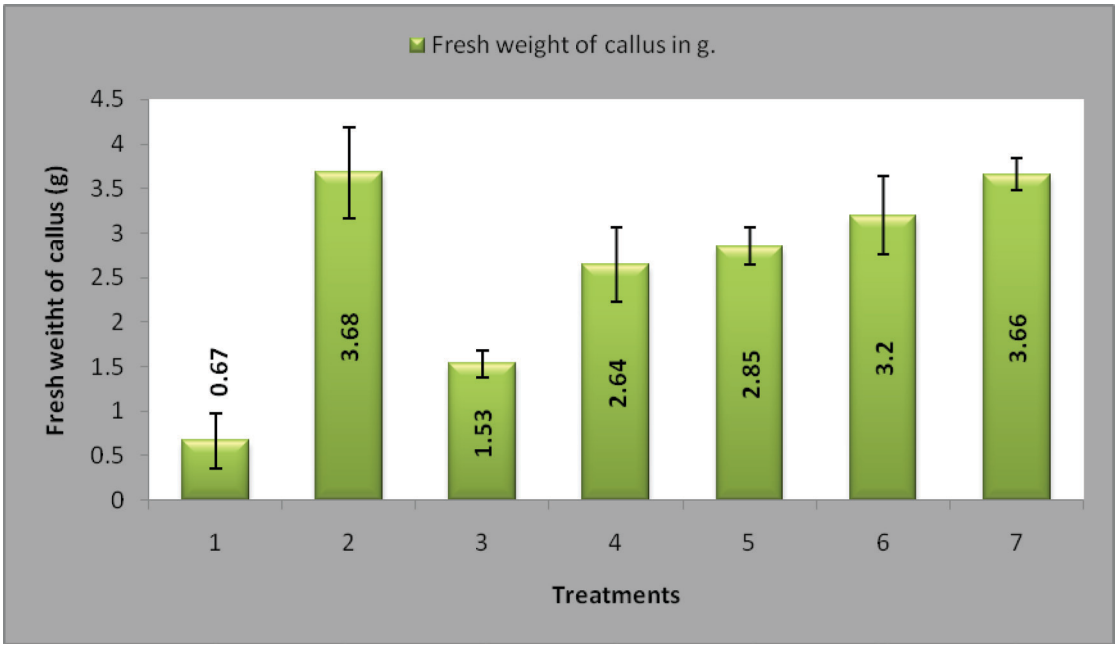


Graph 2: **Effect of various auxins and their different concentration on callus multiplication on MS medium supplemented with additives**
Treatment details: T1-Control, T2-IBA 2.5, T3-IBA 4.0, T4-NAA 2.5, T5-NAA 4.0, T6-2,4-D 1.0, T7-2,4D 2.5, T8-2,4,5T 1.0, T9-2,4,5T 2.5



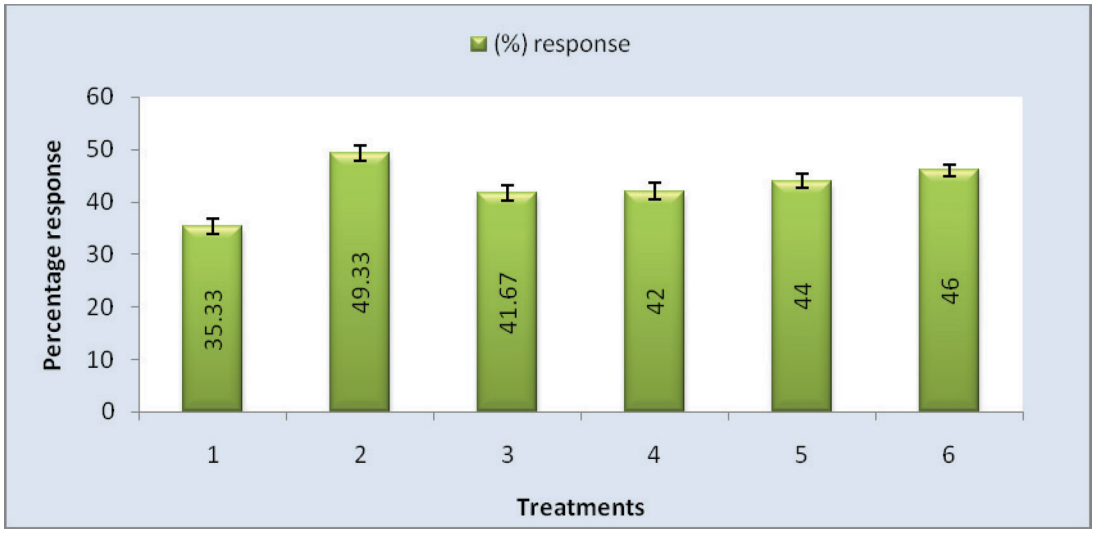
Graph 3: **Effect of type of carbohydrate and their combination on callus multiplication on MS medium supplemented with additives and 2,4,5-T 1.0mg/l**

Treatment details: T1-Control, T2-Sucrose 3%, T3-Glucose 3%, T4-Glucose 4.5%, T5-Glucose 1.5% + Sucrose 1.5%, T6-Glucose 3.0% + Sucrose 1.5%, T7-Glucose 1.5% + Sucrose 3.0%



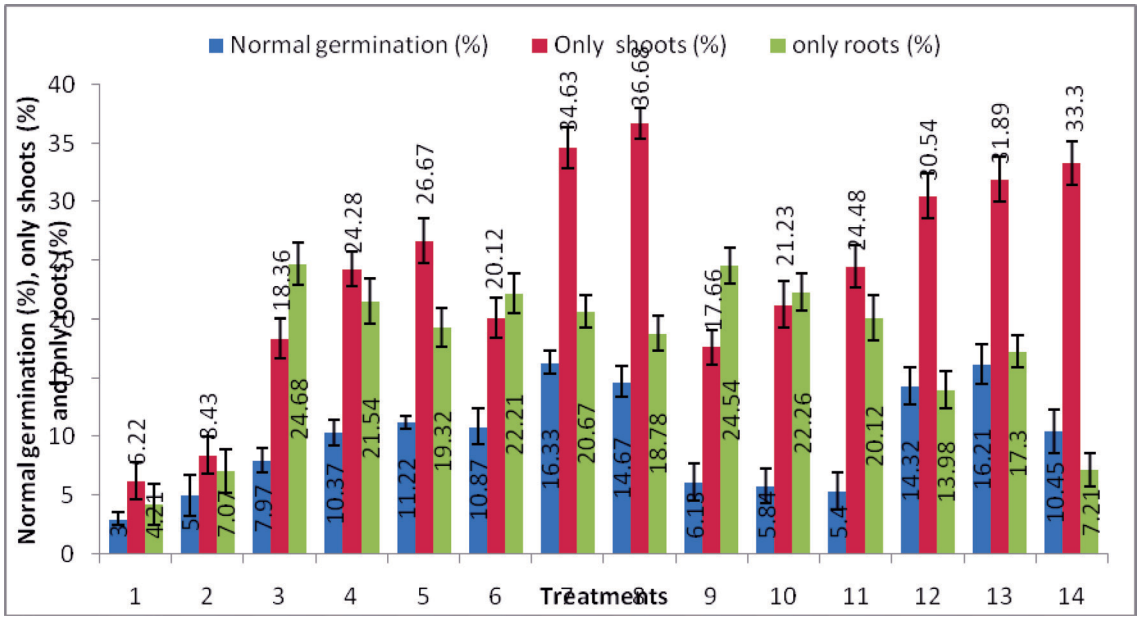
Graph 4: **Effect of various PGRs and their different concentration on somatic embryo induction on MS medium supplemented with additives**

Treatment details: T1-Control, T2-10% CW, T3-Kn 1.0 + NAA 1.0, T4-Kn 2.0 + NAA 1.0, T5-BAP 1.0 + NAA 1.0, T6-BAP 2.0 + NAA 1.0



Graph 5: **Effect of various PGRs and their different concentration in MS medium supplemented with additives on somatic embryo maturation and germination**

Treatment details: T1-Control, T2-10% CW, T3-Kn 1.0 + NAA 1.0, T4-Kn 2.0 + NAA 1.0, T5-Kn 2.5 + NAA 1.0, T6-BAP 1.0 + NAA 1.0, T7-BAP 2.0 + NAA 1.0, T8-BAP 2.5 + NAA 1.0, T9-TDZ 0.1 + NAA 1.0, T10-TDZ 0.2 + NAA 1.0, T11-TDZ 0.25 + NAA 1.0, T12-BAP 2.0 + IAA 1.0, T13-BAP 2.0 + IBA 1.0, T14-BAP 2.0 + NoA 1.0



Regeneration Status of Khasi Pine (*Pinus kesiya* Royle ex. Gordon) in Meghalaya

Nawa Bahar *

INTRODUCTION

The Himalayan species, because of their proximity to western and north-eastern Asian taxa, represent gene pools whose potential is unexplored. Considering the fragile ecosystem the preservation of biological diversity in the Himalaya is a national problem. The technologies for natural and artificial regeneration are therefore required in which studies on seeds are most important (Thapliyal, 1994). Pines are constitute one of the most important groups of species present in the Himalayan region and provide valuable natural resources which contribute significantly to the local and industrial economy of the country and also protect the watersheds which sustains and regulates the water supply for the need of millions that inhabit Himalayan river basins. In this region, pine forests provide for not only timber and fuel needs of the local populations but also support demand for packing wood, stakes for vegetable cultivation and its needles are used as bedding in cattle sheds (Troup, 1921; CABI, 2002). These are the pioneer species over much of their natural range and are active colonizers of degraded sites particularly the drier south facing slope. The species hold thus great promise in afforestation programmes in their natural habitats. Much of the degraded areas in their habitat are deficient in organic matter, water holding capacity, nutrient availability and micro/macro fauna and are thus lacking in almost all the desirable physical, chemical and biological characteristics necessary to support species other than pines, which also do not always respond favourably to afforestation efforts (Tiwari, 1994).

Khasi pine(*Pinus kesiya* Royle ex. Gordon) is a fast growing species and belongs to the family Pinaceae. It is widespread in Southeast between 10°N and 30°N and a longitudinal range between 26°E and 119°E. It grows in India, Myanmar, China, Laos, Vietnam, Thailand, and Philippines. It is the only tropical pine that grows in the eastern Himalayas and it is found in Khasi and Jaintia hills of Meghalaya. It is also found in Arunachal Pradesh, Nagaland and Manipur States. Among various valuable natural resources of conifers, khasi pine has an important role in the north - eastern region of India. It is very popular with the people of Meghalaya. People of this area use every part of the tree. The needles are used for stuffing mattresses, chair cushions, and cheap pillows and even as cementing fibre in the mud plastered wall. The needle litter in the forest is collected, burnt and used as soil correctives in potato beds. The branches and small wood are used as firewood. The knot and the core of the dead branches are collected and used in the destructive distillation for resin. The resinous wood is used as torch wood. The timber is used for house construction and good quality furniture. The increasing demand for various products of khasi pines in the North-East, needs to be matched by increased production to ensure their role in the environmental stability of this area. Khasi pine is an important coniferous species planted extensively in various plantation programmes by government and private planters in North-Eastern States. Forest Survey of India reported about 2.37 thousand ha area under plantation up to the year 1998 with a share of 8.9% in the North-Eastern region (Anon., 1999).

Overviews of this species reveal that meagre work has been carried out on assessment of natural regeneration in this region so far. The natural regeneration methods can provide low cost and effective means for the establishment of the population of this species. In this paper, the preliminary observations on the natural regeneration of khasi pine from Meghalaya state have been described highlighting the research problems and the same is presented in this communication.

MATERIALS AND METHODS

The present investigation was carried out into two locations namely Barapani (Riet Khawn Reserve Forest) and Shillong (North-Eastern Hill University) campus of the Meghalaya state in the month of November, 2010 to evaluate the status of natural regeneration of the species. The growth data on different parameters such as collar diameter, height, density, dry weight and stock quality index of the naturally occurring seedlings at both the locations were recorded. The standard deviation of mean of the character was calculated by prescribed method (Gomez and Gomez, 1984). The stock quality index (SQI) was used to quantify the morphological quality of the seedling as given by Dickson *et al*. (1960):

$$SQI = \frac{\text{Total seedling dry weight (g)}}{\text{Height (cm)/ Diameter (mm)}}$$

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RESULTS AND DISCUSSION

In terms of morphological characteristics of the forests, the population of khasi pine at Barapani area attain an average girth at breast height 63.68± 14.28 cm with 33.52 ±2.87 m height and density was recorded 1022 trees/ha, while, in Shillong area, an average girth at breast height 69.35± 12.84 cm with 32.17 ± 3.43m height and density was recorded 987 tree/ha(Table 1).

Growth performance of the seedlings in cleaned plot at Barapani area attains an average collar diameter 5.11 ± 1.98 mm with 10.36 ± 3.58 cm height, density 22.68 ± 5.22 seedlings/m2, dry weight 12.63 ±3.55g and stock quality index of the seedling was recorded as 6.25. However, in uncleaned plot, the seedling attains an average collar diameter 3.46± 0.69 mm with 8.66 ± 2.47 cm height, density 9.24± 4.72 seedlings/m2, dry weight 10.22 ± 1.77g, stock quality index of the seedling 4.08 and litter thickness was recorded 5.26± 1.94 cm (Table 2).

Growth performance of the seedlings in cleaned plot at North-Eastern Hill University campus, attains an average collar diameter 5.21 ± 1.25 mm with 11.45 ± 2.62 cm height, density 29.66± 6.44 seedlings/m^2, dry weight 14.88 ±2.54g and stock quality index of the seedling was recorded as 6.79. However, in uncleaned plot, the seedling attains an average collar diameter 3.77 ± 0.88 mm with 8.92± 2.33 cm height, density 14.38 ± 4.7 seedlings/m2 dry weight 12.47 ± 1.69g ,stock quality index of the seedling recorded 5.28 and litter thickness was recorded as 3.88 ± 1.75 cm (Table 3).

Evaluation of density and growth performance of seedlings in different location in different treatments was indicated that all the growth parameters such as collar diameter, height, density, dry weight and stock quality index were higher in cleaned plot as compare to uncleaned plots, because uncleaned plot in pine forests have thick ground vegetation, which hinders the emergence percentage of the seeds during germination process. The radicles do not contact the soil directly and unable to take the moisture and other nutrients from the soil. In case of a cleaned plot, the seeds of this pine germinate and their radicle is in direct contact with the mineral soil. Due to this the seedlings develop vigorously in cleaned plot. Secondly, some allelopathic effect of pine needle also impact on seed germination. Seed of this species are light as compared to other pines in weight, they get attached to the litter in forest and after sometimes the seeds lose their viability. But few seeds get attached to the mineral soil and germinate and establish themselves in the forest areas. The similar investigation has also been carried out in chir pine forest in Uttarakhand (Bahar*et al.,* 2003; Srivastava and Nawa Bahar, 2007).

Heavy weed growth in khasi pine forests is considered to be the most important adverse factor for its natural regeneration in both the locations. Weeds check the seedling growth through root competition and suppression. Perennial weeds form a thick mat of roots and offer severe root competition. Tiny seedlings, when covered under a thick mat of stalks of weeds, are killed. This process is repeated every year and does not allow natural regeneration to establish. In certain areas undergrowth consists of thick grass. Dense grass is generally very harmful to natural regeneration and in order to reduce its harmful effect, it has to be cut regularly. Light grass is however, not harmful and khasi pine regenerates well under light grass. Similar studies have been also reported in chir pine forests (Khanna, 1984).

Fire is also a factor in hindrance to natural regeneration. Many grass species invite fire. Moreover, these grasses get burnt or have to be control burnt every year to safeguard the forests against accidental fires. Whatever tiny seedlings might come up, get killed in such fires. In a few pine species, which grow in fire prone areas, shoot elongation is suppressed for one to several years whilst the seedling develops a thick carrot - like root a dense cover of needles. This so called grass stage is common in *Pinuskesiya, P. merkusii*and *P. roxburghii* (Turakka*et al.,* 1982; Koskela*et al.,* 1995).The young crop needs protection from fire, but older trees are more fire resistant, due to the thick bark that develops with age (Troup, 1921). The sprouting ability of the natural occurring seedlings of chir pine has been investigated in Uttarkashi, Uttarakhand and it is evident from the results that seedlings of pine have the capability to develop it in forests (Nawa Bahar, 2008). The growing stock in khasi pine forests is mostly mature and over matures. Such trees are reported to produce inadequate quantities of seed for natural regeneration. Heavy grazing does more harm than good as the seedlings are trampled and killed. Due to very slow rate of decomposition the debris continue accumulate on the forest floor and affects the natural regeneration adversely.

CONCLUSION

The present investigation revealed that the growth performance and density of naturally occurring seedlings in cleaned plots were found better than the uncleaned plots in both studied sites. Litter thickness in Barapani location was also found more than in Shillong location. It is also observed that stand density may nave important role in establishment of natural regeneration. On the basis of stock quality index, it is concluded that clean plots favoured more regeneration in both the studied sites.

ACKNOWLEDGEMENTS

Author wishes to record sincere gratitude to the field staff of Meghalaya Forest Department and also wishes to thank the staff members of Silviculture and Forest Management Division, Rain Forest Research Institute, Jorhat, for providing facilities in the present study.

Tables 1: **Description of khasi pine forests in different locations of Meghalaya**

Characters	Barapani	Shillong
GBH (cm)	63.68± 14.28	69.35± 12.84
Height (m)	33.52 ±2.87	32.17± 3.43
Density (tree/ha)	1022± 133	987± 107
Altitude (m)	1120	1680
Latitude(°N)	25° 30′	25° 39′
Longitude(°E)	91° 30′	90° 35′

GBH -girth at breast height

± is Standard deviation of mean

Tables 2: **Seedling characteristics at Barapani area (Meghalaya)**

Characters	Cleaned plot	Uncleaned plot
Collar diameter (mm)	5.11 ± 1.98	3.46 ± 0.69
Height (cm)	10.36 ± 3.58	8.66 ± 2.47
Density (seedlings/m2)	22.68 ± 5.22	9.24± 4.72
Dry weight (g)	12.63 ± 3.55	10.22 ± 1.77
Stock quality index(SQI)	6.25	4.08
Litter thickness (cm)	---	5.26 ± 1.94

± is Standard deviation of mean

Tables 3: **Seedling characteristics at Shillong area (Meghalaya)**

Characters	Cleaned plot	Uncleaned plot
Collar diameter (mm)	5.21 ± 1.25	3.77 ± 0.88
Height(cm)	11.45 ± 2.62	8.92± 2.33
Density(seedlings/m2)	29.66 ± 6.44	14.38± 4.75
Dry weight(g)	14.88 ± 2.54	12.47 ± 1.69
Stock quality index(SQI)	6.79	5.28
Litter thickness(cm)	---	3.88 ± 1.75

± is Standard deviation of mean

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Natural regeneration of khasi pine in Meghalaya

Plate -1

Plate -2

Screening of Teak (*Tectona grandis* L.) Clones *vis-à-vis* Defoliator (*Hyblaea puera*) in Gujarat

Prajapati, V. M.; Kukadia, M.U. and Sushil Kumar *

INTRODUCTION

The teak tree (*Tectona grandis* L. F.), is well known for its versatile timber. Its heartwood combines several qualities like termite, decay resistance, lightness, strength, drying without wrapping, splitting, easy workability and attractive appearance making it one of the world's finest timbers. Teak position among timbers has been similar to that of gold among metals and diamond among precious stones. Among the various insect-pests infesting teak trees, 136 are defoliators, of these the most important is teak defoliator, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) causing defoliation and browning of teak leaves which adversely affect teak growth (Mathur and Singh, 1960). The pest is oligophagous and teak (*Tectona grandis*) is the principal host plant, however, it has also been observed on many plants belonging to the family Verbenaceae and Bignonaceae (Mohandas, 1936). It is observed in attacking stage with the onset of rainy season which coincides with emergence of new flush of leaves and shoots in south Gujarat. The newly hatched (including neonate) larvae feed during night under cover of silken strands on young soft tissues of foliage by nibbling it and making shallow depression on leaf surface. Later instars of larvae cut a portion of the leaf in semicircular or rectangular flap at the edge and thereafter they fold or roll over and fasten it with silken strands causing extensive damage by means of defoliation during the active growth period of the host plant.

In south Gujarat, the average incidence of defoliator has been recorded up to 9.68% indicating peak status (79.50%) during July at Dharampur taluka in Valsad District of Gujarat state (Kumar and Prajapati, 2008). In natural forest, spraying of chemical insecticides are not recommended owing to risk of environmental and pollution hazards coupled with possible damage on diversity of natural forest, natural enemies and pollinators. So, the main focus of pest management is based on biological, silvicultural, evaluation and selection of resistant or relatively less susceptible trees. Therefore, the experiment was planned and carried out for screening teak clones against teak defoliator (*Hyblaea purea*) during 2007- 2009 under field condition.

MATERIALS AND METHODS

The experiments were conducted during 2007-2008 and 2008-2009 at Rajpipla in Narmada district in Gujarat state located at 21° 53' N latitude, 73° 31' E longitude and 45 meters above the mean sea level. For experimental purpose, eighteen teak clones (TCR-1 to TCR-18) were selected with four replications of each in Randomized Block Design. In each replication, two trees were selected for recording observations at monthly interval. The leaf damage was counted from North-South and East-West parts of the lower tree canopy. For this purpose, five terminal twigs from each section were selected randomly and were subsequently observed for leaf damage by counting total and damaged leaves which was later calibrated into percent leaf damage. On the same twig, larvae of the concerned insect-pest were also counted. Degree of resistance/susceptibility to leaf defoliator (*Hyblea purea* Cramer) was assessed on the basis of susceptibility ratings which are as under:

Table 1: **Degree of resistance/susceptibility to leaf defoliator (*Hyblaea purea* Cramer)**

Degree	Leaf damage (%)	Susceptibility ratings
Immune/Free/Escape	0	R_0
Resistant	10-20	R_1
Moderately resistant	21-45	R_2
Least resistant	46-55	R_3
Moderately susceptible	56-70	S_1
Susceptible	> 70	S_2

RESULTS AND DISCUSSION

Results based on leaf damage and larval population of *H. purea* in various teak clones indicated lowest leaf damage (15.75%/tree - pooled value) in TCR-2 indicating resistant reaction (R_1) followed by TCR-3 (15.86) which was not significantly different from it, whereas TCR-12 was ultimately the most susceptible entry (S_2) indicating highest leaf damage of 74.87%, though it was not significantly different from TCR-13 (72.66) (S_2). These ratings have also been conformed on the basis of standard deviation (17.25 in pooled value) which happened to be higher than the mean values (15.75 and 15.86) obtained in TCR-2 and TCR-3 entries, conforming less susceptibility to leaf defoliator as compared to the other entries tested in the investigation (Table 2).

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Similarly, lowest larval population (1.39/leaf/tree - pooled value) was observed in TCR-3 followed by TCR-2 (1.52) which did not differ significantly from it. Whereas, TCR-12 was the most susceptible entry (12.13 larvae), though it did not differ significantly from TCR-13 (11.09). These results have also been conformed on the basis of standard deviation (2.86 pooled value) which was higher than the mean values (1.39 and 1.52) obtained in resistant entries (TCR-3 and TCR-2). Remaining clones also recorded moderately susceptible (S_1), least resistant (R_3) and moderately resistant (R_2) rating as given in table 2 on the basis of leaf damage. Not much work has been done on clonal screening of teak clones against leaf defoliator, however, Jacob and Balu (2007) identified three clones (MYSA-3, APKKA-1 and APAKB-1) showing minimum damage. Similarly Jain *et al*. (2002) identified ORANR-3 and APT-14 as resistant and susceptible teak clones on the basis of lowest and highest leaf area consumption of leaf defoliator which were later categorized as resistant and susceptible entries, respectively.

Thus, in the present study conducted during 2007-2009 at clonal seed orchard Rajpipla in Narmada district of Gujarat, lowest leaf damage was observed in TCR-2 (15.75%) and TCR-3 (15.86%) entries, while it remained highest in TCR-12 (74.87%). Similarly, larval population was lowest in TCR-3 (1.39/leaf/tree) and TCR-2 (1.52), while it was highest in TCR-12 (12.13). So, on the basis of these results, TCR-2 and TCR-3 were identified as tolerant teak clones against teak defoliator (*Hyblaea purea*) and rest of the clones possibly played a crucial role in host plant preference by the larvae of the pest under discussion.

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Table 2: **Leaf damage and larval population of leaf defoliator (*Hyblaea purea* Cramer) in various teak clones at clonal teak seed orchard Rajpipla during 2007 - 2009**

Clone	Leaf damage /tree (%)			Susceptibility grade	Larval population/leaf/tree		
	2008	2009	Pooled		2008	2009	Pooled
TCR-1	24.66	27.95	26.31^{c*}	R_2	2.33	2.83	2.58^{abc*}
TCR-2	19.55	11.94	15.75^{a}	R_1	1.33	1.70	1.52^{ab}
TCR-3	16.39	15.32	15.86^{ab}	R_1	1.46	1.31	1.39^{a}
TCR-4	59.86	58.35	59.11^{l}	S_1	9.37	5.93	7.65^{fghij}
TCR-5	62.01	32.43	47.22^{fg}	R_3	8.96	7.08	8.02^{ghijklm}
TCR-6	36.08	59.80	47.94^{fghi}	R_3	4.35	6.36	5.36^{de}
TCR-7	64.92	60.90	62.91^{mn}	S_1	8.46	7.67	8.07^{ghijklmn}
TCR-8	48.17	50.49	49.33^{fghijk}	R_3	5.25	5.20	5.23^{d}
TCR-9	36.63	59.17	47.90^{fgh}	R_3	7.56	6.31	6.94^{fg}
TCR-10	40.28	31.15	35.72^{d}	R_2	6.85	6.03	6.44^{def}
TCR-11	67.52	64.59	66.06^{nop}	S_1	9.75	6.23	7.99^{ghijkl}
TCR-12	77.40	72.34	74.87^{qr}	S_2	12.65	11.60	12.13^{qr}
TCR-13	74.52	70.79	72.66^{q}	S_2	11.78	10.39	11.09^{q}
TCR-14	42.42	35.73	39.08^{de}	R_2	7.74	6.34	7.04^{fgh}
TCR-15	43.89	59.73	51.81^{ghijkl}	R_3	6.58	7.58	7.08^{fghi}
TCR-16	65.19	26.06	45.63^{f}	R_3	9.41	8.80	9.11^{op}
TCR-17	66.26	62.10	64.18^{no}	S_1	8.94	8.00	8.47^{o}
TCR-18	64.27	31.66	47.97^{fghij}	R_3	9.41	6.49	7.95^{ghijk}
Mean	50.56	46.14	48.35		7.36	6.44	6.90
SD	18.91	19.40	17.25		3.27	2.63	2.86
SEm ±	1.77	1.67	1.72		0.27	0.55	0.43
CD at 5 %	5.08	4.81	4.86		0.78	1.59	1.25
CV (%)	6.05	6.27	6.16		6.36	14.87	10.93

** Ranking as per DMRT. Treatments having similar letters are not statistically different from each other.*

Optimization of DNA Extraction Protocol of *Pongamia pinnata* Linn.

Shruti Sharma, Shivani Dobhal and Ashok Kumar *

INTRODUCTION

Pongamia pinnata (L.) Pierre, indigenous to the Indian subcontinent and South-East Asia is a drought resistant semi-deciduous and leguminous tree of family Fabaceae. It is a medium sized tree ranging from 12-15 m in height (Troup, 1921) and grown for ornamental purposes, as shade tree and as a wind break in the plantations. The plant has been used as a source of traditional medicines, green manure, timber, fish poison and fuel besides being cultivated along roadsides, canal banks and open farm lands. Its dense network of lateral roots enables it to control soil erosion and bind sand dunes. The mature tree can withstand water logging and slight frost and highly tolerant to salinity. It is one of the few nitrogen fixing trees and can help in restoration of fertility. Extracts from the plants are known to have medicinal properties. It flowers in April-May and fruits mature in January-February. It produces seeds which are exploited for extraction of non-edible oil commercially known as 'Karanja oil'.

The species has been recognized as an important source of biodiesel and therefore the efforts are being directed towards its genetic improvement. Recent studies in plant molecular biology and biotechnology has opened-up interesting and challenging possibilities so that its potential could easily be utilized to its maximum through understanding genetic diversity of the species. To screen high oil yielding genotypes and to analyze genetic diversity at an early age itself, the preparation of quality DNA has become a major concern. So in this study, method for DNA extraction from *Pongamia pinnata* was optimized for achieving quality DNA. It is however clarified that a single DNA protocol might not be suitable for isolation of optimal genomic DNA (Weishing *et al*., 1995).This method involved a modified CTAB procedure of Doyle and Doyle (1990) and Stange *et al.* (1998).

MATERIALS AND METHODS

The juvenile leaves were collected from the progeny trials established by Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun, and subjected to protocols given by Doyle and Doyle (1990) and Stange *et al*. (1998) with some modifications for extraction of total genomic DNA.

Young leaves (500 mg) without mid-rib and margins were ground to fine powder using liquid nitrogen in an autoclaved pre-chilled mortar and pestle. The fine powder was transferred to 2 ml Eppendorf tubes containing 1 ml of cold extraction buffer (100 mM Tris HCl, pH 8.0; 20 mM EDTA; 1.42 mM NaCl; 5 mM ascorbic acid; 2% w/v PVP) and 3μl β-mercaptoethanol. The mixture was gently mixed by inversion and incubated for 10 min at 4^{0}C in refrigerator. After incubation, it was centrifuged for 5 min at 6000 rpm at 4^{0}C. Supernatant was discarded and to the remaining plant tissue 1 ml of pre-heated (at 60^0 C) CTAB extraction buffer (100 mM Tris HCl, pH 8.0; 20 mM EDTA; 1.42 mM NaCl; 5 mM ascorbic acid; 2% CTAB; 2% w/v PVP) and 3 μl β-mercaptoethanol was added. The mixture was gently mixed and incubated for 45 min at 60^0 C in water bath. After incubation equal volume of chloroform : isoamyl alcohol (24:1) (v/v) was added and mixed by inversion. It was then centrifuged for 5 min at 13000 rpm at 25^0 C. The upper aqueous phase was carefully transferred to a fresh 1.5 ml Eppendorf tube and about 500μl of chilled isopropanol (-20^0 C) was added to precipitate DNA. The mixture was incubated for 1-2 hours at -20^0 C to allow proper precipitation of DNA and was pelleted by centrifugation for 15 min at 13000 rpm at 4^0 C. DNA pellet was washed by adding 998 μl of 76% ethanol and 2 μl of ammonium acetate (5M) and placing it for 45 min on a gel rocker. The mixture was again centrifuged for 5 min at 13000 rpm where supernatant was discarded and pellet was washed with 70% ethanol, followed by centrifugation for 15 min at 13000 rpm. Supernatant was discarded and pellet was dried for 5-10 min in vacuum drier. The dried genomic DNA pellet was finally resuspended in 100 μl of Tris-EDTA buffer (100mM Tris HCl; 1mM EDTA; pH 8.0) and stored at -20^0 C. The yield and purity of DNA isolated was checked by reading their absorbance at 260 nm and the ratio of absorbance at 260/280 nm respectively (Sambrook, 1989). The deviation above and below from value 1.80 at 260/280 nm absorbance ratio determines RNA and protein contamination in the extracted DNA, respectively (Linacero, 1998)

RESULTS AND DISCUSSION

Plants produce secondary metabolites such as polyphenols and polysaccharides which interfere with genomic isolation procedures (Amani *et al.*, 2011). Polyphenols are powerful oxidizing agents and can reduce the yield and purity of extracted DNA (Porebski *et al.*, 1997). Excess cell debris and proteins may inhibit polymerase chain reaction (Saiki, 1990).

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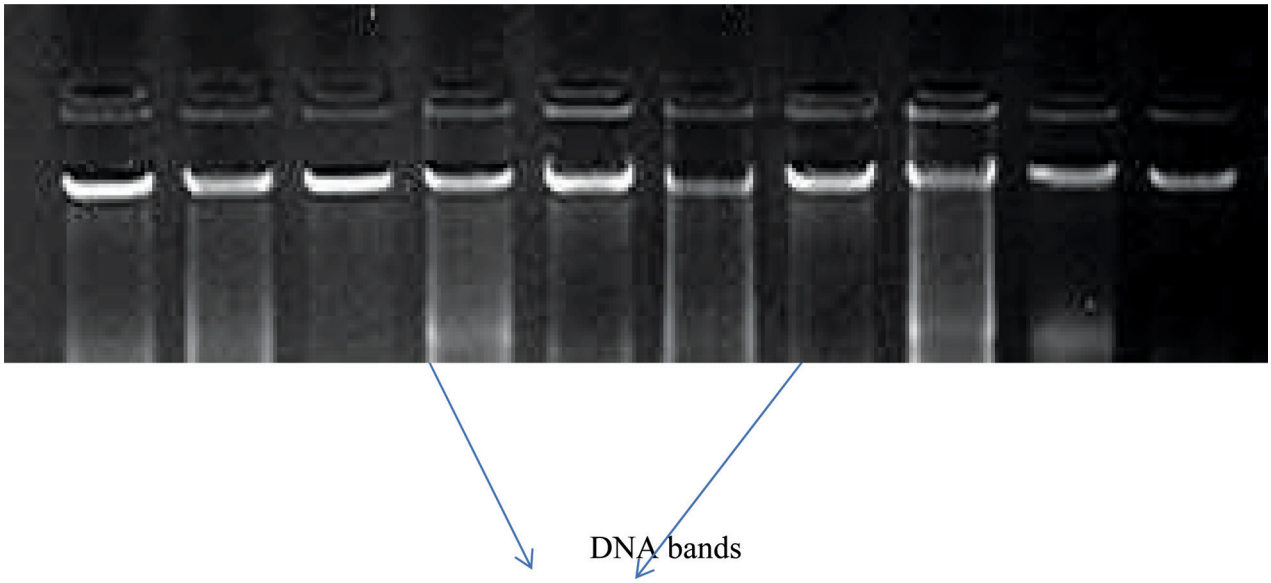

The use of fresh and young leaf tissue has been emphasized by Sytsma *et al.* to obtain good quality DNA. With maturity, leaves contain increased quantities of polyphenols and polysachharides that prevent extraction of good quality genomic DNA (Ginwal and Maurya, 2010). Therefore this new protocol based on modification of CTAB method given by Doyle and Doyle (1990) and Stange *et al.* (1998) was optimized for the extraction of quality DNA from leaves of *Pongamia pinnata* and was found suitable for extraction of desired quantity and quality of genomic DNA. The modifications in this method included the use of two extraction buffers one without CTAB and the other with CTAB, use of increased concentration of polyvinyl pyrrolidone (3% instead of 2%), extended time for incubation with extraction buffer in water bath (45 minutes instead of 30 minutes) and extended time of washing of DNA pellet with alcohol (45 minutes instead of 30 minutes). High PVP concentration removes polyphenols on complexing with them through hydrogen bonds and allows them to be separated from the DNA thus reducing levels of polyphenol in the product (Maliyakal, 1992). This method thus helped in removal of excessive polyphenols from the leaf samples and a clear DNA pellet was obtained which was suitable to be used in RAPD, ISSR and SSR analysis.

SUMMARY

Pongamia pinnata (L.) Pierre is an important bio-diesel producing tree species besides other uses. The potential could easily be utilized to its maximum by understanding genetic diversity of the species. This would help in screening high oil yielding genotypes which could be multiplied and planted on commercial scales. It is however essential that the genetic diversity is analyzed at an early age itself adopting advanced tool of biotechnology. Therefore, optimization of DNA extraction protocol for this species was done for achieving quality DNA. Quality DNA was achieved with a modified protocol for isolation of total genomic DNA from *Pongamia pinnata* based on CTAB (Cetyl trimethyl ammonium bromide) method. Experimental material used in the current study was young leaves collected from the progeny trials established in different geographic locations by the Division of Genetics and Tree Propagation, Forest Research Institute, Dehradun. Approximately, 500 mg of young leaves were subjected to different established protocols for isolation of total genomic DNA. Modifications were carried out by adding or not adding CTAB. The modifications were also made with use of 3% polyvinyl pyrrolidone and increased time for incubation with extraction buffer in water bath (45 minutes) and washing of DNA pellet with alcohol (45 minutes). The yield of DNA obtained using this modified method ranged from 221.3 to 3392.2 g per 500 mg of leaf. This modified method helped in removal of excessive polyphenols from the leaf samples and was found to be suitable for the extraction of desired quantity and quality of genomic DNA. This total genomic DNA obtained was suitable to be used in DNA based molecular marker analysis

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Eucalyptus Improvement in Southern India

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INTRODUCTION

In India, during 1790 about sixteen species of *Eucalyptus* were introduced from Australia by Tippu Sultan in Mysore in Nandi Hills near Bangalore. Over the years, a highly adopted land race evolved from these introductions was planted throughout India and called *E. hybrid* (widely referred as Mysore gum). The early introduction of *E. tereticornis* and *E. camaldulensis* to India was from southern temperate localities in Australia rather than the northern tropical regions where the climatic conditions closely resemble the areas available in India because of the inaccessibility and difficulties in collecting seeds. Results from different provenance trials indicated the superiority of the northern provenances of both the species of eucalypts to the southern provenances. The local E. hybrid seed lots performed poorly (7m^3 ha^{-1} $year^{-1}$) in comparison to the "Petford" and "Katherine" provenances of *E. camaldulensis* (20-25 m^3 ha^{-1} $year^{-1}$) and "Laura River" and "Kennedy River" seed lots of *E. tereticornis* (12-25 m^3 ha^{-1} $year^{-1}$).

Provenance trials established during early 80s in Tamil Nadu revealed the superiority of certain provenances namely Kennedy River, Morehead River, Laura River, Petford, Katherine, Gilbert River, Gibb River and Irvinebank. (Brooker and Kleinig, 1994; Doran and Burgess, 1993). Based on the growth performance of the different provenance trials, Institute of Forest Genetics and Tree Breeding in collaboration with CSIRO, Australia initiated a comprehensive breeding programme during 1995. Identified family seed collections from selected native provenances of Australia for both ***E. tereticornis*** (42 families) and ***E. camaldulensis*** (132 families) were used to establish provenance cum progeny trials.

Davidson (1998) listed different short term strategies followed for obtaining eucalypt seeds for routine plantations in Asia-Pacific countries. Often seeds are either imported from native stands of Australia or collected from the plantations raised from native seed. Provenance trials are also used as seed stands after removal of inferior provenances.

Provenance trials of ***E. camaldulensis*** were established in three different sites during 1981, 1982 and 1986 in Tamil Nadu and assessed in collaboration with IFGTB which revealed the superiority of the provenances namely Petford, Katherine, Gilbert River, Gibb River and Irvinebank. Amongst the best performing ***E. tereticornis*** provenances viz., Kennedy River, Morehead River and Laura River were designated as ***E. camaldulensis*** (Brooker and Kleinig, 1994; Doran and Burgess, 1993).

About fifty good trees were selected from the four outstanding provenances (Kennedy river – 15; Laura river – 14; Mt. Carbine-11 and Lake land down - 10) identified in two provenance trials located at Pudukkottai and Karaikudi in Tamilnadu state. Seeds were collected from the 13 year old trees during 1995 and seedling seed orchards were established as progeny trials at two locations namely Pondicherry and Pudukkottai in South India. Progenies of Kennedy river selections had best growth followed by Laura River and Lake Land down selections.

Based on the growth performance of the different provenance trials, Institute of Forest Genetics and Tree Breeding in collaboration with CSIRO, Australia initiated a comprehensive breeding programme during 1995. Un-pedigreed and pedigreed seed orchards were established with about 200 family identified seed collections from selected native provenances in Australia for both *E. camaldulensis* and *E. tereticornis* by including the superior provenances.

TREE IMPROVEMENT AT IFGTB

E. tereticornis

1. Provenance cum progeny trial

One provenance cum progeny trial was established in 1997 with seeds received from CSIRO. The trial has 42 families comprising of 17 provenances. The trial was established at Karunya, Coimbatore during 1997. The trial was subsequently thinned twice during 2000 and 2002 and converted into Seedling Seed Orchard and seeds are being collected since 2002 onwards.

2. Seed production area

Two seed production areas were established 1995 using the seeds received from CSIRO from 506 trees from 21 provenances bulk. These orchards were established one in Panampally (Near Coimbatore) and another one at Pudukottai (each with 2 ha area). These seed orchards were also thinned during 2000 and seeds are being collected since 2002.

E. camaldulensis

1. Provenance cum progeny trials

Three provenance cum progeny trials were conducted using the seeds received from CSIRO during 1996. There are 132 families comprising of 13 provenances. The trials are placed in Panampally, Pudukottai (Tamil Nadu) and Sathyavedu (Andra

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Pradesh). All these trials were evaluated and converted into seed orchards during 2002 and seeds are being collected.

2. Seed production area

Two seed production areas were established 1995 using the seeds received from CSIRO from 514 trees from 11 provenances bulk. These orchards were established one in Panampally (Near Coimbatore) and another one at Pudukottai (each with 2 ha area). These seed orchards were also thinned during 2000 and seeds are being collected since 2002.

Clone trials for *Eucalyptus* spp.

About 100 trees were selected from the provenance cum progeny trials and seed production areas of *E. tereticornis* and *E. camaldulensis* during 2000. These trees were cut and clonally multiplied and three clonal trials were established in 2001. These trials were placed in Karunya (Coimbatore), Sathyavedu (Andra Pradesh) and Kulathupuzha (Kerala). The evaluation was completed during 2008. Though not significantly different, *E. tereticornis* clones had comparatively less growth at 36 months than *E. camaldulensis* clones which were on par with the commercially planted clones. The commercial ITC and KFRI clones also performed on par with the tested clones. About 30 clones selected from different SPAs and SSOs of *E. camaldulensis* and *E. tereticornis* were on par with the best performing commercial clone (ITC-3) which is reported to be stable in several locations. These clonal trials were subsequently converted into clonal seed orchards and seeds are being collected for sale.

Based on the growth superiority, four clones of E. *camaldulensis* were released for commercial cultivation in the states of Tamil Nadu and Andhra Pradesh. Clonal trials were also established in Hyderabad, Rajahamundri, Warangal, Nellore, Salem, Karur and Tirupathi with promising thirty clones during 2009.

Genetic gain trials for *Eucalyptus* spp.

Bulked Seeds (from 25 randomly selected trees) were collected at four years of age from four orchards (unpedigreed orchards of *E. camaldulensis* from Pudukkottai and Panampalli, to establish genetic gain trials in three locations (Karunya, Dandeli and Kulwalli). A local seed lot (land race) and a bulked natural Australian provenance seed lot were used as control. The trials were evaluated for tree growth (height and dbh) at three years of age. In general the difference in growth (height and dbh) between the progeny of unpedigreed orchards was not very marked. About 17% gain in height and 14% gain in DBH was achieved by the seed orchard progeny over the local seed lot.

Progeny trials for *Eucalyptus* spp.

Seeds were collected from fifty selected clones placed in clonal trials converted to CSOs during 2008 and progenies were raised and established progeny trials at Hyderabad (Andhra Pradesh) and Pudukottai. During 2009, seeds were collected from selected 50 clones and progenies were raised and established progeny trials at Hyderabad, Pudukottai, Nellore, Karaikudi, Thiyagadurgam, Marakkanam, Chennai and Coimbatore. The clonal and progeny trials were established with locally grown seedlots and commercially available clones for the purpose of comparison. The preliminary results showed positive response of selected clones and families.

VMG for *Eucalyptus* spp.

One VMG at Bharathiar University campus, Coimbatore was established with 45 selected clones for mass multiplication during 2008. The VMG has a capacity to produce 5000 plant propagules per day.

Research trials established under Eucalyptus Improvement programme

No.	Species	Name of trial	Year	Area (Ha)	Location	Details
1	*E.camaldulensis*	SPA	1996	1.0	Panampally	11 provenance; 514 trees bulk
2	*E.camaldulensis*	SPA	1996	2.0	Pudukkottai	11 provenance; 514 trees bulk
3	*E. tereticornis*	SPA	1996	1.0	Panampally	21 provenance; 506 trees bulk
4	*E. tereticornis*	SPA	1996	2.0	Pudukkottai	21 provenance; 506 trees bulk
5	*E. tereticornis*	SSO	1996	1.0	Karunya Nagar	9 provenances; 42 families
6	*E.camaldulensis*	SSO	1996	2.0	Panampally	20 provenances; 132 families
7	*E.camaldulensis*	SSO	1996	2.5	Sathyavedu	-do-
8	*E.camaldulensis*	SSO	1996	2.0	Puthukottai	-do-
9	*E. tereticornis*	SSO	1996	1.0	Pondicherry	50 families of PRS, Puthukottai
10	*Eucalyptus spp.*	Gain trial	2004	0.5	Karunya Nagar	5 seed orchards
11	*Eucalyptus spp.*	Clonal trial	2000	1.0	Karunya Nagar	100 clones
12	*Eucalyptus spp.*	Clonal trial	2000	0.8	Sathyavedu	80 clones
13	*Eucalyptus spp.*	Clonal trial	2000	0.5	Kulathupuzha	50 clones
14	*Eucalyptus spp.*	VMG	2008	1.0	Bharathiar	22 clones
15	*Eucalyptus spp.*	Progeny trial	2009	2.0	Hyderabad	54 families

16	*Eucalyptus spp.*	Progeny trial	2009	2.0	Pudukkottai	56 families
17	*Eucalyptus spp.*	CSO	2009	2.0	Salem	18 clones
18	*Eucalyptus spp.*	SSO	2009	1.0	Nellore	54 families
19	*Eucalyptus spp.*	CSO	2009	1.0	Nellore	15 Clones
20	*Eucalyptus spp.*	SSO	2009	1.0	Karaikudi	60 families
21	*Eucalyptus spp.*	SSO	2009	1.0	Thiyagadurgam	56 families
22	*Eucalyptus spp.*	SSO	2009	1.0	Marakkanam	51 families
23	*Eucalyptus spp.*	Progeny trial	2009	2.0	Karur	40 families
24	*Eucalyptus spp.*	SSO	2010	3.0	Chennai	60 families
25	*Eucalyptus spp.*	SSO	2010	2.0	Coimbatore	45 families
26	*Eucalyptus spp.*	CSO	2010	7.0	Nellore	45 clones
27	*Eucalyptus spp.*	Clonal demo	2010	2.0	Athipalayam, Karur	4 Released clones of IFGTB
28	*Eucalyptus spp.*	Clonal demo	2010	2.0	Aravakuruch, Karur	4 Released clones of IFGTB
29	*Eucalyptus spp.*	Clonal demo	2010	2.0	Nellore R.L.	4 Released clones of IFGTB
30	*Eucalyptus spp.*	SSO	2011	1.0	Udumalaipettai	66 families
31	*Eucalyptus spp.*	CSO	2011	1.0	Udumalaipettai	40 clones
32	*Eucalyptus spp.*	Clonal trial	2011	1.0	Udumalaipettai	25 clones

All India coordinated Eucalyptus Improvement Programme

Eucalyptus is cultivated throughout India and hence the Indian Council of Forestry Research and Education (ICFRE) has proposed an All India Coordinated Programme for Genetic Improvement of Eucalyptus by creating a network. IFGTB been working on this species for the last two decades is designated as the lead institute for this programme and the partners include four other ICFRE institutes, Forest Research Institute, Dehradun, Institute of Wood Science and Technology, Bangalore and Institute of Forest Productivity, Ranchi and Forest Research Centre, Hyderabad, two agricultural universities, Tamil Nadu Agricultural University, Pandit Jawaharlal Nehru College of Agriculture and Research Institute (PAJANCOA and RI), Karaikkal, State Forest Departments of Andhra Pradesh and two paper industries, M/s Tamil Nadu Newsprint and Papers Limited and Seshasayee Papers and Boards Limited.

The programme is proposed with the following objectives,

- To improve the productivity of eucalyptus plantations through selection
- To improve the productivity of eucalyptus plantations through inter-specific hybridization
- Characterization of clones/ full-sibs for morphological, anatomical, physiological, biochemical and pulpwood traits
- Production and supply of quality planting stock / seeds
- Bio-technological interventions to improve the productivity of Eucalypts plantations
- Breeding for special traits like pulpwood traits, salinity tolerance, drought tolerance pest and disease resistant

Clone release

About 102 clonal entries were selected from the first generation seed orchards. The selections were carried out basically through index selection method. The height, diameter at breast height and stem straightness were the main characters for selection. The selected individuals were coppiced and clonal plants were raised. During 2000, three clonal trials were established in Coimbatore (Tamil Nadu), Sathyavedu (Andhra Pradesh) and Kulathupuzha (Kerala).

The trials at Coimbatore, Sathyavedu and Kulathupuzha were represented by 100, 80 and 50 clonal entries respectively including the control clones and seedlots. About 33 clones were replicated in all the three clonal trials and tested for their superiority. About 10 commercial clones and seed origin plants of *Eucalyptus camaldulensis* (3 entries) and *E. tereticornis* (2 entries) and 5 clones of Kerala Forest Research Institute (KFRI) were also included in the experiment for the purpose of comparison. All the trials were laid in Randomized Block Design with five replications. Each replication was represented by three ramets except Kulathupuzha where two ramets per replication were maintained.

Care was taken in selection of site for establishment of these trials. The trials were conducted in divergent climatic and edaphic conditions. The rainfall and temperature and soil characters are different in each of the selected sites. At the same time, uniformity within the trials was ensured. Trees were planted at 3 x 2 meter spacing. One guard row of Eucalyptus clonal plants (local clone) was planted along the perimeter. Weeding was carried out once in a year before rain. Watering was restricted to planting and initial period of establishment. Fertilization and soil amendments were also restricted to initial planting season.

Every year, growth traits were recorded before rain. However, periodic inspections were carried out once in two months for observing any other pest and disease incidence. Observations on flowering and fruiting were also recorded after third year. Straightness was scored at seventh year.

Statistical analysis for growth and tree form traits were carried out and the clones were ranked according to their superiority based on growth traits and stability. Four clonal entries viz., IFGTB- EC1, IFGTB-EC2, IFGTB-EC3 and IFGTB-EC4 were found to be superior in their growth performance over the seed control and commercial clones.

As per the "Guidelines for testing and releasing of tree varieties and clones" of the Indian Council of Forestry Research and Education, it was recommended to release four clones by Variety Releasing Committee (VRC) constituted by ICFRE for the dry tracts of Tamil Nadu and Andhra Pradesh.

GROWTH, YIELD AND ECONOMICS

Very high productivity is possible under favourable conditions: growth of 70 m^3 ha^{-1} yr^1 of wood in a four-year-old plantation at 3 m x 2 m spacing on a fertile, well-watered site has been recorded (Zohar, 1989). Seldom are these conditions duplicated on the broad scale, and yields are generally much less. In the drier tropics, yields of 5-10 m^3 $ha^{-1}yr^{-1}$ on are common, whereas in moister regions up to 30 m^3 $ha^{-1}yr^{-1}$ may be achieved (Evans, 1982). In Southern Vietnam, overall yield for the species is 12 m^3 $ha^{-1}yr^{-1}$ at four years but better adapted provenances give yields of 20 m^3 $ha^{-1}yr^{-1}$. Tree breeding and better husbandry will quickly enhance these yields.

In Tamil Nadu, about 25-30 t/ha at a rotation of 6-7 years was realized through seed raised plantations during early 1990's. Introduction of clones increased the yield up to 60-70 t/ha in six years rotation. With introduction of new clones, it is expected that the yield can go up to 70-80 t/ha in six years rotation.

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Characters of the approved clones at the age of seven years

Clone No.	Height (m)	DBH (cm)	Avg. Single tree volume (m^3)	Descriptors
IFGTB-EC-1	15.17	13.53	0.120	1. Smooth light brown colour fresh bark turning to brownish grey and thereafter it peels off showing dark brown. 2. Oval shape branch scar with embossed upper lid. The branch scar does not close.
IFGTB-EC-2	15.68	12.92	0.113	1. Smooth bark with light grey colour fresh bark turning to brownish grey and thereafter it peels off after 3-4 months showing brown in colour. 2. Round shape branch scar with prominent smooth mound. The branch scar was closed.
IFGTB-EC-3	13.48	11.68	0.097	1. Very smooth bark with silver grey colour fresh bark turning to light grey and thereafter it peels off after showing light brown colour. 2. Round in shape branch scar with smooth mound. The branch scar was closed.
IFGTB-EC-4	15.24	11.78	0.0914	1. Rough bark with light green to white in colour fresh bark turning to light grey and thereafter it peels off showing brown colour. 2. Bell shaped branch scar with totally embossed scar. The branch scar was closed.

Development of Genetic Linkage Map in *Eucalyptus camaldulensis* X *E. tereticornis* using Microsatellite Markers

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INTRODUCTION

The simple sequence repeats (SSRs) are ubiquitously present in eukaryotic genomes and over the last few decades they has been used in genetic improvement of many crop and tree species. SSRs were very efficiently used in the development of linkage map for many plant species (Gupta et al. 2005). Linkage map construction of any plant species will assist in the better study of plant genetics and assists in improvement of the species. Linkage and QTL mapping using microsatellites support early selection of plants with desired traits providing better germplasm for propagation. Linkage map is particularly valuable for breeding programmes of woody plants because conventional selection in tree species is delayed due to long reproductive cycle and also the complex traits like wood properties, disease and pest control and tolerance to abiotic stresses makes it difficult in achieving significant improvements.

Genetic mapping in tree species holds the constraints like high levels of heterozygosity, generation of suitable pedigrees and inbred lines which are limited due to long generation intervals and genetic load. Hence pseudo testcross strategy, an alternative approach involving segregation of markers similar to a testcross configuration has been employed in many tree species (Grattapaglia and Sederoff 1994; Scalfi et al 2004; Yin et al. 2001). Several genetic and QTL maps were developed for inter-specific hybrids of eucalypts and the information generated were utilized for breeding programmes successfully.

In *Eucalyptus* linkage map has been constructed using dominant markers like RAPD and AFLP (Grattapaglia *et al* 1994, Marques *et al* 1998, Agrama *et al* 2002, Freeman *et al* 2006). Brondani et al (1998) initially characterized and mapped SSR loci in *E. grandis* x *E. urophylla* and an integrated consensus linkage map with 230 microsatellites was developed (Brondani *et al* 2006). The present study discusses the prospects of developing genetic and QTL map for salinity tolerance for the interspecific cross *E. camaldulensis* x *E. tereticornis*.

MATERIALS AND METHODS

Selection of parents

Highly productive clones of *Eucalyptus* including 14 clones of *E. camaldulensis* and 2 clones of *E. tereticornis* were used for screening salinity tolerance. Sodium chloride concentrations with a range varying from 100 to 450 mM were tested for salt tolerance. From these clones screened, the most tolerant clone of *E. camaldulensis* and most susceptible clone of *E. tereticornis* were chosen as parents and subjected to control hybridization.

Controlled Hybridization

Controlled hybridization was carried out using *E. camaldulensis* as the seed parent and *E. tereticornis* as the pollen parent. Hybridization was carried out in the trees located in the Clonal Seed Orchard (CSO) at Karunya. After seed set, the seeds were collected and germinated in the sand bed. After one month the seedlings were transferred to the polybags and the three months old plants were field planted.

DNA isolation

Two hundred F1 individuals were randomly selected from the cross of *E. camaldulensis* X *E. tereticornis* for the development of linkage map with microsatellites. Fresh juvenile leaves collected from three month old F1 hybrids and from the parent trees were used for DNA isolation. 100 mg of the leaf tissue was used for isolating DNA using Qiagen D'Neasy Plant Mini Kit. The extracted DNA quantity was assessed using agarose gel electrophoresis with ethidium bromide staining and the quantity of DNA was confirmed using Pico drop spectrophotometer (Picodrop microlitre spectrophotometer version 3.01, UK). DNA samples were diluted to get the final concentration of 10ng/μl using sterile water.

Screening of primers

Two hundred and twelve primers developed from various species like *E. grandis, E. nitens E. camaldulensis, E. tereticornis* and *Corymbia citriodora* were used for cross amplification. Initial screening of primers was carried out using the two parents. Out of these 212 primers, 185 were developed from *E. grandis* (Brondani *et al* 2006), 1 EMCRC SSR from *Corymbia* (Sheperd *et al* 2008), 10 genomic SSRs were from *E. nitens* (*Thamarus et al 2002*) 6 genomic SSRs were from *E. camaldulensis* (Motta da Silva *et al* 2009) and 10 EST-SSRs were from *E. tereticornis* (Yasodha *et al* 2008).

Screening of primers for polymorphism between the two

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parents was carried out in 5% denaturing polyacrylamide gels of size 21 cm X 50 cm (Sequi-Gen GT System, BIO-RAD, USA) containing 7 M urea and 1 x TBE buffer, and visualized by silver staining. Screened primers which gave a proper banding pattern without any non specific amplification were used for the further amplification of the F1 individuals. Two hundred F_1 individuals were amplified with 6 SSRs and size separated using ABI 3500 Genetic Analyzer, USA, employing Schuelke (2000) three primer strategy. The scoring data of the parents were used to estimate Polymorphic Information Content, observed and expected heterozygosity using the software Powermarker.

Amplification reactions for microsatellite markers were carried out with a total volume of 10 μl containing the following constituents: 10 x buffer containing 100 mM Tris–HCl pH 8.3, 500 mM KCl and 15 mM $MgCl_2$, 125μM dNTP mix, 0.1 pmol of forward primer with M13 tail at 5' end, 0.4 pmol of reverse primer and 0.2 pmol of fluorescently labelled universal M13 primer, 10 ng of template and 1 U of Taq DNA polymerase. PCR conditions include initial denaturation of 94°C for 5 min, 30 cycles of 94°C for 45 sec, annealing temperature varying from 48°C to 60°C for 30 sec and 72°C for 1 min and an elongation step at 72°C for 15 min. Incorporation of fluorescently labelled dye into the PCR product employed extra 20 cycles consisting of 94°C for 30 sec, 50°C for 45 sec and 72°C for 45 sec, and final extension of 72°C for 30 min. Amplification is carried out using Veriti PCR of Applied Biosystems, USA. 1 μl of PCR product was mixed with 0.2μl of GeneScan 600 Liz size standard and 8.8 μl of Hi- Di formamide (ABI) and the mixture was electroinjected in an ABI 3500 genetic analyzer. The data collected under dye set Ds33 was analyzed with Genemapper software version 4.1. The allele size data obtained from Genemapper software was used to determine its segregation type. The data showed two types of segregation pattern, maternal informative (ab x bb) and fully informative (ab x bc and ab x cd). Because the parents were heterozygous, there were two possibilities for single-locus segregation—those which segregated in only one of the parents (1:1) (heterozygous for one parent and homozygous for the other parent), and those which segregated in both parents (1:2:1 or 1:1:1:1) (heterozygous in both parents). Codominant markers segregating according to the latter possibility can be used as genetic bridges for aligning the linkage information from each parental dataset to produce a consensus linkage map (Grattapaglia and Sederoff 1994) A chi-square test was performed to check the segregation distortion.

RESULT AND DISCUSSION

Transferability of microsatellites

The microsatellites developed from other species of Eucalyptus and *Corymbia* were used for cross amplification and hence required standardization for achieving proper amplification of the loci in the targeted species. PCR conditions were standardized with modification in annealing temperature and time, extension time and number of cycles for incorporation of labelled primer. PCR additives like Dimethyl Sulphoxide and betaine were added to avoid non specific amplification. PCR components like dNTPs, primer concentration were also modified to complete the 50 cycles reaction resulting in high yield of labelled products.

Among 212 loci screened with the parents, 139 primers (65%) gave good banding pattern without any non specific amplification. Nineteen primers which showed monomorphic banding pattern were excluded for further amplification of the F1 individuals. Transferability microsatellites across species of the subgenus *Symphyomyrtus* varies between 80 and 100%, for species of different subgenera was reported to be 50–60% and for related genus like *Corymbia* was 25% (Kirst et al. 1997; Faria et al. 2010). The heterozygosity generated using Powermarker was found to be 0.65 and polymorphic Information Content was found to be 0.5 which has been reported between 0.2 to 0.6 (Faria et al. 2010)

Segregation pattern

A total of 120 primers finally were chosen for the amplification of 200 F1 hybrids. Among 200 individuals around 12 showed alleles of different size for each loci which were considered to be contaminants and they were excluded from the study. Twenty five individuals could not be amplified. Analyzing the data of six primers using Chi–square test revealed that four of the primers (EMBRA12, EMBRA 147, EMBRA 50 AND EMBRA 122) segregated in the ratio 1:1 and other two primers (EMBRA 101 and EMBRA 36) were not. The segregation pattern of EMBRA 36 and EMBRA 12 were found to be fully informative pattern. EMBRA 36 segregated with the pattern ab x cd 4 different alleles in the F1 individuals.

In pseudo testcross based linkage map analysis markers segregating in test cross pattern and with intercross pattern could be used for linkage map construction (Wu et al.2010). Hence these SSR markers could be used for linkage map construction.

Phenotyping of the parents with hybrids will be performed for QTL mapping. The plants will be treated with different concentration of sodium chloride and morphological, anatomical, physiological and biochemical parameters will be analyzed in parents and hybrids. Phenotyping and genotyping data will be used to position the QTL responsible for salinity tolerance.

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Influence of Time of Collection on Cone Characteristics in Blue Pine

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INTRODUCTION

The blue pine (*Pinus wallichiana*, A.B. Jacks) is an evergreen tree species occurs throughout north-west Himalayan region at an altitude ranging between 2000-3000m. In spite of its tendency to form pure crops, it frequently occurs mixed with other species such as *Abies pindrow, Cedrus deodara, Picea smithiana, Quercus* species etc. *Pinus wallichiana* is a tall tree and found in cool and moist places. It has a conical crown with whorled and spreading branches. Adult needles are 12.5 – 20.00 cm long, cylindrical and bluish-green in colour. The female cones 15-30 cm long, cylindrical in shape and light brown in colour. Scales are arranged in spirals of 5x3, about 5 cm long and 2.5 – 3.75 cm broad at the end. The wood of blue pine is moderately hard and heavy. It is easy to saw and used in packing cases, furniture, planking, shutters, window frames and pulp and paper industry. Wood is also used as a fuel and for manufacturing of charcoal. This coniferous tree species provides raw materials to various forest based industries besides conserving soil and water in the hills. The present study was, therefore, undertaken to find out the impact of time of collection on cone characteristics in *Pinus wallichiana*.

MATERIAL AND METHODS

Trees for cone collection were selected in Harsil forest area at an altitude of 2700 m in Gangotri Range of Uttarkashi forest division, on the basis of clear and straight bole, compact crown and were free from pests and diseases. Ten trees were selected randomly and marked at a distance of about 100 m in the selected forest stand. Thus, Cone collection was made at an interval of 15 days till some of the cones started opening. Cones collection of *Pinus wallichiana* was started on the 15th September, second on 30th September, third on 15th October and fourth on 30th October during two years. 50 cones were collected on each collection date from the marked standing trees by climbing the trees. The collected cones were brought to the laboratory for further measurements.

The cone diameter was measured in centimetres by fixing the middle portion of the cone in digital vernier calliper (D_1) and again turning the middle portion at right angle (D_2). Average of these two measurements was taken as cone diameter.

To determine the cone specific gravity, the fresh weight of the each cone was recorded on an electronic balance and the volume of each cone was measured by water displacement method (Oliver, 1974). The specific gravity was determined by dividing fresh weight of cone (g) by its volume of cone in cubic centimetres as per following formula:

$$\text{Specific gravity} = \frac{\text{Fresh weight of cone}}{\text{Volume of cone}}$$

All the cones were broken open for the extraction of the seeds. During this extraction total number of scales and number of fertile scales per cone was recorded. The statistical analysis was done on the mean values and analysis of variance (ANOVA) was performed following the method of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Table 1 presents the data on mean mid diameter, fresh weight, volume, specific gravity, and number of scales/cone of *Pinus wallichiana* cones for different cone collection dates for both the collection years. The lowest cone diameter was measured 2.74 cm for first collection date and highest 3.01 cm for fourth collection date during the 1st collection year. The cone diameter recorded from fourth collection date of October 30 was statistically higher than cone diameter of other collection dates except third collection date (October 15). During the 2nd collection year the mid cone diameter varied from 2.81 to 3.12 cm, the minimum was recorded for first collection date of September 15 and maximum for October 30 collection date, respectively. The cone diameter of fourth collection date differed significantly with cone diameter of other cone collection dates. The similar results have also been reported by Seth and Agrawal (2003) in *Pinus wallichiana* and Kumar (2000) in *Pinus roxburghii*. The increase in the mid cone diameter might be attributed to the growth and development of the cones and consequently the seeds (Troup, 1921).

The fresh weight/cone varied from 94.63 to 131.82 g and 88.32 to 136.19 g for the 1st and 2nd collection years. The heaviest cones (131.82 g) were obtained from second collection date while, lightest (94.63 g) from fourth collection date of 1st year and these differed significantly. In the 2nd collection year, heaviest cone with 136.19 g weight and lightest cone with 88.32 g were collected from September 15 collection date and October 30 collection dates, respectively. The cone weight recorded for first collection date was higher and differed significantly with the cone

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weight of third and fourth collection dates. There was a significant difference between second and third and third and fourth dates of cone collection. Kumar (2000) reported in *Pinus roxburghii* that cone fresh weight tended to increase from first date of cone collection (mid November) to fourth date of cone collection (7th February) and then it decreased. In early stages, the increase in cone weight and volume might be due to growth and development of the cones. Later on, the cone weight and volume decreased due to the reduction in moisture content. The reduction in cone moisture content with maturity has been reported by Pandit and Ram (2004).

The cone volume/cone increased up to second collection date then decreased till final date of cone collection (30th October) during both the collection years. Lowest cone volume of 113.50 cc and 103.90 cc. was recorded for first and fourth collection dates, respectively while highest cone volume was recorded 132.80 cc and 130.45 cc from second collection date during the 1st and 2nd cone collection years, respectively. The cone volume of second collection date of 1st year differed significantly with cone volume of other collection dates except third collection date of 1st year but in 2nd year, the maximum cone volume of second collection date differed significantly with other cone collection dates except first collection date of 2nd year.

The specific gravity/cone indicated the decreasing trend from first to fourth collection date for both the collection years. The highest specific gravity/cone was recorded 1.03 and 1.05 for the first cone collection date in 1st year while lowest 0.83 and 0.85 for the fourth collection date of 30th October during 1st and 2nd cone collection years, respectively. The specific gravity of fourth collection collected cones was statistically significantly different with specific gravity of other collection dates except third collection date of 1st year. However, the specific gravity of fourth collection differed significantly with each other. Lavania *et al*. (2008) also observed that fruit characteristics influenced with date of collection in *Robinia pseudoacacia*.

During the collection year in 1st year the minimum number of scales/cone was 71.45 and maximum 77.50 scales/cone. Similarly during the 2nd year collection minimum 75.68-scales/cone and maximum 81.71scales/cone were recorded from the cones collected on 30th October and 15th September collection dates. However, there was no significant difference in the number of scales/cone between different collection dates during both the collection years. The number of fertile scales/cone and percentage of fertile scales/cone ranged from 58.10 to 63.69 and 81.28 to 85.33 during the 1st collection year and were statistically at par with each other. During the 2nd collection year, no significant difference was recorded between the collection dates for number of fertile scales and percentage of fertile scales/cone. Similar results have also been found by Lavania *et al*. (2009) in *Picea smithiana*.

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Table 1: **Mid diameter, fresh weight, volume and specific gravity of cones, total number of scales/ cone and number of fertile scales/cone for different cone collection dates in *Pinus wallichiana***

Time of collection	Cone mid diameter (cm.)	Cone fresh weight (g)	Cone volume (cc)	Cone specific gravity	Total number of scales/ cone	Number of fertile scales/cone	Percentage of fertile scales/cone
15th September, Ist year	2.74	116.39	113.50	1.03	71.45	58.10	81.32
30th September, Ist year	2.83	131.82	132.80	0.99	73.79	62.29	84.42
15th October, Ist year	2.92	112.20	124.75	0.90	74.64	63.69	85.33
30th October, Ist year	3.01	94.63	114.20	0.83	77.50	62.99	81.28
Mean	2.88	113.76	121.31	0.94	74.35	61.77	83.09
C.D.(5%)	0.17	7.35	11.86	0.13	NS	NS	NS
15th September, IInd year	2.81	136.19	129.70	1.05	75.68	59.23	78.26
30th September, IInd year	2.90	133.06	130.45	1.02	79.44	63.63	80.10
15th October, IInd year	2.98	109.56	111.80	0.98	79.56	59.14	74.33
30th October, IInd year	3.12	88.32	103.90	0.85	81.71	64.75	79.24
Mean	2.95	116.78	118.96	0.98	79.10	61.69	77.98
C.D.(5%)	0.13	12.40	10.65	0.10	NS	NS	NS

A Complete Protocol for the Native Biodiesel Plant - *Pongamia Pinnata* Using Low Cost Alternatives for Development of High Frequency Micropropagation

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INTRODUCTION

The major application of plant tissue culture lies in the production of true-to-type high quality planting material that can be multiplied under aseptic condition on a year round basis anywhere irrespective of seasonal variations and weather conditions. Micropropagation is a capital-intensive technology involving energy and labour (Raghu *et al.,* 2007). The need for low-cost plant tissue culture systems, applicable for micropropagation and *in vitro* conservation of plant genetic resources, has been emphasized to allow the large-scale application and adaptability of such technology in developing countries (IAEA, 2004). This problem has been addressed by inventing reliable cost effective tissue culture methods without compromising on quality of plants. Cost of chemicals, media, energy, labour and capital affects the production cost. Low cost technology means an advanced generation technology in which cost reduction is achieved by improving process efficiency and better utilization of resources (Savangikar, 2002). Low cost option should lower the cost of production without compromising the quality of the micropropagation and plants (Anonymous, 2004). Recurring costs of micropropagation and *in vitro* conservation include those for chemicals that are used in culture media, i.e., carbon sources, gelling agents, inorganic and organic supplements, and growth regulators. In plant production through micropropagation, media chemicals cost a little less than 15% of the total cost (Prakash *et al*., 2004). Out of all component used in a media, gelling agents such as agar contribute 70% to the total cost of media (Gaur and Kant, 2011). Here we present the results of a study on the effectiveness of low cost alternatives of agar, in the development of a cost effective *in vitro* clonal micropropagation protocol of *P. pinnata*.

Pongamia pinnata L. Pierre, commonly known as Karanja, is a medium-sized glabrous tree mainly found in tribal forests of India and South-east Asia (Punitha *et al.,* 2006). *Pongamia pinnata* is one of the non-edible oil yielding leguminous tree species of Indian origin. Seed of *P. pinnata* contain bitter, red brown, thick non drying, non edible oil (27-39%), used for tanning leather, soap making, to treat various ailments (Meera *et al*., 2003) and as an illuminating oil in some parts of rural India. Various plant parts including oil are known to have value in folk medicine. (Burkill, 1966) (Muthu *et al.,* 2006). Seed oil contains two flavonoids, Pongamol and Karanjin (Parmar *et al.*, 1976), which makes it unsuitable for edible purpose. Pongam oil has been recognized as "Biodiesel", as several key parameters related to combustion efficiency of diesel and *P. pinnata* oil are comparable (Shrinivasa, 2002). Biodiesel from these seeds is fast emerging as a viable alternative to fossil fuel. The commercial cultivation of *Pongamia* is lucrative as the plants are high yielding and easily adapted to semiarid marginal land, the oil recovery is high from seeds, and the oil is of high quality (Karmee and Chadha, 2005). Protocols for micropropagation of this plant from seedling derived axillary meristem (Sugla *et al.,* 2007 and Shrivastava and Kant, 2010), *de novo* organogenesis (Sujatha *et al.,* 2008) and mature tree derived nodal meristem (Sujatha and Hazra 2006, 2007) are available. However, there is no report on *in vitro* plant regeneration of *Pongamia* utilizing low cost alternatives aimed at cost reduction. We describe a reproducible protocol for micropropagation of *P. pinnata* through mature nodal segments.

MATERIALS AND METHODS

Plant material

Twigs of *Pongamia* were collected from mature trees growing locally. Nodal explants of approximately 3-4 cm in length were excised and washed thoroughly. Explants were treated with 2% tween 80 detergent solutions and rinsed thoroughly with distilled water thrice, to remove traces of detergent. These explants were then treated with alcohol for 3-5 minutes, and rinsed with distilled water. The explants were then dipped in solution of Bavistin (systemic fungicide) and Streptomycin (antibiotic) for 15 minutes, followed by washing with distilled water. The explants were surface sterilized with 5% NaOCl for 5 minutes, (4-6% available chlorine) followed by four rinses with sterile water. The explants were trimmed to the appropriate size, making them ready for inoculation.

Culture media and condition

Full strength MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose (HiMedia, India) and (0.8% w/v) (HiMedia, India) agar was used during the study. MS media were

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used in all the experiments to evaluate efficacy of low cost alternatives of agar. The cost of MS media and agar-agar was calculated using price list of HiMedia Laboratories (India). To determine the optimum concentration of low cost gelling agents required for solidifying the media, different concentration were tested. To replace the agar (HiMedia, India) low cost alternatives such as sago powder, guar gum powder and isabgol-husk (purchased from a local market at Jodhpur, India) were added to the media. Thirty milliliter of medium were dispensed per conical flask (100 ml and 150 ml of Borosilicate), and 20 ml per Borosilicate culture tube. Cotton plugs made up of non-absorbent cotton were used throughout. The glassware, forceps, scalpels, media and distilled water were autoclaved. The pH of the medium was adjusted to 5.8 prior to autoclaving at 120° C and 104 Kpa (15 psi) for 15 min. The cultures were incubated at 25±2 °c under a 16-h photoperiod with 35μ mol $m^{-2}s^{-1}$ photon flux density provided by cool white fluorescent tubes.

Full strength MS medium supplement with 2.2 μM BAP was used as the initiation medium. After 4 weeks of inoculation, nodal explants collected from the established cultures were taken out and sub cultured in full strength MS medium supplemented with 8.8 μM BAP for studying the in vitro multiplication responses. Sucrose was used as the carbon source in all the combinations. All media combinations were solidified by adding 0.8% agar (w/v). The low cost media were solidified with sago powder, guar gum and isabgol in place of agar. All cultures were maintained under similar condition as described earlier. The frequency of explants producing shoots, number of shoots per explants and shoot length were scored after 8 week of culture. Sprouting frequency and number of shoots produced per explants were noted after 8 week of culture initiation. The elongated shoots, 3-4 cm. in length, were isolated from the cluster and cultured individually for rooting on half strength MS medium supplemented with 9.8 μM Indole-3-butyric acids (IBA) solidified with sago powder, guar gum or isabgol and also on liquid medium with coconut coir providing a substratum for shoots. Observation on percent rooting, number of roots per shoot and root length were recorded after 4 weeks. Rooted shoots were taken out from the culture tubes; well-rooted plantlets were washed with sterilized distilled water and transferred to vermiculite moistened with half strength MS medium. After 4 weeks, acclimatized plantlets were transferred to thermacol cups containing vermiculate and maintained in greenhouse under high humidity and desired temperature. Later they were transferred to plastic bags with garden soil. In each experiment 10-12 explants were used and all experiments were repeated thrice. Mean and standard error were determined and were subjected to analysis of variance (ANOVA). The differences among the treatment means were tested using Duncan multiple range test (DMRT) at a significance level of P = 0.05. The results were analyzed statistically using SPSS ver. 17 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Pongamia nodal segment (containing axillary buds) cultured on MS + 2.2 μM BAP started sprouting within a week. In 3-4 wk, 1-2 cm long shoots were obtained; these shoots were sub-cultured on MS medium supplemented with 8.8 μM BAP repeatedly to obtain sufficient stock of explants for *in vitro* plantlet regeneration and conservation experiments using low cost-media. Sago powder (15%), guar gum (5%) and isabgol (3.5%) media solidified faster than agar gelled (0.8%w/v) media (Fig. 2 A-D). Both types of media were firm enough to hold the explants vertically. Out of different gelling agents tested for complete protocol of *P. pinnata* agar (control) was found to be the best media gelling agents. Agar is one of the major components to be swapped with low cost gelling alternatives to be able to achieve cost reduction.

Figure 1 (A – D): **Effect of gelling agents at various *in vitro* stages in *Pongamia pinnata* supplemented:**

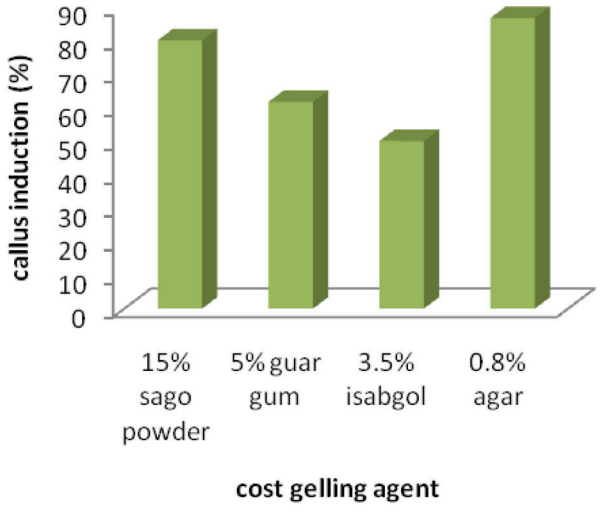

Fig. 1 (A): **Effect of low cost gelling agents on callus induction in *P. pinnata***

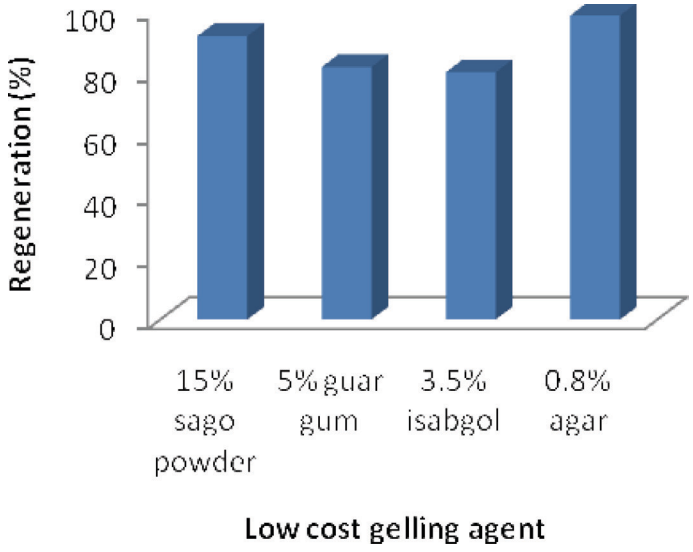

Fig. 1 (C): **Effect of low cost gelling agents on number of shoot produced /explants in *P. pinnata***

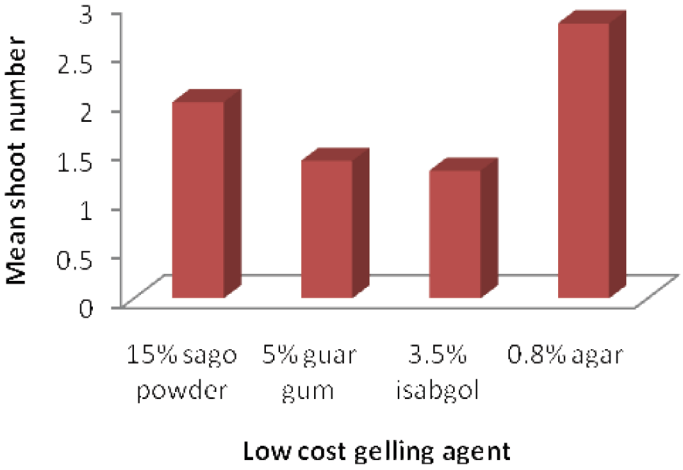

Fig. 1 (B): **Effect of low cost gelling agents on regeneration in *P. pinnata***

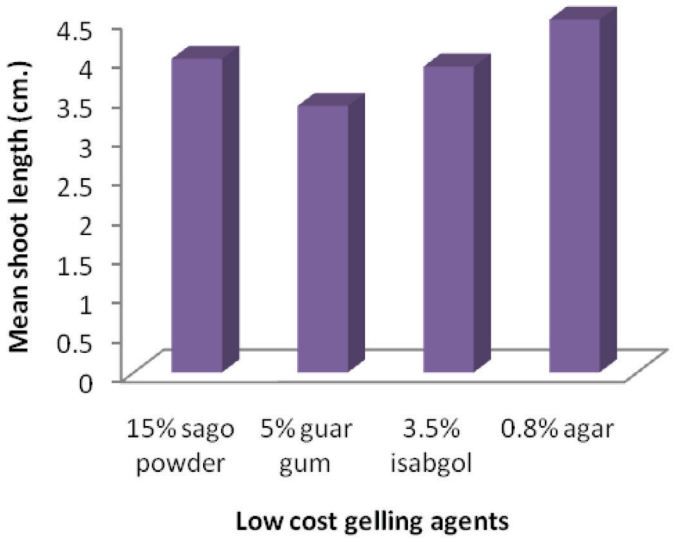

Fig 1. (D): **Effect of low cost gelling agents on shoot length (cm.) in *P. pinnata***

Attempts have been made earlier to replace agar. A few low cost gelling agents had been tested in several laboratories viz., sago powder, isabgol husk, guar gum, cassava flour and xantham gum etc (Maliro and Lameck, 2004; Jain and Babbar, 2005,2006). Moreover, the exclusive use of agar may result in over exploitation of its resources (Jain and Babbar, 2005; Deb and Pongener, 2010). Different materials such as isabgol (Jain and Babbar, 2011, Saqlam and Cifict, 2010, Babbar and Jain 1998; Jain *et al*., 1997), sago (Bhattacharya *et al*., 1994) and guar gum (Babbar *et al*, 2005; Jain *et al*., 2005) were used as alternative gelling agents. Other researchers evaluated poly-urethane foam, coconut coir and betel nut coir in liquid medium as alternative to the agar (Fig. 2 I) (Deb and Pongener, 2010; Temjensangba and Deb, 2005, Deb and Temjensangba, 2006). The performance of these low cost gelling agents was found satisfactory and could compare well with agar. The axillary bud break percentage response was highest in agar (96%), followed by isabgol (83%) and sago powder (80%) and lowest in guar gum (66%). Cultures in media supplemented with BAP alone resulted in increased shoot length and shoot elongation. After induction on medium Containing 2.2 μM BAP, shoot along with original explants were sub-cultured on media containing 8.8 μM BAP and this was repeated at a 4 weeks interval. This induction was found to be, the highest shoot regeneration frequency and callus induction in sago powder (91.66±6.0) (Fig. 1 A-B), number of shoots per explants (2.0±0.2) (Fig. 1 C) and shoot length (4.0±0.1) after agar (Fig. 1 D). Root initiation occurred after 10 days of culture at 9.8μM IBA. Presence of IBA in the medium induced brown compact callus at the base of micro shoots. Thus rooting occurred from the base of the callus and not directly from the cut ends of micro shoots (Gadidasu *et al*., 2011). Same observations were also made on other species by our group. Our study reveals that incorporation of guar gum and isabgol as a gelling agents reduce the percentage of callusing by 30% and 33% respectively, which is favourable for the process of rooting (Fig .2 E-H). A rooting percentage of 86% in guargum, 83% isabgol and 76% were obtained with sago powder. Highest root frequency (number of roots per shoots) of 2.7±0.2 and root length of 1.3±0.1 were obtained in guar gum (Table 1). For guar gum; rooting percentage was comparable to that of isabgol. Isabgol was reported to be highly cost-effective gelling agents compared to agar (Bhattacharya *et al*., 1994). They studied the effect of these gelling agents on the micropropagation of *chrysanthemum* (*Dendranthema grandiflora Tzvelev*) plantlets. These results are also in agreement with those reported by Jain and Babbar (2005) who indicated that the cost of isabgol and guar gum/ liter of medium is about 2.5-13 times less than different brands of agar; these are two highly cost-effective gelling agents which could be used for reducing the cost of *in vitro*-propagated orchid *(Dendrobium chrysiotoxum*) plants. Both, being of plant origin, are biodegradable and do not pose any threat to the environment once disposed after use. Guar gum was used as a gelling agent in an *in vitro* study of *Linum usitatissimum* and *Brassica juncea* (Babbar *et al*., 2005). IBA has been widely used as a root-inducing hormone in difficult-to-root plants both under *in vitro* and *ex vitro* conditions (Fig. 2 J-K). Successful *ex vitro* rooting further reduced the cost of micropropagation George *et al*. (2004) and Tiwari *et al*. (2000) reported the easiness of field establishment *C. asiatica*. Successful *ex vitro* rooting and field establishment was reported in several other species (Sujatha and Chandran, 1997; Geetha *et al*., 2005) (Fig.2 L). The cost effectiveness of these low cost alternative media constituents was established by this study on *P. pinnata*.

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Table 1: **Effect of gelling agents on rooting of micro shoots of *Pongamia pinnata***

Gelling agents	Rooting (%)	No. of root/shoot	Root length (cm.)	Callusing (%)
15% sago powder	76.67±7.8^{b}	1.6±0.2^{b}	.88±0.1^{b}	60.00±9.0^{c}
5% guar gum	86.67±6.3^{bc}	2.7±0.2^{c}	1.3 ±0.1^{cd}	30.00 ±8.5^{a}
3.5% isabgol	83.33 ±6.2^{bc}	1.4 ±0.1^{b}	1.1 ±0.1^{c}	33.33 ±8.7^{ab}
Liquid media with coir	43.33 ±9.2^{a}	.63 ±0.14^{a}	.46 ±0.1^{a}	56.67 ±9.2^{bc}

Observations were made after 4 weeks of culture. Values are Mean ± SE of three independent experiments each with 10 replicates. Treatment means followed by same letter within columns are not significantly different from each other (P=0.05) comparison by Duncan's multiple range test

Figure 2: **Low cost micropropagation system for *Pongamia pinnata*; (A) Initiation from a mature nodal segment of *P. pinnata* on MS+BAP (2.2 µM) gelled with Agar (0.8%), guar gum (5%), sago powder (15%) and isabgol (3.5%); (B) Proliferation of shoots on MS+BAP (8.8 µM) gelled with different low cost alternatives; (C-D) Elongation and proliferation of multiple shoots on MS+BAP (8.8 µM) with low cost alternative medium gelled with agar (control), guargum, isabgol and sago powder after 8 weeks; (E-I) *In vitro* rooted planted on MS+IBA (9.8 µM) with low cost alternative medium gelled with agar (control), guargum, isabgol, sago powder and liquid medium supported by coconut coir; (J) *In vitro* rooted plantlet in vermiculate; (K) *Ex vitro* rooted plants; (L) Hardened plants in polythene bags.**

Effect of Crown Position on Cone, Seed and Germination Characteristics in Himalayan Cedar (*Cedrus deodara* royle ex d. Don)

VIRENDRA SINGH, S.K. LAVANIA, V.K. SAH AND AJAY KUMAR SINGH *

INTRODUCTION

Himalayan Cedar (*Cedrus deodara* Royle ex D. Don) occurs throughout the temperate regions of Western Himalayas. It grows predominantly between 1750 m and 2500 m and occasionally, is also found growing up to about 3,000 m. The most frequent companions of Himalayan Cedar among conifers are blue pine (*Pinus wallichiana*) and spruce (*Picea smithiana*). At higher elevation, it is found sometimes mixed with silver fir (*Abies pindrow*). Ban oak (*Quercus leucotrichophora)* and Moru oak (*Q. floribunda)* are common associates of Himalayan Cedar at lower and higher reaches among broadleaved tree species. It is not only a valuable timber tree species but also provides attractive urban landscape (Pijut, 2000). Western Himalayas (Himachal Pradesh, Jammu and Kashmir and Uttarakand) cover an estimated area of 2, 03,263 ha under deodar forest, out of which Uttarakhand covers an area of 20,391 ha only (Luna, 2005) and thus there is an ample scope of bringing more area under deodar. In response to the decisive state of deforestation in the country and associated ecological and socio-economic disaster, the artificial regeneration/ plantations have become mandatory if trees are to be raised in non forest areas or in forest areas where natural regeneration is absent, scanty or inadequate. Plantations cover an area of more than 264 million hectare worldwide that account for 7.00% of global forest area but have the potential to provide two third of the global industrial round wood demand (FAO,2010). That is why the interests in plantations are increasing day by day. In view of the increased reliance on artificial regeneration, the forest stands of this species are generally obtained by artificial regeneration. This requires collection of such cones from the crown of the tress that can provide quality seeds. The use of superior quality seed in forestry assumes greater importance than in agriculture because the mistake of using poor quality seed in forestry plantations can only be detected after a few decades when the crop raised turns out to be of poor quality and incapable of giving the expected yield (Singh *et al.*, 1970). Therefore, need of the day is to use superior quality seeds in artificial regeneration to save the time, money and efforts as well. Therefore, the study on the effect of crown position on cone, seed and germination characteristics in Himalayan Cedar was undertaken.

MATERIAL AND METHODS

The stand in the natural forests of *Cedrus deodara* were selected at Buranskhanda compartment 4b that lies between 30° 26' N Latitude and 78° 14' E Longitude at an altitude of 2385 m. The Buranskhanda compartment 4b falls under Dhanolti Range of Mussoorie Forest Division. After selecting the stand, 10 trees with straight clean bole, compact crown and apparently free from insect pest and disease were selected. The crown of the trees was divided into lower crown, middle crown and upper crown by ocular estimate. The cones were collected from selected trees on 1st October at a time when the natural seed dispersal just started. Four cones each from upper, middle and lower crown were collected randomly from each tree and thus, initially a total of 40 cones each from upper, middle and lower crown were collected. The cones were brought to the laboratory and all the damaged or apparently insect infested/ diseased cones were discarded and 25 cones each from upper, middle and lower crown were taken for further study.

The cones in each category were measured for cone length and cone diameter. The cone length was measured by scale and the cone diameter by digital vernier calliper. The cones were broken open manually in each category to count the number of infertile scales, fertile scales, total scales, number of seeds/cone, and weight of seed/cone. The seed length, seed width and seed thickness was measured by digital vernier calliper. For determining 100 seed weight, eight replicates of 100 seeds were drawn randomly from the extracted seed and weighed on electronic balance. The average of eight replications was taken as 100 seed weight. The moisture content of the seed was determined by following rules of I.S.T.A. (1993). Seed germination test in the laboratory was carried out on 400 seeds divided into four replicates of 100 seeds for each category (I.S.T.A., 2010). The seeds were sterilised by soaking in 0.01% mercuric chloride solution for five minutes and then rinsed twice in distilled water before placing for germination test in petridishes. The seeds in petridishes were placed on moist filter paper at a constant temperature of 20 ^{0}C ± 0.5. (Singh *et al.*1997). The protrusion of radicle was taken as the criterion of germination (Copeland and McDonald, 1985, Lavania *et al*. 2009) and the germination counts were made daily after the commencement

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of germination and continued for 28 days or till no germination occurred continuously for five days. The germination of the seed was also carried out under nursery condition. The seeds of each category were sown in the nursery beds of $1m^2$ containing 4 lines spaced 20 cm apart at 10 mm depth (Chandra and Ram, 1980). The germination value of the seed was calculated by following the method of Czabator (1962). The method of Kendrick and Frankland, (1969) was used to determine the germination index. Germination speed was worked out by the method of Maguire (1962).

The angular transformation of the percentage data was carried out before putting the data to analysis. The statistical analysis was done on the mean values and analysis of variance (ANOVA) was performed following the method of Snedecor and Cochran (1989). The critical difference was calculated as CD = SEd x $t_{0.05}$ where SEd is the standard error of difference calculated as SEd = 2Me/r, where Me is the mean sum of square due to error and r is the number of replications.

RESULTS AND DISCUSSION

The results of cone length, cone diameter, number of infertile scales, number of fertile scales, number of total scales, number of seeds/cone and weight of seeds/cone have been presented in Table 1.

The results revealed that the cone length and cone mid diameter varied between 11.54 cm to 9.31 cm and between 5.64 cm to 6.10 cm. The maximum cone length of 11.54 cm and cone mid diameter of 6.10 cm were recorded from the cones collected from upper crown position of the tree. The maximum cone length and cone diameter recorded from the cones collected from upper crown position differed significantly with the cone length and cone mid diameter of cones collected from either middle crown position or lower crown position at 5% level of significance. The minimum cone length of 9.31 cm and minimum cone diameter of 5.64 cm were found in the cones collected from lower crown position, however, the difference in cone length and cone mid diameter in the cones collected from lower and middle crown position was not significantly different. The results showed that the number of infertile scales/cone varied from 35.32 to 37.00 but showed no significant difference. The number of fertile scales/cone differed from 246.96 to 172.00 in the cones collected from different crown position. The maximum number of fertile scales/cone was found to be 246.96 in the cones collected from upper crown position that differed significantly with the number of fertile scales of middle or upper crown position. The number of total scales/cone varied from 209.00 to 282.28. The maximum number of scales/cone was obtained from the cones collected from upper crown position followed by middle and lower crown position. However, the number of total scales/cone from middle and lower crown position were statistically non significant. The number of seeds/cone and the weight of seeds/cone were highest in the cones collected from upper crown that showed a significant difference from middle and lower crown position.

The data on seed length, seed width, and seed thickness, seed fresh weight, seed dry weight and moisture content is presented in Table 2.

The data in Table 2 indicated that the maximum seed length of 15.45 mm was obtained from the cones collected from upper crown followed by 13.14 mm from middle crown and 12.85 from lower crown position. The seed length from upper crown was significantly different from the seed length of seeds of either middle crown or lower crown. Like that of seed length, the minimum seed width of 4.80 mm and minimum seed thickness of 3.23 mm was also recorded from cones collected from lower

Table 1: **Effect of crown position on cone length, cone diameter, number of infertile scales, fertile scales, total scales, number of seeds and weight of seeds/cone**

Crown position	Cone length (cm)	Cone mid diameter (cm)	No. of infertile scales	No. of fertile scales	Total No. of scales	No. of seeds/ cone	Weight of seeds/ cone (g)
Upper	11.54	6.10	35.32	246.96	282.28	472.27	71.50
Middle	9.38	5.90	35.04	186.08	221.12	359.28	48.95
Lower	9.31	5.64	37.00	172.00	209.00	304.12	40.40
CD (0.05)	0.63	0.36	2.86	12.84	13.27	24.18	3.50

Table 2: **Seed length, width, thickness, 100 seed weight and moisture content as influenced by crown position**

Crown position	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	100 seed fresh weight of (g)	100 seed dry weight (g)	Moisture content (%)
Upper	15.45	5.01	3.50	15.04	11.72	22.07
Middle	13.14	4.90	3.48	13.65	10.58	22.49
Lower	12.85	4.80	3.23	13.30	10.31	22.44
CD (0.05)	0.91	NS	NS	0.34	0.22	NS

position of the crown, however the seed width and seed thickness showed no significant difference.

The fresh weight of 100 seed was 15.04 g collected from upper crown, 13.65 g from middle crown and 13.30 g, from lower crown respectively. The dry seed weight was 10.31 g, 10.58 g, and 11.72 g of the seeds collected from lower, middle and upper crown. The 100 fresh and dry seed weight was highest and significantly different of the seeds extracted form cones collected from upper crown. The moisture content of seeds varied from 22.07% to 22.49% and was statistically non significant.

The germination of seeds collected from different crown position under laboratory condition has been prearranged in Table 3.

The results have revealed that the maximum germination of 64.00% was obtained from the seed extracted form the cones collected from upper crown. The germination of 56.25% and 48.75% was obtained from the seeds collected from middle and lower crown of the trees. The germination of seeds of upper crown has shown a significant difference from the seed germination of lower crown position at 0.05% level of significance. The germination value of seeds from upper, middle and lower crown was 16.22, 13.99 and 11.12, respectively. The germination value of the seeds from upper crown differed significantly with each other. However, germination index of the seeds from upper, middle and lower crown was statistically non significant. The maximum speed of germination of seeds from upper crown was 12.36 that differed significantly only with speed of germination of seeds obtained from lower crown.

The germination of seeds collected from different crown position under field condition has been prearranged in Table 4.

The results of the seed germination under field condition have revealed that the maximum seed germination of 54.75% was obtained from upper crown followed by middle and lower crown. However, the germination of the seeds of upper and middle crown was not significantly different with each other (Table 4). The germination value of upper, middle and lower crown varied from 1.59 to 1.12 but was non significant. However, the germination index and speed of germination were significantly affected by different crown position.

The results of above studies are in conformity with the results reported by other workers. The best cones and seeds were found in the upper and middle portion of the crown in conifers (Matthews, 1963). The study of an open grown *Pseudotsuga menziessii* stand showed that the upper and middle south side of the crown had more cones and greater seed contents (Winjum and Johnson, 1964). Grayson *et.al*., (2002) reported that lower crown position in *Pinus echinata* produced significantly lighter cones. Pandit (2002) has reported in *Cupressus torulosa* that the cones and seeds collected from upper canopy were found superior in cone size, seed weight and germination as compared to cones and seeds collected from lower canopy. .

It can be concluded from the present study that cones should be procured from the upper crown of the trees to get the superior quality seeds.

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Table3: **Seed germination, germination value, germination index and speed of germination as influenced by crown position under laboratory condition**

Crown position	Germination %	Germination Value	Germination index	Speed of germination
Upper	64.00 (53.13)*	16.22	6.71	12.36
Middle	56.25 (48.62)	13.99	6.20	9.51
Lower	48.75 (44.31)	11.12	4.89	6.96
CD (0.05)	5.41	0.49	NS	3.61

** Figures in parenthesis are the arc sine transformed value of germination*

Table 4: **Seed germination, germination value, germination index and speed of germination as influenced by crown position under nursery condition**

Crown position	Germination %	Germination value	Germination index	Speed of germination
Upper	54.75 (47.75)*	1.59	2.21	2.38
Middle	45.75 (42.59)	1.34	1.70	1.74
Lower	39.25 (38.82)	1.12	1.50	1.44
CD (0.05)	5.41	NS	0.32	0.29

** Figures in parenthesis are the arc sine transformed value of germination*

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Estimation of Location and Scale Parameters of Lognormal Distribution Using Ranked Set Sampling

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1. INTRODUCTION

A lot of work has been done in the area of parameter estimation for many distributions using simple random sampling (SRS). In many environmental situations, it has been observed that the data obtained from the site generally follows a distribution with heavy right tail, such as a lognormal distribution. The average concentration of an air pollutant such as sulfur dioxide, carbon monoxide, nitrous oxides, etc., is approximately lognormally distributed (see, Larsen (1969)). When the parameters of such distributions are completely unknown, the researcher faces a great challenge to estimate these parameters with minimum cost and maximum precision. The RSS, introduced by McIntyre (1952), is another technique to estimate the parameters, that can be advantageous when measurements of all sampling units is costly, but small sets of units can be ranked according to the value of the characteristic under study by means of visual inspection or other methods not requiring actual measurements. For applications, we refer to Halls and Dell (1966), Martin *et al.* (1980), Cobby et al. (1985), Stokes and Sager (1988) and Sinha *et al.* (1996).

The basic concept behind RSS can be briefly described as follows. Suppose (X_1, X_2,... X_n) is a simple random sample from F(*x*)with a mean μ and a finite variance σ^2. Then a standard unbiased estimator of μ is

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i$$

with $\text{var}(\bar{X}) = \sigma^2 / n$.

In contrast to SRS, RSS starts with taking a simple random sample of size k from the population and the k sampling units are ranked on the basis of personal judgment or a concomitant variable, say X, without actual measurement. Then the unit with rank 1 is identified and taken for the measurement and the remaining units of the sample are discarded. Next, another simple random sample of size k is drawn and the units of the sample are ranked by judgment, the unit with rank 2 is taken for the measurement and the remaining units are discarded. This process is continued until a simple random sample of size k is taken and ranked and the unit with rank k is taken for the measurement. This whole process is referred to as a cycle. The cycle then repeats m times to get a ranked set sample of size $n=km$ from the population of size $N = k^2 m$. Throughout this paper, we consider the case $m = 1$.

McIntyre (1952) proposed

$$\hat{\mu}_{rss} = \frac{1}{n}\sum_{i=1}^{n} X_{(i)} \quad (1.1)$$

as a rival estimator of μ as opposed to $\bar{X}$, where $X_{(ii)}$ denote the measured unit for the i^{th} rank order. It is easy to verify that $E(\hat{\mu}_{rss}) = \mu$, and $Var(\hat{\mu}_{rss}) < Var(\bar{X})$. Dell (1969) and Dell and Clutter (1972) observed that

$$Var(\hat{\mu}_{rss}) = \frac{\sigma^2}{n} - \frac{1}{n^2}\sum_{i=1}^{n}(\mu_{(i)} - \mu)^2 \quad (1.2)$$

where $\mu_{(i)}$ is the mean of $X_{(i:n)}$, the i^{th} order statistic in a sample of size n from $F(x)$.

Many other aspects of RSS have been studied in the literature. For details, one may refer to Takahashi and Wakimoto (1968), David and Levine (1972), Ridout and Cobby (1987), Muttlak and McDonald (1990), Stokes (1977), Takahashi (1969, 70), Yanagawa and Shirahata (1976), Yanagawa and Chen (1980) and Bohn and Wolfe (1992).

Considerable work has already been done on estimation of parameters using RSS. Lloyd (1952) has discussed the estimation of location and scale parameters, based on an ordered sample. Lieblein and Zelen (1956) derived the best liner unbiased estimators (BLUEs) of location and scale parameters, $\hat{\mu}$ and $\hat{\sigma}$, for sample size $n \leq 6$ based on the ordered observations by using Lloyd's (1952) method. Lieblein (1962) presented tables of coefficients for computing $\hat{\mu}$ and $\hat{\sigma}$, and the variances and covariances of $\hat{\mu}$ and $\hat{\sigma}$, for sample size up to 6. White (1964) extended these tables for $n \leq 20$, and Balakrishnan and Chan (1992b) extended them for sample sizes up to 30. Balakrishnan and Chan (1992a) also give the extensive tables of means, variances and covariances of order statistics from the extreme value distribution, which were used in computation of the coefficients. Downton (1954) obtained least square estimates explicitly for a class of two-parameter distributions having the form $f\{(x-\mu)/\sigma\}/\sigma$. Bhoj and Ahsanullah (1996) derived the minimum variance linear unbiased estimators for the parameters of the generalized

geometric distribution using RSS. Lam, Sinha and Wu (1994) used the RSS on estimating parameters in a two-parameter exponential distribution. Fei et al. (1994) addressed the problem of estimation of the parameters of a two-parameter Weibull distribution and Extreme-value distribution using RSS and partial RSS (PRSS). For additional applications of RSS and its multivariate considerations, one may refer to Johnson, Patil and Sinha (1993), Patil, Sinha and Taillie (1994) and Gore et al. (1993).

In this paper, we consider the estimation of the parameters of lognormal distribution, which depends on location (μ) and scale (σ) parameters only. In Section 2, we discuss in brief the least square estimation of location and scale parameters. These parameters are estimated by SRS in Section 3. Section 4 is devoted to the estimation of these parameters based on McIntyre's RSS as well as a partial RSS (PRSS). In Section 5, we compare the RSS and PRSS estimates with SRS estimates of μ and σ for sample size n = 2, 3.....10.

2 LEAST SQUARE ESTIMATION OF LOCATION AND SCALE PARAMETERS USING ORDER STATISTICS

In this Section, we will discuss in brief the technique of least square estimation of location and scale parameters using order statistics, given by Lloyd (1952). Suppose μ and σ (not necessarily the mean and standard deviation) are the location and scale parameters of a variate X whose distribution depends on only these two parameters. Let $(X_1, X_2, ...X_n)$ be a sample of n independent observations on X. Arrange these observations $(X_i's)$ in ascending order of magnitude and denote the ordered set by $(Y_1, Y_2, ...Y_n)$ so that $Y_1 \le Y_2 \le ..... \le Y_n$. The parameters μ and σ are estimated by applying general least-squares theory to the ordered sample $(Y_1 \le Y_2 \le ..... \le Y_n)$, the resulting estimates being unbiased, linear in the ordered observations, and of minimal variance. To begin with, let us introduce the standardized variates

$$U_i = \frac{X_i - \mu}{\sigma},$$

which may be regarded as independent observations on the standardized variable

$$U = \frac{X - \mu}{\sigma},$$

whose distribution is parameter free. We arrange the U_i in ascending order of magnitude, denoting the ordered set by

$(V_1, V_2, ...V_n)$. Then

$$V_i = \frac{Y_i - \mu}{\sigma} \text{ and } V_1 \le V_2 \le ..... \le V_n.$$

Let $E(V_i) = c_{i:n}$
(2.1)

$Var(V_i) = d_i$, and $Cov(V_i, V_j) = d_j$
(2.2)

The quantities $E(V_i)$, $Var(V_i)$ and $Cov(V_i, V_j)$ have known values depending on the form of the parent distribution but not on the parameters.

Using equations (2.1) and (2.2) to the original ordered observations, we clearly have

$$E(Y_i) = \mu + \sigma c_{i:n}$$
(2.3)

$$Var(Y_i) = \sigma^2 d_i \text{ \& } Cov(Y_i, Y_j) = \sigma^2 d_j$$
(2.4)

Since the ordered observations have expectations which are linear functions of the parameters μ & σ, with known coefficients, variances and covariance which are known up to a scalar factor σ^2, Gauss and Markoff theorem of least squares can be applied to them. The parameters are therefore estimable by unbiased linear functions of Y_i, with minimal variance. Equation (2.3) can be rewritten in the matrix from, as follows

$$E(\mathsf{Y}) = \mu\mathsf{1} + \sigma\mathsf{c}$$
(2.5)

where Y is the vector of the Y_i, c the vector of the $c_{i:n}$ and $\mathsf{1}$ a vector with unit elements. Again equation (2.5) can be written as

$$E(\mathsf{Y}) = \mathsf{p}\theta$$
(2.6)

where p is the $(n \times 2)$ matrix $(\mathsf{1}, \mathsf{c})$ and $\theta' = (\mu, \sigma)$. The variance-covariance matrix of the Y_i is

$$Var(\mathsf{Y}) = \sigma^2\mathsf{d}$$
(2.7)

where d is the $(n \times n)$ symmetric positive-definite matrix of d_{ij}.

Using Gauss and Markoff theorem of least squares, the required estimator of the vector $\grave{e}$ of parameters is given by

$$\hat{\theta} = (\mathsf{p}'\mathsf{D}\mathsf{p})^{-1}\ \mathsf{p}'\mathsf{D}\mathsf{Y}$$
(2.8)

where $\mathsf{D} = \mathsf{d}^{-1}$.

The variance-covariance matrix of the estimates is $(\mathsf{p}'\mathsf{D}\mathsf{p})^{-1}\sigma^2$, where

$$\mathsf{p}'\mathsf{D}\mathsf{p} = \begin{pmatrix} \mathsf{1}'\mathsf{D}\mathsf{1} & \mathsf{1}'\mathsf{D}\mathsf{c} \\ \mathsf{1}'\mathsf{D}\mathsf{c} & \mathsf{c}'\mathsf{D}\mathsf{c} \end{pmatrix}$$
(2.9)

The inverse of this matrix is

$$\left(\mathbf{p}'\mathbf{D}\mathbf{p}\right)^{-1} = \frac{1}{\Delta}\begin{pmatrix} \mathbf{c}'\mathbf{D}\mathbf{c} & -\mathbf{1}'\mathbf{D}\mathbf{c} \\ -\mathbf{1}'\mathbf{D}\mathbf{c} & \mathbf{1}'\mathbf{D}\mathbf{1} \end{pmatrix}$$

(2.10)

where Δ is the determinant of the matrix $\mathbf{p}'\mathbf{D}\mathbf{p}$.

Using these results in (2.8) we find the estimates

$$\hat{\mu} = -\mathbf{c}'\,\Gamma\mathbf{Y} \;\;\&\;\; \hat{\sigma} = \mathbf{1}'\Gamma\mathbf{Y},$$

(2.11)

where Γ is the skew-symmetric matrix defined by

$$\Gamma = \mathbf{D}\left(\mathbf{1}\,\mathbf{c}' - \mathbf{c}\mathbf{1}'\right)\mathbf{D} / \Delta.$$

The variances and covariance of these estimates are given by

$$Var(\hat{\mu}) = \mathbf{c}'\mathbf{D}\mathbf{c}\;\sigma^2 / \Delta$$

(2.12)

$$\mathrm{var}(\hat{\sigma}) = \mathbf{1}'\mathbf{D}\mathbf{1}\;\sigma^2 / \Delta$$

(2.13)

$$Cov(\hat{\mu}, \hat{\sigma}) = -\mathbf{1}'\mathbf{D}\mathbf{c}\;\sigma^2 / \Delta$$

(2.14)

3 ESTIMATION OF PARAMETERS USING SRS

Let us consider a lognormal distribution with pdf

$$f\left(x \,|\, \mu, \sigma\right) = \frac{1}{x\sigma\sqrt{2\pi}}\;\exp\left[-\left(\frac{\log \mathrm{x}-\mu}{\sigma}\right)^2\right],\; x > 0,\; -\infty < \mu < \infty,\; \sigma > 0$$

(3.1)

where μ and σ are the location and scale parameters respectively.

Let $X_1, X_2, \ldots\ldots X_n$ is a SRS of size n from (3.1). After arranging these observation in ascending order, suppose

$$X_{1:n}, X_{2:n}, \ldots\ldots X_{n:n}$$

be the order statistics. Then

$$U_{i.n} = \frac{X_{i.n} - \mu}{\sigma},\quad i = 1, 2\ \ldots\mathrm{n}$$

are the order statistics from a SRS of size n from the standard lognormal distribution. In our notation

$$E\left(U_{i.n}\right) = c_{i:n},\; i = 1, 2, \ldots\ldots n.$$

(3.2)

$$Var\left(U_{i:n}\right) = d_i,\; Cov\left(U_{i:n}, U_{j:n}\right) = d_j,\; i, j = 1, 2, \ldots\ldots n.$$

(3.3)

$$\mathbf{d} = \left(d_{ij}\right)_{n\times n},\; \mathbf{D} = \mathbf{d}^{-1} = \left(d^{j}\right)_{n\times n}.$$

(3.4)

After simplifying the equation (2.11) and using the notation

$$\mathbf{X}' = \left(X_{1:n}, X_{2:n}, \ldots\ldots X_{n:n}\right)$$

instead of $\mathbf{Y}' = \left(Y_{1:n}, Y_{2:n}, \ldots\ldots Y_{n:n}\right)$, we get the unique minimum variance linear unbiased estimator (UMLUE) of μ and σ based on $X_1, X_2, \ldots\ldots X_n$, given by

$$\hat{\mu} = \sum_{i=1}^{n} a_i\; \mathrm{X_{in}}$$

(3.5)

$$\hat{\sigma} = \sum_{i=1}^{n} b_i X_{i:n}$$

(3.6)

The coefficients a_i and b_i are obtained as

$$a_i = A_n \sum_{j=1}^{n} d^j + B_n \sum_{j=1}^{n} c_{j:n} d^j$$

(3.7)

and

$$b_i = B_n \sum_{j=1}^{n} d^j + C_n \sum_{j=1}^{n} c_{j:n} d^j$$

(3.8) where

$$A_n = \frac{1}{\Delta}\sum_{i=1}^{n}\sum_{j=1}^{n} c_{i:n}\mathrm{c_{jn}}\, d^j$$

(3.9)

$$B_n = -\frac{1}{\Delta}\sum_{i=1}^{n}\sum_{j=1}^{n} c_{i:n} d^j$$

(3.10)

and

$$C_n = \frac{1}{\Delta}\sum_{i=1}^{n}\sum_{j=1}^{n} d^j$$

(3.11)

Here,

$$\Delta = \left(\sum_{i=1}^{n}\sum_{j=1}^{n} d^{ij}\right)\left(\sum_{i=1}^{n}\sum_{j=1}^{n} c_{i:n} c_{j:n} d^j\right) - \left(\sum_{i=1}^{n}\sum_{j=1}^{n} c_{i:n} d^j\right)^2$$

(3.12)

The variances of $\hat{\mu}$ and $\hat{\sigma}$ and $Cov(\hat{\mu}, \hat{\sigma})$ can be written in simplified form as:

$$Var(\hat{\mu}) = A_n \sigma^2$$

$$Var(\hat{\sigma}) = C_n \sigma^2$$

$$Cov(\hat{\mu}, \hat{\sigma}) = B_n \sigma^2$$

The values of $c_{i:n}$'s and d_{ij}'s are provided in Gupta et al. (1974). To facilitate computations of the estimators $\hat{\mu}$ and $\hat{\sigma}$, the coefficients a_i and b_i in (3.5) and (3.6) are provided in Tables I and II of Appendix for sample size n = 2, 3 ...10.

4 ESTIMATION OF PARAMETERS USING RSS AND PRSS

This is well known that in contrast to SRS, RSS uses only one observation each from n sets of observations, each of size n, namely $X_{1:n} \equiv X_{(1)}$,

the lowest observation from the first set of n observations, then $X_{2:n} \equiv X_{(2)}$, the second lowest from another independent set of n observations, and so on, and finally $X_{n:n} \equiv X_{(n)}$, the largest observation from the last set of n observations. The important point to be emphasized is that although RSS requires identifications of as many as n^2 sampling units, only n of them namely,

$$\left(X_{(1)}, X_{(2)}, \ldots\ldots\ldots X_{(m)}\right),$$

are actually measured. It is obvious that the elements of the resultant ranked set sample

$$\left(X_{(1)}, X_{(2)}, \ldots\ldots\ldots X_{(m)}\right)$$

are independent but not identically distributed. Moreover, marginally, $X_{(ii)}$ is distributed as $X_{i:n}$, the i^{th} order statistic in a sample of size n from (3.1). Therefore, we have

$$E(X_{(ii)}) = \mu + \sigma \, E(Z_{i:n}) = \mu + c_{i:n}\sigma, \qquad i = 1, 2, \ldots\ldots n \quad (4.1)$$

$$Var(X_{(ii)}) = \sigma^2 \, Var(Z_{i:n}) = \sigma^2 d_i, \qquad i = 1, 2, \ldots\ldots n \quad (4.2)$$

$$Cov(X_{(ii)}, X_{(jj)}) = 0, \; i \neq j, \qquad i, j = 1, 2 \ldots n. \quad (4.3)$$

In our usual notations,

$$X' = \left(X_{(1)}, X_{(2)}, \ldots\ldots\ldots X_{(m)}\right) \quad (4.4)$$

$$d = \begin{pmatrix} d_1 & 0 & \ldots\ldots 0 \\ 0 & d_2 & \ldots\ldots 0 \\ \vdots & \vdots & \vdots \\ 0 & 0 & \ldots\ldots d_n \end{pmatrix}_{n \times n} \quad (4.5)$$

$$p = \begin{pmatrix} 1 & c_{1:n} \\ 1 & c_{2:n} \\ \vdots & \vdots \\ 1 & c_{n:n} \end{pmatrix}_{n \times 2} \quad and \quad \theta = \begin{pmatrix} \mu \\ \sigma \end{pmatrix} \quad (4.6)$$

$$E(X) = \begin{pmatrix} \mu + c_{1:n}\sigma \\ \mu + c_{2:n}\sigma \\ \ldots\ldots\ldots\ldots\ldots \\ \mu + c_{n:n}\sigma \end{pmatrix}_{n \times 1} = p\theta \quad (4.7)$$

where

$$p = \begin{pmatrix} 1 & c_{1n} \\ 1 & c_{2n} \\ \vdots & \vdots \\ 1 & c_{nn} \end{pmatrix}_{n \times 2} \quad and \quad \grave{e} = \begin{pmatrix} \mu \\ \sigma \end{pmatrix} \quad (4.8)$$

After simplifying the equation (2.11), using above expressions and replacing Y by X **(**given in (4.4)**),** we obtain the unique minimum variance linear estimators of μ and σ based on

$\left(X_{(1)}, X_{(2)}, \ldots\ldots\ldots X_{(m)}\right)$ as:

$$\mu^* = \sum_{i=1}^{n} v_i X_{(i)} \quad (4.9)$$

$$\sigma^* = \sum_{i=1}^{n} w_i X_{(i)}, \quad (4.10)$$

where

$$v_i = \frac{U_n}{d_i} + V_n \frac{c_{i:n}}{d_i} \quad (4.11)$$

$$w_i = \frac{V_n}{d_i} + W_n \frac{c_{i:n}}{d_i} \quad (4.12)$$

$$U_n = \frac{1}{\rho}\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}$$

(4.13)

$$V_n = -\frac{1}{\rho}\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}$$

(4.14)

$$W_n = \frac{1}{\rho}\sum_{i=1}^{n}\frac{1}{d_i}$$

(4.15)

and

$$\rho = \left(\sum_{i=1}^{n}\frac{1}{d_{ii}}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2$$

(4.16)

Now the simplified form of μ^* and σ^* can be written as

$$\mu^* = \frac{\left(\sum_{i=1}^{n}\frac{X_{(ii)}}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}X_{(i)}}{d_i}\right)}{\left(\sum_{i=1}^{n}\frac{1}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2}$$

(4.17) and

$$\sigma^* = \frac{\left(\sum_{i=1}^{n}\frac{1}{d_{ii}}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}X_{(i)}}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)\left(\sum_{i=1}^{n}\frac{X_{(i)}}{d_i}\right)}{\left(\sum_{i=1}^{n}\frac{1}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c^2{}_{i:n}}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2}$$

(4.18)

The variance of the estimators μ^* and σ^* are

$$Var(\mu^*) = \sigma^2\frac{\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right)}{\left(\sum_{i=1}^{n}\frac{1}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2}$$

(4.19)

and

$$Var(\sigma^*) = \sigma^2\frac{\left(\sum_{i=1}^{n}\frac{1}{d_{ii}}\right)}{\left(\sum_{i=1}^{n}\frac{1}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2}$$

(4.20)

Also the simplified from of $Cov(\mu^*, \sigma^*)$ is

$$Cov(\mu^*, \sigma^*) = -\sigma^2\frac{\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}}{\left(\sum_{i=1}^{n}\frac{1}{d_i}\right)\left(\sum_{i=1}^{n}\frac{c_{i:n}^2}{d_i}\right) - \left(\sum_{i=1}^{n}\frac{c_{i:n}}{d_i}\right)^2}$$

(4.21)

The values of the coefficient v_i and w_i for computations of the estimators μ^* and σ^* for sample size n = 2, 3 ...10, are given in Tables III and IV of Appendix.

Incidentally, we can also derive the BLUEs of μ and σ based on a PRSS, namely,

$\left(X_{(1)}, X_{(2)}, \ldots\ldots\ldots X_{(l)}\right)$ for l < n.

It is easy to verify that the BLUEs of μ and σ based on the PRSS (denoted by $\mu^*(l)$ and $\sigma^*(l)$) are given exactly by (4.17) and (4.18) except that in all the summations, n is replaced by l. Moreover, the corresponding variances of these BLUEs and covariance between them are given by (4.19), (4.20) and (4.21), respectively with n replaced by l in all the summations.

5 COMPARISONS OF RSS AND PRSS ESTIMATES WITH SRS ESTIMATES

In this Section we compare the variances of estimates of μ and σ based on RSS and PRSS with the variances of estimates of μ and σ based on SRS in terms of relative precision (RP). We are also comparing the generalized variance of

$\hat{\mu}$ and $\hat{\sigma}$ $\left(GVar(\hat{\mu}, \hat{\sigma})\right)$ with generalized variance of μ^* and σ^* $\left(GVar(\mu^*, \sigma^*)\right)$.

Table 1 shows the RP of RSS estimate of μ in (3.1) with respect to its SRS estimate. This table indicates that if the location parameter μ of lognormal distribution is unknown to us and we want to estimate it, ranked set sampling is the most powerful tool for sample size n>6. As the sample size n increases the corresponding RP also increases. In Table 2, the RP of RSS estimate of σ with respect to its SRS estimate is given. From this table it is clear that ranked set estimator of σ, σ^* is more efficient than $\hat{\sigma}$ for $n \geq 4$. In this case, the gain in precision is as much as the one attained in estimating μ.

Table 1: **Comparison of $Var(\hat{\mu})$ with $Var(\mu^*)$ in terms of RP for sample size n = 2, 3 ...10.**

n	$\frac{Var(\hat{\mu})}{\sigma^2}$	$\frac{Var(\mu^*)}{\sigma^2}$	RP $= \frac{Var(\hat{\mu})}{Var(\mu^*)}$
2	1.710832	2.701752	0.633231
3	0.446235	0.654136	0.682175
4	0.207827	0.269088	0.772339
5	0.123217	0.141657	0.869826
6	0.083219	0.086012	0.967521
7	0.060932	0.057290	1.063564
8	0.047115	0.040704	1.157494
9	0.037879	0.030325	1.249076
10	0.031365	0.023426	1.338894

Table 2: **Comparison of $Var(\hat{\sigma})$ with $Var(\sigma^*)$ in terms of RP for sample size n = 2, 3 ...10.**

n	$\frac{Var(\hat{\sigma})}{\sigma^2}$	$\frac{Var(\sigma^*)}{\sigma^2}$	RP $= \frac{Var(\hat{\sigma})}{Var(\sigma^*)}$
2	2.171198	2.671196	0.812818
3	0.932705	0.937293	0.995105
4	0.577602	0.469045	1.231442
5	0.414025	0.278605	1.486066
6	0.320993	0.183529	1.749011
7	0.261325	0.129604	2.016337
8	0.219958	0.096211	2.286204
9	0.189598	0.074158	2.556689
10	0.166520	0.058858	2.829180

In Table 3, we have calculated the generalized variance of $\hat{\mu}$ and $\hat{\sigma}$ as well as of μ^* and σ^* and the corresponding RP in the lines of Bhoj (1997) and Bhoj and Ahsanullah (1996). The generalized variances of the two sets of estimators are given as.

$$GVar(\hat{\mu},\hat{\sigma}) = Var(\hat{\mu})Var(\hat{\sigma}) - (Cov(\hat{\mu},\hat{\sigma}))^2 \quad (5.1)$$

and

$$GVar(\mu^*,\sigma^*) = Var(\mu^*)Var(\sigma^*) - (Cov(\mu^*,\sigma^*))^2 \quad (5.2)$$

The gain in precision in terms of the generalized variance is considerably higher than the individual gains in estimating μ and σ.

Table 3: **Comparison of $GVar(\hat{\mu},\hat{\sigma})$ with $GVar(\mu^*,\sigma^*)$ in terms of RP for sample size n = 2, 3 ...10.**

N	$\frac{GVar(\hat{\mu},\hat{\sigma})}{\sigma^4}$	$\frac{GVar(\mu^*,\sigma^*)}{\sigma^4}$	$RP = \frac{GVar(\hat{\mu},\hat{\sigma})}{GVar(\mu^*,\sigma^*)}$
2	1.153133	1.337241	0.862322
3	0.182924	0.149327	1.224992
4	0.060887	0.035037	1.737784
5	0.028032	0.011878	2.360025
6	0.015461	0.005023	3.077912
7	0.009562	0.002462	3.884254
8	0.006398	0.00134	4.77457
9	0.004532	0.000789	5.743882
10	0.003354	0.000494	6.794048

The Table 4 provides the minimum values of l for n=8, 9 and 10 for which

$$RP = \frac{Var(\hat{\mu})}{Var(\mu^*(l))} > 1,$$

and shows that often a PRSS combined with optimum weight does better than a SRS of size n. For example, $\mu^*(7)$ based on a PRSS of size l=7 is as efficient as the BLUE of μ based on a SRS of size n=10. Table 5 provides the minimum values of l for n=5, 6, 7 and 8 for which dominance of $\sigma^*(l)$ over $\hat{\sigma}$ holds, and clearly demonstrates the superiority of PRSS method. For example, $\sigma^*(6)$ based on a RSS of size 6 is efficient as $\hat{\sigma}$ based on n=10.

Table 4: **Minimum value of l and n for which**

$$RP = \frac{Var(\hat{\mu})}{Var(\mu^*(l))} > 1 .$$

N	l	$\frac{Var(\hat{\mu})}{\sigma^2}$	$\frac{Var(\mu^*(l))}{\sigma^2}$	$RP = \frac{Var(\hat{\mu})}{Var(\mu^*(l))}$
8	7	0.047115	0.044039	1.069832
9	7	0.037879	0.036500	1.037761
10	7	0.031365	0.031353	1.000398

Table 5: **Minimum value of l and n for which**

$$RP = \frac{Var(\hat{\sigma})}{Var(\sigma^*(l))} > 1 .$$

n	l	$\frac{Var(\hat{\sigma})}{\sigma^2}$	$\frac{Var(\sigma^*(l))}{\sigma^2}$	$RP = \frac{Var(\hat{\sigma})}{Var(\sigma^*(l))}$
5	4	0.414025	0.381092	1.086418
6	5	0.320993	0.229315	1.399794
7	5	0.261325	0.215586	1.212163
8	5	0.219958	0.210675	1.044064
9	6	0.189598	0.139577	1.358375
10	6	0.166520	0.137843	1.208040

Finally, Table 6 gives the minimum values of l for n= 4, 5, 6, 7, 8, 9 and 10 for which

$$RP = \frac{GVar(\hat{\mu},\hat{\sigma})}{GVar(\mu^*(l),\sigma^*(l))} > 1.$$

Table 6: **Minimum value of l and n for which**

$$RP = \frac{GVar(\hat{\mu},\hat{\sigma})}{GVar(\mu^*(l),\sigma^*(l))} > 1.$$

n	l	$\frac{GVar(\hat{\mu},\hat{\sigma})}{\sigma^4}$	$\frac{GVar(\mu^*(l),\sigma^*(l))}{\sigma^4}$	$RP = \frac{Var(\hat{\sigma})}{Var(\sigma^*(l))}$
4	3	0.060887	0.055174	1.103546
5	4	0.028032	0.016302	1.719479
6	4	0.015461	0.010071	1.535237
7	4	0.009562	0.007039	1.358306
8	4	0.006398	0.003288	1.945874
9	5	0.004532	0.002327	1.947001
10	5	0.003354	0.001872	1.791774

APPENDIX

Table I: **The values of a_i for sample size n = 2, 3 ...10.**

i / n	1	2	3	4	5	6	7	8	9	10
2	1.460615	-0.460615								
3	1.506860	-0.395733	-0.111127							
4	1.451750	-0.238131	-0.165442	-0.048177						
5	1.392381	-0.141015	-0.135598	-0.088905	-0.026863					
6	1.339388	-0.077831	-0.104560	-0.084579	-0.055210	-0.017208				
7	1.293104	-0.033797	-0.078686	-0.073692	-0.057296	-0.037600	-0.012033			
8	1.252538	-0.001557	-0.057373	-0.062492	-0.053645	-0.041233	-0.027303	-0.008934		
9	1.216897	0.022927	-0.040611	-0.051503	-0.047949	-0.040944	-0.031208	-0.020683	-0.006926	
10	1.185619	0.041167	-0.025549	-0.042510	-0.042508	-0.038365	-0.031678	-0.024265	-0.016361	-0.005551

Table II: **The values of b_i for sample size n = 2, 3 ...10.**

i / n	1	2	3	4	5	6	7	8	9	10
2	-0.582642	0.582642								
3	-0.914086	0.718865	0.195221							
4	-1.044603	0.585137	0.356371	0.103095						
5	-1.109760	0.476317	0.351076	0.216525	0.065843					
6	-1.145690	0.393258	0.324143	0.233986	0.147638	0.046666				
7	-1.166016	0.328298	0.294691	0.231179	0.168201	0.108330	0.035318			
8	-1.176979	0.275953	0.266706	0.221908	0.173267	0.127569	0.083615	0.027963		
9	-1.182298	0.233197	0.241963	0.209677	0.170491	0.136367	0.101046	0.066689	0.022869	
10	-1.184878	0.198331	0.218820	0.197888	0.167265	0.137598	0.108836	0.081830	0.055126	0.019183

Table III: **The values of v_i for sample size n = 2, 3 ...10.**

i / n	1	2	3	4	5	6	7	8	9	10
2	1.460615	-0.460615								
3	1.379206	-0.221159	-0.158047							
4	1.254452	0.002994	-0.182414	-0.075031						
5	1.139986	0.136377	-0.112439	-0.121324	-0.042600					
6	1.042283	0.214460	-0.041982	-0.104767	-0.082937	-0.027057				
7	0.959667	0.260809	0.014270	-0.072305	-0.084606	-0.059285	-0.018549			
8	0.889402	0.288405	0.057022	-0.039391	-0.070781	-0.067112	-0.044108	-0.013436		
9	0.829059	0.304497	0.089196	-0.010241	-0.052337	-0.062438	-0.053667	-0.033923	-0.010145	
10	0.776708	0.313297	0.113431	0.014428	-0.033585	-0.052459	-0.053562	-0.043535	-0.026812	-0.007911

Table IV: **the values of w_i for sample size n = 2, 3 ...10.**

i / n	1	2	3	4	5	6	7	8	9	10
2	-0.582642	0.582642								
3	-0.789727	0.548798	0.240929							
4	-0.854418	0.347298	0.380083	0.127037						
5	-0.861581	0.182972	0.349163	0.252290	0.077156					
6	-0.844902	0.064144	0.280961	0.273332	0.175106	0.051359				
7	-0.818429	-0.020795	0.212503	0.253421	0.209653	0.127205	0.036442			
8	-0.788402	-0.082049	0.152617	0.220457	0.211246	0.163030	0.096000	0.027100		
9	-0.757714	-0.126748	0.102369	0.185176	0.198119	0.173884	0.129290	0.074735	0.020889	
10	-0.727722	-0.159703	0.060756	0.151618	0.179000	0.171488	0.143798	0.104527	0.059675	0.016562

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National Forest Inventory- A GIS based Approach

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INTRODUCTION

Forest Survey and inventory started in 18th century in Europe originally with a view to assess availability of fuelwood. Initially measurements were based on ocular estimations. Large forest areas were divided into smaller units and volume was estimated and then all estimates were added to get an estimate of volume and stocking of estimates. In the 19th century, with the availability of measurement equipment, new relationships between, diameter, height and volume were developed and used for quick estimation of larger forest areas. By the end of 19th century sample based techniques using statistical methods were developed and in the 20th century statistical based methods have been used for forest survey and inventory. Now the technology has further improved with the availability of computer based programmes which have made the sampling and assessment more efficient.

The main objective of preparation of National Forest Inventories has been to provide information about the state of given the Nation's forest resources for sustainable development. The information mainly includes species composition, species wise number of stems per unit area and volume as well as other related information like basal area, site quality, volume equation of important species, demand of timber and fuelwood etc. National Forest Inventories provide major inputs at national level planning and management of forest resources.

NATIONAL FOREST INVENTORY IN INDIA

In India, first efforts towards taking up forest inventory in selected areas of the country started in 1965 by Pre Investment Survey of Forest Resources (PISFR) under a collaborative project between the Government of India and UNDP (United Nations Development Programme) with a view to assess availability of forest resources for establishment of wood based industries in the country. In 1981, the PISFR was reorganized as Forest Survey of India (FSI) and given a new mandate. It was to monitor periodically the changing situation of land and forest resources on a ten year cycle and present the data for national level planning. The mandate was revised in 1986, and one of the activities of FSI was to take up forest inventory in selected States and Union Territories (UTs). Before 1981, PISFR carried out inventory in selected areas of the country covering an area of 0.23 million km^2. FSI covered more than 0.5 million km2 of forest area till the year 2000. In early nineties FSI started inventory of tree resources in area outside forests, now called TOF (trees outside forests) also in addition to regular inventory in forest area.

SAMPLING DESIGN OF FOREST INVENTORY BY FSI

Prior to the year 2000, the inventory design used by FSI aimed at preparing District wise reports. The sampling design was based on systematic random sampling with 0.01% sampling intensity. The SOI (Survey of India) toposheets covering the district on 1:50,000 scale, were divided into $2^{1/2'}$ x $2^{1/2'}$ grids. Each grid represents about 18-20 km^2 area. In each grid, two plots of 0.1 ha were marked on the toposheets. Selection of the first plot was random and the second plot was the mirror image of the first one. Inventory was carried out in the plots falling within the forest area. Results of inventory were within 95% confidence limit with ± 10% standard error. A District inventory report gives details of area estimates, topographic description, classification of forests into strata, composition of species in 10 cm diameter classes, state of forests (healthy or degraded), ownership pattern, estimation of volume and other growth parameters such as height and diameter in different types of forests, estimation of growth, regeneration and mortality of important species, volume equation and wood consumption of the inventoried area. More than 80% of country's forest area was covered till 2000.

From 1991-92, FSI took up inventory of trees falling outside conventional forest areas in the country. The main objective was to assess the extent of plantations raised under different social forestry schemes by various agencies. The sampling design adopted for this inventory was a two-stage stratified sampling. Sampling in the first stage was a district and in the second stage it was a village. While taking up the survey of a State, optimum number of villages to be surveyed was determined on the basis of data obtained from a pilot survey. The number of villages to be inventoried in a district was decided according to proportional allocation. Trees standing in the selected villages were enumerated and measured. Planted trees were classified in eight categories: Farm forestry, village woodlots, block plantation, roadside, pond side, rail side and canal side plantations and others (miscellaneous). In each village, 100% enumeration of trees was done and then information on number of trees in different girth class in each category and volume was generated. Based on village data, information at District level and State level was then generated.

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CURRENT INVENTORY DESIGN OF FSI

The earlier design was good to generate estimate at district level but it could not generate a reliable estimate at national level as the collection of inventory data was spread over a very long time period. An attempt was made in 1995 to generate estimate of above ground woody volume of forests using remote sensing data (both satellite and aerial photograph based) and the inventory data (FSI 1995). After the year 2000, FSI focused on generating estimate of forest resources for the entire country by modifying the sampling design within a reasonable time period. The new design was based on a combination of stratified and systematic random sampling. The country is divided into 14 physiographic zones (FSI 2001) based on topography, latitude and altitude, besides climatic and soil properties, and broad similarities in factors responsible for the growth of tree vegetation. Within each strata (physiographic zones), districts were considered first sampling unit. Total 10% districts (60 districts) were selected in each cycle of two years for inventory and generating national estimate in such a way that at least two districts are selected in each physiographic zone so that each zone is adequately represented in the sampling. Within each district, same design is followed as was used earlier except that the grids of $2^{1/2\prime} \times 2^{1/2\prime}$ are subdivided into four subgrids of $1^{1/4\prime} \times 1^{1/4\prime}$ and of these two subgrids are selected randomly. The centre point of each sub-grid is taken to lay out a plot of 0.1 ha for collection of field data. The sampling intensity remains 0.01%. Using this design FSI has so far surveyed 180 districts and generated three biennial estimates (FSI 2003, 2005 and FSI 2009).

THE LIMITATIONS AND SHORTCOMINGS

The current inventory design of forest inventory was initially appeared as a well thought design for generating a national level estimate for species wise on number of tree per ha and volume. It provided important information at national level planning as no reliable information on growing stock of forests in India was available, but subsequent inventory exercises carried out by the FSI using the same design showed serious limitations and shortcomings some of which are listed as under:

- The Strata are based only on physiographic considerations and cannot be considered homogenous strata in true sense. Neither species composition nor forest density had been taken into consideration. Both these parameters have very important bearing on estimation of growing stock. It was simply presumed that each physiographic zone has similar vegetation/forest type and the density of forests is also uniform. These assumptions are not true as each of the 14 physiographic zones covers very large area with a lot of variation in species composition/ forest types and density of forests.
- Even if it is presumed that the estimates generated at physiographic zone level are fairly accurate but the estimates at State or UT level cannot be considered accurate. It is because of the reason that the Districts for taking up field inventory are selected randomly within a physiographic zone and the Districts so selected may not represent the real physiographic picture of the State. The State may comprise parts of more than one physiographic zone and the some physiographic zones in a State may not at all be represented in the inventory by the districts selected. One example had been the State of Kerala where the estimate for the State was prepared in 2003 assessment projecting data of a single district for the entire State.
- In some small state/UT where no district was selected, estimate was generated based on the selected districts falling in the nearest similar physiographic zone. This has led to generation of errors in the estimate. One classic example is Andaman and Nicobar Islands where inventory was not carried out in any District but the estimates have been generated using inventory carried out in district of adjoining States falling within similar physiographic zone and stated to have been validated using old inventory data of these Islands. In the draft inventory report prepared by FSI recently for these Islands, many important tree species like Padauk, Gurjan, etc., which constitute a significant percent of forest crop are missing. Basis of providing estimate of number of trees per ha in different diameter classes without actually taking up inventory is also not known.
- So far FSI has generated three biennial estimates using different sets of districts and no comparison can be made between two different estimates. If there is change in the estimates of a particular physiographic zone or a State in two different cycles, it is not possible to spatially locate the area where changes have taken place. As a result, while these estimates have limited applications in national level planning and hardly any utility at State or district level planning and management of forest resources.
- In every biennial assessment, inventory data of Districts taken up in the previous cycle is also added to work out fresh estimation of growing stock at National level as well as for State level. This affects the accuracy of results as annual increment is not taken into consideration while using data of earlier inventory.
- Estimates may not depict correct picture of NE State where shifting cultivation is a major land use.

PROPOSED GIS BASED APPROACH

The methodology proposed here is GIS (Geographic Information System) based and is an improvement over the methodology used by FSI in 1995. It involves following steps:

1. Creation of a geo-referenced vector layer of grids of $2^{1/2\prime} \times 2^{1/2\prime}$ (already available in FSI) covering the entire country.
2. Overlaying of latest forest cover over and selection of grids having forest cover.
3. Overlaying of forest types maps recently prepared by FSI
4. Overlaying of State and UT boundary on the spatial maps generated in step 1, 2 and 3 above.
5. The first sampling unit will be the State or UT. The sampling design in each State will be a combination of stratified and systematic sampling. The strata should be identified using forest types and the three canopy density classes as per classification adopted by FSI. Similar forest subtypes can be merged to minimize number of strata.

A simple example of Andaman and Nicobar Islands can be used to illustrate the classification of forest strata for the purpose of

taking up forest inventory. In A & N Islands there are following four main forest types:

a. Tropical evergreen forests
b. Tropical semi-evergreen forests
c. Tropical moist deciduous forests
d. Littoral and swamp forests

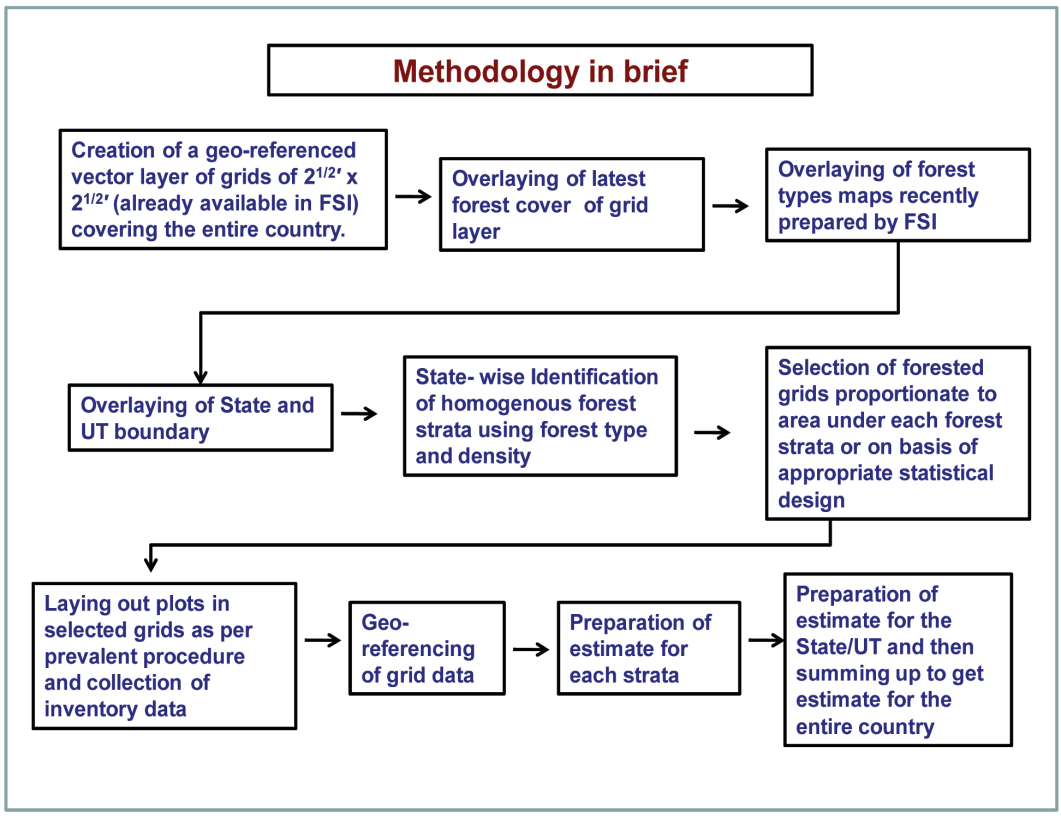
These four forest types constitute around 98% of total forest cover in these Islands. Other forest types covering very small areas can be grouped with the four main types depending upon similarity to any of these four forest types. Now each forest type can further be classified based on the canopy density of the forest therein. There are three density classes in the canopy density classification adopted by the FSI- Very Dense forest (VDF: with a canopy density of 70% and above)), moderately dense forests (MDF: with canopy density between 40% and 70%) and open forests (OF: with canopy density between 10% and 40%). With this 12 forest strata will be generated based on forest types and canopy density. Each stratum will be homogenous from the point of view of species composition, physiographic considerations and canopy density. All the forested grids of A & N Islands can now be classified into these 12 forest strata and in each stratum appropriate number of grids can be selected for field inventory so as to generate fairly accurate estimate on information on species wise number of stems in different girth classes and growing stock for each forest type, each density class, for each strata, each forest Division, District and at State level.

6. Once the strata are identified in a State, appropriate number of forested grids can be worked out in such a way that each strata gets adequate representation.
7. Inventory in each selected grid to be carried out as per the present inventory methods by laying out two plots of 0.1 ha in each grid. Additional information like number of villages around the grid, human and cattle population, requirement of small timber, fuelwood, fodder etc should also be collected to generate information having utility in planning and management.
8. Once the data is collected it can be suitably processed to generate estimates at state level and also GIS based maps depicting spatial distribution of information so generated.
9. District or forest divisional level information can be generated from the spatial maps so generated by overlaying district or forest division boundary.
10. National estimates can be generated by adding data of all constituent States/UTs

This methodology if adopted will have following advantages:

i) The methodology if adopted may take a few years in the first assessment but subsequent assessments will be less time consuming. Once first assessment is complete, subsequent assessments will include inventory of only those grids where any change in forest cover is detected based on biennial

Figure 1: **Advantages and future scope of the proposed GIS based Methodology**



assessment of forest cover done by FSI. A few grids showing no change in each stratum should also be taken for inventory annually in order to generate average annual increment for each stratum. Thus, there will be considerable saving of time, human efforts and financial resources, and FSI will be able to generate estimate of growing stock of forest resources biennially at national as well as State/UT level.

ii) Since inventory information will be spatially available, it will be possible to locate changes if any in the subsequent assessments. This will help in planning and management of forest resources at all levels.

iii) Grid level additional information like number of adjoining villages, human and cattle population, demand of small timber, fuelwood, fodder, NTFP (Non Timber Forest Resources) etc will be helpful in finding out reasons of changes if any in the growing stock of the grid.

iv) The information will also have its application in generating biennial estimates of biomass and carbon in forest areas.

v) This methodology will provide reasonably good estimates for NE region also

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