THEME 3

Expanding Frontiers in Forestry Sciences

3.1 – Geomatics - Applications and opportunities:

3.2 – Managing forest resources: scientific base

3.3 – Forest genetics and biotechnology

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Geomatics in Sustainable Management of Forests

1st Indian Forest Congress, 22-25 November 2011

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Geomatics is relatively new as a scientific term.

Geomatics also known as **geospatial technology** is the discipline of gathering, storing, processing, and delivering geographic information, or specially referenced information.

It includes the tools and techniques used in land surveying, remote sensing, cartography, geographic information system (GIS), global navigation satellite systems (GPS, GLONASS, Galileo, Compass), photogrammetry, geography and related forms of earth mapping.

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Modern tools & technology used for SFM

- Computer
- Remote sensing
- Geographic Information System (GIS)
- Global Positioning System (GPS)

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Monitoring forest area change using remote sensing imagery

- **Monitoring of changes of forest areas- deforestation**
- **Monitoring of increase of forest area- forestation**
- **Monitoring of forest area change within forests- forest degradation**

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Considerations essential for monitoring on a scientifically credible basis

- The national circumstances, particularly existing definitions and data sources
- Selection and acquisition of satellite imagery and coverage
- Available skilled staff and soft and hardware resources
- Sampling based or wall to wall coverage
- Image interpretational technique
- Accuracy assessment

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Optical mid-resolution (10-60 m) sensors presently available

Nation	Satellite &sensor	Resolution and coverage	Cost for data acquisition	Features
USA	Landsat-5 TM	30 m 180 X 180 km^2	All data archived at USGS is free	Images down loadable to any satellite receiving station at repetivity of 16 days
USA	Landsat-7 ETM+	30 m 60 X 180 km^2	All data archived at USGS are free	Data gaps outside of the central portion of the images due to failure of scan line corrector in April 2003
USA/Japan	Terra ASTER	15 m 60 x 60 km^2	60 US$/scene	Data acquired on request and is not routinely collected for all areas
India	IRS-P6 LISS-III AWIFS	23.5 m 141 X141 km^2 56 m 740 X 740 km^2	152 US$/scene 322 US$/scene	Images available from 2003 from NRSC. Images of earlier satellites IRS IC/ID with same resolution also available since 1997
China/Brazil	CBERS-2 HRCCD	20 m	Free in Brazil and Potentially for other developing countries	Experimental: Brazil uses on demand to bolster their coverage
France	SPOT-5 HRVIR	10-20 m 60 X 60 km	2000 €/scene	Commercial, Indonesia and Thailand uses along with Landsat

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• Other types of sensors such as Radar (ERS1/2 SAR, JERS-1, ENVISAT-ASAR and ALOS PALSAR) and Lidar are potentially useful and appropriate.

- Coarse resolution (250 m – 1km) data available from 1998 (SPOT – VGT) or 2000 (MODIS) have utility because high temporal resolution (1 to 2 day)
- Fine resolution data obtained from IKONOS, QuickBird, Worldview, Geoeye-I, Cartosat but expensive to cover large areas- used to calibrate algorithm and ground truthing

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Wall to wall or sampling approach?

- Wall to wall approach- covers the full spatial extent of the forested areas and is a common approach
- A few large countries like India and Brazil have established operational wall-to-wall system since 1980s based on mid-resolution satellite imagery (India-biennial and Brazil- annual and sensitive regions on short intervals)
- If resources are insufficient, the sampling approach is equally efficient specially for large countries.
- The recommended sampling approaches are systematic and stratified sampling.

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Analysis of the satellite imagery

- **The selection of the method depends on the available resources including software for image processing.**
- **A combination of automated methods (segmentation or classification) and visual interpretation gives the best result.**
- **An independent accuracy assessment is an essential component to link area estimates to a crediting system.**

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Monitoring of forest area change- special situations

- **Monitoring of increase of forest area- forestation**
  - Identifying increase in forest area with satellite imagery is generally difficult canopy closure slow- better with high resolution
- **Monitoring of forest area change within forests- forest degradation**
  - Only those areas can be identified by satellite- where intensity of degradation is high and but not all of them
  - Demands use of more sophisticated algorithm and high resolution imagery
  - Spectral mixture analysis (SMA) has been found to be the robust technique.

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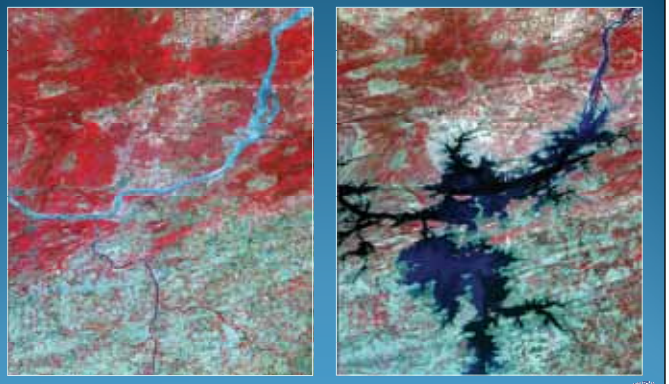
Application of Remote Sensing for Sustainable Forest Management in India

- Nation-wide Forest Cover Mapping on two a year cycle
- Assessment of Trees Outside Forests
- Forest Fire Monitoring
- Forest Type Mapping of the country's forests
- Assessment of encroachments and damages due to disasters
- Preparation of Forest Management Plan for local level operational
- Stratification for Forest Inventory
- Assessment of Carbon in India's Forests
- Wildlife census and management of National Parks and other protected areas

Submergence of Forest Area in Harda, East Nimar & Dewas Districts



2003 2005

CURRENT USE OF GIS IN INDIA

- Urban Planning
- Infrastructure development
- Census
- Disaster management
- Maintenance of Land records
- Forestry
- Land and Water Resources management
- Traffic control and locating criminals hideouts
- Election
- Energy distribution, monitoring and maintenance
- Demarcating costal zones

GIS IN FORESTRY

- Forest cover assessment and change analysis
- Assessment of trees out side forests
- Preparation of management plan of forests
- Forest fire risk zonation
- Site suitability for setting up water harvesting (watershed analysis)
- Mapping Ecotourism sites

GIS IN FORESTRY

- Establishing patrolling camps and mapping road network for protection
- Wildlife habitat mapping and biodiversity characterization
- Preparing of management plan of PAs- boundaries of all PAs have been digitized at WII
- Mapping of the non wood forest resource
- Site suitability for plantations and online nursery information system as well as assessment of plantation areas

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Agencies using GIS in forestry in India

- Forest Survey of India (FSI)
- Some State Forest departments
- NRSA and Regional Remote Sensing Centers and SAC of Deptt of Space
- Private agencies

GIS in regular works of FSI

- Manual GIS since early 1980s with start of national forest cover assessment using Landsat data
- Digital image processing began in 1992 but it was limited to only one or two states.
- Project level GIS studies were initiated in 1994/95
- National scale application of GIS began in 1998 with introduction of national level DIP for forest cover assessment

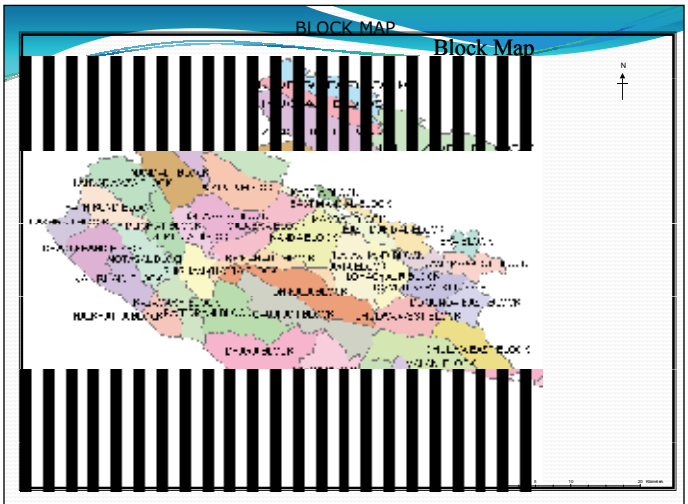
GIS BY STATE FOREST DEPARTMENTS

- Andhra Pradesh (1994-WB)
- Chhattisgarh (1998-WB)
- Maharashtra (1998 State fund)
- Tamil Nadu (2000-JBIC)
- Kerala (1999-WB)
- West Bengal (1999-State fund)
- Karnataka (1998-DFID/JBIC)
- Orissa
- Gujarat
- Madhya Pradesh
- Uttarakhand
- Meghalaya
- Sikkim

GIS in preparation of Working Plans

Generation of basic input layers for preparation of Working Plan

- Contour (100 m interval),
- Road network
- Drainage
- Village locations
- Division boundary
- Range boundaries
- Block and beat boundaries
- Reserved forest boundaries
- Compartment boundaries



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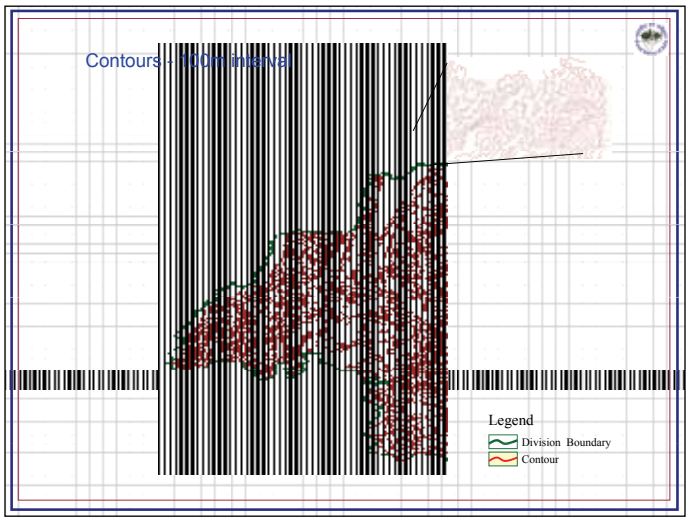

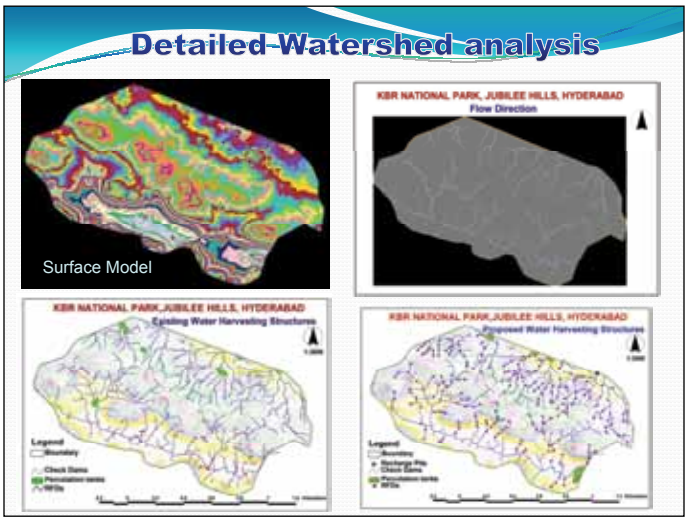

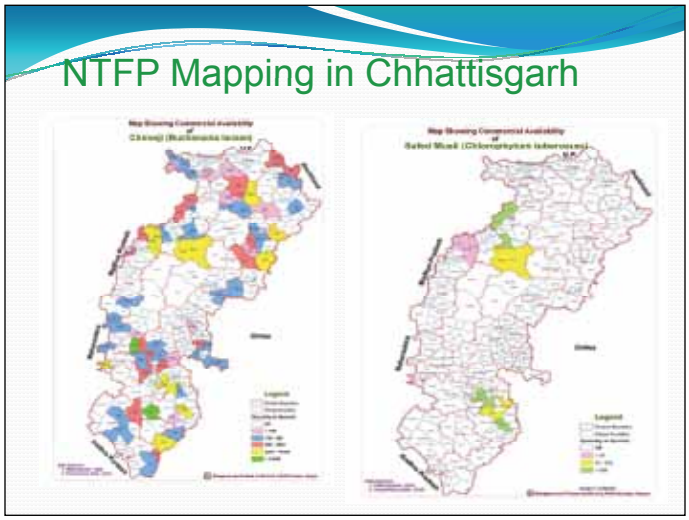

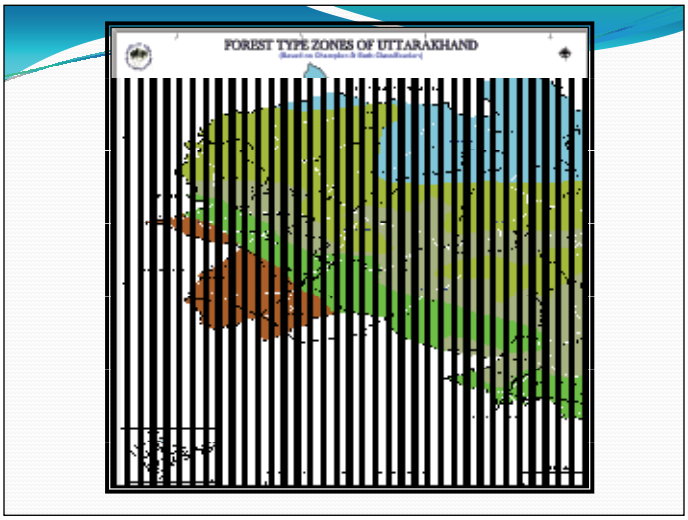

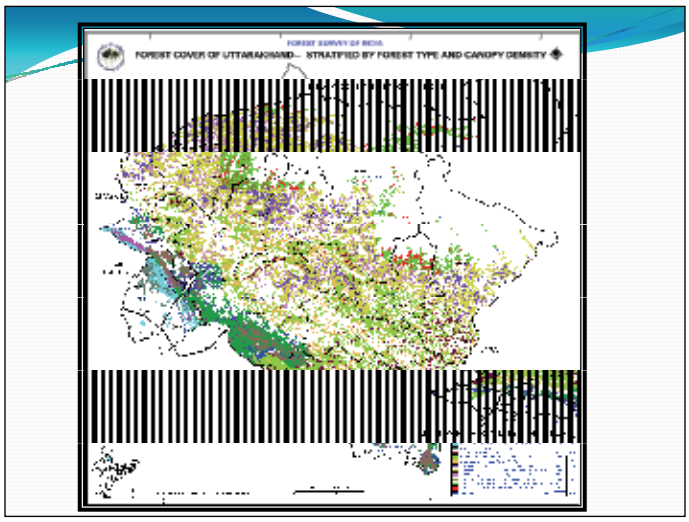

Deciding area & boundary of forest land for grant of right under Forest Right Acts 2006 to forest dwelling communities

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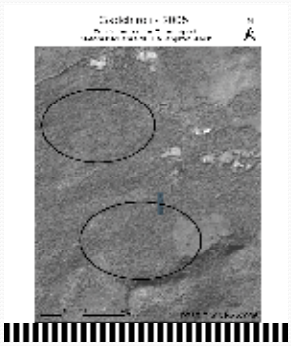
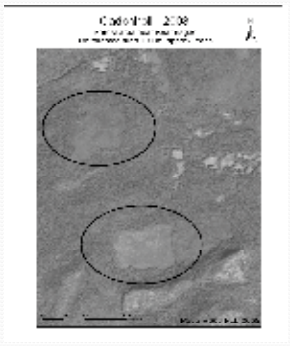
Gadchiroli-Bamni village

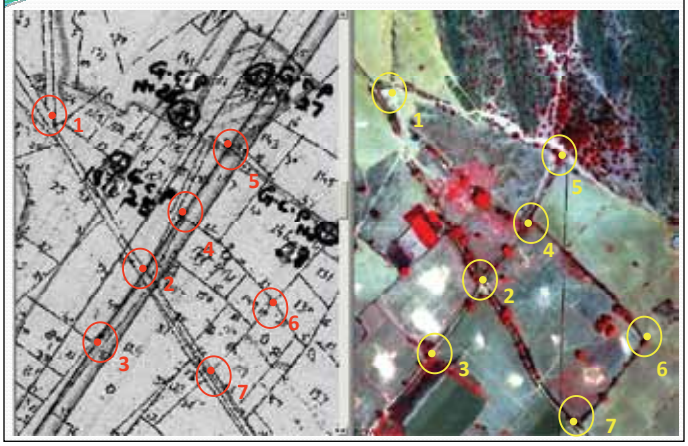
Amravati-forested areas claimed

Gadchiroli - change in vegetation





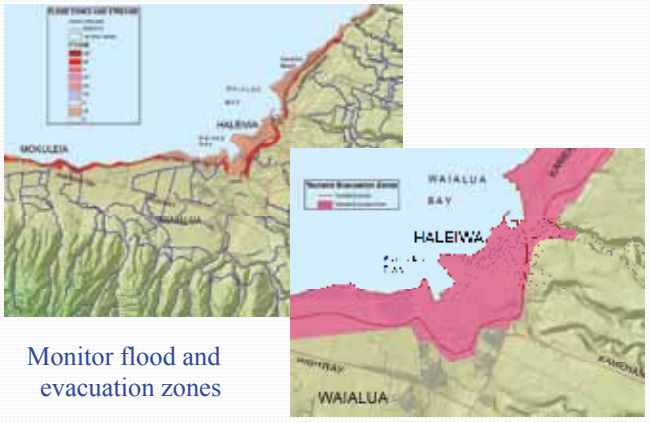
GEOREFERENCING OF RECTIFIED VILLAGE MAP



GIS in Coastal Zone Regulation

- Mapping and demarcation of coastal zone lines which includes High Tide Line (HTL), Low Tide Line (LTL), 200 m and 500 m lines from HTL in case sea, bays, estuaries, creeks, rivers and back waters in India which are influenced by tidal action
- Demarcating areas likely to be affected by flood and needs to be evacuated

Coastal zone management



Monitor flood and evacuation zones

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Coastal Zone Regulation: POSCO case - NIO

- The limits of the CRZ lines drawn on 1:5,000 scale maps by NIO at 500 m towards the northern portion of POSCO site and at 150 m on the creek side are not very clear. The limits and extent upto which these lines exists should have been well defined by the geo-coordinates in the maps as well as in the text.

ISSUES IN GIS APPLICATION

- **The appreciation of the technology is still to pick up fully**
- **Lack of dedicated skilled human resource to use the technology**
- **At places there are mismatch between land notification /maps and existing boundaries**

ISSUES IN GIS APPLICATION

- **Government Map policy do not allow digitization of maps by other than a few designated government agencies**
- **Standards and protocols are yet to be setup for interoperability of the digital maps**
- **The technology to be made cheaper and customized for easy operability/user friendly.**
- **Early operationalization of NSDI (National Spatial Data Infrastructure)**

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Geomatics: Applications and Opportunities

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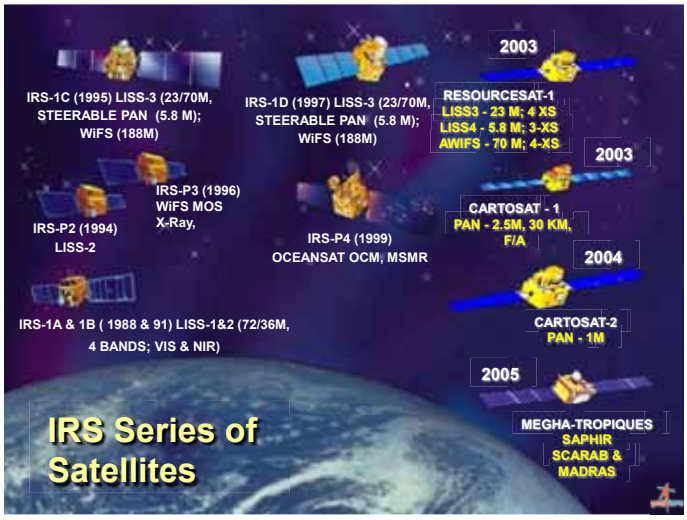

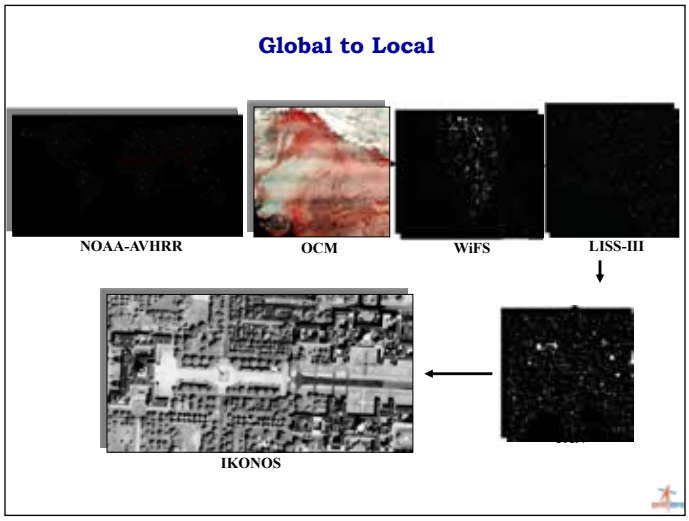

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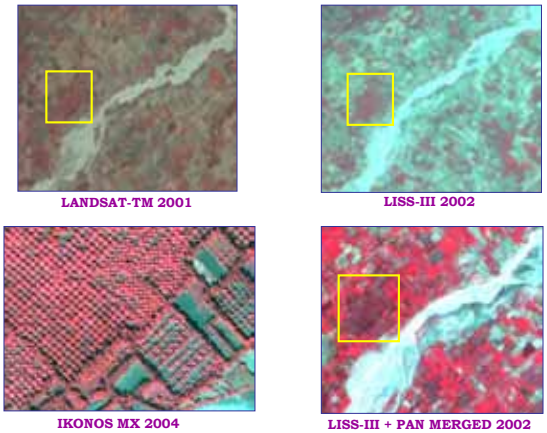
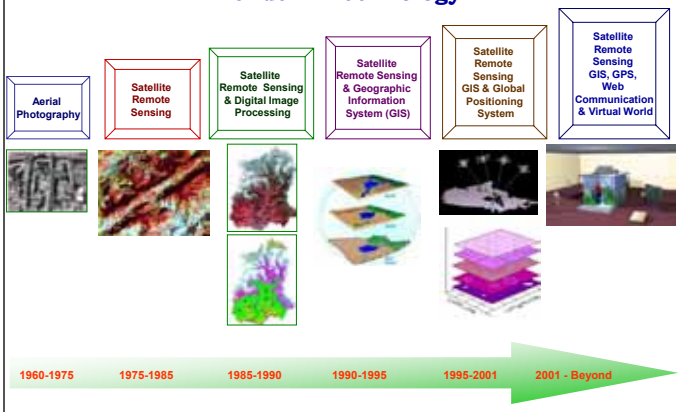
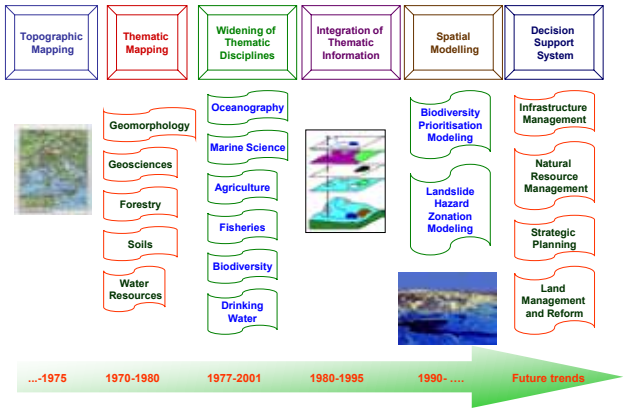
Progress in Imaging Technology



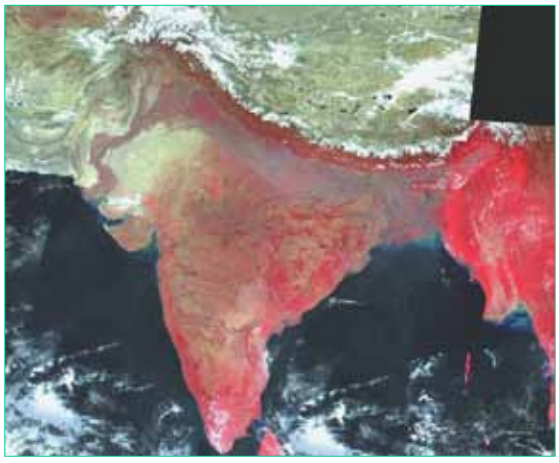
Trends in Technology



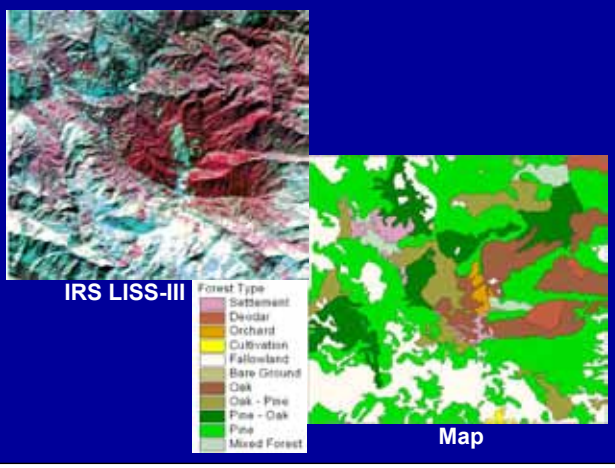
Trends in Applications



Large Area Montoring



Visual Interpretation of Imagery (Ranikhet)



Satellite Image Mosaic of Arunachal

(Mosaic of 21 LISS-III Scenes)

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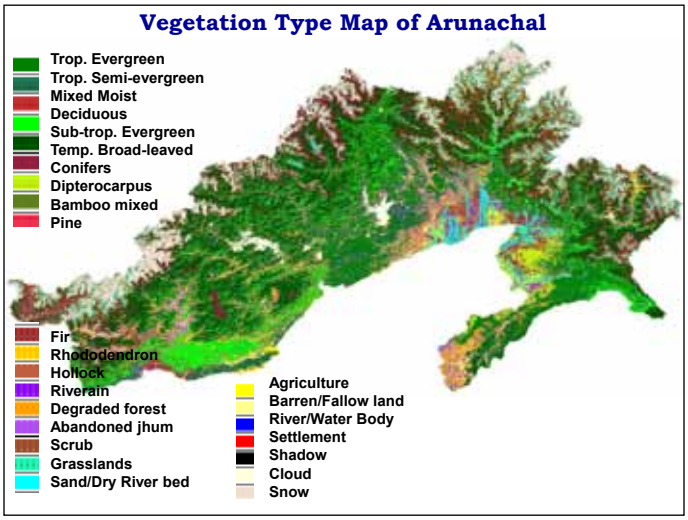

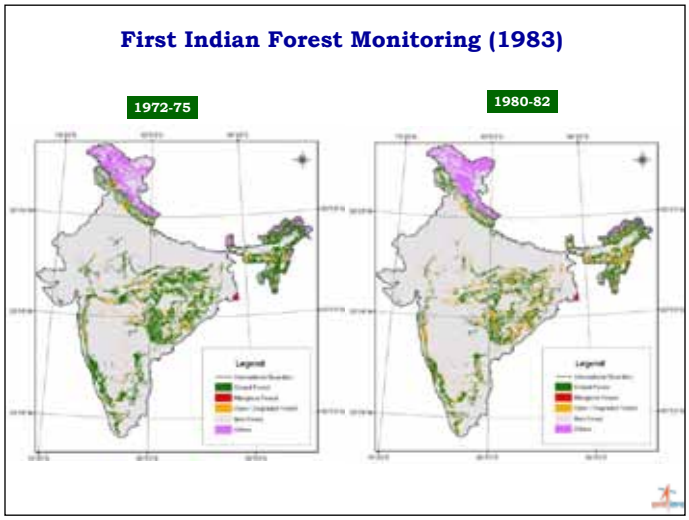

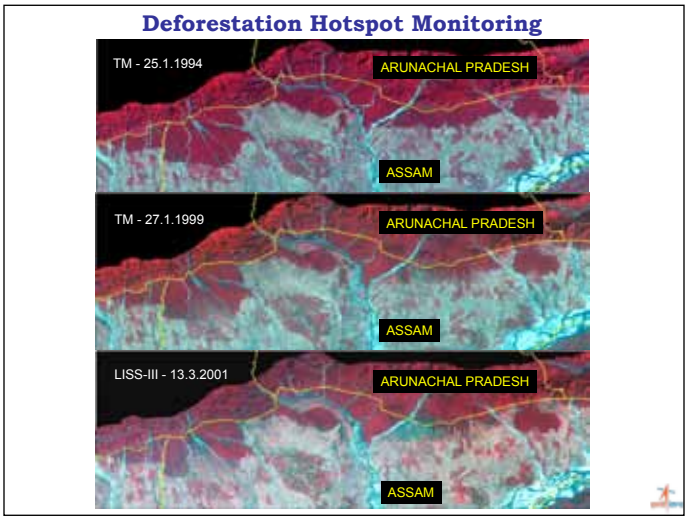

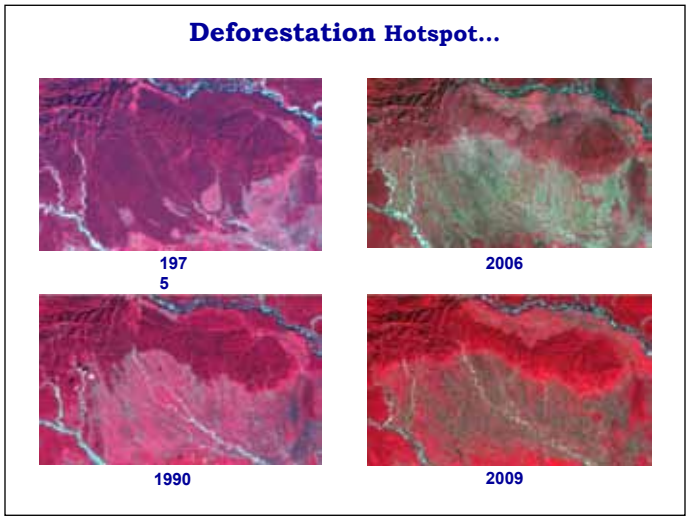

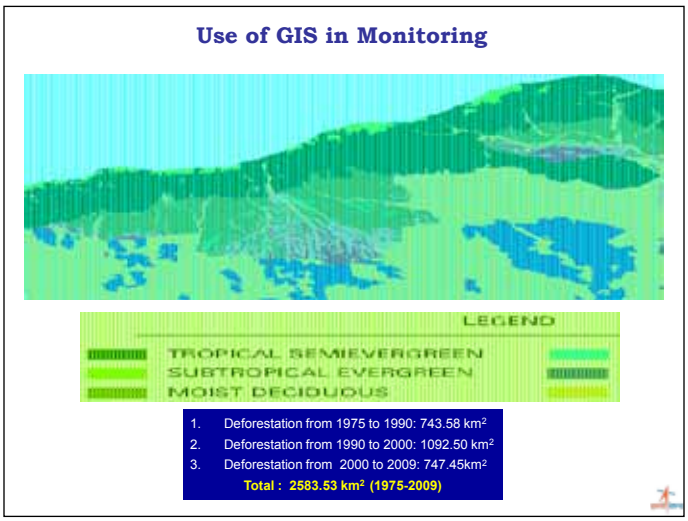

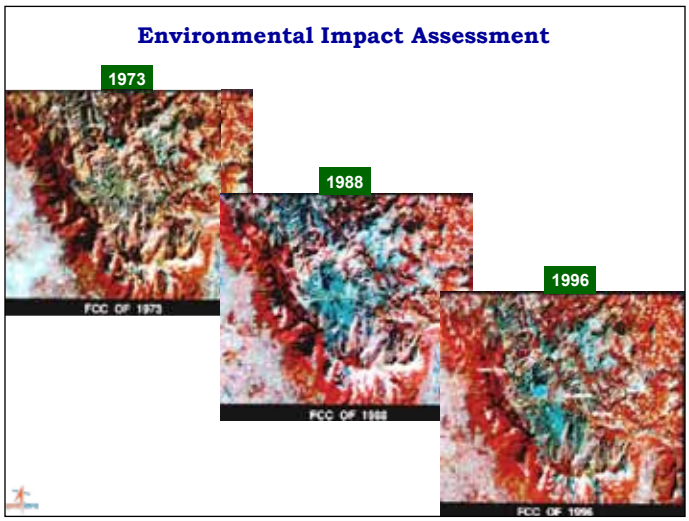

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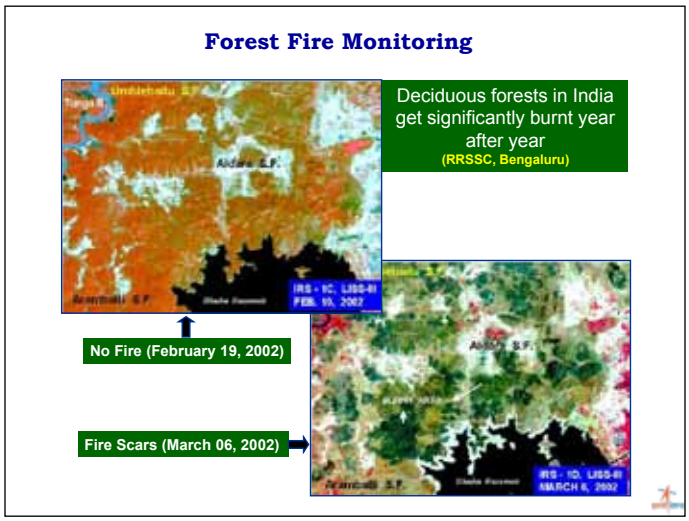

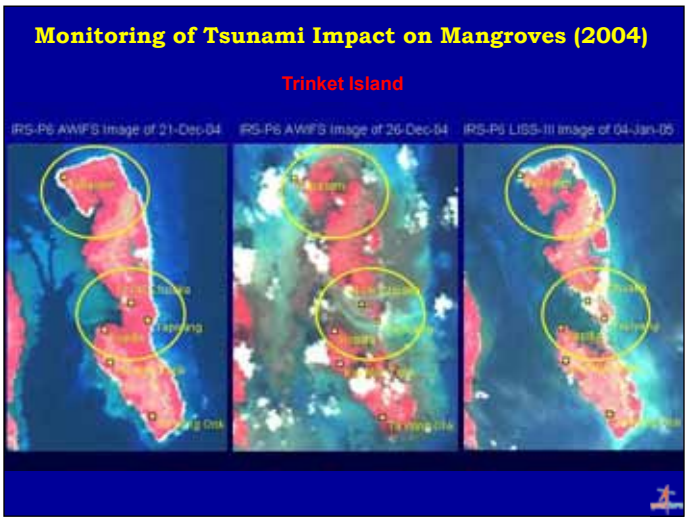

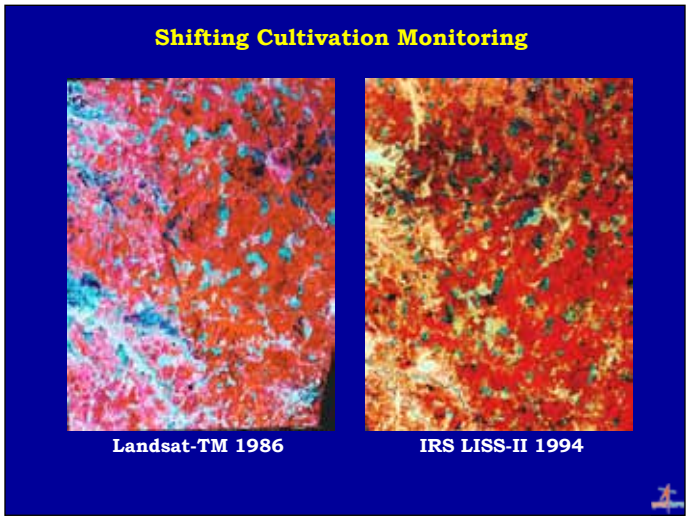

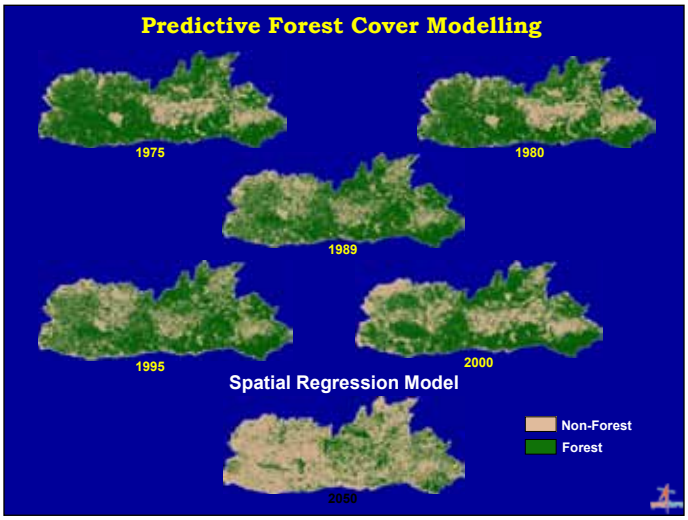

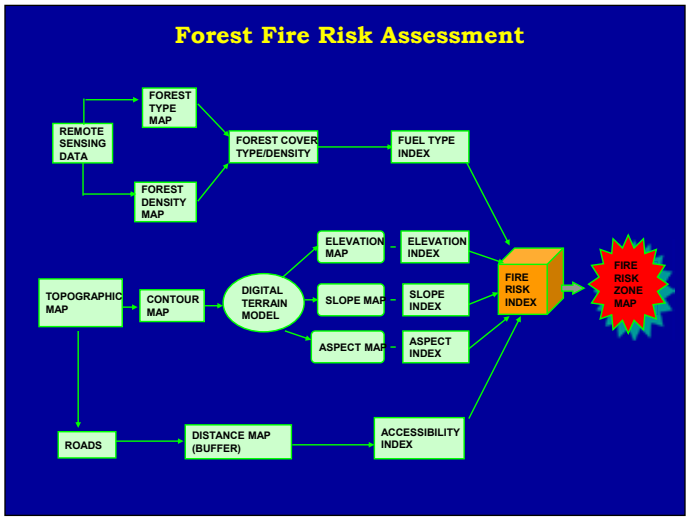

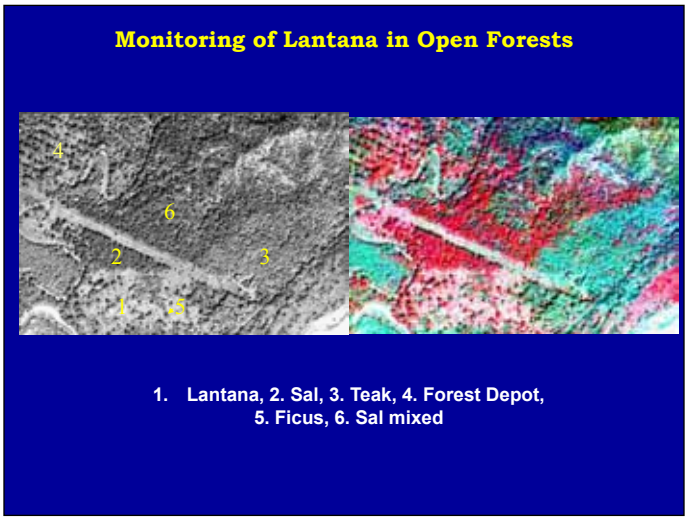

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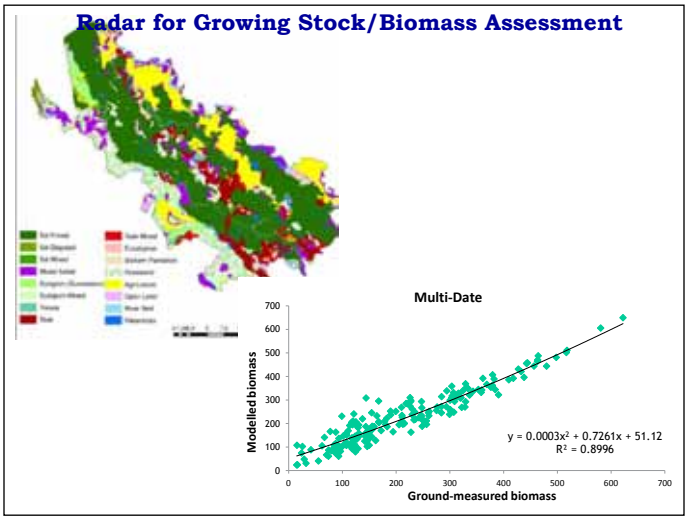
Emerging Remote Sensing Tools

IKONOS Image of Part of the Doon Valley

Avenue Tree Mapping using Image Segmentation

Radar for Growing Stock/Biomass Assessment



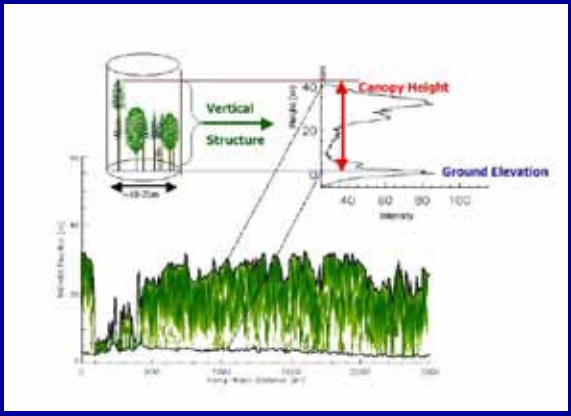
LiDAR Sensing of Forests

- The distribution of LiDAR measurements throughout the canopy contains information relating to forest structure in both vertical and horizontal dimensions
- Small-footprint, discrete return LiDAR measurements can be modelled as observations from a stochastic process
- Large-footprint, continuous waveform LiDAR has been used successfully to characterize forest structure patterns (Lefsky *et al.*, 2002).

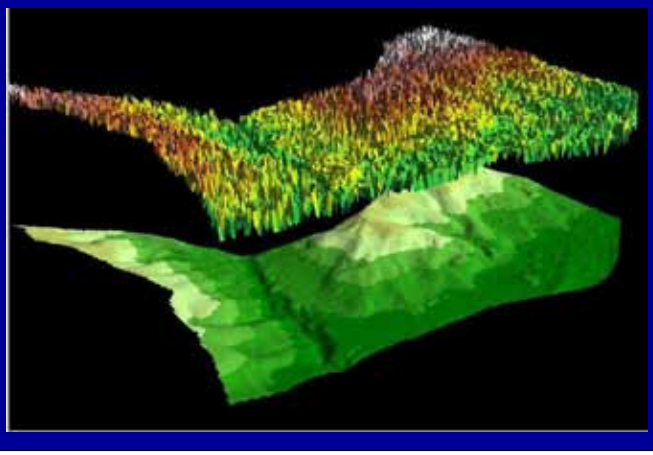
LiDAR (Light Detection And Ranging)

- Active airborne sensor emits several thousand infrared laser pulses per second.
- Operates on principle that if location and orientation of laser scanner is known, we can calculate a range measurement for each recorded echo from a laser pulse.
- Components of system include INS (inertial navigation system), airborne differential GPS, and laser scanner.
- Range measurements are post-processed and delivered as x,y,z coordinates.

Principles of LiDAR Sensing



DSM and DTM from LiDAR



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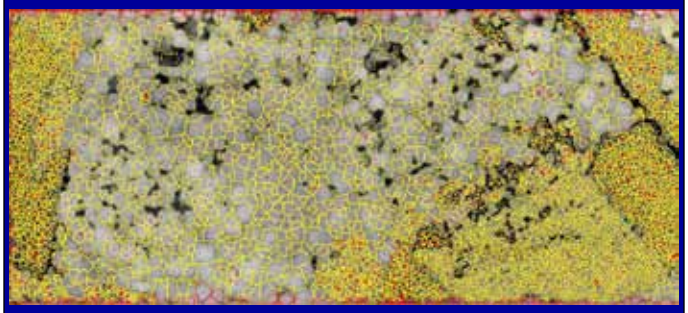
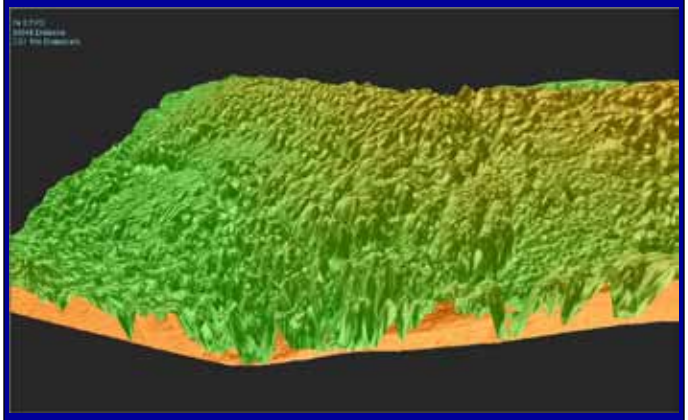
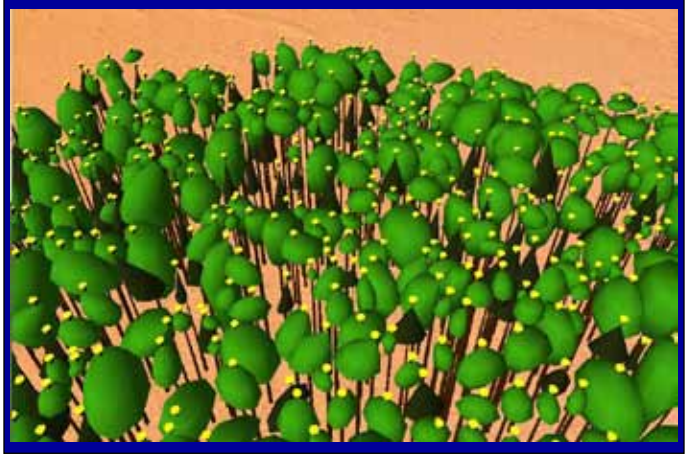
Crown Shapes and Sizes from LiDAR Data

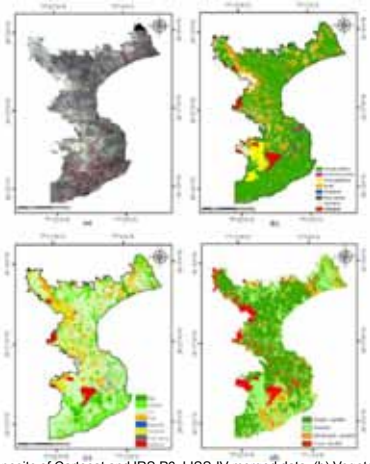


Digital Surface Model of a Forest Area



Stand Height Visualization by *TreeVIS*





(a) False colour composite of Cartosat and IRS P6, LISS-IV merged data, (b) Vegetation cover/land use map, (c) Forest density map and (d) Habitat suitability map of nilgai in Asola-Bhatti WLS, Delhi

"The satellite imagery and related technology is one of the top ten advances in forestry in the past one hundred years"

- Society of American Foresters

"Remote sensing can play a prominent role in promoting growth for sustainable Development"

- Rio Conference, 1992

Constraints

- ❑ Cloud, Fog, Snow, Rain, Dust Storm, Smoke with optical data.
- ❑ Negligible information about under storey vegetation.
- ❑ Reflectance weakly related to wood/woody biomass.
- ❑ Tree height retrieval from optical satellite data- LiDAR
- ❑ Species identification in mixed forests often a problem.

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COST EFFECTIVE MICROSATELLITE GENOTYPING OF *EUCALYPTUS* MAPPING POPULATION

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Division of Plant Biotechnology
Institute of Forest Genetics and Tree Breeding
Coimbatoe-641002

MICROSATELLITES FOR GENOTYPING

- Genotyping using SSRs discovered by Litt and Luty
- Simple sequence repeats are short tandem repeats of 1-6 bases
- Coding and non coding region of genome
- Highly reproducible
- Multiallelic in nature
- Co dominantly inherited
- Relatively abundant
- Have good genome coverage

TECHNIQUE FOR SSR GENOTYPING

Conventional method

- Denaturing Poly Acrylamide Gel Electrophoresis (PAGE)
  - 5% PAGE
  - Denatured to unfold the DNA and to remove the influence of shape on their mobility
  - 7M Urea - denaturing agent
- Silver staining
- Limitations
  - Time consuming technique
  - Involves labor cost
  - Chemical cost
  - Manual scoring errors

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LABELING UNIVERSAL PRIMER

- Automated detection
- Fluorescence based detection
  - Direct labelling
  - Limitation
    - Primer labeling cost is very expensive while labeling 100s of primers for mapping study
- M13 labelled sequence used for laser detection - three primer strategy
  - First reported by Oetting et al. (1995), Neilan et al. (1997) and Schuelke (2000)
  - In Common bean (Oblessuc et al, 2009)
  - In jellyfish tree Medusagyne oppositifolia (Finger et al, 2010)
  - In Cajanus sp. (Bohra et al, 2011)

OBJECTIVE

- To optimize a cost effective genotyping using SSR markers in *Eucalyptus* species

MATERIALS AND METHODS

- Plant material
- DNA isolation

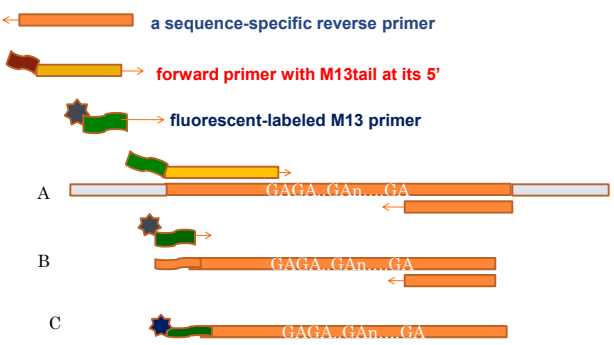
Juvenile leaves of *E. camaldulensis, E. tereticornis, E. grandis* and F1s of *E. camaldulensis* x *tereticornis* and *E.tereticornis* X *grandis*

- SSR primer synthesis and amplification

1. Forward primer synthesized with m13 sequence
2. Reverse primer
3. Universal m13 sequence labelled with a fluorescent dye FAM-5' TGT AAA ACG ACG GCC AGT-3'

SSRs cross amplified from other species of Eucalyptus like *E. camaldulensis , E. grandis, E. nitens, E. tereticornis* and from *Corymbia* were used.

Three Primer Strategy



A- Incorporation of M13 sequence,
B- Universal M13 primer incorporates FAM, no complimentary with the target genome
C- FAM Labeled product

PCR COMPONENTS

In PAGE	In Genetic Analyser (Three primer strategy)
10X buffer	10X buffer
dNTPs - 100 µM each	dNTP mix- 125µM each
Each primer - 4 pmol	Forward primer with M13 tail at 5' end- 0.1pmol
Template DNA - 20 ng	Reverse primer - 0.4pmol
Taq DNA polymerase - 1 unit	Labeled universal M13 primer - 0.2 pmol
	Template DNA- 10ng
	Taq DNA polymerase - 1 unit.

PCR CONDITION

In PAGE

- 94°C for 5 min(Initial denaturation)
- 30 cycles of
  - 94°C for 1 min
  - T_a for 30 or 60sec
  - 72°C for 2 min
- 72°C for 7 min (final extension)

In Genetic Analyzer (Three primer strategy)

- First sequence of reaction
  - 94°C for 5 min
  - 30 cycles of
    - 94°C for 45sec,
    - T_a for 30 sec
    - 72°C for 1 min
  - 72°C for 15 min(elongation step)
- Second sequence of reaction
  - 20 cycles of
    - 94°C for 30sec
    - T_a of labelled M13 at for 45sec
    - 72°C for 45sec
  - 72°C for 30 min (final extension)

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ELECTROINJECTION AND DATA ANALYSIS

- Along with the sample GeneScan 600 Liz size standard and Hi- Di formamide (ABI) mixture was electroinjected in a 8 capillary ABI 3500 genetic analyzer having POP7 polymer
- Data collected using data collection software and analyzed (includes scoring of allele size) with Genemapper software version 4.1

RESULT

- Genotyping of the parents through 5% PAGE , showed out of 212 SSRs, 139 SSR loci polymorphic between the parents
- 38 loci were initially used for genotyping of F1 hybrids and their parents using the Genetic Analyser

USE OF DENATURING PAGE

EMBRA139 EMBRA89

EMBRA 149

- Amplification
- Polymorphism

Ambiguity in scoring

3 monomorphic SSR loci misinterpreted as polymorphic when genotyped with PAGE

OPTIMIZATION

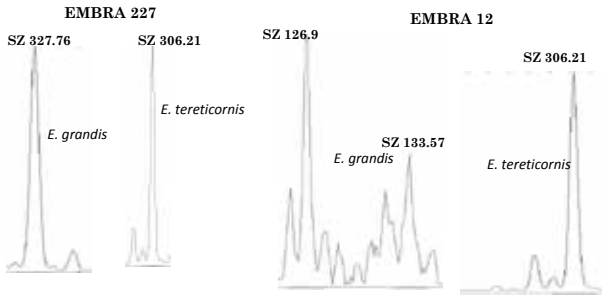
- Stutter peaks were reduced by increasing the annealing temperature of the locus specific primers.
- Loci with no amplification was tried with decreasing the annealing temperature
- PCR enhancers like DMSO and betaine, which reduce the secondary structure formation in GC rich primers, did not produce positive results

STANDARDISATION

Table showing details of the Embra primers used for the study and modification in annealing time and temperature according to the primer

S.No	Microsatellite	Brondani et al. 2006 Annealing Temp.(°C)	Modified A.temp.(°C) in PAGE	Modified A.temp.(°C) in GA(A. time 30 sec)	Allele size range	M13 A. temp. (°C)	Pattern in GA
1	EMBRA98	56	56(30s)	57	220-270	50	Polymorphic
2	EMBRA36	56	55 (30s)	56	130-155	50	polymorphic
3	EMBRA28	56	56(30s)	56	180-200	50	polymorphic
4	EMBRA147	56	56(30s)	56	190-230	53	polymorphic
5	EMBRA148	62	62(30s)	62	215-230	50	polymorphic
6	EMBRA149	56	56(30s)	56	130-145	50	Monomorphic
7	EMBRA153	56	56(30s)	56	225-240	50	polymorphic
8	EMBRA154	60	60(30s)	60	240-260	50	polymorphic
9	EMBRA156	60	54(30s)	54	110-130	50	Monomorphic
10	EMBRA122	54	56(30s)	58	220-255	50	polymorphic
11	EMBRA101	58	55 (30s)	55	120-145	50	polymorphic
12	EMBRA63	58	58(30s)	58	165-225	50	polymorphic

Peaks showing two SSR loci



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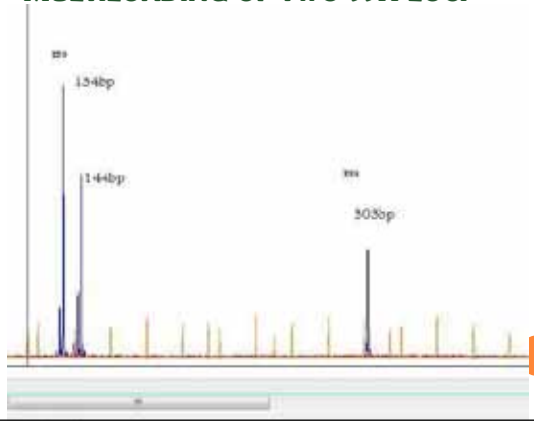
MULTILOADING

- Multiloading of SSR loci performed having amplification range difference of 25 bp
- Multiloading with two primer is optimum which do not produce off scale peaks
- Additional labelling dyes such as VIC (green), PET (RED) and NED (Yellow) allow detection of at least 8 loci in a single injection
- Seven or more SSR loci can be multiloaded in a single capillary (Bohra et al, 2011)

ELECTROPHEROGRAM DISPLAY FOR MULTILOADING OF TWO SSR LOCI



DISCUSSION

- Three primer strategy with multiloading will be cost effective since only the M13 universal primer alone need to be labelled
- Single labelled universal loci can be used along with the other species specific primers for genetic studies

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Potential Use of Some Plants Extracts Against Wood Rotting Fungi *Coriolus versicolor*

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E-mail: skbmdu66@gmail.com

"TREES IN DIRTH ENDANGER EARTH"

- They have high residual activity and lead to the contamination or pollution of the soil and water, hence causing environmental damage.
- Contamination of food materials, which ultimately enter the food chain and when consume by human beings lead to various disorders and complication to human health.
- These chemicals have broad spectrum of toxicity and hence non-specific in their action and also results in the loss/killing of useful organisms as well.
- Indiscriminate use of these chemicals has resulted in many pathogens becoming insensitive or tolerant to these chemicals.
- They tend to upset the ecological balance, this gives an upper hand to disease causing organisms leading to more frequent outbreak of diseases.

Table: Use of Synthetic Chemicals in India (2005-06)

Sr. No.	State	Synthetic Chemicals (Ton)
1.	Tamil Nadu	12500
2.	Andhar Pardesh	9910
3.	Uttar Pardesh	8480
4.	Maharashtra	6020
5.	Punjab	5770
6.	Gujrat	5500
7.	West Bengal	5000
8.	Haryana	4650
9.	Madhya Pardesh	4500
10.	Rajasthan	2758
11.	Orrisa	1800
12.	Bihar	1700
13.	Kerala	1100
14.	Assam, HP, J&K, Sikkim, Tripura	Minimum Use

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SYNTHETIC CHEMICALS	NEGATIVE EFFECT
Organophosphates: Malathion, Parathion, Trithion, Ethion, TEPP and Fenitrothion.	Organophosphates effect on Nervous System, resulting in convulsions, paralysis, and death. It is similar to Nerve Gas used in World War 11.
Carbamates: Carbaryl (Methyl isocyanate), Carbofuran-Furadan, Aldicarb-Temik and Propoxur-Baygon	Methyl isocyanate (MIC) gas caused Bhopal gas tragedy in 1984.
Dioxin: Herbicides used during Vietnam War to defoliate large area in war zone	Dioxin is extremely toxic to mammals, causing liver disorders, nerve damage and is carcinogen, also damage ecosystems.
Organochlorines: DDT, BHC, Heptachlor, DDE, Chlordane, Lindane, Endosulphan, Aldrin, Dieldrin and Endrin.	Organochlorines act on the Nervous System and also create soil pollution.

BENEFITS

- They have no known environmental hazard.
- They are biodegradable.
- They have very less residual activity.
- They do not cause ecological imbalance.

OBJECTIVES:

- **To determine the antifungal potential of the various plant extracts against plant pathogenic fungi causing diseases in plants.**

MATERIALS AND METHODS:

- **PLANT MATERIALS :**
- Plants materials viz. flower, leaf, root, seed and stem were manually collected from the selected rich sources of plant-diversity areas of Haryana and their neighbor states depending on their periodical and seasonal growth. The collected plant materials were thoroughly washed firstly, with tap water, followed once again with distilled water and then kept at dark in between the filter papers at room temperature for complete dryness. After this, each sample was individually grinded into powder form for further experimental works.

FUNGI: The following different fungi used for experimental work were obtained from the Division of Plant Pathology, IARI, New Delhi. The cultures were maintained at 4°C on Yeast Glucose Agar medium with periodic bi-monthly sub-culturing practices.

- **LIST OF FUNGI:** The fungi experimented with are given below:
- *Alternaria brassicae*
- *Aspergillus oryzae*
- *Chaetomium globosum*
- *Coriolus versicolor*
- *Curvularia lunata*
- *Fusarium moniliforme*
- *Fusarium solani*

ANTIFUNGAL STUDIES:

- **PREPARATION OF PLANT EXTRACT:** Fifteen percent (W/V) plant part extracts was prepared by brewing in hot water for 20 minutes. The assay for antifungal activity of each plant part extract was determined by measuring the growth inhibition as described by Bragulat *et al.*, (1991).

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- A known volume of 15% plant sample extract was supplemented with yeast extract, glucose and agar. The medium was sterilized by autoclaving at 15lb. pressure for 15 minutes. Yeast glucose agar plates, without any plant extract supplementation, was run as a control. The test inoculums consisted of a disc 0.65cm in diameter cut out from the edge of a growing fungal colony on yeast glucose agar medium using a cork borer and placed at the center of the agar medium, in sterile conditions. The experiments were conducted in triplicates along with equal number of controls. The fungi were incubated at 27±1° C and the growth diameters were measured after five days. The percentage inhibition was calculated by the formula as:

  % Inhibition= [(C-T) X 100/C]

  Where C = Diameter of control,

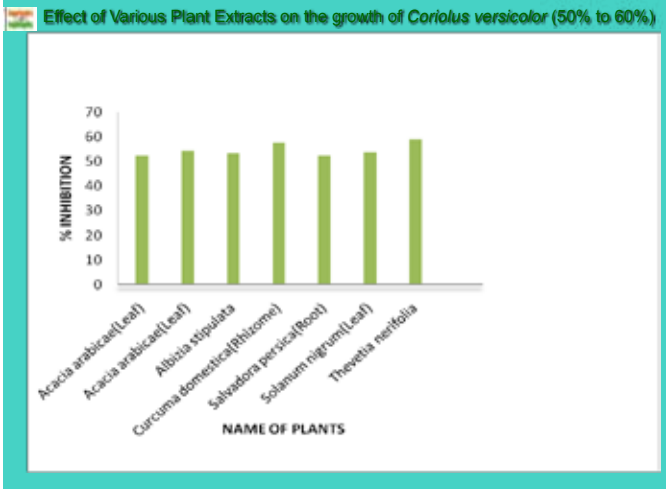
  T = Diameter of test.

Antifungal activity of Plants Extracts against *Coriolus versicolor*:

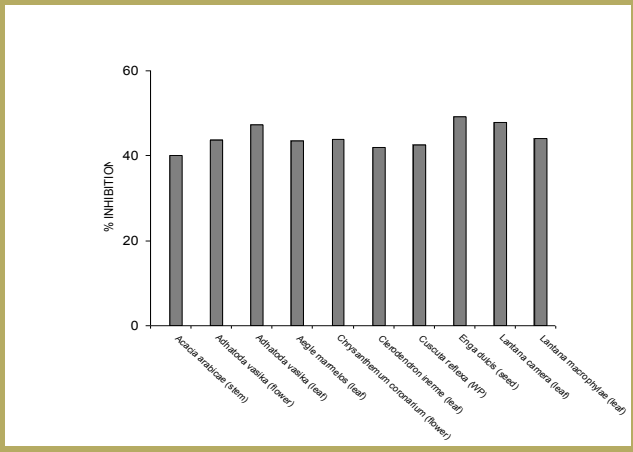
- **Out of 117 plants samples, the reduction in mycelium growth was observed by 77 plants parts extracts which varies in the range of 1.34%(Seed Extracts of *Mimosa hamata*) - 83.72%(Stem Extracts of *Aloe vera*) and the remaining 40 plants parts extracts showed no inhibition.**

Effect of Various Plant Extracts on the growth of *Coriolus versicolor* (60% & above)

Effect of Various Plant Extracts on the growth of *Coriolus versicolor* (50% to 60%)



Effect of Various Plant Extracts on the growth of *Coriolus versicolor* (40% to 50%)



CONCLUSION:

- The study has shown that some plants are very effective in inhibiting the growth of fungus. These plants could be further subjected to field trials to access their effectiveness in field conditions.
- In view of the above facts, the present study has elaborated our knowledge by accessing the antifungal properties among the available local flora which can subsequently be explored for the possibilities towards the identification of the key bioactive agents, through implying modern Microbiology and Biochemical techniques.

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FOREST RESOURCE MANAGEMENT
ON
SCIENTIFIC BASIS

1st INDIAN FOREST CONGRESS
(IFC-2011)
NOVEMBER 22, 2011 NEW DELHI

Dr. G. Kumaravelu
Ex. Full Time Member, State Planning Commission, Tamilnadu

SERVE BACK NATURE

BRING BACK CULTURE

ENSURE OUR FUTURE

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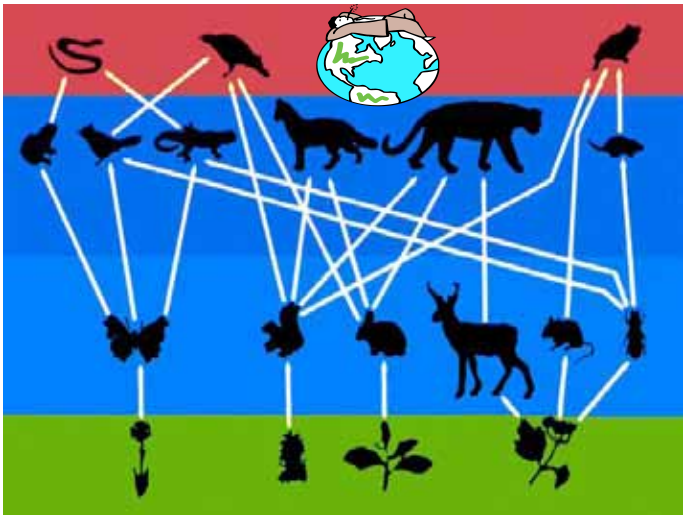

The intrinsic, intimate, interrelationship between the Biotic components consisting of Billion of spp of our planet, in the mantle of abiotic platform, had resulted in Natures stable and self sustaining model and pattern-and is that what we call as 'Ecosystem'

Nature is an infinite sphere of which the centre is everywhere and the circumferences nowhere

- Blaise Pascal,
(French mathematician and Philosopher)

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"Coexistence and harmony
The Balance of Thing"

Our Planetary Ecosystem:

Its Economic Value

All environment goods and services--timber, fishes, watershed functions, soils, climate, biodiversity, etc. are reckoned to be worth

$ 53 trillion per year

or more than the world`s economy of **$ 49 trillion.**

So global natural product is greater than global national product.

The problems we are facing today in Managing Forest Resources can not be solved with the same level of Skill And Will with knowledge, we had at the time of creation of such problems.

We need OUT OF BOX THINKING.

INVESTIGATION, INVENTION AND INNOVATION are the needs of the day.

HEALTH OF THE HILLS
WEALTH OF THE PLAINS

Our Planetary Ecosystem:

Its Economic Value

All environment goods and services--timber, fishes, watershed functions, soils, climate, biodiversity, etc. are reckoned to be worth

$ 33 trillion per year

or more than the world`s economy of **$ 29 trillion.**

So global natural product is greater than global national product.

The considerable array of environmental services generated by Forests is sufficient justification for protecting them.

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A well managed forest should be

Efficient
Orderly
Useful

– **Nancy Langston**
'On Forest Dreams, Forest Night mares'.

As economies keep growing rapidly, they keep on exerting enormous pressure and strain on the finite
'Natural Environmental system that support life on the planet

- Imagine a world without Forests,
- Imagine a world without fishes,
- Imagine a world without Corals,
- Imagine a world where rivers run only in rainy season –

The pity is that it is possible.

Government of developing countries are largely preoccupied with rectifying urban and social problems and often do so, at the expense of environmental degradation.

The extinction of a single species can drive several others to endangered or extinction status .

Cascading effect

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- Ecosystems have to be served back, now, by the humanity – by striking a balance between Ecology,
- Environment,
- Economics,
- Energy and
- Electrons.

We should be a part of the solution and not a part of the problem.

- **CARBON FOOT PRINT**
- **WATER FOOT PRINT.**

Scientific management of Forest resources on sustainable basis lies in stewardship and use of forests in a way, and at a rate, that maintains its biodiversity, productivity, regeneration capacity, vitality and their potential to fulfill, now and in the future, relevant ecological, economic and social functions at local, national and global levels and that does not cause damage to its own and other Ecosystems FAO (1993).

Ecosystem management is atleast as much about managing human activities as it is about managing lands and water

In a scientific and sustainable forest resource management system, rates of tree removal and other managerial activities should be planned according to nutrient budgeting techniques in order to reduce or deter long term degradation of soil nutrients.

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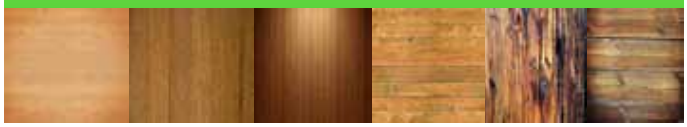
Nutritional status of the soils in the Natural Forest Beats level and its microbial status have to be documented for enhancing the productive potential of the soils

REPLENISHING THE SOIL HEALTH – ORGANIC CARBON AND MINERALS

1.By retaining moribund trees /lops and tops.

2.Substituting easily decomposable wood of equal or more nutrient and mineral value from outside the forest area – (agri-ecosystem)
(Teak can be substituted by trees like Odina wodier, Delonix elata, Ailanthus excelsa, Peltophorum etc)

Analysis of the nutritional status of the secondary fast-woods have to be carried out to compare their status with valuable timbers like teak, rose wood etc.



Fertile soils flourish civilizations

Depleted soils diminish civilization

To reduce the impact of timber harvest on biodiversity, forest management should consider the mosaic of forest patches on the landscape and the connectedness of the habitat for forest species in planning future course of action.

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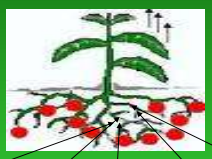
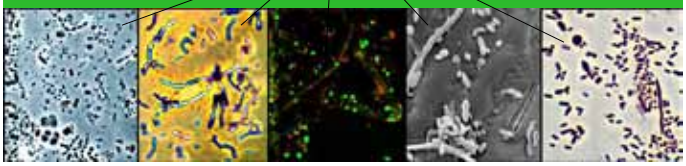
Rhizosphere microflora and microfauna of the main tree species have to be identified, isolated, multiplied and E.M. solution to be sprayed.





BIO DIVERSITY

CONSERVATION

FRAGMENTATION : 'Biodiversity' :

Forest managers must examine effects of fragmentation on a species by species basis with emphasis placed on imperiled species and also `keystone' species that play disproportionally vital role in ecosystem, relative to their abundance and whose removal has large ripple effects on other plants and animals as well as on ecological processes.

1980 – Panama Forest (South America)

19 Trees – 1200 Beetle species.

80% new to science were reported.

Pollinators

- 2,20,000 out of 2,40,000 species of plants require Bees, Butterflies or Birds to get pollinated and set seeds.
- These include both wild plants and 70% of the agricultural crop species that feed the world.
- over 1 lakh different species of Bats, Bees, Beetle, Birds, Butterflies render this vital, life supporting services.

- continuous availability of the diverse forest types, in its climax conditions is essential to sustain viable population of pollinators.
- 1/3rd of human food is derived from plants pollinated by wild pollinators.

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Biological pathogens control

- An estimated 99% of Potential crop pests are controlled by natural enemies,including many Birds, spiders, parasitic wasps, flies and other types of organisms (De Bach 1974).

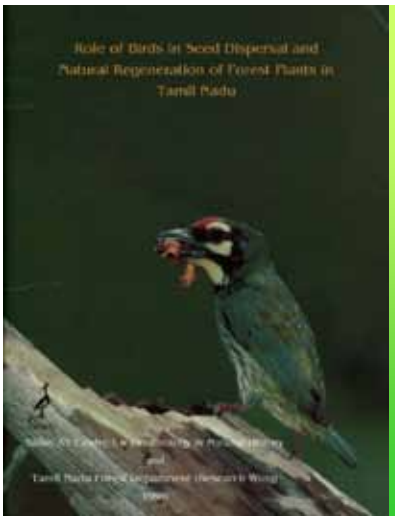
These natural Biological control agents save farmers billions of dollars annually.

POLLINATORS AND SEED DISPERSERS

SKY HIGH FIVE

- Birds
- Bats
- Bees
- Butterflies
- Beetles

Planting of indigenous species yielding food for the pollinators / seed dispersers.



Completed project

(1993-1996)

Collaborative Research

SACON

(Dr.Balasubraniam)

&

Tamil Nadu Forest Department

(Dr.G. Kumaravelu, IFS)

Table 3. Fruiting profile of bird-dispersed plants in the dry mixed deciduous forest (1995-96)

MONTHS

A S O N D J F M A M J J

Table 5. Birds recorded eating fruits in the study area

Family & Species	Common name	Feeding Guild*
BUCEROTIDAE		
Anthracoceros coronatus	Malabar Pied Hornbill	f
CAPITONIDAE		
Megalaima haemacephala	Coppersmith	f
Megalaima viridis	Small Green Barbet	f
Megalaima zeylanica	Large Green Barbet	f
COLUMBIDAE		
Streptopelia chinensis	Spotted Dove	g
Treron pompadora	Pompadour Green Pigeon	f
Chalcophaps indica	Emerald Dove	f
CORVIDAE		
Corvus macrorhynchos	Jungle Crow	o
Corvus splendens	House Crow	o
Dendrocitta vagabunda	Southern Tree Pie	i
CUCULIDAE		
Eudynamys scolopacea	Koel	f
DICAEIDAE		
Dicaeum erythrorhynchos	Tickell's Flower Pecker	n
IRENIDAE		
Chloropsis aurifrons	Gold-fronted Chloropsis	i
Chloropsis cochinchinensis	Gold-mantled Chloropsis	i
Irena puella	Fairy Bluebird	f
MUSCICAPIDAE		
Copsychus saularis	Magpie Robin	i
Turdoides striatus	Jungle Babbler	i
Sylvia curruca	Lesser Whitethroat	i
ORIOLIDAE		
Oriolus oriolus	Golden Oriole	f
Oriolus xanthornus	Black-headed Oriole	f
PSITTACIDAE		
Loriculus vernalis	Indian Lorikeet	g
Psittacula columboides	Blue-winged Parakeet	g
Psittacula cyanocephala	Blossom-headed Parakeet	g
PYCNONOTIDAE		
Hypsipetes indicus	Yellow-browed Bulbul	f
Pycnonotus cafer	Red-vented Bulbul	f
Pycnonotus jocosus	Red-whiskered Bulbul	f
Pycnonotus luteolus	White-browed Bulbul	f
STURNIDAE		
Acridotheres fuscus	Jungle Myna	f
Acridotheres tristis	Common Myna	o
Gracula religiosa	Hill Myna	f
Sturnus pagodarum	Brahminy Myna	f
Sturnus malabaricus	Grey-headed myna	f
NECTARINIDAE		
Nectarinia lotenia	Loten's Sunbird	n
Nectarinia zeylonica	Purple-rumped Sunbird	n
ZOSTEROPIDAE		
Zosterops palpebrosa	White Eye	i

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APPENDIX - A

Bird-dispersed plants recommended for planting

Botanical name	Local name	Habitat
Trees		
Canthium dicoccum	Pepra maram	MDD
Cassine glauca	Perunkil	MDD
Celtis philippensis	Vaka maram	Stream
Carallia integra	Pukkuchati pazham	Stream
Drypetes sepiaria	Vana maram	MDD
Elaeodendron glaucum	.	MDD
Ficus benghalensis	Aala maram	MDD
Ficus virens	Rihi maram	MDD
Ficus microcarpa	Rihi maram	MDD
Grewia orbiculata	Sappa maram	MDD
Ixora pavetta	Cora maram	MDD
Mallotus resinosus	Shiv panara	Stream
Manilkara roxburghiana	Palai	Stream
Morinda coreia	Pachi maram	MDD
Premna serratifolia	Perlia maram	MDD
Strychnos nux-vomica	Kanchara maram	MDD
Schleichera oleosa	Poovathi	Riverine
Santalum album	Chandana maram	MDD
Syzygium cumini	Naval	Riverine
Strychnos potatorum	Dilla maram	MDD
Vitex altissima	Payila maram	Riverine
Zizyphus oenoplia	Soozi	MDD
Shrubs		
Berberis nilagirica	Pala	MDD
Capparis sepiaria	Thoratti	MDD
Carissa spinarum	Siru kila	MDD
Glycosmis pentaphylla	Rata pana	Riverine
Hygrophila spinosa	Nellika kodi	MDD
Pachygone ovata	Javanan kodi	Stream
Scutia myrtina	Thoranti	Stream
Toddalia asiatica	Chedalai mulla	MDD

MDD= Mixed dry deciduous

40

Identification of suitable native bird-dispersed species for the Tamil Nadu Afforestation programme

- **Frugivorous bird species: 66**
- **Bird-dispersed tree species: 219**
  - Lauraceae
  - Euphorbiaceae
  - Moraceae
  - Rubiaceae
  - Meliaceae
  - Myrtaceae
- **Species suitable for different habitats suggested**
- **Fruiting calendar provided**

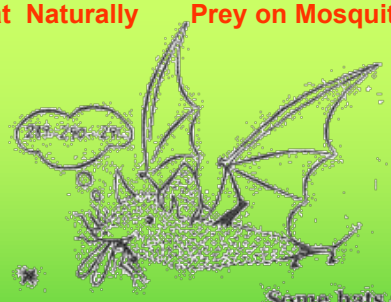
We May Extinct Because of Environmental Degradation

Please . . . . .

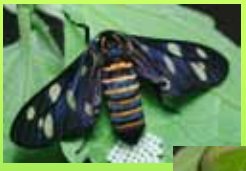
Know About Us

We Need Friends Like You

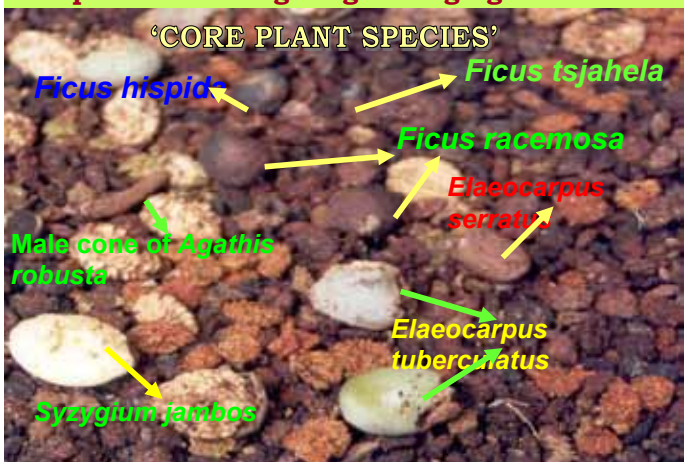
Bats Are Just One of Several Groups of Animals That Naturally Prey on Mosquitoes



Some bats can catch up to 600 mosquitoes in one hour.



To Get Balanced Diet Visit Varieties Of Plant Species Covering Large Foraging Area.



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- Enhancing the photosynthetic efficiency of the Eco systems through increase in the proportion of the juveniles in the population and also by deliberate choice of species to be encouraged by Nat

- More anabolism
- Less Catabolism

Young Forests tend to accumulate more carbon than the mature forests.

Need: Enhance the preponderance of young green leaves in the ecosystem

Endemic Plants Dispersed
by Fruit Bats in Tropical Wet Evergreen Forest

Sl. No	Name of the plant	Habit	Endemic status	Red list status
1	*Elaeocarpus venustus* (Bedd)	Tree	E	EN
2	*Elaeocarpus munroii* (Wt.) Mast	Tree	E	R
3	*Syzygium mundagam* (Bourd.) Chitra	Tree	E	VU
4	*Ensete superbum* (Roxb.) Cheesman	Shrub	E	-
5	*Pallaquim ellipticum* (Daiz.)Baill	Tree	E	-
6	*Aglaia elaeagnioides* (Juss) Benth .var. bourdillonii (gamble) KKN Nair	Tree	E	EN
7	*Garcinia gummi-gutta* (L.) Roxb	Tree	E	EN
8	*Musea ferrea*	Tree	E	-

- **We need them for survival**
- They may not need us.
- If not empathy, at least sympathy.

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WE HAVE TO ASSURE

HAPPY LIFE TO ALL

LIFE FORMS

OWLS:

Though none of the owls in the southern western Ghats are under the threatened category, their ecological function as a top predator and umbrella species in the forest ecosystem implies that it is important for the forest managers and conservationist to study their distribution and population status.

HABITATS

Do what we have undone

- **> Crevices**
- **> Caves**
- **> Holes and Boles in Trees (Wolf Trees)**
- **> Bird Houses simulation in nature**

RE - SEARCH

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BIO – DIVERSITY CONSERVATION

AND

BIO – PRODUCTIVITY ENHANCEMENT

(Forest & Agri Ecosystems)

Every drop of WATER

Every grain of SOIL

Every ray of SUN

- S Y N E R G Y -

VAM using Sand Medium

Production of Azolla

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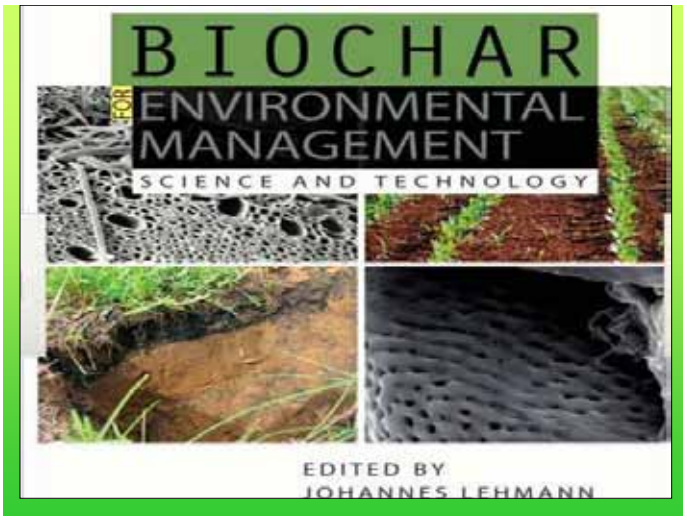
Role of Modern Nurseries in Rural Development

During, 1998-1999 six modern nurseries have been established by the Research wing. The nurseries produce Vermicasting, VAM (Vesicular Arbuscular mycorrhiza) and bacterial bio fertilizers

During the last few years more than 3000 tones of Vermicasting, 800 tones of VAM, 500 tones of Bio fertilizers produced and 5 crores tree seedlings raised by the Department inoculated with bio fertilizers and bio nutrients.

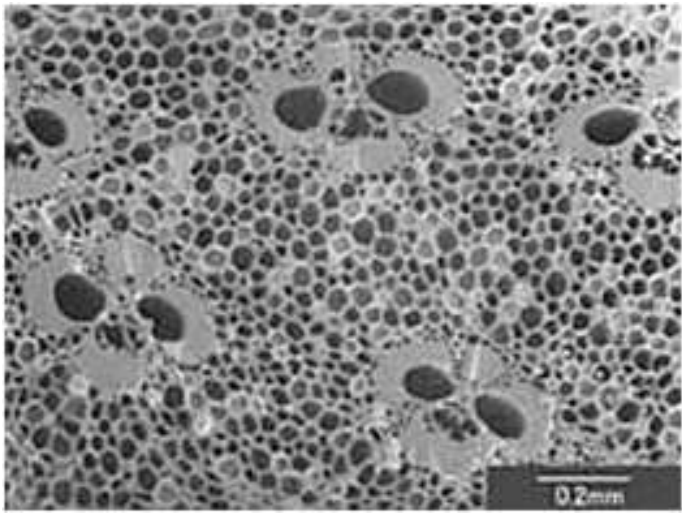
Modern Nursery, Thoppur

Vermicasting production for Tree farming - Employment generation



BIOCHAR

"There is one way we could save ourselves from global warming and that is through the massive burial of charcoal".



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- Water content of charcoal layer in the soil was remarkably higher by 40% even in mid summer compared with 5% in the outside charcoal zone soil mass. (Japan Biochar Association-JBA)

- Growing Trees and burying charcoal is the apt method of carbon sequestration.
  - CARBON FARMING to mitigate GLOBAL WARMING

- In Japan, at least 100 thousand tonnes of Biochar is applied to agricultural lands annually. They contain 80% carbon and so 250 thousand tonnes of CO_2 are shut in the soil and locked without leakage.

  -CARBON FARMING.

INVASIVE EXOTICS

- Bio char
- Briquettes - Pellets
- Biogas
- Bio plastic

Biocoal Thermo hydro carbonization
Biochar
Cellulosic Ethanol/Butanol (Electro Hydrogenesis)
Biomass Humic Acid → (5hrs) Bio char → (8 hrs) Bio coal → (12 hrs)

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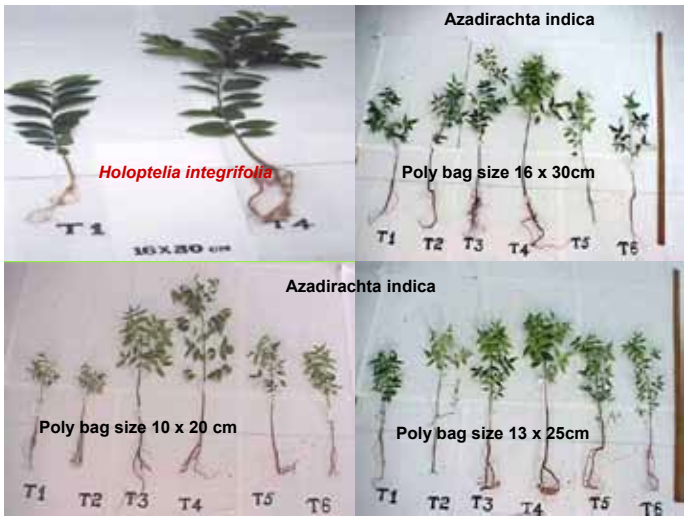
Experiment on dosage of of bio-fertilizer, bio-nutrient inoculation on tree seedlings.

Bag size	T1	T2	T3	T4	T5	T6
10 x 20cm	Control Without addition of Biofertilizers	Vermi - 5gms VAM - 3 gms Azos/Rhizo- 1gm Phospho - 1 gm	Vermi - 10gms VAM - 5 gms Azos/Rhizo- 2gm Phospho - 2 gm	Vermi - 15gms VAM - 7 gms Azos/Rhizo- 3gm Phospho - 3 gm	Vermi - 10gms	DAP - 3gms
13 x 25cm	Control Without addition of Biofertilizers	Ver - 15gms VAM - 5 gms Azos/Rhizo- 3gm Phospho - 3 gm	Ver - 20gms VAM - 7 gms Azos/Rhizo- 4gm Phospho - 4 gm	Ver - 25gms VAM - 10 gms Azos/Rhizo- 5gm	Ver - 20gms	DAP - 7gms
16 x 30cm	Control Without addition of Biofertilizers	Ver - 25gms VAM - 10 gms Azos/Rhizo- 4gm Phospho - 4 gm	Ver - 30gms VAM - 12 gms Azos/Rhizo- 5gm Phospho - 5 gm	Ver - 35gms VAM - 15 gms Azos/Rhizo- 6gm Phospho - 6 gm	Ver - 30gms	DAP - 10gms



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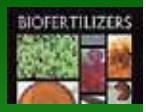
(without bio-fertilizer) (with bio-fertilizer)

ACACIA HYBRID GROWTH AFTER 16 MAP

All the seedling to be planted should be inoculated with appropriate dose of the bio-nutrients and bio-fertilizers.



Cogeneration of Wood and Food

Perennial intercrops, diversified income add value per unit of land, improve cash flow and cause only a limited loss of main crop in Agriecosystems.

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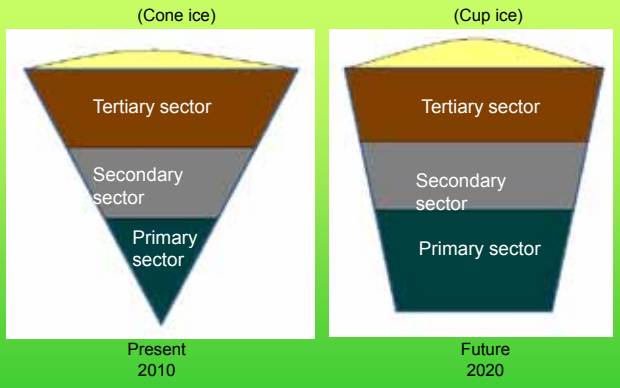
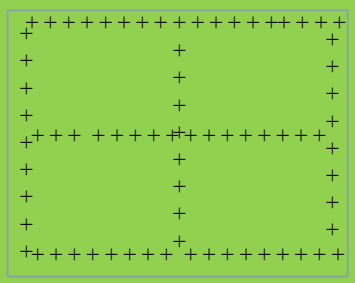
- **Trees to be planted on the Bunds of cultivated lands and inside in rows**

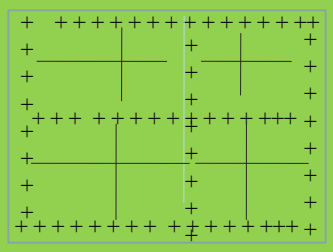
INDIAN ECONOMY



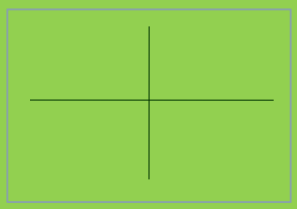
MODEL I
Only bund planting (4 acre plot)



Model II
Bund + '+' model



Only '+' model (1 acre)



- Model-I : Only Bund/acre : 320 Nos.
- Model-II: Bund +, '+' design : 400 Nos.
- Model-III: Only '+' design : 80 Nos.

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- Out of 125 lakh acres of cultivable lands, for growing 50 crore trees in 5 years, we need only 15 lakh acres.
- Income generated per year by the wood crop in the rural areas of TN will be a minimum of Rs.10,000 crores. This will be in addition to the income generated by the food crops.

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ROOT ARCHITECTURE


Drought tolerant Trangenic Plants

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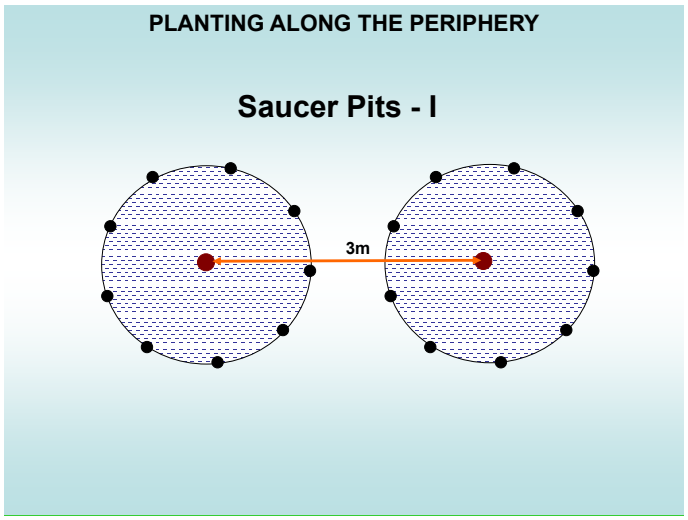

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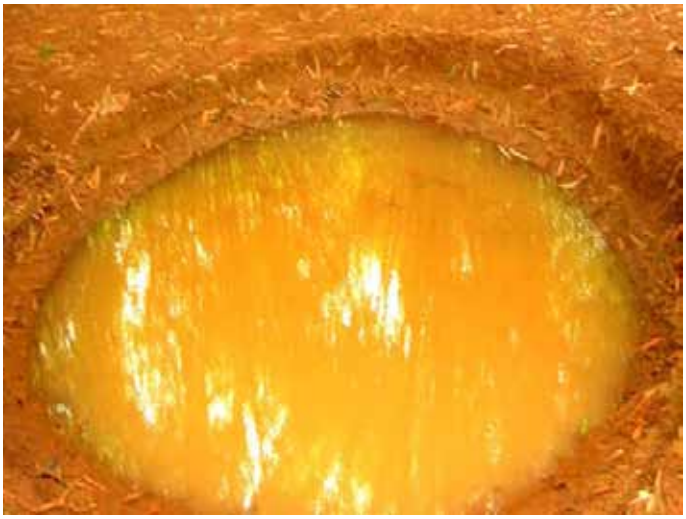
Sand dune afforestation

18 months Appreciable growth of Acacia hybrid in Theri Pattakarai Research center

Eastern ghats

Rain water harvesting



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Saucer : 284 Sq.km
100mts within RF boundary (70000 acres)
10 lakh litter /acre/year /rainwater
7000 crore litter rainwater harvesting

Trench : 5000 crore litter water

Total : 12000 crore rainwater can be harvested

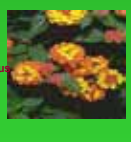
about 3 to 4 lakh acres of Agricultural lands adjoining RF can be irrigated suggested crop.

Suggested crop: Redgram, Kambu, Beans.

Periodic monitoring of forest resource has been made possible with the availability of satellite images of varying resolutions. Maps categorizing forest practices areas help in identifying areas beset with problems.

INVASIVE EXOTICS:

High resolution mapping of the vegetation of the forest areas exhibiting invasion of the exotic weeds can result in GPS based distribution map of the weeds. This can be an excellent tool for scientifically eliminating or curtailing the invasion. Thus Forest Resources could be saved and its dynamics and vitality could be resurrected.



LAND SLIDES: Cause damage to the Forest resources. Prevention of such damages are prudent than repairing. GIS and Remote sensing based approach can be of great utility value. An efficient and accurate method of generating Landslide Hazard Zonation data is very important to mitigate the loss of properties and lives caused by landslides.

The Recreational value of tropical forests has largely been underestimated thus for. Eco tourism is an emerging economic activity with tremendous potential to generate foreign exchange for tropical countries.

Some of the future Research Topics on Forest Resource Management could be as following…..

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- Food-Web, Food-chain Research of each forest types to understand the interrelationship of the Biotic and Abiotic component.
- Ecosystem service Evaluation.
- Carbon sequestration potential of the Forest, the associates, consociates, species and their individual phenotypes and/or genotypes.
- Enhanced 'carbon credit' earning tree species identification. (to be utilized under 'cogeneration of Wood and Food' programmes.)-

Global Warming and Carbon Farming

- Hydrological auditing of Natural Forests and Tree Farms.
- Root architecture studies of tree species to evolve most effective polyculture models that enables appropriate and adequate utilization of every drop of water, every grain of soil and every ray of sun for maximizing the benefit flow.
- Identifying, isolating and multiplying the Rhizosphere micro flora and micro fauna from each of the Forest type soils.

- To enhance the productive potential of soils and also carbon sequestration capacity by the use of appropriate mix of Bionutrients and Biofertilizers

in

degraded forests in the Reserve Forests.
wastelands and wasted lands
rainfed farm lands and
waterbodies like tank foreshores.

Use of
Antitranspirants
in Nurseries

Foliar spray of seaweed extracts in the Nursery

Underexploited
Native
Fodder
Species
(Penning of goat)

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- Where?
- How?
- Who?
- When?
- Cost and Time Factors to be spelt out clearly.

PERFECTION IS A MOVING TARGET

BETTER LATE THAN NEVER OR EVER

To help guide decision Making, on the variety of options available to improve management of Forests, improved valuation is needed.

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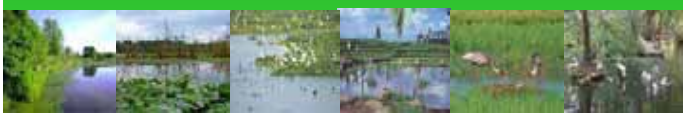
Radio collaring, monitoring and documenting the migratory path, both local and long distances, of animals, all through the year, in various seasons is a must.

Pinch period migration scientific studies can help in an efficient forest management through effective application of the knowledge gained based on carrying capacity assessment.

Vanished Wetlands- should be traced out by application of science, that could enable their resurrection and amplification with all other connected life forms.



Geospatial technology aids policy makers and researchers in the acquisitions of the data that is necessary to further research, manage and recover present and future conditions of the global forests.

The composition and viability of forest may be determined using a combination of remote sensing and geographic information systems (GIS).

Many applications of forestry and natural recourses require accurate change analysis.

Our culture emanated from Nature.
Therefore,
Nature is our culture.
Future is dependent on Nature.
Therefore, Nature is our future.

Future is our choice and not a fate.

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NATURAL FOREST MANAGEMENT – *Issues & Approaches*

Manoranjan Bhanja
APCCF (Research)
Andhra Pradesh

Nov 22, 2011

Widening gap between the societal demands on the forest and the capacity of the forest to supply them on sustainable basis

Challenges for Indian Forestry Sector

- Widening gap between the societal demands on the forest and the capacity of the forest to supply them on sustainable basis
- This widening gap is the major driver of forest degradation and loss of forest biodiversity
- Existing administrative structures & functions, planning & control system and research & training methods should all be geared towards securing a sustained supply of timber and other forest produce.

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Challenges for Indian Forestry Sector

- **The degradation/depletion of the forest is not a specific forestry problem but rather a social problem linked to population growth and poverty.**
- **Why there is a dichotomy between Richness of natural resources and Poverty of the poverty line.**
- **Our strategy is to marry conservation with commercialization, create an economic stake in conservation, make livelihood security and ecological security two sides of the same coin.**

PAST SYSTEM OF MANAGEMENT

- **National Forest Policy 1894 –** commercial interest and development of agriculture. Forest divided into 4 categories:

a) Forest on hill slopes for protection
b) Commercial timber forest for harvest
c) Minor forests for meeting people's needs
d) Pasture & grazing grounds

Peoples' interest were made subservient to State's commercial interest during colonial rule

NATIONAL FOREST POLICY, 1952

Shift from production forestry to focus on meeting objectives of maintaining ecological balance on the one hand and meeting the needs of the stakeholders on the other. Divided the forest into three functional categories:

a) Protection Forests
b) National Forests
c) Village Forests
d) Tree Lands

The reason for ineffectiveness was that this policy was issued as a resolution by Govt. but was not adopted by State Legislatures

NATIONAL FOREST POLICY, 1988

Focused on ecological, economic and social aspects of forest development.

a) Maint. of environmental stability
b) Conservation of natural heritage by preserving the natural forests
c) Meeting the basic needs of people
d) Relationship between tribals & other forest dependent people.

Sustainable management & livelihood security of forest-dependent communities

Participatory Forest Management

- **JFM Resolution, 1990: A paradigm shift in forest management from Govt. management to participatory management with communities**

a) Rehabilitation degraded forest
b) Capacity enhancement
c) Institution building
d) Equity in participation & Benefit sharing

- **JFM Guidelines 2000: Strengthening the mandate, focussing on womens' participation and structural issues**

Net-Change in the Forest Cover since 2001 Assessment

(Area in km^2)

Assessment Year	Dense Forest	Open Forest	Total Forest Cover
2001	416,809	258,729	675,538
2003	390,564	287,769	678,333
Change	- 26,245	29,040	2,795
2005	403,420	286,751	690,171
2007	402,522	288,377	690,899
Change	- 898	1,626	728

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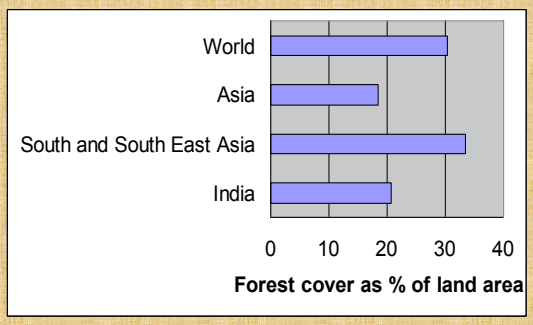
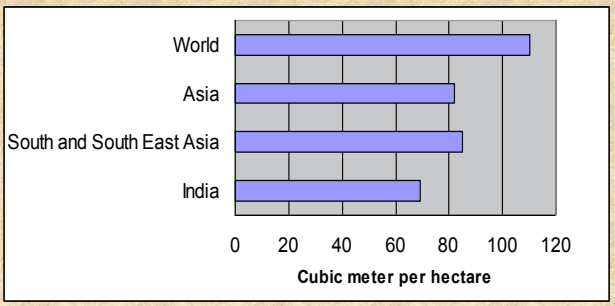
Comparative Figures of Forest cover 2003 Assessment



Comparative Figures of Growing Stock (2003 Assessment)

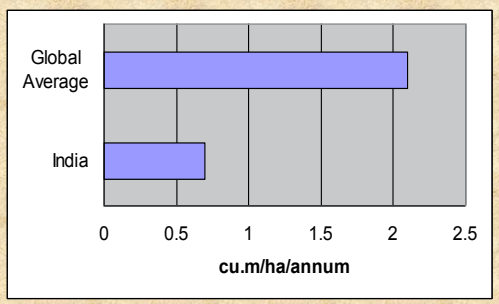


2007 Assessment: Total GS of India is 6098.23 m cum (Inside Forest: 4498.66 m cum & TOF: 1599.57 m cum)

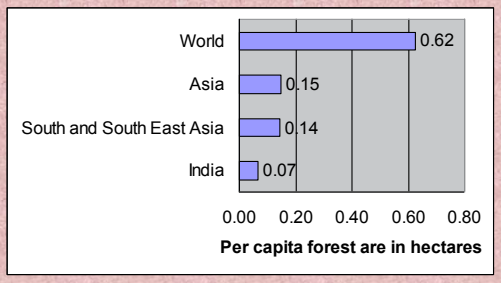
GS in forest for top 10 forest species – 2007 Assessment

Name of the Species	Total Vol %	Total Stem %
Shorea robusta	8.53	8.13
Tectona grandis	4.59	7.32
Pinus roxburghii	3.10	2.14
Terminalia crenulata	3.06	3.74
Anogeissus latifolia	2.80	4.26
Abies pindrow	2.47	0.44
Quercus semicarpifolia	2.15	0.96
Cedrus deodara	2.05	0.59
Pinus excelsa	2.03	0.83
Abies smithiana	1.98	0.20

Forest Productivity – 2003 Assessment



Per Capita Forest Area



Resource Assessment – What is missing from Indian Forest Data

- No reliable assessment of growing stock of trees at state level.
- Other deficits include a lack of data on different forest products from the forests and a lack of increment and biomass data.
- No efficient inventory for 'trees outside forests'

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Resource Assessment – What is missing from Indian Forest Data

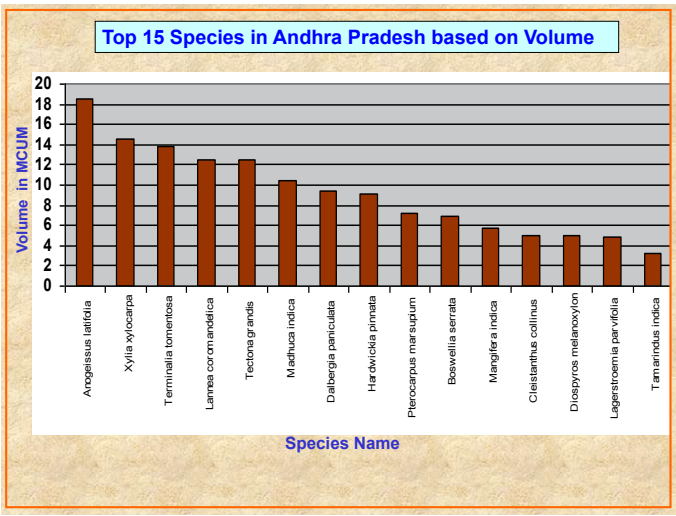
- Many data gaps with respect to the production and consumption of NTFPs.
- No data and statistics on the ecotourism, either in terms of demand or supply.
- No reliable data about changes in the health and vitality of ecosystem including micro-watershed, nutrient status and biodiversity – population size & threat status.

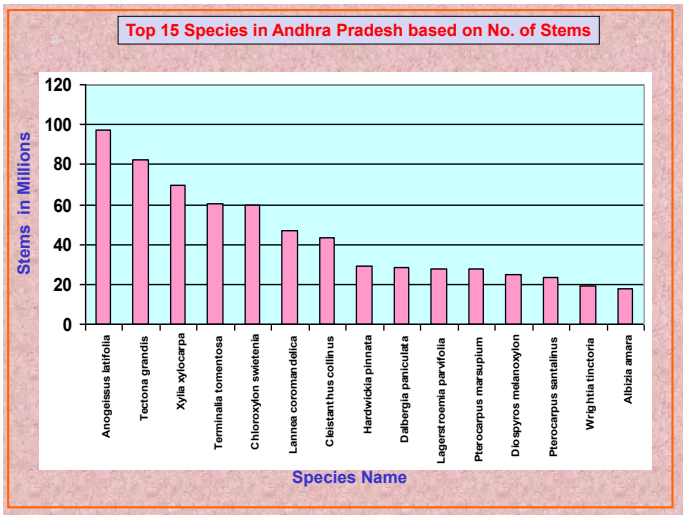
RESOURCE ASSESSMENT

- Already we are getting real time assessment of fire, encroachment etc. However, Real Time Assessment of disease, health of watershed etc. are required which can help to take premptive action
- Can this technology be extended to assessing the regeneration status of various commercial species in natural forest
- Develop the mechanism to estimate the resource potential of NTFP species.
- Predictive growth yield model for different type of forests and commercial valuable species.

NATURAL FOREST MANAGEMENT – Present Scenario

- Practically stagnant or no work is going on silviculture and forest management
- Except Teak and bamboo, not much timber is coming from the forest.
- The inventories suggest that the contribution of commercial timber species to the growing stock of forest is fast decreasing.
- Regeneration is insignificant for these valuable spp.
- Yield of bamboo coupes decreasing due to lack of appropriate clump management interventions in natural bamboo forests.





NATURAL FOREST MANAGEMENT – Present Scenario

- Substantial decrease in the yield of NTFP & medicinal species.
- Retrogressive succession is fast engulfing the different types of forest types giving place to obnoxious weeds.
- Serious impairment of nutrient cycling of the forest ecosystem because of regular incidences of fire, uncontrolled grazing, erosion and compactness of the site.

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NATURAL FOREST MANAGEMENT – Present Scenario

- Advent of JFM & CFM to manage and protect the degraded forest through manipulation of various cultural operations (revitalization of viable rootstock of economically important species, stool coppicing, singling, gap planting; clump management of bamboos; preferential treatment of NTFP species) with intensive smc works.

- No sustainable livelihood base in terms of short-rotation plantations to get continuous annual economic returns

WHAT NEEDS TO BE DONE FOR NFM SECTOR?

- Developing mechanism and models of reversing the trend of retrogressive succession by understanding the site and the vegetation ecology
- Models of appropriate ANR model keeping in view the mandate of increasing the population of economically valuable species.
- What can be done to improve the regeneration status of these important species and make the new recruits / regeneration to go to the pole stage - Regeneration trials of important species
- Revival of existing & setting up of new Preservation / Sample plots

WHAT NEEDS TO BE DONE FOR NFM SECTOR?

- What can be done to improve the nutrient cycling as a tool for productivity enhancement of natural forest
- The silviculture of many of the secondary hardwood species needs to be studied with special emphasis on natural regeneration.
- Focus on coppice management
- How to manage the teak forest after the final harvest – what can be done to restore the vigor of fresh teak plantations?

WHAT NEEDS TO BE DONE FOR NFM SECTOR?

- Can we think up revisiting the existing forest types and assessing the changes therein and re-categorizing the areas.
- Developing key indicators to assess the impact of forest interventions over a period of time
- Developing Predictive Growth Yield Model for the various natural forest & plantations
- Forest certification – How to go about?

MANAGING FORESTS TO PROTECT CATCHMENT FOR WATER

- Whether forested watersheds offer real benefits for water supply?
- If they do, how much forest is required to gain these benefits?
- How forests in watersheds can be managed to protect water supplies?
- Monitoring the over ground water flow in forest streams and the underground water table in the adjacent agriculture fields
- Developing the micro-watershed plan treating the catchment to its full saturation level.

WHAT NEEDS TO BE DONE FOR NTFP

- NTFP potential of the natural forest is overestimated and the management input is virtually nil
- Development of an integrated multipurpose management system of forest resources under a holistic ecosystem approach for wood and non-wood products.
- Need for co-ordinated conservation action based on both *in-situ* & *ex-situ* strategies
- Fixing a limit of harvest for each NTFP species.
- Development of sustainable harvesting protocols for the NTFP species including medicinal plants.

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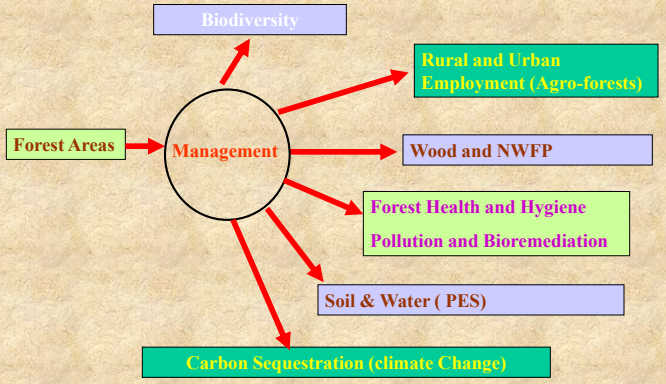
WHAT NEEDS TO BE DONE FOR NTFP

- Development of new and innovative methods of value addition of forest produce
- Encouragement for micro-enterprise development by indigenous & rural communities
- Species domestication & crop variety breeding should be given top priority in NTFP resource management
- Work on identification of potential impt. drug-producing plant resources & development of appropriate biotechnologies to tap these potentialities.

Sustainable Forestry



FORESTRY PLANTATIONS – Present Scenario

- Most technological developments in forestry is focused on forestry plantations – Industry is always in front
- Plantation is confined to very less number of commercially important short-rotation forestry crops and even very less number of long-rotation high-value timber species (except teak) or secondary hardwood species.
- More stress on monoculture in plantation programme

FORESTRY PLANTATIONS – Present Scenario

- No particular emphasis on site management and no crop husbandry protocols are available for forestry crops.
- Lack of effective integrated pest management schedule in large scale plantations.
- Many of the FD plantations are either not productive beyond the maintenance period or struggling to survive.
- Planting stock improvement & Clonal forestry is confined to Euca, *Casuarina* & Poplar.

WHAT NEEDS TO BE DONE IN PLANTATION FRONT

- Identification of 10-15 key farmer-centric high-value short-rotation species for expansion of tree cover outside the forest.
- Tree Improvement Programme should cover many secondary hard wood species and the results of the genetic gain should be very clearly visible in the field for the adoption by user agency.
- The sustainable genetic gain achieved by the Breeders is not passed over to sustainable yield – Needs developing strategies
- All plantations must be developed under high-input-high-output strategy
- Cost-economics of different plantation models should be worked out and demonstrated in the field.

Operationalising new SRFP Models

- Silveroak for timber
- *Eucalyptus & Bamboo* for pulp
- *Ailanthus* and *Melia dubia* for plywood
- *Anthocephalus* with *Gmelina* for plywood
- *Melia dubia* with *Casuarina jhunghuiniana* in high-density plantation

Anthocephalus cadamba

Gmelina arborea

Acacia mangium with Piper longum & Rauvolfia

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ECONOMIC PERFORMANCES OF SRFP SPECIES

Species	Project period (in Yrs.)	Proj. Cost / Proj. Income (in Rs.)	NPV at 15% (in Rs.)	IRR (%)	BCR
Eucalyptus (Clonal)	12 (6 + 6)	83316 / 407560	68176	33.12	1.33:1
Casuarina	4	38532 / 165000	48906	42.00	1.42:1
Bamboo	21	276229 / 682704	39881	24.22	1.24:1
Gmelina (clonal)	12	105040 / 1194320	190243	36.25	1.36:1
Teak (clonal origin)	30	215374 / 4112500	98657	25.26	1.25:1

Eucalyptus *Casuarina* *Gmelina* Bamboo Teak

WHAT NEEDS TO BE DONE IN PLANTATION FRONT

- Bringing more species of quality hardwood species into the realm of clonal forestry and plantation programmes duly reducing gestation of crop & increasing productivity of the spp.
- Develop the multi-tier and mixed plantation models for maximizing the ecosystem value and the economic returns
- Domestication of indigenous fast-growing short-rotation crops

DEPLOYMENT OF NON-TEAK QUALITY HARD WOOD SPECIES IN PLANTATION PROGRAMME

Deploying LRHT species in plantation to improve the economic quality of forests

Tectona grandis

Mitragyna parvifolia

Dalbergia latifolia

Adina cordifolia

Pterocarpus marsupium

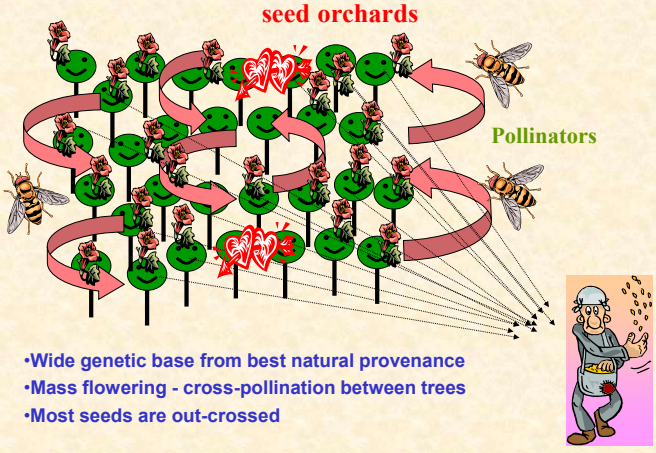
Creation of improved Seed Stands

Development of CSO for high value timber species as a species germplasm security for future and sustained assured supply of quality seeds for plantation programme.

CSO – *Gmelina arborea*

CSO – *Tectona grandis*

High-quality seed from heavy synchronous flowering in seed orchards



- Wide genetic base from best natural provenance
- Mass flowering - cross-pollination between trees
- Most seeds are out-crossed

What forest changes are expected?

What impacts are expected:

- Loss of area under a given forest type and replacement by another type.
- A few species may show a steep decline in populations or may get locally extinct.
- Changes in biodiversity.
- Increased incidence of fire and drought.
- Spread of Invasive species to new areas.

56% of the vegetation grids are projected to undergo change by 2030s. NPP is projected to increase by 57%

Source: INCCA, 2010

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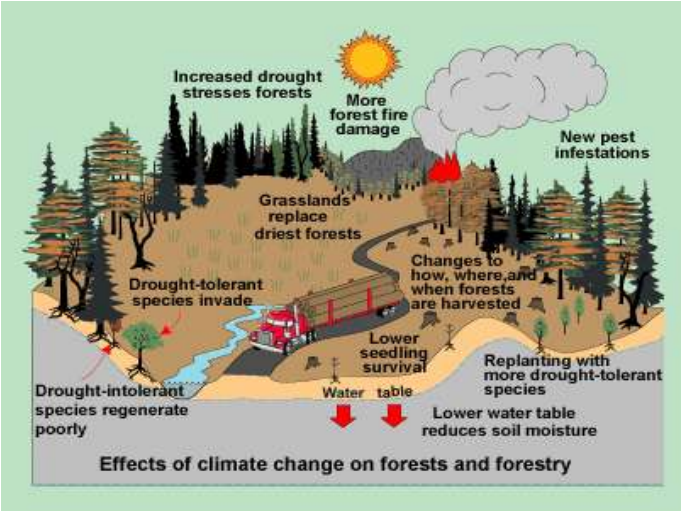

Issues to be addressed in Climate Change

- Monitoring of parameters relevant to impacts on forest vegetation due to changing climate, e.g., phenology, species diversity/composition etc.
- Developing models for assessing carbon sequestration and mitigation potential of major species & different forestry and plantation activities.
- Developing simple methods for determining rates of changes in carbon pools under different forest and plantation systems for formulation of CDM projects.

Issues to be addressed in Climate Change

- Long-term monitoring of ecological processes and changes.
- Standardization of credible and efficient method of valuation of environmental services of different ecosystems
- How climate change will have effect on tree growth and wood formation, possible physiological and anatomical changes

CONSERVATION ISSUES

- Periodical inventory of flora and fauna on regional basis or ecotype basis and studying the various biodiversity parameters.
- Development of Biodiversity database for major forest groups and types.
- Documentation of traditional knowledge and attempt to obtain IPRs of for the benefit of the community & nation

CONSERVATION ISSUES

- Assessing threat status of some of the vulnerable species in various biodiversity hot spots.
- Developing species recovery plan for the RET species.
- Implementing various *ex-situ* conservation strategies for all valuable and vulnerable species. Regional Arboreta or Plant Resource Centers in various places of the country should be established

LOOKING AHEAD

- *To achieve enhanced productivity, there is a need for increased scientific intervention using available genetic material and biotechnologies coupled with introduction of plantation models involving high -input - high - output strategy.*
- *Also there is a need for effective appropriate low-cost management intervention in natural forest which may reverse the succession stage of degraded forest and thereby increase the quality and value of growing stock.*

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The Fractionation of Pectin for the period of fruit-ripening in *Diospyros peregrina*

CHEMISTRY DIVISION
FOREST RESEARCH INSTITUTE, DEHRA DUN

RESEARCH SCHOLAR :

DEEPIKA CHAUHAN

SUPERVISOR :

DR. P.K.GUPTA

intention

To scrutinize pectin as of fruits of *Diospyros peregrina* all the way through fractionations at some stage in fruit-ripening

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IMPLICATION OF PECTIN

- Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic acid residues.
- Pectin is a structural heteropolysaccharide enclosed in the crucial cell walls of terrestrial plants.
- It is created commercially as a white to light brown powder, essentially extracted from citrus fruits and is used in food as a gelling agent particularly in jams and jellies.
- It is also used in fillings, sweets as a preservative in fruit juices and milk drinks and as a resource of dietary fiber.

DIOSPYROS PEREGRINA

- *Diospyros* is a large genus of shrubs and trees comprising of 500 species distributed in the warmer regions.
- It belongs to the family Ebenaceae.
- About 41 species occur in India mostly on evergreen forests of Deccan, Assam, and Bengal; only few are found in North India.
- Common Name: Kalatendu

DIOSPYROS PEREGRINA

LEACHING OUT OF TANNINS WHICH GIVE TARNISH MANIFESTATION TO THE FRUITS

EFFUSIVE RIPE FRUITS OF *DIOSPYROS PEREGRINA*

UNRIPE FRUITS OF *DIOSPYROS PEREGRINA*

GREEN SCIENCE OF PECTIN

- Pectin is present all over primary cell walls but also in the middle lamella between plant cell walls where it helps to unite cells together.
- Pectin is a innate ingredient of human diet, but does not contribute drastically to nutrition.
- The scheduled ingestion of pectin as of fruit and vegetables can be predictable to be around 5g(assuming consumption of approximately 500g fruit and vegetable per day).

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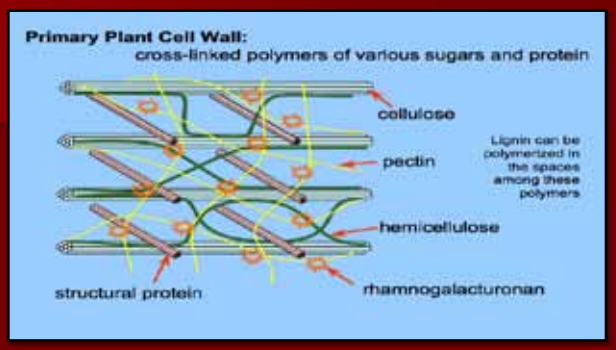
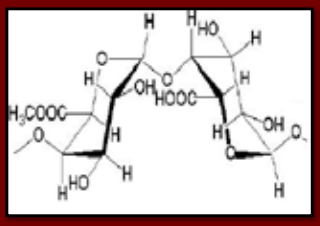


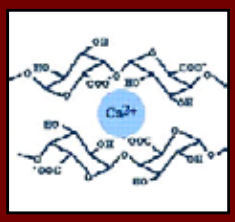
Pectin is a complex carbohydrate, which is found both in the cell walls of plants, and between the cell walls, helping to regulate the flow of water in between cells and keeping them rigid.

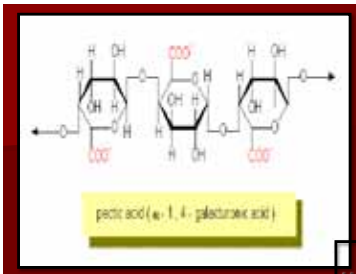
CHEMISTRY OF PECTIN

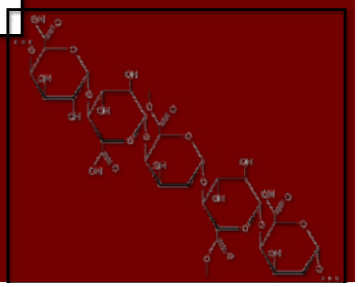
- The attribute structure of pectin is a linear chain of alpha-(1-4)-linked D-galacturonic acid that forms the pectin-backbone,a homogalacturonan.
- The non-esterified galacturonic acid in units can be either free acids(carboxyl grps.) or salts with Na,K or Ca.
- Some of the galacturonic acid is renewed with ammonia to carboxylic acid amide.

The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5% The salts are called pectates, the insoluble acid form, pectic acid.









IMPACT OF PECTIN

Pectin provides contour to the soft non-woody parts of the plant.

Pectin in plant cell walls plays as vital role in the ripening, texture, and storeroom qualities of fruits and vegetables.

KINDS OF PECTINS AND THEIR USES

Rapid Set Pectin – traditionally used for jams and marmalades.

Slow set Pectin – used for jellies and for some jams and preserves, especially using vacuum cooking at lower temperatures. Also important for higher sugar products like bakery and biscuit jams, sugar confectionary.

Stabilising Pectins – used for stabilising acidic protein products such as yoghurts, whey, and soya drinks against heat processing.

Low methyl ester and aminated Pectins – used in a wide range of lower sugar products, reduced sugar preserves, fruit preparations for yoghurts, dessertsgels and topppings, and savoury applications .

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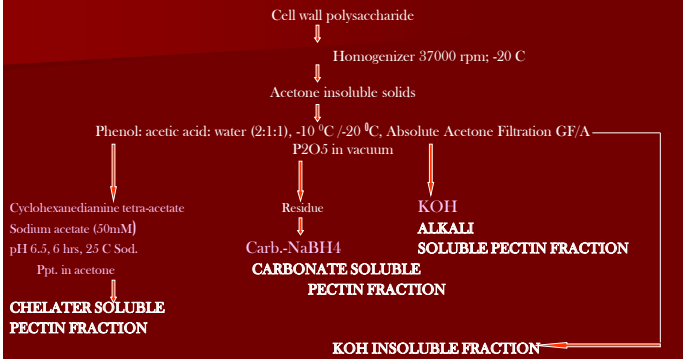
INDUSRIAL USES OF PECTINS

- To a food manufacturer, pectin is a natural fruit polysaccharide, used because of its ability to gel. The commercially important pectins derive in the primary cell wall of fruits (citrus, apple etc.).
- Pectins are used as an emulsion stabilizer.
- Pectins are employed as a therapeutic agent and a potentiator of drug as an ingredient in its grounding and as a food addition with a explicit therapeutic value.
- Pectin are used in the construction of jams and jellies. For superlative competence pectins with the degree of methylation of above 60% are used.

outlook



	RIPE FRUITS	UNRIPE FRUITS
CHELATER SOLUBLE PECTIN FRACTION	04.0 ± 0.2%	01.8 ± 0.1 %
CARBONATE SOLUBLE PECTIN FRACTION	20.2 ± 1.8 %	24.7 ± 2.1 %
ALKALI SOLUBLE PECTIN FRACTION	9.38 ± 0.8 %	6.90 ± 0.6 %
KOH INSOLUBLE FRACTION	67.38 ± 2.8 %	66.93 ± 2.9 %

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NATIONAL BUREAU OF FOREST GENETIC RESOURCES FOR ECONOMIC AND ECOLOGICAL SECURITY

Dr. N. Krishna Kumar &

R. Anandalakshmi

Institute of Forest Genetics and Tree Breeding

Coimbatore

What is Biodiversity?
What are genetic resources?

CBD

The **Convention on Biological Diversity** (**CBD**) an international legally binding treaty has 3 main goals:

- conservation of **biological diversity** (or biodiversity)
- **sustainable use** of its components &
- fair and equitable sharing of benefits arising from **genetic resources**

Objective : **To develop national strategies for the conservation and sustainable use of biological diversity.**

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Definitions in CBD

Article 2. Use of Terms

- **"Biological diversity"** means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.
- **"Genetic resources"** means genetic material of actual or potential value.
- **"Genetic material"** means any material of plant, animal, microbial or other origin containing functional units of heredity.

Definitions pertaining to forestry

Genetic resources are elements of genetic variability that are (or might be) used to meet human needs and objectives.

In forestry, the term covers naturally occurring populations and individuals, plantations, and collections, which carry currently or potentially valuable genetic information, and their protection is considered necessary from standpoints of economics, ecology, or conservation.

(Source: Encyclopedia of Forest Sciences, 2004, Elsevier)

FOREST RESOURCES VS. FOREST BIODIVERSITY

- In many fora, the term "**biological diversity**" ("biodiversity"), is increasingly used to refer to the management and use of forest resources rather than to biological diversity in forest ecosystems.
- For example, reference to, "harvesting of forest biodiversity", "management of forest biological diversity" and "forest biodiversity products" (CBD 2002,2005), **leaves the impression that "diversity" is synonymous with "resources"**.
- **This is clearly not correct**; resources are managed and harvested, and products are obtained from the resources, while biological diversity denotes "the variability among living organisms" (FAO 2003)

Forest Genetic Resources Working Papers:Technical review of status and trends of the world's forest genetic resources (FAO, 2007)

FOREST GENETIC RESOURCES

Forest genetic resources (FGR): genetic variability that is of actual or potential value for human well-being.

- **source for improvement of traits of commercial, subsistence or other importance**
- **source of raw material for adaptation to environmental change**
- **source of resistance/tolerance to insects, diseases, climatic extremes**

"Genetic resources are the living material that local communities, breeders and researchers use to adapt to changing socio-economic needs and ecological challenges."

Threats to FGR

The global average surface temperatures will rise about 1.8 to 4.0 °C during the 21st century, and up to 30% of the world's species will be at increased risk of extinction (IPCC, 2007)

The net decrease in global forest area between 2000 and 2005 was estimated to be 7.3 million hectares (FAO, 2006)

Habitat loss and deforestation can lead to fragmentation of remaining native stands, which can contribute to the decline of those stands by disrupting natural patterns of gene flow and reducing effective population sizes.

Natural disturbances such as disease, insects, fire, and extreme weather

…..coupled with over exploitation, pollution…….

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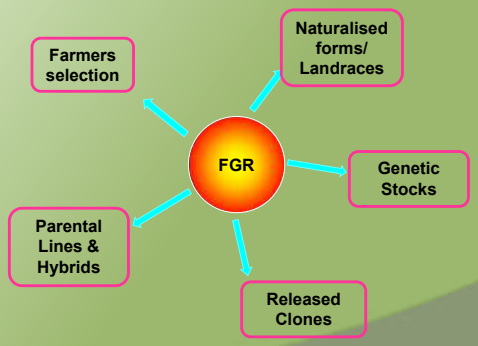
Components of Forest Genetic Resources



What is happening internationally in FGRM ?

- Commission on Genetic Resources for FAO- preparing the **State of the World's Forest Genetic Resources** to be completed in 2013.
- Country reports are to be prepared in 2010 and 2011.
- FGR National Focal Points have been identified for the purpose. Country Report submission deadline- 1.1.2012 followed by compilation and release by FAO in 2013

What is happening regionally ?

- Asia Pacific Forest Genetic Resources Programme **(APFORGEN)** initiated in 2003 with 14 participating countries including India, with the following objectives:
  - 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
- National Status Report prepared *(Katwal et al, 2004; Rawat and Ginwal, 2009)*
- 52 priority species identified (species for tree improvement and species for conservation)

What is happening nationally ?

- MoEF has formulated an Action Plan to Enhance Forestry Science (with 5 components)
  - Forestry Fellowship Programme
  - National Forestry Knowledge Forum
  - National Forestry Information Network
  - IT for fire monitoring
  - **National Bureau for Forest Germplasm**

Agriculture sector

ICAR has major steps for conservation

- *National Bureau of Plant Genetic Resources- New Delhi*
- *National Bureau of Fisheries Genetic Resources- Lucknow*
- *National Bureau of Agriculturally Important Microorganism – Mau*
- *National Bureau of Animal Genetic Resources- Karnal*
- *National Bureau of Agriculturally Important Insects- Bangalore*

National Bureau of Plant Genetic Resources- New Delhi

Germplasm Conservation: Network Approach

Base collection (LTS)

- National Genebank at NBPGR, New Delhi

Active collections (in MTS/Field genebanks)

- NBPGR Regional Stations (10 stations)
- NAG Sites: 57
- SAUs
- Other stakeholders (State Departments/ MoEF/ DRDO/ CSIR,NGOs etc.)

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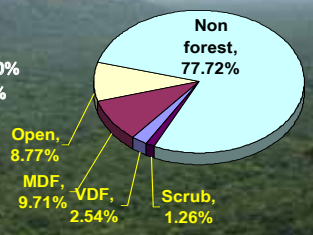
India's forests and biodiversity

Total forest & Tree cover- 23.84%

Total forest cover – 21.02%
Tree cover (Tree patches >1ha & canopy >10%) -2.82%

VDF - canopy density > 70%
MDF – canopy density between 40% - 70%
OF – canopy density between 10% - 40%



Country of Diversity

- India is the seventh largest country in area (328.73 million ha)
- Second largest in human population (more than 1.00 billion).
- Has 2.5% of the world's geographical and 1.8% of the forest area.
- Country at present is supporting 16% of the world's population and 18% of the domestic cattle population.
- India represent 8% of world's biodiversity, and one of the twelve mega biodiversity countries of the world.
- Two global terrestrial biodiversity hot spots – the North-eastern States and the Western Ghats.

Legal Framework in India

- Indian Forest Act, 1927
- Forest Conservation Act, 1980
  - In 1988, the act was amended to make the existing provisions more stringent
- Biological Diversity Act, 2002
- Protection of Plant Varieties & Farmers' Rights Act, 2001
- Seed Bill, 2004
- Wild Life (Protection) Act, 1972
- National Forest Policy, 1988

CONSERVATION

The heritable variations found between and within species can be conserved through a network of managed areas called *in situ* conservation and/or through *ex situ* conservation mode

in situ	*ex situ*
✓ Biosphere reserves	✓ Seed gene banks
✓ National parks	✓ *In vitro* gene banks
✓ Sanctuaries	✓ Cryo gene banks
✓ Preservation plots	✓ Seed orchards
✓ Seed stands	✓ Clonal repositories
✓ Sacred Groves	✓ Arboreta
✓ Community reserves	✓ Plantation
	✓ Herbal gardens
	✓ Botanical gardens

Germplasm Conservation

CRYOGENEBANK

IN VITRO GENEBANK

SEED GENEBANK

FIELD GENE BANK

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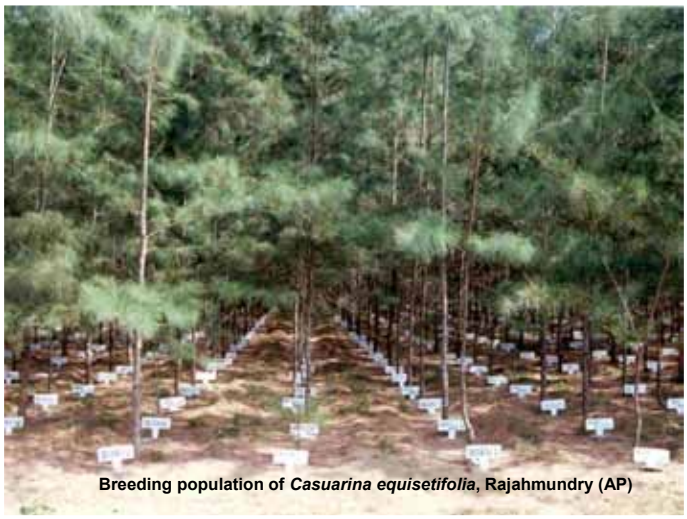

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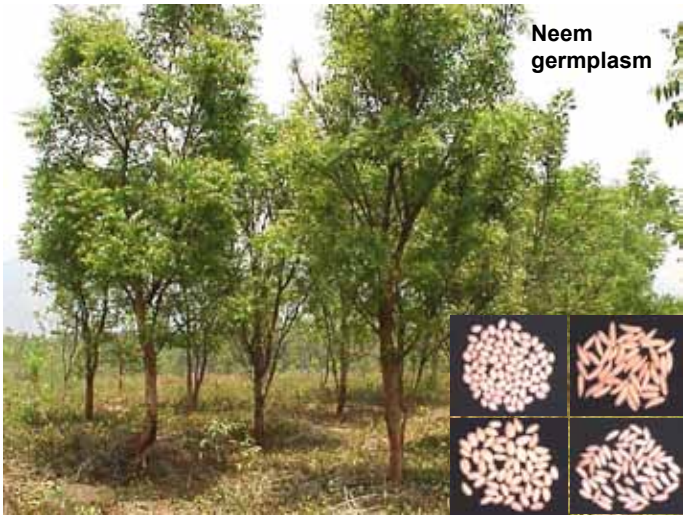

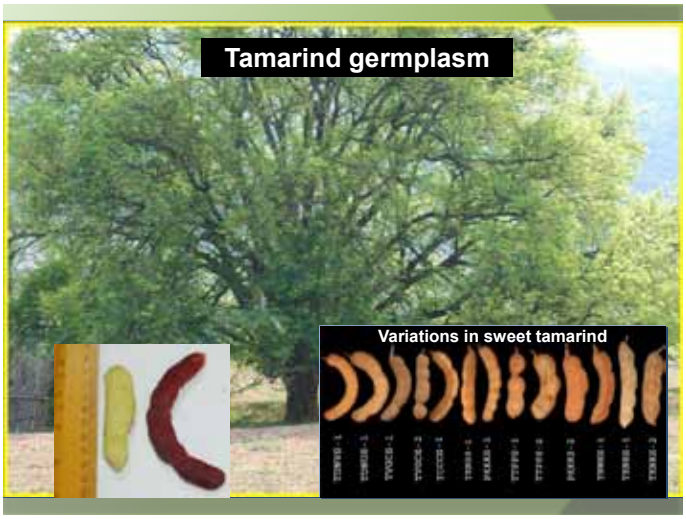

Seed Bank

Walk in cold chamber for seed storage

Contains over
110 species of trees,
91 medicinal plants, bamboos and ornamental plants.

Serves as *ex situ* conservation area for endemic, rare and threatened species.

Recognized by the Botanical Gardens Conservation International (BGCI) and Indian Botanical Garden Network (IBGN)

Alpinia calcaricata

Calophyllum inophyllum

Cordia obliqua

Limonia acidissima

Cardiospermum halicacabum

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Fischer Herbarium (FRC)

The Herbarium has 15,881 plant specimens of 3259 species belonging to 1329 genera and 172 families.

A century old collections by eminent botanists including CEC Fischer, T.F. Bourdillon, M. Rama Rao, P.F.Fyson, A.W. Lushington and C.A. Barber are maintained.

These reference collections are invaluable for taxonomic and biodiversity research.

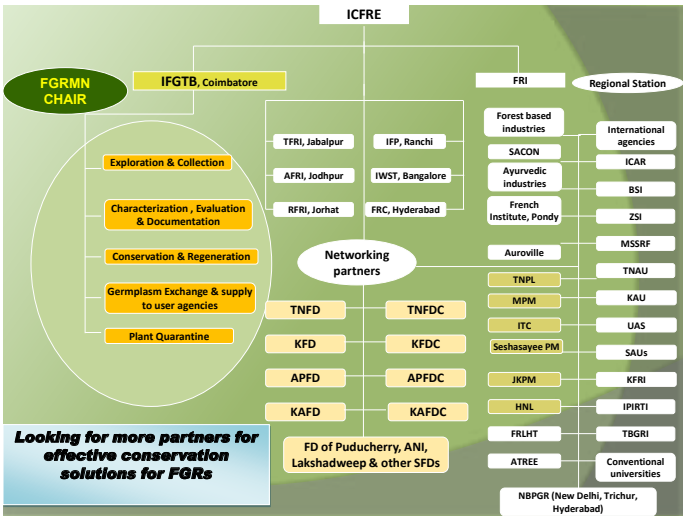
Digitization of specimens has been completed in collaboration with the University of Agricultural Sciences, Bangalore

MANDATE- FGRMN

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.

Objectives of FGRMN

- 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 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.



Species prioritized for FGRMN with identified partners

Phase I

S. No.	Prioritized Species	Networking partner for species
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

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Its following components/ programs would constitute its activities,

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

Role & Challenges of Forest Departments

- 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 program
- Forest species needs priority setting for conservation based on economic and ecological significance.
- Programs 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 programs 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 eg. Medicinal Plants Conservation Area (MPCA), Permanent Preservation Plots (PPP) to protect genetic diversity, inorder 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.

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THE PATH AHEAD……

FGRMN will pave way to a larger agency "National Bureau for Forest Genetic Resources-NBFGR"

Concerted effort and co-operation from all the stakeholders of the forests are required for effective conservation and sustainable utilization of the forest genetic resources of India

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ADDITION OF NEW HOST RECORDS TO LARVAL PARASITOIDS: *APANTELES* SPP. AND THEIR ROLE IN MANAGEMENT OF TEAK LEAF SKELETONIZER, *EUTECTONA MACHAERALIS* (WALKER) IN INDIA

By

***Mohd. Yousuf and Neetu Vaishy**

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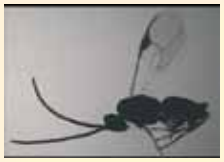
Apanteles Foerster:

- ***Apanteles*** **spp. are larval parasitoids which control teak pests in nature.**
- **These are larval parasitoids of insect pests belonging to the orders Lepidoptera, Hemiptera, Diptera, Coleoptera etc.**
- **These are Ideal biocontrol agents of insect pests.**



- **Mostly solitary endoparasitoids of Lepidopterous pests.**
- **Characterized by: small size 2- 4 mm, black appearance, reduced wing venation and 18-segmented antennae.**
- **66 species of *Apanteles* have been reported from India.**
- **Selected species have been released for biological control.**
- **27 species of *Apanteles* have been recorded from Central India.**

Life cycle of *Apanteles* species

- Females insert their eggs inside the skin of host larvae.
- Eggs of *Apanteles* spp. hatch and larvae feed on contents of the host larvae.
- Mature larvae pupate and form cocoons outside the host larvae, attached to host larvae or separately.

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Teak Skeletonizer : *Eutectona machaeralis*

- ***Eutectona machaeralis*** **is commonly known as Teak skeletonizer.**
- ***Eutectona machaeralis*** **causes skeletonization in teak up to 100 % in some severely attacked areas of central India (Madhya Pradesh, Chhattisgarh, Maharashtra and Orissa).**

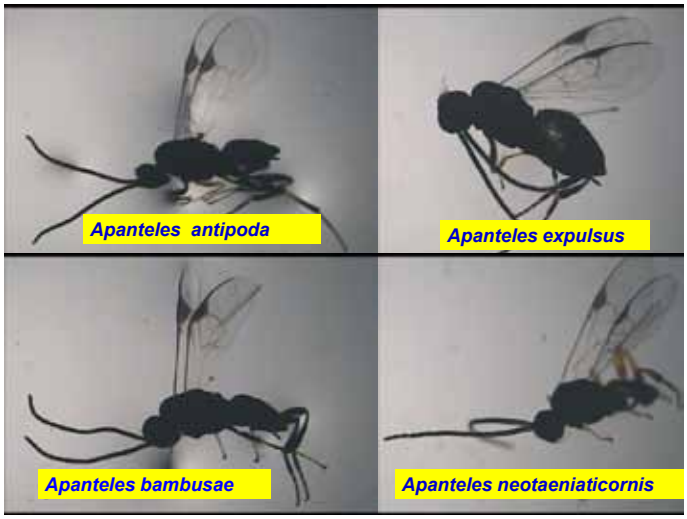
Eutectona machaeralis

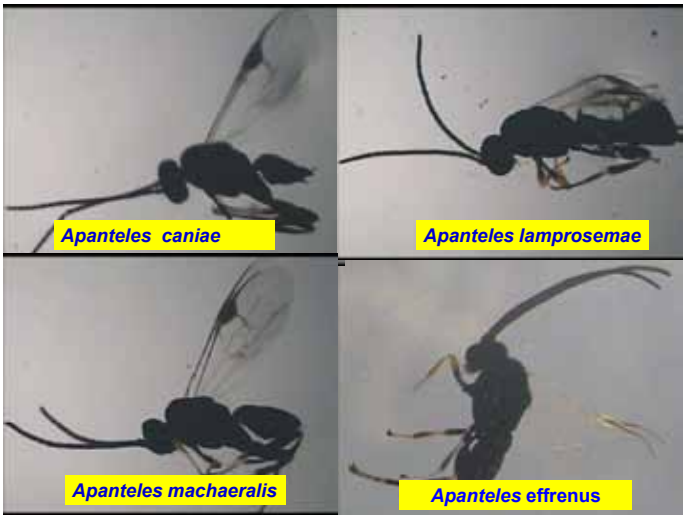
Biological control of teak defoliator & teak skeletonizer:

- **Braconids are the major parasitoids of teak pests.**
- ***Cedria paradoxa*** **was first Braconid, which was mass reared in India for control of *Eutectona machaeralis*. In 1937 over 40,000 adults were released in eleven localities in Nilambur teak plantation, Kerala.**
- ***Apanteles malevolus*** **was imported from Myanmar to Nilambur in 1937-38 and it was also released in 1938 against teak defoliator, *Hyblaea puera* at Nilambur. This species is indigenous in Myanmar and north India.**
- **Chatterjee and Misra (1974) cited parasitisation record of Indian species, *Apanteles machoeralis* and *Apanteles ruidus*, parasitising *Eutectona machaeralis* from central India.**
- **Yousuf (2008) recorded *Apanteles machaeralis* & *A. tachardiae* parasitizing *Eutectona machaeralis* .**
- **Yousuf and Puja (2010) recorded parasitisation of *Apanteles antipoda* and *A. machoeralis* on *Eutectona machaeralis*, from Maharashtra.**

Apanteles spp., from Orissa, on *Eutectona machaeralis*:

S. No.	Teak leaf skeletonizer	Month of collection	% of parasitization	Name of parasitoids *Apanteles* spp.
1.	*Eutectona machaeralis*	August 2010	5. 00 %	*Apanteles effrenus*
2 .	*Eutectona machaeralis*	August 2010	10. 00 %	*Apanteles lamprosemae*
3 .	*Eutectona machaeralis*	August 2010	12. 00 %	*Apanteles expulsus*
4.	*Eutectona machaeralis*	August 2010	22.72 %	*Apanteles expulsus*
5.	*Eutectona machaeralis*	August 2010	16. 00 %	*Apanteles expulsus*
6.	*Eutectona machaeralis*	August 2010	18.75 %	*Apanteles expulsus*
7.	*Eutectona machaeralis*	August 2010	26. 66 %	*Apanteles antipoda*
8.	*Eutectona machaeralis*	September 2010	33.33 %	*Apanteles effrenus*
9.	*Eutectona machaeralis*	December 2010	6.66 %	*Apanteles neotaeniaticornis*
10.	*Eutectona machaeralis*	December 2010	5.00 %	*Apanteles machaeralis*
11.	*Eutectona machaeralis*	December 2010	10.00 %	*Apanteles expulsus*
12	*Eutectona machaeralis*	December 2010	10.00 %	*Apanteles expulsus*
13.	*Eutectona machaeralis*	December 2010	25.00 %	*Apanteles bambusae*
14.	*Eutectona machaeralis*	December 2010	10.00 %	*Apanteles belippae*
15 .	*Eutectona machaeralis*	December 2010	10.00 %	*Apanteles caniae*





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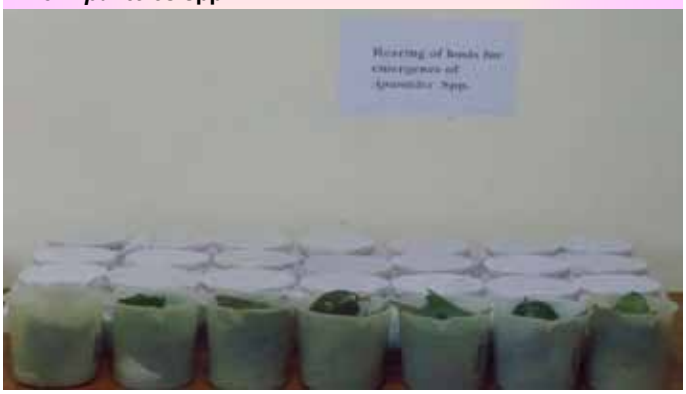
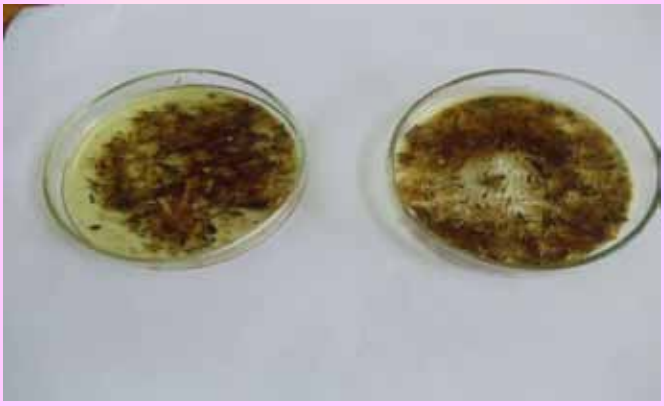
Methods of Collection of *Apanteles* species:

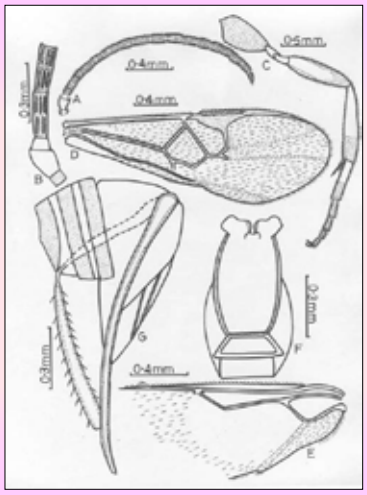
1. Host collection and laboratory rearing for emergence of *Apanteles* spp.



2.Sweeping method and sorting of *Apanteles* species



Identification of *Apanteles* spp.



1. *Apanteles antipoda* Ashmead

***Apanteles antipoda* Ashmead, 1900: 355.**

Diagnosis: Fore-wings with first abscissa of radial is equal to transverse cubital, shorter than recurrent and the breadth of stigma; longer than apical portion of first abscissa of cubital; transverse cubital longer than pigmented portion; recurrent equal to breadth of stigma. Stigma is shorter than metacarp. The hind legs with two tibial spurs sub-equal and about half the length of hind basitarsus. Ovipositor sheaths not longer than hind-tibial spur.

Hosts: ***Agrotis ypsilon, Helicoverpa armigera, Hypsipyla robusta, Perigea capensis, Spodoptera mauritia*** (Chatterjee & Misra 1974); ***Eutectona machaeralis*** (Yousuf and Puja 2010); also recorded during present study.

Distribution: India (Uttarakhand: Dehra Dun; Bihar: Pusa; Tamil Nadu: Coimbatore; Madhya Pradesh: Chhindwara, Khandwa, Khargon, Ratlam, Raisen Chhattisgarh: Durg, Kanker; Maharashtra: Aurangabad, Ahmad Nagar, Buldhana, Yavatmal; Orissa: Ganjam, Sambalpur, Sonepur, Parbhani).

Material examined: INDIA: Orissa: Sonepur, Khambeshri Pali 1♀, 5.XII.2007; Ganjam (Patra Teli) 1♂ 21.XII.2010 , sweeping; Parbhani, Ganga khed,1♀1♂, 17.IX.2009, Sambalpur (Pradhanpali) 2♀2♂ 7.VIII.2010, Ex. *Eutectona machaeralis,* M. Yousuf.

2. *Apanteles bambusae* Wilkinson

Apanteles bambusae Wilkinson, 1928: 129.

Diagnosis: Fore-wings with breadth of stigma, first abscissa of radial, of transverse cubital and recurrent all nearly equal; apical portion of first abscissa of cubital shorter than transverse cubital but longer than the pigmented portion of second abscissa of cubital, and also longer than the upper portion of basal vein; stigma shorter than metacarp. First abdominal tergite is parallel sided, nearly twice as long as broad. Ovipositor sheaths are shorter than hind femora.

Hosts: ***Cosmopteryx bambusae*** (Chatterjee & Misra, 1974) and ***Eutectona machaeralis***.

Distribution: India (Bihar,Pusa; Chattisgarh, Koriya; Orissa, Angul and Nawapara.

Material examined: INDIA: Orissa: Angul, Ranibhuin 1♀ 22.XII.2010; Nawapara, Sameshwar 1♀ 24.XII.2010, Ex. *Eutectona machaeralis,* M. Yousuf.

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3. *Apanteles belippae* Rohwer

Apanteles belippae Rohwer, 1918: 566.

Diagnosis: Fore-wings with first abscissa of radial longer and sharply angled with transverse cubital which is just shorter than recurrent; breadth of stigma is nearly equal to the first abscissa of radial; length of stigma is longer than metacarp; pigmented portion of second abscissa of cubital equal to apical portion of first abscissa of cubital. Longer tibial spur of hind legs is half while shorter tibial spur is less than half the length of basi-tarsus. First metasomal tergite about 3 times as long as wide.
Ovipositor sheaths about half the length of abdomen.

Hosts: *Belippa lohor* (Wilkinson,1928a), *Eutectona machaeralis.*

Distribution: India (Chhattisgarh: Surguja; Orissa: Kalahandi, Phulbani).

Material examined: INDIA: Orissa: Kalahandi (Bhawanipatna) 1♂ 23.XII.2010, Ex. *Eutectona machaeralis*, Phulbani (Tikawalikoha) 1♂ 4. XII. 2007, cocoon of *Apanteles* on teak, M. Yousuf.

4. *Apanteles caniae* Wilkinson

Apanteles caniae Wilkinson, 1928: 126.

Diagnosis: Fore-wings with first abscissa of radial equal to transverse cubital, longer than apical portion of the first abscissa of cubital, shorter than recurrent which is equal to the breadth of stigma. Pigmented portion of the second abscissa of cubital just longer than upper portion of basal vein. Length of stigma is a bit shorter than metacarp.
In hind legs, longer tibial spur is longer than half while shorter spur is two-fifth the length of hind basi-tarsus.
Ovipositor sheaths are shorter than hind femora.

Hosts: *Cania bilinea* (Wilkinson 1928b); and *Eutectona machaeralis.*

Distribution: INDIA:(Chattisgarh, Dantewara, Korba, Raigad, Raipur, Surguja; Orissa: Kalahandi).

Material examined: INDIA: Orissa: Kalahandi (Bhawanipatna), (Seinpur) 1♀1♂ 23.XII.2010, Ex. *Eutectona machaeralis,* M. Yousuf.

5. *Apanteles effrenus* Wilkinson

Apanteles effrenus Wilkinson, 1928: 103.

Diagnosis: Fore wings with first abscissa of radial about equal to the breadth of stigma, but longer than recurrent; transverse cubital shorter than recurrent, nearly equal to the apical portion of first abscissa of cubital; the latter is longer than pigmented portion of the second abscissa of cubital; upper portion of basal vein longer than pigmented portion of second abscissa of cubital; length of stigma is equal to metacarp. In hind legs longer tibial spur about two-third while shorter spur is more than half the length of hind basi tarsus. First metasomal tergite is about two times as long as wide. Ovipositor sheaths shorter than shorter hind tibial spur.

Hosts: *Caviria ochripes*, *Pygospila tyres*, *Sylepta derogata* (Chatterjee & Misra, 1974), *Catopsilia pyranthe* (Yousuf & Puja 2010), *Eutectona machaeralis.*

Distribution: India (Uttarakhand: Dehra Dun; Mysore; Orissa, Angul, Ganjam and Kalahandi).

Material examined: INDIA: Orissa: Ganjam, Gaya ganda, 1♂, 4.XII.2007, sweeping; Angul, Rani *Bhuin* 1♀ 6.VIII.2010 ; Kalahandi (Seinpur) 1♀ 23.IX.2010, Ex. larvae of *Eutectona machaeralis*, M. Yousuf.

6. *Apanteles expulsus* Turner

Apanteles expulsus Turner, 1918: 346.

Diagnosis: Fore-wings with first abscissa of radial just longer than recurrent and just shorter than the breadth of stigma, longer than transverse cubital; first abscissa of radial and transverse cubital evenly rounded; apical portion of first abscissa of the cubital is shorter than transverse cubital, just longer than the pigmented portion of the second abscissa of cubital; the latter is longer than half length of transverse cubital and longer than the upper portion of basal vein; pterostigma is shorter than metacarp. In hind legs, longer tibial spur just less than half while shorter spur is one-third the length of basal joint of hind tarsus.
Ovipositor sheaths are just shorter than basal joint of hind tarsus.

Hosts: *Anticarsia irrorata* (Wilkinson1928a) and *Eutectona machaeralis*.

Distribution: India (Chhattisgarh, Bastar; Orissa, Angul and Kalahandi)

Material examined: INDIA: Angul (Jorapoda) 3♀, (Ranibhuin) 4♀9♂ 6.VIII.2010,1♀ 22.XII.2010, Kalahandi (Karni Semal) 1♀1♂, 22.12.2010, Kumar Basa 1♀, Salepada 1♂ 23.12.2010, Ex. larvae of *Eutectona machaeralis*, M. Yousuf.

7. *Apanteles lamprosemae* Wilkinson

Apanteles lamprosemae Wilkinson, 1928: 88.

Diagnosis: Fore-wings with first abscissa of radial and transverse cubital evenly rounded. Upper portion of basal vein shorter than recurrent, nearly equal or longer than apical portion of first abscissa of cubital which is longer than pigmented portion of second abscissa of cubital. Width of stigma longer than recurrent vein. Stigma is shorter than metacarp. In hind legs, longer tibial spur two-third and shorter spur half of length of the basal joint of hind tarsus. Ovipositor sheaths equal to the shorter hind tibial spur.

Hosts: *Lamprosema diemenalis* (Wilkinson,1928a) and *Eutectona machaeralis*.

Distribution: India (Chhattisgarh, Bastar, Raipur; Orissa, Angul) .

Material examined: INDIA: Orissa: Angul (Ranibhuin) 1♂ 6.VIII.2010, Ex. larvae of *Eutectona machaeralis*, M. Yousuf.

8. *Apanteles machaeralis* Wilkinson

Apanteles machaeralis Wilkinson, 1928: 123.

Diagnosis: Fore-wings with stigma equal to the metacarp. First abscissa of the radial is quite rounded so that the point of junction with the transverse cubital is difficult to determine; apical portion of first abscissa of cubital shorter than recurrent. Ovipositor sheaths just shorter than hind tibiae. Hind legs with longer tibial spurs 2/5 th and shorter tibial spur 1/4 th of hind basitarsus.

Hosts: *Agrotera basinotata*, *Eutectona machaeralis*, *Diaphania bicolor*, *Glyphodes conclusalis*, *Hyblaea puera* (Chatterjee & Misra 1974).

Distribution: India (Uttarakhand: Dehra Dun; Kerala: Nilambur; Karnataka: Mysore; Madhya Pradesh: Seoni, Rahatgaon, Hosangabad; Uttar Pradesh: Saharanpur; Chhattisgarh: Kawardha; Orissa: Angul and Kalahandi).

Material examined: INDIA: Orissa, Angul (Ranibhuin) 1♂ 22.XII.2010, Kalahandi (Karni Semal) 1♂ 22.XII.2010, (Kumar Basa) 1♂23.XII.2010, 1♀24.XII.2010, Ex. larvae of *Eutectona machaeralis*, M. Yousuf.

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9. *Apanteles neotaeniaticornis* Yousuf & Puja Ray

Apanteles neotaeniaticornis Yousuf & Puja Ray 2010: 5.

Diagnosis: Female, fore wings with first abscissa of radial slightly curved, just shorter than breadth of stigma; its point of junction with transverse cubital well-marked; transverse cubital straight, about equal to apical portion of first abscissa of cubital, rather longer than upper portion of basal vein; Width of stigma longer than recurrent vein. Stigma shorter than metacarp; hind legs with longer tibial spur three-fifth and shorter tibial spur about one-third the length of hind basi-tarsus. First metasomal tergite about two times as long as its maximum breadth and three times of its apical width; Ovipositor sheaths about as long as hind femur.

Hosts: *Eutectona machaeralis.*

Distribution: India (Chhattisgarh, Koriya; Maharashtra, Beed; Orissa, Nawapara).

Material examined: INDIA: Orissa: Nawapara (Gurla para) 1♂ (Lakhandi forest)1♂ 22.XII.2010, Ex. larvae of *Eutectona machaeralis*, M. Yousuf.

Apanteles machaeralis *Apanteles bambusae* *Apanteles effrenus*

Conclusion:

- These *Apanteles* species are indigenous.
- These *Apanteles* species can play important role in controlling teak skeletonizer at larval stage, if these are released after mass multiplication.
- Biological control by *Apanteles* species will be an eco-friendly approach, free from Human health hazards.

Results :

- During the course of present study several *Apanteles* species were reared / emerged from teak skeletonizer, *Eutectona machaeralis*:
- Nine species of *Apanteles: A. antipoda*, *A. bambusae*, *A. belippae, A. caniae, A. expulsus, A. effrenus, A. lamprosemae, A. machaeralis* and *A. neotaeniaticornis* have been recovered from the larvae of teak leaf skeletonizer *E. machaeralis,* collected from teak forest areas of Orissa.

Apanteles antipoda *Apanteles expulsus* *Apanteles neotaeniaticorni*

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PRESENT STATUS OF INDIAN SPECIES OF *TRICHOGRAMMA* AND THEIR APPLICATION IN BIOLOGICAL CONTROL OF FOREST INSECT PESTS

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Introduction

- The genus *Trichogramma* consists of an economically important group of Hymenopterous egg-parasitoids of size ranging from 0.4-0.6 mm.
- They attack a broad range of host species, covering several insect pests, mostly belonging to Lepidoptera and Hemiptera.
- Species of the genus *Trichogramma* have been utilized in biological control of insect pests all over the world.
- These parasitoids have been utilized against the pests of agricultural crops, commercial cash crops, orchards and forest insect pests as well.
- Twenty eight species of *Trichogramma* (*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)* have been recorded from India.

Host -range of Indian species of *Trichogramma*

S. N.	*Trichogramma* sp.	Host-range (Host-insects)
1	*Trichogramma achaeae*	*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*	*Agrius convolvuli* and *Corcyra cephalonica.*
3	*Trichogramma breviciliata*	*Corcyra cephalonica, Eutectona machaeralis, Hyblaea puera* and *Hasora alexis.*
4	*Trichogramma brevifringiata*	*Chilo infuscatellus.*
5	*Trichogramma chilonis*	*Achaea janata, Acherontia styx, Acigona steniellus, Acrobasis caryae, Aglossa dimidiata, Agraulis vanillae, Agrius cingulata, Agrius juglandis, Agrius convolvuli, Ampillia dioscoridea, Anomis flava, Arctia coerulea, Argyroploce schistaceana, Ascotis selenaria dianeria, 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 atrilineata, 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, Prodesata kurosawai, Psara spp., Samia cynthia, Scirpophaga exceptalis, Scirpophaga incertulas, Scirpophaga innotata, Scirpophaga nivella, Scirpophaga sp., Sesamia inferens, Sitotroga cerealella, Spilactia oblique, Spodoptera litura, Spodoptera mauritiana, Tiracola plagiata, Trichoplusia ni, Tryporyza inceretulas,* and Unidentified Lycaenid, Noctuid, Pyralid and Sphingid eggs.

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6	*Trichogramma chilotraeae*	*Agrius convolvuli, Bactra sp., Chilo infuscatellus, Chilo partellus, Chilo suppressalis, Corcyra cephalonica, Helicoverpa armigera, Pelopidas mathias, Ostrinia furnacalis* and *Trichoplusia ni.*
7	*Trichogramma convolvuli*	*Agrius convolvuli* and *Corcyra cephalonica.*
8	*Trichogramma cuttackensis*	*Psalis* sp.
9	*Trichogramma danausicida*	*Corcyra cephalonica* and *Danaus chrysippus.*
10	*Trichogramma danaidiphaga*	*Danaus chrysippus.*
11	*Trichogramma flandersi*	*Agrius convolvuli, Chilo infuscatellus* and *Corcyra cephalonica.*
12	*Trichogramma giriensis*	Undetermined lepidopterous eggs.
13	*Trichogramma hebbalensis*	*Corcyra cephalonica.*
14	*Trichogramma hesperidis*	*Corcyra cephalonica, Pelopidas mathias* and Hesperiid eggs.
15	*Trichogramma japonicum*	*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 plumbellis, Sepedon sauteri, Sepedon sphegens, Sepedon violaceua, Sitotroga cerealella, Spodoptera mauritiana,Spilarctia obliqua, Susumia exigua, Trichoplusia ni, Tryporyza incertulas, Tryporyza innotata* and *Tryporyza novella.*

16	*Trichogramma kankerensis*	*Corcyra cephalonica.*
17	*Trichogramma kashmirica*	Eggs of unidentified Sciomyzid.
18	*Trichogramma latipennis*	*Corcyra cephalonica.*
19	*Trichogramma manii*	*Deudorix isocrates.*
20	*Trichogramma pallidiventris*	*Corcyra cephalonica* and *Scirpophaga incertulas.*
21	*Trichogramma pieridis*	*Catopsilia pyranthe.*
22	*Trichogramma plasseyensis*	*Chilo auricilius, Chilo infuscatellus, Chilo terrenellus, Chilo tumidicostalis, Corcyra cephalonica, Eutectona machaeralis* and *Hyblaea puera.*
23	*Trichogramma poliae*	*Chilo auricilius, Chilo infuscatellus, Chilo tumidicostalis, Clostera cupreata, C. Fulgurita* and *Corcyra cephalonica.*
24	*Trichogramma rabindrai*	Unidentified eggs of sphingid
25	*Trichogramma raoi*	*Achaea janata, Corcyra cephalonica, Eutectona machaeralis, Hyblaea puera* and *Naranga aenescens.*
26	*Trichogramma sankarani*	*Agrius convolvuli* and *Corcyra cephalonica.*
27	*Trichogramma semblidis*	*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 stultana, Polychrosis botrana, Rhychites botrana , Rhychites auratus, Sialis californica, Sialis flavilaterata, Sialis ifiumata, Sialis lutaria,* *Sialis rotunda* and *Tabanus macer.*
28	*Trichogramma thalense*	*Diatraea grandiosella, Heliothis zea, Trichoplusia ni, Venessa sp.* and Noctuid eggs .

Application of *Trichogramma* spp in Biological Control of Forest Insect Pests

- Record on release of *Trichogramma* in forests goes as early as 1937 when *Trichogramma chilonis* was released at Nilambur, 9250 parasitoids against *Hyblaea puera* in teak forest of Kerala (Beeson, 1941).
- Patil and Thontadarya (1983, 1984) carried out laboratory efficacy of nine exotic *Trichogramma* species against *Eutectona machaeralis* and 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.
- Ahmad (1990) tested laboratory efficacy of *Trichogramma japonicum*, *T. confusum* and *T. brasiliensis* against teak defoliator, *Hyblaea puera* and teak skeletonizer, *Eutectona machaeralis*. Ahmad (1992) carried out also the laboratory testing of seven *Trichogramma* spp. against poplar defoliator, *Clostera cupreata*.
- Ramachandra *et al.* (2001) recorded the field efficacy of *Trichogramma* spp. against *Eutectona machaeralis*.
- Yousuf (2005) carried out the laboratory testing of four exotic species of *Trichogramma* (*T. brasiliensis*, *T. chilonis*, *T. japonicum* and *T. pretiosum*) and one indigenous species *Trichogramma raoi* against *Eutectona machaeralis* and *Hyblaea puera.*
- Yousuf (2005) also carried out the field efficacy of 5 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.
- Joshi *et al.* (2007) carried out the field efficacy of *Trichogramma brasiliensis* against *Eutectona machaeralis* and concluded that the lowest effective quantity of *T. brasiliensis* was @ 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, *Hyblaea puera* , teak leaf skeletonizer, *Eutectona machaeralis* and *Hasora alexis*.

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 obliqua*; Arjun defoliator, *Lymantria* spp.*;* Lagerstroemia defoliator, *Achaea janata*; Cassia defoliator, *Catopsilia crocale* etc.
- Controlling of these key insect pests in large forestry and agro-forestry areas by application of chemical pesticides is not only expensive but also environmentally unsafe.
- Biological control by *Trichogramma* species play the key role for controlling these key forest insect pests.

Acknowledgement

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Indian Forest Congress

(November 22-25, 2011)

Theme: Expanding Frontiers of Forestry Sciences

Subtheme: Managing forest resources: Scientific base

Laboratory antifungal guided identification of foliar chemical constituents from the hybrid bred from *Eucalyptus citriodora x E. torelliana* and its parental taxa conferring resistance to *Cylindrocladium quinqueseptatum*

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Background information

- Hybrid breeding of eucalyptus is a common procedure in silviculture to maximize tree performance by combining the desirable traits of different species *(Assis, 2000)*
- Traits for improvement through hybridization include
  - growth rate
  - ability to coppice and propagate
  - pulp yield
  - wood density
  - resistance to frost, drought, salinity, pests and diseases *(Dale and Dieters, 2007; Potts and Dungey, 2004)*

Background information contd...

- Eucalyptus is a rich source of terpenoids and phenolics which convey some potentially interesting interactions.
- These secondary metabolites are putative defensive chemicals.
- The fungi, insects and vertebrate herbivores of eucalyptus are reported to be deterred by a range of these secondary metabolites *(Keszei et al 2008)*
- An understanding of how characters important to plant herbivores and fungi (for instance, secondary chemicals and physical leaf characteristics) vary between species and their hybrids contributes to understanding of the mechanisms of host choice and selection of resistance to the insect pests *(Nahrung et al. 2009, Hallgren et al. 2003).*

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Background information contd…

- The plant secondary metabolites are being researched to develop chemical markers for their application in predicting indirect selection response in primary traits such as yield, form, quality, or insect and disease resistance to increase effectiveness and efficiency of tree breeding programmes.
- Because tree breeders are vitally concerned with developing methods for early selection and evaluation of progeny performance, chemical markers are of direct benefit to them.

Background information contd…

- *Cylindrocladium quinqueseptatum* (CQ), the most destructive pathogen of Eucalyptus, is wide spread and occurs on eucalyptus seedlings in nurseries, plantations or in small trial plots. This fungus causes cylindrocladium leaf and seedling blight (CLSB) disease and is most often fatal.
- A hybrid of *E. citriodora* (EC) and *E. torelliana* (ET) bred at Forest Research Institute, Dehra Dun has significant advantages in biomass accumulation.
- The hybrid and one of its parents ET have been observed resistant to the CLSB in the field *(Tewari 1992)*.
- This resistance, was however, subjective and it was **hypothesized** that the foliar resistance of the hybrid to CQ may be derived from foliar chemical constituents.

Objective

- To analyze laboratory antifungal assay directed foliar chemical attributes of the hybrid (EC X ET) and its parental taxa (EC and ET).
- To identify the chemical constituents in the hybrid conferring resistance to CQ
- To study the variations of the active constituents in each of the taxon.
- To estimate the heritability of the active constituents

Isolation of essential oils, their analysis for monoterpenes, and their laboratory antifungal assay

Foliage

↓ Hydrodistillation using Clevenger apparatus ; 3-4h

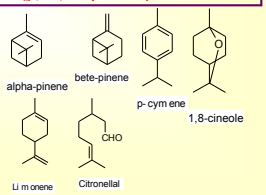
Essential oil [EC (1.2%), ET (0.03%) , EC X ET (0.03%)]

GC-MS analysis

Hewlett Packard 5890 Series II gas chromatograph (GC) and Hewlett Packard 5971 Series Mass Selective Detector. Column : J&W Scientific Durabond – 5MS column (30m x 0.25mm x 0.25 µm). The GC conditions used were initial temperature 50°C followed by a rate of 5oC min-1 up to a final temperature of 210°C (6 min), carrier gas helium at constant flow 1.5 mL min-1, split ratio 1:100, transfer line temperature 250°C. The MS was held at 250°C in the ion source with one scan per minute acquired.

Characterization: By comparison of RT of the peaks with those of commercial standards (Sigma Aldrich) and the National Institute of Standards and Technology (NIST) mass spectral library.

Monoterpene	RT (in minutes)	EC	EC XET	ET
	Mean precentage area			
α-pinene	6.3	0.14	0.77	0.14
β- pinene	9.3	0.22	0.41	0.06
p-cymene	10.8	0.16	0.19	Not Detected
1,8-cineole	11.0	1.85	Not Detected	Not Detected
Limonene	13.9	0.32	0.41	0.04
Citronellal	14.9	74.65	0.45	0.24



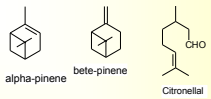
Laboratory antifungal assay of monoterpenes

Monoterpenes

↓

Cylindrocladium quinqueseptatum using poison food technique; incubated in dark at 27° C and 70% relative humidity

S.No.	Monoterpene	MIC (%)
1.	α-pinene	0.50
2.	β- pinene	0.25
3.	Citronellal	0.13



No reduced dose dependent activity was observed.

Isolation of foliage extractives

Foliage

↓ Soxhlet extraction Petroleum ether (60-80°C) ; 6 h

Extract (LPET)

Residue

↓ Soxhlet extraction Acetone : 6 h

Extract (LACE)

Residue

↓ Soxhlet extraction 70% aquous MeOH 6 h

Extract (LMET)

Residue

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Laboratory antifungal assay of extractives

Extract (LPET) | Extract (LACE) | Extract (LMET)

Cylindrocladium quinqueseptatum using poison food technique; incubated in dark at 27° C and 70% relative humidity

LPET	LACE	LMET
Inactive	MIC 1.0%	MIC 0.12%

No reduced dose dependent activity was observed.

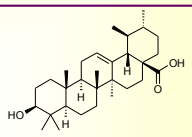
Isolation of ursolic acid and its antifungal assay

LACE

Column Chromatography on Silica gel

Eluant: Chloroform : methanol

Ursolic acid (UA)



Ursolic acid

Cylindrocladium quinqueseptatum using poison food technique; incubated in dark at 27° C and 70% relative humidity

UA MIC 1.0%

HPTLC analysis of foliage for estimation of UA content- *Method validation*

Ursolic acid

CONDITIONS *CAMAG* **HPTLC system**

STANDARD SOLUTION

45mg of UA was dissolved in 50 ml of MeOH. Standard solutions containing 240, 360, 480, 600, 720, 840, 960, 1200 µg/mLwere prepared by diluting 2,3,4,5,6,7,8,10 mLof the stock solution with 5 mL methanol separately.

LAYER

Silica gel 60 F254 TLC precoated aluminum plates (20 x 10cm, layer thickness 0.2mm, E. Merck)

SAMPLE APPLICATION

6mm bands (slit dimension 5.00 x 0.45mm, micro) using the Linomat 5 applicator, application volume 2-12µL; application position 8mm, starting 15mm from the edge of the plate

CHROMATOGRAPHY

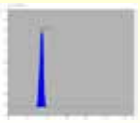
In twin trough chamber with 25 % ethyl acetate : hexane, presaturated (filter paper) for 20min migration distance 80mm from the lower plate edge; drying in air at ambient temperature, derivatization with 10 % methanolic H_2SO_4 followed by heating at 120°C for 2 minutes

EVALUATION

scanning and quantification at 515 nm in absorbance/ reflectance mode using the TLC scanner 3 with winCATS 3.2.1 software incorporating track optimizing option.

HPTLC analysis of foliage for estimation of UA content- *Method validation* contd…..

Parameter	UA
Specificity	Specific
Dynamic range (ng / spot)	240-1200
Instrumental precision (%RSD) n=3	0.79
R_F Value	0.22
Linearity (r; %RSD)	0.999; 1.18
Accuracy	Accurate
Spikked amount (ng)	120, 240
Mean recovery (%) (n-3)	100



HPTLChoromatogram of the reference ursolic acid

Densitogram of the reference ursolic acid

Calibration plot obtained by linear regression analysis for the reference ursolic acid

Quantification of ursolic acid in the foliage

Ursolic acid

Foliage

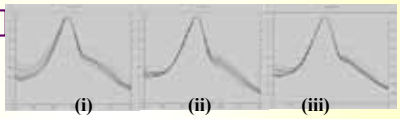
Petroleum ether :acetone (4:1) 4 h

Extract (10 mg in 5 mL MeOH)

HPTLC analysis

Taxon	UA (%)
EC	0.99
ET	1.07
EC X ET	1.09

Taxon	r	% RSD
EC	0.999	1.10
ET	0.999	0.59
EC X ET	0.999	1.07



(i) (ii) (iii)

Overlaid UV absorption spectra obtained from reference ursolic acid and from the corresponding peak from the extract of foliage of taxa (i) EC, (ii) ET and (iii) EC X ET

(i) (ii) (iii)

HPTLChromatograms of reference ursolic acid and extracts of the foliage of taxa (i) EC, (ii) ET and (iii) EC X ET

Fractionation of LMET and antifungal assay of the fractions

LMET

Suspended in water

EtOAc

Extract (LMETEA) | Residue

n-BuOH

Residue | Extract (LMETBU)

Cylindrocladium quinqueseptatum using poison food technique; incubated in dark at 27° C and 70% relative humidity

LMETEA	LMETBU
MIC 0.12%	Inactive

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HPTLC examination of LMETEA

LMETEA

Ethylacetate : formic acid: acetic acid: water (100:11:11;27)
1%Vanillin H_2SO_4

Flavonoid type	Color	$UV_{\lambda max}$	R_F
Catechin / epicatechin	Red	228 (sh), 279	0.95
Flavonols	Yellow-orange	266, 353 269, 367 273, 365	0.74 0.64 0.51
Flavanols	Red	281 276	0.38 0.29

- Several polyphenolic compounds such as flavonoids (flavones, flavanones, dihydroflavonols, flavonols and flavanols) and phenolics acids (*Conde et al. 1997, Horn et al. 1964,, Lamberton 1964, Hillis & Isoi 1965, Wollenweber & Kohorst 1981)* have been reported in leaves of different Eucalyptus species.
- Total phenolics contents (TPCs) in foliage of each taxon were, therefore, determined and compared.

Determination of Total Phenolic Contents (TPCs)

Powdered foliage
→ Hexane
→ Hexane Extract
→ Defatted material (5 g)
→ Folin –Ciocalteau method
→ 50% acetone water at ambient temperature for 24 h

Taxon	TPC s (g GAE / 100 g of the leaves)
EC	0.51
ET	0.51
EC X ET	0.57

Heritability (% broadsense) estimates of the bioactive constituents

- Heritability (% broadsense) of the three bioactive monoterpenes, UA and the total phenolics was estimated using GENSTAT 5.
- Amongst the monoterpenes, only β- pinene was highly heritable (H 90.6%) while α-pinene and citronellal were not heritable.
- Heritability of UA was found to be relatively low (H 37.06%) whereas total phenolics demonstrated high heritability (H 93.98%).

- Studies of susceptibility of plant species and the hybrids to pests and diseases have been done considerably.
- Hybrid susceptibility (arising either through dominance to a susceptible parent, or a hybrid that is more susceptible than either parent) appears the most common pattern while hybrid resistance (arising either through dominance to a resistant parent, or a hybrid that is more resistant than either parent) appears to be reasonably rare while in some of the studies an additive pattern, whereby hybrid traits are intermediate between the two parental types, and almost no difference between parents and hybrids has been found *(Fritz et al. 1999, Dungey & Potts 2003, Hallgren et al.2003, O' Reilly Wapstra et al. 2005).*

- The hybrid (EC XET) exhibited traits superior to the parent species for the foliar chemical characteristics investigated.
- The concentration of the foliar constituents (monoterpenes-α-pinene, β- pinene and citronellal, UA, and total phenolics) conferring resistance to fungi, CQ in laboratory bioassays were higher (monoterpenes and total phenolics) in the hybrid than either parent or equivalent (UA) to parent ET

Taxon	α-pinene	β- pinene	Citronellal	UA (%)	TPC s (g GAE / 100 g of the leaves)
EC	0.14	0.22	74.65	0.99	0.51
ET	0.14	0.06	0.24	1.07	0.51
EC X ET	0.77	0.41	0.45	1.09	0.57

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- Monoterpenes have found applications in forest genetics as biochemical markers in chemotaxonomy and in selecting less susceptible chemotypes to pests and diseases (*Baradat et al. 1991, Hanover 1992, Michelozzi et al. 1995, Hanover 1992, Michelozzi et al. 1999)*
- Within eucalyptus, terpenes have been implicated in many ecological interactions including resistance to pests and diseases (*Morrow & Fox 1980, Lawler et al. 1999, Eyles et al. 2003, Alves et al. 2004).*
- UA, a triterpene occurring in concentration upto 2.5% in the eucalyptus foliage has been reported to possess an array of biological activities including antifungal activity *(Shukla et al. 1992, Dayal 1982).*

- Although hybrid susceptiblity to herbivores is predicted in eucalyptus *(Dungey & Potts 2003; Potts & Dungey 2004),* the hybrid taxon displayed resistance pattern in our study.
- Our findings also suggest a possible chemical basis for the hybrid resistance to CQ.
- Heritability estimates of the active constituents also show and that use of the contents of β- pinene, ursolic acid and total phenolics is possible for screening of CLSB resistant progeny in EC X ET system.

Conclusion

- Three monoterpenes (α-pinene, β- pinene and citronellal), ursolic acid, and total phenolics conferring resistance to fungi, CQ were identified.
- Concentration of these active constituents of the hybrid was higher (monoterpenes- α-pinene, β- pinene and citronellal, and total phenolics) than either parent or equivalent (ursolic acid) to parent ET thus suggesting an resistance pattern of hybrid.
- β- pinene, ursolic acid and total phenolics were found to be heritable.
- The findings suggested a possible chemical basis for the hybrid resistance to CQ and that use of the contents of β- pinene, ursolic acid and total phenolics is possible for screening of CLSB resistant progeny in EC X ET system

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BIOPESTICIDAL PROPERTIES OF *A.MARMELOS* AGAINST *HYBLAEA PUREA* AND *SPODOPTERA LITURA*

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Different parts of this species are being extensively used as they possess

analgesic, antipyretic, anti-inflammatory properties

due to the presence of alkaloid, marmeline, lignan-glucosides and anthraquinone

investigations on efficacy of secondary plant derivatives of *A. marmelos* has not been carried out

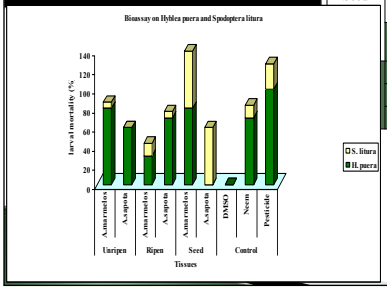
in respect important insect pests of forest tree species grown in forest nurseries

Bioassay on *H. puera* & *S. litura*

Aqueous
MeOH
EtOAc
Hexane
1, 2, 5, 10%

Control (DMSO)
Neem control
Pesticide control
0.5%
0.05 %

Over all COMPARISION OF LARVAL MORTALITY



Tissues	Treatments (%)	*H. puera*	*S. litura*
		Larval mortality (%)	
Unripen (Half fruit)	*A. marmelos* (1% C)	80 (EtOAc)	6.6 (water)
	A. Sapota (1%)	60 (MeOH)	No effect
Ripen (Fruit)	*A. marmelos* (10% C)	30 (water)	13.3 (EtOAc)
	A. Sapota (5% C)	70 (MeOH)	6.6 (water)
Seed	*A. marmelos* (1%C)	80 (MeOH)	60 (Hexane)
	A. Sapota	70 Hexane)	60 (Hexane) (1% C)
	DMSO	0.00	0.00
	Neem	70.0	13.3
	Pesticide	100	26.6

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EFFECT OF DIFFERENT ALCOHOLIC FRACTIONS OF *A.MARMELOS* TISSUES ON *H. PUERA* LARVAL MORTALITY

	Hexane fraction				Methanol fraction				Ethylacetate fraction			
	AMS	AMUR	AMR	ASS	AMS	AMUR	AMR	ASS	AMS	AMUR	AMR	ASS
T1 (250 PPM)	40 ± 2.52	30± 1.68	20 ± 0.90	15 ± 1.23	80 ± 4.48	5 ± 0.26	10 ± 0.65	10 ± 0.56	80 ± 4.88	30 ± 1.59	0 ± .65	10 ± 1.02 1
T2 (500 PPM)	70 ± 4.97	50 ± 3.10	25 ± 1.00	20 ± 1.20	80 ± 4.88	5 ± 0.27	10 ± 0.56	10 ± 1.14	20 ± 1.00	60 ± 3.18	40 ± 2.24	10 ± 1.11
T3 (750 PPM)	70 ± 4.34	60 ± 3.72	40 ± 2.48	30 ± 1.22	80 ± 4.96	10 ± 0.65	20 ± 1.10	15 ± 1.10	20 ± 1.14	60 ± 3.84	20 ± 1.14	10 ± 0.66
T4 (1000 PPM)	80 ± 4.88	80 ± 4.48	40 ± 2.52	30 ± 2.13	80 ± 5.12	10 ± 0.56	20 ± 1.14	20 ± 1.14	40 ± 2.52	50 ± 3.65	50 ± 2.65	15 ± 1.01
DMSO	13.0 ± 0.18				20 ± 1.09				40 ±2.25			
Neem	95 ± 4.80				95 ± 4.80				95 ±4.8			
Pesticide	80 ± 4.00				80 ± 4.00				80 ±4.00			

EFFECT OF DIFFERENT ALCOHOLIC FRACTIONS OF *A.MARMELOS* TISSUES ON *S.litura* LARVAL MORTALITY

	Hexane fraction		Methanol fraction				Ethylacetate fraction			
	AMS	ASS	AMS	AMUR	AMR	ASS	AMS	AMUR	AMR	ASS
T1 (250ppm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T2 (500ppm)	13.3 ±1.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T3 (750ppm)	40.0 ±2.49	0.00	6.66±0.58	0.00	13.3±1.08	0.00	0.00	0.00	0.00	0.00
T4 (1000ppm)	60.0 ±2.96	20.0 ±1.53	6.66 ±0.58	0.00	13.3±1.08	0.00	6.66 ±0.49	0.00	13.3 ±1.09	5.32 ±0.55
DMSO	0.00		0.98 ± 0.36				0.98±0.24			
Neem	13.3 ± 1.09		13.3 ± 0.68				13.3 ±0.54			
Pesticide	26.6 ± 1.23		26.6 ±1.56				26.6 ±1.34			

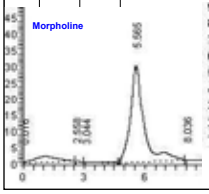
Lab Experiment conducted in Nilambur – Confirmation

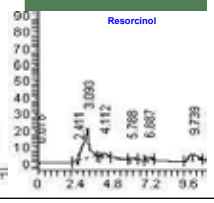
Treatments	%Mortality		
	Mortality after feeding leaf disc	Mortality out starvation	%Total Mortality
Quinalphos (25 EC)	82	5	87
A. marmelos oil			
2000ppm	40	0	40
5000ppm	47	7	54
10000ppm	63	37	100
Formulation 6			
2000ppm	22	11	33
5000ppm	30	40	70
10000ppm	42	48	90
Formulation 7			
2000ppm	33	0	33
5000ppm	44	11	55
10000ppm	57	43	100
Formulation 8			
2000ppm	33	11	44
5000ppm	22	22	44
10000ppm	20	30	50

The fractionated phenols, phenolics and polyphenols through TLC-UV –HPLC method were further

fractionated and about 11 fractions of each tissue (total 30 fractions) were purified by HPLC and GC-MS-MS and identified 13 natural plant secondary compounds

	Rt. (mins)	Area (%)	Compound Identified
S1B1	3.09	66	Resorcinol
S1B2	3.118 5.425	55 21	Catechic acid Vanillic acid Syringic acid Cinnamic acid
S1B3	3.06 3.27	45 24	Pyrogallol Ferulic acid
S2B5	2.96 4.1	87 6	Gallic acid
S2B10	3.06 5.41 10.4	40 22 30	Pyrogallol Cinnamic acidSamples





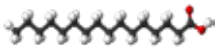
Samples	Retention time (min)	Area (%)	Compound identified
S2B1	5.46	83	Cinnamic acid
S2B2	5.56	89	Morpholine
S2B3	5.42 6.43	10.59 89.28	Cinnamic acid
S2B4	5.32 6.41	8.00 91.99	Morpholine
S2B5	5.02 6.34	93.68 6.23	Chlorogenic acid
S2B6	5.10 5.40 6.26	24.5 47.1 19.87	7-Hydroxy coumarin-3-carboxylic acid Cinnamic acid
S2B7	5.16 6.80	69.75 27.66	7-Hydroxy coumarin-3-carboxylic acid
S2B8	5.76 7.13	94.49 5.5	Morpholine
S3B2	5.15	97.68	7-Hydroxy coumarin-3-carboxylic acid
S4B1	5.05 5.32 6.75	39.99 39.00 8.10	Chlorogenic acid Morpholine

individual compounds were tested for biopesticidal effect

Four compounds are showing biopesticidal effect

Plant compounds	24 hrs (Larval mortality %) (ppm)				48 hrs (Larval mortality %)				72hrs (Larval mortality %)			
	250	500	750	1000	250	500	750	1000	250	500	750	1000
Gallic acid	10	20	30	20	30	20	10	50	50	20	10	70
Tannic acid		10	20	10	10	40	40	40	20	50	70	70
Resorcinol	20	10	20	10	20	20	30	10	30	40	30	10
Pyrogallol	10	10	10	30	10	20	20	30	30	20	10	20
Cinnamic acid	40	30	30	30	70	30	50	60	80	30	70	80
Chlorogenic acid + Elagic acid	40	30	30	20	50	20	35	30	50	20	45	40
Vanillic acid +Syringic acid	33	30	33	16	50	16	70	16	50	16	75	16
Morpholine	16	20	30	33	33	25	16	16	33	50	83	50
Ferulic acid +Catechic acid	60	40	40	50	70	33	70	16	75	35	70	30

HPLC Analysis for Free Fatty acids

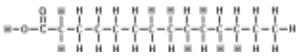


Palmitic acid

Lauric acid

Free Fatty acids (Biologically not active)

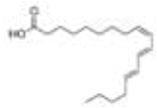
Stearic acid



Myristic acid

Free Fatty acids (Biologically active)

Linoleic acid



Linolenic acid

Oleic acid

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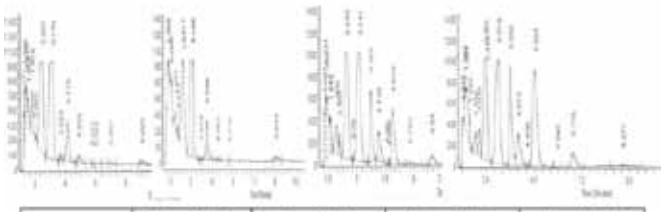
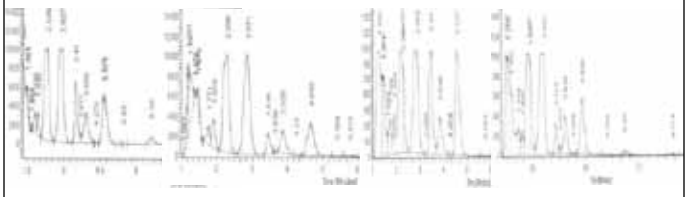
HPLC ANALYSIS OF PREFORMULATED OIL FOR FREE FATTY ACID



Name of the compound	Formulation 1		Formulation 2		Formulation 3		Formulation 4	
	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)
Stearic acid	1.334	3.537	1.33	0.136	1.343	2.009	1.381	7.184
Palmitic acid	1.555	0.990	1.398	1.009	1.612	2.397	1.553	1.176
Linolenic acid	2.307	26.691	2.420	1.999	2.449	32.825	2.414	3.824
Linoleic acid	3.178	33.713	1.389	3.614	1.388	7.088	–	–
Oleic acid	3.683	0.945	3.676	0.345	3.707	0.519	3.592	11.872
Lauric acid	4.116	8.040	4.105	0.136	4.549	0.802	4.423	0.8370
Myristic acid			3.102	42.643				



Name of the compound	Formulation 5		Formulation 6		Formulation 7		Formulation 8	
	Rt (min.)	Area (%)	Rt (min.)	Area (%)	Rt (min.)	Area (%)	Rt (min.)	Area (%)
Stearic acid	1.503	1.755	1.479	1.868	1.471	4.640		
Palmitic acid	1.795	2.200	1.770	3.071	1.639	0.814	1.749	2.934
Linolenic acid	2.335	28.413	2.295	26.429	2.29	4.569	2.287	7.680
Linoleic acid	1.451	0.641	1.371	0.779	1.412	3.711	1.397	8.884
Oleic acid	3.895	7.639	3.856	5.520	3.837	4.528	3.838	5.753
Lauric acid	4.598	1.251	4.654	9.972	4.537	14.021	4.635	9.083
Myristic acid	2.93	29.739	2.870	28.673	2.868	17.351	2.880	23.612

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Forests seed certification

Problems, Limitations and Needs

Dr. Nawa Bahar

Scientist

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Forests seed certification

Seed certification is a legally sanctioned system designed to control and maintain high - purity seed and for propagating material of genetically distinct crop varieties.

Forests seed certification

Seed certification allows one to check on the origin of seed and trueness to its cultivar purity, to evaluate the growing crop and supervise the pre - harvest, harvest and post - harvest operations during seed production and processing, as well as conduct sample inspection (laboratory test), bulk inspection for homogeneity, and controlled plot testing.

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Forests seed certification

- Unlike several attributes of seed such as purity, germination per cent, moisture content and health etc., which can be assessed in the laboratory, one attribute of prime importance- varietal purity- cannot be assessed in the laboratory.
- 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.

Forests seed certification

- 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

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).

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.

Classes and sources of certified seed

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.

Classes and sources of certified seed

Source - identified seed

Source-identified seed shall be seed from

- Natural stands with the geographic origin known and
- From plantation of known provenance, as specified in the standards of the various certifying agencies.

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Classes and sources of certified seed

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.

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.

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(ISTA) or appropriate State or Governmental Laboratories as determined by the certifying agency

Labeling 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.

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.

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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

- 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).
- The identification of the exact origin of seeds collected from wild stands, however, may be more difficult and more expensive.

Role of the seed testing laboratory

- 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.

Role of the seed testing laboratory

- 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.
- Development of suitable methods of pre treatments.
- 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.

Profile of Speaker

Name: Dr. Nawa Bahar
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Date of Birth: 01-06-1965
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Publications:

Papers: More than 80 research papers published in national and international journals of repute.
Book: (One)
Handbook: (One)
Booklet: (One)
Brochure (One)
Award: Brandis Prize in the field of forestry for the year 2000.

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SCREENING FOR RESISTANCE AGAINST SOME COMMON DISEASES IN *DALBERGIA SISSOO*

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- RAISING DISEASED PLANTING MATERIAL - COLOSSAL WASTE OF TIME, SPACE AND MONEY
- IT LEADS TO

1) SLOW GROWTH AND VIGOUR,
2) REDUCED VOLUME PRODUCTION,
3) DEFORMED STEMS,
4) REDUCED WOOD PROPERTIES,
5) DISEASE SPREAD, AND
6) REDUCED YIELD OF CHEMICALS.

Therefore,
attempts should be made to raise plants free from diseases through

- manipulation of pathogen,
- host and/or
- environment

Options available

- use of pesticides,
- cultural practices,
- biological control and
- resistant plant material (an economical and long-term measure for effective disease management)

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- In natural ecosystem balance in hosts and pathogens
- In artificial systems like plantations this balance is disturbed
- Monucultures more prone to diseases due to uniform crop of hosts, narrow genetic variability as in CSOs and SSPAs

- From disease resistant point of view, it is to be mentioned here that while selecting source material in natural stands (seeds, vegetative propagation material), the disease factors are seldom considered in our country.
- This leads to the spread of the pathogen along with the seeds to a locality where the disease is absent.
- In Tree Improvement Programme of FREEP-WB Project, CSOs and SSPAs of *Dalbergia sissoo* were estblished

Hypothesis

- In a heavily diseased area it is not unusual to find a few disease-free individuals
- The freedom from infection may be due to escape or due to inherent character for resistance in the host
- Selection of such disease-free individuals and testing their progeny by raising them in the heavily diseased locality or through inoculations which will eliminate the escapes

Selection of Diseases of *D. sissoo* Considered in the Study

Leaf & Petiole Rust

Leaf blight caused by *Colletotrichum gloeosporioides*

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Leaf blight caused by *Rhizoctonia solani*

Leaf blight caused by *Rhizoctonia solani*

Twig blight caused by *Helminthosporium dalbergiae*

Stem canker caused by *Phoma nivea*

Stem canker -causing wind breakage

Pod blight caused by *Collectotrichum gloeosporioides*

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Methodology

- As per the design observation on each plant in the rows, to note diseases in 3-5 plants per clone - Lacchiwala, Poanta Sahib, Chandigarh
- Block (clone) of 9 plants - observations of 3 plants per block (3 replications) - Hoshiyarpur, Hissar, Chandiarh
- Observations of each plant (33 clones) 10 replications at Mirpur
- Disease incidence recorded from 2002 - 2007

Observations

Hedge Garden, Brandis Road

Screening against Leaf & Petiole Rust

Clones screened - 52

Resistant - 66, 192

Most susceptible - 10, 24, 41, 42, 112, 86

BHITMERA, HISSAR

CLONE 57- SHOWING RESISTANCE AGAINST LB, PB, TB, CAB, CAS

Disease	Clones showing resistance in CSO	Clones showing resistance in SSPA
Pod blight	25, 34, 41, 57, 81, 82, 84, 88, 90, 94, 103, 123, 189, 192, 194, 196, 199, 204, 219, 242, 255	None
Twig Blight	20, 25, 88, 252	272, 291, 297, 299, 295, 293, 302, 303, 304
Cankers	12, 25, 33, 41, 81, 82, 85, 86, 88, 90, 94, 151, 189, 192, 194, 199, 200, 219, 242, 255	294, 297
Leaf blight	85, 88, 219	None

MIRPUR

Clones showing resistance against canker disease - 5, 32

No clone showing resistance against- LB, TB

PB- absent

CHANDIGARH

Clones showing resistance against canker disease - 13, 15

No clone showing resistance against- LB

Clones showing resistance against twig blight - 1, 2, 5, 6, 7, 9, 11, 14, 17, 20, 26, 29, 31, 33, 35, 45

PB- absent (no pod formation)

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Hoshiyarpur

Clones showing resistance against canker disease - 85

No clone showing resistance against- LB

PB- absent

Poanta Sahib

Clones showing resistance against canker disease - 36, 49, 51, 236, 237

Clones showing resistance against LB - 103, 237

Clones showing resistance against twig blight - 86, 203

PB- absent

Lacchhiwala

Clones showing resistance against canker disease - 16

Clone showing resistance against LB - 16

Clones showing resistance against *Rhizoctonia solani* leaf blight - 15, 18, 26, 30, 31

PB- absent (no pod formation)

Testing Resistance - through Inoculations

Developing a protocol for inoculation of rust in *D. sissoo*

Performance of different clones of *D. sissoo* against induced rust disease

Clone No./Source	Disease Score							Resistance class
	Exp.	R1	R2	R3	R4	R5	Mean	
S-57; Khalawala Range, Ambala Division, Haryana	1	1	0	0	0	0	0.20± 0.45	R
	2	0	0	0	0	0	0	
S-24; C. B. Ganj, Bareilly Division, Uttar Pradesh (U.P.)	1	80	76	71	75	76	75.6± 3.21	S
	2	85	77	79	81	77	79.8± 3.35	
S-66; Chhichrauli Range, Yamunanagar Division, Haryana	1	17	19	16	20	14	17.2± 2.39	MS
	2	19	21	18	15	24	19.4± 3.36	
S-41; Hasnapur, Tulsipur range, Gonda Division, U.P.	1	9	11	7	4	14	9± 3.81	MR
	2	11	15	14	20	9	13.8±4.21	
S-361; Sohelwa Wildlife Division, Gonda, U.P.	1	6	3	9	8	4	6± 2.55	MR
	2	7	7	9	11	4	7.6± 2.61	
S-10; Pathri Range, Haridwar Division, Uttaranchal	1	11	12	11	15	7	11.2± 2.86	MR
	2	9	13	14	17	11	12.8± 3.03	
S-51; Birpur beet, Bhamar range, Gonda Division, U.P.	1	17	11	24	23	11	17.2± 6.26	MS
	2	11	12	17	16	14	14± 2.55	
S-374; Tulsipur range, Sohelwa Wildlife Division, Gonda, U.P.	1	7	4	10	12	2	7± 4.12	MR
	2	11	7	9	10	8	9± 1.58	
S-106; Birdwal range, Hanumangarh Division	1	0	0	0	0	1	0.20 ± 0.45	R
	2	0	1	0	0	0	0.20± 0.45	
S-124; Kosi riverbank, Sunsaria Inerva, Nepal	1	0	1	0	0	0	0.20 ± 0.45	R
	2	1	0	0	0	0	0.20± 0.45	
S-19; Shahmansoorpur range, Khanpur Division, Saharanpur, U.P.	1	186	170	190	200	183	185.8± 10.92	S
	2	172	192	180	176	176	179.2± 7.69	
S-14; Pathri Range, Haridwar Division, Uttaranchal	1	22	17	27	29	15	22± 6.08	MS
	2	26	28	20	30	22	25.2± 4.15	
S-89; Hanumangarh range, Comptt. 54 D, Nausand Desal, Shergarh Division, Punjab	1	106	117	96	99	110	105.6± 8.44	S
	2	118	122	95	101	108	108.8± 11.30	
S-44; Trilokpur, Tulsipur range, Gonda Division, U.P.	1	7	5	9	3	11	7± 3.16	MR
	2	10	9	12	8	13	10.4± 2.07	
S-167; Rajaji National Park Chilla, Kunau range, Uttaranchal	1	0	0	0	0	0	0	R
	2	0	0	0	0	0	0	

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Screening against leaf blight by *Colletotrichum gloeosporiodes*




Results of screening against Leaf Blight

Clones showing resistance

9 = Pathri Range, Haridwar

41=Hasnapur, Tulsipur Range, Gonda

66= Chhachhrauli Range, Yamunanagar

113= Hitauda Campus, Nepal

106=Hanumangarh, Birdwal

Most susceptible clone

84=Hanumangarh, Ganganagar

Clone No	Percent infection
84	71.47
203	58.2
266	40
62	33.3
57	32.5
49	23.18
121	14.6
19	18.4
94	16.5
36	6.7
10	5.9
144	5.8
14	4.1
106	3.4
113	2.5
9	0
41	0
66	0

Screening against leaf blight by *Rhizoctonia solani*



Ten clones tested- none found resistant

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INDIAN FOREST CONGRESS, 2011
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MINISTRY OF ENVIRONMENT & FOREST

Interaction between *Ganoderma lucidum* and *Fusarium solani* – two serious root pathogens of *Dalbergia sissoo* mortality

by
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INTRODUCTION

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Dalbergia sissoo Roxb. ex DC

(A) (B)

Ganoderma lucidum (Leyss.) Karst.

- *Ganoderma lucidum* belongs to family Ganodermataceae and is a cosmopolitan basidiomycetes which causes white rot of hardwoods.
- The name is derived from the Greek word *ganos* = brightness, sheen and derma = skin, and the species epithet *lucidum* in Latin is for "shining".
- *Ganoderma lucidum* is considered to be one of the most beautiful shelf fungi and distinguished by its varnished red surface when it is young.
- Although *Ganoderma* is a important plant pathogen but the fruit bodies are popular as, and have long been used in traditional medicinal material in Asian countries and known as **Ling zhi** in China and **Reishi** in Japan.

Macromorphology

Fruiting body of *Ganoderma lucidum*

Micromorphology

(A) (B) (C)

(A) Culture of *Ganoderma lucidum* on PDA Plate (B) Fungal Mycelium (C) Chlamydospores

- **Host Range:** 64 host tree species
- ***Damage:*** components of cell wall e.g. cellulose and lignin are utilized and the resultant rot is termed white rot.
- **Symptoms:**

Stag-headed symptoms in *Dalbergia sissoo*

Fusarium solani (Mart.) Sacc.

- *Fusarium* is among the commonest of fungi, as well as being of great economic importance, and every mycologist or plant pathologist at one time or another encounters it.
- The fungus is facultative parasite inhabiting soil and possesses wide power of saprophytic colonization.

Macroscopic and Microscopic morphology

(A) (B) (C)

(A) Culture of *Fusarium solani* on PDA Plate (B) Fungal Mycelium & (C) Conidia

- **Host Range:** *Dalbergia sissoo*, *Azadirachta indica*, *Eucalyptus alba*, *Mangifera indica*, *Acacia nilotica*, *Ficus bengalensis*, etc.
- **Damage:** It causes moisture stress and plug the vessels resulting into wilt.
- **Symptoms:**

Wilting Symptom in *Dalbergia sissoo*

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Interaction between soil microbes

- On the basis of relative advantage to each partner i.e. host and microorganism, the relationships are basically of three types:

  (a) *neutralism* (b) *mutualism* and (c) *parasitism*

- *Ganoderma lucidum* and *Fusarium solani* cause root rot and wilt diseases, respectively in *Dalbergia sissoo* plants. They both share a common host and both attack the roots. It has not been reported wheather they live together in a friendly way or they check the growth of one another.
- The interaction between these two pathogens of a common host sharing same niche has not been reported earlier. So the present study has been planned with the following objectives:
  - ✓ To study interaction between *Ganoderma lucidum* and *Fusarium solani in vitro.*
  - ✓ To confirm the interaction in simulated environment.

MATERIALS AND METHODS

Collection of test fungus

National Type Culture Collection (NTCC), Forest Pathology Division, FRI, Dehradun (U.K.)

Experiment – I

To study the interaction of *F. solani* and *G. lucidum* in solid medium by dual culture method

Procedure

- Plate of MEA medium was prepared.
- Mycelial discs of *G. lucidum* and *F. solani* was placed with the help of inoculating needle in the opposite side of the plate. Control was also prepared in the same manner for each culture.
- Plates were incubated at 25±1°C in a BOD incubator.

Experiment: II

To study the interaction of *F. solani* and *G. lucidum* in broth medium

In liquid Medium

- Cell free culture filtrate of each culture was prepared and inoculated by opposite culture in two sets.
- One set was incubated in BOD incubator and second in shaking incubator for 15 days at 27 ± 2ºC.

Well in Agar method

- Culture filtrate of each culture was prepared in same manner in two set. One set was filtered by Whatman No. 1 filter paper and them with a bacterial syringe filter and second set was filtered by Whatman No. 1 paper only.
- Four agar plugs were cut with the help of cork borer and removed from MEA plates and filled with both type of culture filtrate of each culture separately.
- These plates were then inoculated by opposite fungus culture in center along the 4 wells.
- Plates were incubated in a BOD incubator at 25±1ºC for 15 days.

Experiment: III

To study the interaction on wood chips in soil

Procedure

- Flasks of 50 ml capacity were taken, half filled with soil, plugged with cotton and autoclaved.
- These flasks were first inoculated by wood chips which were already inoculated with *G. lucidum* culture. Spore suspension of *F. solani* was also mixed in the flasks.
- Flask were incubated for 21 days at 25± 1ºC.
- After 21 days inner most region of these wood chips was placed in PDA plates and cfu of *Fusarium* were counted on FSM medium plate by serial dilution method.

Experiment: IV

To study the interaction on spore germination

Procedure

- Chlamydospores of *G. lucidum* and conidia of *F. solani* were inoculated in five types of media :
  - ✓ Control (1% glucose solution)
  - ✓ Culture filtrate of *G. lucidum*
  - ✓ Cell free culture filtrate of *G. lucidum*
  - ✓ Culture filtrate of *F. solani*
  - ✓ Cell free culture filtrate of *F. solani*
- Observation for spore germination was started after 6 h and continued till 48h.

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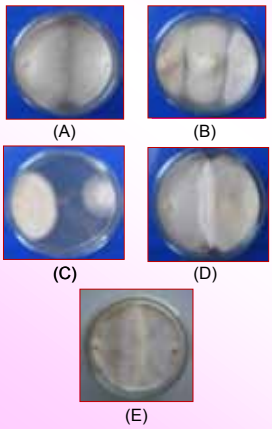
RESULTS & DISCUSSION

Experiment -I: Interaction of *F. solani* and *G. lucidum* in solid medium by dual culture method



Interaction on solid media. Control *Ganoderma lucidum* (A), Control *Fusarium solani* (B), Interaction between *Ganoderma lucidum* and *Fusarium solani* after three days (C) after seven days (D) and after fifteen days (E)

Number of spores from different area of the plate

Area of Sampling		No. of Conidia / Chlamydospores	
		F. solani	*G. lucidum*
At Growth Area of *F. solani*	At point of inoculation	35 x 10^5/ml	2 x 10^5/ml
	0.5cm away from inoculation point	1 x 10^5/ml	0.5 x 10^5/ml
	1.5 cm away from inoculation point	1.25 x 10^5/ml	2.25 x 10^5/ml
	2.5 cm away from inoculation point	1.5 x 10^5/ml	2.5 x 10^5/ml
At Interaction Point of Two Fungi		1.58 x 10^5/ml	2.25 x 10^5/ml
At Growth Area of *G. lucidum*	At point of inoculation	1.5 x 10^5ml	9.5 x 10^5/ml
	0.5 cm away from inoculation point	2 x 10^5/ml	5 x 10^5/ml
	1.5 cm away from inoculation point	2.25 x 10^5/ml	1.75 x 10^5/ml
	2.5 cm away from inoculation point	2.75 x 10^5/ml	2.25 x 10^5/ml

Both the fungi were able to sporulate in their area of growth as well as in the growth area of each other. Maximum sporulation was found at the point of inoculation for both fungi. However, the number of spores was found minimum when one was near to the point of inoculation of another. At the zone of interaction of two fungi spores of *G. lucidum were* more in number than those of *F. solani.*

Experiment – II: Interaction of *F. solani* and *G. lucidum* in broth medium

Mycelial weight of *Ganoderma lucidum* in broth in agitated condition (mean of three replicates)

Mycelial Weight of *G. lucidum*	Control (MEA broth)	Culture Filtrate of *F. solani*	
		Autoclaved	Unautoclaved
	0.14	0.096	0.070

Mycelial weight of *Ganoderma lucidum* in broth in stationary condition (mean of three replicates)

Mycelial Weight of *G. lucidum*	Control (MEA broth)	Culture Filtrate of *F. solani*	
		Autoclaved	Unautoclaved
	0.14	0.060	0.056

***G. lucidum* exhibited more growth under agitated condition than under stationary condition, however, growth was less in unautoclaved flasks.**

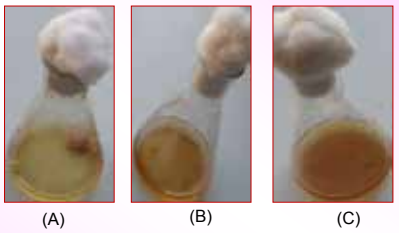
Mycelial weight of *Fusarium solani* in broth in agitated condition (mean of three replicates)

Mycelial Weight of F. solani	Control (MEA broth)	Culture Filtrate of *G. lucidum*	
		Autoclaved	Unautoclaved
	0.205	0.086	0.090

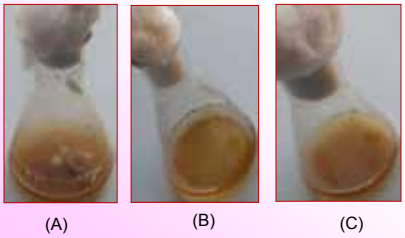
Mycelial weight of *Fusarium solani* in broth in stationary condition (mean of three replicates)

Mycelial Weight of *F. solani*	Control (MEA broth)	Culture Filtrate of *G. lucidum*	
		Autoclaved	Unautoclaved
	0.15	0.103	0.083

***F. solani* exhibited more growth under agitated condition than stationary condition, however, mycelial weight was less in autoclaved flasks in agitated culture but it was more under stationary condition in autoclaved flasks.**



Interaction in liquid medium (A) Control *Ganoderma lucidum* (B) *Ganoderma lucidum* in autoclaved culture filtrate of *Fusarium solani* (C) *Ganoderma lucidum* in unautoclaved culture filtrate of *Fusarium solani*



Interaction in liquid medium. (A) Control *Fusarium solani* (B) *Fusarium solani* in autoclaved culture filtrate of *Ganoderma lucidum* (C) *Fusarium solani* in unautoclaved culture filtrate of *Ganoderma lucidum*

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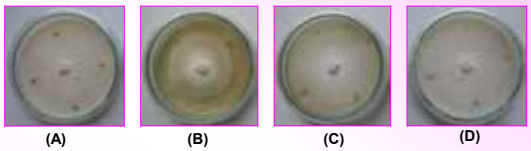
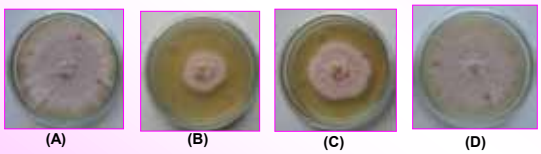


(A) (B) (C) (D)

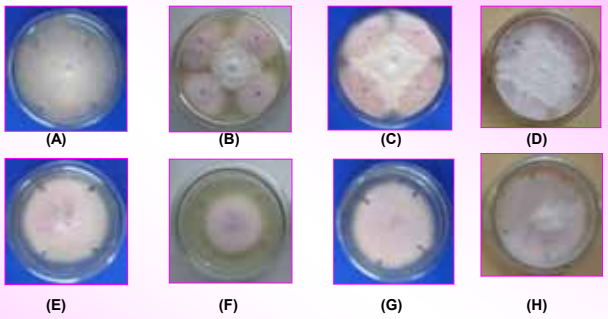
Well in Agar method (In cell free culture filtrate). Control *Ganoderma lucidum* (A), *Ganoderma lucidum* with *Fusarium solani* culture filtrate after three days (B), after seven days (C) and after fifteen days (D)



(A) (B) (C) (D)

Well in Agar method (In cell free culture filtrate). Control *Fusarium solani* (A), *Fusarium solani* with *Ganoderma lucidum* culture filtrate after three days (B), after seven days (C) and after fifteen days (D)

This experiment shows that cell free culture filtrates of both the fungi (*F. solani* and *G. lucidum*) do not impart any effect on the growth of one another.



(A) (B) (C) (D)

(E) (F) (G) (H)

Well in Agar method (In with-cell culture filtrate). Control *Ganoderma lucidum* (A), *Ganoderma lucidum* with *Fusarium solani* culture filtrate after three days (B), after seven days (C) and after fifteen days (D). Control *Fusarium solani* (E), *Fusarium solani* with *Ganoderma lucidum* culture filtrate after three days (F), after seven days (G) and after fifteen days (H)

Experiment – III: Interaction on wood chips in soil

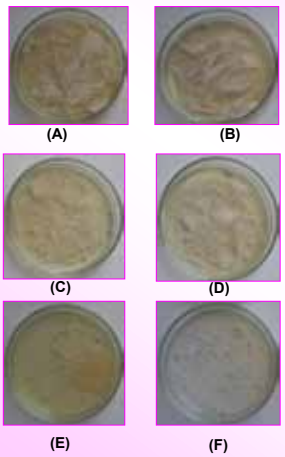
(A) (B) (C)

Interaction on wood chips in soil. (A) Control of *Ganoderma lucidum* (B) Control of *Fusarium solani* (C) Interaction between *Ganoderma lucidum* and *Fusarium solani*

Colony Forming Units of *F. solani* on *Fusarium* Specific Medium (mean of three replicates)

cfu of *F. Solani*	Dilution	Control	Interaction (*F. solani* and *G. lucidum*)
	10^{-1}	1.2×10^3	1.95×10^3
	10^{-2}	7.1×10^3	9.03×10^3
	10^{-3}	34×10^3	54×10^3

In the soil flask experiment conducted with *G. lucidum* colonized wood chips, it was found that the number of cfu(s) of *F. solani* increased as compared to control without *G. lucidum* chips. On isolation from the wood chips *G. lucidum* was obtained in cultures.



(A) (B) (C) (D) (E) (F)

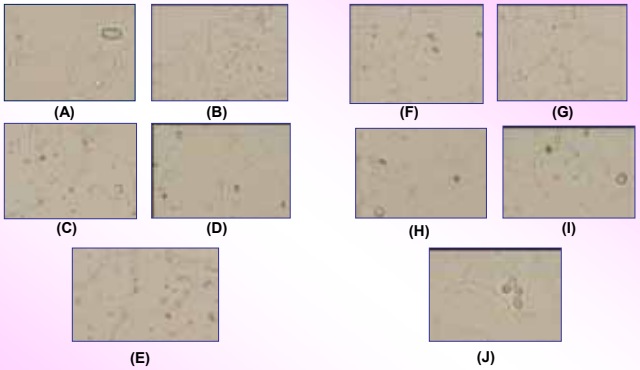
Colony Forming Units of *Fusarium solani* on Fusarium Specific Medium in different dilutions. (A) Control of *Fusarium solani* in 10^{-1} (B) *Fusarium solani* in 10^{-1} (C) Control of *Fusarium solani* in 10^{-2} (D) *Fusarium solani* in 10^{-2} (E) Control of *Fusarium solani* in 10^{-3} (F) *Fusarium solani* in 10^{-3}

Experiment – IV: Interaction on spore germination

Observation of spores in cavity slides

Medium	Germination percentage in *G. lucidum* spores		Germination percentage in *F. solani* spores	
	In light	In dark	In light	In Dark
Control (1% Glucose solution)	25	65	82.58	50.58
In culture filtrate of *G. lucidum*	68	60.37	52	33.84
In culture filtrate of *F. solani*	59	67.18	36	47.80
In cell free culture filtrate of *G. lucidum*	53	37	83.69	72.27
In cell free culture filtrate of *F. solani*	40	37	87	51.61

Germination of spores of *G. lucidum* and *F. solani* was favoured less in dark than under light. Culture filtrate of *G. lucidum* favoured spore germination of *F. solani* more than *vice versa*. Spore germination of *F. solani* was found more in cell free culture filtrate of both *G. lucidum* and *F. solani* as compared to control (1% glucose), in latter it was more than the former. In absence of light *G. lucidum* cell free filtrate favoured germination of *F. solani* spores than that of *F. solani* cell free filtrate. Maximum spore germination of *G. lucidum* was found in *G. lucidum* culture filtrate followed by *F. solani* filtrate.



(A) (B) (C) (D) (E) (F) (G) (H) (I) (J)

Spore germination in *Ganoderma lucidum*. (A to E in light) and (F to J in dark). (A) Control (1% Glucose solution) (B) In culture filtrate of *G. lucidum* (C) In culture filtrate of *F. solani* (D) In cell free culture filtrate of *G. lucidum* (E) In cell free culture filtrate of *F. solani*. (F) Control (1% Glucose solution) (G) In culture filtrate of *G. lucidum* (H) In culture filtrate of *F. solani* (I) In cell free culture filtrate of *G. lucidum* (J) In cell free culture filtrate of *F. solani*.

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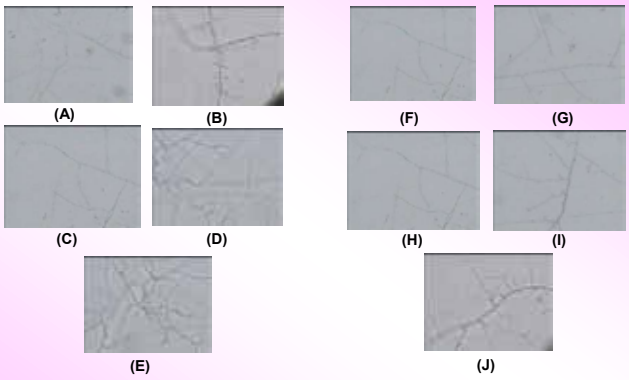

Spore germination in *Fusarium solani.* (A to E in light) and (F to J in dark). (A) Control (1% Glucose solution) (B) In culture filtrate of *G. lucidum* (C) In culture filtrate of *F. solani* (D) In cell free culture filtrate of *G. lucidum* (E) In cell free culture filtrate of *F. solani.* (F) Control (1% Glucose solution) (G) In culture filtrate of *G. lucidum* (H) In culture filtrate of *F. solani* (I) In cell free culture filtrate of *G. lucidum* (J) In cell free culture filtrate of *F. solani.*

CONCLUSION

- ✓ On the basis of the results it can be concluded that both the fungi can co-exist with each other at the same time in the soil and cause disease.
- ✓None of the fungus adversely affects the growth of another fungus, instead they favour the growth of each other. It can be summarized that both fungi have synergistic effect on each other.
- ✓It can be interpreted that both pathogens can cause disease in *Dalbergia sissoo* trees independently depending on conditions favouring them.

ACKNOWLEDGEMENT

With profound pleasure and gratitude, I would like to acknowledge Dr. S. S. Negi, IFS, Director, Forest Research Institute , Dehradun for his continuous encouragement and support.

My sincere thanks to Dr. N.S.K. Harsh, Scientist-F, Head, Forest Pathology Division, Forest Research Institute, Dehradun for his valuable guidance and constant supervision.

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DESCRIPTORS OF CASUARINAS FOR REGISTRATION OF NEW VARIETIES

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R. Anandalakshmi and V. Sivakumar

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Coimbatore

Introduction

IPR protection

Protection of Plant Varieties and Farmers' Rights Act, 2001

DUS Characters ;
D-Distinctiveness
U- Uniformity
S- Stability

Tree varieties - clones

Plant parts used for developing DUS characters

Tree habit(traits=1)
Stem (traits= 1)
Bark(traits=11)
Branch (traits= 4)
Cladodes (traits= 10)
Inflorescence (traits=4)
Flower (traits= 7)
Fruit (traits= 13)

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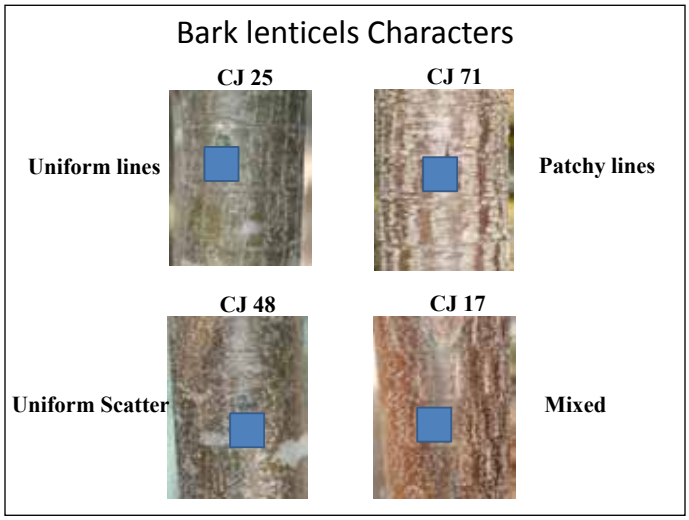
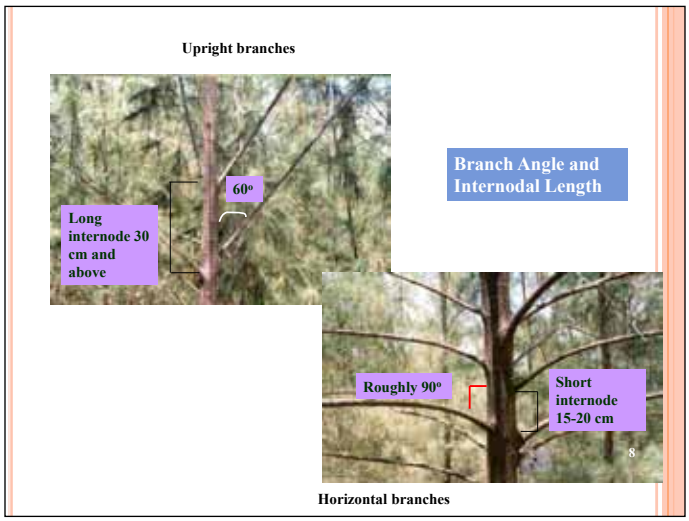
CASUARINA

Characteristic	State	Note	Example clone	Stage of observation	Type of assessment
Tree habit	Erect	3	51,87	24	VG
	Conical	5	140,49		
	Spreading	7	74,75,101		
Stem circularity	Circular	1	51	24	VS
	Non-circular	9	90,100		
Bark Texture	Smooth	1		36	VS
	Fissured	9			
Bark colour	light grey	1	90	36	VG
	purple	2	51		
	pinkish purple	3	134		
	dark grey	4	100		
	brown	5	206		
Pruning Scars	Isosceles triangular	3	51	36	VS
	Equilateral triangular	5	59		
	Scalar triangular	7	74		
Grouping of lenticels	Uniform lines	3	40, 63	36	VS
	Patchy lines	5	109		
	Uniform Scatter	7	51		
	Patchy Scatter	9	49		
Lenticels distribution	Uniform	1		36	VS
	Patchy	9			



CASUARINA

Characteristic	State	Note	Example clone	Stage of observation	Type of assessment
Lenticel shape	Round	1		36	MS
	Oval	2			
	Irregular	3			
Lenticels density	Very Low (<20 per sq. cm)	1		36	MS
	Low (21-30per sq. cm)	3			
	Intermediate (31-40per sq. cm)	5			
	High (41-50per sq. cm)	7			
	Very High (>51 per sq. cm)	9			
Lenticel Size	Small (≤0.5 mm)	3	63	36	MS
	Medium (0.5- 1.5 mm)	5	51		
	Large (≥1.5 mm)	7	140		
Knots	Present	1	108	24	VS
	Absent	9	51		
Leaf tip marks	Present	1		36	VS
	Absent	9			



CASUARINA

Characteristic	State	Note	Example clone	Stage of observation	Type of assessment
Branching pattern	Single	1		24	VG
	Paired	2			
	Others	3			
Branch angle	upright - angle ≥60	1	51	24	VS
	Horizontal angle> 60-90	9	14,111		
Branch Thickness	Thick (>2.5 cm)	3	51	24	VS
	Medium (1.5-2.5 cm)	5	111		
	Thin (<1.5 cm)	7	134		
Protrusions on primary branches at the point of occurrence of secondary branches	Present	1	61	24	VS
	Absent	9	51		
Cladode Colour	dark green	1	108	6	VS
	light green	2	51,140		
	bluish green	3	203		
	yellowish green	4			

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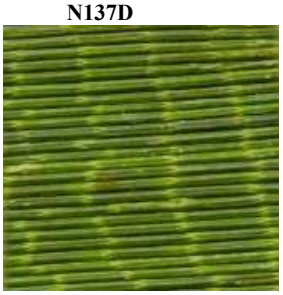
Protrusions on primary branches

Colour of Deciduous Branchlet

Dark Green

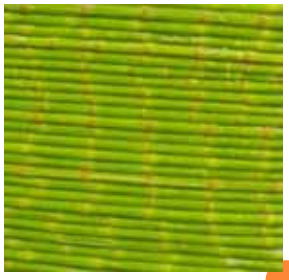
RHS Green Group N137D



CE25

Yellow Green

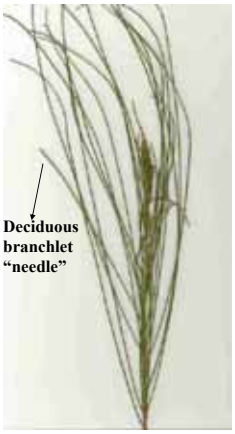
RHS Yellow - Green Group N147A

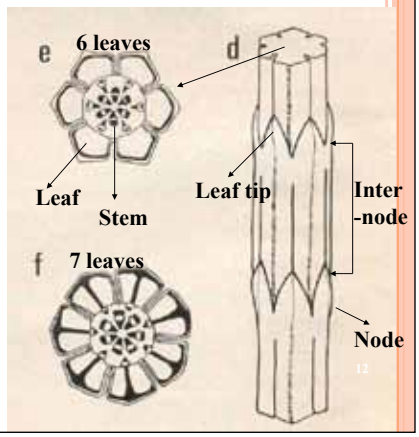


CJ9

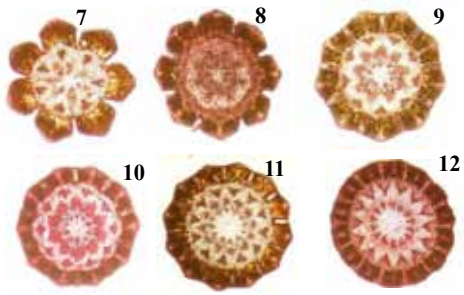
"Needle" Structure

The deciduous branchlet is a fusion of stem and leaves

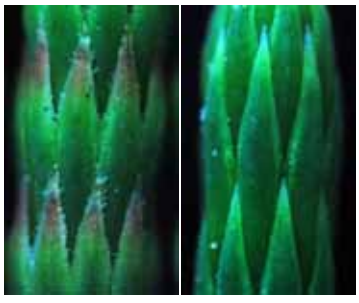




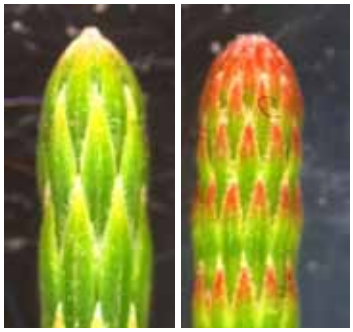
Number of leaves per node



Pubescence at leaf tips



Leaf tip colour



Casuarina equisetifolia *Casuarina junghuhniana*

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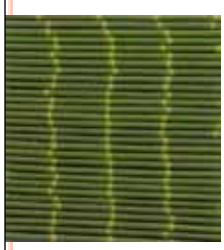
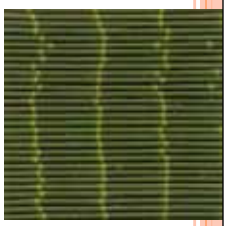
Descriptors on needle thickness and internodal length



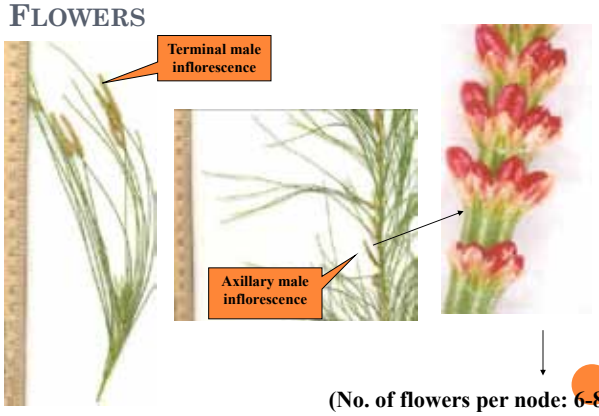
CJ56

CJ57



CJ58

Reproductive Characters: Male Flowers



(No. of flowers per node: 6-8)

Length of the male inflorescence	Short (<3 cm)	3		24	MS
	Intermediate (3-8 cm)	5			
	Long (>8 cm)	7			
No. of male flowers per whorl	6	1		24	MS
	7	2			
	8	3			
	Others	4			
Length of the filament at the time of anthesis	Long	1		24	MS
	Short	9			
Colour of the female flower	Light pink	1		24	VS
	Dark pink	2			
	Red	3			
Flower size	Small (<0.5 cm)	1		24	MS
	Large (>0.5 cm)	9			
No. of female flowers per whorl	6	1		24	MS
	7	2			
	8	3			
	Others	4			

Male Inflorescence – Filament length

Cone Characters- Peduncle length

CJ18

CJ55

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MANAGEMENT OPTION FOR FLOWERING IN *Bambusa tulda* Roxb. - A Case Study Under Chhotanagpur Agro- Climatic Zone

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BACKGROUND

- Flowering in bamboo is a rare phenomenon.
- Inventory on flowering bamboo is not adequate in our country for ensuring seed availability and maintenance of bamboo germ plasm.
- Information on bamboo flowering and seed setting in Jharkhand is almost absent except that in *Dendrocalamus strictus.*

BACKGROUND

- Both sporadic and gregarious flowering in *D. strictus* with successful seed formation have been noted from various parts of Jharkhand.
- Though sporadic flowering in *Bambusa nutans* has been noted in the state, seed setting is very rare.
- *Bambusa tulda* Roxb. is a sympodial bamboo endemic to Eastern and North-eastern states of India and grows well in humid tropical and subtropical regions of high rainfall having fine textured alluvial soil. Flowering cycle of *B. tulda* varies from 30 to 60 yrs (Seethalakshmi & Muktesh Kumar, 1998).

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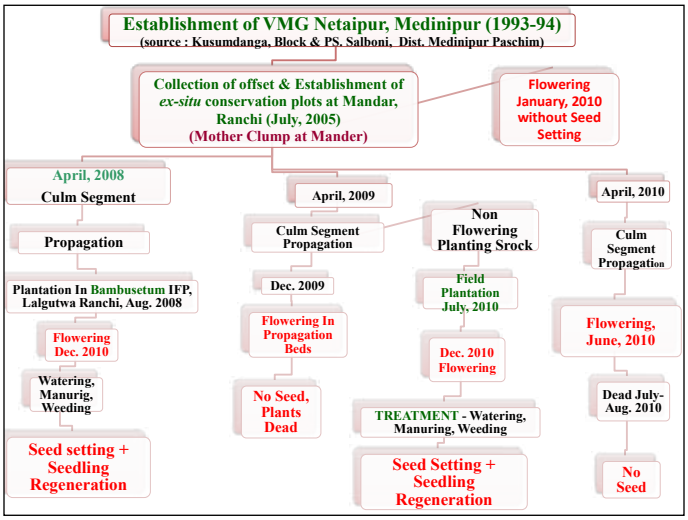
BACKGROUND

- It has recently been introduced in Chhotanagpur plateau region within the **BAMBUSETUM** and ***ex-situ*** **conservation garden** of Institute of Forest Productivity, Ranchi, Jharkhand.
- During vegetative propagation from culm cuttings and in one clump at the IFP bambusetum flowering in the species has been noted.

OBJECTIVES

- To understand the flowering behaviour of *B. tulda* under Chhotanagpur climatic condition.
- To study the effect of soil work, irrigation and manuring on seed setting in *B. tulda*.

FLOWERING BEHAVIOUR

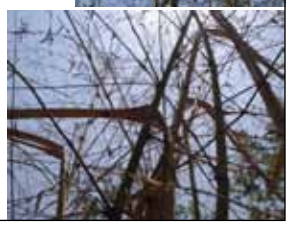


Flowering pattern of *B. tulda* in Jharkhand

Sporadic & culm flowering pattern –

Some culms flowering and die – other grow continuously but whole clump die after every culm has flowered.

The sequential flowering incidence supports the culm flowering pattern in the species



INFLORESCENCE

- **A leafless panicle** with branching pattern similar to that of vegetative culm bearing interrupted clusters of 2-3 long spikelets at nodes supported by chaffy bracts.
- **Rachis** smooth and striate.

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INFLORESCENCE

- **Spikelets** variable in length 20-40 mm long, 6-7 mm broad, sessile, glabrous, cylindrical, acute, when young after that they divide into 7-9 bisexual flowers or florets separated by conspicuous rachillae.

INFLORESCENCE

- The **spikelets** are subtended first by 1-2 **bracts**, 20-40 mm long, the 5 lowermost florets are reduced to empty **glumes** 5-9 mm long, acute and many nerved; followed by 4-6 **fertile florets** 17-20 mm long and 2mm broad at the base, acuminate, mucronate, glabrous, many nerved. **Lemma** 11mm long acuminate, mucronate, concave, bright green when fresh, overlapping with palea,

Bracts

Lemma & Palea

Single Fertile Floret

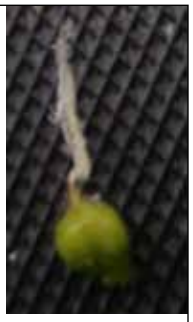
INFLORESCENCE (contd...)

- **Palea** shorter than lemma 9-10 mm long, 3-4 mm broad, boat shaped, 2-keeled, with long ciliae on keels, membranous, subtending a bisexual florets.
- **Stamens** 6 in number long and exserted, anthers 4.5-6.0 mm long, purple in colour, basifixed, blunt at tip, with a linear dehiscence, filament thread like.

INFLORESCENCE (contd...)

Ovary obovate -2 mm long, short style 1-2 mm long, divided into 3 plumose wavy stigma.

- **Lodicules** 3 found at base of ovary, 2.5-3.5 long cuneate, oblong, hyaline, upper part long, white fimbriate, 5 nerved.



Ovary with style

Lodicules

SEED

- **The seeds are caryopsis 0.94-1.05 cm long oblong, hirsute at apex and furrowed. The mid width of seeds ranges from 0.23-0.29 cm and thickness of 0.17- 0.20 cm.**
- **Dry 10 seeds weigh 0.422 g i.e, 23696 seeds kg^{-1}.**
- **Laboratory tests shows 97% germination.**

EFFECT OF SOIL WORK, IRRIGATION AND MANURING ON SEED SETTING

Materials & Methods :

- The study site is situated in *Chhotanagpur Plateau* (21°58' to 25°30' N Lat and 83°22' to 87°40' E Long) within agro-climatic zone 7 i.e., *Eastern plateau & hills region* and has the forest type *Northern tropical dry deciduous forests.* It experiences annual precipitation, maximum and minimum temperatures of 1246 to 1400 mm, 35.5 to 43.3°C and 5.6 to 11.5°C.

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EFFECT OF SOIL WORK, IRRIGATION AND MANURING ON SEED SETTING

Materials & Methods:

- Twelve clumps planted during July, 2010 (at 5m x 5m) developed through culm cuttings (during April 2009 - but not flowered in propagation beds during Nov.- Dec., 2009) have been selected for the trial after simultaneous appearance of inflourescence in them during Dec., 2010.

EFFECT OF SOIL WORK, IRRIGATION AND MANURING ON SEED SETTING - Materials & Methods :

The clumps were treated during Feb., 2011 as below taking 3 clumps for each treatment.

T_1 - Only Soil Work (Tillage operation surrounding the base of clumps at a radius of 1.0 m)

T_2 - T_1 + Irrigation (Fortnightly)

T_3 - T_2 + Manuring + Fert. (2.0 kg FYM + 0.25 kg DAP)

T_4 - T_2 + Manuring + Fert. (5.0 kg FYM + 0.50 kg DAP)

EFFECT OF SOIL WORK, IRRIGATION AND MANURING ON SEED SETTING

Materials & Methods (contd...)

- **Culm parameters viz., culm numbers, culm length & diameter, number of nodes, branching nodes etc. have been recorded.**
- **Three culms from each clump and three nodes from mid culm of each have been selected and marked.**
- **Number of spikelet bearing rachis & spikelets per node and fertile florets per spikelet have been recorded.**
- **Regular observations have been made to ascertain the seed setting since April, 2011.**
- **During May & June, 2011 after browning of the spikelets, seeds were collected and counted separately from each marked nodes.**

Clump parameters of Flowering *B. tulda*

Treatment		Clump No.	Clump parameters					
			No of Total Culm	Culm length (m)	Coll. Dia (cm)	No. of Nodes	No. of branching nodes	Rachis per node
T_1	Soil Work (no irrigation)	1	12	3.34	2.55	15	9	3
		2	7	2.83	2.14	12	7	4
		3	10	2.47	1.86	11	6	3
			Mean	2.88	2.18	12.67	7.33	3.33
T_2	T_1 + Irrigation	1	11	3.25	2.23	14	8	3
		2	13	3.45	2.65	15	9	3
		3	8	2.63	2.17	13	7	5
			Mean	3.11	2.35	14.00	8.00	3.67
T_3	T_2 + 2.0 kg FYM + 0.25 kg DAP	1	6	3.54	2.55	16	9	6
		2	8	2.75	1.86	12	6	4
		3	13	2.87	2.2	11	8	3
			Mean	3.05	2.20	13.00	7.67	4.33
T_4	T_2 + 5.0 kg FYM + 0.50 kg DAP	1	11	3.21	2.32	12	7	4
		2	10	2.88	2.25	10	8	3
		3	11	2.75	1.86	11	6	5
			Mean	2.95	2.14	11.00	7.00	4.00

Influence of Clump Treatments on Spikelets formation during Flowering in *B. tulda*

Treatment		Clump No.	Spikelets per node at Mid height			
			1st Node	2nd Node	3rd Node	Mean
T_1	Soil Work (no irrigation)	1	35	25	36	32.0
		2	25	21	42	29.3
		3	30	38	26	31.3
		Mean	30.00	28.00	34.67	30.89
T_2	T_1 + Irrigation	1	26	48	39	37.7
		2	52	42	29	41.0
		3	43	52	36	43.7
		Mean	40.33	47.33	34.67	40.78
T_3	T_2 + 2.0 kg FYM + 0.25 kg DAP	1	56	47	46	49.7
		2	42	49	53	48.0
		3	54	43	38	45.0
		Mean	50.67	46.33	45.67	47.56
T_4	T_2 + 5.0 kg FYM + 0.50 kg DAP	1	55	47	43	48.3
		2	45	54	48	49.0
		3	52	47	51	50.0
		Mean	50.67	49.33	47.33	49.11

Influence of Clump Treatments on Fertile Florets & Seed Setting in Flowering *B. tulda*

Treatment		Clump No.	Fertile Florets/spikelets				No of Seeds Collected			
			1st Node	2nd Node	3rd Node	Mean	1st Node	2nd Node	3rd Node	Total
T_1	Soil Work (no irrigation)	1	4	3	4	3.67	0	0	4	4
		2	5	4	5	4.67	2	0	1	3
		3	4	5	4	4.33	2	3	0	5
		Mean	4.33	4.00	4.33	4.22	1.33	1.00	1.67	4.00
T_2	T_1 + Irrigation	1	4	6	3	4.33	13	9	6	28
		2	5	5	6	5.33	9	5	6	20
		3	5	3	5	4.33	6	8	7	21
		Mean	4.67	4.67	4.67	4.67	9.33	7.33	6.33	23.00
T_3	T_2 + 2.0 kg FYM + 0.25 kg DAP	1	6	3	7	5.33	9	13	15	37
		2	4	5	6	5.00	12	11	7	30
		3	4	7	7	6.00	9	5	11	25
		Mean	4.67	5.00	6.67	5.44	10.00	9.67	11.00	30.67
T_4	T_2 + 5.0 kg FYM + 0.50 kg DAP	1	7	5	7	6.33	11	19	12	42
		2	6	5	8	6.33	9	11	9	29
		3	5	5	3	4.33	12	7	8	27
		Mean	6.00	5.00	6.00	5.67	10.67	12.33	9.67	33.67

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CONCLUSION

- Sporadic flowering may give rise to seed setting in isolated clumps which could be utilized for future propagation and subsequent plantation.
- Further, for setting of seeds in such situations of sporadic flowering, silvicultural management of clumps is obligatory with proper irrigation and manuring/fertilization.

CONCLUSION

- A moderate tillage of operation (soil working up to 15 cm deep) followed by manuring @ 2.0 to 5.0 kg FYM and 0.25 to 0.50 Kg DAP per clump favoured seed setting and regeneration of wild seedlings at the clump floor.
- The seedlings with known flowering cycle could be safely utilized for large scale plantation as well as clonal propagation.

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STEM & BRANCH WOOD VOLUME EQUATIONS AND VARIABLE DENSITY YIELD MODEL FOR *DALBERGIA SISSOO* PLANTATIONS IN IGNP AREA OF RAJASTHAN

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Institute of Wood Science and Technology

Bangalore

INTRODUCTION

- Forest yield tables are an essential source of information to forest management and forest planning.
- Their predictions make it possible to develop sustainable yield plan and to optimize silvicultural management.
- 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.
- 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.

INTRODUCTION

- To combat the desertification, the State Forest Department has taken up massive afforestation activities along the IGNP canal by planting various tree species like *A. nilotica*, *D. sissoo*, and *E. camaldulensis*.
- The plantations were raised throughout the area at different sites with varying stand density.
- The objective was to develop total wood, stem & branch volume equations and variable density yield model for *D. sissoo* for the productivity/yield estimation and management of plantations of this species in the area.

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YIELD TABLES

- A yield table is essentially a tool of long term planning and usually refer only to even-aged stands.
- It is a type of growth or 'experience' table which lists expected productivity/volume yield for a given age, site or crop quality and sometimes other indices like density.
- The main purpose of yield tables is to provide estimates of present yield and future increment and yield.
- 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, and applies to fully stocked (or normal) stands.
- 'Normal' is an unfortunate term as fully stocked stands are rather unusual.

  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.
- 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.

- It is always difficult to locate fully stocked stands or representative average stocked stands from which to collect the basic data as stocking may not have always been 'fully stocked' or 'average'.
- This led to the development of techniques for compiling tables by including stand density as the third variable; termed as variable density yield tables.
- Basal area/ha, mean diameter or stand density indices are used to define the density classes.

Study Area

- maximum daily summer temperature often exceeds 46-48°C.
- Night temperature occasionally touches 0°C owing to cold waves associated with the western disturbance causing frost conditions.
- mean monthly max. temperature varies between 39.5°C & 42.5°C.
- mean monthly minimum temperatures vary between 14-16°C.
- mean annual rainfall in the area varies from 120 mm to 300 mm.
- number of rainy days varies from 8 to 17 days.
- mean monthly relative humidity in the IGNP area fluctuates largely during the year from 15 to 80 %.
- The mean evaporation varies from 2.7 to 4.7 mm per day in winter and 13.2 to 15.3 mm in the summer.

Study Area

- 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.

Data and Field Procedures

- A total of 30 ample plots were laid out at various locations throughout the IGNP area, covering the available age groups, stand densities and sites, using stratified multistage sampling.
- 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.

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Summary statistics for the pooled data of the 30 plots of *D. sissoo*

Attribute	Minimum	Maximum	Mean	Std. Dev.
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
Quad. 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

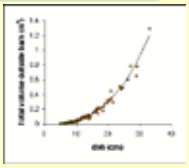
Site index, the dominant height of the trees in a stand at a given reference age, has been the most widely used means for estimating potential forest site productivity.

The dominant height is practically independent from the stand density and thus is used as an indicator of the site productivity.

For estimating site index, the base-age was selected as 15 years.

Volume Equations Tested

Equation type
$V = a+bD^2H$
$V = a+bD^2$
$V = a+bD+cD^2$
$V = a+bH+cD+dD^2+eD^2H+fDH$
$V = aD^b$
$V = aD^bH^c$



The error structure in volume estimation never happens to be homogeneous since the observations are not measured with equal precision.

Thus, ordinary least squares no longer yields parameter estimates of the linear regression models with minimum variances.

Hence weighted least square fitting technique was applied for fitting the first four equations.

It was not necessary for the last two equations as they were fitted with non-linear technique.

Variable Density Yield 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* :

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

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).

Model Fitting & Validation

The data was randomly divided into two sets.

The models were fitted to the first set consisting of 70% of the data and the second set, consisting of 30% of the data, was used for validation purposes.

The coefficient of determination (R^2) and the root mean square error (RMSE) were used to determine the quality of fit.

With small data sets, there are chances that assignment of trees to the validation data set may be poor.

Therefore iterative validation procedure was also adopted to avoid this problem.

Here the regression equations were compared against each other for estimating volume from sample data by using cross-validated simulation study.

The data were randomly partitioned into 5 different subsets.

In turn, each of the 5 data sets containing 20% of the data was set aside for validation, and the remaining 80% of the data were used to fit each equations.

The fitted models were then used to estimate the volume for each of the 5 validation subsets.

The standard error of estimate (SEE) and the average bias (B), were used as evaluation criteria for model validation.

The SEE is given as

$$SEE = [\sum(V_i-V_i)^2/(n-p-1)]$$

The average bias is calculated as

$$B = \sum(V_i-V_i)/n$$

where, p = number of model parameter; V_i, V_i, and n are as given above.

In the cross validation study, the average prediction bias was given by

$$B = (1/5)\sum B$$

Similarly the standard error (SEE) was also computed over the five validation subsets.

Results

The data collected was used to develop stem and branch & total wood volume equations:

Total wood

$V = -0.0023 + 0.0000364\ D^2H$; $R^2 = 0.992$; RMSE = 0.00006

$V = 0.01328 - 0.00538\ D + 0.000760\ D^2$; $R^2 = 0.961$; RMSE = 0.00005

Stem wood

$V = -0.001337 + 0.00003399\ D^2H$; $R^2 = 0.991$; RMSE = 0.00012

Branch wood

$V = -0.000373 + 0.000002459\ D^2H$; $R^2 = 0.938$; RMSE = 0.00015

Variable density yield model was developed taking volume/ha as regressor variable and age, dominant height, stems/ha, and BA/ha as predictor variables

$$V = \text{Exp}[2.0593 + 0.1215*\ln(TH/A) - 0.2477*\ln(N) + 1.4835*\ln(BA)]$$

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Discussion & Conclusions

- A regional yield model is a useful tool for evaluating the effects of different harvest levels on a given age-class distribution.
- 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.
- The first step towards refinement should be involving a method for considering the effects of different levels of stand density.
- Projections based on yield tables need to be adjusted for variable density which may be done using tables of reduction factors.

Discussion & Conclusions

- VD density 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.
- 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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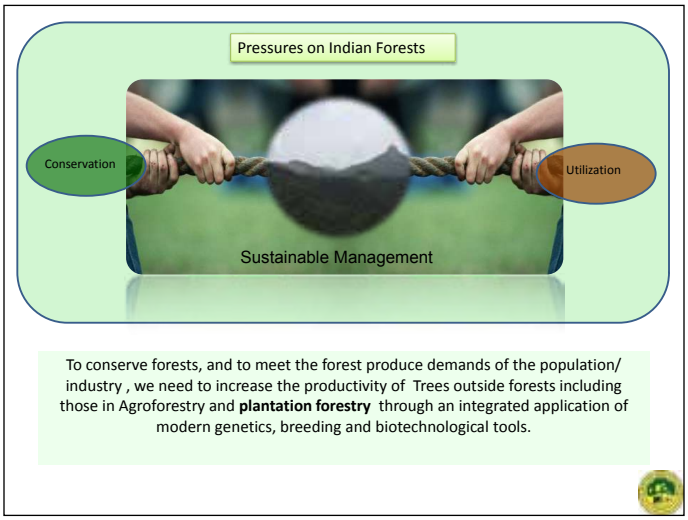
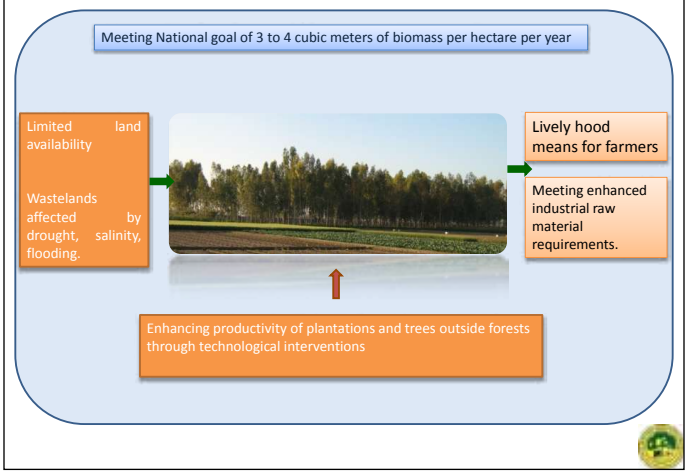
Dr. N. Krishna Kumar, IFS, Director,
Institute of Forest Genetics and Tree Breeding, Coimbatore



To conserve forests, and to meet the forest produce demands of the population/ industry , we need to increase the productivity of Trees outside forests including those in Agroforestry and **plantation forestry** through an integrated application of modern genetics, breeding and biotechnological tools.



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Tree improvement programmes

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.**

Components of Tree Breeding

- Provenance tests
- Selection of plus trees
- Vegetative propagation and nursery techniques
- Establishment of gene banks
- Clonal and seedling seed orchards
- Controlled pollination / hybridisation
- Half sib progeny tests
- Full sib progeny testing

Tree Improvement: Different phases

- 1930-60s:
- Seed origin plot of Chir Pine by Laurie in FRI (1925).
- All India Teak Seed Origin Cooperative Experiment (1930) by Sir Harry Champion at FRI.
- Research on Vegetative propagation, chromosome numbers, tree breeding (1950s)

- 1960s: Based on the "Programme of Forest Genetics and Forest Tree Breeding Research" by Mr. J.D Mathews, and Under the Stewardship of Dr. S. Kedharanath, the Forest Geneticist of FRI, **Plus trees of teak** were identified in the states of TN, Kerala, AP, and Karnataka. **Clonal Seed orchards** were established in these states by deploying these selections.

- 1970s: "Indo Danish Project on Seed Procurement and Tree Improvement" came into existence with its base in Hyderabad and Centres at Dehra Dun and Coimbatore.
  - Emphasis on Teak, Rosewood, Gmelina and Bombax improvement.
  - Plus trees Identified, Orchards and Seed Production Areas established
  - Emphasis on "Certification of Forest Reproductive Material"

- Sandal Research Centre , Bangalore
  - *Elimination of the sandal spike disease.*
  - *Selection of sandal Plus trees and establishment of seed orchards*
- Tropical Pine Centre, Kodaikanal
  - Introduction and evaluation of provenances of *Pinus carribea, Pinus keysia, Pinus oocarpa*

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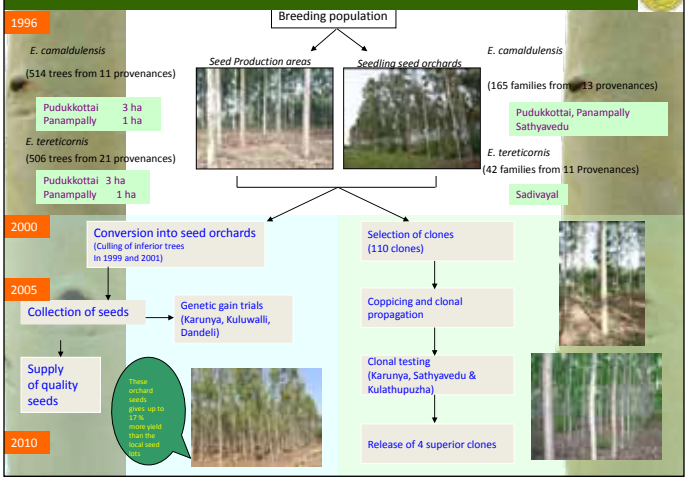
- Eucalyptus Research Centre, Ooty
  - Introduction and evaluation of Eucalyptus species and provenances. These trials formed the base for large scale plantations of Eucalyptus in Southern States, and were the source for many commercially planted clones.
- India also participated in the International Provenance trial of Gmelina and Teak during 1981-83. (Teak International Provenance trial at Maredumili, Andhra Pradesh)

International Neem Network coordinated by FAO

Seedlots from six countries were received and trails were established at India. Similarly seeds from 10 locations from India were collected and given to participating counties for establishment of trials through FAO collaboration

- 1990s : World Bank aided Forestry Research, Education and Extension Project (FREEP) by ICFRE.
  - Under this project IWST, Bangalore and IFGTB, Coimbatore established SPA, Seed Orchards for various species in collaboration with the State Forest Departments.

Eucalyptus Improvement Programme in IFGTB



Ramets of a shortlisted clone

Growth performance of tested clones

Clone	GBH (cm)	ht (m)	Clone	GBH (cm)	ht (m)
1	37.58	16.30	101	30.42	17.30
7	34.46	13.75	111	33.95	14.44
9	35.14	16.31	115	34.40	16.60
10	29.45	15.65	116	31.11	16.56
17	36.58	16.17	123	29.50	14.34
19	31.4	16.50	124	30.71	16.13
31	34.43	16.67	131	34.57	15.63
53	36.50	17.50	136	34.64	15.11
66	33.67	16.29	154	35.38	15.63
69	35.54	18.30	186	34.38	16.72
75	27.30	16.06	187	30.33	15.88
76	32.42	17.50	188	32.83	16.00
88	32.61	15.08	191	32.13	16.50
94	31.69	16.42	196	33.59	17.44
100	29.32	11.61	198	31.59	16.78

Mass plantations of such high biomass clones have become possible due to the use of novel vegetative propagation methods like minicutting technique.

Clone	GBH (cm)	ht (m)
ITC-3	32.5	17.3
ITC-7	30.2	14.6
ITC-10	38.1	16.9

Hybrids of Eucalypts

- Intra-specific crosses were produced between *E. tereticornis* and *E. camaldulensis, E. grandis, E. pellita, E. urophylla, E. alba,* to combine growth with wood density and Growth with form
- Collaborative projects with ITC has been initiated for hybridization of the related *Corymbia torelliana* x *C. citriodora.*

Panampally

- 36 months growth data reveal full sib families of *E. tereticornis x E.pellita, E.grandis* promising

E. tereticornis x E. pellita

E. tereticornis x E. grandis

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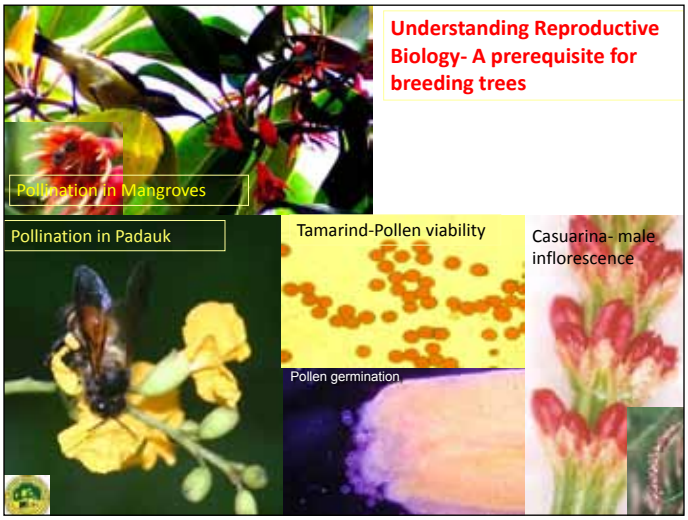

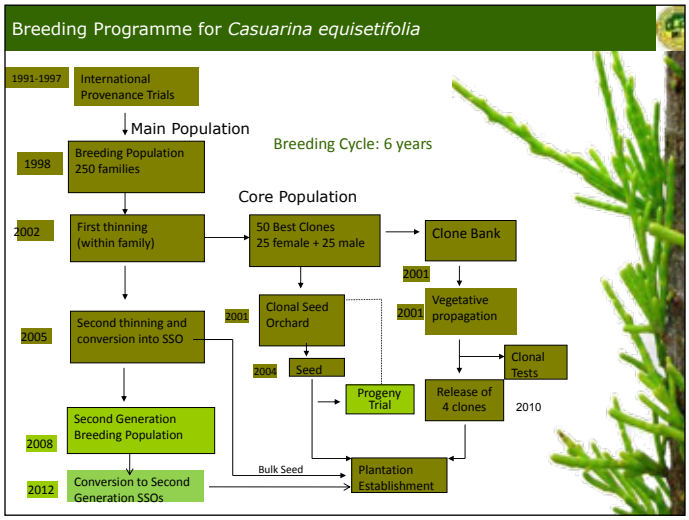

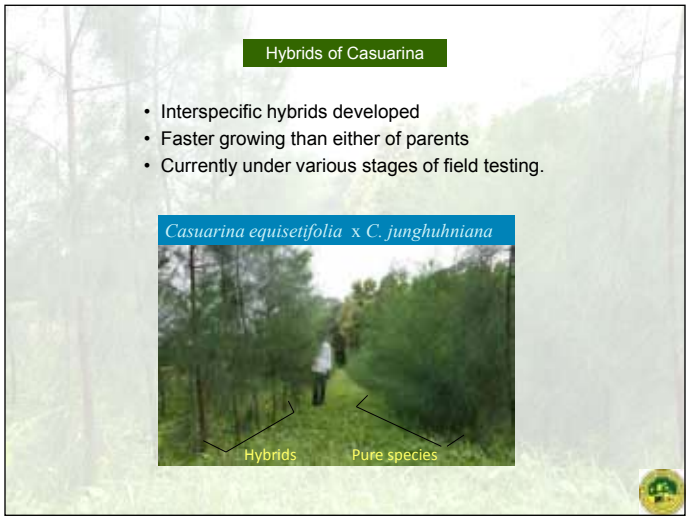

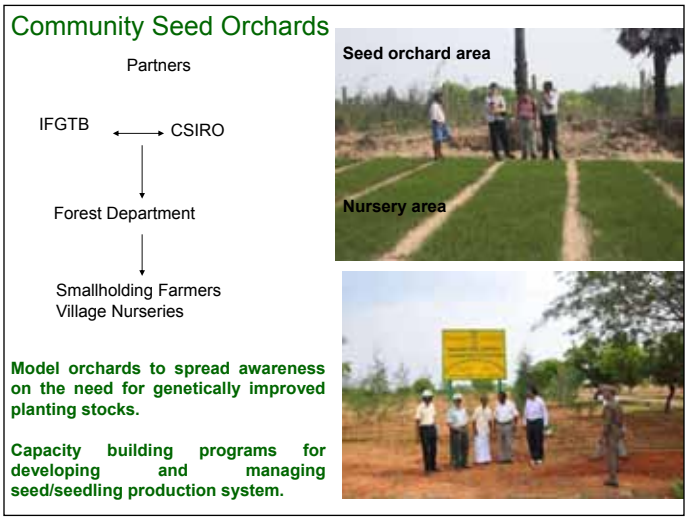

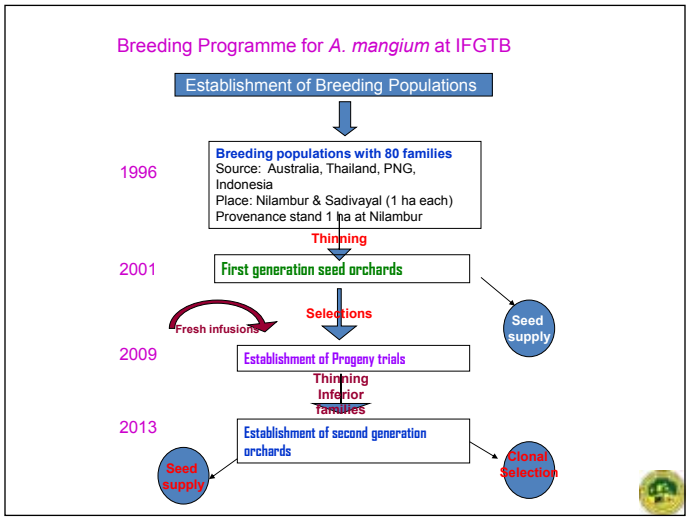

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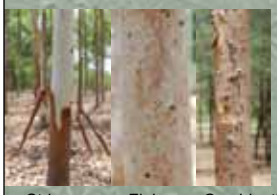
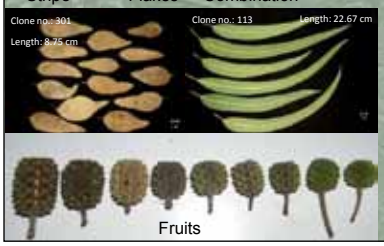
Developing strategies for describing, testing and registering varieties of four forest tree species in India



- Develop species level descriptors for 2 Eucalyptus and 2 Casuarina species.
- Develop provenance level descriptors based on the variability in populations in two Eucalyptus and two Casuarina species
- Develop clonal descriptors for Eucalyptus and Casuarina.

Stripe Flakes Combination



Casuarina junghuhniana *C. equisetifolia*

Genetic Improvement of Indigenous Trees-Species for research

- ***Ailanthus excelsa***
- ***Ailanthus triphysa***
- *Dalbergio sissoo*
- ***Gmelina arborea***
- ***Melia dubia***
- ***Neolamarkia cadamba***
- *Tectona grandis*
- ***Thespesia populnea***
- *Tamarindus indica*

- *Artocarpus spp.*
- *Azadirachta indica*
- *Bamboo spp.*
- *Calophyllum inophyllum*
- *Dalbergia latifolia*
- *Pterocarpus marsupium*
- *Pterocarpus santalinus*
- *Sapindus emarginatus*
- *Santalum album*
- *Terminalia spp*

Genetic improvement of Teak

SPAs have been established in the states of Tamil Nadu and Kerala

Cost-effective clonal technology and tissue culture protocols have been developed for mass production of superior planting stock.

Understanding low fruitset, Seed Production and Germination from CSOs and SPAs of Teak

Pollinator and hybridisation studies

Studies on impact of continuous moisture on growth, flowering, seed production and wood characteristics of canal teak plantations in Tamil Nadu

Entomopathogenic fungi and biopesticides like NPV have been used to control teak stem borer.

Genetic Variability In *Artocarpus* Species

- Genetic Variability in *A. heterophyllus* Lam., *A. hirsutus* Lam. (wild jack, *A. gomezianus* Wall. ex Trec ssp. *zeylanicus* has been studied.

An *ex situ* conservation stand cum Seed Production Area (10Ha) has been established for *A. hirsutus* –an endemic threatened species of Western Ghat.

Lessons from Gall Outbreak in Eucalyptus

Use of diverse germplasm in clonal plantations

Large scale plantations of Eucalyptus using limited clones have resulted in unprecedented outbreak of the insect pest *Leptocybe invasa*.

The need for a multiclonal approach using diverse clones to deal with pest outbreaks.

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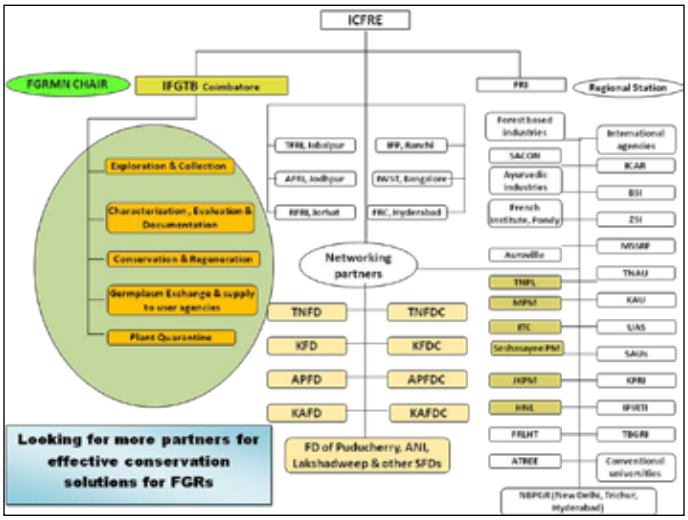
Genetic Resources for Sustainable Breeding

– Breeding towards trait improvement: targeted breeding for desirable trait
  - Pulping and Biomass/ biofuel traits- High cellulose, low lignin
  - Pest tolerance
  - Salt , drought, flooding and metal contamination tolerance.
  - Flowering traits.
  - Coppicing and rooting potential

– Molecular markers help in both characterisation and rational management of germplasm resources

Forest Genetic Resource Management

- With breeding programmes increasingly moving from selection for enhanced biomass to breeding for desired traits, availability of germplasm resources characterized for these traits, therefore, becomes important.
- In this direction, ICFRE has embarked on a Forest Genetic Resource Management Network.



Species prioritized for Forest Genetic Resource Management Network

S. No.	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

S. No.	Prioritized Species		Networking partner for species
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 species identified by NMBA)	Indigenous	IFGTB, IWST, RFRI, TNFD, KFD, APFD, KAFD, MFD, TNPL, KFRI, CARI, FCRI, TBGRI

Germplasm Conservation at NBPGR*

CRYOGENEBANK
8,804 accessions
722 species

SEED GENEBANK
~ 3,70,208 accessions
~1300 species

(*as on 31st March 2009)

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DNA STORAGE

Long-term storage of DNA of different genotypes would also provide an alternative space saving method for conserving genes from valuable genotypes making it possible for later use in transgenic programmes.

In-vitro plant culture: Applications in FGR and Tree Breeding

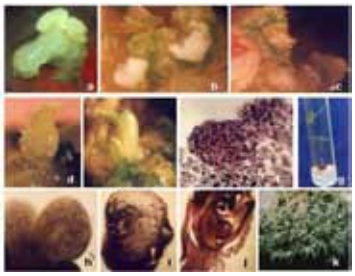
In- vitro culture of plants provide controlled condition platform for conservation of a large number of precious genotypes growing in diverse climatic conditions.

Teak

Different techniques of tissue culture

- Tissue and cell culture
- Meristem culture
- Pollen culture
- Embryo culture
- Protoplast culture
- Somatic hybridization

Somatic embryogenesis protocols allows for reprogramming of juvenile phase in addition to fixing of desirable traits.



Various stages of somatic embryogenesis in bamboo, *Dendrocalamus strictus*

D. giganteus

Species Multiplied

- *Eucalypts*
- Teak
- *Acacia* hybrid
- *Bambusa bambos*
- *B. bambos var. giganteus*
- *B. nutans*
- *Dendrocalamus strictus*
- *D. stocksii*

***E. torelliana X E. citriodora* hybrid**

Field demonstration trial of *D. stocksii*

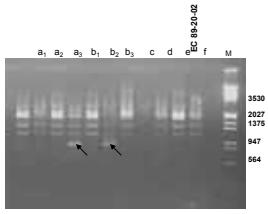
Field trials of Tissue cultured Bamboo

Species taken up for trials

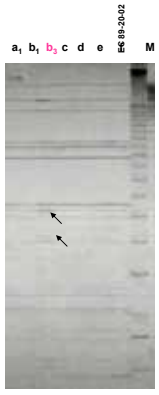
- *Bambusa bambos*
- *Bambusa balcooa*
- *Bambusa tulda*
- *Bambusa vulgaris*
- *Bambusa nutans*
- *Dendrocalamus strictus*
- *Dendrocalamus stocksii*

Total area – 100 ha

Quality control for genetic uniformity of micropropagated plants using molecular markers



RAPD profiles (using primer OPE-13) of Plantlets a_1, a_2, a_3, b_1, b_2, b_3, c, d, e and f derived from EC 29-20-2. Lane M is lambda *Hind*III/*Eco*RI digest.

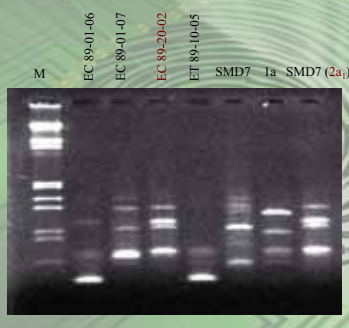


AFLP analysis with primer pair E_{ACC} and M_{CAC}. Lane M is 25 bp ladder.

Tripathi *et al.*, 2006

Rational Management of Germplasm Resources:

Identification of mislabelled clones in germplasm collection



RAPD analysis with primer OPB-04 to compare the doubtful clones (1a and $2a_1$) with other clones maintained in germplasm viz. EC 89-01-06, EC 89-01-07, EC 89-20-02, ET 89-10-05 and SMD 7. The profiles of $2a_1$ matched exactly with that of EC 89-20-02.

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Germplasm characterization: Advanced tools

Characterization	Technologies
Tree form factor, branch angle, branch thickness, crown length and width	Digital image analysis
Eucalyptus Pulping quality, Fibre characteristics	NIR spectroscopy, Acoustics, Silviscan
Diversity and other desirable traits	DNA markers
Medicinal properties	Metabolomics

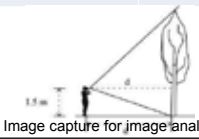


Image capture for image analysis

Characterization of valuable plant metabolites and *in vitro* production

Source of Forty five percent of the best selling allopathic drugs is from natural products or their derivatives (Cragg *et al.*, 1997).

Plant source	Phytochemical	Pharmacological activity
Catharanthus roseus G.Don	Vincristine, Vinblastine	Anticancer
Digitalis purpurea L.	Digitoxin	Cardiovascular
Digitalis lanata L.	Digoxin	Cardiotonic
Dioscorea deltoidea Wall.	Steroids from Diosgenin	Antifertility agent
Rauwolfia serpentina L.	Reserpine	Antihypertensive
Catharanthus roseus	Ajmalicine	Antihypertensive
Colchicum autumnale L.	Colchicine	Antigout
Papaver somniferum L.	Morphine	Analgesic
Papaver somniferum L.	Codeine	Antitussive
Cinchona ledgeriana Moens ex Trimen	Quinine	Antimalarial
Atropa belladonna L.	Atropine	Anticholinergic
Hyoscyamus niger L.	Hyoscyamine	Anticholinergic
Datura metel L.	Scopolamine	Anticholinergic
Pilocarpus sp.	Pilocarpine	Cholinergic

Biochemical characterization of valuable plant metabolites and *in vitro* production

- *Premna serratolia*
- *Saraca asoca*
- *Mimusops elengi*
- *Commiphora mukul*
- *Mallotus phillippensis*
- *Stereospermum sp.*
- *Albizia amara*
- *Oroxylum indicum*

Coumarin profile of *Aegle marmelos*

Medicinal trees species from which Bark, root , heartwood are extracted

Hairy root cultures have been used to produce important alkaloids from transformed medicinal plants like *Atropa belladonna, Catharanthus tricophyllus* and *Datura candida* (Saito *et al.*, 1992; Sevon, 2002).

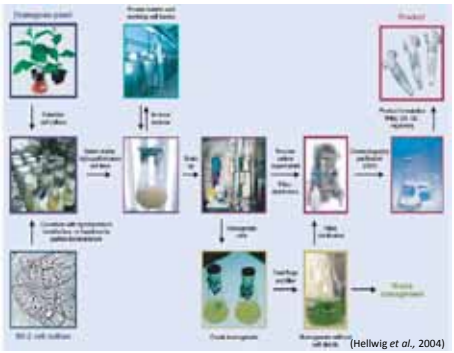
Metabolic pathways could be engineered for

- **more of a desired compound.**
- **production of new compounds .**
- **reduced levels of compounds.**

Hairy root cultures of *Atropa belladonna*.
(Courtesy: www.mbc.ntu.edu.tw/faculty/teachers/LeeKT2.htm)

Metabolic engineering

Metabolic engineering for large scale **non-destructive production** of phytochemicals like taxol, from transgenic plant, cell and hairy root cultures are being developed.



(Hellwig *et al.*, 2004)

Overview of phytochemical production using transgenic plant cell cultures

DNA fingerprinting applications in Tree Breeding

– Provenance trials

- – **Understanding the existing diversity of natural and breeding populations to** making rational selections-What to plant and what to thin
- – **Early selection – tagging genes responsible for desirable traits like pest resistance, low lignin.**

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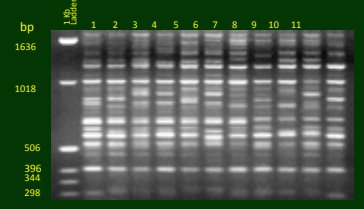
DNA fingerprinting applications in Tree Breeding

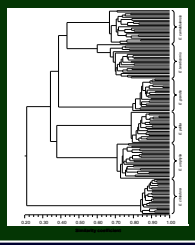
– Seed orchards
  – Maintaining diversity during establishment.
  – Mating patterns within seed orchards.
    » Estimating out crossing and selfing rates,
    » Identification of trees contributing to the gene flow
    » quantifying genetic drift in seed orchards-Study changes in allele frequencies under various selection pressures

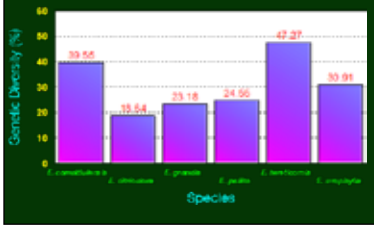
– Hybridization
  – Identification and parentage determination of hybrids.

Molecular markers help in accelerated breeding

Genetic diversity within *Eucalyptus* species



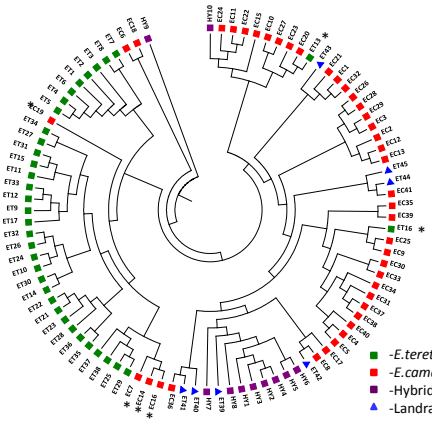




AMOVA analysis for Genetic diversity

Source of variation	Percentage of variation
Among populations	15.65
Within populations	31.03

Species differentiation in *E.camaldulensis* and *E.tereticornis* using microsatellite (SSR) markers

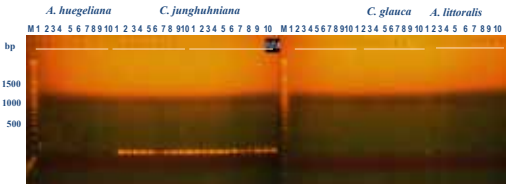


- 62 EMBRA SSRs were used
- Pure ET and EC were separated
- Landraces and Hybrids grouped with EC
- Queensland provenances (naturally intergrade between EC and ET) and bulked seedlot showed mixed clustering

-*E.tereticornis*
-*E.camaldulensis*
-Hybrids
-Landraces

DNA MARKERS FOR SPECIES/ HYBRID AUTHENTICATION

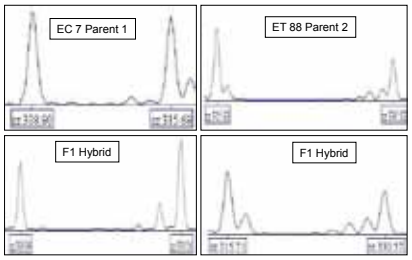
Species diagnostic marker in *C. junghuhniana*



Species diagnostic marker in *C. equisetifolia*

High throughput analysis of populations

Genetic Analyser – 8 capillary system used for microsatellite analysis



F1 Hybrids showing the parent alleles obtained using EMBRA SSR 9

Development of markers for wood and rooting traits

Development of mapping populations

A networking program on Genetic improvement of *Eucalyptus* through QTL and Association mapping has received financial support from DBT, GOI

- Generation of genome wide linkage maps using SSR markers for the eucalypts mapping populations
- Identification of QTLs for wood properties and adventitious rooting traits

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Next Generation Sequencing Era

With the

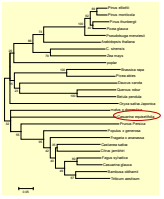
- *release of the 691 MB Eucalyptus grandis genome sequence*
- *advent of high throughput Next Generation Sequencing techniques using 454, Solexa and Solid systems*

the tree genomics studies are poised for a quantum jump providing insights on a number of genes that govern desirable traits.

Bioinformatics based Gene Mining Program

Availability of genome sequence information in tree species necessitates use of Bioinformatic tools for annotation of novel genes from these species.

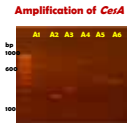
Pathogen defense- related genes (chitinase, *chi1* and glucanase, *glu1*) have been isolated, cloned and characterized from *Casuarina equisetifolia*.



Phylogenetic analysis of *CeChi1*

Bioinformatics based Gene Mining Program

Gene	Organism	trait
CesA	*E. tereticornis*	Cellulose synthesis
HKT1	*E. tereticornis*	Sodium transport
Chitin Synthase	*Leptocybe invasa*	Insect chitin synthesis
Chitin Synthase	*Hyblea purea*	Insect chitin synthesis

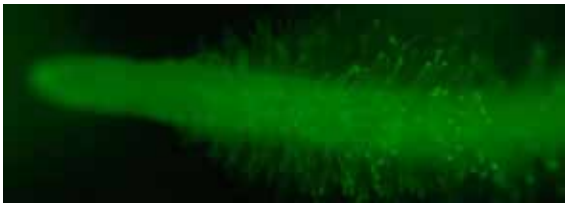


Transgenics for gene function analysis

Validating that the gene sequences functionally contribute to the desired trait is a prerequisite for their development as molecular markers.

- Transgenics are being used for understanding the function of genes determining valuable traits.
  - heterologous expression
  - silencing genes

Composite transgenics as *in vitro* systems for gene function analysis



Induction of transgenic hairy root from *in vitro* micropropagated *E. tereticornis*

Transgenics for breeding desirable traits

- *Field selections provide a rapid method for identification of trees with high biomass.*
- *Incorporation of other desired traits in these identified selections could be brought out using transgenic technologies.*

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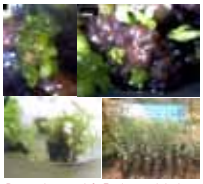
Transgenic programme at IFGTB

- Transgenic programme for enhancing productivity of Eucalyptus in salt affected lands are ongoing. Putative *AtNHX1* transgenic Eucalyptus plantlets have been generated.
- Project on development of gene silencing approaches for control of the Gall pest " *Leptocybe invasa" is ongoing.*

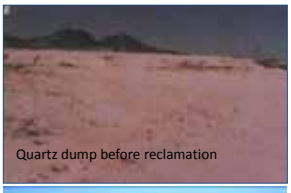


Regeneration protocols for Eucalyptus optimized

Genetic transformation , regeneration, selection and PCR confirmation of transformants

Eco-restoration of problem sites

Problem soils like quartz dumps, magnesite / lime stone/ bauxite mine spoils were reclaimed using suitable tree species and proper soil amendments. Transgenic approaches could be used for bioremediation of problem sites.



Quartz dump before reclamation

Reclaimed quartz dump

Reclamation of bauxite mine spoils after 1 year

Reclamation of bauxite mine spoils after 2 years

Transgenic trees in field trials in different parts of the world

S.No	Name of the tree species	Gene	Properties	Institute/ Company	Trial release date	Place of release
Abiotic stress tolerance						
	Metal Bioremediation					
1	*P. deltoides*	Mercuric ion reductase (MerA) , Organomercury lyase	Bioremediation of mercury contaminated soils	Applied PhytoGenetics Inc.	2004	USA
	Cold tolerance					
2	*E.camaldulensis*	CBF3 gene	Cold tolerant	Arborgen (USA)	2005	South carolina
	Drought tolerance					
3	*P. tremula x P. alba*	Vacuolar pyrophosphatase	Drought	University of Connecticut	2005	USA
	Herbicide tolerance					
4	*P. tremula x P. alba*	Phosphinothricin acetyl transferase	Glufosinate tolerant	University of Connecticut	2005	USA
5	*E. grandis*		Glyphosate tolerance	Shell Research Ltd		UK
6	Roundup Ready Eucalyptus clones SFR10 and SFR11	HPPD inhibitors	Glyphosate tolerance	Monsanto do Brasil Ltda Brazil	1999	Brazil
Biotic stress tolerance						
	Insect tolerance					
7	*P. deltoides*	*cry3Aa gene-*	Resistant to insects especially Coleoptera	Swedish University of Agricultural Sciences, Umeå	2008	Umea

S.No	Name of the tree species	Gene	Properties	Institute/ Company	Trial release date	Place of release
Other desired traits						
	Lignin modification					
8	*Eucalyptus urophylla*		Reduced lignin	International Paper do Brasil Ltda.	Approved 2006	Brazil
9	*P. deltoides*	*INRA717-1-B4 gene-*	Reduce lignin content and improves pulp performance	Swedish University of Agricultural Sciences	2008	Umea
10	*Populus alba x populus tremula-*	*CCoAOMT (Caffeoyl coenzymeA O-methyl transferase*	Modified lignin due to the decreased activity of an enzyme of the lignin biosynthetic pathway	Vlaams Interuniversitair Instituut voor Biotechnologie , Belgium.	2009	University of Ghent Zwijnaarde
11	Poplar WT52-3	*Cinnamoyl coenzymeA reductase gene*	Modified lignin (a major constituent of wood) due to the decreased activity of an enzyme of the lignin biosynthetic pathway-	Vlaams Interuniversitair Instituut voor Biotechnologie , Belgium.	2009	University of Ghent, Zwijnaarde
12	*Populus alba x populus tremula-*	LtAPS1, LtCAAT1, LtCAAT1/LtAPS1, LtPPS1, PAAS, PAR1, Peroxidae, TP60, PtNAC1 transcription factor, PtNAC13 transcription factor, PtNAC17 transcription factor, PtNAC6 transcription factor, PtNAC7 transcription factor, PtriADT1, PtriADT2, PtriADT3	OO-Lignin Content Alteration, OO-Phenylalanine Synthesis, OO-Synthesis Of 2-Phenylacetaldehyde, OO-Synthesis Of 2-Phenylethanol, OO-Synthesis Of Allylpenols, OO-Synthesis Of Propenylpenols, OO-Wood Development Altered	University of Washington	2010	USA
13	*P. alba x P. tremula*	Cinnamoyl CoA reductase and o-methyl-transferase	Modified lignin	Institut National de la Recherche Agronomique (INRA)		France
	Altered fertility					
14	*E.camaldulensis*	CBI	Altered fertility	Arborgen (USA)	2004	Florida and south carolina

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Challenges to be addressed for transgenic trees

- Lack of pure lines, identified desired genotypes.
- Lack of efficient regeneration protocols.
- Lack of knowledge on the genes governing the traits in tree species.
- Long generation time.
  - Stability of transgenes over a long period of time.
  - Development of pest resistance.
  - Difficulties in assessment of ecological risks.
- Public concern on transgenic technologies

Forensic Sciences

IFGTB has taken NDF study for *Pterocarpus santalinus* an endangered species of eastern ghats.

DNA Barcoding techniques for determining country of origin need to be developed for prevention of illegal exports from the country

BIOTECHNOLOGICAL TOOLS IN CONSERVATION

- Identification of diversity hot spots.
  - **Sites having highest level of variability have been identified as sites having highest conservation priority**

- Narrowing down to core collections for germplasm conservation.
  - **Most divergent genotypes are identified for core collections for *ex situ* conservation.**
  - **Capturing the extant diversity in *ex-situ* conservation programmes.**
- Tissue culture tools could be tapped for *ex situ* conservation.

CONCLUSIONS

- *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.*
- *To translate available results, there is a need for networking among stakeholders and decentralizing the planting stock production through concepts like Community Seed Orchards.*

- *High end technologies should be taken up for solving the most pressing issues of the rural society for which benefits will be beyond what the current technologies would be envisaged to provide.*
- *Mission mode approach for these pressing problems.*
- *Investment in understanding fundamental pathways governing these desired traits.*
- *Funding concerted Cross-institutional research and breaking transnational boundaries for bringing together domain experts.*

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Forest Genetic Resource Conservation and Improvement: Aspects & Prospects

Dr. H.S.Ginwal

Forest Research Institute

(Indian Council of Forestry Research & Education)

Dehradun

Forest Genetic Resources

- Are the foundation for food, nutritional and environmental security of any country
- FGR assessment, improvement and conservation is an emerging concept of world wide
- Maintaining a wide basket of Forest genetic diversity in era of climate change has become an essential component of Forest planning

Forest Genetics and Tree Improvement in India

- Forest Research Institute initiated work on Forest Genetics and Tree Improvement in India
- Prof. Champion in 1930 established a provenance trial of Chir pine at New Forest, Dehradun
- In 1961 Prof. Mathews prepared action plan for tree improvement in India.
- Four species were initially identified : Teak, Eucalyptus, Pine and Semul for genetic improvement

Plus tree of Teak

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Major Tree Improvement Programs

- PINE IMPROVEMENT
- POPLAR IMPROVEMENT
- EUCALYPTUS IMPROVEMENT
- TEAK IMPROVEMENT
- SHISHAM IMPROVEMENT
- SEMUL IMPROVEMENT

Introduction of Germplasm

- POPLARS
- TROPICAL PINES
- EUCALYPTUS
- PAULONIA
- ACACIA


Progeny trial of Eucalyptus

Current Major Activities

- Tree Improvement and Breeding
  - Seed source evaluation
  - Identification of superior trees
  - Production of quality seeds
  - Development of new clones & varieties
- Establishment of breeding & production populations
- Micro-propagation
- Molecular characterization
- Population & Conservation genetics

Organizations involved

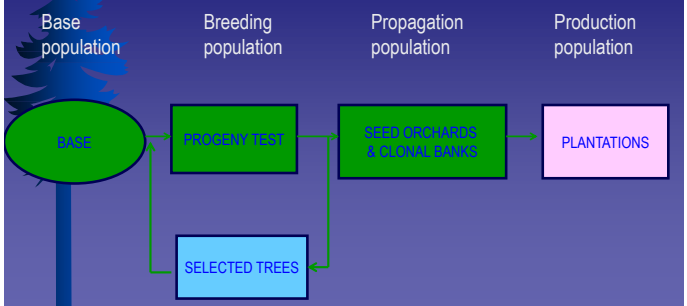
ICFRE institutes : FRI, HFRI, TFRI, AFRI, RFRI

Universities : UHF, PAU, GBPUAT, JNKVV, RAU, HAU,

Industries & private organizations : Star paper Mill, WIMCO, Pragati Biotech, etc.

State Forest Departments

Breeding Strategy



Seed source/provenance evaluation

- Conducted in major species
- Utilization of Best provenances ?

Species : Eucalyptus, Acacia, Dalbergia, Azadirachta, Albizia, Casuarina, Prosopis, Tecomella, Ailenthus, Pongamia, Teak, Pinus etc.

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Eucalyptus improvement

- Breeding was initiated beginning from the year 1965 and continued till now.
- Introduced the germplasm of Eucalyptus of Australian origin under evaluation trials
- Inter specific F1 hybrids were developed by using different combinations.
- Species used for hybridization are :
  E.tereticornis
  E.camaldulensis
  E.citriodora
  E.torelliana
  E.grandis

F1 hybrids FRI 15
Age 20 years

F2 recombinants Showing clean bole and vigorous growth

Poplar Improvement

- During 1997 open pollinated seeds of 103 CPTs of *P.deltoides* collected from 8 states of U.S.A.
- Field trial resulted in 26 highly promising clones : (FRI-AM-58 m.a.i. 43.25 m^3/ha/yr in comparison with 28.75 m^3/ha/yr recorded by G48)
- *Populus ciliata and Populus deltoides:* UHF, Nauni has identified clones and hybrids for various zones
- Hybrids *P. deltoides* and *P. euphratica*

Field trial in distt. Hoshiarpur,
Planting time : Feb 2002
105 clones
Concluded in March 2008.

POPLAR IMPROVEMENT

Field trial of FRI clones in Punjab (2002-2008)

Out of 105 clones evaluated (US origin), after 6 years best 5 clones are:

FRI PD-AM-58 (vol. 0.519 cum/tree)
FRI PD-AM- 51 (vol. 0.458 cum/tree)
FRI PD-AM- 41 (vol. 0.452 cum/tree)
FRI PD-AM- 32 (vol. 0.448 cum/tree)
FRI PD-AM- 54 (vol. 0.447cum/tree)

Clone G48 (control clone) is at rank 27 (vol. 0.345 cum/tree)

Shisham (*Dalbergia sissoo*) Improvement

- Provenance trials conducted in U.P., Haryana, J&K and M.P.
- Selected 351 CPTs covering entire range of distribution of species including Nepal
- Established progeny trials and studied breeding system of the species
- Developed about 200 clones
- Established seed orchards of advanced generation for supply of quality seeds

Teak (*Tectona grandis*) improvement

- Conducted provenance trials & plus trees identified
- 31 genotypes having high combining abilities were selected out of 94 CPTs
- Reproductive biology of teak studied
- Developed vegetative propagation technique through cuttings
- Established germplasm banks and breeding populations

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Melia composita improvement

Identified candidate plus 55 trees of *Melia composita*

Field trials of the selected material established in the states of Punjab, Haryana, U.P and Uttarakhand

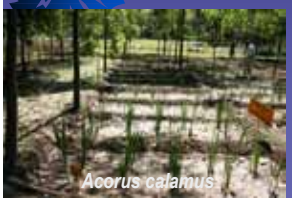
New clones are in the process of development for use under clonal forestry program after field evaluation

One year old plant of Melia in a clonal test

Genetic improvement of medicinal plants

Identified populations of *Acorus calamus* possessing low concentration (16 – 25 %) of the β –asarone

Identified high saponin (> 3 %) containing accessions of *Asparagus racemosus*



Acorus calamus

Asparagus racemosus

Efforts for production of quality seeds

Establishment of seed production area
Establishment of seed orchards

Seed Production Areas

Species: *Pinus roxburghii*

Area: 50 ha in HP
Kopra Forest (Nurpur): 10.52 ha
Bairkot Forest (Sunder Nagar): 22 ha
Dibkan Forest (J. Nagar): 18.44 ha
15 ha Marghana Forest (Udhampur J&K)

SEED PRODUCTION AREA

SPECIES : CHIR PINE, Soni (Uttarakhand)

SEED PRODUCTION AREA : CEDRUS DEODARA

Area: 50 ha in HP & J&K
Cheog Forest (Theog Forest Division): 25.00 ha
Nankhari Forest (Rampur Forest division): 15.00 ha
Neeru Forest (Bhadrawah Forest Division J &K): 10.00 ha

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Seed Production Area of *Eucalyptus*

Plus trees selection

No. of Species: 11

No. of Plus Tree selected: 4033

Expected genetic gain: 10-20%

Species :

***Tectona grandis, Azadiracta indica, Dalbergia sissoo, Cedrus deodara, Pinus roxburghii, Gmelina arborea, Albizia procera*, Eucalyptus, Dipterocarpus etc.**

PLUS TREE SELECTION

Teak =700
Simbal = 62
Gmelina = 60
Sandal = 30
Pterocarpus = 258
Sissoo = 53 (>100)
Deodar = 100
Populus = 60
Pinus = 200

Seed Orchards

- Established primarily for the production of seed of proven genetic quality
- Ex-situ conservation
- Part of long-term conservation management programme and breeding programme

Present position of seed orchards

State	Species with area in hectare
Arunachal pradesh	*Tectona grandis-17, Gmelina arborea-3, Bombax ceiba-4, Terminalia myriocarpa-2, Phoebe goalparensis-1, Duabanga grandiflora-1, Michelia champaca-1, Chukrasia tabularis-0.75.*
Bihar	*Tectona grandis-134.43, Dalbergia sissoo-1.65*
Chhattisgarh	*Eucalyptus 15.0, Tectona grandis 98.0, Gmelina arborea 39.0, Emblica officinalis 20.0,*
Haryana	*Total area 43 ha (species Dalbergia sissoo, Tectona grandis, Azadirachta indica, Ficus benghalensis, F.religiosa, Eucalyptus spp., Populus deltoides Acacia nilotica and Melia azedarach), D. sissoo-4, E. tereticornis-12*
Jharkhand	*Total 60.00 ha (Species Acacia catechu, Cassia siamea, Tectona grandis and Dalbergia sissoo)*
Karnataka	*Tectona grandis-109.8, Eucalyptus 18.0,*
kerala	*Tectona grandis-50.65*
Madhya pradesh	*Tectona grandis-113*
Marashtra	*Tectona grandis-234.65, Dalbergia sissoo-1.11*
Manipur	*Tectona grandis-0.3, Pinus kesiya-0.5*
Orissa	*Tectona grandis 12.37,*
Punjab	*D. sissoo-4,*
Tamil Nadu	*Tectona grandis-18.7, Santalum album-2.4, Eucalyptus tereticornis-1.5, Casuarina equisetifolia-5, Anacardium occidentel-12,* Terminalia *sp. 6.00, Pterocarpus marsupium 2.0.*
Tripura	*Tectona grandis-5, Gmelina arborea-5*
Uttarakhand	*Total 216 ha (Species Cedrus deodara, Pinus roxburghii, P.wallichiana, Ficus micrantha, Juglans regia and Abies pindrow)*
Uttar Pradesh	*Bombax ceiba-7, Tectona grandis 3, D. sissoo 16.00, Tectona grandis 3, D. sissoo 95, Acacia nilotica 6.0,*
West Bengal	*Total 54.0 ha with various species*

SEED ORCHARDS

Teak 800 ha
Shisham 30 ha
Gmelina 50 ha
Eucalyptus 56 ha

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Clonal Seed Orchard (*Dalbergia sissoo*)
Total Area: 8 ha
Sites: Bir Plasi, Nalagarh (1.5ha)
Paonta Sahib (3 ha)
Laliyal J& K(3.5 ha)

Clonal Seed Orchard (Advanced Generation) of Shisham at Pindori Mindo Mind (Hoshiarpur)

Up gradation of Clonal Seed Orchard of Shisham at Pindori Mindo Mind (Hoshiarpur)

Improved seed

Great demand for seeds of Eucalyptus hybrids and seed orchards by SFDs, industries and farmers

A few example :

2008-09	**30 kg seed of these hybrids was sold**
2009-10	**65 kg seed of these hybrids sold to Star Paper Mill, Saharanpur & farmers**

Use by farmers (in Punjab)

Farmers are raising seedlings of Eucaluptus hybrid on commercial scale

To cite an example these two farmers are using FRI seed for raising seedlings to a tune of about 11 lakh per year for sale

Ashok Kumar Agnihotri
V.P.O. Tuto Mazra
Dist. Hoshiarpur (Punjab)
Ph. 09463280002

Surinder Pal
V.P.O. Jassowal
The. Garhshankar
Dist. Hoshiarpur (Punjab)
Ph. 9815750191

Surinder Pal Ashok Kumar

Production from Seed Orchards (in Haryana)

Shisham

Year 2009	5000 Kg pods
Year 2010	2500 Kg pods collected

Eucalyptus

Year 2008-2009	**133 Kg**

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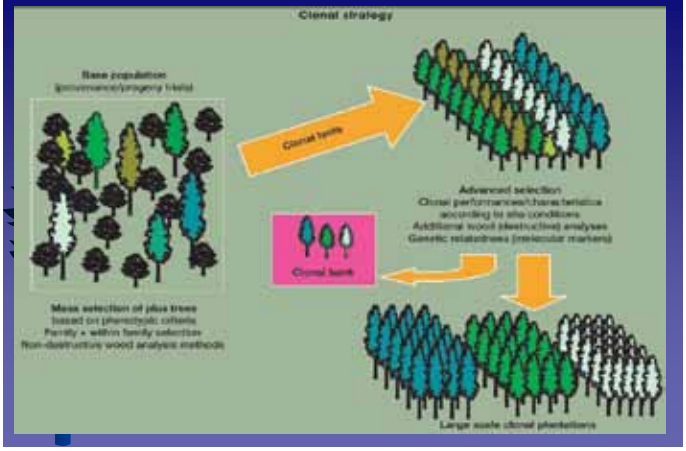
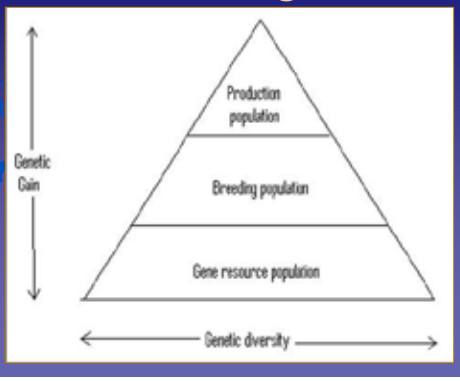
Development of new clones and varieties

Development of Tree Varieties and Clones

1. Approved guidelines are in place for Testing and Releasing of Tree Varieties and Clones, first time in India.
2. ICFRE institutes are the nodal agency for proper testing and release of tree varieties and clones for commercial production



Genetic gains



Release of clones

After comprehensive multi-location field testing :

- A clone of Eucalyptus hybrid (*Eucalyptus camaldulensis* Dehn. X *E. tereticornis* Sm.) has been released by the Variety Release Committee of the MoEF
- One productive and resistant clone (against wilt disease) of *Dalbergia sissoo* has been identified and has been released by the Variety Release Committee of the MoEF

New clones

Development of clones is a dynamic process

New series of clones are in the process of development and filed testing in following species :

Eucalyptus	: 24 clones by FRI
Poplars	: 26 clones by FRI
Shisham	: 100
Melia	: 10
Salix	: 15
Gmelina	: 20

Industries and SFDs also have their own programs for development of new clones particularly of Eucalyptus & Poplars

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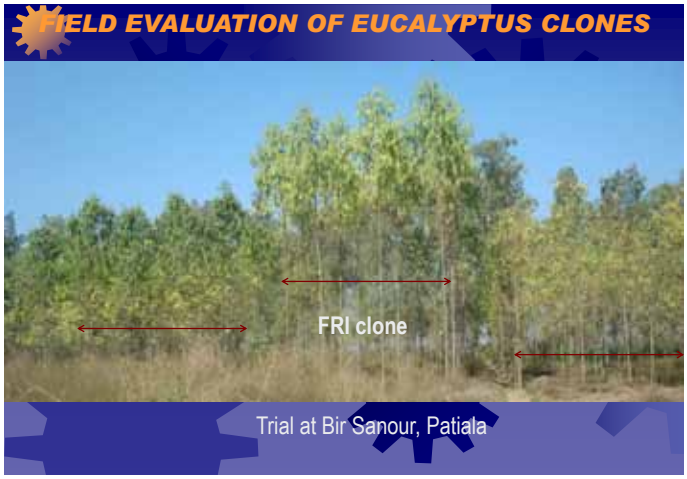

LIMITATIONS

- Biological features of many species remains unknown.
- Selection work slow process
- Dependence on conventional techniques
- Long generation period of trees thus time consuming
- Slow rate of growth
- Low effectiveness of selection for many character due to low heritability
- Poor juvenile adult correlation.

Intervention of Biotechnology

- Support to long term strategic research
- Genetic mapping to understand genetic control of important traits, such as disease resistance
- Maker-assisted selection
- Functional genomics
- Genetic engineering

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Biotechnological tools used in forestry

- Micro-propagation *and in vitro* selection
- Use of molecular marker
- Cryo-preservation & *in vitro* storage
- Genetic engineering

Micropropagation

Technology developed for 30 species

Useful in those tree species which are either endangered or where conventional means of multiplication have limitations

Mass production of quality planting material
Rejuvenation of adult trees
Exploitation of hybrid vigour
Production of haploid for hybridization

In Vitro Selection

- **Disease resistance**
- **Tolerance to:**
  - **Salt**
  - **Draught**
  - **Cold**
  - **Water logging**
  - **Metals**

Conservation of Forest Genetic Resources

Conservation of genetic diversity is of major global concern and is important to :

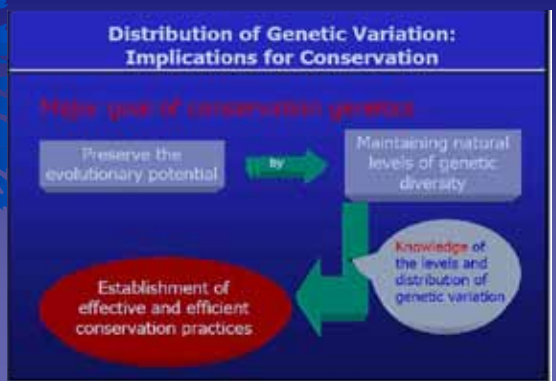
- Maintain the health and function of forest ecosystems
- Sustain the genetic diversity of noncommercial species that may eventually have economic value

Conservation Biology

"To conserve a plant species, conservation programme must be guided by the biological attributes of the species"

We cannot conserve what we do not understand

Developing conservation priorities and Developing management plans of forests.



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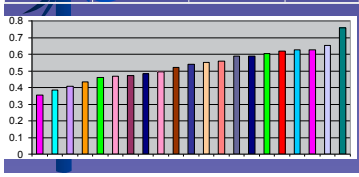
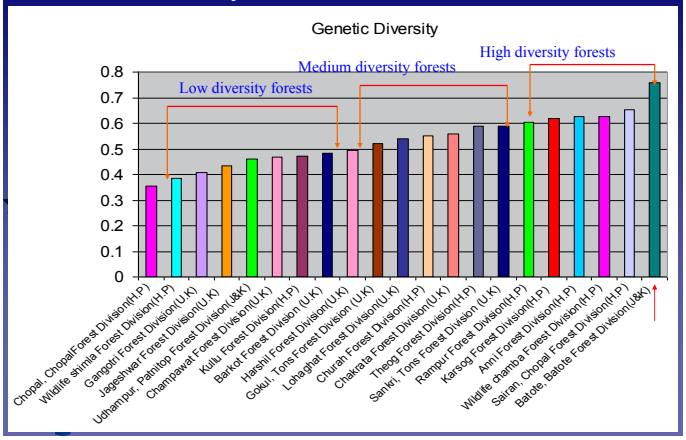
Himalyan Deodar (*Cedrus deodara*)

Genetic diversity studied in natural Himalayan Deodar (*Cedrus deodara*) forests of Uttarakhand, H.P. and J&K. through SSR DNA markers

Total gene diversity HT	Gene diversity within populations HS	Genetic differentiation GST/ FST	Gene flow (*Nm*)
0.666	0.527	0.209	1.896



Gene diversity in different Deodar Forests



Himalyan Chir Pine (*Pinus roxburghii*)

Genetic diversity studied in Himalayan Chir Pine (*Pinus roxburghii*) forests of Uttarakhand, H.P., J&K and North East through SSR DNA markers

Populations – 55
Markers used – SSR
States covered – Uttarakhand, H.P., J&K, Assam

Total gene diversity HT	Gene diversity within populations HS	Genetic differentiation GST/ FST	Gene flow (*Nm*)
0.746	0.401	-	0.581

Indian batch (*Acorus calamus*)

Population genetics and genetic diversity studied in *Acorus calamus* of Uttarakhand, H.P., J&K and North East through SSR markers

Populations – 50
Markers used – SSR
States covered – Uttarakhand, H.P., J&K, Assam

Total gene diversity HT	Gene diversity within populations HS	Genetic differentiation GST/ FST	Gene flow (*Nm*)
0.53	0.140	0.735	0.179

Shisham (*Dalbergia sissoo*)

Genetic diversity studied in clones of *Dalbergia sissoo* through RAPD & SSR markers

Clones – 67
Markers used – RAPD & SSR
Origin of clones – Uttarakhand, H.P., U.P., Nepal

Total gene diversity HT	Gene diversity within populations HS	Genetic differentiation GST/ FST	Gene flow (*Nm*)
0.276	0.240	0.132	-

Teak (*Tectona grandis*)

Population genetics & Genetic diversity studied in populations of *Tectona grandis* through ISSR markers

Populations – 29
Markers used – ISSR
Origin – A.P., Kerala, Karnataka, M.P., Maharashtra, Orissa, Rajastahan, Tamilnadu

Total gene diversity HT	Gene diversity within populations HS	Shannon's information index 'I'	Gene flow (*Nm*)
0.41	-	0.45	-

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Prospects & Priorities

- Regional and national action plan for priority species
- Institutional capacity building
- Guidelines and strategies for tree breeding and FGR conservation
- Exchange of genetic material
- Intervention of biotechnology
- Evaluation, characterization and documentation of FGR

Challenges

- Financial sustainability to long term breeding programs
- Networking
- Utilization of the improved germplasm
- Germplasm exchange
- Promotion and strengthening conservation of wild crop relatives, medicinal & fodder species
- Technical expertise

Future Line of Action

- FGR conservation & management
- Hybrids for specific traits
- Advance generation seed orchards
- Site matched clones with traits
- Cautious evolvement of transgenic approaches
- QTL mapping
- Greater understanding of the genomes
- Collaboration with international research group
- Timber forensics and molecular taxonomy
- Population genetics of natural forests

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Evaluation of *Anogeissus latifolia* (Roxb.) Wall ex. Bedd. gum for authentic characteristic identification

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- Plant gums are one of the important Non-Wood Forest Produce's of India
- They are plant exudates, oozing out partly as natural phenomena and also as the result of disease or injury in the bark of stem
- The gum exudates vary considerably with different botanical sources and there is even substantial difference in gum from the same species when collected from plant growing under different climatic conditions

- *Anogeissus latifolia* (Roxb.) Wall ex. Bedd. is a medium to large sized tree, distributed throughout India in dry deciduous forests and in the sub-Himalayan region and hills of South India up to 1300 meters.
- It grows up to 30 m in height with a clear bole of up to 15 m and with greenish or greyish white smooth bark exfoliating in irregular thin scales.

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- The tree is the main source of Indian gum, also known as Ghatti
- The gum exudes practically throughout the year, but its collection is done during the month of September to June
- The gum is mainly the calcium salt of a complex, high molecular weight polysaccharic acid (ghattic acid)

Uses of Ghatti gum

General

- Traditional food
- Pharmaceutical preparations
- Emulsifier in butter and butterscotch

Industrial

- In drilling mud's to reduces the viscosity by absorbing water

Rational of the Investigation

- Demand of ghatti gum
- Collectors often collect and sell the less important gums of botanical origin claiming it as that of ghatti
- Lack of authentication, proper characterization and identification
- Physical and chemical properties and physical characteristics play a pivotal role in authentic identification and determining their commercial value

Materials and Methods

Plant Material

- ✓ Materials comes from Barha experimental area, TFRI, Jabalpur, M.P India
- ✓ Ten trees were randomley selected
- ✓ Artificial incision was done in tree bark.
- ✓ Collection of gum was done through hand picking.
- ✓ The collected gum was laid to dry in the sun for several days.
- ✓ After drying, gum was sorted (on the basis of gums with bark and without bark),
- ✓ Then graded (according to color and purity) and stored in air tied glass bottles for the further study

1. **Physical appearance:**
   - Recorded by the method of Glickman, 1969
2. **Characteristic reaction with different reagents**
   - Tested as per Bureau of Indian Standards (1988) and Glickman (1969)
3. **Physico-chemical properties**
   - Impurities, Moisture, total ash and acid insoluble ash in ghatti gum were determined as per IS: 6795-1972
   - **Pentosan** - method of AOAC
   - **Total carbohydrate** - Anthrone method (Hodge and Hofreiter, 1962)
   - **Methyl sugar** - Aniline phthalate method

   Viscosity determination
   - Brookfield Digital Viscometer (RVT) Model 84 using Spindle No 21 and 27 at 25^0C

Results

Physical characteristics of gum of *Anogeisus latifolia*

State	Solid
Shape	Rounded tears less than 1 cm in diameter but more often occurs in large vermiform masses
Colour	Light to dark brown
Texture	Amorphous, glossy
Brittleness	Glassy fracture
Odour	Odourless
Exposed surface	Translucent
Solubility in water	Dissolves to form almost clear solution but some insoluble material may remain as fine suspension
pH in aqueous solution	Acidic
Solubility in alcohol	Insoluble

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Behaviour of the gum of *Anogeissus latifolia* with various reagents

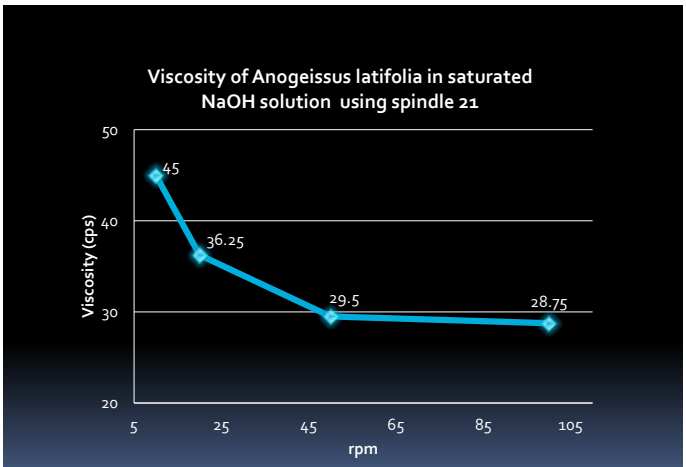
Reagent	Precipitate
Basic lead acetate	Translucent flocculent precipitate
Potassium hydroxide (10%)	Negative
Ferric chloride (5% solution)	Negative
Sodium tetra borate (4% solution)	Negative

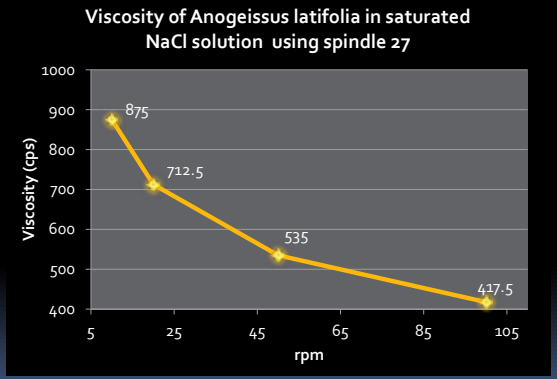
Physico-chemical properties of ghatti gum in fresh and one year old sample

Physical properties	Fresh sample in %	One year old sample in %
Impurities	8.45	8.45
Moisture content	16.03	13.14
Total ash, percent by mass	-	4
Acid insoluble ash, percent by mass	-	0.50
Pentosan	-	15.78-16.88
Total Carbohydrate	39.11	43.71
Methyl sugar	3.94	-

Viscosity of ghatti gum in 2 concentrations in 3 grades in 8 months old samples

rpm	Viscosity(cps) of gum ghatti					
	Ist Grade	IInd Grade	IIIrd Grade	Ist Grade	IInd Grade	IIIrd Grade
	5% concentration			10% concentration		
10	60-70	145-170	1300-1350	465-505	415-465	--
20	40-45	112.5-122.5	925	262.5-275	332.5	--
50	29-30	91	590	202	274	--
100	24.5-25.5	79-79.5	425-435	176.5	238	--





To conclude .....

- ✓ **The present investigation characterization of *Anogeissus latifolia* gum has been determined and which is of immense practical significance.**
- ✓ **Authentic characteristic identification and aid in detection of adulteration.**
- ✓ **Characteristics of gum dhawara are somewhat similar to gum arabic and hence it finds application as its viable substitute.**

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Species improvement programme of *Dipterocarpus retusus* Bl. syn. *D. macrocarpus* Vesque: Progeny analysis after seven years

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2. Biotechnology and Genetics Division, Rain Forest Research institute, PB-136, Jorhat (Assam)

Introduction of work

This project was started with the objective of genetic improvement of this species and has a bi-directional approach : -

- ⇨ Seedling Seed Orchard (SSO)/Progeny Trials
- ⇨ Standardization of Vegetative multiplication Techniques (Presented in poster)

Dipterocarpus retusus Bl. syn. *D. macrocarpus* Vesque commonly known as hollong is a climax species of Assam valley tropical wet evergreen forest (1B/c1). It grows more than 48 m and clear bole height sometimes attained 40 m, attributes it as the most desirable tree species for commercial plywood of this region.

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Selection of Plus Trees

- Plantations surveyed: Eighteen even aged
- 102 candidate plus trees (CPTs) selected
- Plus tree selected 89 trees

Selection of Plus Trees

Seed Collection of *Dipterocarpus retusus*

Progeny Trials

- Deovan (Jorhat) May 1999
- 17 progenies and designed in RBD with 3 replication and 5 plants per plot

Progeny Trials

Accumulated analysis of variance

	d.f.	s.s.	m.s.	v.r.	F pr.
Progenies	17	678676	39922	2.4	0.003
Replication	2	94526	47263	2.8	0.065
Within plot	4	3383	846	0.1	0.995
Residual	150	2543383	16956		
Total	173	3319969	19191		

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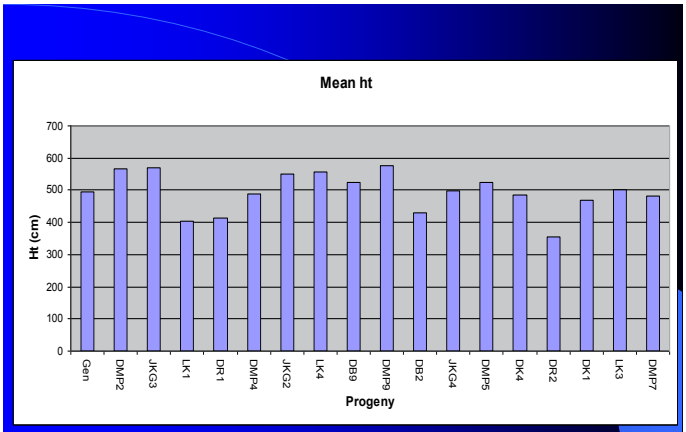
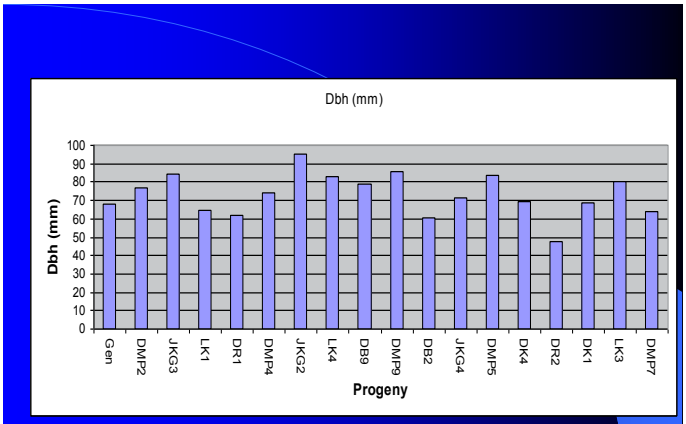
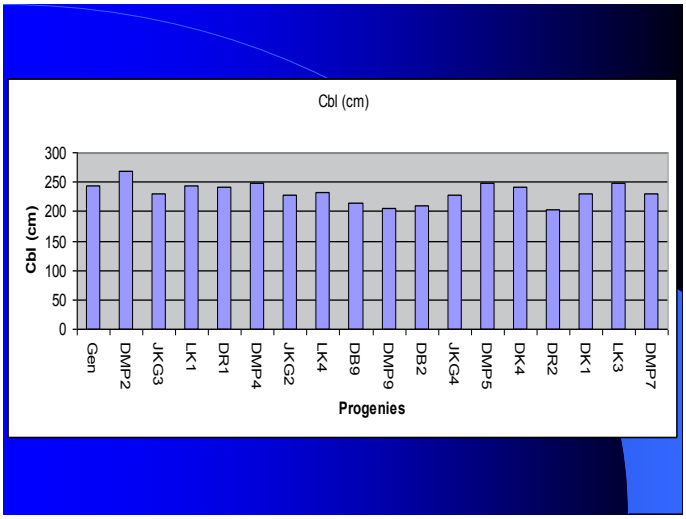


	d.f.	s.s.	m.s.	v.r.	F pr.
Progenies	17	21395.7	1259	2	0.02
Replication	2	3622.1	1811	3	0.06
Within plot	4	1131.9	283	0	0.77
Residual	148	93099.6	629		
Total	171	119249	697		



	d.f.	s.s.	m.s.	v.r.	F pr.
Progenies	17	47770	2810	1.32	0.188
Replication	2	717	359	0.17	0.845
Within plot	4	3810	952	0.45	0.774
Residual	144	306537	2129		
Total	167	358834	2149		



Fixed model:	Constant		
Random model:	Prog + Repl		
Residual term has been added to model			

Heritabilty	
Height	0.092
Dbh	0.1413
Cbl	0.046

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Conclusion

- After seven year; overall mean height, diameter at breast height (dbh) and clear bole length after seven years was 4.94 m, 7.3 cm and 2.3 m respectively. There was significant variation among families for height and diameter at breast height but not for clear bole length. Best performing families were DMP-9 for height (5.77m), JKG-2 for dbh (9.5 cm) and DMP-2 for clear bole length (2.7m) which was better from their respective means by 17%, 30 % and 17%.

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EFFICACY OF IDS TECHNIQUE ON IMPROVING THE QUALITY OF *JATROPHA CURCAS* SEEDLOT

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(DBT funded project)

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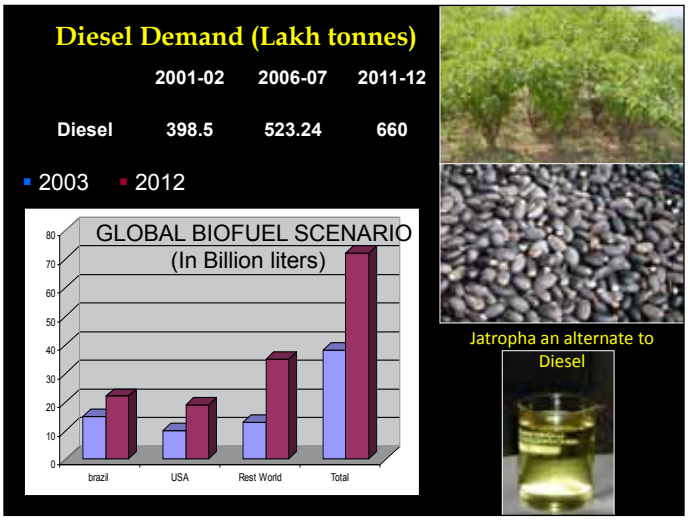

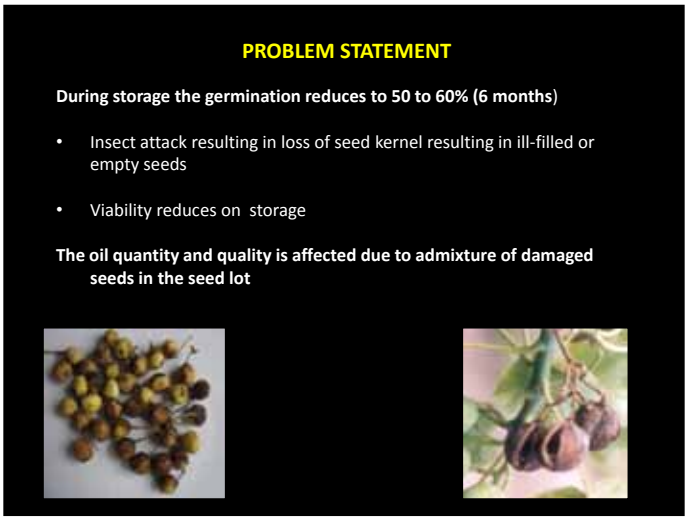

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Materials and Methods

One year old seedlot of Sathyamangalam -TZ test revealed that the seed lot had viability of only 58%.

X-ray images of the sampled seeds were taken using a Faxitron model x-ray unit (19 kvp with an exposure time for 2 minutes.)

Divided into 4 sublots with 800 seeds per sublot & each subjected to different regimes of IDS treatments,

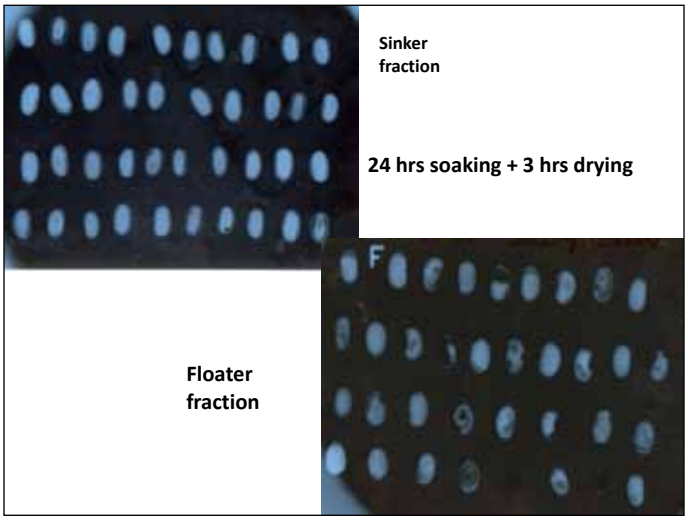
T1- Control- 24 hrs. soaking in water + 0 hr. drying
T2- 24 hrs. soaking in water + 1 hr. drying
T3- 24 hrs. soaking in water + 2 hrs. drying
T4- 24 hrs. soaking in water +3 hrs. drying

Following drying, the seeds were separated into floaters and sinkers using water as separation medium

Number of floaters and sinkers in each treatment counted. The individual identities of the separated seeds marked and subjected to X-radiography and then tested for germination

Results

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



Conclusion

- soaking Jatropha seeds for 24 hours in water followed by 3 hours drying helps in recovery of sound seeds

-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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Descriptors for Registration of Eucalyptus Clones for IPR Rights in India

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A.Nicodemus
R.R.Warrier

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(INDIAN COUNCIL OF FORESTRY RESEARCH & EDUCATION)
P. B. No. 1061, R.S.Puram, HPO, COIMBATORE – 641 002
Tamil Nadu, India

Introduction

- Forestry crops are in their early stages of domestication.
- Some of the species like Eucalyptus, Casuarinas and Populus tree improvement programs are in place and clones are being developed.
- Among the many species planted in India, Eucalyptus is planted widely (4 million ha).
- The act of Protection of Plant Varieties and Farmers' Rights, 2001 is an effective system for protection of plant varieties, the rights of farmers and plant breeders
- The DUS characters have been developed for many self pollinated agriculture and horticulture crops in India and plant varieties are being registers by PPV&FR Authorities, New Delhi.
- In tree crops, which are predominantly cross pollinated, DUS characters have not been developed. Attempts were made for developing DUS characters in Eucalyptus using leaf and bark characters.

Clonal Trials

Location	Clones	Rep.	Ramets/ rep.
Karunya, Tamil Nadu	100	5	3
Sathyavedu, Andra Pradesh	80	5	3
Kulathupuzha, Kerala	50	5	2

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S. No.	Characteristics	State	Notes	Example clone	Stage of observation	Type of assessment
1*	Tree character					
1.1	Tree: Clear bole	<50% of the tree height 50-70% of the tree height >70% of the tree height	3 5 7	Clone 154 Clone 111 Clone 94, 19	24	VG
2*	Crown character					
2.1	Crown: Shape	Lanceolate Conical Columnar	1 2 3	Clone 111 Clone 69 Clone 154	24	VG

S. No.	Characteristics	State	Notes	Example clone	Stage of observation	Type of assessment
3*	Stem characters					
3.1	Stem-scar: Type	Open Close	1 9	Clone 7 Clone 123	36	VG
3.2	Stem-scar: Shape	Oval Round Bell	1 2 3	Clone 53 Clone 123 Clone 1	36	VG
3.3	Stem- scar: Periphery	Totally prominent Partly prominent Flat	1 2 3	Clone 53 Clone 69 Clone 111	36	VG
4*	Branch characters					
4.1	Branch: Self pruning	Absent Present	1 9	Clone 154 Clone 69	36	VS
4.2	Branch: Thickness	Small (Diameter <1.5 cm) Medium (Diameter 1.5 to 3.0 cm) Thick (Diameter > 3.0 cm)	3 5 7	Clone 111 Clone 123 Clone 154,53	36	VS
4.3	Branch: Angle	Acute Perpendicular Drooping	1 2 3	Clone 69 Clone 111 Clone 276	24	VS

5*	Bark characters					
5.1	Bark: Texture	Rough Smooth	1 9	Clone 1 Clone 17	36	VG
5.2	Bark: Peeling	Absent Present	1 9	Clone 76 Clone 100	36	VG
5.3	Bark: Peeling type	Strip Flakes Combination of Strip & flakes	1 2 3	Clone 14 Clone 188 Clone 198	36	VG
5.4	Bark: Thickness	Thin (< 7 mm) Medium (7 to 9 mm) Thick (> 9)	3 5 7	Clone 17 Clone 53 Clone 111	36	MG
5.5	Fresh bark: Colour	Light yellow Light green Light brown Light grey Dark grey	1 2 3 4 5	Clone 7 Clone 17 Clone 53 Clone 124 Clone 1	36	VG
5.6	Dry bark: Colour	Light green Light brown Dark brown Light grey Dark grey	1 2 3 4 5	Clone 94 Clone 1 Clone 14 Clone 16 Clone 188	36	VG
5.7	Peeled bark: Colour	Light brown Dark brown Dark grey	1 2 3	Clone 63 Clone 94 Clone 75	36	VG

6*	Leaf characters					
6.1	Leaf: Shape	Lanceolate Ovate Linear	1 2 3	Clone 231 Clone 154 Clone 276	12	VG
6.2	Leaf: Margin	Entire Wavy	1 9	Clone 154 Clone 196	12	VG
6.3	Leaf base: Symmetry	Oblique Symmetric	1 9	Clone 110, 86 Clone 93,100,231	12	VG
6.4	Leaf base: Shape	Acute Attenuate Obtuse	1 2 3	Clone 121 Clone 172 Clone 154	12	VG
6.5	Leaf apex: Shape	Acuminate Acute Obtuse	1 2 3	Clone 86 Clone 88 Clone 157	12	VG
6.6	Leaf: area	Very small (<20.5 mm^2) Small (20.6 to 29 mm^2) Medium(29.1 to 37.5 mm^2) Large(37.6 to 46.0 mm^2) Very large (>46 mm^2)	1 3 5 7 9	Clone 3 Clone 33 Clone 22 Clone 206 Clone 169	12	MG

6.7	Leaf : Length	Very short (<12 cm) Short (12 to 15 cm) Medium (15.1 to 18 cm) Long (18.1 to 21 cm) Very long (>21 cm)	1 3 5 7 9	Clone 172 Clone 207 Clone 206 Clone 22 Clone 136	12	MG
6.8	Leaf: Breadth	Very narrow (<2.5 cm) Narrow (2.5 to 3.1 cm) Medium (3.2 to 3.8 cm) Wide (3.9 to 4.5 cm) Very wide (>4.5 cm)	1 3 5 7 9	Clone 207 Clone 172 Clone 169 Clone 206 Clone 204	12	MG
6.9	Leaf: Perimeter	Very short (<27.1 cm) Short (27.1 to 33.9 cm) Medium (34 to 40.6 cm) Long (40.7 to 47.4 cm) Very long (>47.4 cm)	1 3 5 7 9	Clone 207 Clone 172 Clone 206 Clone 22 Clone 136	12	MG
6.10	Leaf: Roundness gradation	Near round (<2.5) Moderate round (2.5 to 3.4) Intermediate (3.5 to 4.3) Moderate far (4.4 to 5.2) Far from round (>5.2)	1 3 5 7 9	Clone 172 Clone 12 Clone 207 Clone 22 Clone 15	12	MG
6.11	Leaf: Aspect ratio	Very low (< 3.4) Low (3.4 to 4.6) Medium (4.7 to 5.8) High (5.9 to 7) Very high (>7)	1 3 5 7 9	Clone 204 Clone 206 Clone 169 Clone 207 Clone 136	12	MG
6.12	Petiole: Length	Short (<1.5 cm) Intermediate (1.5-2.5 cm) Long (>2.5 cm)	3 5 7	Clone 206 Clone 9 Clone 10	12	MG

7+	Flower character					
7.1	Flower: Calyptra	Short (<5 mm) Long (>5 mm)	1 9	Clone 9 Clone 217	36	VS
8+	Fruit characters					
8.1	Fruit: Basal shape	Spherical Oblate Conical	1 2 3	Clone 131 Clone 17 Clone 154	40	VS
8.2	Fruit: Prominent rim	Absent Present	1 9	Clone 63 Clone 26	40	VS
8.3	Fruit pedicel length	Short (<4 mm) Medium (4 to 8 mm) Long (>8 mm)	3 5 7	Clone 1 Clone 63 Clone 23	40	VS

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Most discriminating characters

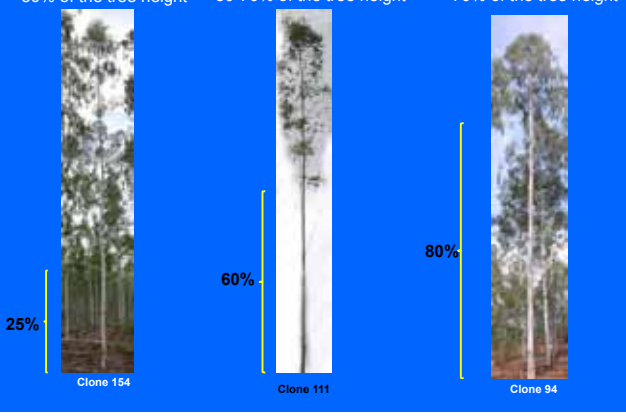
Tree character

1.Tree: Clear bole

<50% of the tree height

50-70% of the tree height

>70% of the tree height



2. Stem scar: Periphery

Totally prominent

Partly prominent

Flat

Clone 53

Clone 69

Clone 111

3. Branch thickness

Small (<1.5 cm)

Medium (1.5 to 3.0 cm)

Thick (> 3.0 cm)

Clone 111

Clone 123

Clone 53

4. Branch angle

Acute

Perpendicular

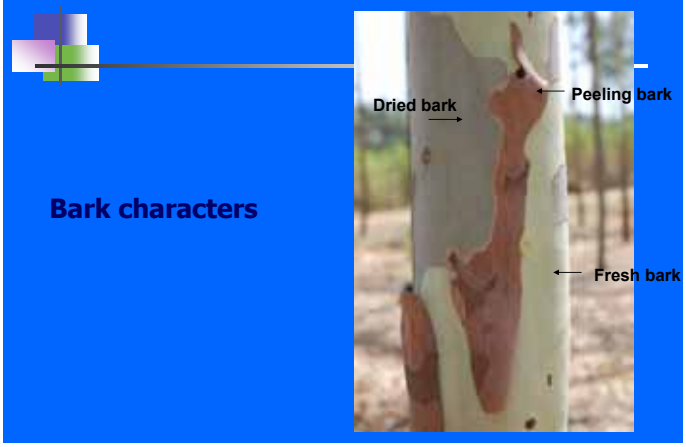
Drooping

Clone 69

Clone 111

Clone 276

Growth stages of bark



Bark characters

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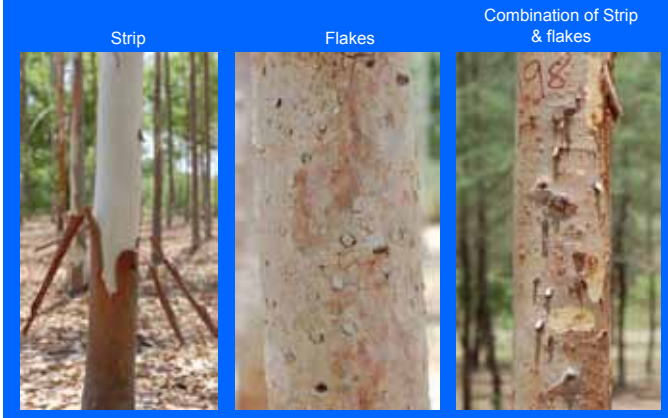
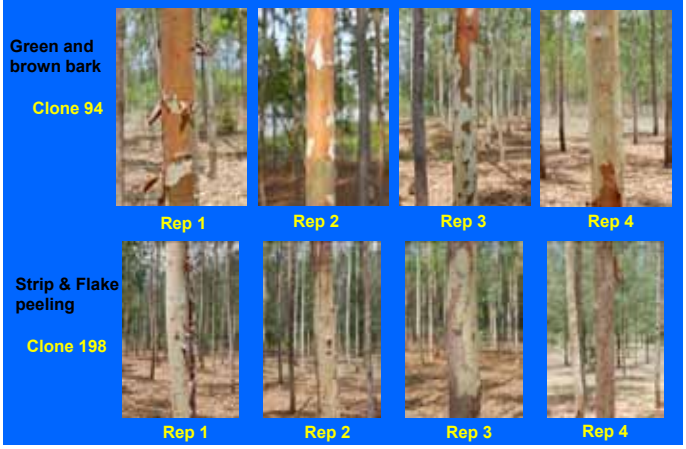
5. Bark: Peeling type



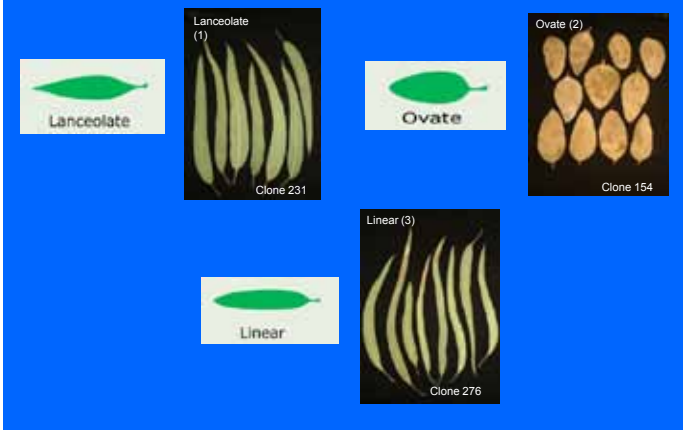
6. Fresh bark: Colour

Uniformity in bark characters across replications



Leaf Characters

7. Leaf: Shape

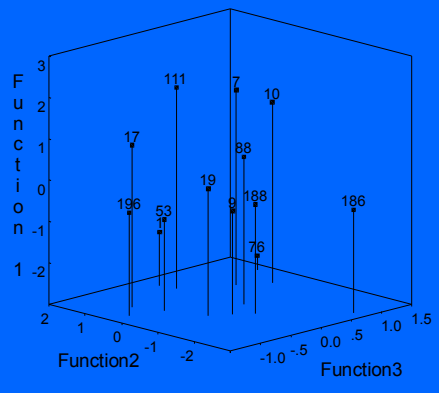


Quantitative character analysis and discrimination of clones using leaf traits

Number of clones used: 13

surface area (cm^2),
length (cm),
breadth (cm),
equivalent diameter (cm),
perimeter (cm),
convex perimeter (cm),
curve length (cm),
convex area (cm^2),
roundness,
aspect ratio and
fullness ratio

Scatter diagram of clones based on Canonical Discriminant Analysis



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Classification of clone membership through canonical discriminant function analysis

Clone	Predicted Group Membership													Total
	1	7	9	10	17	19	53	76	88	111	186	188	196	
1	**100**	0	0	0	0	0	0	0	0	0	0	0	0	100
7	0	**100**	0	0	0	0	0	0	0	0	0	0	0	100
9	0	0	**66.7**	16.7	0	0	0	16.7	0	0	0	0	0	100
10	0	0	0	**87.5**	0	0	0	0	0	12.5	0	0	0	100
17	0	0	0	0	**100**	0	0	0	0	0	0	0	0	100
19	0	0	0	0	0	**77.8**	0	0	0	0	22.2	0	0	100
53	0	0	0	0	0	0	**100**	0	0	0	0	0	0	100
76	14.3	0	0	0	0	0	0	**85.7**	0	0	0	0	0	100
88	0	0	0	0	0	0	0	0	**100**	0	0	0	0	100
111	0	0	0	0	0	0	0	0	0	**100**	0	0	0	100
186	0	0	0	0	0	0	0	0	0	0	**100**	0	0	100
188	0	25	25	0	0	25	0	0	0	0	0	**25**	0	100
196	0	0	0	0	0	14.3	0	0	0	0	0	0	**85.7**	100

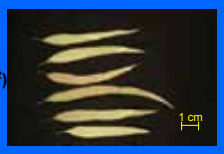
89.1% of selected original grouped cases were classified correctly

Classification of clone membership under different data sets

Type of data	Both location combined
Individual leaf (13 clones)	43.4%
Replication average (13 clones ; 10 leaves)	89.1%

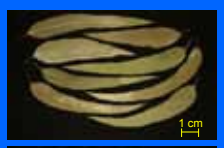
8. Leaf: Area

Very small (<20.5 cm²) Clone 3



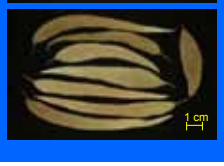
Large (37.6 to 46.0 cm²) Clone 206

Small (20.6 to 29 cm²) Clone 33



Very large (>46 cm²) Clone 169

Medium (29.1 to 37.5 cm²) Clone 22



9. Leaf: Perimeter

Very short (<27.1 cm) Clone 207

Long (7) (40.7 to 47.4 cm) Clone 22

Short (3) (27.1 to 33.9 cm) Clone 172

Very long (9) (>47.4 cm) Clone 136

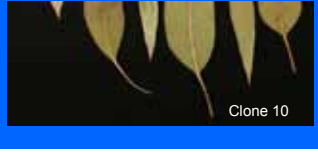
Medium (5) (34 to 40.6 cm) Clone 206

10. Petiole length

Short (<1.5 cm)

Intermediate (1.5-2.5 cm)

Long (>2.5 cm)



Flower characters

11. Calyptra length

Short (<5 mm)

Clone 217

Long (>5 mm)

Clone 9

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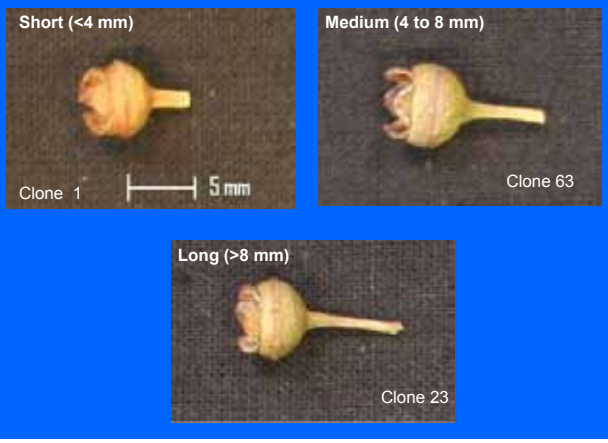
12. Fruit peduncle length



Most discriminating characters

1. Tree habit (Characteristic 1.1)
2. Stem: Scar (Characteristic 3.1)
3. Branch: Thickness (Characteristic 4.2)
4. Branch: Angle (Characteristic 4.3)
5. Bark: Peeling type (Characteristic 5.3)
6. Bark: Fresh bark colour (Characteristic 5.5)
7. Leaf: Shape (Characteristic 6.1)
8. Leaf: Area (Characteristic 6.6)
9. Leaf: Perimeter (Characteristic 6.9)
10. Leaf: Petiole length (Characteristic 6.12)
11. Flower: Calyptra length (Characteristic 7.1)
12. Fruit: Peduncle length (Characteristic 8.3)

Thank You

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Evaluation of plus trees of *Pongamia pinnata* (L.) Pierre for oil content and germination pattern

By

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Forest Research Institute
Dehradun

Pongamia pinnata (Linn) Pierre

Member of

- Family: Leguminosae
- Sub Family: Papilionoideae

Multipurpose tree species

- Important bio-diesel plant
- Used as a source of traditional medicine
- Green manure and pesticide

Methodology

Selection of Plus Trees

- A total of 312 candidate plus trees were selected from different geographical locations of Northern India
- Selection was done on the basis of index method
- The status of fruit formation and incidences to disease and insect were also considered

Oil Extraction

- The oils were extracted using non-polar solvents through Soxhlet apparatus
- The solvent was evaporated and weight of solvent free oil was determined

Germination Experiment

- Twenty seeds of each progeny in three replications planted in the polybags and counted for germination from tenth day onwards
- Rate of germination was calculated by counting the fresh emergence each day till the final count

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Germination Percentage

- **The Progeny FRI-63 showed the maximum germination (96.67%) originated from Phagwara , Punjab**
- **The Progeny FRI-54 Showed the minimum germination (50%) originated from Phagwara, Punjab**
- **The average germination percentage for 61 progenies was 82.33 %**
- **Out of 61 progenies, 35 progenies showed higher germination percentage than average value**
- **26 progenies showed lower germination than the average value**

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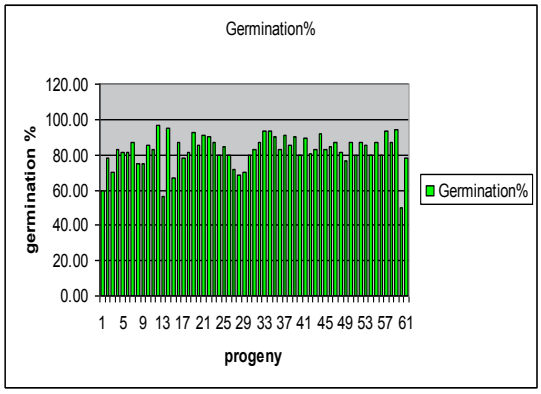
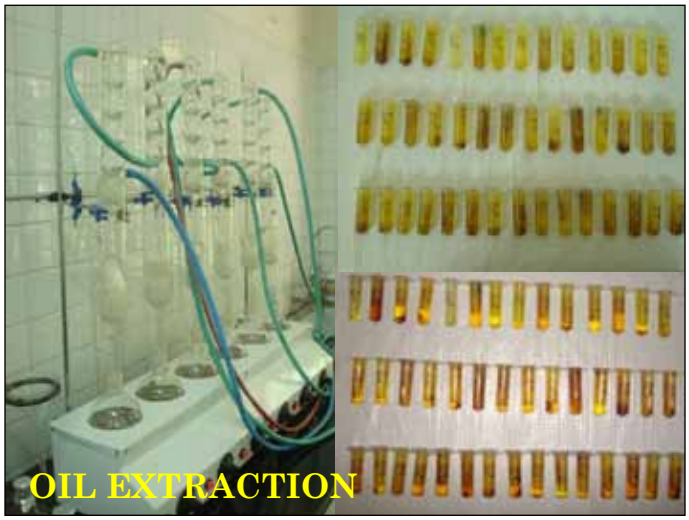
GERMINATION PERCENTAGE

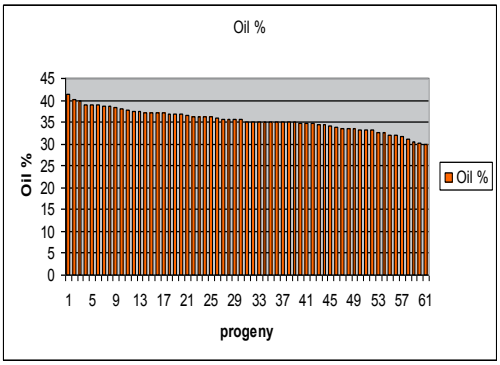


OIL PERCENTAGE

- **The Progeny FRI-81 showed the maximum Oil percentage (41.43%) originated from Fatehabad , Haryana**
- **The Progeny FRI-69 Showed the minimum Oil percentage (29.77%) originated from Jalandhar, Punjab**
- **The average Oil percentage for 61 progenies was 35.46 %**
- **Out of 61 progenies, 30 progenies showed higher Oil percentage than average value**
- **31 progenies showed lower Oil percentage than the average value**



PERCENTAGE



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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

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Forest Research Institute,
Dehradun

In this investigation, we tested potential and utility of Inter Simple Sequence Repeat (ISSR) markers to study the genetic variation among three common species of bamboos.

INTRODUCTION

- Bamboos are members of the sub-family Bambusoideae within the grass family Poaceae with a total of 1400 species.
- Among bamboo species, the vegetative growth phase varies from 1 year to 120 years, so it is very difficult to get genetic information from the traditional means. Classification systems proposed to date need further support, and taxonomic delineation at lower levels often lack sufficient resolution.
- The tremendous advancement of molecular marker technologies holds the promise to address different needs of bamboo taxonomy (systematics and identification) and diversity studies. There is need to develop barcodes for taxonomic identification of Bamboos.

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MATERIALS

S.No.	Species	Code	Location
1	Bambusa bambos	Bb1	Pantnagar, Uttarakhand
2	Bambusa bambos	Bb2	TERI, New Delhi
3	Bambusa bambos	Bb3	TERI, New Delhi
4	Bambusa bambos	Bb4	Itanagar, Arunachal Pradesh
5	Bambusa bambos	Bb5	Ambiwala, Dehradun
6	Bambusa bambos	Bb6	Navada, Dehradun
7	Bambusa balcoa	Bba1	Kishanpur, Uttarakhand
8	Bambusa balcoa	Bba2	Nagao, Assam,
9	Bambusa balcoa	Bba3	Gangapur, Uttarakhand
10	Bambusa balcoa	Bba4	Uday nagar,Uttarakhand
11	Bambusa balcoa	Bba5	Gadapur, Ward No. 5 Uttarakhand
12	Bambusa balcoa	Bba6	Lalkuan, Uttarakhand
13	Bambusa balcoa	Bba7	Kalinagar, Uttarakhand
14	Bambusa vulgaris	Bv1	Dehrdun
15	Bambusa vulgaris	Bv2	Seetpur, Dineshpur, Uttarakhand
16	Bambusa vulgaris	Bv3	Pantnagar, Chandan nagar, Dineshpur
17	Bambusa vulgaris	Bv4	Dehradun
18	Bambusa vulgaris	Bv5	Pantnagar, Uttarakhand

ISSR primers screened:

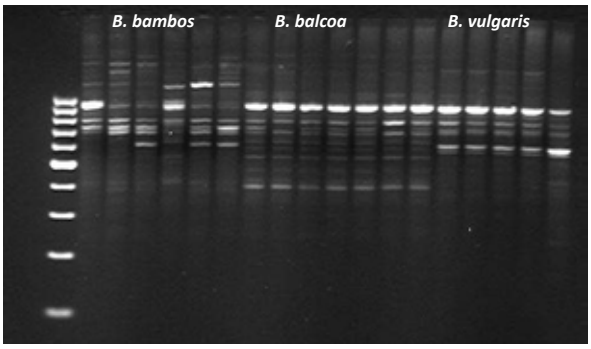
S.NO.	ISSR Primer code	Tm	sequence(5'-3')
1	ISSR-1	24.9	ATATATATATATATATG
2	ISSR-2	23.8	ATATATATATATATATC
3	ISSR-3	43.3	GAGAGAGAGAGAGAGAC
4	ISSR-4	44.3	GAGAGAGAGAGAGAGAA
5	ISSR-5	54.3	GTGTGTGTGTGTGTGTYG
6	ISSR-6	44.5	TCTCTCTCTCTCTCTCRA
7	ISSR-7	48	TCTCTCTCTCTCTCTCRG
8	ISSR-8	51.9	ACACACACACACACACYT
9	ISSR-9	49.8	ACACACACACACACACYA
10	ISSR-10	53.7	ACACACACACACACACYG
11	ISSR-11	54.3	TGTGTGTGTGTGTGTGRT
12	ISSR-12	54.9	TGTGTGTGTGTGTGTGRC
13	ISSR-13	52.2	TGTGTGTGTGTGTGTGRA
14	ISSR-14	67.1	ACCACCACCACCACCACC
15	ISSR-15	89.3	CCGCCGCCGCCGCCGCCG
16	ISSR-16	46.7	AAAAAAAAAAAAAAAA
17	ISSR-17	81.3	CCCCCCCCCCCCCCCC

S.No.	Primer	Primer	Primer sequence(5'-3')
1	ISSR-3	UBC811	GAGAGAGAGAGAGAGAC
2	ISSR-4	UBC812	GAGAGAGAGAGAGAGAA
3	ISSR-8	UBC855	ACACACACACACACACYT
4	ISSR-9	UBC856	ACACACACACACACACYA
5	ISSR-11	UBC858	TGTGTGTGTGTGTGTGRT

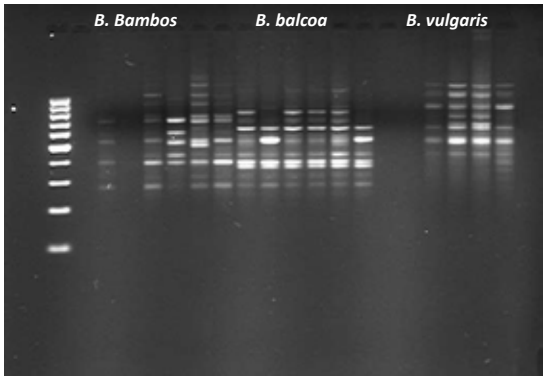
List of ISSR primers used in the study

S.No.	Primer	Total no. of bands	Polymorphic bands	% polymorphism	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					

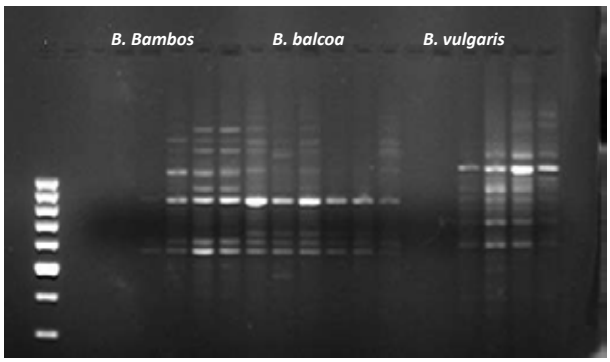
Total number of bands, polymorphic bands, polymorphism, PIC, EMR, MI, and RP obtained from 5 ISSR markers.



ISSR primer-UBC811 on 1.5 % agarose gel



ISSR primer-UBC812 on 1.5 % agarose gel



ISSR primer-UBC856 on 1.5 % agarose gel

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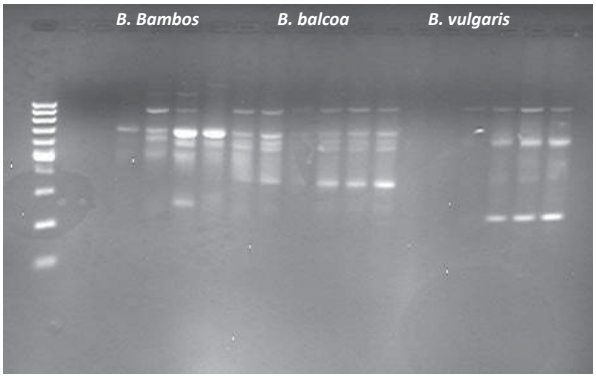
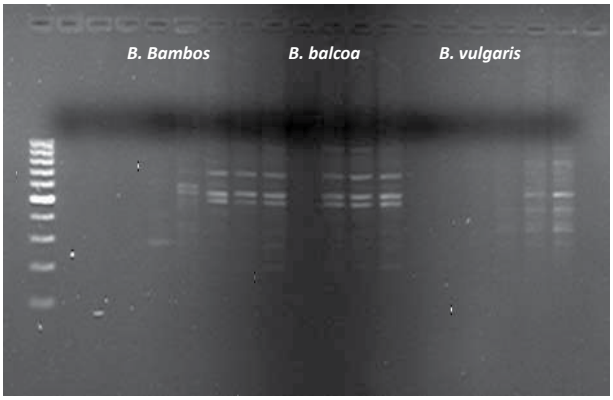


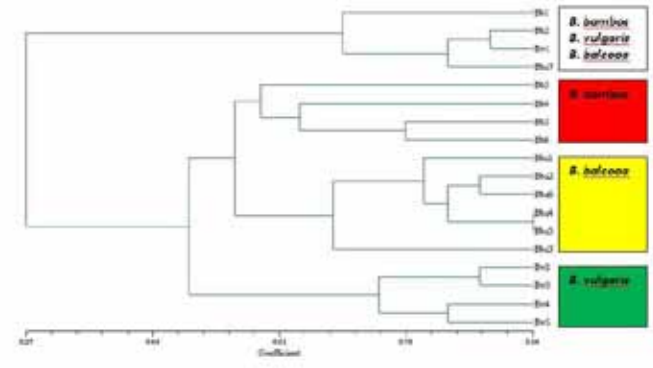
ISSR primer- UBC855 on 1.5 % agarose gel



ISSR primer- UBC858 on 1.5 % agarose gel

Jaccard similarity matrix table based on cluster analysis of three (3) 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



The ISSR markers are a useful simpler and cheaper molecular marker tool for the identification of germplasm and analysis of genetic relationships between and within the bamboo species.

Thanks

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MOLECULAR CHARACTERIZATION OF HIGH AND LOW RESIN YIELDING GENOTYPES OF *PINUS ROXBURGHII* SARG. USING MICROSATELLITE MARKERS

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DEHRADUN

INTRODUCTION

- Family- Pinaceae
- The genus *Pinus* contains around 100 species world over.

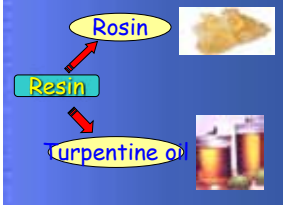
Species found in India:

Pinus roxburghii
Pinus wallichiana
Pinus gerardiana
Pinus kesiya
Pinus merkussi

Pinus roxburghii Sarg.

- *Pinus roxburghii* (named after William Roxburgh) is distributed predominantly in Himalaya. Range extends from Northern Pakistan, across India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim) and Nepal to Bhutan. Occurs upto 2300m (asl)

Commercially important for Resin



- Paper manufacturing and paper sizing
- Chemicals and pharmaceuticals
- Ester gums and synthetic resins
- Paint and varnishes, printing inks
- Soap, rubber, surface coatings
- Linoleum and floor coverings
- Adhesives and plastics
- Oil and greases

- Used as a solvent in industries
- Used as an inhaler for nasal and throat ailments
- Gums and synthetic resins
- Synthesis of fragrant chemicals
- Insecticides and disinfectants
- Asphaltic products
- Adhesives and plastics

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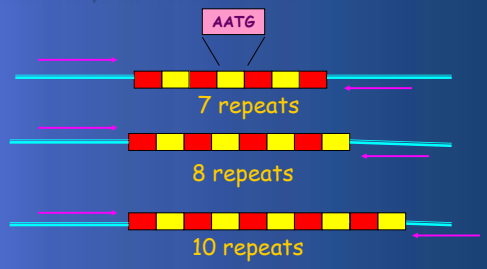
SSR_S / STR_S

SSRs are simple sequence repeats or short tandem repeats in which the repeat region is variable between samples while the flanking regions where PCR primers bind are constant



Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

VARIATION IN RESIN YIELD

Site	Location	Minimum Resin Yield	Maximum resin yield
A_1S_1	Chatra	1.4 kg	8.0 kg
A_1S_2	Chatra	0.25 kg	6.4 kg
A_2S_1	Chatra	2.25 kg	5.6 kg
A_2S_2	Chatra	0.9 kg	5.6 kg

Individuals showing significant variation in resin yield were used for molecular characterization

MATERIAL

	ample	Location	Yield (gm)	Longitude (^{0}E)	Latitude (^{0}N)	Altitude (m asl)
	A-1	Aspect 01, Site Quality 01; Chatra	2.2	77^{0}56' 16.9"E	30^{0}58' 02.3"N	1301
2	A-10	Aspect 01, Site Quality 01; Chatra	3.3	77^{0}56' 18.4"E	30^{0}58' 02.2"N	1298
3	A-12	Aspect 01, Site Quality 01; Chatra	8	77^{0}56' 42.9"E	30^{0}56' 54.9"N	1437
4	A-13	Aspect 01, Site Quality 01; Chatra	3.35	77^{0}9' 58.0"E	30^{0}51' 26.2"N	1203
5	A-19	Aspect 01, Site Quality 01; Chatra	5.2	77^{0}56' 17.4"E	30^{0}58' 02.7"N	1307
6	A-2	Aspect 01, Site Quality 01; Chatra	6.5	77^{0}56' 42.9"E	30^{0}56' 53.3"N	1423
7	A-24	Aspect 01, Site Quality 01; Chatra	2.8	77^{0}56' 20.3"E	30^{0}58' 01.0"N	1292
8	A-25	Aspect 01, Site Quality 01; Chatra	2.9	77^{0}56' 20.3"E	30^{0}58' 1.6"N	1292
9	A-28	Aspect 01, Site Quality 01; Chatra	6.2	77^{0}56' 43.3"E	30^{0}56' 52.9"N	1421
10	A-3	Aspect 01, Site Quality 01; Chatra	0.9	77^{0}56' 43.0"E	30^{0}56' 53.3"N	1423
11	A-6	Aspect 01, Site Quality 01; Chatra	1.4	77^{0}56' 43.2"E	30^{0}56' 53.6"N	1425
12	A-7	Aspect 01, Site Quality 01; Chatra	4.1	77^{0}56' 18.0"E	30^{0}68' 03.3"N	1293
13	A-9	Aspect 01, Site Quality 01; Chatra	5.8	77^{0}56' 42.5"E	30^{0}56' 54.4"N	1432
14	B-10	Aspect 01, Site Quality 02; Chatra	2	77^{0}56' 47.4"E	30^{0}57' 48.2"N	1454
15	B-12	Aspect 01, Site Quality 02; Chatra	4.6	77^{0}56' 48.4"E	30^{0}56' 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	77^0 56' 47.6"E	30^{0}56' 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	77^{0}56' 22.1"E	30^{0}57' 57.6"N	1315
20	B-2	Aspect 01, Site Quality 02; Chatra	2.5	77^{0}56' 46.4"E	30^{0}57' 47.5"N	1450
21	B-24	Aspect 01, Site Quality 02; Chatra	5.7	77^{0}56' 46.6"E	30^{0}56' 47.1"N	1438
22	B-25	Aspect 01, Site Quality 02; Chatra	6.4	77^{0}56' 46.8"E	30^{0}56' 42.2"N	1437
23	B-26	Aspect 01, Site Quality 02; Chatra	2.8	77^{0}56' 21.7"E	30^{0}57' 58.3"N	1306
24	B-3	Aspect 01, Site Quality 02; Chatra	4.9	77^{0}56' 46.7"E	30^{0}56' 47.4"N	1450
25	B-4	Aspect 01, Site Quality 02; Chatra	0.8	77^{0}56' 22.7"E	30^{0}57' 58.0"N	1316
26	B-6	Aspect 01, Site Quality 02; Chatra	4.5	77^{0}56' 47.0"E	30^{0}56' 47.3"N	1452

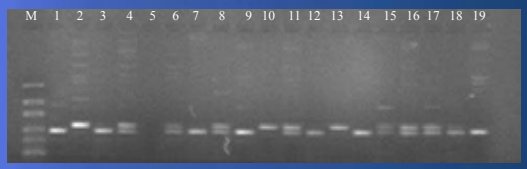
MATERIAL

S.No.	Sample	Location	Yield (gm)	Longitude (^{0}E)	Latitude (^{0}N)	Altitude (m asl)
27	B-7	Aspect 01, Site Quality 02; Chatra	4.3	77^{0}56' 21.6"E	30^{0}57' 58.0"N	1324
28	B-8	Aspect 01, Site Quality 02; Chatra	2.25	77^{0}56' 47.3"E	30^{0}57' 48.5"N	1462
29	B-9	Aspect 01, Site Quality 02; Chatra	4.7	77^{0}56' 47.0"E	30^{0}56' 48.3"N	1454
30	C-1	Aspect 02, Site Quality 01; Chatra	4.2	77^{0}56' 36.5"E	30^{0}57' 9.8"N	1379
31	C-10	Aspect 02, Site Quality 01; Chatra	2.25	77^{0}56' 48.6"E	30^{0}56' 47.9"N	1406
32	C-12	Aspect 02, Site Quality 01; Chatra	2.25	77^{0}56' 37.2"E	30^{0}57' 10.1"N	1364
33	C-15	Aspect 02, Site Quality 01; Chatra	2.9	77^{0}56' 47.7"E	30^{0}56' 49.3"N	1404
34	C-20	Aspect 02, Site Quality 01; Chatra	2.9	77^{0}56' 46.6"E	30^{0}56' 50.3"N	1401
35	C-3	Aspect 02, Site Quality 01; Chatra	5	77^{0}56' 47.8"E	30^{0}56' 46.1"N	1404
36	C-4	Aspect 02, Site Quality 01; Chatra	2.7	77^{0}56' 37.2"E	30^{0}57' 9.8"N	1379
37	C-7	Aspect 02, Site Quality 01; Chatra	4.5	77^{0}56' 48.4"E	30^{0}56' 47.7"N	1404
38	C-8	Aspect 02, Site Quality 01; Chatra	4	77^{0}56' 38.8"E	30^{0}57' 11.2"N	1360
39	C-9	Aspect 02, Site Quality 01; Chatra	5.6	77^{0}56' 49.5"E	30^{0}56' 47.5"N	1405
40	D-11	Aspect 02, Site Quality 02; Chatra	2.6	77^{0}56' 30.4"E	30^{0}58' 4.9"N	1171
41	D-14	Aspect 02, Site Quality 02; Chatra	2.3	77^{0}56' 31.0"E	30^{0}58' 5.1"N	1171
42	D-24	Aspect 02, Site Quality 02; Chatra	1.75	NA	NA	NA
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	77^{0}56' 38.2"E	30^{0}57' 4.4"N	1360
45	D-29	Aspect 02, Site Quality 02; Chatra	2.1	77^{0}56' 38.0"E	30^{0}57' 6.2"N	1353
46	D-30	Aspect 02, Site Quality 02; Chatra	1.6	77^{0}56' 38.3"E	30^{0}57' 6.5"N	1355
47	D-31	Aspect 02, Site Quality 02; Chatra	4.2	77^{0}56' 43.7"E	30^{0}56' 53.9"N	1396
48	D-33	Aspect 02, Site Quality 02; Chatra	5	77^{0}56' 42.4"E	30^{0}56' 53.5"N	1393
49	D-37	Aspect 02, Site Quality 02; Chatra	4.5	77^{0}56' 42.4"E	30^{0}56' 54.1"N	1432
50	D-38	Aspect 02, Site Quality 02; Chatra	4	77^{0}56' 43.2"E	30^{0}56' 53.4"N	1423
51	D-39	Aspect 02, Site Quality 02; Chatra	5.6	77^{0}56' 42.2"E	30^{0}56' 54.3"N	1421
52	D-7	Aspect 02, Site Quality 02; Chatra	1.3	77^{0}56' 38.8"E	30^{0}57' 4.5"N	1370
53	D-8	Aspect 02, Site Quality 02; Chatra	1.1	NA	NA	NA

Details of SSR primer pairs used for the study

S.No.	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	$(GA)_8G_3(GA)_5$
		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	$(AT)_3(GT)_{10}(AT)_2$
		Reverse	ATTAAAGGTTATATGGGGCT			
12	PCP26106	Forward	AATCCGACACAAAAAGATTCGG	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	$(T)_{11}$

GEL PHOTOGRAPHS



SSR primer pm 05 on 3% metaphor agarose gel

SSR primer PCP 1289 on 8% PAGE

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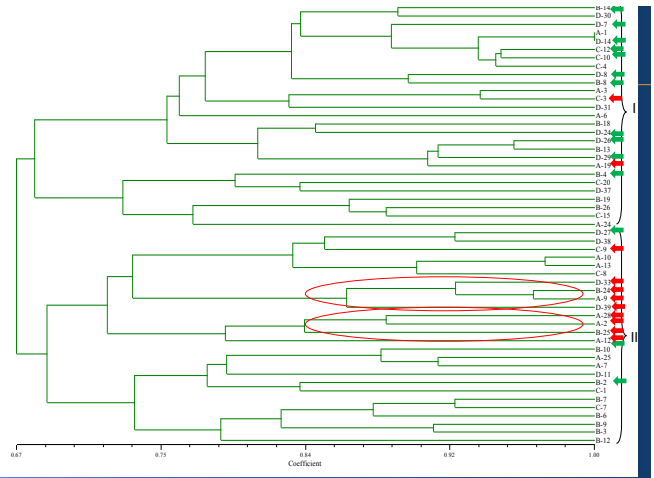
RESULTS

S. No.	Primer code	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
	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

Total number of alleles, Polymorphic alleles, Polymorphism, PIC, EMR, MI and Rp obtained for the 19 SSR primers



Dendrogram of 53 *Pinus roxburghii* genotypes based on Jaccard's similarity coefficient.

CONCLUSION

SSR markers tested clustered the genotypes into two major clusters with majority of high and low resin yeilders in separated clusters. Eight high resin yielders were clustered in two distinct minor clusters.

Thanks

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Establishment of nodulation and Nitrogen fixation in *Casuarina junghuhniana* Miq. rooted stem cuttings with *Frankia* under aseptic conditions

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Scientist D
Institute of Forest Genetics and Tree Breeding
Coimbatore – 641 002.

Casuarina junghuhniana Miq

- Wind break
- Life fencing
- Building material
- Paper & Pulp
- Agro Forestry crop
- High calorific value (7180 kcal kg-1)
- *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.

Frankia

Filamentous Gram+ Actinomycete
Symbiotic association with *Casuarina spp.*

Allocasuarina spp,
Alnus spp,
Hipphophae rhamnoides,
Eleagnus angustifolia,
Ceanothus spp.

Major role in Biological Nitrogen fixation

Frankia **fixes atmospheric nitrogen through root nodules in Casuarinas**

Early establishment of *Frankia* in seedlings and cuttings is essential otherwise the root Nodules may not be formed particularly in rooted stem cuttings of Casuarinas.

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• Farmers usually applied crushed nodules of casuarinas in the seedlings for nitrogen fixation but often un successful as the nodules contains dead or inactive *Frankia*

• Farmers applying 150 Kg of DAP/acre for casuarinas per year (Nicodemus, 2009)

•To find an alternate solution for use of chemical fertilizers for the rooted stem cuttings of *C. junghuhniana* during plantation. We attempted to improve the rooted stem cuttings of *C. junghuhniana* in terms of growth, biomass and nodulation through inoculation of *Frankia* so as to reduce the use of chemical fertilizers

Isolation and culture of *Frankia*

Strain No.	Place	Soil type	Source of Nodules	Nodules Colour	Nodules diameter
CeCO1	Cuddalore (T.N) Coastal zone	Sandy clayloam	Coastal plantations of *Casuarina junghuhniana*	Brown	1 – 1.5 cm

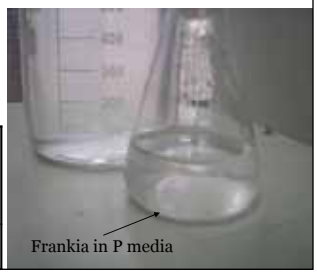
One litre of P medium (Shipton and Burgraff, 1983) : 10g $CaCl_2,2H_2O$, 20g $MgSO_4$, 0.46g Propionic acid, 0.15g H_3BO_3, 0.15g $ZnSO_4.7H_2O$, 0.45g $MnSO_4.H_2O$, 0.004g $CuSO_4.5H_2O$, 0.028g $Na_2MoO_4.2H_2O$, 0.009g $CaCl_2.6H_2O$, 0.04g Biotin, 100g K_2HPO_4, 67g $NaH_2PO_4.2H_2O$, 0.1g FeNa EDTA, and 8.g agar. The pH of the medium was adjusted to 6.8.

Frankia

Thick-walled Vesicles

Hyphae

Strain No.	Hyphal width (in µm @ 40x)	Vesicle dimension (in µm @ 40x)	Sporangia shape	No. of days grown in media
CECo1	1- 1.5	2-3	Circular	25 days

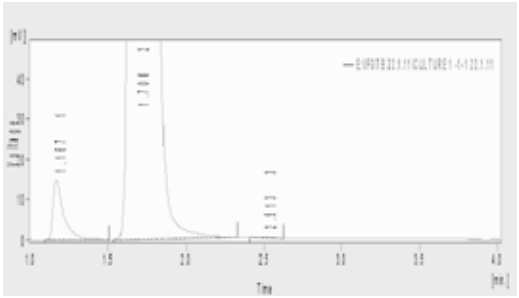


Frankia in P media

Acetylene Reduction Assay (ARA) for measuring Nitrogenase Activity by using Gas Chromatography (Hardy *et al.*, 1975)

Operating Conditions:

Nucon Model 91098 Gas Chromatograph
Column : Poropak – Q (2M, 2.1mm stainless steel, 80-100 mesh)
Detector : Flame Ionizing Detector (FID)
Injector temperature : 50^0 C
Oven temperature : 70^0C
Column temperature : 80^0 C
Detector temperature : 120^0C
Carrier gas : Nitrogen
Flow rate : 30 ml sec^{-1}
Sample injection volume : 100µL
Standard injection volume : 500µL



RETENTION TIME	PEAK AREA
1.167 (unknown)	468.092
1.700	4045.401
2.513	85.941

ARA: 158.23n mol.

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

- Clone No. Cj 18
- Treated with 2000ppm of IBA
- Placed in 100cc root trainer
- Maintained in Poly tunnels
- *Frankia* inoculated @ 5ml/ rooted stem cuttings

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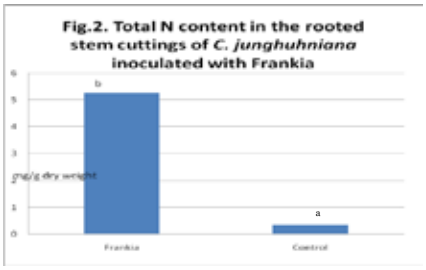
Table. 3. Growth and bio mass of *C. junghuhniana* rooted stem cuttings to *Frankia* inoculation at 90 days under nursery conditions.

Clone No.	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.											



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

Results

- Increased the growth and biomass
- Increased Nutrient (N) content
- Lower R/S ratio
- Increased nodule number and nodule biomass in rooted stem cuttings of *C. junghuhniana*

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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 *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.

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GENOTYPE X ENVIRONMENTAL ANALYSIS FOR DIFFERENT CLONES OF *DALBERGIA SISSOO* ROXB.

BY

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BRIEF HISTORY OF THE CLONE

- *Genetic improvement of the species started during 1994 under World Bank Funded FREE project*
- *The plus trees (~300) were selected in entire growing range of the species*
  - *Uttar Pradesh (Undivided)*
  - *Haryana*
  - *Rajasthan*
  - *Punjab*

  India
  - *Nepal*
- *Clones and progenies raised at different locations*
- *The VMG and production populations (CSO, SSO, gene banks) raised over the locations*
- *Further selection carried out to establish present trials*
- *Trials have also been established in the other states viz. Haryana, Uttar Pradesh, Uttarakhand*

LIST OF 36 CLONES UNDER EVALUATION

S. No.	Clone No.	Source of the stock
1	1	Sabalgarh, Chirapur, Bijinour, U.P.
2	2	Sabalgarh, Chirapur, Bijinour, U.P.
3	3	Trilokpur, Gonda, Bijinour, U.P.
4	4	Sabalgarh, Chirapur, Bijinour, U.P.
5	9	Sabalgarh, Pathri, Haridwar, Uttarakhnad
6	10	Sabalgarh, Pathri, Haridwar, Uttarakhnad
7	12	Sabalgarh, Pathri, Haridwar, Uttarakhnad
8	14	Sabalgarh, Pathri, Haridwar, Uttarakhnad
9	19	Shah Mansurpur, Saharanpur, Uttar Pradesh
10	24	C. B. Ganj, Barielly, Uttar Pradesh
11	33	Bhainsasur, Tulisipur, Gonda, Uttar Pradesh.
12	36	Hasanpur, Tulisipur, Gonda, Uttar Pradesh
13	41	Hasanpur, Tulisipur, Gonda, Uttar Pradesh
14	43	Trilokpur, Tulisipur, Gonda, Uttar Pradesh
15	49	Trilokpur, Tulisipur, Gonda, Uttar Pradesh
16	51	Birpur, Bhambar, Gonda, Uttar Pradesh
17	57	Dinsia, Khalawala, Ambala, Haryana
18	66	Chhirauli, Yamunanagar, Haryana
19	124	Kosi River Bank, Inerwa,
20	128	Maherdragarh,
21	168	Kanau, Chaur, Chila, Bairaj, Chila Rajaji, Chila, U.P.
22	174	Kanau, Chaur, Chila, Bairaj, Chila Rajaji, Chila, U.P.
23	192	Hasanpur 2, Tulisipur, Gonda, U.P.
24	198	Hasanpur 2, Tulisipur, Gonda, U.P.
25	201	Hasanpur 2, Tulisipur, Gonda, U.P.
26	204	Hasanpur 2, Tulisipur, Northgonda, U.P.
27	232	Birpur 4 (Near Limla Khonder), Bhambar, Gonda, U.P.
28	235	Bankatwa, 2, Gonda, U.P.
29	237	Bankatwa, 2, Gonda, U.P.
30	243	Bankatwa, 2, Gonda, U.P.
31	247	Bankatwa, 2, Gonda, U.P.
32	5038	BBC, 72-73 I /S, Sangrur,
33	5039	Pir Mashala Compartment-II, Dera Bassi, ,
34	5040	Amargarh-Chaunada Road, Km 6-7, I/S, Sangrur, Punjab
35	5041	GBC 194-95, R/S Leharganga, Sangrur, Punjab
36	5042	Bir Mattiwara Com., 25, Ludhiana, Punjab

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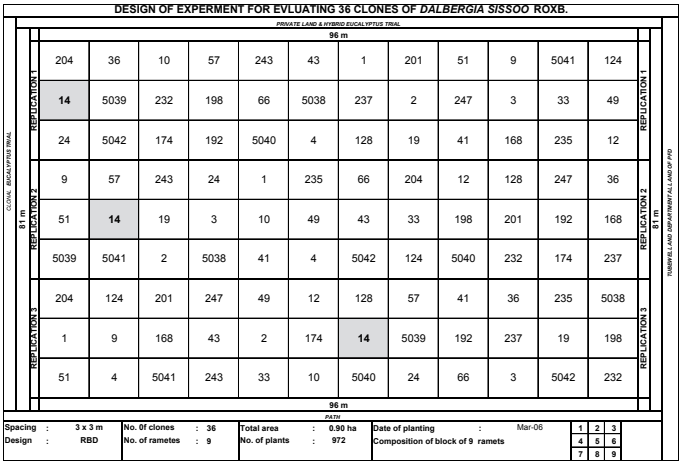
GEOGRAPHICAL LOCATION OF EXPERIMENTATION

S. NO	NAME OF SITES			LATITUDE	LONGITUDE
	LOCATION	DISTRICT	STATE		
1	Mattewara	Ludhiana	Punjab	30°59′08.3″N	75°59′11.4″E
2	Pindori Mindo Mind	Hoshiarpur	Punjab	31°33′33.0″N	75°49′02.3″E
3	Bir Sanour	Patiala	Punjab	30°19′36.0″N	76°24′01″E



CLONAL TRIAL OF SHISHAM AT PATIALA

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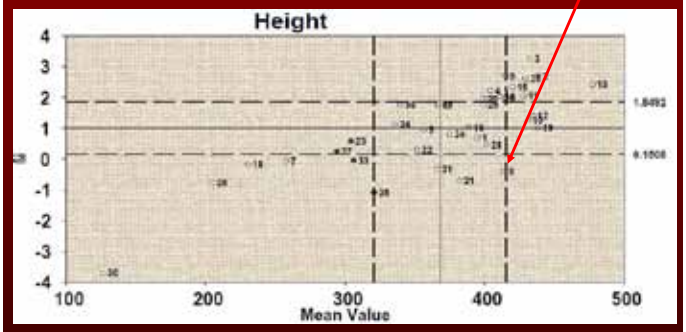
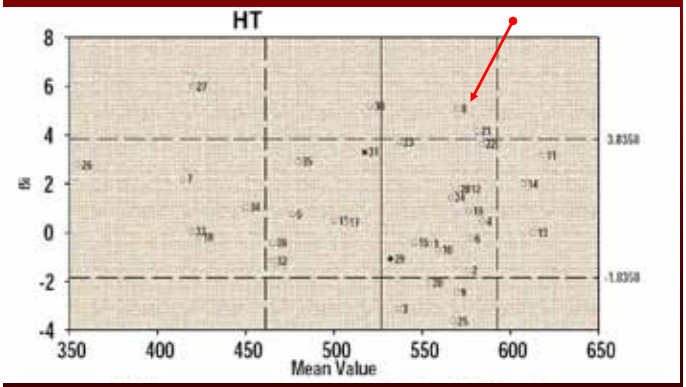
GENETIC ANALYSIS

- Adaptability and stability among the clones to understand G x E interactions
- Clustering of different clones for understanding the genetic diversity among the clones
- Principal component analysis to understand contribution of each character towards the genetics diversity
- Development of genetic correlations for developing future strategy for breeding and hybridization

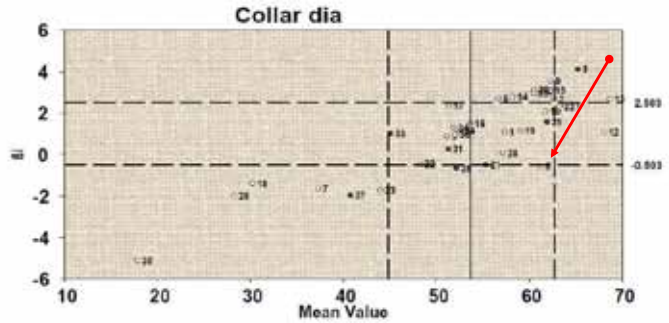
ADAPTABILITY & STABILITY FOR HEIGHT
(2.5 YEARS)



ADAPTABILITY & STABILITY FOR HEIGHT
(4 YEARS)



ADAPTABILITY & STABILITY FOR COLLAR DIAMETER
(2.5 YEARS)



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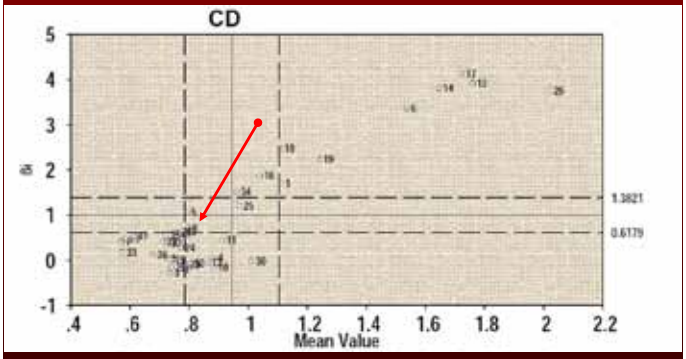
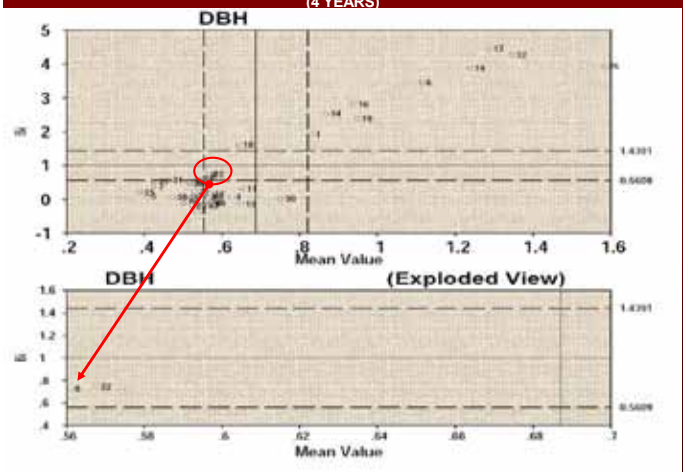
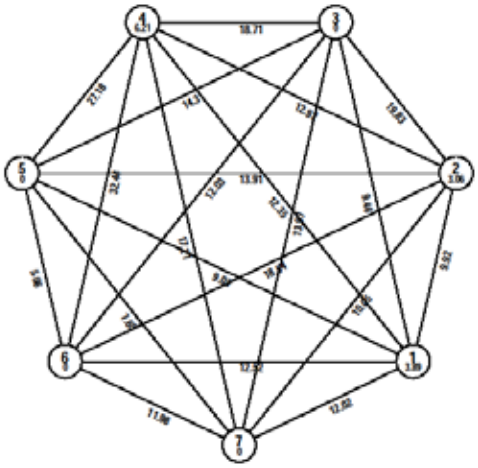
ADAPTABILITY & STABILITY FOR COLLAR DIAMETER
(4 YEARS)



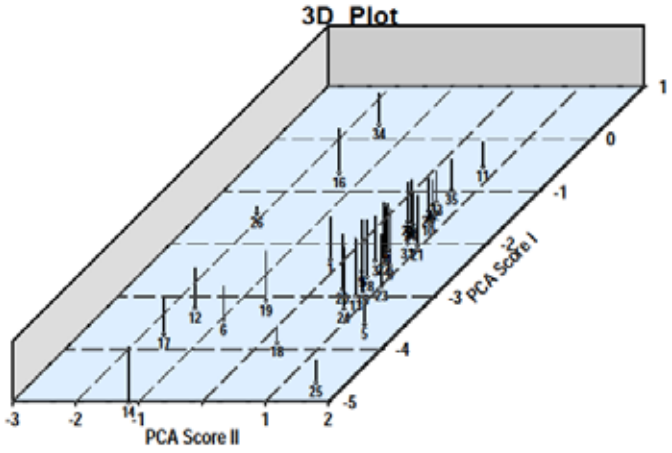
ADAPTABILITY & STABILITY FOR DIAMETER AT BREAST HEIGHT
(4 YEARS)



CLUSTERING AT 4 YEARS OF AGE



PRINCIPAL COMPONENT ANALYSIS



CONTRIBUTION OF IMPORTANT CHARACTERS DURING PRINCIPAL COMPONENT ANALYSIS

Determinant of Error Matrix			54724.1016E+3
Determinant of Error + Variety Matrix			-13987.21326E+11
Wilk's Criterion			39124.37782E-12
M	82.5	V statistics	1407.16300
Degree of Freedom	245	Probability	0.00000

ANOVA for DISPERSION

Source of Variations	df	Sum of Squares	Mean Squares	F Ratio	Probability
Varieties	35	- 1.3987E15	- 3.9963E13	- 5.039E07	0.00000 **
Error	69	5.4724E07	7.9310E05		
Total	104	- 1.3987E15	- 1.3449E13		

Source	Times Ranked 1st	Contribution %
1 HT	75	11.90 %
2 CBH	88	13.97 %
3 DBH	145	23.02 %
4 CD	142	22.54 %
5 Crown dia	38	6.03 %
6 Straightness	86	13.65 %
7 Branching	56	8.89 %

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Results

The inoculated plants started exhibiting wilt disease symptoms after a month (Fig. 6). The results of the screening tests by using direct injection method have been presented in Table 5. Clone No. 14, 11, 6, 9 and 1 were the resistant clones against all the virulent isolates of *F. solani*, whereas Clone No. 3, 10, 12, 15 and 18 were the susceptible clones. Clone No. 14 was the most promising clone from disease resistant point of view as it exhibited stabilized resistant reaction after two months of inoculation which was followed by Clone No. 6.

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IN VITRO CLONAL PROPAGATION OF *COMMIPHORA WIGHTII* (GUGGAL) – A MEDICINALLY IMPORTANT TREE SPECIES OF THE ARID REGION

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JODHPUR

GUGGUL: PRESENT SCENARIO

Guggal – belongs to family Burseraceae

Guggal once a luxuriantly growing species in the arid and semi arid areas has become a threatened species due to excessive tapping for extraction of gum. Plant dies after tapping for gum.

Seed germination is very poor and takes time to produce plants from stem cuttings.

Gum guggul is the oleoresin of *Commiphora wightii*.

Commiphora wightii (Arn.) Bhandari

- Member of family Burseraceae
- Locally known as Guggul, Gogil, Gugar and Mukul
- Chiefly known for oleo-gum-resin

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VALUE

Guggal – belongs to family Burseraceae

- *Commiphora wightii* is well known and over exploited for its oleo-gum-resin which has very high medicinal value.
- The oleo-gum-resin is a complex mixture made up of various useful secondary metabolites.
- Two isomeric forms of a steroid - guggulsterone-E and guggulsterone-Z are the most sought after by the drug industry.
- These compounds are frequently used with combination of other supplements in curing the patients of cardiac dysfunction.

Eyebrow raising facts

- **Plant with high potential for increase production with some R&D efforts.**
- **Domestic demand is 2548.9 tonnes and is 0.9% share quantity wise and 1.6% price-wise of total medicinal demand of the country.**
- **Demand supply gap is 1489.7 tonnes.**
- **Supply is from wild and import.**

GUGGUL: NEED FOR TISSUE CULTURE

GUGGUL: NEED FOR TISSUE CULTURE

- The major advantage of the *in vitro* system
  - ✓ Reproducible and rapid rate of multiplication of rare and endangered species.
  - ✓ Pathogen free saplings.
  - ✓ By-pass system for such species that are difficult to propagate by vegetative methods or by seeds.
  - ✓ Production of plantlets all around the year uninterruptedly without any seasonal constraints.

GUGGUL: NEED FOR TISSUE CULTURE

Micro propagation has tremendous scope for further expansion and gainful utilization as-

➔Production of 'synthetic' seeds from somatic embryos

➔The increased variability observed in plants regenerated in tissue culture via callus phase, could be utilized in exploiting the somaclonal variations.

➔Can be used for germplasm conservation as well.

DEVELOPMENT OF TISSUE CULTURE PROTOCOLS OF GUGGAL

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PROTOCOL -1

Micropropagation through cotyledonary node culture

THROUGH COTYLEDONARY NODE CULTURE

Fruit selection process:

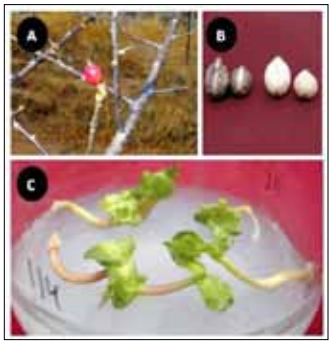
- The mature fruits collected (both white and black and from winter and summer season) were subjected to a '*water submergence selection process*'.
- The fruits either remained submerged or floated and were accordingly classified as sinkers and floaters.
- Sinkers were used for germination while floaters were rejected as they were mostly found to have empty locules with small, improperly developed or no seed.

THROUGH COTYLEDONARY NODE CULTURE

Seed Germination for obtaining explant:

- Isolated seeds after surface sterilization were inoculated on full and half strength salt concentrations of MS (Murashige & Skoog 1962) and B_5 media (Gamborg's 1968).
- Half strength B_5 media was found to be the most suitable medium for *in vitro* germination of seeds.
- Isolated seeds were treated with BAP during germination (pre-conditioning).
- Such screened and treated seed resulted in 100% germination

THROUGH COTYLEDONARY NODE CULTURE



- A- mature fruit on guggal plant;
- B- seeds with black and white endocarp;
- C- germination after BAP pretreatment

MICROPROPAGATION THROUGH COTYLEDONARY NODE CULTURE

Bud break and proliferation:

- Cotyledonary node explants harvested from BAP-preconditioned seedlings responded better as compare to those without the treatment.
- Multiple shoots were produced only on preconditioned explants.
- Excellent growth of micro-shoots was observed on both the BAP combinations with IBA.
- A maximum of upto six micro-shoots per cotyledonary node explant were obtained.

Rooting of microshoots.

- Hormone- free White's root culture media (White, 1954) with 5% AC was found best for rooting. Roots were white and produce secondary roots. No callusing was observed during rooting

MICROPROPAGATION THROUGH COTYLEDONARY NODE CULTURE

- A- micro-shoot multiplication;
- B- *in vitro* rooting of cotyledonary node derived micro-shoots of guggal

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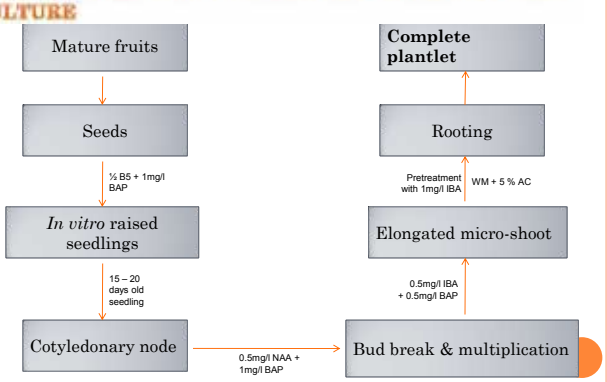
MICROPROPAGATION THROUGH COTYLEDONARY NODE CULTURE



PROTOCOL -2

Micropropagation through axillary bud proliferation

Axillary shoot proliferation

Explant- Mature Nodal segments

Table : Axillary shoot proliferation on MS medium supplemented with different concentration of hormones and additives

S. No.	Media	% bud break response	No. shoots induction
1.	BAP (1.0 mg/l) and NAA (0.5 mg/l), (Prajapati , 2008)	40.0%	1
2	BAP(4.0 mg/l) + Kinetin (4.0 mg/l) + additives (Barve and Mehta, 1993)	72.0%	2
3.	MS + BAP (2.0 mg/l) and IAA (0.1 mg/l) + additives	**84.5%**	2

These axillary shoots were transferred with mother explants for multiplication on the same medium and lower concentration of BAP. But multiplication was not observed. More experiments will be carried out for improving the response of multiplication.

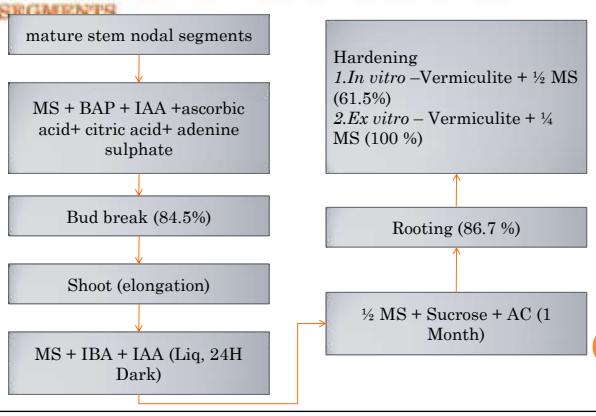
Rooting of micro-shoots

1. Pulse treatment for rooting- liquid MS medium + IBA/ IAA (1 mg/l) for 24 hrs in dark.

2. MS + activated charcoal (0.5% w/v) + Sucrose (2%) + Agar (0.8%) + pH 5.8

Observations and results: The micro-shoots were subcultured and maintained for further elongation on the same medium for 4 weeks. Best rooting was obtained when the shoots were initially given a 24 hours pulse treatment in liquid MS medium supplemented with 1 mg/l each of IBA and IAA under dark condition, followed by transfer to semi-solid half-strength hormone-free MS medium supplemented with 2% (w/v) sucrose and 0.5% (w/v) activated charcoal. High (86.7%) percent rooting was achieved after 4-5 weeks with 3-4 multiple adventitious roots of 5-6 cm length

MICROPROPAGATION FROM MATURE STEM NODAL SEGMENTS



PROTOCOL -3

In vitro propagation through Somatic embryogenesis

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Somatic Embryogenesis (SE)

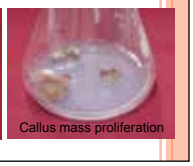
Establishment of SE cultures

Explant- Immature seeds

Media- B5 + 2,4-D Hormone (0.5mg/l) +Agar (0.8%) + Sucrose (3%) + pH 5.8

Immature fruits were collected from different locations of Rajasthan such as AFRI Nursery, Kayalana (Jodhpur), Mangliyavas (Ajmer) and Charbhuja (Rajsamand). Settled fruits were used as a source of immature seeds. Good callus multiplication in terms of callus mass was achieved and subcultured for callus mass proliferation on the same medium for 3-4 weeks. Callus turned embryogenic after subculturing on hormone free B5 medium. Embryogenic callus with different stages of embryos were seen and converted to further advanced stages.

Callus induction from immature seed



Callus mass proliferation

Multiplication of SEs :

Explant - Embryogenic callus

Media- MMS + activated charcoal (0.5%) or without activated charcoal +Hormone- BAP (0.25mg/l) and IBA or IAA (0.1mg/l) and hormone free + Sucrose (3%) + pH 5.8

Observation and results :

A clump (with 4-5 SEs) of embryogenic callus was used to check the response of multiplication.

After one month interval, fast multiplication was obtained on activated charcoal, IBA and BAP supplemented medium.

Embryogenic callus

Multiplication of Embryogenic callus

Maintenance of embryogenic cultures (solid and liquid medium):

Explant- Embryogenic callus and SEs.

Media-

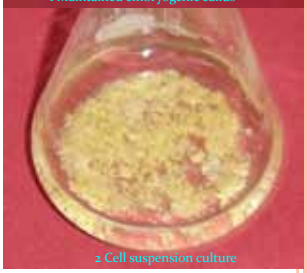
•Modified MS medium+ Activated charcoal (0.5%) + IBA (0.1mg/l) and BAP (0.25mg/l) + Agar (0.8%)

•Modified MS medium+ Activated charcoal (0.5%) + Agar (0.8%)

•Modified MS medium+ IBA (0.1mg/l) and BAP (0.25mg/l) [Liq. Medium for suspension culture]

•Modified MS medium+ Sucrose (3%) [Liq. Medium for suspension culture]

1 Maintained embryogenic callus



2 Cell suspension culture

Cell Suspension culture

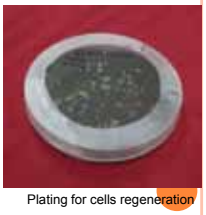
Explant - Embryogenic callus

Media- Liquid media (MS and MMS) + Hormone IBA (0.1mg/l) and BAP (0.25mg/l) and hormone free + Sucrose (3%) + pH 5.8

Observation and Result :

A clump of embryogenic callus was used for initiating the cell suspension culture. Suspension cultures have helped in synchronization of SE stage and further is helping in SE maturation and germination increasing the frequency to a greater extent.

Establishment of Cell suspension culture



Plating for cells regeneration

Maturation of SEs

Late torpedo and early coteyledonary stages of somatic embryogenesis were used for maturation of SEs. Depleted modified MS medium without any PGRs were used for maturation for reducing the water content available in SEs which results in better desiccation and dehydration of SEs. It was observed that somatic embryos turned whitish and enlarged in size. They were seen completely dehydrated and desiccated after 6-8 weeks. Further, they were harvested for germination.

Maturated Somatic embryos

SE Germination & Hardening of plantlets

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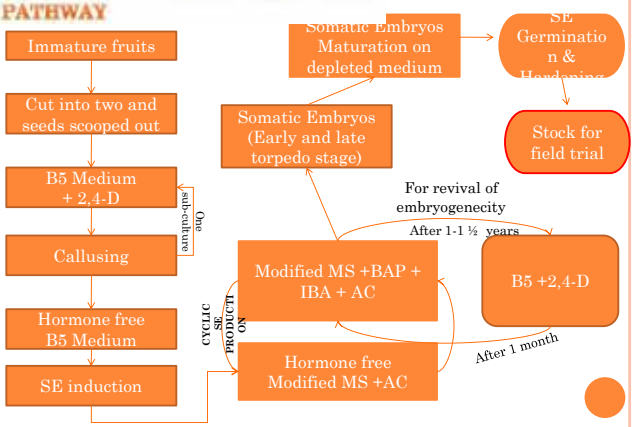
SE-Derived *In Vitro* Hardened Plants Showing Well Developed Root System

SOMATIC EMBRYOGENESIS BASED PATHWAY



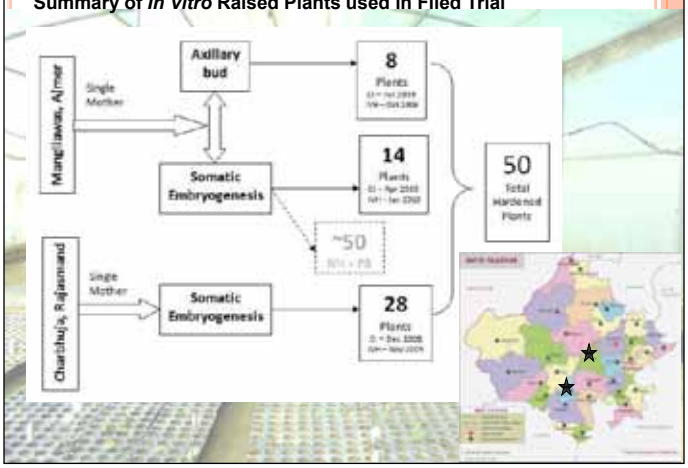
Potting mixture trials during hardening

Treatment	Sand ©	Sand : FYM 1: 5	Sand : Vermicompost 1: 5	Vermiculite : FYM 1: 5	Vermiculite : Vermicompos t 1: 5
Response	IV	III	III	II	I

Hardenin

- ✓ **50 plants have been hardened and transferred to field**
- ✓ **A batch of more than 50 plants is in *ex vitro* hardening stage which will soon be transferred to field for second trial.**

Summary of *In Vitro* Raised Plants used in Filed Trial



Field Trial Layout Design of *In Vitro* Raised Plants

Control (Seedlings)	Lane 1	Lane-2	Lane-3	Lane-4	Lane-5
S1	A1	B2	A2	B17	C7
S2	A7	B1	A9	B24	C3
S3	A6	B8	B26	B3	C15
S4	B7	B14	B21	C1	C21
S5	A8	B25	B15	B12	B18
S6	B9	B19	B6	C4	C14
S7	B10	B11	B27	C10	C5
S8	A5	B22	B20	C2	C13
S9	B13	A4	B16	C12	C25
S10	B4	B23	B5	B29	C6

Legends

A: Axillary shoot-derived Plants from mother plant growing in Mangliyavas, Ajmer

B: SE-derived plants from mother plant growing in Charbhuja town, Rajsamand district

C: SE-derived plants from mother plant growing in Mangliyavas, Ajmer

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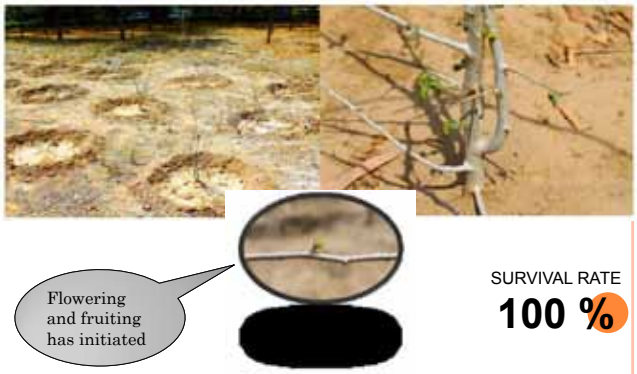
Field Trial – Laid out on 28th July 2010 at AFRI Campus, Jodhpur

50 *In vitro* raised *Commiphora wightii* plants
- 8 Axillary-bud derived
- 42 Somatic embryo derived

Field growing tissue culture raised plants after 5 months in field condition (December 2010)

Field growing tissue culture raised plants after 10 months in field condition (April 2011)

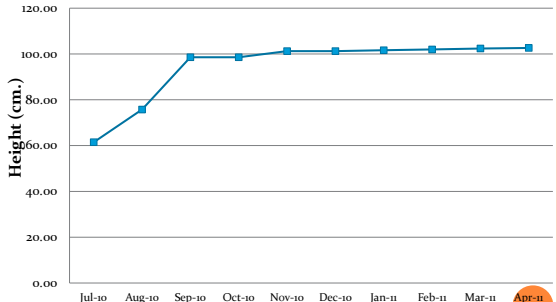


Flowering and fruiting has initiated

SURVIVAL RATE
100 %

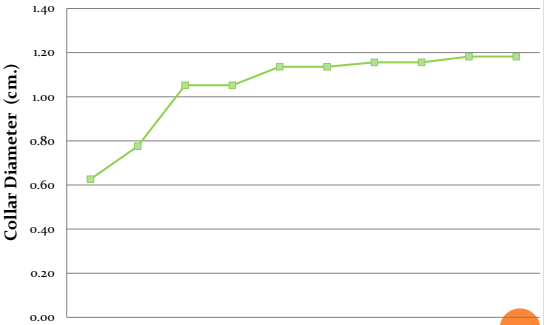
Field growing tissue culture raised plants after 14 months in field condition (September 2011)

Growth performance of TC plants in terms of height over a period of 10 months under field condition



	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11
Height of TC Plants (cm.)	61.48	75.74	98.58	98.58	101.26	101.26	101.64	101.98	102.41	102.65

Growth performance of TC plants in terms of collar diameter over a period of 10 months under field condition



	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11
Mean CD (cm.) of TC plants	0.63	0.78	1.05	1.05	1.14	1.14	1.16	1.16	1.18	1.18

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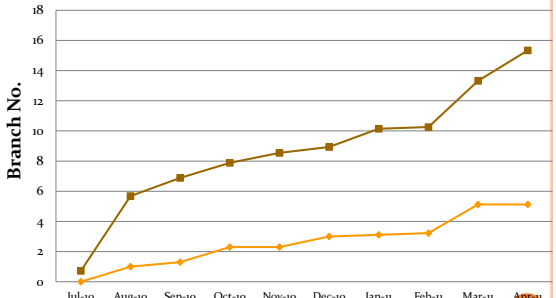
Growth performance of TC plants in terms of branching over a period of 10 months under field condition



	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11	Mar-11	Apr-11
Mean Br. No. (TC Plants)	0.72	4.68	5.58	5.58	6.24	5.94	7.03	7.03	8.20	10.21
Mean Br. No. (Cntrol)	0	1	1.30	2.3	2.30	3	3.11	3.22	5.12	5.12

New batch of hardened tissue culture raised plants in open nursery condition, ready for field plantation

ECONOMIC SIGNIFICANCE

- ✓ Per plant cost from these protocols has been calculated using the equation formulated by Tomar *et al.,* (2007). This formula takes into account all the stages from initiation to acclimatization up to the plantable size.
- ✓ The cost of single plant produced through somatic embryogenesis pathway, is equivalent to Indian Rupees (INR) 19, while that produced through cotyledonary node and axillary shoot proliferation protocols is INR 27 (Kant *et al,* 2010a).
- ✓ This indicates the applicability and benefits of using tissue culture technology to assist in conservation of *C. wightii*.

International Publications

In vitro propagation as a viable conservation strategy for *Commiphora wightii*, an endangered medicinally important desert tree, India

EFFICIENT MICROPROPAGATION FROM COTYLEDONARY NODE CULTURES OF *COMMIPHORA WIGHTII* (ARN.) BHANDARI, AN ENDANGERED MEDICINALLY IMPORTANT DESERT PLANT

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In vitro propagation of *Dendrobium bensoniae* Rchb.f. an important orchid of North Eastern India.

By
Babita Rani, T.S.Rathore
& K.S. Shashidhar
Arid Forest Research Institute, Jodhpur

Introduction

- Orchids are outstanding ornamentals due to its diverse colors, shapes, forms and long lasting flowering (Toukuhara and Mii, 2001).
- The genus *Dendrobium,* is the second largest genus in the orchid family consisting more than 1000 species.
- *Dendrobium bensoniae* have an epiphytic growth habit.
- It blooms in the spring with one to three inflorescences of wide fragrant flowers and requires warm to hot temperatures and medium amounts of light.
- It is found in NE India, Burma and Thailand at the elevations of 450 to 1550 meters.
- Traditionally, It is propagated by division of clumps/rhizomes/ cuttings/separation of offshoots .

Methodology & Results

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Source of Explant:

- Closed green capsules of *Dendrobium bensoniae* obtained from Nagaland.

Surface sterilization :

- Tween 80 : 10 min
- Bavistein (0.25%) : 10-12 min
- Ethanol (70%) : 1 min.
- $HgCl_2$ (0.2%) : 12 min.

Effect of Additives and Plant growth regulators on seed germination:

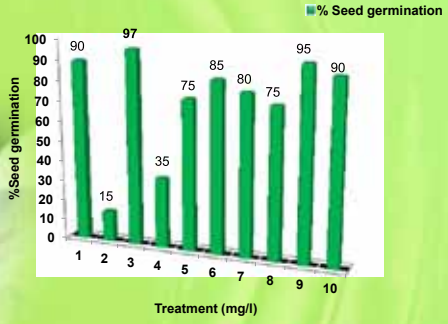
- The immature seeds were scooped out by using sterilized forceps from the capsules and small mass of aggregated seeds were spreaded uniformly for germination on full strength MS (Murashige and Skoog 1962) mediia supplemented with 3% (w/v) sucrose and solidified with 0.6% Agar.
- Media was enriched with ascorbic acid (50mg/l), citric acid (25 mg/l), cysteine (25 mg/l) and glutamine (100mg/l).
- Medium was incorporated with different PGRs (auxin; NAA 0.1-1.0mg/l and 2, 4-D,1.0mg/l with or without cytokinins; BAP,1.0-2.0mg/l; TDZ, 0.1-0.25mg/l and Kn, 0.5mg/l), and without hormones used as a control.
- Coconut water (10%v/v) and Banana homogenate (10% w/v) were also tested for germination.
- The pH of the medium was adjusted to 6.2

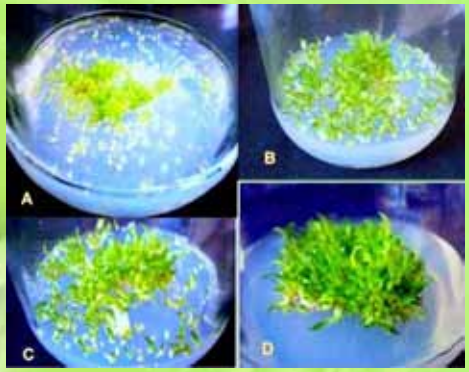
Results:

- Seed started germination with the swelling after 6-8 week of inoculation and globule shape protocorm like formation became distinct at 8-10 weeks of inoculation.
- MS medium supplemented with 10% CM proved the best with 97% of germination.
- Treatment consisted MS + NAA 1.0 mg/l + BAP 1.0 mg/l, also favored development of protocorms like structure.
- After 12-14 weeks of inoculation two and four leaves structure were seen protruding from the PLBs.

T. No.	Treatment (PGRs mg/l)
1	HF
2	2,4-D 1.0
3	**CM 10 %**
4	2,4-D 1.0 + CM10%
5	NAA 1.0 + Kn0.5
6	NAA1.0 + BAP 1.0
7	NAA0.25 + TDZ 0.1
8	NAA0.25+TDZ 0.25
9	Banana homogenate 10%
10	Banana homogenate 10% + CM 10%

Effect of additives and PGRs on seed germination in *Dendrobium bensoniae.*





Asymbiotic Germination of *D. bensoniae*

A. Globule shape PLBs after 8-10 week of inoculation
B & C. Two leaf structure after 3-4 week of subculture
D. Four leaf structure after 3-4 week of subculture

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Effect of various additives and plant growth regulators (PGRs) in the medium on growth of seedlings:

- After 20 week old culture, seedlings derived from seeds of mature capsules were transferred to fresh MS basal medium enriched with ascorbic acid (50,mg/l) + citric acid (25, mg/l)+ cystein (25,mg/l)+ glutamine (100,mg/l).
- Different auxins (NAA, 0.1-1.0,mg/l and 2, 4-D, 1.0mg/l) were used either individually or in combination with cytokinins (BAP,1.0-2.0 mg/l; TDZ, 0.1-0.25mg/l and Kn, 0.5 mg/l).
- Coconut water (10%, v/v) and Banana homogenate (10%, w/v) were used either alone or in combinations.

Results

- MS basal medium incorporated with NAA 1.0 mg/l and BAP 2.0mg/l proved the best for shoot multiplication (80%) with shoot length of 6-7 cm in 4-5 weeks.

Effect of various additives and plant growth regulators (PGRs) for rooting:

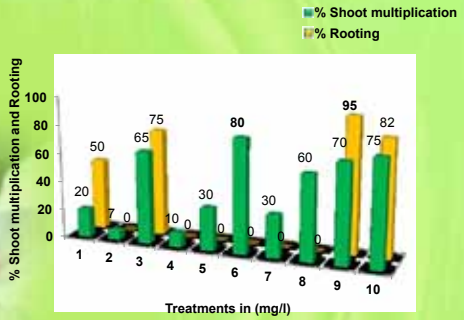
- Multiple shoot of length 4-5 cm. were used for rooting on MS basal medium with 3% of sucrose and solified with 0.6 % Agar.
- Different auxins (NAA 0.1-1.0,mg/l and 2, 4-D, 1.0mg/l) were used either individually or in combination with cytokinins (BAP,1.0-2.0 mg/l; TDZ, 0.1-0.25 mg/l and Kn , 0.5 mg/l).
- Coconut water (10%, v/v) and Banana homogenate (10%, w/v) were used either alone or in combinations.

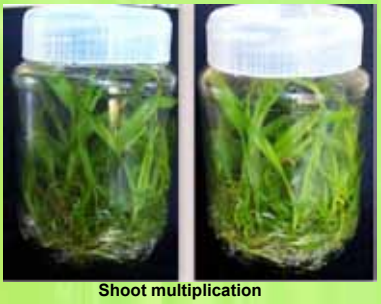
Results:

- Banana homogenate containing MS basal medium supported vigorous growth as shown by increased shoot length and well developed roots (97%) in 5-6 weeks.

T. no.	PGR's/Additives
1	HF
2	2,4-D 1.0
3	CM10%
4	NAA 1.0 + Kn0.5
5	NAA1.0 + BAP 1.0
6	NAA1.0 + BAP 2.0
7	NAA0.25 + TDZ 0.1
8	NAA0.25+TDZ 0.25
9	Banana homogenate 10%
10	Banana homogenate 10% + CM

Effect of additives and PGR's on shoot multiplication and Rooting in *Dendrobium bensoniae.*





Shoot multiplication

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Hardening:

- Well developed plantlets with 5-6 cm. shoot length were transplanted in 50 cell block type trays.
- Potting mixture like charcoal, bricks and cocopeat were used in different ratio.
- Transplanted plants were kept to green house under the polyglobule for first 7 days and slowly after one day of interval it has been exposed to open green house system to open shed system.

Result

- Potting mixture like charcoal, bricks and cocopeat in the ratio1:1:4 (v/v) was found to be the best.
- Hardening was found essential for 6-8 weeks for high rate of survival.

Incubation Condition & Statistical analysis:

- All cultures were incubated at 25 ± 2ºC temperature in culture room at 2500 lux intensity of light provided by the cool white fluorescent tube with 16 h photoperiod.
- All the data were analyzed statistically by ANOVA (one way).

A. Growth of the Seedling
B. 5-6 cm. length of shoot used for hardening

A. Hardening in poly-tunnel
B. Hardening of plants in trays with potting mixture

Hardened Plant of *D. bensoniae*

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Utilization of Tissue Culture Technique for propagation of *Melia dubia* Cav

BHIMI RAM, Research Scholar, F. R. C. Hyderabad

Dr. T. S. Rathore, Director, A. F. R. I. Jodhpur

Dr. G. R. S. Reddy, F. R. C., Hyderabad

Mr. D. S. Rajput, Research Scholar, I. W. S. T., Bangalore

INTRODUCTION

- *Melia dubia* Cav. commonly known as Malabar neem and locally called as Hebbevu in Kannada, belongs to the family Meliaceae

Plus tree of *M. dubia*

- It is an industrially and economically important fast growing tree
- Bears clean cylindrical bole, usually 15-20ft and sometimes up to 40ft with big branches
- The species is originated from southern Asia (India-Pakistan-Iran). It has been introduced and widely cultivated in South Africa, Middle East, America (Bermuda, Brazil and Argentina), Australia and Europen countries.
- It requires deep red gravelly soil, rainfall of about 800-1000mm and an elevation of 800-1000mtrs.

INTRODUCTION contd…..

Clean bole of *M. dubia*

- Timber is in high demand for plywood industries due to termite and fungal resistant (Suprapti *et al*., 2004).
- It has great potential for its Biomass power plants (power generation).
- The wood is mainly used for packing cases, cigar boxes, ceiling planks, pencil, match boxes, furniture, agricultural implements and house construction.
- Used for afforestation and land rehabilitation (Langenberger *et al*., 2005)
- It has various medicinal properties like antiviral (Vijayan *et al*., 2004), bacteriostatic and fungistatic (Nagalakshmi *et. al*., 2003), antifeedant activity (Koul *et. al*., 2002) etc.
- Oil is used for variety of purposes like; soaps industries, as a lubricants and illuminants.

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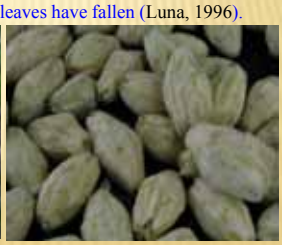
LIMITING FACTORS OF *M. dubia* PROPAGATION AND IMPROVEMENT

- The seeds are very hard and hardly germinate without any treatment.
- limited period of viability.
- Seeds take one year to ripen.
- Fruit drop is limited and ripe fruits clings to the branches for several months even after the leaves have fallen (Luna, 1996).

So, Production of planting material become a problem. Only 14% germination reported (**Nair *et al* 2005**)

Present Research status on *M. dubia*

Clonal propagation

Vegetative propagation were reported in *M. dubia* using coppice shoot

76-94% rooting by using 5% IBA (Manjunatha, 2007)

Drawbacks of clonal propagation by using coppice shoot

- Less number of explant material
- Destruction of whole mother plant
- Small scale commercial production

As such there is no report on micropropagation of *M. dubia*

Shoot initiation

Collection and processing of the explants

Newly grown shoot segments with 6-8 nodal segments were collected during morning hours of March from experimental field station of Karnataka State Forest Department Nallal, Bangalore

↓

Shoots were cut into 2.5-3.0 cm long nodal segments (explants)

↓

Explants were washed under tap water for half an hour

↓

All subsequent operations were carried out inside a laminar air-flow cabinet

↓ 15 min

liquid detergent solution Tween-80 (0.1% v/v)

Washed 3-4 ↓ 10 min

0.1% (w/v) solution of Bavistin (systemic fungicide)

Collection and processing of the explants contd….

Washed 3-4 ↓ 50 seconds

Surface sterilized with a quick dip in 70 %(v/v) ethanol 50 seconds

Washed 3-4 ↓ 8-10 minutes

0.1% (w/v) $Hgcl_2$ based on an explants used

Washed 6-7 times ↓

Exposed end of explants on either side were trimmed aseptically and then inoculated vertically on shoot induction medium

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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]
0.5	0.1	-	90.00	1.10±0.10[b]
0.5	0.25	-	95.00	2.80±0.80[a]
0.5		0.1	82.42	1.53±0.06[b]
0.5		0.25	75.43	1.20±0.20[b]

Shoot multiplication

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)
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]

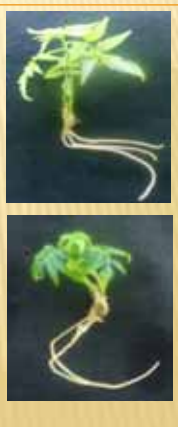
Effect of GA_3 on Shoot elongation

T. No.	Treatments	% of multiple shoot production	Shoot no /shoot clump	Shoot length (in cm)	Remarks
T1	BAP, 0.5 + GA_3, 1.5	86.03[d]	9.61[c]	2.44[d]	Healthy shoot
T2	BAP, 0.5 + GA_3, 2.5	94.00[b]	11.33[b]	4.43[b]	Healthy shoot

In vitro rooting

Rooting response of shoots in MS/2 medium supplemented with different concentration of IBA after 4 weeks

Treatments	% root induction	Average Root length (cm)
1/2MS+ IBA 0.1	76	3.23±0.12[d]
1/2MS + IBA 0.3	90	3.6±0.48[c]
1/2MS + IBA 0.5	98	5.6±0.48[a]
1/2MS + IBA 1.0	85	4.1±0.48[b]



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Hardening

Hardening of rooted shoots was found essential for 4 weeks in polytunnel in green house and four weeks inside the green house without polytunnel, before keeping in shade house for one month to obtain high rate (> 95%) of survival of the micropropagated plants.

HARDENING OF MICROPROPAGATED PLANTS

Direct Organogenesis by using leaf explant on MS medium supplemented with BAP (1.0 mg/l) + Kn (0.1 mg/l) + AS (3.0 mg/l)

Microscopic view

Direct Organogenesis from leaf explant

ACKNOWLEDGEMENT

Authors are thankful to ICFRE, Dehradun and IWST, Bangalore for providing financial assistance and Karnataka State Forest Department for providing plant material.

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Growth performance of industrially important bamboo species in two different agro climatic conditions

Chethan, K S

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Bangalore-560 003

Why bamboo?

- Cheap
- Renewable
- Fast growing
- Short rotation
- Wide adaptation
- Grow in poor soil and low rainfall
- Rehabilitation of degraded land
- High social, economic & environmental values
- Substitute of timber
- Requires less energy for processing

Major uses of bamboo

- Pulp, paper and rayon (major industrial uses)
- Agriculture & Handicrafts: Bamboo baskets, stacking material, agriculture implements and structural material.
- Bamboo houses, disaster resistant bamboo buildings, walling, roofing and structural material
- Sericulture, Fisheries, Medicinal
- Bamboo seeds and shoots used as food and leaf as fodder
- **Panels as substitute of traditional timber spe:** Plywood, Particle Board, Hard board, Medium Density Fiber board
- **Other import uses:** Carbon sequestration, checking soil erosion, water conservation, wind barrier, bio-fencing, restoration of degraded land, important species in social forestry and agroforestry

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BIO-RESOURCE OF BAMBOO (selected countries)

Country	Number of Genera	Number of Species
Global	**75**	**1250**
Asia	**65**	**900**
China	**26**	**300**
Malaysia	10	44
India	**23**	**130**
Thailand	12	41
Vietnam	16	92
Indonesia	-	65
Nepal	11	53
Bangladesh	8	20
Sri lanka	7	1

Flowering and regeneration

Flowering : Sporadic / Gregarious

Flowering cycle : 3-120 years

Natural regeneration : Seed & rhizome

Vegetative regeneration : Culm , branch, rhizomatous cutting

Micropropagation : **Axillary shoot proliferation** and **Somatic embryogenesis**

Limitations of traditional methods

Seed base

- Lack of seed availability
- Short viability period
- Exhibit variation in progenies

Cutting base

- Bulk requirement of source material
- Availability of right stage of material for a limited period of time
- Low production potential

Selected Bamboo species for the field trials

1. *Bambusa balcooa*
2. *B. nutans*
3. *Dendrocalamus asper*
4. *D. stocksii*
5. *D. hamiltonii*
6. *Guadua angustifolia*

METHODOLOGY

- *Bambusa nutans, Dendrocalamus asper, D. stocksii* and *Guadua angustifolia* raised in Tissue culture lab, IWST, Banglore. Whereas *Bambusa balcooa* and *Dendrocalamus hamiltonii* were outsourced from Growmore Biotech, Hosur (T.N.) and IHBT, Palampur (H.P.), respectively.
- 5-6 months old hardened plant with 25-35 cm in height and 2.0 to 3.0 number of tiller were used for the field trials.
- Site preparation was carried out during June, 2007. Pit size of 1cum was made at spacing of 5x5m.

- At the time planting 10kg FYM + 100g neem cake + 50g SSP were used in each pit.
- Planting was carried out during August 2007 at Chintalpuddi, Eluru, AP and at Navtoor, Shimoga, Karnatka.
- After planting, 0.1% (v/v) chloropyrophos solution was applied in each pit as prophylactic measures.
- Weeding, soil working and watering operations were done as and when required.
- Growth parameters such as survival rate, height (cm) and culm number were recorded at six months intervals.

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Experimental Details

Spacing = 5x5m
Treatment = 6
Replication = 3 (20 plants/rep)
No. of plants/species = 60
Date of start = July-August, 2007
Design = Randomized Block Design (RBD)
Area = 10 ha

R 1	R 2	R 3
A	C	F
B	D	E
C	E	D
D	F	C
E	A	B
F	B	A

Treatments:

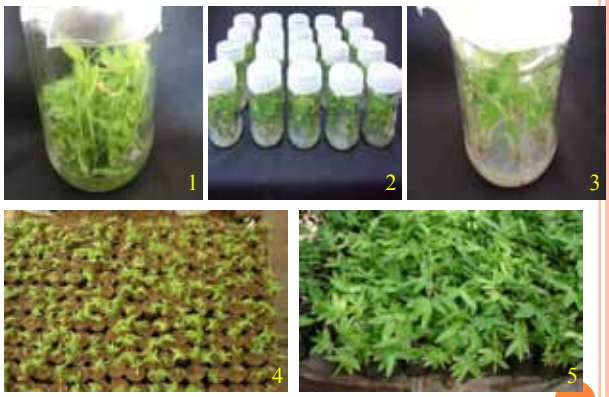
A= B. balcooa
B= B. nutans
C= Dendrocalamus asper
D= D. stocksii
E= D. hamiltonii
F= Guadua angustifolia

STUDY SITES

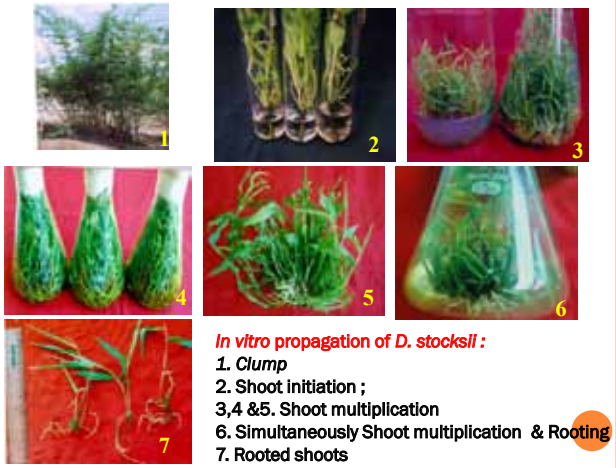
Climate data

Locations	Geographical location	Altitude (m)	Rain fall (mm)	Temperature (° C)
Shimoga, Karnataka	14° 03' 25.77" N 75° 22.41' 87" E	2410	2848	Maximum: 33 Minimum: 13
Chintalapudi, Andhra Pradesh	17° 19.66' 01" N 80° 98.33' 01" E	482	858	Maximum: 48 Minimum: 17.7

B. nutans: 1. Shoot initiation; 2 &3. Shoot multiplication; 4. *In vitro* rooted shoots; 5. Hardening of plantlets



D. asper : 1.Shoot multiplication;2&3. Rooted shoots; 4. Hardening stage; 5. Hardened plants



In vitro propagation of *D. stocksii* :
1. Clump
2. Shoot initiation ;
3,4 &5. Shoot multiplication
6. Simultaneously Shoot multiplication & Rooting
7. Rooted shoots

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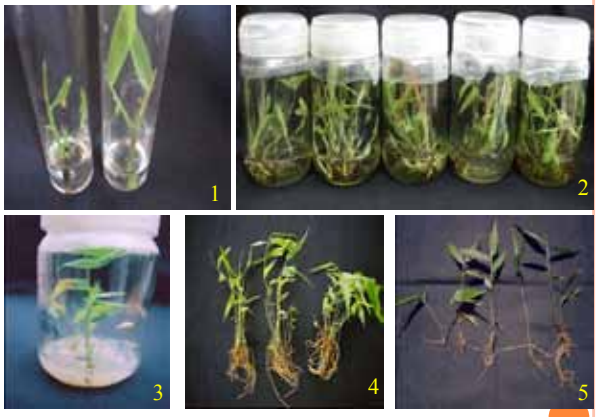

G. angustifolia: 1.Shoot initiation; 2.Shoot multiplication 3&4.*In vitro* rooted shoots ; 5.*Ex vitro* rooted shoots

Ex vitro rooting & hardening in Green house

Hardening of *D.asper* in Green house

Primary harden plants of *B.nutans under shade*

Harden plants of *G. aungustifolia*

Micropropagated bare root plants for field trials ; 1.*B. balcooa* 2. *D. asper* 3. *D. hamiltonii* 4. *G. aungustifolia* 5. *D. stocksii* and 6. *B. nutans*

Results

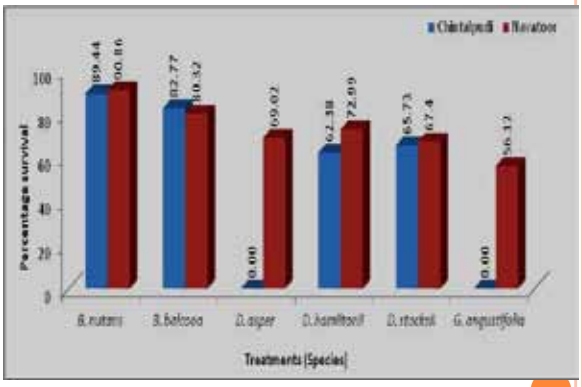

Fig 1. Survival percentage of various species at Chintalpudi, Andhra Pradesh and Navatoor, Karnataka after 30 months.

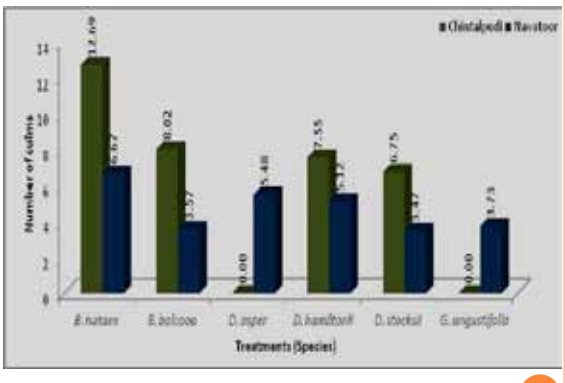

Fig 2. Number of culms in various species at Chintalpudi, Andhra Pradesh and Navatoor, Karnataka after 30 months.

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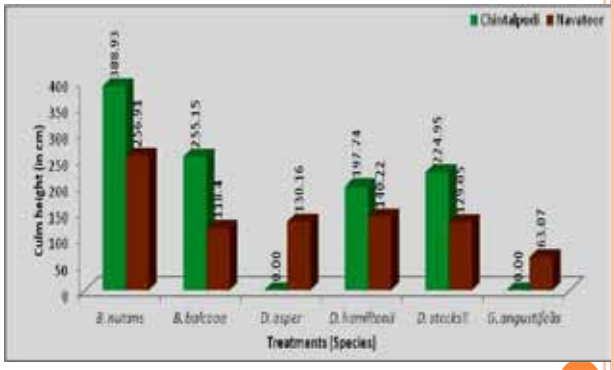

Fig 3. Culm height (in cm) in various species at Chintalpudi, Andhra Pradesh and Navatoor, Karnataka after 30 months.

30 month old plants of 1) *Dendrocalamus stocksii,* 2) *D. hamiltonii,* 3) *Bambusa balcooa* & 4) *B. nutans* at Chintalapudi, Andhra Pradesh.

Overview of plantation at Chintalapudi, Andhra Pradesh

30 month old plants of A) *B. balcooa*, B) *B. nutans*, C) *D. asper*, D) *D. hamiltonii*, E) *D. stocksii* and F) *G. angustifolia* at Navatoor, Karnataka

CONCLUSIONS

- Observation showed that *B. balcooa* and *B. nutans* were the two most suited species followed by *D. hamiltonii* and *D. stocksii* for these areas in terms of initial survival and subsequent growth.
- Whereas both excotic species (*D. asper* and *G. angustifolia*) are not suited in Chintalapudi, AP because of the fact that, they are prone to termite attack and need intensive management.

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ACKNOWLEDGMENTS

- DBT, Govt., India for funding
- Director, IWST, Bangalore
- IHBT, Palampur, HP
- APFDC and KFD

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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 a multipurpose and economically important tree.
- Wood is strong & durable equivalent to teak.
- Also used in Ayurvedic medicines.
- It is threatened due to overexploitation.
- Large genetic variation among the trees population.
- Clonal propagation for higher potential of genetic gain and genetic uniformity.

METHODOLOGY FOR MICROPROPAGATION:

EXPLANT COLLECTION & STERILIZATION:

- 10-15 year old healthy trees of *T. undulata* were selected from AFRI field. Nodal part used as explant were thoroughly washed with tap water followed by treating with 2-3 drops of detergent Tween-80 followed by treatment 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.

INOCULATION & SUBCULTURING:

- The explants were inoculated on Murashige and Skoog (MS) The regenerated shoots were excised and inoculated in to the subculturing medium. The healthy shoot cultures were maintained by repeated subculturing of the stock after 3-4 weeks on fresh MS medium.

IN VITRO ROOTING:

- Elongated Shoots of (3-5cm) length were isolated from shoot multiplication cultures and used for *in vitro* rooting.
- To initiate rooting two step procedures was adopted. In the first step the microshoots were given treatment of autoclaved IBA and NAA (100 mg/l) solution for 15 minutes and then transferred to the hormone free medium.

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METHODOLOGY FOR MACROPROPAGATION:

COLLECTION AND PREPARATION OF CUTTINGS:

- 15-16 year trees were selected and coppiced, pollard and lopped. Stem cuttings were collected from coppiced, pollard and lopped branches. The branches were cut into approximately 8.0-12.0 cm long shoot segments with 3-5 nodes.

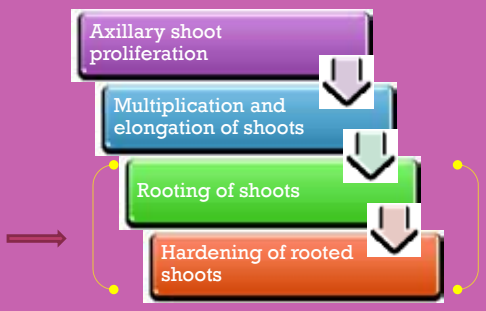
TREATMENT AND POTTING MIXTURE:

- The lower portions of stem cuttings were treated with auxins. Upper portions of cutting were covered with choupatia paste. in the ratio 2:1:1 to prevent fungal attack.
- A mixture of sand: compost (3:1) ratio was filled in root trainers Hyko trays. These root trainers were placed in a raised platform inside polyhouse/mist chamber.

ENVIRONMENTAL CONDITIONS:

- Average temperature and relative humidity maintained in polyhouse was 30±2 °C and 70±5% respectively. 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.

STAGES OF MICROPROPAGATION



PREVIOUS WORK ON MACRO AND MICROPROPAGATION:

- No work was available on macropropagation of *T. undulata.*
- Work has been done by various workers on micropropagation of *T. undulata.* The previous work was taken as base for further studies.
- Experiments have been done to get more repeatable statistically sound data.

RESULTS (MICROPROPAGATION):

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

Months	Explant number	Responding explant % ± SE	Bud length ± SE
January-February	30	75^{c} ± 7.8	4.0^{d} ± 0.4
March-April	29	40^{ab} ± 9.3	2.0^{c} ± 0.5
May-June	27	43^{ab} ± 9.7	1.7^{bc} ± 0.4
July-August	31	23^{a} ± 7.6	0.5^{a} ± 0.1
September-October	28	27^{a} ± 8.7	0.6^{ab} ± 0.2
November-December	26	63^{bc} ± 9.8	3.4^{d} ± 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} ± 0.3	8^{a} ± 1	++
MS + BA (1 mg/l)	15	1.8^{a} ± 0.1	22^{b} ± 2.1	+++
MS + BA (2 mg/l)	17	2.6^{b} ± 0.3	15^{c} ± 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

Two step procedure.-15 minute treatment of different auxins for *in vitro* rooting.

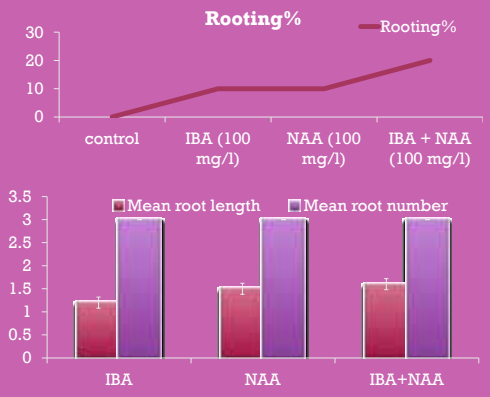


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Table 3. Rooting response of *Tecomella undulata* shoots on different media after treatment of IBA + NAA solution (100 mg/l) for 15 minutes.

Treatment	Explant number	Rooting%	Root length Mean ± SE	Root number Mean ± SE
½ MS	23	17.4[a]	3.2[a] ± 0.8	2.8 ± 0.8
½ B5	25	43.4[b]	2.8[a] ± 0.4	3.1 ± 0.3
½ WPM	23	4.3 [a]	0.5[b] ± 0	1.0 ± 0
Hoagland	23	4.3 [a]	3.6 [a] ± 0.1	1.3 ± 0.3

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

STAGES OF MICROPROPAGATION

RESULTS (MACROPROPAGATION):

EFFECT OF DIFFERENT SEASON ON SPROUTING & ROOTING RESPONSE OF STEM CUTTINGS

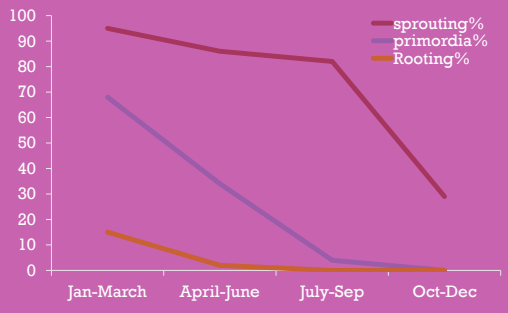

Table4. Effect of stem cutting collected from different trees on sprouting and rooting response.

Different genotype	Number of Cuttings	Sprouting % ±SE	Primordia % ±SE	Rooting%
Tree No 09	135	99[b] ± 7.4	48.1[b] ± 4.3	10.37[b]
Tree No 12	135	93[a] ± 2.2	7.4[a] ± 2.2	0.74[a]
Tree No 17	135	92[a] ± 2.3	4.4[a] ±1.8	0.74[a]
Tree No 21	135	97[ab] ± 1.4	5.9[a] ± 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 5. 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] ± 6.7	13.3[ab] ± 9	0[a]	6.7
Middle crown	45	33.3[b] ± 12	13.3[ab] ± 9	6.7[a] ± 6.7	17.8
Bottom crown	45	0[a]	20[ab] ± 10	0[a]	6.7

STAGES OF MACROPROPAGATION

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CONCLUSIONS:

❑ Season plays important role in *in vitro* shoot establishment. The cultures can be raised through out the year but maximum response was in winters for this tree species.

❑ Rooting is very difficult in this species but we have achieved a little success.

❑ Macropropagation of *T. undulata* is possible from mature tree & rooting in stem cuttings is also influenced by season.

❑ Individual genotype show different rooting response. The branch position of stem cuttings also influences the rooting response as the cuttings taken from the middle crown position had the best rooting percentage.

❑ The research emphasis is needed to improve rooting and hardening success by understanding more factors influencing the stages of micro and macropropagation.

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TISSUE CULTURE METHOD FOR MULTIPLICATION OF FRI HYBRIDS OF EUCALYPTUS AND THEIR FIELD TRIALS

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NEED OF FRI HYBRID TISSUE CULTURE

- Yield of hybrid is 3-5 times more for biomass production
- Represents characteristics of both the parents
- Superior in growth parameters i.e shows positive hybrid vigourness
- Need to capture the hybrid vigoursity in true sense
- Multiplication through seeds results segregation of characters

Problems associated with conventional propagation of Eucalyptus hybrids

- ✓ Limited number of hybrids are available.
- ✓ Difficult rooting of cutting as these hybrids are 28-30 years old.
- ✓ Multiplication by seeds - F_2 generation show a lot of segregation.
- ✓ Thus conventional methods are not possible for its large scale multiplication.

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Develop Tissue Culture Protocol for Rapid Mass Multiplication of Eucalyptus Hybrids

FRI-5 (*E.camaldulensis x E.terticornis*)

FRI-10 (E. grandis x E. teriticornis)

FRI-13 (E. camaldulensis x E.teriticornis x E.grandis)
FRI-14 (E. *toerelliana x E. citriodora*)
FRI-15 (E. citriodora x E. torelliana)

Methodology

The plant material of this study was collected from Eucalyptus hybrids planted in experimental area of FRI campus, Dehradun.

Collection of Explant

Axillary buds were collected from 28-30 years old tree of Eucalyptus hybrid of FRI-10.

Surface sterilization

Different surface sterilizing agents like $HgCl_2$, NaOCl and H_2O_2 were used for surface sterilization of explant and followed by 3-5 times washing with autoclaved distilled water.

0.1% $HgCl_2$ for 10 minutes in FRI- 10 gave maximum 62.09% aseptic cultures.

Mother plant of F1 hybrid of Eucalyptus FRI -10

Axillary bud induction on MS medium supplemented with Kn

Effect of cytokinin (BAP) in MS medium on axillary bud induction using nodal segments of FRI-14.(*E. toerelliana x E. citriodora*) Data was recorded after 5 weeks.

BAP (mg/l)	Response %	Mean shoot number	Mean shoot length (cm)
Control	8.33 ± 0.012	0.30 ± 0.02	0.23 ± 0.17
0.1	12.50 ± 0.006	0.80 ± 0.30	0.43 ± 0.16
0.5	45.83 ± 0.006	1.80 ± 0.30	0.85 ± 0.03
1.0	65.00 ± 0.029	4.30 ± 0.50	0.88 ± 0.06
1.5	55.00 ± 0.029	2.70 ± 0.30	0.68 ± 0.03
2.0	41.66 ± 0.006	1.80 ± 0.30	0.76 ± 0.05
2.5	25.00 ± 0.029	1.50 ± 0.02	0.78 ± 0.07
3.0	16.66 ± 0.006	1.20 ± 0.03	0.58 ± 0.13
Significance	***	***	***
CD at 5%	0.05	0.92	0.29

Effect of Kn in MS medium on axillary bud induction using nodal segments of FRI-14. Data recorded after 5 weeks.

Kn (mg/l)	Response %	Mean shoot number	Mean shoot length (cm)
Control	4.16 ± 0.01	0.17 ± 0.16	0.08 ± 0.08
0.1	5.14 ± 0.02	0.83 ± 0.31	0.53 ± 0.17
0.5	20.83 ± 0.05	1.83 ± 0.30	0.68 ± 0.07
1.0	33.33 ± 0.07	1.82 ± 0.31	0.63 ± 0.04
1.5	58.33 ± 0.01	2.17 ± 0.33	0.79 ± 0.05
2.0	33.35 ± 0.01	1.50 ± 0.22	0.73 ± 0.07
2.5	20.83 ± 0.02	1.33 ± 0.21	0.75 ± 0.06
3.0	12.50 ± 0.03	1.32 ± 0.21	0.57 ± 0.04
Significance	***	***	***
CD at 5%	0.02	0.74	0.24

*- Significance at 5% **- Significance at 1% ***-Significance at 0.1%

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Effect of combination of cytokinin and auxin (BAP+NAA) in MS medium on axillary bud induction using nodal segments of FRI-10. Data recorded after 5 weeks.

(BAP + NAA) (mg/l)	Axillary buds inoculated	Response %	Mean shoot number
0.1+ 0.1	24	0.00 ± 0.00	0.00 ± 0.00
0.1 + 0.5	24	0.00 ± 0.00	0.00 ± 0.00
0.1 + 1.0	24	0.00 ± 0.00	0.00 ± 0.00
0.1 + 1.5	24	0.00 ± 0.00	0.00 ± 0.00
0.5 + 0.1	24	8.33 ± 2.41	2.00 ± 0.58
0.5 + 0.5	24	14.67 ± 4.17	2.56 ± 1.00
0.5 + 1.0	24	12.50 ± 4.16	2.00 ± 1.00
0.5 + 1.5	24	13.67 ± 4.18	2.35 ± 0.99
1.0 + 0.1	24	8.33 ± 3.67	2.00 ± 1.15
1.0 + 0.5	24	39.33 ± 2.41	4.00 ± 1.53
1.0 + 1.0	24	12.50 ± 2.78	3.00 ± 0.58
1.0 + 1.5	24	16.67 ± 2.41	3.00 ± 2.31
1.5 + 0.1	24	9.72 ± 3.67	2.33 ± 0.88
1.5 + 0.5	24	8.33 ± 2.41	2.00 ± 0.58
1.5 + 1.0	24	10.11 ± 2.78	2.67 ± 0.67
1.5 + 1.5	24	4.17 ± 2.41	1.00 ± 0.58
Significance		NS	**
CD at 5%			1.93

Axillary bud induction on MS medium supplemented with BAP+NAA in FRI-10

IN VITRO SHOOT MULTIPLICATION OF FRI-14

Effect of cytokinin (BAP) in MS medium on shoot multiplication. Data was recorded after 5 weeks.

BAP (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	12.25 ± 0.84	0.73 ± 0.02	2.04 ± 0.14
0.5	40.42 ± 1.48	1.15 ± 0.01	6.37 ± 0.25
1.0	62.67 ± 1.08	1.30 ± 0.12	10.54 ± 0.18
1.5	40.58 ± 0.98	0.92 ± 0.05	8.76 ± 0.16
2.0	30.92 ± 0.81	1.01 ± 0.09	6.15 ± 0.14
2.5	26.75 ± 1.75	0.88 ± 0.10	5.56 ± 0.29
3.0	25.08 ± 1.52	0.79 ± 0.05	4.18 ± 0.25
Significance	***	***	***
CD at 5%	3.38	0.21	0.58

In vitro shoot multiplication on MS medium supplemented with BAP in FRI-10

Optimal *in vitro* shoot multiplication on MS medium supplemented with 1.0mg/l BAP of FRI-14

Effect of Kn in MS medium on shoot multiplication. Data was recorded after 5 weeks

Kn (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	9.50 ± 1.56	0.45 ± 0.05	1.58 ± 0.24
0.5	27.17 ± 1.77	0.65 ± 0.07	4.53 ± 0.30
1.0	39.67 ± 1.49	1.00 ± 0.12	6.61 ± 0.25
1.5	27.92 ± 1.76	0.74 ± 0.12	4.65 ± 0.29
2.0	24.58 ± 2.34	0.73 ± 0.09	4.10 ± 0.39
2.5	23.67 ± 1.92	0.72 ± 0.12	3.94 ± 0.32
Significance	***	NS	***
CD at 5%	5.25		0.89

*- Significance at 5% **- Significance at 1% ***-Significance at 0.1%

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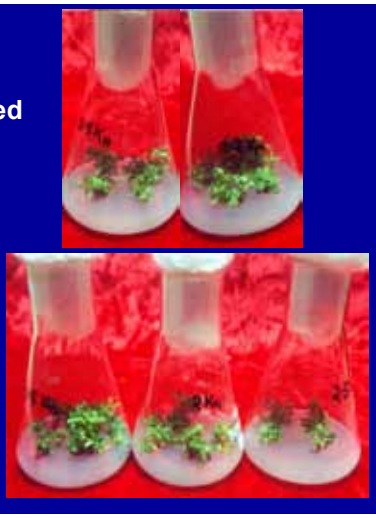
***In vitro* shoot multiplication on MS medium supplemented with Kn**



Effect of hormonal interaction (BAP + NAA) was studied for *in vitro* shoot multiplication. Data was recorded after 5 weeks.

(BAP + NAA) (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
1.0 BAP + 0.1NAA	35.42 ± 1.74	0.66 ± 0.05	5.90 ± 0.29
1.0 BAP + 0.5NAA	46.58 ± 2.02	0.70 ± 0.07	7.76 ± 0.34
1.0 BAP + 1.0NAA	28.08 ± 1.18	0.61 ± 0.09	4.68 ± 0.20
1.0 BAP + 1.5NAA	22.75 ± 1.24	0.65 ± 0.06	3.79 ± 0.21
1.0 BAP + 2.0NAA	20.17 ± 0.91	0.53 ± 0.08	3.36 ± 0.15
Significance	***	NS	***
CD at 5%	4.10		0.70

Effect of different basal media and their strength supplemented with 1.0mg/l BAP for shoot multiplication of FRI-10.

Media	Mean shoot number	Mean shoot length (cm)	Multiplication rate
MS - 2 x	30.58 ± 2.31	0.87 ± 0.06	5.10 ± 0.39
1 x	55.58 ± 2.85	1.80 ± 0.05	9.26± 0.48
½ x	24.75 ± 2.85	1.24 ± 0.11	4.13 ± 0.48
¼ x	10.92 ± 0.99	0.75 ± 0.07	1.82 ± 0.16
B_5 - 2 x	26.92 ± 2.99	0.60 ± 0.05	4.49 ± 0.50
1 x	14.25 ± 1.08	0.82 ± 0.07	2.38 ± 0.18
½ x	10.67 ± 1.55	0.52 ± 0.03	1.78 ± 0.26
¼ x	7.25 ± 0.76	0.39 ± 0.05	1.21 ± 0.13
WPM - 2 x	40.33 ± 1.21	0.65 ± 0.04	6.72 ± 0.20
1 x	29.67 ± 0.76	0.48 ± 0.04	4.94 ± 0.13
½ x	11.92 ± 1.41	0.49 ± 0.03	1.99 ± 0.23
¼ x	6.83 ± 0.75	0.37 ± 0.02	1.14 ± 0.12
Significance	***	***	***
CD at 5%	5.10	0.18	0.86

Effect of sucrose concentration on *in vitro* shoot multiplication of FRI-10. Shoots were cultured on MS + 1.0 mg/l BAP.

Sucrose concentration	Mean shoot number	Mean shoot length (cm)	Multiplication rate
0 %	10.67 ± 0.69	0.52 ± 0.06	1.78 ± 0.11
1 %	21.00 ± 1.85	0.75 ± 0.04	3.50 ± 0.31
2 %	36.00 ± 1.88	0.83 ± 0.08	6.00 ± 0.31
3 %	59.67 ± 2.44	1.75 ± 0.11	9.94 ± 0.41
4 %	43.83 ± 1.77	0.80± 0.03	7.31 ± 0.30
5 %	39.75 ± 2.15	0.65 ± 0.06	6.63 ± 0.36
6 %	35.08 ± 1.83	0.55 ± 0.05	6.00 ± 0.33
Significance	***	***	***
CD at 5%	5.06	0.22	0.90

Effect of shoot multiplication rate on liquid and semi solid medium. Shoots were cultured on MS +1.0mg/l BAP

Media	Mean shoot number	Mean shoot length (cm)	Multiplication rate
1.0mg/l BAP (Liquid)	53.75 ± 1.41	0.96 ± 0.03	8.96 ± 0.21
1.0 mg/l BAP (Semi solid)	59.52 ± 1.56	1.73 ± 0.45	9.92 ± 0.37
Significance	***	***	***
CD at 5%	1.32	0.87	0.69

Comparision of shoot multiplication rate on semi solid and liquid medium.

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Effect of no. of shoots in a propagule for *in vitro* shoot multiplication. Shoots were cultured on MS+1.0 mg/l BAP.

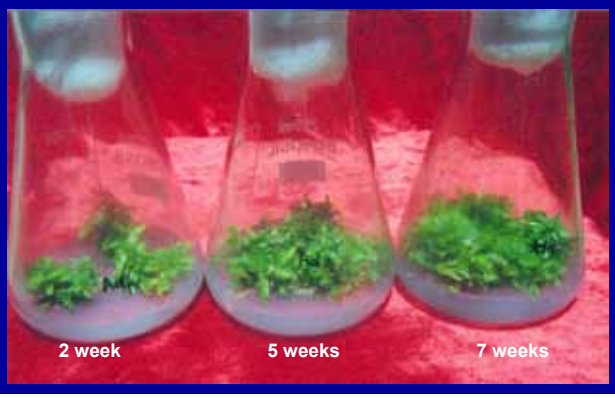
No. of shoots in a propagule	Mean shoot number	Mean shoot length (cm)	Multiplication rate
1	3.33 ± 1.02	0.66 ± 0.03	0.56 ± 0.17
2	9.83 ± 1.30	0.82 ± 0.04	1.64 ± 0.22
3	17.67 ± 1.94	0.91 ± 0.06	2.94 ± 0.32
4	25.50 ± 1.88	0.96 ± 0.01	4.25 ± 0.31
5	50.50 ± 1.95	1.12 ± 0.06	8.24 ± 0.32
6	57.50 ± 1.23	1.14 ± 0.01	9.58 ± 0.21
7	71.50 ± 2.22	1.36 ± 0.04	11.92 ± 0.37
Significance	***	***	***
CD at 5%	4.81	0.12	0.82

Effect of no. of shoots in a propagule for *in vitro* shoot multiplication on MS medium with 1.0mg/l BAP.

Effect of subculture duration on *in vitro* shoot multiplication of FRI-10. Shoots were cultured on MS + 1.0mg/l BAP.

Subculture duration	Mean shoot number	Mean shoot Length (cm)	Multiplication rate
1 Weeks	9.50 ± 0.99	0.76 ± 0.07	1.58 ± 0.17
2 Weeks	18.80 ± 1.64	0.74 ± 0.08	3.14 ± 0.27
4 Weeks	50.30 ± 1.65	1.12 ± 0.10	8.39 ± 0.26
5 Weeks	54.68 ± 2.43	1.68 ± 0.04	9.11 ± 0.41
7 Weeks	62.80 ± 2.04	1.19 ± 0.07	10.47 ± 0.34
Significance	***	***	***
CD at 5%	5.44	0.23	0.88

Effect of subculture duration for *in vitro* shoot multiplication in FRI-10



Effect of myo-inositol concentration on shoot multiplication of FRI-10. Shoots were cultured on MS +1.0mg/l BAP.

Myo-inositol conc. (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	8.50 ± 1.26	0.79 ± 0.07	1.42 ± 0.21
50	30.70 ± 0.88	0.79 ± 0.06	5.11 ± 0.15
100	53.00 ± 1.83	1.70 ± 0.18	8.83 ± 0.30
150	41.50 ± 0.85	1.38 ± 0.19	6.92 ± 0.14
200	29.20 ± 0.95	0.81 ± 0.08	4.88 ± 0.16
Significance	***	***	***
CD at 5%	3.62	0.38	0.58

Effect of adenine sulphate on shoot multiplication of FRI-10. Shoots were cultured on MS + 1.0mg/l BAP

Adenine sulphate conc. (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	48.70 ± 1.02	0.84 ± 0.03	8.11 ± 0.17
25	51.50 ± 0.92	0.89 ± 0.07	8.58 ± 0.15
50	86.52 ± 1.26	1.35 ± 0.08	14.42 ± 0.21
75	97.98 ± 0.77	1.31 ± 0.12	16.33 ± 0.13
100	103.98 ± 0.70	1.28 ± 0.12	17.64 ± 0.12
Significance	***	***	***
CD at 5%	2.87	0.27	0.46

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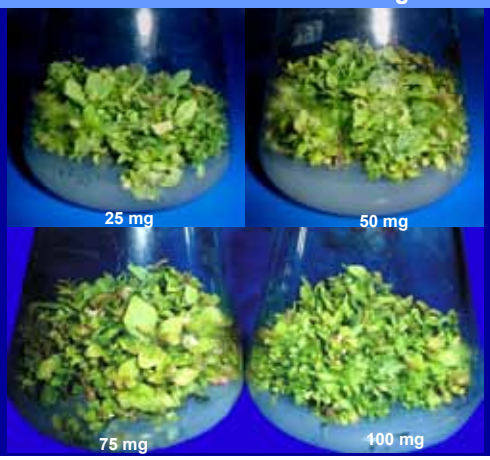
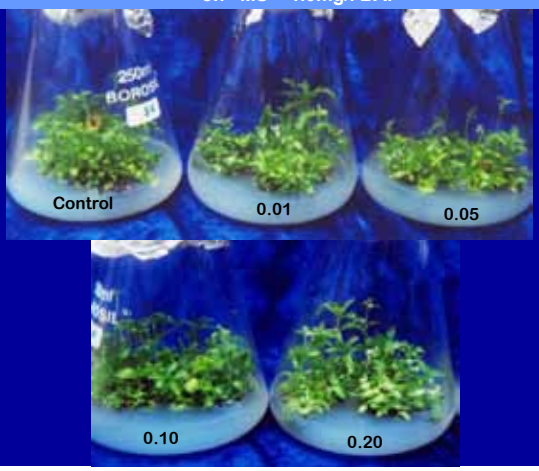
Effect of adenine sulphate on shoot multiplication of FRI-10. Shoots were cultured on MS+1.0mg/l BAP



Effect of GA_3 on *in vitro* shoot elongation of FRI-10. Shoots were cultured on MS + 1.0mg/l BAP

GA_3 conc. (mg/l)	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	21.00 ± 1.53	1.06 ± 0.08	2.50 ± 0.25
0.01	22.70 ± 1.71	1.14 ± 0.05	2.78 ± 0.28
0.05	22.50 ± 1.12	1.91 ± 0.13	3.75 ± 0.19
0.10	23.00 ± 1.59	2.24 ± 0.14	5.83 ± 0.27
0.20	18.30 ± 1.41	1.93 ± 0.07	3.05 ± 0.23
Significance	**	***	***
CD at 5%	3.71	0.31	0.77

Effect of GA3 on shoot elongation of FRI-10. Shoots were cultured on MS + 1.0mg/l BAP



In vitro rooting in FRI hybrids

In vitro rooting was obtained on ½ MS medium supplemented with auxins (IBA, NAA and IAA) were tried for *in vitro* rooting.

Effect of IBA in ½ MS medium on *in vitro* rooting of FRI-10. Data was recorded after 5 weeks

IBA (mg/l)	Rooting %	Mean root number	Mean root length (cm)
Control	12.50 ± 2.42	1.62 ± 0.24	0.82 ± 0.11
0.1	20.83 ± 2.38	9.00 ± 0.70	0.83 ± 0.12
0.5	62.50 ± 2.40	16.40 ± 1.03	1.05 ± 0.14
1.0	65.17 ± 2.39	19.38 ± 1.03	1.28 ± 0.14
1.5	85.67 ± 2.42	20.21 ± 0.86	2.20 ± 0.18
2.0	55.83 ± 2.40	11.00 ± 0.70	1.72 ± 0.16
Significance	***	***	***
CD at 5%	7.28	3.04	0.41

In vitro rooting on ½ MS medium with IBA

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Optimal *in vitro* rooting on ½ MS medium with 1.5mg/l IBA

Effect of NAA in ½ MS medium on *in vitro* rooting of FRI-10. Data was recorded after 5 weeks

NAA (mg/l)	Rooting %	Mean root number	Mean root length (cm)
Control	11.67 ± 1.86	1.60 ± 0.52	0.66± 0.34
0.1	20.83 ± 1.92	3.40 ± 0.97	0.69 ± 0.35
0.5	50.00 ± 1.86	7.00 ± 0.91	1.44 ± 0.10
1.0	75.00 ± 2.01	15.60 ± 0.67	1.50 ± 0.10
1.5	65.00 ± 2.02	12.00 ± 1.82	1.44 ± 0.25
2.0	52.50 ± 1.86	8.80 ± 0.75	1.10 ± 0.12
Significance	***	***	***
CD at 5%	6.21	2.21	0.25

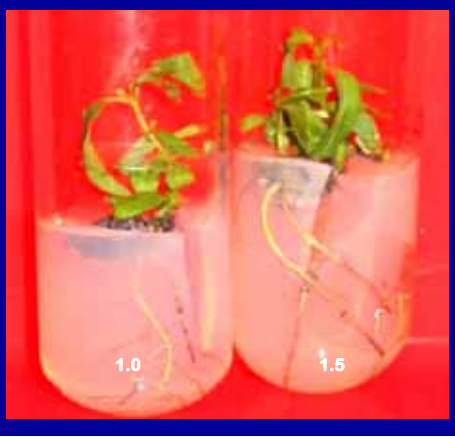
***In vitro* rooting on ½ MS medium supplemented with NAA**

Effect of IAA in ½ MS medium on *in vitro* rooting of FRI-10. Data was recorded after 5 weeks

IAA (mg/l)	Rooting %	Mean root number	Mean root length (cm)
Control	1.39 ± 1.86	0.85 ± 0.34	0.98 ± 0.06
0.1	8.33 ± 1.92	1.00 ± 0.44	0.20 ± 0.05
0.5	12.50 ± 1.86	1.30 ± 0.68	1.44 ± 0.04
1.0	20.83 ± 2.01	2.00 ± 0.45	1.44 ± 0.02
1.5	25.17 ± 2.02	3.00 ± 0.43	1.50 ± 0.04
2.0	16.67 ± 1.86	1.00 ± 0.46	1.10 ± 0.05
Significance	**	NS	**
CD at 5%	11.01		0.14

*- Significance at 5%
NS- Non significant
**- Significance at 1%
***- Significance at 0.1%

***In vitro* rooting on ½ MS medium supplemented with IAA**



HARDENING AND ACCLIMATIZATION

In vitro rooted plantlets need to be hardened and acclimatized before their field transplantation. All attempts were made for direct transfer of tissue culture raised plantlets in the field failed. They can not withstand the environmental conditions without proper hardening and acclimatization.

- ✓ *In vitro* rooted plantlets were transferred to autoclaved culture bottles containing soilrite, supplied with ½ MS medium twice a week.
- ✓ After 2 weeks hardened plantlets transferred to mist chamber for 3 weeks and then transferred in net house into polybags containing soil, sand and FYM in 1:1:1 ratio.
- ✓ 80-90% hardening and acclimatization was achieved along with 85% field survival rate of T. C. raised plants of FRI-10.

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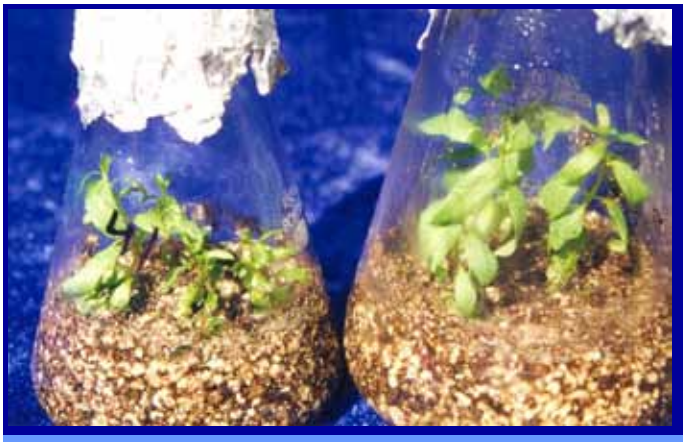

In vitro hardening of T.C. plantlets of FRI- 14 with soilrite in culture room

Acclimatization of Hardened plantlets of FRI-14 in mist chamber

Tissue culture raised plants in polybags

Six months old tissue culture raised plant of FRI-14 in field

One year old tissue culture raised plantation of FRI-15 (*E. citriodora X E. toerelliana*)

In vitro propagation of FRI-13

FRI-13 (Trihybrid)

(*E. camaldulensis* x *E. tereticornis*) x *E. grandis*

FRI-13 is the only tri hybrid available in India and is widely adaptable and drought resistant hybrid.

Planted in central nursery of FRI campus, Dehradun

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Mother plant of Trihybrid of Eucalyptus FRI -13

Axillary bud induction in FRI-13

Effect of BAP in MS medium on axillary bud induction using nodal segments of FRI-13.

BAP (mg/l)	Response %	Mean shoot number	Mean shoot length (cm)
Control	5.56 ± 3.67	0.33 ± 0.02	0.14 ± 0.17
0.1	12.50 ± 4.14	0.67 ± 0.30	0.24 ± 0.16
0.5	58.33 ± 4.17	1.67 ± 0.30	0.87 ± 0.03
1.0	60.25 ± 4.80	3.50 ± 0.30	0.95 ± 0.03
1.5	62.00 ± 4.81	4.30 ± 0.50	1.10 ± 0.06
2.0	50.00 ± 4.20	1.85 ± 0.30	0.80 ± 0.05
2.5	29.17 ± 2.41	1.80 ± 0.02	0.74 ± 0.07
3.0	16.67 ± 4.16	1.50 ± 0.03	0.70 ± 0.13
Significance	***	***	***
CD at 5%	12.38	1.08	0.24

Axillary bud break in FRI-13

Axillary bud break on MS medium supplemented 1.5 mg/l BAP

Effect of Kn in MS medium on axillary bud induction using nodal segments of FRI-13.

Kn (mg/l)	Response %	Mean shoot number	Mean shoot length (cm)
Control	7.17 ± 2.41	0.20 ± 0.16	0.08 ± 0.08
0.1	8.50 ± 4.17	0.70 ± 0.31	0.37 ± 0.17
0.5	25.00 ± 4.15	1.70 ± 0.30	0.70 ± 0.06
1.0	33.33 ± 2.44	1.65 ± 0.31	0.78 ± 0.08
1.5	56.50 ± 4.82	2.10 ± 0.22	1.09 ± 0.07
2.0	45.83 ± 2.41	1.75 ± 0.33	0.80 ± 0.03
2.5	25.00 ± 4.17	1.30 ± 0.21	0.70 ± 0.06
3.0	12.50 ± 4.17	0.70 ± 0.21	0.50 ± 0.16
Significance	***	**	***
CD at 5%	11.16	0.83	0.29

Axillary bud induction on MS medium supplemented 1.5 mg/l Kn.

IN VITRO SHOOT MULTIPLICATION

Effect of BAP in MS medium on shoot multiplication of FRI-13. Data was recorded after 5 weeks.

BAP (mg/l)	Mean shoot number	Mean shoot Length (cm)	Multiplication rate
Control	12.33 ± 0.78	0.68 ± 0.04	2.06 ± 0.13
0.5	24.08 ± 1.39	1.02 ± 0.09	4.01 ± 0.23
1.0	44.08 ±1.21	1.55 ± 0.03	7.35 ± 0.20
1.5	57.54± 1.61	2.32 ± 0.04	9.59 ± 0.27
2.0	38.22 ± 1.09	2.30 ± 0.08	6.37 ± 0.18
2.5	26.10 ± 1.41	2.00 ± 0.04	4.35 ± 0.23
3.0	20.75 ± 0.62	1.70 ± 0.08	3.43 ± 0.10
Significance	***	***	***
CD at 5%	3.33	0.19	0.55

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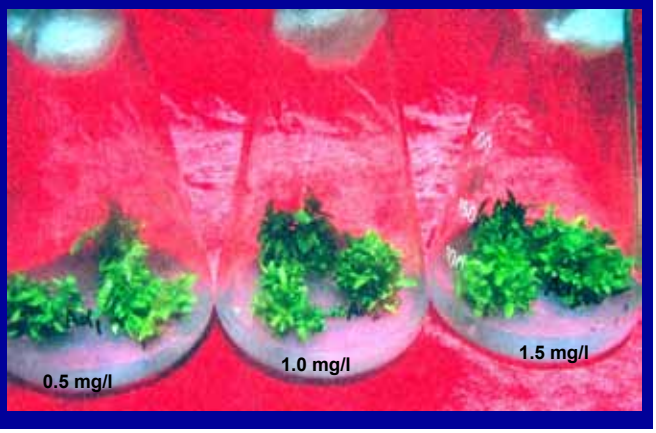
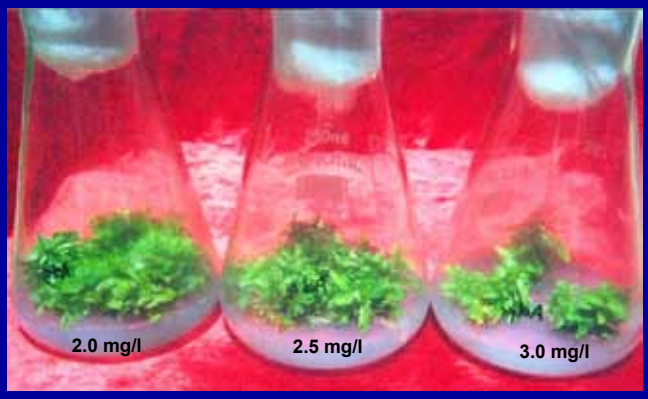
In vitro shoot multiplication on MS medium supplemented with BAP 0.5 to 1.5 mg/l



In vitro shoot multiplication on MS medium supplemented with 2.0 to 3.0 mg/l BAP

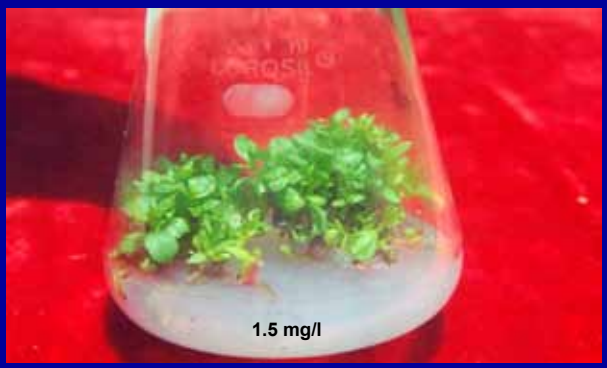


Optimal *in vitro* shoot multiplication on MS medium supplemented with 1.5mg/l BAP in FRI-13

Effect of Kn in MS medium on shoot multiplication of FRI-13. Data was recorded after 5 weeks.

Hormonal conc. Kn (mg/l)	Mean Shoots Produced	Mean Shoot length (cm)	Multiplication Rate
Control	10.33 ± 0.78	0.65 ± 0.06	1.72 ± 0.11
0.5	16.70 ± 1.21	0.98 ± 0.10	2.78 ± 0.20
1.0	27.20 ± 1.69	1.59 ± 0.19	4.53 ± 0.28
1.5	40.80 ± 1.02	2.07 ± 0.10	6.85 ± 0.26
2.0	27.90 ± 1.82	1.48 ± 0.15	4.65 ± 0.30
2.5	26.40 ± 1.26	1.17 ± 0.14	4.40 ± 0.21
3.0	23.70 ± 1.71	0.97 ± 0.15	3.94 ± 0.28
Significance	***	***	***
CD at 5%	4.15	0.45	0.70

In vitro shoot multiplication on MS medium supplemented with 1.5mg/l Kn



Effect of sucrose conc. on *in vitro* shoot multiplication of FRI-13. Shoots were cultured on MS+1.5 mg/l BAP.

Sucrose conc. in %	Mean Shoots Produced	Mean Shoots length (cm)	Multiplication Rate
0 %	9.30 ± 0.71	0.86 ± 0.05	1.54 ± 0.12
1 %	19.10 ± 1.78	1.06 ± 0.03	3.18 ± 0.30
2 %	34.80 ± 1.52	1.23 ± 0.07	5.81 ± 0.25
3 %	49.90 ± 1.16	1.53 ± 0.07	8.32 ± 0.19
4 %	44.30 ± 1.43	1.20 ± 0.03	7.39 ± 0.24
5 %	35.00 ± 1.20	1.08 ± 0.05	5.83 ± 0.20
6 %	19.70 ± 1.37	0.97 ± 0.03	3.28 ± 0.23
Significance	***	***	***
CD at 5%	3.62	0.14	0.62

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Effect of myo-inositol conc. on shoot multiplication of FRI-13. Shoots were cultured on MS +1.5mg/l BAP.

Myo-inositol conc. (mg/l)	Mean Shoots Produced	Mean Shoots length (cm)	Multiplication Rate
Control	8.80 ± 0.60	0.71 ± 0.04	1.47 ± 0.10
50	26.00 ± 1.29	0.78 ± 0.04	4.33 ± 0.22
100	48.20 ± 1.17	1.22 ± 0.06	8.03 ± 0.19
150	38.20 ± 0.79	1.20 ± 0.04	6.36 ± 0.13
200	24.50 ± 1.77	0.93 ± 0.05	4.03 ± 0.29
Significance	***	***	***
CD at 5%	3.61	0.15	0.58

In vitro rooting in FRI-13

***In vitro* shoots were cultured on ½ MS medium supplemented with Auxins like IBA, NAA and IAA**

Effect of IBA in ½ MS medium on *in vitro* rooting in FRI-13. Data was recorded after 5 weeks

IBA (mg/l)	Response %	Mean root number	Mean root length (cm)
Control	0.00± 0.00	0.00 ± 0.00	0.00± 0.00
0.1	2.00± 0.06	0.83± 0.54	0.10± 0.06
0.5	5.00± 0.09	1.67± 0.49	0.35± 0.08
1.0	18.50± 0.03	3.17± 0.31	1.37± 0.02
1.5	30.00± 0.29	4.50± 0.76	1.51± 0.07
2.0	25.00± 0.27	3.67± 0.33	1.48± 0.03
Significance	***	***	**
CD at 5%	0.53	1.36	0.15

***In vitro* rooting on ½ MS medium supplemented with IBA in FRI-13**

Effect of NAA in ½ MS medium on *in vitro* rooting in FRI-13. Data recorded after 5 weeks

NAA (mg/l)	Response %	Mean root number	Mean root length (cm)
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	2.00 ± 0.06	0.50 ± 0.34	0.58 ± 0.32
1.0	4.00 ± 0.06	0.67 ± 0.33	0.15 ± 0.07
1.5	6.00 ± 0.29	1.33 ± 0.49	0.24 ± 0.08
2.0	14.00 ± 0.58	2.33 ± 0.56	0.31 ± 0.07
Significance	**	**	NS
CD at 5%	0.82	1.04	

*- Significance at 5% **- Significance at 1% ***-Significance at 0.1%

***In vitro* rooting on ½ MS medium supplemented with different conc. (0.1 to 2.0 mg/l) of NAA in FRI-13**

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Effect of IAA in ½ MS medium on *in vitro* rooting in FRI-13. Data was recorded after 5 weeks

IAA (mg/l)	Response %	Mean root number	Mean root length (cm)
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.0	2.00 ± 0.06	0.50 ± 0.34	0.21 ± 0.13
1.5	4.00 ± 0.28	0.67 ± 0.42	0.28 ± 0.11
2.0	7.00 ± 0.29	1.17 ± 0.48	0.57 ± 0.12
Significance	**	*	NS
CD at 5%	0.52	0.85	

*- Significance at 5% NS- Non significant **- Significance at 1% ***-Significance at 0.1%

***In vitro* rooting on ½ MS medium supplemented with IAA in FRI-13**

HARDENING AND ACCLIMATIZATION

In vitro rooted plantlets were transferred to autoclaved cultured bottles containing soilrite, supplied with ½ MS medium twice a week.

After 2 weeks hardened plantlets were transferred to mist chamber for 3 weeks and then transferred in net house into polybags containing soil, sand and FYM in 1:1:1 ratio.

After hardening and acclimatization 83% field survival rate was achieved.

***In vitro* hardening of T.C. plantlets of FRI-13 in autoclaved soilrite with ½ MS medium in culture room**

Hardened and Acclimatized plants of FRI-13 in mist chamber

Tissue culture raised plants in polybags in net house

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Stability Analysis in Clones of *Casuarina equisetifolia*

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Coimbatore

The Species

Casuarina equisetifolia **is a tree of multiple end uses and is the most widely planted species of Casuarina in India.**

Casuarinas have been the farmers favourite in south India as they fit well in agrarian ecosystems.

The Species

It is being used for construction, pulpwood, fuelwood and for ecorestoration activities.

It is also highly preferred for planting in various agroforestry systems.

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The Species

Casurina exhibits substantial variation in growth and form characteristics.

The Species

- Clonal propagation offers tremendous possibility to explore this variation for large scale production of end-use specific planting materials.
- Systematic tree improvement programmes are underway at IFGTB over a decade.
- A clone bank consisting of 106 accessions of *C. equisetifolia* selected from Chidambaram, Chengalpet and Tiruchendur was established.

Experimental Materials

33 clones selected from Chidambaram / Chengalpet & 43 from Tiruchendur, Tamil Nadu.

Observations recorded over **6 years (Age 3 to Age 8)** were used for the study.

Design

RCBD with 6 Replications.

Stability Analysis

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.

It is always advisable that genetic tests be established in multiple environments.

Environments may consist of different locations, different years or different site preparation or management treatments.

Stability Analysis

Stability parameters were estimated using the model proposed by Eberhart and Russel.

According to them, a high yielding genotype with unit regression coefficient (bi=1) and the deviation from regression not significantly different from zero ($s^2di=0$) is considered as the stable one.

Stability Analysis

Group	Mean	Regression Coefficient 'bi'	Deviation from Regression 's^2di'
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.

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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

Stability Analysis

The CAI for total height, DBH, CDM, FV and volume index over 5 growth periods were subjected to stability analysis and the variance due to clone x growth period interaction was found significant for total height, FV and volume index in case of CH / CP clones.

Clones CP 4202, CH 3002, CH 2803 and CP 3903 were found to be stable for total height (Placed in Group I)

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 FV and volume index.

Eventhough, clones CH 3004 and CH 2703 exhibited superior growth, they could not register favourable values for stability parameters

Tables

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	-

Stability Analysis

In TCR clones, clone x growth period interaction was significant for CAI of all the characters except volume index.

Among the 15 clones, which recorded high mean values for total height, 10 were found to be highly stable over the growth periods

TCR 060101, TCR 030202 and TCR 030101 exhibited high stability for all the four traits.

TCR 040204 which registered superior growth characteristics was unpredictable across the environments due to the significant deviation from regression for height and CDM.

TCR 120102 which ranked first for most of the characters exhibited instability for DBH, CDM and FV.

No clone was found suitable for stress or favourable growth phases in both the groups.

Tables

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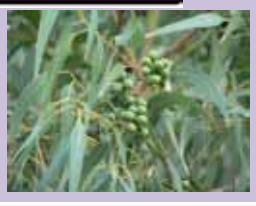
Differential Expression of *Cellulose Synthase* Gene Families in *Eucalyptus tereticornis*

***Karpaga Raja Sundari B.*, SRF and**
Modhumita Dasgupta, Scientist E
Division of Plant Biotechnology,
Institute of Forest Genetics and Tree Breeding,
Coimbatore

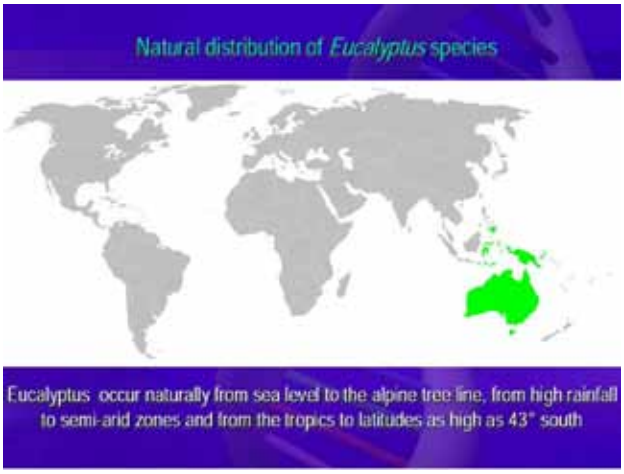
Eucalyptus -Fact sheet

- Long-lived, evergreen species
- Eucalypts- dominant or co-dominant in almost all vegetation type where they occur
- Keystone species for ecological studies



- ✓Division: Magnoliophyta
  - ✓Class: Magnoliopsida
    - ✓Order : Myrtales
      - ✓Family: Myrtaceae
        - ✓Genus: Eucalyptus

About 13 subgenera
Subgenus:
Symphyomyrtus- 29



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Eucalyptus spp.

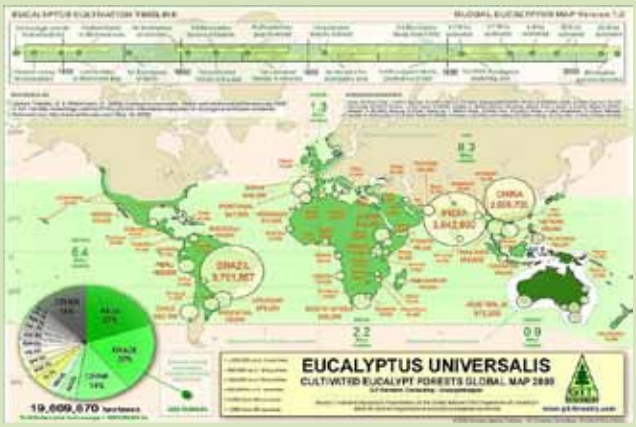
Native to Australia with a few species indigenous to its adjacent islands

- 800 species
- Fast growing
- Short rotation
- Pulp wood

- World-wide – 20.07 M ha
- India is the largest planter with 8 M ha and average productivity is 20 m^3/ha/yr
- Brazil has 3 M ha with average productivity of 45–60 m^3/ha/yr

Next to *Populus*, *Eucalyptus* has high genetic and genomic resources

INDIA- Leading in Eucalyptus plantation



Genetic improvement in Eucalypts

- *Eucalyptus* is a potential out-crosser
- Genetic improvement includes
  - Selection of elite plants for clonal propagation
  - establishment of seed orchards and hybridization
- Two main areas to accelerate improvement of specific traits include
  - Exploitation of genetic diversity in breeding programs
  - Genetic modification, by introducing new genes into already existing elite genotypes

Importance of molecular marker for tree improvement

Limitations in trait improvement in tree species

- Long generation times
- Highly heterozygous
- Few extended pedigrees
- Late expression of important traits

Most valuable contribution of molecular markers would be :

- Early identification of parents that will yield superior progeny for hybridization program
- Reduce the time of selection
- cost reduction in tree breeding programs
- importance for paper and other forest product based industries, by guaranteeing quality wood products

Genomic Platform in Tree improvement

- Genomics currently represents- expanding area of biotechnological research.
- In forestry- genomic research on high throughput gene discovery and function elucidation.
- Dramatic improvements in genomic technologies spurred by
  - Development of next generation sequencing and
  - High-throughput genotyping platforms.
- Development of bioinformatic tools and breeding theory – improves our knowledge of genes and genomes in forest trees.
- MIGRM (marker informed gene resource management) may play an expanding role in tree breeding and ecosystem management.
- Genomics of woody Perennials - Populus as model system

Eucalyptus, a second tree genome –For comparative, perennial plant genomics

- A second tree genome sequence of *Eucalyptus* -provide extraordinary opportunities for comparative genomic analysis with the *Populus* genome
- Full release of *E. grandis* genome sequence(11th April2011) EUCAGEN- publicly available as the first *Eucalyptus* reference sequence for future genomic undertakings
- Eucagen-further extend unique facets of tree biology including perennial growth habit, extensive formation of secondary xylem (wood) & juvenile-mature phase change

Significance of Eucalyptus Genomics

Establishment of a regional network for genomic application and association mapping of Eucalyptus forests to explore genetic base for wood development with industrial and energetic purposes

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EucaWood *Genomics* one more tool available to the breeder

➲EucaWood– has increased global demand in paper industry due to

- ☙ fiber's unique characteristics
- ☙ paper with high opacity, softness & good absorption qualities that are important to tissue, printing and specialty paper manufacturers

➲Rengel *et al.,* 2009- identified a set of wood related *Eucalyptus* unigenes called EUCAWOOD-valuable resource for functional genomics studies of wood formation and molecular breeding in Eucalypts

Annotated EUCAWOOD sequence

- ▪Instrumental for candidate gene approaches
- ▪marker-assisted selection programs aimed at improving and modulating wood properties

Candidate gene based association studies in Eucalyptus

❖Immediate applications of genomics
- •Identification of candidate genes for association studies
- •targets for genetic modification studies.

❖Ideally suited for the dissection of complex quantitative traits such as wood properties to reveal the genes and allelic variations in these traits in forest trees.

❖Two of the most commonly used tools for dissecting complex traits
- • QTL analysis and
- • association mapping.

Selection of candidate genes involved in wood formation is tedious.

❖All genes for expression of a trait may be candidate genes- but only genes with polymorphisms influencing the trait are accessible to the geneticist.

❖Differentially regulated Genes- identified during wood formation, clustered into groups or identified as candidate genes based on their expression pattern.

Role of Cellulose synthase gene in wood fomation

Significance of Cellulose Synthase gene

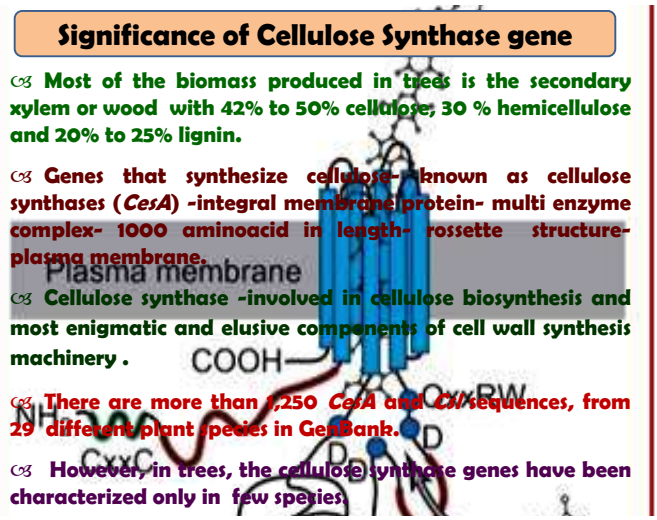
☙ Most of the biomass produced in trees is the secondary xylem or wood with 42% to 50% cellulose, 30 % hemicellulose and 20% to 25% lignin.

☙ Genes that synthesize cellulose- known as cellulose synthases (*CesA*) -integral membrane protein- multi enzyme complex- 1000 aminoacid in length- rossette structure- plasma membrane.

☙ Cellulose synthase -involved in cellulose biosynthesis and most enigmatic and elusive components of cell wall synthesis machinery .

☙ There are more than 1,250 *CesA* and *Csl* sequences, from 29 different plant species in GenBank.

☙ However, in trees, the cellulose synthase genes have been characterized only in few species.



Cellulose synthase gene in different tree species

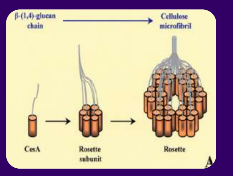
Eucalyptus
- 6 *CesA* gene- identified in *Eucalyptus grandis.*
- 5 *CesA* gene reported in *E. camaldulensis.*
- 3 *CesA* gene reported in *E.globulus*

Populus
- *CesA* & hemicellulose related *Csl* genes-present
- 7 *CesA* genes, 4 *Csl* genes- xylem specific-synthesis of wood

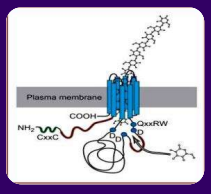
Pinus
- 3 *CesA* gene in *Pinus taeda*
- *Xylem specific CesA genes*

Structural features of *CesA* genes

- *CesA* is a member of protein complex- rosette structure in surface of plasma membrane
- Six large subunit-arranged in hexagonal pattern
- Aminoterminus region- protein-protein interaction in *CesA* complex
  - Hypervariable region followed by two trans-membrane domain
  - Globular soluble domain- has glycosyl transferase activity



Rosette structure of CesA



Conserved region in *CesA* proteins

❖ CesA protein in higher plants-contain certain defining region:

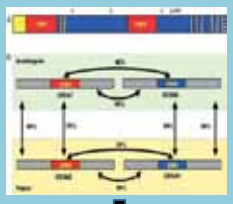
↳ N- terminus- motif similar to ring finger domain- involved in oligomerisation of cesa protein.

↳ Class specific region (CSRI & CSR II)- shows limited conservation among CesA family members from same species.

↳ CSR II -highly conserved in CesA orthologs from different species.

↳ CSRII- helps in distinguishing individual family members & serve as starting point for full length cDNA isolation.

Major regions of Plant CesA proteins



CONSERVED REGION AMONG ORTHOLOGS

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Plant Material for study

15 years old *E. tereticornis*
(South of Helenvale Provenance,
CSIRO seed lot no. 12944)
SEEDLING SEED ORCHARD
Karunya ResearchPlot,
Coimbatore
Tamil Nadu

Tissues
Leaves
internodes
developing xylem
mature xylem tissues

Fresh tissues were harvested and immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

Research Methodology

Ascertain differential expression of CesA gene families using quantitative Real Time PCR (qRT-PCR).

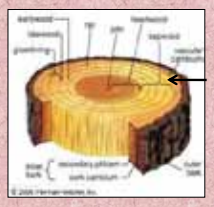
RNA isolation from all the tissue samples
cDNA synthesis

Designing of Primer pairs targeting CSR II region of *CesA*
Amplification of *CesA* genes in all tissues

qRT-PCR reactions in biological replicates
Selection of endogenous reference gene

Differential expression studies by qRT -PCR

Different layers of wood

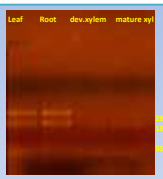


Results

In silico sequence analysis of cellulose synthase super family members to design specific primers

Conserved CSRII region was identified and ten primer pairs were designed for *CesA* using Primer3

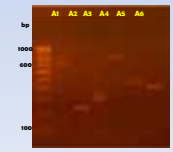
Total RNA from different tissues



Primers used for amplification of CSR II regions of *CesA* families

PRIMER ID	SEQUENCE
A1 CSR IIF:	AATGCCGCCATCTTCAACCTTGGA
A1 CSR IIR:	GATCGCAGTGATCGATATG
A2 CSR IIF:	ACATGTGATTGCTGGCCTTC
A2 CSR IIR:	TTCTGAGACATGAGCGATGA
A3 CSR IIF:	CTGTGATTGCTGCCCATGCT
A3 CSR IIR:	CGCCACCCTGTTCCATCAAA
A4 CSR IIF:	AGCCAAAGCAGAGAAAGTCA
A4 CSR IIR:	ATAAGCGTAGAGGCCACAAA
A5 CSR IIF	GGGAAGGGTGCAATAAGAA
A5 CSR IIR	CTAAGCCAGTCAGAGGACAA
A6CSR IIF	CAAGTCATCCACACCATCTCTTC
A6CSR IIR	TGGCGATCCATCTCTTGCCTATGC

Amplification of *CesA* genes in cDNA

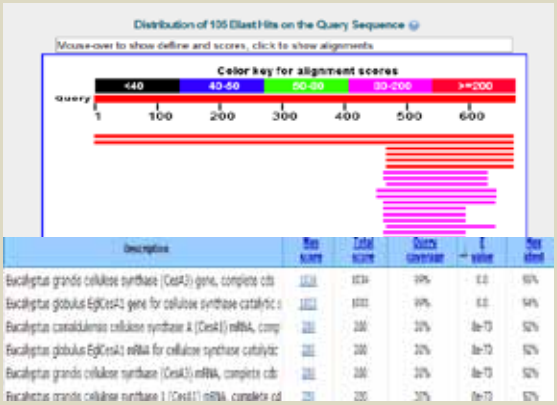


- Total RNA isolation was optimized using the in-house protocol from different tissues.
- Six classes of *CesAs* expressed in the *E. tereticornis* were amplified in cDNA in the size range of 200-800 bp
- The sequences showed similarity to *CesAs* from *E. grandis and E. globulus*

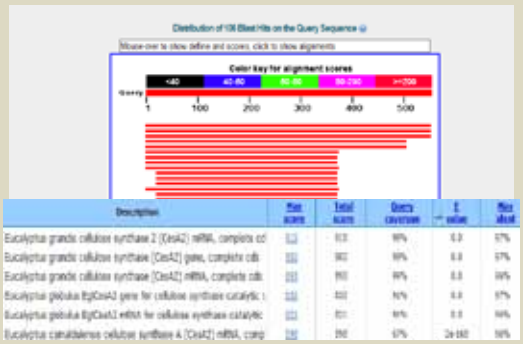
Sequence Homology

Seq. ID	Amplicon Size	Similarity	Percentage of similarity
EtCesA1	700bp	*Eucalyptus grandis* cellulose synthase gene *CesA1* (E- 6e-73)	92%
EtCESA2	250bp	*Eucalyptus grandis* cellulose synthase gene *CesA2*(E- 8e-73)	97%
EtCESA3	300bp	*Eucalyptus grandis* cellulose synthase gene *CesA3* (E- 6e-19)	88%
EtCESA4	400bp	*Eucalyptus grandis* cellulose synthase gene *CesA4* (E- 2e-10)	83%
EtCESA5	300bp	*Eucalyptus grandis* cellulose synthase gene *CesA5* (E-2e-08)	90%
EtCESA6	300bp	*Eucalyptus grandis* cellulose synthase gene *CesA6* (E-9e-50)	96%

Blast Hits of EtCesA1



Blast Hits of EtCesA2



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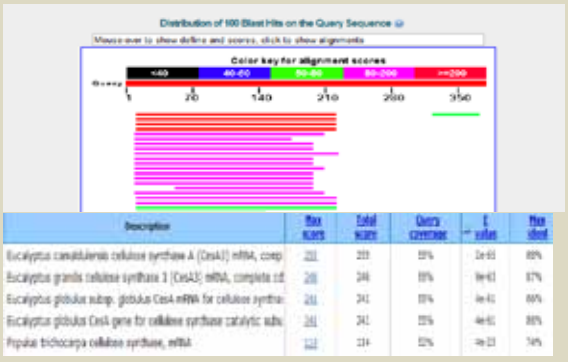
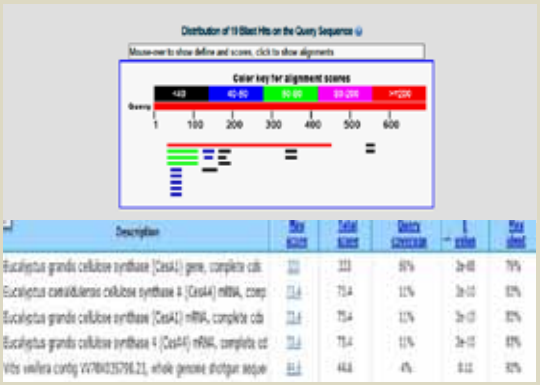
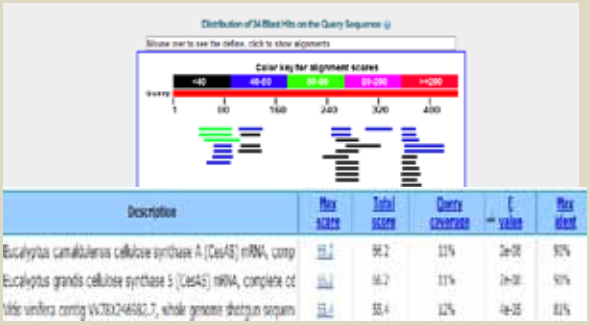
Blast Hits of EtCesA3



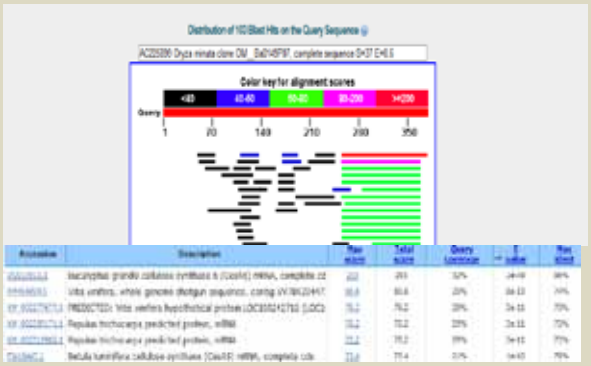
Blast Hits of EtCesA4



Blast Hits of EtCesA5



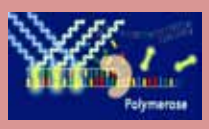
Blast Hits of EtCesA6



Differential expression studies by quantitative real time PCR

Quantitation OF mRNA

- ***Northern blotting***
- ***Ribonuclease protection assay***
- ***in situ hybridization***
- ***PCR***
  - ***most sensitive***
  - **can discriminate closely related mRNAs**
  - **technically simple**
  - **difficult to get truly quantitative results using conventional PCR**
- **Advantage of Real Time PCR**
  - **To quantitate differences in mRNAexpression**
  - **using SYBR green chemistry**



Differential expression of *CesA* gene families in different tissues of *Eucalyptus tereticornis*

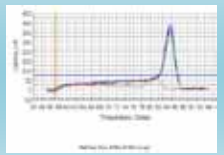
Relative expression of *EtCesA1, 2, 3, 4, 5* and 6 was analyzed in different tissues of *E.tereticornis* like leaf, internodes, developing xylem and mature xylem tissues .

RNA was extracted from all the tissue samples using the in-house protocol.

The quantity and purity of RNA was quantified and first strand cDNA was synthesized from all tissues using cDNA synthesis kit (Fermentas, USA)

Real time PCR was conducted in ABI PRISM 7500 Step one plus Sequence Detection System (Applied Biosystems, USA) based on SYBR Green chemistry.

Melt curve analysis



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Selection of reference genes by Real time PCR

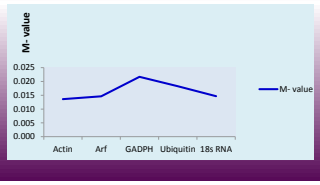
Five candidate reference genes including Actin, ADP- ribosylation factor (*Arf*), *GAPDH,* Ubiquitin & 18s rRNA screened in different tissues of *Eucalyptus tereticornis* like leaf, internodes, developing xylem and mature xylem.

Actin-suitable refernce gene along with *Arf* for normalizing *EtCesA* genes using geNorm, BestKeeper and Norm finder programs..

Reference gene selection by geNORM

Amplification of *Actin* transcript in Secondary xylem

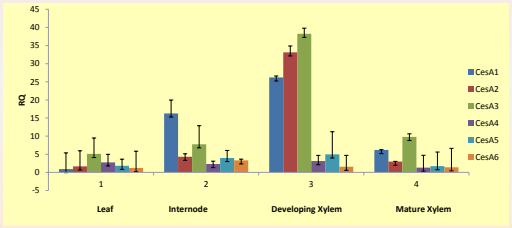
Reference gene selection by Normfinder



Differential Expression analysis

Relative expression analysis obtained- delta-Ct method using Actin as endogenous control & Arf - calibrator

- *EtCesA1, 2 and 3* - higher in developing xylem and mature xylem tissues – *indicating* secondary wall *specificity*
- *EtCesA4, 5 and 6*- predominantly in leaf and internodes –indicating primary wall specificity



Significance of the study

- Aimed at identifying suitable reference genes in different tissue types for normalization of qRT-PCR conditions *in Eucalyptus tereticornis.*
- Accurate normalization of qRT-PCR experiments in this important species of Eucalypts used in paper and pulp industries.
- Multi enzyme complex Cellulose synthase *(CesA) is* regulated through tissue specific and differential gene expression during development.

The authors acknowledge

- Research grant from Indian Council of Forestry Research and Education, Govt of India
- Dr. Viswanathan, R, Principal Scientist & Head (Plant Pathology), Sugarcane Breeding Institute, Coimbatore for Real time PCR facility

Thank you

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Characterisation of commercially important eucalyptus germplasm using molecular markers for identification of redundant accessions and prediction of susceptibility to *Leptocybe invasa* infestation

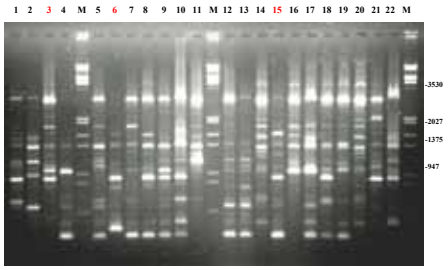
Mathish Nambiar-Veetil[1*], Shashi Bhushan Tripathi [1,2], Balu Ayyapillai[1] , Rajarishi Rajappan[1], Vimal Panneerselvam[1] and Gurumurthi Krishnamurthy[1]

1. Institute of Forest Genetics and Tree Breeding, Coimbatore
2. Presently in The Energy and Resources Institute, New Delhi

PLANT Material used for the study

Clones	Economically important characters
ITC 3, ITC 7, ITC 93, ITC 132	Good tree form
ITC 3, ITC 7, ITC 10, ITC 93, ITC 99, ITC 105, ITC 128, ITC130	Faster growth (based on high MAI)
ITC 6, ITC 74, ITC 93, ITC 147	Clones suitable for pulping (based on fiber length, fiber diameter, wall thickness, lumen diameter, felting coefficient, Isenberg coefficient, Runkel's ratio)
ITC 6, ITC 10, ITC 99, ITC 105	Clones suitable for solid wood products (high wood density).
ITC 7, ITC 10, ITC 130	Clones with high cellulose content and longer fibers

Ajith, 2001



RAPD analysis of twenty two ITC clones of Eucalyptus with OPB 04. Lane 3, Lane 6 and Lane 15 representing ITC6, ITC10 and ITC105 showing unique fingerprints. Lane M is lambda *Hind*III/*Eco*RI digest.

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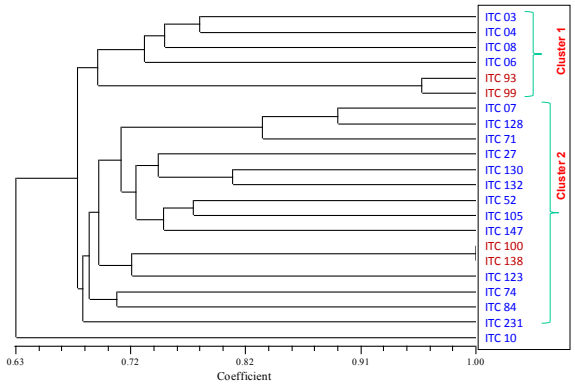
	Presence	Combined presence	Combined absence
Clone 132	$OPB03_{695}$ $OPB03_{812}$	-	-
Clone 10	$OPB04_{400}$	-	-
Clone 105	$OPB04_{1750}$	-	-
Clone 6	$OPB04_{954}$	-	-
Clone 27	$OPB17_{624}$	-	-
Clone 74	$OPB01_{1120}$	-	$OPB17_{624}$
Clone 84	$OPB05_{820}$	-	$OPB04_{954}$
Clone 128	$OPB08_{567}$	$OPB18_{730}$	-

Key developed for markers identifying the clones

Identification of Redundant clones



Leptocybe invasa emerges as a Major Pest of Eucalyptus

Photo Courtesy: Dr. J.P.Jacob

Status of 38 ITC clones for Gall Insect attack

S. No.	Location	Infection status of Clones			
		Negligible	Low	Moderate	High
1	Coimbatore	122, 227	4, 6, 228, 248, 268, 285, 290	116, 259,264	8, 74, 339
2	Satyavedu	1	7, 231, 251	3, 72, 130, 161, 256	10, 27, 71, 83, 99, 128, 132, 148, 242, 286, 351, 399,404,419

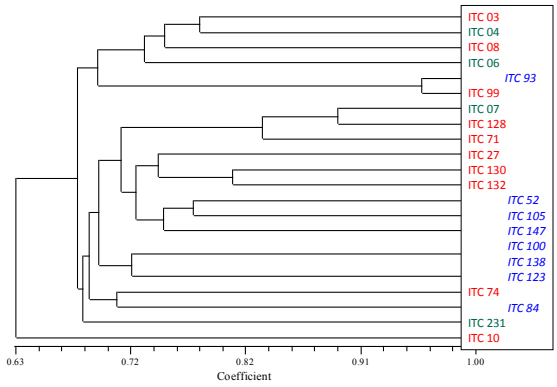
Based on the amount of foliage affected by gall formation, the severity scales were fixed and the clones categorized under four groups viz., Negligible (only with Ovipositional scars), less susceptible (< 25% of the foliage affected by gall formation) moderately susceptible (25-50% of the foliage affected by gall formation) and Highly susceptible (>50% of the foliage affected by gall formation).

ITC clones for which DNA fingerprint data are available

Infection status of Clones		
TOLERANT	SUSCEPTIBLE	Data unavailable
4, 6, 7, 231	3, 8,10, 27,71,74,99,128,130,132	52, 84, 93, 100,105,123,138,147

Question :

What recommendation could be given to the planters on the probability of Leptocybe infestation for the clones for which no data on pest incidence have been studied?



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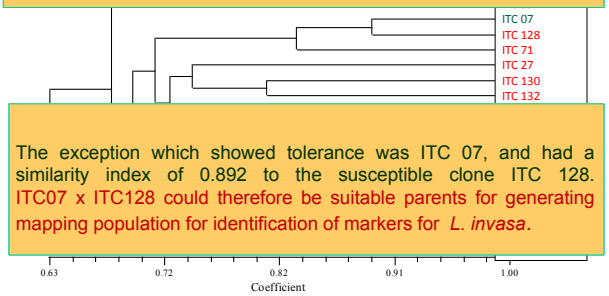
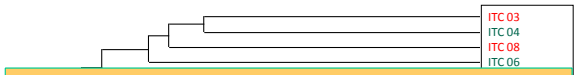
Six clones with a similarity index between 0.74 - 0.84 showed 83.3 % prediction accuracy for tolerance to *L. invasa*.

Tolerance Prediction for SI between 0.74-0.84: 83.3 %



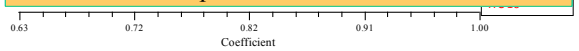
The exception which showed tolerance was ITC 07, and had a similarity index of 0.892 to the susceptible clone ITC 128. ITC07 x ITC128 could therefore be suitable parents for generating mapping population for identification of markers for *L. invasa*.



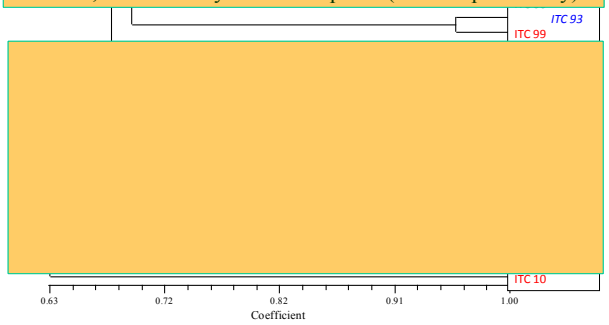
Four Clones with a similarity index between 0.73 - 0.78 showed 50% accuracy in prediction of Tolerance/ susceptibility.

Tolerance Prediction for SI between 0.73-0.78: 50%

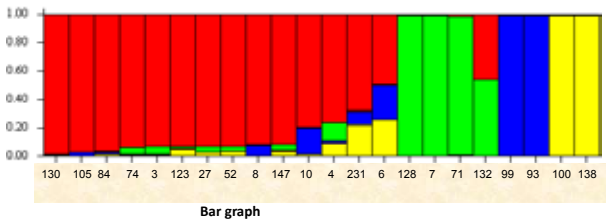
With this data lets examine what rational recommendations could be given for clones for which no data on pest tolerance is available.



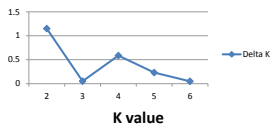
ITC 99 and ITC 93 have a similarity index of 0.95 and therefore, ITC 93 likely to be susceptible (> 85 % probability).



Structure analysis revealed that the clones are derived from 4 populations



Bar graph



K value

Significant association was found with certain bands for Leptocybe tolerance

Primer	Band Size	Chi Square	Probability
OPB4	1571	16	0.001
OPB4	337	9.375	0.01
OPB11	645	6.00	0.01

Significant association was found with certain bands for Leptocybe tolerance

Primer	Band Size	Chi Square	P value	Clones		
				Tolerant	Susceptible	Tolerance Data unavailable
OPB4	1571	16	0.001	4, 6, 231		
OPB4	337	9.375	0.01	7	8, 27, 71,74, 99, 128,130,132	52, 93,105
OPB11	645	6.00	0.01		3,8,10,74,99	93,105,147

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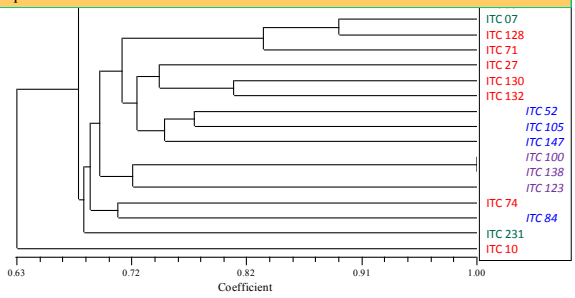
We predict that the clones **ITC 52, 105, 147** have **> 50 %** probability of being susceptible (SI ~0.73 with susceptible clones + OPB4, 11 association data) .

Similarly, **ITC 100, 138, 123, 84** (SI ~ 0.7) have **<50%** probability of being susceptible.



Salient Findings

- RAPDs were used to assess the diversity and discriminate commercially planted Eucalyptus clones and redundants were identified.

  ITC 93 and 99 are likely to be siblings.
  ITC 100 and 138 are clones with the same name.

- Genetic relatedness was used to make recommendations on the probability of clones being tolerant/ susceptible to *L. invasa*.

  Tolerance Prediction for SI between 0.74-0.84: 83.3 %
  Tolerance Prediction for SI between 0.73-0.78: 50 %

- Probability of susceptibility of clones for which no pest infestation data is available

ITC93	(SI ~0.95)	> 85 %
ITC 52, 105, 147	(SI ~ 0.73 + Association data)	**> 50 %**
ITC 100, 138, 123, 84	(SI ~ 0.7)	**<50 %**

Conclusions

- Important to profile DNA of germplasm collections for understanding the genetic relatedness.
- Important to Characterise Germplasm for other desired traits.
- Genetic relatedness could be an important criteria for prediction of susceptibility to insect pests.

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Theme 3. Expanding frontiers of Forestry Sciences

Sub Theme 3: Forest Genetics and Biotechnology

Effect of Donor age and genotype on coppicing and rooting ability in *Dalbergia sissoo* Roxb.

By

Meena Bakshi

Plant Physiology, Botany Division

Forest Research Institute, Dehradun

Introduction

- ***Dalbergia sissoo*** **Roxb. commonly named as "Shisham or sissoo" is an multipurpose tree species known for its variety of adaptive and economic importance in India.**
- **It is widely distributed in sub-himalayan tract upto 900m occasionally ascending to 1500m.**
- **The species has certain positive features such as nitrogen fixation, growth in hardy condition, quality wood, good fodder and fuel value, nutrient rich and fast decomposing leaves which makes it fit for agro forestry, social forestry, biomass production and timber plantations.**
- **Most favored species for afforestation and reforestation programmes and is being commercially exploited for its hard, strong and durable timber.**

WHY CLONING?

- **Poor stem form with generally crooked and forked bole is the stumbling block of sissoo which deteriorates its timber quality.**
- **A very high number of trees in natural populations have crooked and forked stems with a very small proportion of straight boles (Bangarwa *et al.*, 1994).**
- **During last few decades, heavy mortality of Shisham was registered in almost all shisham growing states of India.**
- **Inspite of its timber use , most of the planting stock is still produced from seeds of unselected sources which show remarkable variations in growth and stem form.**

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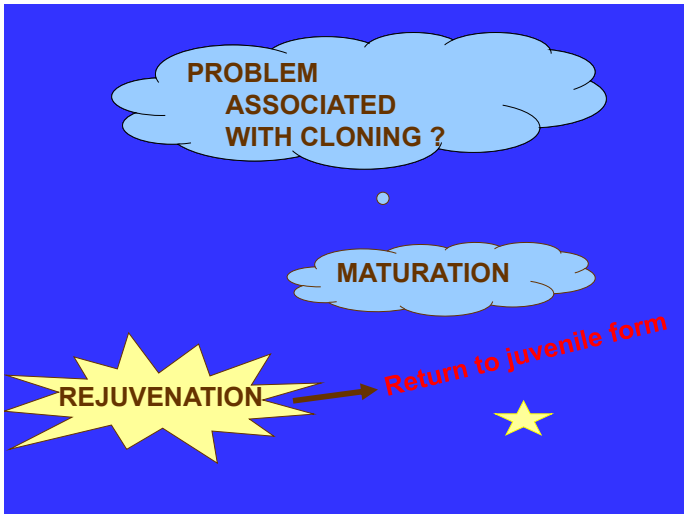
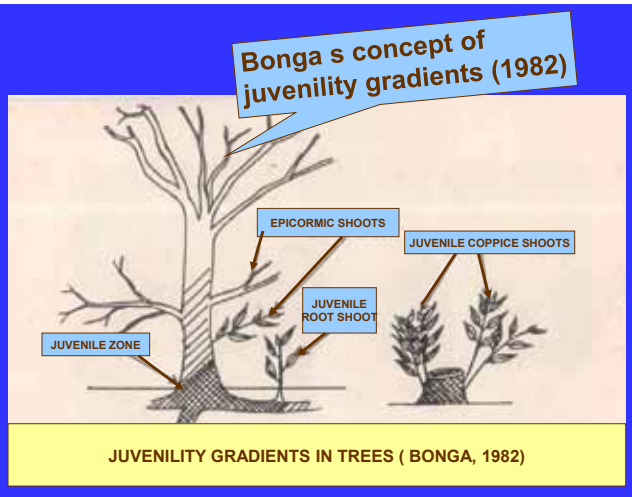
❖ Concerted efforts are required to improve the genetic quality of planting stock as well as to produce disease resistant varieties.

❖ Clonal propagation is a proven technique for mass production of superior planting stock of forest tree species. The species can be multiplied clonally and thus true nature of selections can be maintained in plantations.



Cloning in Shisham

- ❖ Mature Hardwood cuttings
- ❖ 20-25 cm long, 1-2cm dia.
- ❖ IBA 100 ppm (24h.)
- ❖ Dip method
- ❖ 2:1:1 Soil : Sand:Manure



• In Shisham, rooting of juvenile shoot cuttings is the most acceptable approach to obtain high quality and uniform , disease resistant planting material for establishment of commercial plantations.

• Uninterrupted supply of juvenile shoots is possible establishment of Vegetative Multiplication Garden /Hedge Garden. For how long these Hedge gardens be maintained till the rooting and subsequent growth of rooted propagules is not affected is an intrinsic question

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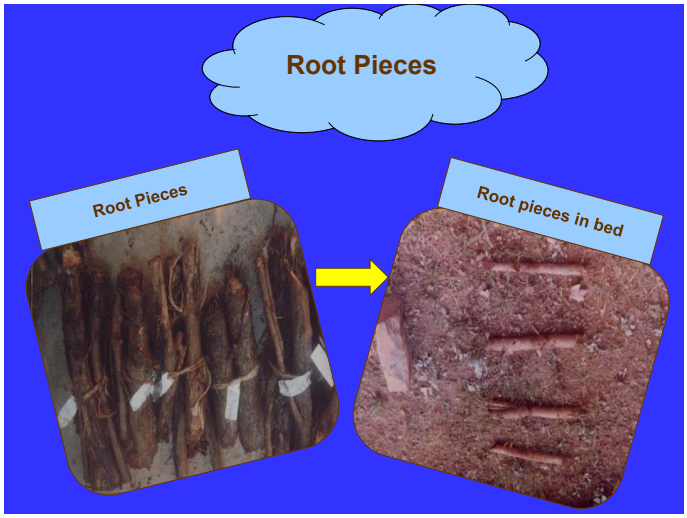

How VMGs established ?

Selection of Plus Tree → Root Pieces excised (50-60cm) in Jan- Feb → Planting of root pieces in beds

Primary Shoot developed from Root Pieces called Suckers

These suckers are regularly hedged

Lateral juvenile shoots developed from these hedges known as Coppiced Shoots

These coppiced shoots are further used to establish VMGs.

VMG (Vegetative Multiplication Garden)

Living collection of selected biotypes which are regularly hedged to produce juvenile shoots for rooting and vegetative propagule production for establishment of Plantations.

Three different VMGs used for present study are :-

- ❖ VMG established during 1994 (14 years old)
- ❖ VMG established during 1998 (10 years old)
- ❖ VMG during 2003 (5 years old)

FAO CONSULTANTS
DR. MENZIES AND FAULDS
FRI, NEW ZEALAND

'Sissoo appears to age very rapidly with hedging.The juvenile cuttings are showing variable performance as has been collected from all over hedges. Trials are needed to determine how quickly sissoo hedges age'

❖ **The present work was therefore undertaken to study the effect of age of Vegetative multiplication garden on rooting and subsequent growth of rooted propagules in Shisham.**

MATERIAL AND METHODS

Experimental site: *The trials were laid down at FRI, Dehra Dun.*

Selection and marking of clones : Five diverse clones represented in all three VMGs were selected and marked for the present study.

Table 1 . List of 5 selected clones in three VMGs

Clone No.	Location	State
C09	Pathri (Haridwar)	Uttarakhand
C41	Tulsipur (Gonda)	Uttar Pradesh
C49	Trilokpur(Gonda)	Uttar Pradesh
C66	Chichraulli (Yamunanagar)	Haryana
C88	Hanumangarh	Rajasthan

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Hedging operations
• Dec.- Jan.
• Coating

Antifungal treatment

VEGETATIVE MULTIPLICATION GARDEN

Hedged Orchard

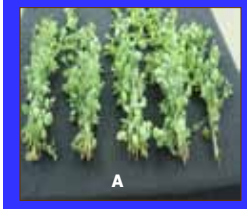
VMG after 6 weeks of Hedging

SHOOT DEVELOPMENT IN VMG

Collection of juvenile coppice shoots

- The coppiced shoots produced after 8-12 weeks were then exploited for rooting of cuttings .
- Data was collected on days of first shoot emergence, Number of coppice shoots produced and length of coppice shoots in three VMGs.

Juvenile shoot multiplication



A

B

C

- 6-7 cm single nodal cuttings and treated with Bavistin (0.1%)
- IBA 1000ppm
- Dry smear
- Vermiculite

D

E

Continued.....

Preparation and treatment of cuttings

Continued.....

Planting Operations

Continued.....

[432]

Misting duration
½ hr On delay 1 min
1 hr , 1 min

Planting of cuttings in beds

Continued.....

ROOTING

DIFFERENT STAGES OF ROOTING

A ROOTED PROPAGULE AFTER 6 WEEKS

A ROOTED PROPAGULE READY FOR TRANSPLANTING

Weaning & Hardening

The rooted cuttings were transplanted in polybags filled with soil , sand and compost (2:1:1) and kept for weaning under mist for a period of one week .

CUTTINGS KEPT IN MIST CHAMBER FOR WEANING

HARDENING OF CUTTINGS IN SHADE HOUSE

The rooted cuttings were then gradually exposed to natural conditions.

The periodical data on subsequent growth of rooted propagules was undertaken for one year.

RESULTS

Table 2 : Interaction Cl x VMG in days of first Shoot emergence after Hedging

Clone No.	VMG I (14 yr.)	VMG II (10 yr.)	VMG III (5 yr.)	Mean
C9	33	28	30	30.3
C41	31	27	28	28.7
C49	34	25	28	29.0
C66	36	28	32	32.0
C88	31	26	30	29.0
Mean	33	26.8	29.6	29.8

Table 3: Interaction CLx VMG in coppice shoot production / stump after hedging

Cl No.	VMG I (14 yr.)	VMG II (10 yr.)	VMG III (5 yr.)	Mean
C9	18	16	16	16.3
C41	19	19	12	16.7
C49	26	25	09	20.0
C66	24	11	08	14.3
C88	19	25	13	19.0
Mean	21.6	19.2	11.6	17.5

Table 3: Interaction Cl x VMG in mean coppice shoot length .

Clone No.	VMG I (14 yr.)	VMG II (10 yr.)	VMG III (5 yr.)	Mean
C9	54.20	37.36	30.43	40.66
C41	23.70	57.40	27.30	36.13
C49	18.12	68.75	26.00	37.62
C66	27.25	36.20	19.00	27.48
C88	18.40	51.50	33.40	34.43
Mean	28.33	50.24	27.23	35.27

Table 5. Critical difference of studied parameters

Critical difference 9			
Source of variation	Mean days of shoot emergence	Mean no. of shoots/stump	Mean shoot length(cm.)
VMG	2.00***	0.66***	4.59***
Clone	1.58**	0.85**	5.92***
VMG*Clone	NS	NS	NS

Table 7: Interaction of Clone x VMG on Sprouting and Rooting Percentage (days of root initiation) of juvenile cuttings

Clone	14 yr. old		10 yr. old		5 yr. old	
	Sprouting %	Rooting %	Sprouting %	Rooting %	Sprouting%	Rooting %
C9	38	25(19)	60	55(9)	63	60(10)
C41	65	45(18)	65	55(12)	65	85 (12)
C49	30	50(15)	60	60 (7)	90	90(8)
C66	75	40(20)	80	75(11)	70	50(10)
C88	75	45(24)	65	60(12)	90	80(12)
Mean	56.6	41(19.2)	66	61 (10.2)	75.6	73 (10.4)

Table 8 : Interaction of Clone x VMG in sprout no. / sprouted cutting and root no./ rooted cutting

Clone	14 yr. old		10 yr. old		5 yr. old	
	Sprout No.	Root No.	Sprout No.	Root No	Sprout No.	Root No.
C9	1.00	2.28	1.12	4.39	1.14	3.02
C41	1.17	3.92	1.22	3.93	1.30	3.91
C49	1.00	2.22	1.00	3.86	1.00	6.17
C66	1.00	3.02	1.00	3.81	1.00	2.90
C88	1.00	1.97	1.26	4.77	1.08	3.49
Mean	1.03	2.68	1.12	4.15	1.10	3.90

Table 9: Interaction of Clone x VMG in mean sprout length/ sprouted cutting and root length/rooted cutting

Clone	14 yr. old		10 yr. old		5 yr. old	
	Shoot Length	Root Length	Shoot Length	Root Length	Shoot Length	Root Length
C9	1.18	6.00	1.18	2.12	1.47	2.96
C41	0.99	5.50	1.31	4.37	1.50	5.70
C49	1.23	6.28	1.41	4.31	1.63	5.17
C66	1.17	5.27	1.28	3.20	1.37	4.44
C88	1.61	3.83	1.13	8.42	1.56	5.55
Mean	1.236	5.376	1.262	4.484	1.506	4.764

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Table 10: Variations in Sprouting and Rooting parameters as affected by clone

Clone	Sprouting %	Rooting %	Sprout Number	Root Number	Sprout length	Root length
C9	53.7	46.7 (12.7)	1.08	3.23	1.27	3.69
C41	65.0	63.3 (14.0)	1.23	3.92	1.27	5.19
C49	60.0	67.0 (10.0)	1.00	4.08	1.43	5.25
C66	75.0	63.3 (13.7)	1.00	3.24	1.27	4.29
C88	77.0	65.0 (16.0)	1.11	3.41	1.43	5.91
Mean	52.4	58.32 (13.28)	1.08	3.576	1.33	4.87

Table 11: Variations in Sprouting and Rooting parameters as affected by VMG

	Sprouting %	Rooting %	Sprout Number	Root Number	Sprout length	Root length
VMG I	56.6	41(19.2)	1.03	2.68	1.236	5.376
VMG II	66.0	61(10.2)	1.12	4.15	1.262	4.484
VMG III	75.6	73(10.4)	1.10	3.90	1.506	4.764
Mean	66.06	58.3(13.26)	1.08	3.57	1.33	4.870

Table. 12. ANOVA for rooting and sprouting parameters in different clones and VMGs

Source of variation	Sprouting %	Rooting %	Mean no. of sprout	Mean leng. sprout	Mean no. of root	Mean leng. root
VMG	3.11***	3.34***	0.09***	0.12***	369***	0.43***
Clone	4.02***	4.32***	0.11***	NS	0.47***	0.56***
Clone * VMG	6.96**	7.48**	0.20***	0.28***	0.82***	0.97***

Figure . Intercional variations in height and collar diameter of rooted propagules at 12 months of age as affected by age of VMG

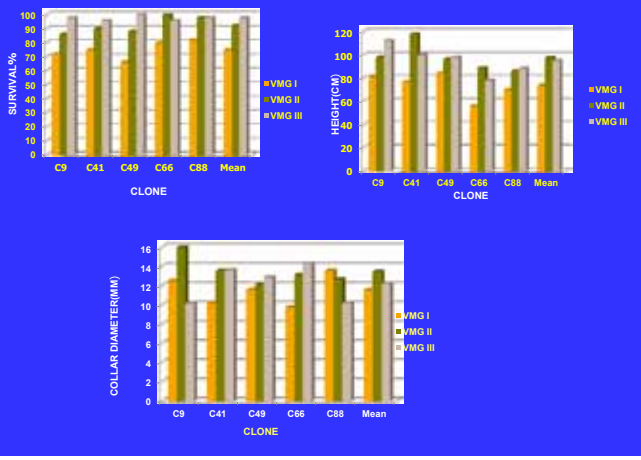

Table 11a: ANOVA for different variables with respect to Height

Effect	Degree of Freedom	MS	F	p
Intercept	1	2298651	9681.618	0.000000
VMG	2	2834	11.937	0.000019
CLONE	4	6933	29.203	0.000000
VMG*CLONE	8	2237	9.423	0.000000
Error	120	237		
Height	4	136457	1256.865	0.000000
Height *VMG	8	1010	9.302	0.000000
Height *CLONE	16	793	7.300	0.000000
Height*VMG*CLONE	32	294	2.709	0.000003
Error	480	109		

Table 11b: ANOVA for different variables with respect to collar diameter.

Effect	Degree of Freedom	MS	F	p
Intercept	1	45846.54	10857.50	0.000000
VMG	2	59.26	14.03	0.000003
CLONE	4	9.74	2.31	0.062029
VMG*CLONE	8	35.53	8.41	0.000000
Error	120	4.22		
DIAMETER	4	2097.69	846.95	0.000000
DIAMETER*VMG	8	27.56	11.13	0.000000
DIAMETER*CLONE	16	3.57	1.44	0.117299
DIAMETER*VMG*CLONE	32	8.31	3.36	0.000003
Error	480	2.48		

Summary of Findings

- Marked inter clonal and intra clonal variations in days of shoot emergence, shoot no. and shoot length were discernible which were statistically significant.
- The minimum time (25 days) for first shoot emergence was taken by C49 in 10 year old hedges while maximum time(36) days was taken by c66 in 14 year old hedges. Overall, minimum days (28.7) of first shoot emergence was noticed in clone 41 at par with c49 while maximum time (32 days) was taken by C66. Regarding age of VMG, mature hedges took more time (33 days) for shoot initiation than young hedges which took only 26.8 days.
- Significant differences were also observed in shoot production capability with maximum (20) shoots in c49 and minimum in c66. Overall, max. 26 shoots were obtained by C49 in 14 years old and min. 8 no. by c66 in 5 years old. Different aged VMG revealed maximum shoots (21.6) in 14 year old hedges in contrast to 11.6 in 5 year.

Continued...

- Shoot length showed max. value (68.7cm) in c49 in 10 year old hedges in contrast to 18 cm in same clone in 14 year. Significant diffrences in shoot length were also discernible in clone as well as VMG with maximum length (40.66 cm) in clone 09 and 50.24 cm in 10 year old hedges.
- The differences in sprouting and rooting % were also significant with maximum (90 %) in c49 in 5 year old hedges while in aggregate 73.6 % sprouting and 73% rooting was observed in 5 year old hedges. The minimum time (7 days) was taken to first root initial was by c49 in 10 year old hedges.
- Sprout and root number also revealed significant variations among clone and VMG with maximum sprout No. (1.3 cm) was observed in c41 and maximum root no. (6.17) in c49 in 5 year old hedges.
- Regarding root length and sprout length the maximum values 6.28 cm and 1.63 cm were discernible in c49 in 14 year and 5 year old hedges respectively which were significantly variable.

Continued...

- Overall, 75% sprouting was discernible in C66 , 67% rooting by c49, 1.23 sprout no. by c41 and 4.08 root number by c49, 1.43 cm sprout length by c49 and 5.91 root length by c88 which were the maxima.
- Overall age effect revealed a maxima of 75.6 % sprouting and 73 % rooting in 5 year old hedges,1.12 sprout number and 4.15 root number in 10 year old hedges and 1.5 cm sprout length in 14 year and 5.4 cm root length in 5 year old hedges.
- The survival % of rooted propagules was maximum 100% in C49 and c66 in 10 year and 5 year old respectively which declined to 60% in 14 year old hedges in the same clone.
- Highly significant variations were observed in height of rooted propagules at 12 months of age with maximum value 118 cm in c41 in 10 year old hedges followed by c09 in 5 years which falls to 56 cm in c66 in 14 years age.
- Highly significant variations were also noticed with regard to collar diameter which showed a maxima of 15.8mm in c09 at 10 years of age and minimum 9mm in c66 at 14 years.
- Overall, 5 year old hedges revealed maximum survival followed by 10 years old hedges . With regard to height and collar diameter, the maximum values were discernible in 10 year old hedges.

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In a nutshell, C49 (Gonda) was the best in early and maximum shoot production, rooting, survival and subsequent growth of rooted propagules hence, could be selected for future multiplications. With respect to age, 10 year old hedges were the best in early shoot formation , coppice shoot production capability and rooting and subsequent growth which declines abruptly with increasing age of VMG hence rejuvenation of the old hedges is essential.

Technology to rejuvenate old hedges

Exposure and incision operations of roots

Continued...

Continued...

Juvenile orthotropic sucker production

CONCLUSION

A well defined Technique for rejuvenation and mass multiplication of *D. sissoo* was developed at Forest Research Institute, Dehra Dun. Through this technology superior and disease resistant genotypes can also be multiplied on a mass scale which is the need of hour today looking into severe mortality problem in shisham during last few decades. Efforts were also made to rejuvenate old hedges which could be retained to generate juvenile shoots for further multiplications. Mass multiplication of superior selected clones for timber industry and other user needs would fetch enormous revenue and increase productivity in short duration which is not possible through seed plantations.

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Monitoring genetic fidelity of somatic embryo regenerated plants of *Bambusa bambos* by RAPD and ISSR markers

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Tree Improvement and Propagation Division

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Malleswaram, 18th Cross,
Bangalore

Introduction

- Bamboo are fast growing, short rotation, woody grasses belonging to **Poaceae** family with **125 species** in **23 genera** found in India (FSI, 2003).
- Bamboo, the grass of hope is also called as "**Green gold**" due to its colossal applications in every aspects of life.
- Recent advances in processed bamboo products would soon replace the so called poor mans timber by "**rich mans decorum**".

Traditional bamboo baskets

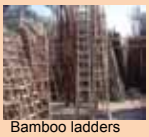
Advanced processed bamboo flooring

- There is a national drive for the **commercialization of bamboo** within India to convert natural stands into commercial ventures with standardized quality control (FAO, 2008).

Industrial applications of bamboo products

- Pulp & paper
- Flooring , lamination
- Low cost houses
- Food items
- Chopsticks
- Planting purpose
- Landscape enhancement
- Medicine
- Handicrafts

Bamboo food items



Bamboo ladders

Bamboo interiors

Bamboo scaffoldings

Bamboo fencing

Bamboo architecture marvels

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Introduction cont...

- The **increasing demand of bamboo** and its products drives the development of **micropropagation technologies** (Gillis, *et al.,* 2007).
- The **maintenance of genetic integrity of micropropagated perennial species** (Gamborg, 1993) is the most crucial concerns for uniform quality of plantlets (Lark and Scowcroft, 1983; D'Amato, 1985).
- Only few reports are available on genetic fidelity studies by Negi and Saxena (2009; 2010) in *B. balcooa* and *B. nutans* using ISSR markers in the plants regenerated through axillary shoot proliferation. And by Agnihotri *et al.* (2009) in *D. hamiltonii* in axillary shoot proliferation plants using RAPD markers.
- Only Mehta *et al.* (2010) reported low level of variation in *B. nutans* somatic embryo regenerated plants using AFLP.

Bambusa bambos (L.) Voss

- **Distributed through** out the country
- Occupies **second position** after *D. strictus* in terms of total bamboo forest area
- Attains a height of 15-30 meters, internode long (20-40 cm), thick walled
- Flowering cycle is 44-49 years
- **Gregarious flowering** seen in Coorg district of Karnataka during 1977-79 (Singhal and Gangopadhyay, 1999)
- **Traditional propagation** through seeds and **vegetative propagation** by offset cutting, rhizome, culm and branch cuttings

Somatic embryogenesis

Callus was initiated from the nodal shoot segments of *in vitro* axillary shoots, initiated from the mature CPC-1 material from bamboo germplasm bank, IWST field research station, Gottipura.

Experiments carried out:

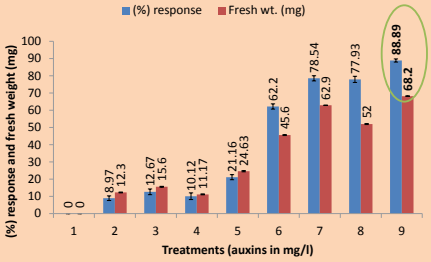
1. Effect of **auxins and their different concentration** on **callus induction**
2. Effect of **auxins and their different concentration** on **callus multiplication**
3. Effect of **types of carbohydrates and different concentration** on **callus multiplication**
4. Effect **of different sucrose concentration** on **callus multiplication**
5. Effect of **various PGR's and their different concentration** on **somatic embryo induction**
6. Effect of various **PGRs and their different concentrations** on **embryo maturation and germination**

Callus induction

1. Effect of **auxins and their different concentration** in MS medium with additives* on **callus induction** from nodal shoot segments

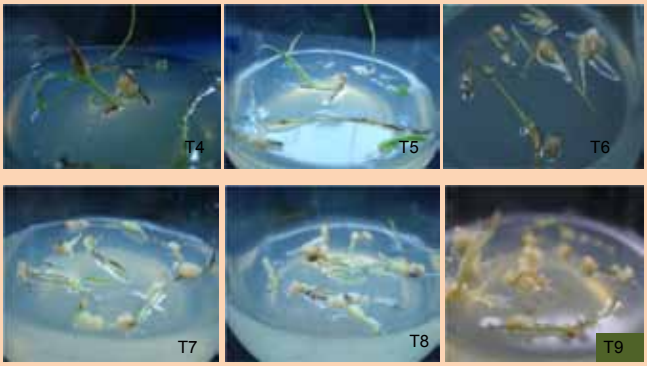
T. No.	Auxins (mg/l)
T1	HF
T2	IBA 1.0
T3	IBA 2.5
T4	NAA 1.0
T5	NAA 2.5
T6	2,4-D 1.0
T7	2,4-D 2.5
T8	2,4,5-T 1.0
T9	2,4,5-T 2.5

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



T9 (2,4,5-T, 2.5mg/l) proved best (88.89 %) for callus induction with **fresh weight of 68.2 mg.**

Callus initiation from nodal shoot segment on MS medium with additives + auxins



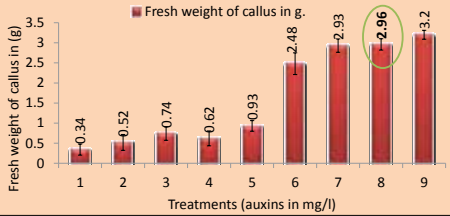
T4	NAA 1.0	T5	NAA 2.5	T6	2,4-D 1.0
T7	2,4-D 2.5	T8	2,4,5-T 1.0	T9	2,4,5-T 2.5

Callus multiplication

2. Effect of **various auxins** and different concentrations on **callus multiplication** in MS medium with additives*

T. No.	Auxins (mg/l)
1	HF
2	IBA 1.0
3	IBA 2.5
4	NAA 1.0
5	NAA 2.5
6	2,4-D 1.0
7	2,4-D 2.5
8	2,4,5-T 1.0
9	2,4,5-T 2.5

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



T8 (2,4,5-T 1.0 mg/l) proved best for callus multiplication with **fresh weight of 2.96 g.**

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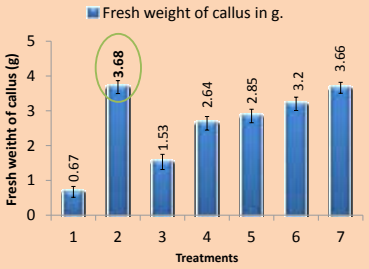
Callus multiplication

3. Effect of **carbohydrates on callus multiplication** in MS medium with additives* + 2,4,5-T 1.0 mg/l

T. No.	Carbohydrate (%)
T1	Control (Without carbohydrates)
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%

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



Among carbohydrates **3% sucrose** proved best for callus multiplication with fresh weight of **3.68 g**.

Callus multiplication

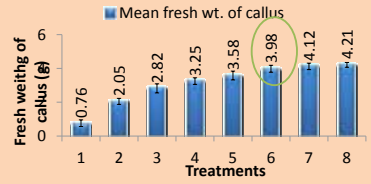
4. Effect of **sucrose concentration** on **callus multiplication** on MS medium with additives* + 2,4,5-T 1.0 mg/l

Callus morphology

1. Mucilagenous
2. Compact

T. No.	Sucrose concentration (%)
T1	Control (Without sucrose)
T2	Sucrose 2%
T3	Sucrose 3%
T4	Sucrose 4%
T5	Sucrose 5%
T6	Sucrose 6%
T7	Sucrose 7%
T8	Sucrose 8%

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



6% sucrose proved best for callus multiplication with fresh weight of 3.98 g.

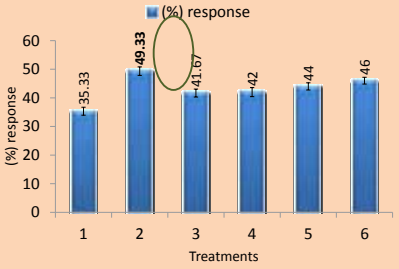
Mucilageous callus Embryogenic callus

Somatic embryo induction

5. Effect of various **PGRs, CM (10%)** and their concentration on **somatic embryo induction** on MS medium with additives*

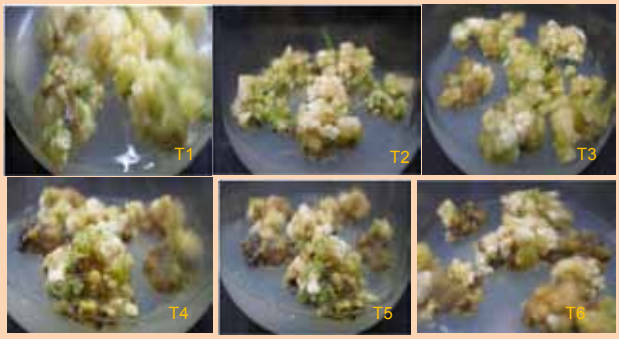
T. No.	Treatments (PGRs) in mg/l
T1	HF
T2	10% CM
T3	Kinetin 1.0+NAA 1.0
T4	Kinetin 2.0+NAA 1.0
T5	BAP 1.0+NAA 1.0
T6	BAP 2.0+NAA 1.0

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



Maximum **(49.33%) somatic embryo induction** was in MS medium with **10 % CM.**

Effect of various PGRs and their concentration on somatic embryo induction on MS medium with additives*



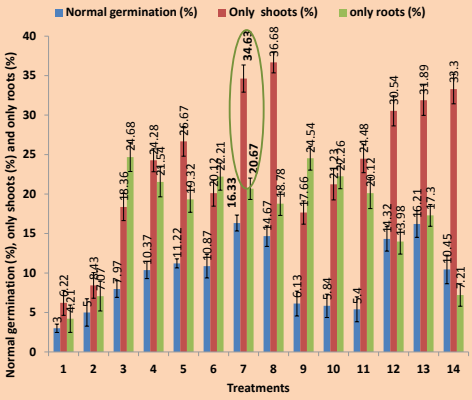
T1	HF	T2	10% CM	T3	Kinetin 1.0+NAA 1.0
T4	Kinetin 2.0+NAA 1.0	T5	BAP 2.0+NAA 1.0	T6	BAP 2.0+NAA 1.0

Somatic embryo maturation and germination

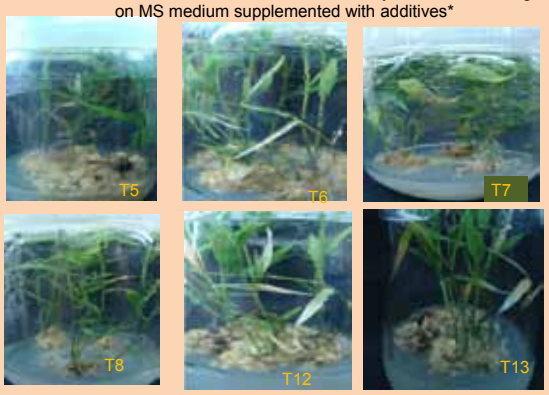
Effect of various **PGRs and their concentrations** on **embryo maturation and germination** on MS medium supplemented with additives*

T. No.	Treatments (CM in % and PGRs in mg/l)
T1	HF
T2	HF+ 10% CM
T3	Kinetin 1.0+NAA 1.0
T4	Kinetin 2.0+NAA 1.0
T5	Kinetin 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 2.5+IAA 1.0
T13	BAP 2.5+ IBA 1.0
T14	BAP 2.5+NOA 1.0

*Additives: Ascorbic acid 50 mg/l, Citric acid 25 mg/l, Cystine 25 mg/l and Glutamine 100 mg/l



Effect of various PGRs and their concentrations on embryo maturation and germination on MS medium supplemented with additives*



T5	Kinetin 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	T12	BAP 2.5+IAA 1.0	T13	BAP 2.5+ IBA 1.0

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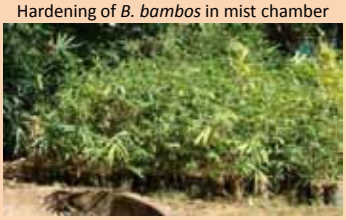
Hardening of micropropagated plants in mist chamber

- Transplanting was done in potting media containing sand: soil: compost (4:1:5)
- 100 % survival was observed in mist chamber

Hardening of *B. bambos* in mist chamber



Hardened *B. bambos plants* at open nursery stage

Different stages of Somatic embryogesis from embryogenic callus

Callus initiation | Callus multiplication | Somatic embryo induction | Somatic embryo maturation and germination | Somatic embryo plantlet

Genetic fidelity using RAPD and ISSR markers over a period of two years

The callus initiated was sub cultured periodically at every 25-30 days on callus multiplication medium for a period of 24 months

At every 6 months interval, somatic embryo regeneration was undertaken to evaluate the genetic fidelity studies at 6th, 12th, 18th and 24th month time intervals

- **DNA extraction**
- DNA of the mother plants (Germplasm bank, Gottipura field research station, IWST).
- 10 % of the micropropagated plants were randomly selected for DNA extraction from mist chamber hardened plantlets of both the species
- DNA was extracted at 6th, 12th, 18th and 24th months interval

RAPD and ISSR PCR reaction mixture and PCR cycles

PCR reaction mixture (25 µl)			
Sl. No.	Master mix	Volume	Conc.
1	Water	13.7 µl	-
2	PCR buffer (10X) (sigma, USA)	2.5 µl	1 X
3	dNTPs (sigma, USA)	1.0 µl	10 mM
4	$MgCl_2$ (sigma, USA)	2.5 µl	2.5 mM
5	Primer (sigma, USA)	2.5 µl	10 mM
6	Taq polymerase (sigma, USA)	0.3 µl	1.2 Units
7	Genomic DNA	2.5 µl	30 ng
	Total volume	**25 µl**	

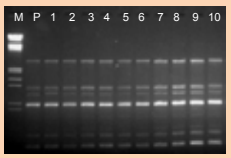
RAPD Cycles	ISSR Cycles
40	35

Primer details of amplified DNA of *B. bambos*

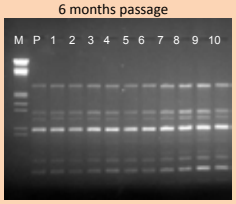
B. bambos	ISSR	RAPD	
	UBC-800 series	OPR	OPD
Total Number of primers used for screening	51	20	20
Number of primers amplified	31	18	10
Total number of loci	188	51	86
Lowest no. of bands (Primer no.)	2 (UBC – 842)	2 (OPR – 5)	1 (OPR - 15)
Highest no. of bands (Primer no.)	12 (UBC – 816)	10 (OPR-12)	9 (OPR - 3)
Average no. of bands	6.06	2.83	8.6

Genetic fidelity of *B. bambos* plants raised through somatic embryogenesis with ISSR UBC 817 primer representing monomorphic banding pattern

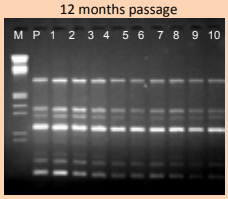
Monomorphic banding pattern at 6 months passage



Monomorphic banding pattern at 12 months passage



Monomorphic banding pattern at 18 months passage



Monomorphic banding pattern at 24 months passage

M	100bp ladder
P	Parent
1-10	progeny

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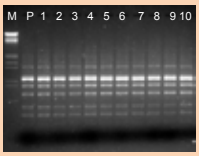
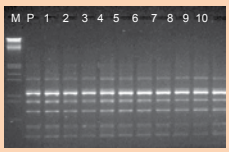
Genetic fidelity of *B. bambos* plants raised through somatic embryogenesis with ISSR UBC 841 primer representing monomorphic and polymorphic banding pattern

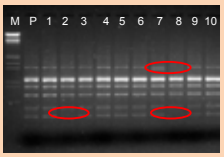


Monomorphic banding pattern at 6 months passage

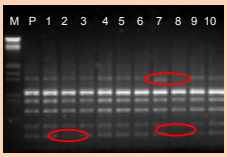


Monomorphic banding pattern at 12 months passage

M	100bp ladder
P	Parent
1-10	progeny



Polymorphhic banding pattern at 18 months passage



Monomorphic banding pattern at 24 months passage

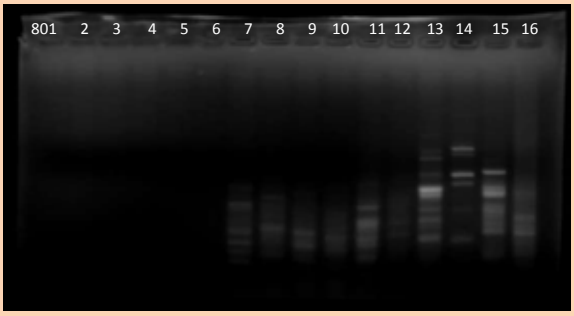
(%) of genetic stability at 6th, 12th, 18th and 24th months of passage by ISSR and RAPD markers

Species	Micropropagation method	DNA marker	(%) of genetic stability at different stages			
			6 months	12 months	18 months	24 months
B. bambos	Somatic embryo planelets	ISSR	100.0	100.0	97.82	97.12
		RAPD	100.0	98.23	97.65	96.97

Screening of ISSR UBC 800 primers series in genomic DNA of *B. bambos*

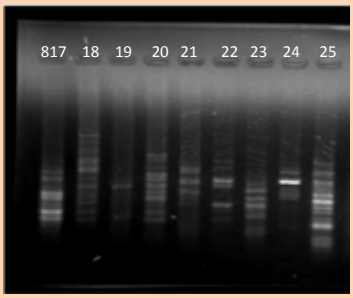
Sl. No.	Primer	Sequence	Tm	No. of bands amplified in screening
1	UBC-801	ATA TAT ATA TAT ATA TT	24.2	0
2	UBC-802	ATA TAT ATA TAT ATA TG	24.9	0
3	UBC-803	ATATATATATATATATC	23.8	0
4	UBC-804	TAT ATA TAT ATA TAT AA	23.2	0
5	UBC-805	TATATATATATATATAC	21.9	0
6	UBC-806	TAT ATA TAT ATA TAT AG	22.5	0
7	UBC-807	AGA GAG AGA GAG AGA GT	42.5	5
8	UBC-808	AGA GAG AGA GAG AGA GC	46.8	7
9	UBC-809	AGA GAG AGA GAG AGA GG	46.6	2
10	UBC-810	GAGAGAGAGAGAGAGAT	42.9	0
11	UBC-811	GAG AGA GAG AGA GAG AC	43.3	7
12	UBC-812	GAG AGA GAG AGA GAG AA	44.3	0
13	UBC-813	CTCTCTCTCTCTCTCTT	43.5	7
14	UBC-814	CTC TCT CTC TCT CTC TA	41.3	7
15	UBC-815	CTCTCTCTCTCTCTCTG	44.9	3
16	UBC-816	CACACACACACACACAT	51.1	12
17	UBC-817	CACACACACACACACAA	52.7	6
18	UBC-818	CACACACACACACACAG	52.1	8
19	UBC-819	GTGTGTGTGTGTGTGTA	47.6	0
20	UBC-820	GTGTGTGTGTGTGTGTC	50.3	6
21	UBC-821	GTG TGT GTG TGT GTG TT	49.9	3
22	UBC-822	TCT CTC TCT CTC TCT CA	45.8	6
23	UBC-823	TCTCTCTCTCTCTCTCC	47.5	5
24	UBC-824	TCTCTCTCTCTCTCTCG	49	3
25	UBC-825	ACACACACACACACACT	49.2	11
26	UBC-826	ACA CAC ACA CAC ACA CC	53.3	11
27	UBC-827	ACACACACACACACACG	54.9	0
28	UBC-828	TGTGTGTGTGTGTGTGA	53.2	10
29	UBC-829	TGTGTGTGTGTGTGTGC	56.3	0
30	UBC-830	TGTGTGTGTGTGTGTGG	56.1	0
31	UBC-831	ATA TAT ATA TAT ATA TYA	25.8	0
32	UBC-832	ATA TAT ATA TAT ATA TYC	27.6	0
33	UBC-833	ATA TAT ATA TAT ATA TYG	28.6	0
34	UBC-834	AGA GAG AGA GAG AGA GYT	45.4	5
35	UBC-835	AGA GAG AGA GAG AGA GYC	45.6	8
36	UBC-836	AGAGAGAGAGAGAGAGYA	43.3	9
37	UBC-837	TATATATATATATATART	25.8	0
38	UBC-838	TATATATATATATATARC	25.4	0
39	UBC-839	TATATATATATATATARG	26.5	0
40	UBC-840	GAG AGA GAG AGA GAG AYT	45.8	6
41	UBC-841	GAG AGA GAG AGA GAG AYC	46	7
42	UBC-842	GAGAGAGAGAGAGAGAYG	47.2	2
43	UBC-843	CTC TCT CTC TCT CTC TRA	44.3	2
44	UBC-844	CTCTCTCTCTCTCTCTRC	46.5	8
45	UBC-845	CTCTCTCTCTCTCTCTRG	47.7	4
46	UBC-846	CAC ACA CAC ACA CAC ART	53.7	0
47	UBC-847	CACACACACACACACARC	54.7	0
48	UBC-848	CAC ACA CAC ACA CAC ARG	55.5	4
49	UBC-849	GTGTGTGTGTGTGTGTYA	50.5	2
50	UBC-850	GTGTGTGTGTGTGTGTYC	53	3
51	UBC-852	TCT CTC TCT CTC TCT CRA	44.5	9
		Total Bands ampilifed		188

Screening of ISSR 800 series primers of genomic DNA of *B. bambos*



M	100bp ladder
801 to 16	UBC primer number

Screening of ISSR 800 series primers of genomic DNA of *B. bambos*



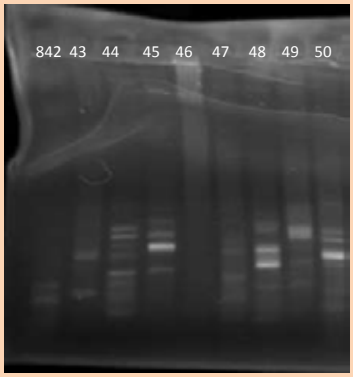
M	100bp ladder
817 to 25	UBC primer number

Screening of ISSR 800 series primers of genomic DNA of *B. bambos*

M	100bp ladder
817 to 25	UBC primer number

[440]

Screening of ISSR 800 series primers of genomic DNA of *B. bambos*



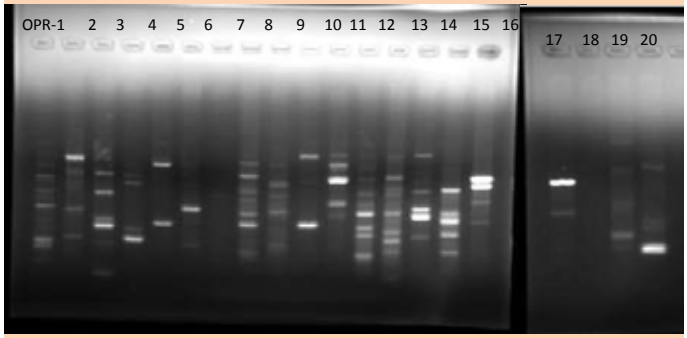
M	100bp ladder
817 to 25	UBC primer number

Screening of RAPD primers of OPR & OPD series in genomic DNA of *B. bambos*

Sl. No.	Primer	Sequence	Tm	No. of bands amplified in screening
1	OPD-01	ACCGCGAAGG	37.8	0
2	OPD-02	GGACCCAACC	33.1	0
3	OPD-03	GTCGCCGTCA	38.2	9
4	OPD-04	TCTGGTGAGG	29.4	0
5	OPD-05	TGAGCGGACA	34.4	8
6	OPD-06	ACCTGAACGG	32	8
7	OPD-07	TTGGCACGGG	37.8	0
8	OPD-08	GTGTGCCCCA	36.7	6
9	OPD-09	CTCTGGAGAC	27.1	0
10	OPD-10	GGTCTACACC	27.6	0
11	OPD-11	AGCGCCATTG	32	4
12	OPD-12	CACCGTATCC	32	0
13	OPD-13	GGGGTGACGA	34	4
14	OPD-14	CTTCCCCAAG	32	0
15	OPD-15	CATCCGTGCT	32.9	1
16	OPD-16	AGGGCGTAAG	31.3	1
17	OPD-17	TTTCCCACGG	32.4	0
18	OPD-18	GAGAGCCAAC	29.7	6
19	OPD-19	CTGGGGACTT	29.6	0
20	OPD-20	ACCCGGTCAC	36.1	4
		Total amplified bands		51

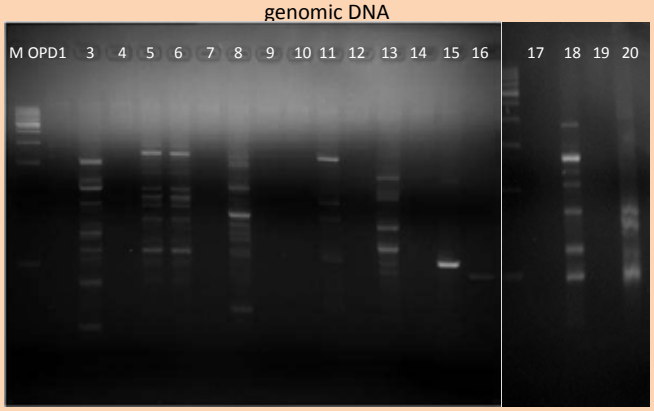
Sl. No.	Primer	Sequence	Tm	No. of bands amplified in screening
1	OPR-1	TGCGGGTCCT	38.1	8
2	OPR-2	CACAGCTGCC	35.5	5
3	OPR-3	ACACAGAGGG	30	4
4	OPR-4	CCCGTAGCAC	34.1	4
5	OPR-5	GACCTAGTGG	26.8	2
6	OPR-6	GTCTACGGCA	31.8	2
7	OPR-7	ACTGGCCTGA	32.8	0
8	OPR-8	CCCGTTGCCT	37.1	8
9	OPR-9	TGAGCACGAG	32.6	8
10	OPR-10	CCATTCCCCA	30.2	2
11	OPR-11	GTAGCCGTCT	31	5
12	OPR-12	ACAGGTGCGT	35.6	10
13	OPR-13	GGACGACAAG	30.4	8
14	OPR-14	CAGGATTCCC	27.8	5
15	OPR-15	GGACAACGAG	30.4	5
16	OPR-16	CTCTGCGCGT	38.7	4
17	OPR-17	CCGTACGTAG	29.2	2
18	OPR-18	GGCTTTGCCA	33.8	0
19	OPR-19	CCTCCTCATC	26.8	2
20	OPR-20	ACGGCAAGGA	34.7	2
		Total amplified bands		86

RAPD DNA banding pattern of *D. stocksii* of OPR 1-20 primers of genomic DNA



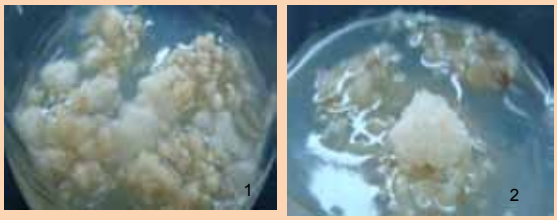
M	100bp ladder
OPR 1-20	OPR primer number

RAPD DNA banding pattern of *D. stocksii* of OPD 1-20 primers of genomic DNA



M	100bp ladder
OPD 1-20	OPD primer number

Morphological variation in *B. bambos* callus after 20 months of sub culture



1. Conversion of compact to friable callus; 2. Growth of friable callus

Comparison of genomic DNA of mother plants, compact callus and friable callus with ISSR UBC primer series in *B. bambos*

M	100bp ladder	1	Mother plant	2	Compact callus	3	Friable callus

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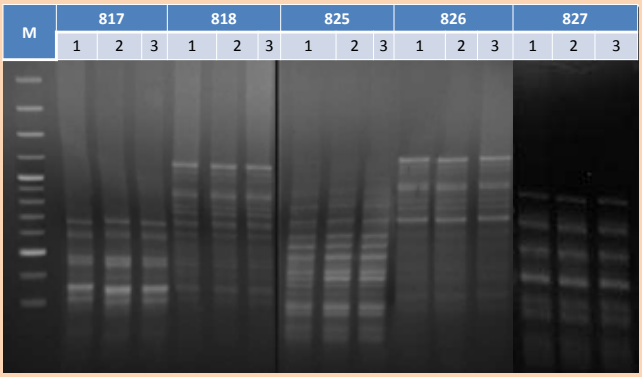
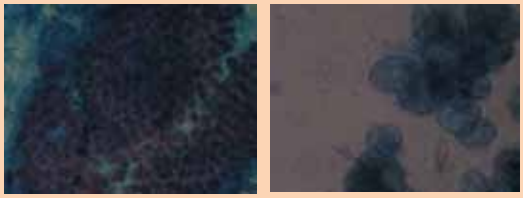
Comparison of genomic DNA of nursery plants, compact callus and friable callus with ISSR primers *B. bambos*



M	100bp ladder	1	Mother plant	2	Compact callus	3	Friable callus

Embryogenic and non embryogenic callus distinguish by double staining

Gupta and Homstrom (2005) reported distinguishing of embryogenic callus and non embryogenic callus by double staining by acetocarmine and Evan's blue



Embryogenic callus showing prominent nucleus in compact callus

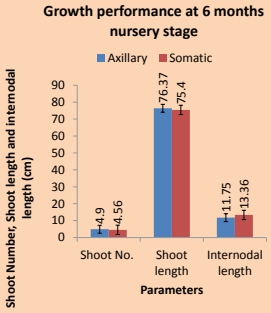
Non-embryogenic callus showing nucleus in compact callus

The prominent nucleus of embryogenic callus is stained by Acetocarmine, whereas, non embryogenic cytoplasm get stained by Evan's blue

Morphological parameters

- Growth performance at nursery stage
- Chlorophyll content
- Leaf area

Comparison of growth performance of axillary and somatic embryogenesis micropropagated plants at 6 months nursery stage



Chlorophyll content (mg/g of dry wt.)	Chlorophyll A	Chlorophyll B	Total Chlorophyll content
Axillary	8.74	2.78	11.53
Somatic embryo	8.57	2.93	11.41

Leaf area	Axillary proliferated	21 cm^2
	Somatic embryo proliferated	20.72 cm^2

No morphological variations was observed in plantlets raised by axillary and somatic embryogenesis after 6 months at nursery

Nursery plants

6 months old *B. bambos* plants at nursery

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Regeneration status of Khasi pine in Meghalaya

Dr. Nawa Bahar
Scientist

Silviculture Division
Forest Research Institute
Dehradun

Brief description about the species

- Khasi pine is an important tree species of North - Eastern Region and belongs to the family Pinaceae.
- Khasipine 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 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.

Khasi pine

Pinus kesiya (Khasi pine) is becoming a very important timber wood producer, particularly in southern Africa and other tropical regions. Wood properties of straight trees are often superior to those of other pine species. Selective breeding can reduce the problems associated with the species, such as poor form and coarse branches. Because the tree grows in a variety of soil and is tolerant to various pests and climatic conditions, it is possible to grow the tree widely in subtropical and tropical areas. The production rate is moderately high, due to its rapid growth rate, vigorous germination and propagation in favourable conditions. This species is not very successful in areas that have hot humid climates that are at low altitudes.

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Area under Khasi pine

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 khasi pine plantation up to the year 1998 with a share of 8.9% in various plantation programme in the region.

Uses of Khasi pine in North - East

- Khasi pine is very popular with the people of Meghalaya.
- People of the area use every part of the tree.
- Needles are used for stuffing mattresses, chair cushions, and cheap pillows and even as cementing fibre in the mud plastered wall.
- Needle litter in the forest is collected, burnt and used as soil correctives in potato beds.
- 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.
- Resinous wood is used as touch wood.
- Timber is used for house construction and cheap furniture.

Countries where Khasi pine planted

India (Andhra Pradesh, Arunachal Pradesh, Kerala, Manipur, Orissa and Tamil Nadu). Bangladesh, China, Taiwan, Malaysia, Peninsular Malaysia, Sabah, Sarawak, Philippines, Sri Lanka and Thailand, Angola, Cameroon, Congo, Cote d'Ivoire, Ethiopia, Madagascar, Malawi, Mali, Nigeria, South Africa, Swaziland, Tanzania, Togo, Uganda, Zambia and Zimbabwe, Jamaica and Puerto Rico, El Salvador, USA, Hawaii, Brazil, Minas Gerais, Sao Paulo, Guyana ,Venezuela,Australia, Australian Northern Territory, New South Wales, Queensland, New Caledonia, Papua New Guinea and Vanuatu.

Common names

- **English:** Khaya pine, Khasi pine, Benguest pine
- **French:** pin-a-trois-feuilles
- **India**: dingsa, ding-se, dieng-kysi, far, saral
- **Myanmar:** tinyu
-
- **Other names used:** *Pinus insularis* Endl
- *Pinus khasya*,F., orth.var.
-
- **Trade names**: Khasi pine.

Needles of Khasipine

A

B

C

Needles of this species are dark green in colour, soft, usually with 3 needles in a fascicle at the tip of short twigs. Needles of adult trees are 10 - 20 cm. long, bright green, margin finely serrated, stunted on shorter trees, slender, in fascicles of three (rarely two or four).

Bark of Khasi pine

Bark is brownish, splitting or flaking in old trees. Adult trees have 2.5 - 4.5 cm thick bark, deeply fissured and pinkish to reddish – grey, flaking in small, thick and irregular.

Natural population of khasipine

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Natural population of Khasi pine


Cone characteristics of Khasi pine

Maturation period of cones

February - March

Source: Nawa Bahar(2010). Seed biology of Indian pines, Ann. For., 18(1): 39 - 46.

Immature cones of Khasi pine


Cone characteristics of Khasi pine

- Cone length (cm) = 8.60
- Cone width(cm)=4.85
- Cone fresh wt (g) = 65.95
- Number of scale/cone = 74.80
- Specific gravity = 0.85
- Number of seed/cone = 72.40

Source: Nawa Bahar(2010). Seed biology of Indian pines, Ann. For., 18(1): 39 - 46.

Note: Mean value of characters

Matured cones of Khasipine



Seed biology of Khasi pine

- Seeds extracted/cone (g) = 3.88
- Seed yield(%) = 5.88
- Seed pure line (%) = 90.05
- Seed length (mm) = 7.65
- Seed breadth(mm) = 4.33
- 1000 seed wt(g) =16.93
- Purity (%) = 92.60
- Number of seeds /kg = 59,066

Source: Nawa Bahar(2010). Seed biology of Indian pines, Ann. For., 18(1): 39 - 46.

- Note: Mean value of characters

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Seed germination stages

- Germination (%) = 97.25
- Germination value = 27.42
- Mean germination time (days)= 7.25

Source: Nawa Bahar(2010). Seed biology of Indian pines, Ann. For., 18(1): 39 - 46.

Note: Mean value of characters

Seedling vigour index (SVI)

- Seedling collar dia(mm) = 2.11
- Shoot length(cm) = 4.53
- Root length (cm) = 5.87
- Number of cotyledons = 8.44
- Dry matter production/seedling(mg) = 82.0
- Seedling vigour index(length basis) = 1011
- Seedling vigour index(wt. basis) = 7974

Source: Nawa Bahar(2010). Seed biology of Indian pines, Ann. For., 18(1): 39 - 46.

Note: Mean value of characters

REGENERATION

"Renewal of a forest crop by natural or artificial means."

Natural regeneration

(by self –sown seed/coppice/root sucker)

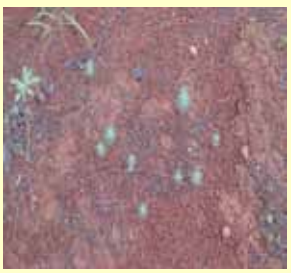
Artificial regeneration

(sowing, planting or other artificial methods/ plantation).

Natural regeneration of Khasi pine

Natural regeneration of Khasipine

Natural regeneration of Khasipine



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Seedling vigour index


Natural Regeneration of Khasipine

Natural regeneration


Natural regeneration


Natural regeneration


Natural regeneration


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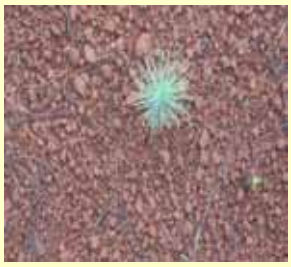
Seedling characters

Normal Seedlings

The healthy seedlings with all the essential structures viz., root, hypocotyle, shoot apex and cotyledons developed in proper proportions.



Seedlings growth of Khasi pine

Growth of apical buds

Natural regeneration at NEHU Campus

- Collar diameter (mm) = 4.25
- Height (cm) = 8.66
- Biomass (g) = 7.84
- Density (Seedling / m2) = 22.36
- Name of site = NEHU
- Altitude (m) = 1680
- Status of plot = Cleaned

Natural regeneration at Barapani

- Collar diameter (mm) = 5.11
- Height (cm) = 10.36
- Biomass (g) = 12.68
- Stock Quality Index = 6.25
- Density (seedling / m2) = 22.68
- Altitude (m) = 1120
- Latitude (°N) = 25° 30′
- Longitude (°E) = 91° 30′

Natural regeneration at Barapani

- Collar diameter (mm) = 6.58
- Height (cm) = 11.84
- Biomass (g) = 19.36
- Density (seedling / m2) = 9.24
- Altitude (m) = 1180
- Litter thickness (cm) = 5.25

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Characteristics of natural seedling

- Collar diameter (mm) = 3.85
- Height (cm) = 6.85
- Biomass (g) = 6.54
- Density (seedling / m2) = 35.68
- Name of site = NEHU
- Altitude (m) = 1685
- Status of plot = Cleaned

Natural regeneration at NEHU campus

- Collar diameter (mm) = 5.21 ± 1.25
- Height (cm) = 11.45 ± 2.62
- Biomass (g) =14.88 ± 2.54
- Stock Quality Index = 6.79
- Density (Seedling / m2) =29.66 ± 6.44
- Name of site = NEHU
- Altitude (m) =1680
- Latitude (°N) = 25° 39′
- Longitude (°E) = 90° 35′
- Status of plot = Cleaned

Natural regeneration of Khasipine

- Collar diameter (mm) = 4.36 ± 1.98
- Height (cm) = 9.68 ± 2.14
- Biomass (g) = 9.55
- Density (seedling / m2) = 19.50
- Name of site = NEHU
- Altitude (m) = 1745
- Status of plot = Cleaned

Bud bursting of khasi pine

Root shoot ratio of seedling

Adverse factor for natural regeneration

Undergrowth

- Heavy weed growth in khasipine forests is considered to be the most important adverse factor for its natural regeneration.
- Weeds check the growth of the seedling through root competition and suppression.
- Perennial weeds form a thick mat of root and offer severe root competition.
- Tiny seedlings, when covered under a thick mat of stalks of weeds, are killed. This process repeated every year and does not allow natural regeneration to establish.
- Dense grass is generally very harmful to natural regeneration and in order to reduce its harmful effect, it has to be cut regularly.

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Adverse factor for natural regeneration

Poor seed production

The growing stock in khasi pine forests is mostly mature and over mature. Such trees of this pine are reported to produce inadequate quantities of seed for natural regeneration.

Adverse factor for natural regeneration

Grazing

Heavy grazing does more harms than good as the seedlings are trampled and killed.

Fire

Dry grasses get burnt and tiny seedlings might come up get killed in such fire.

Adverse factor for natural regeneration

Debris accumulation

Due to very slow rate of decomposition the debris continue accumulate on forest floor and affects the natural regeneration adversely.

Natural regeneration

Some others adverse factors:

- Quantity of seed collection by right holders.
- Seedling trampled by Sheep and Goats.
- Drought.
- Habitat degradation.
- Allelopathic effect.
- Seed eaten by Squirrel and Rats.
- Soil eroson
- Precipitation: Its germination and establishment is highly dependent on local precipitation and temperature and locally variable summer rains can also influence the distribution of seedling establishment.

Contribution in employment regeneration

Contribution in employment regeneration

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Contribution in employment regeneration


Contribution in employment regeneration


Contribution in employment regeneration


Contribution in employment regeneration


Contribution in employment regeneration


Contribution in employment regeneration


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Contribution in economy

Khasi pine also produces resin of a high quality, but it is not widely tapped because the tree does not yield very freely. The oleoresin is rich in pinenes, which comprise 21 per cent turpentine oil and 79 per cent rosin (Luna, 1996).

Source: Luna, R.K. (1996). Plantation Trees. International Book Distributors, Dehra Dun.

Profile of Speaker

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Designation: Scientist-B
Qualification: M.Sc. Ph.D (Botany)
Nationality: Indian
Postal Address: Forest Research Institute, Dehradun
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Publications:

Papers: More than 80research papers published in national and international journals of repute.
Book: (One)
Handbook: (One)
Booklet: (One)
Brochure (One)
Award: Brandis Prize in the field of forestry research

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

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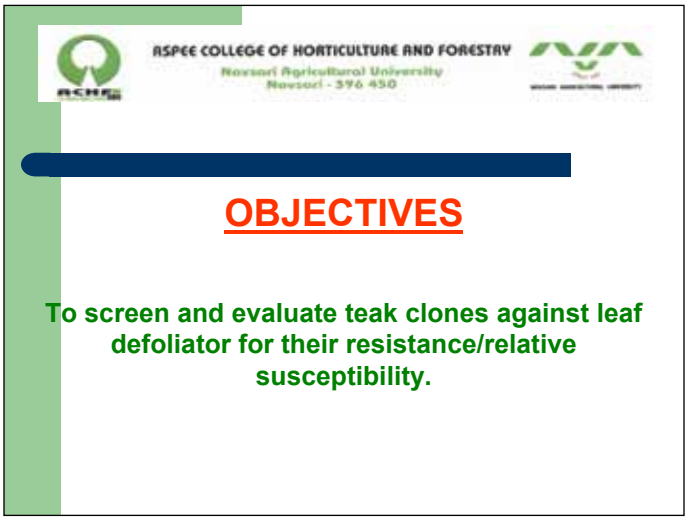

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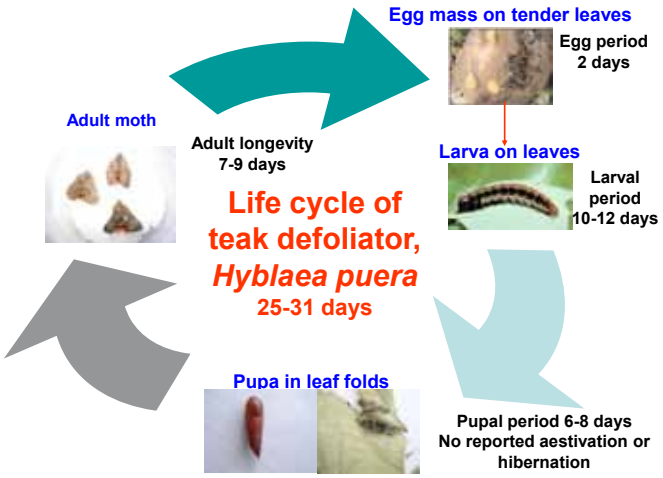
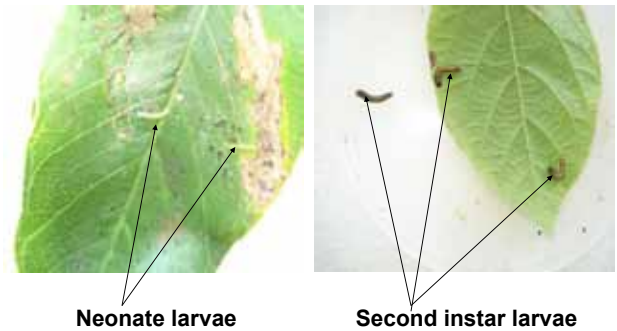
ASPEE COLLEGE OF HORTICULTURE AND FORESTRY

INTRODUCTION

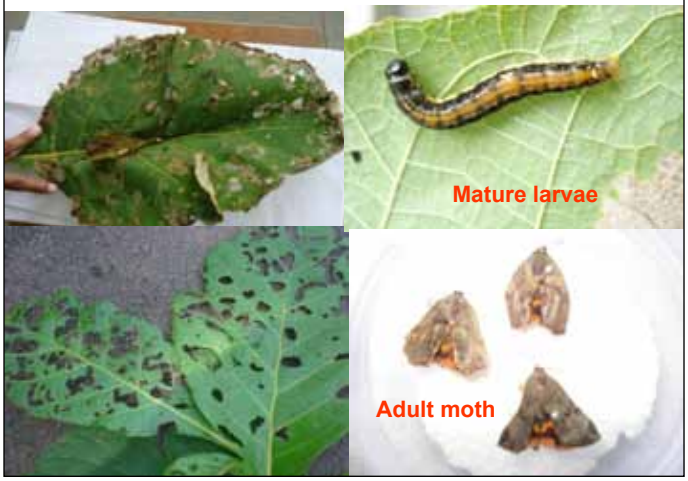
- The teak trees are attacked by 136 species of defoliators. The most important is teak defoliator, *Hyblaea puera* Cramer (Lepidoptera : Hyblaeidae) (Mathur and Singh, 1960).
- The pest is oligophagous and teak (*Tectona grandis*) is the principal host plant. (Mohandas, 1936).
- The attacking stage (Larva or caterpillar) of the pest coincides with the onset of rainy season and emergence of new flush in 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.

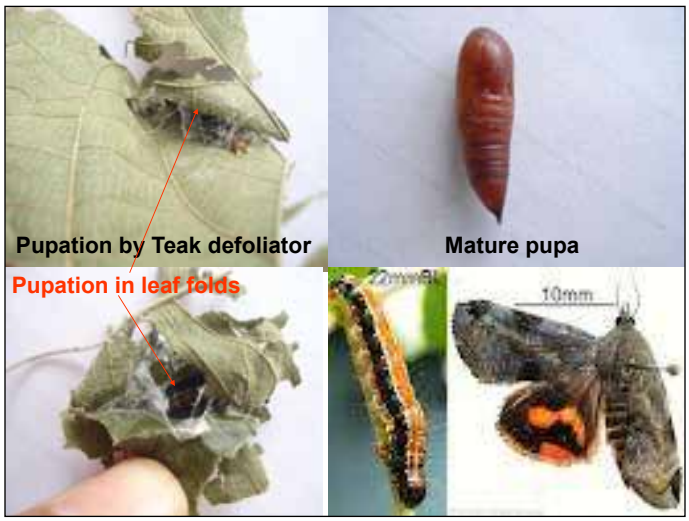


Young larvae of teak defoliator



FOLIAGE PEST –TEAK DEFOLIATOR





ASPEE COLLEGE OF HORTICULTURE AND FORESTRY

MATERIALS AND METHODS

- The experiments were conducted during 2007-2009 at Rajpipla in Narmada district of Gujarat state located at 21° 53' N latitude, 73° 31' E longitude and 45 meters above the mean sea level using 18 teak clones (TCR-1 to TCR-18) each replicated four times (2 trees/replication) in Randomized Block Design.
- Observations based on leaf damage were recorded from N-S and E-W parts of the lower tree canopy at fortnightly interval during June - December and at monthly interval during January – May.
- Five terminal twigs from each tree section were selected randomly and were observed for leaf damage by counting total and damaged leaves which was later calibrated into per cent leaf damage. On the same twig, larvae of the pest were also counted.

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RSPEE COLLEGE OF HORTICULTURE AND FORESTRY

MATERIALS AND METHODS (CONTD.)

Degree of resistance/susceptibility to leaf defoliator (*Hyblaea purea* Cramer) was assessed on the basis of susceptibility ratings.

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

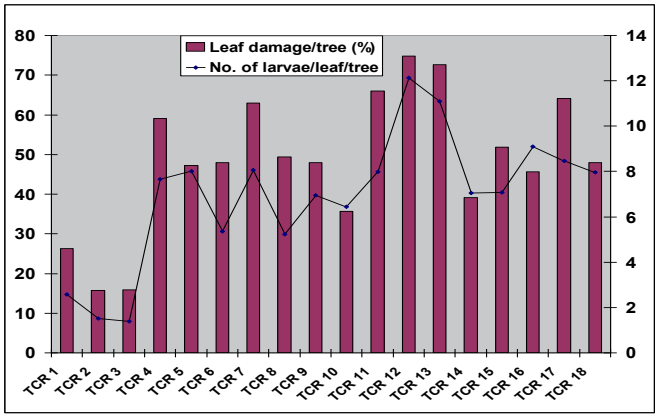
Table 2 : Leaf damage and larval population of leaf defoliator (*Hyblaea purea* Cramer) in teak clones at clonal teak seed orchard Rajpipla during 2007- 2009

Teak clone	Leaf damage /tree (%)	Susceptibility grade	No. of larvae /leaf/tree
TCR-1	26.31^{c*}	R_2	2.58^{abc*}
TCR-2	15.75^{a}	R_1	1.52^{ab}
TCR-3	15.86^{ab}	R_1	1.39^{a}
TCR-4	59.11^{l}	S_1	7.65^{fghij}
TCR-5	47.22^{fg}	R_3	8.02^{ghijklm}
TCR-6	47.94^{fghi}	R_3	5.36^{de}
TCR-7	62.91^{mn}	S_1	8.07^{ghijklmn}
TCR-8	49.33^{fghijk}	R_3	5.23^{d}
TCR-9	47.90^{fgh}	R_3	6.94^{fg}
TCR-10	35.72^{d}	R_2	6.44^{def}

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

Teak clone	Leaf damage /tree (%)	Susceptibility grade	No. of larvae /leaf/tree
TCR-11	66.06^{nop}	S_1	7.99^{ghijkl}
TCR-12	74.87^{qr}	S_2	12.13^{qr}
TCR-13	72.66^{q}	S_2	11.09^{q}
TCR-14	39.08^{de}	R_2	7.04^{fgh}
TCR-15	51.81^{ghijkl}	R_3	7.08^{fghi}
TCR-16	45.63^{f}	R_3	9.11^{op}
TCR-17	64.18^{no}	S_1	8.47^{o}
TCR-18	47.97^{fghij}	R_3	7.95^{ghijk}
S.Em ±	1.72	* Ranking as per DMRT.	0.43
C.D. at 5 %	4.86		1.25
C.V. (%)	6.16		10.93

Evaluation of teak clones against teak defoliator



RSPEE COLLEGE OF HORTICULTURE AND FORESTRY

CONCLUSIONS

- Lowest leaf damage (15.75 per cent) was observed in TCR-2 indicating tolerant/resistant reaction (R_1); through it did not differ significantly from TCR-3 (15.86) (R_1).
- TCR-12 remained most susceptible entry on the basis of highest leaf damage (74.87) indicating susceptible reaction (S_2), though it was similar to TCR-13 (72.66) (S_2).
- Lowest larval population (1.39) was observed in TCR-3 indicating tolerant/resistant reaction (R_1) followed by TCR-2 (1.52)(R_1).
- TCR-12, remained most susceptible entry harboring highest number of larvae (12.13/leaf/tree) indicating susceptible reaction (S_2), followed by 11.09 in TCR-13 (S_2).
- TCR-2 and TCR-3 were categorized as tolerant/resistant entries while TCR-12 was categorized as the most susceptible entry.

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Analysis of epigenetic changes in Jatropha using methylation sensitive AFLP

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New Delhi

Jatropha curcas

- *Jatropha curcas* belongs to family *Euphorbiaceae* having chromosome number 2n=22
- Identified as a major bio fuel crop by Planning commission of India (National biofuel mission-2003)
- It can be grown on arid and semiarid conditions
- Seeds contain non-edible oil (30-35%)
- Bio diesel is produced by trans-esterification of oil extracted from seeds
- Seed cake is used as manure

Cytosine methylation of DNA

- It is the widely studied epigenetic modification
- It is the modification of cytosine molecule by the transfer of methyl group from S-adenosyl methionine to the 5C position of the cytosine pyrimidine ring

Cytosine 5-Methyl Cytosine

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Methylation sensitivity of MspI and HpaII

MspI and HpaII show differential sensitivity to DNA methylation and display polymorphism in the digested DNA fragments through methylation sensitive- AFLP (MSAP)

Methylation	*Msp*I	*Hpa*II
mCCGG	No cleavage	No cleavage
CmCGG	Cleavage	No cleavage
CCGG	Cleavage	Cleavage
hmCCGG	No Cleavage	Cleavage

In MSAP, no cleavage = band absent

Using methylation sensitive AFLP in Jatropha- Questions

- Does MSAP show higher polymorphism than AFLP?
- What is the inheritance pattern of cytosine methylations?

Materials

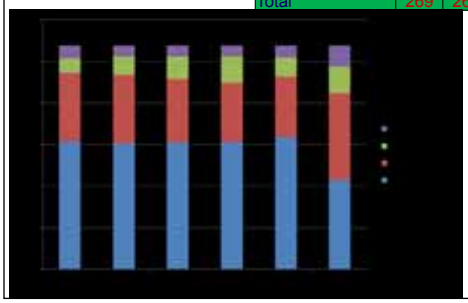
- **Dataset 1-** Diverse *Jatropha curcas* accessions (JIP74, 75, 76, 77, 07, 40)
- **Dataset 2-** *J. curcas, J. integerimma* and their interspecific hybrids (F1s)

Methods

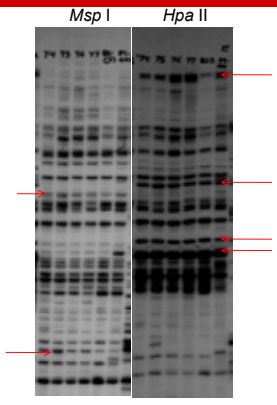
- **DNA isolation**: based on CTAB DNA isolation method, Doyle and Doyle,1990
- **AFLP analysis**: Modified Vos *et al.,*1995
- **Restriction enzymes**: *Eco*RI (rare cutter) and *Msp*I *and Hpa*II (methylation sensitive frequent cutter)
- **Selective amplification**: with 32P labelled *Eco*RI primers containing 2 to 3 selective nucleotides
- Polyacrylamide gel **electrophoresis** and **autoradiography**
- **Scoring** of binary data and analysis

Intra-specific diversity for cytosine methylation

	J74	J75	J76	J77	BL-3	T40
No site	154	151	152	152	159	107
Unmethylated	82	83	77	72	73	105
Methylated	18	22	27	32	23	32
Hemimethylated	15	13	13	13	14	25
Total	269	269	269	269	269	269



AFLP profiles of diverse accessions



AFLP profile of Jatropha showing methylation sensitivity of isoschizomers

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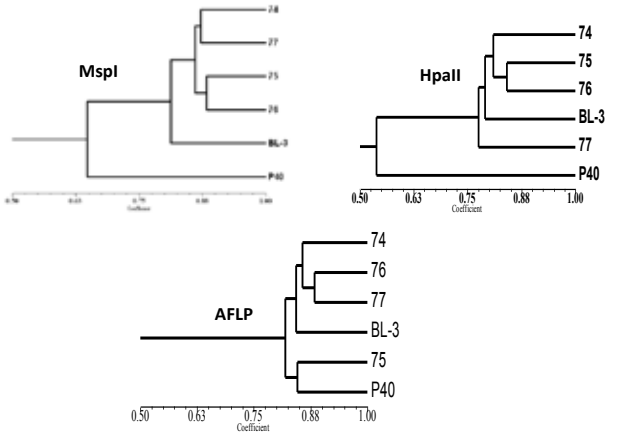
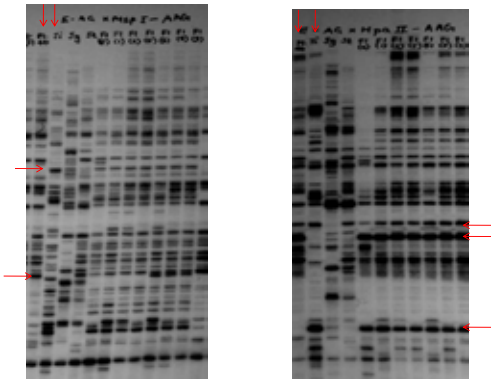
MSAP shows higher diversity than AFLP



Inheritance pattern of parental bands



AFLP profile of Jatropha showing methylation sensitivity of isoschizomers

Experimental findings

•A total of 212 bands were scored. Each band was present at least in one of the parents

•A total of 43 sites were methylated in the F1 hybrid. For 29 sites of these, at least one of the parents had methylations

•Two of these sites were hemimethylated in the parents

•Twelve sites were either not present in one of the parent or were demethylated in the parents

•Out of total 19 sites which were hemimethylated in the F1, 18 were also hemimethylated in one or both parents

Contd..

Contd..

- A total of 83 sites were unmethylated in the F1. Out of these 77 were either unmethylated in both the parents or were absent in one parent and unmethylated in the other.
- 91 out of 155 sites in F1 had same methylation status as that of *J. curcas* parent
- 76 out of 151 sites in F1 had same methylation status as that of *J. integerrima* parent

Inheritance of methylation pattern seems to be more similar to *J. curcas* parent

Conclusion

- MSAP shows higher diversity than AFLP. Some of these methylations have implications in phenotype also
- The inheritance of methylation pattern seems to be more similar to *J. curcas* parent

Thank you…

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Identification of chloroplast & nuclear microsatellite markers in *Pinus roxburghii*, *Pinus kesiya*, *Pinus wallichiana* and *Pinus gerardiana* through cross-species amplification

Priti Chauhan

Division of Genetics & Tree Propagation
Forest Research Institute
Dehradun

Simple sequence repeats (SSR)/ Microsatellites

Simple sequence repeats are present in the genomes of all eukaryotes and consist of repeats of 1-6 nucleotide motifs

Mono : A, T Di : AT, GA Tri : CGA Tetra: ATGC

AAAAAAAAAAAAAA = (A)15

ATATATATATAT = (AT)6

CGACGACGACGA = (CGA)4

ATCGATCGATCG =(ATCG)3

At a given microsatellite, different individuals can have different number of repeats.

Changes in the number of repeats result from mutation.

Trans-specific amplification

The highly polymorphic nature of microsatellites, frequent occurrence and an even distribution throughout the nuclear genome, presence in chloroplast (Vendramin *et al.*, 1996) and mitochondrial genomes (Soranzo *et al.*, 1999) has made microsatellites as the marker of choice in many diversity studies.

The usefulness of genomic SSRs is well established, development of SSRs from genomic DNA is costly, labour intensive, time consuming, and in some cases, the primers for PCR amplification are species specific.

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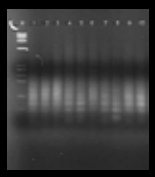
Objectives

Identification and development of microsatellite (SSR) markers for *P. roxburghii, P. kesiya, P. wallichiana* and *P. gerardiana* through trans-specific microsatellite amplification.

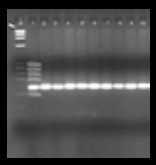
For amplification a total of 80 primer pairs comprising of 33 cpSSRs and 47 nuclear SSRs were tested on *Pinus roxburghii, Pinus kesiya, Pinus wallichiana and Pinus gerardiana*

Species	Author	cp/nuclear
Pinus thunberghii	Vendramin *et al.*, 1996	cpSSR
Pinus sylvestris	Provan *et al.*, 1999	cpSSR
Pinus resinosa	Boys *et al.*, 2005	Nuclear SSR
Pinus taeda	Zhou et al., 2002; Chagne *et al.*, 2004; Elsik *et al.*, 2000	Nuclear SSR
Pinus merkussi	Nurtgahjaningsih *et al.*, 2005	Nuclear SSR
Pinus densiflora	Watanabe *et al.*, 2006	Nuclear SSR

- Protocols by



Dzialuk and Burczyk (2005)



Vendramin *et al.*, 1996

Optimization of reaction components
- DNA template concentration
- $MgCl_2$ Concentration
- Primer concentration

Optimization of thermal cycling parameters
- Annealing Temperature
- Number of cycles

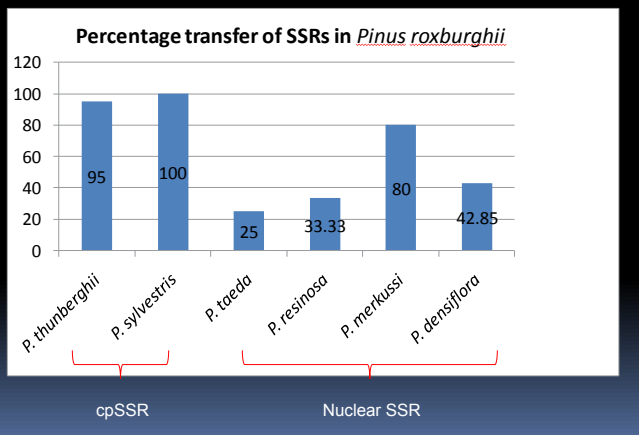
- PCR Amplification was performed as follows

- Step 1:
  Initial Denaturation : 5 min. at 95^{0}C 1 X

- Step 2:
  a. Denaturation : 1 min. at 94^{0}C
  b. Annealing : 1 min. at ^{0}C 30 X
  c. Extension : 1 min. at 72^{0}C

- Step 3:
  Final extension : 8 min. at 72^{0}C 1 X

Amplification products were then separated on 8% PAGE gels, stained with Ethidium Bromide and viewed on a U.V Transilluminator.



Gel image showing positive trans-specific amplification in *P. roxburghii* with primer Pt71936. Lane M: GeneRuler™ 100bp ladder, Lanes 1-10: individuals of *P. roxburghii*. The amplified product was in range of the product size (148bp) reported with primer Pt71936

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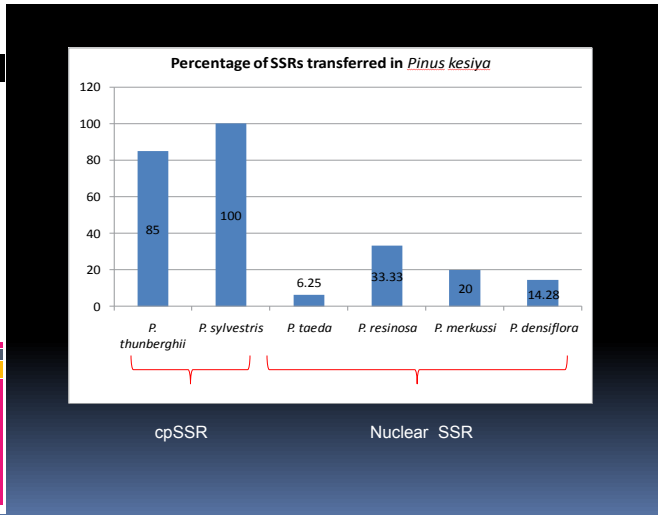
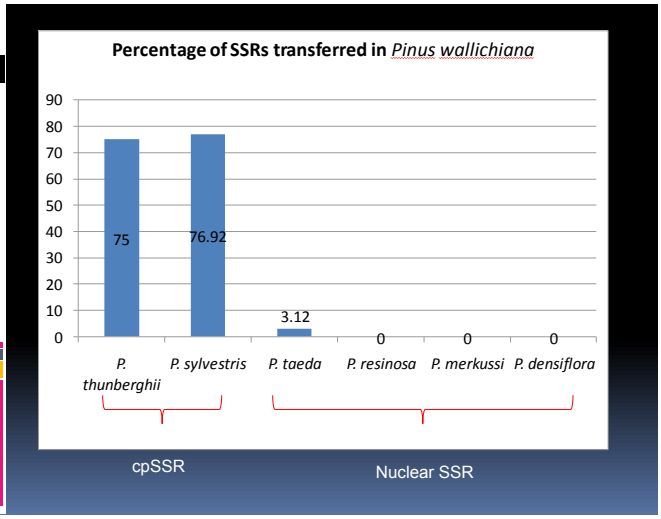
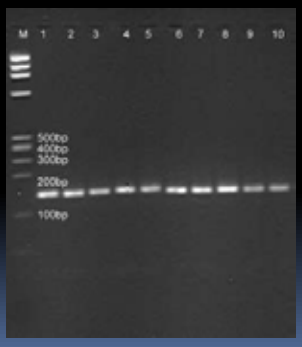
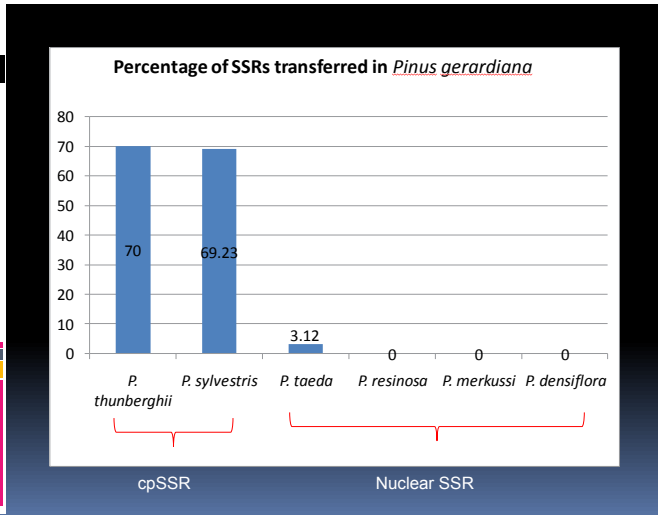


Gel image showing positive amplification in *P. kesiya* with primer PCP9434 developed for *P. sylvestris*. Lane M: O'GeneRuler™ Ultra low range DNA ladder, Lanes 1-10: individuals from *P. kesiya*. The amplified product was in range of the product size (131bp) reported with primer PCP9434

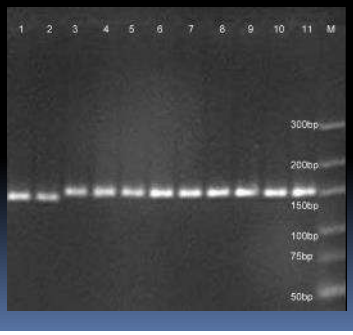


Gel image showing positive trans-specific amplification in *P. wallichiana* with primer PCP26106. Lane M: GeneRuler™ 100bp, Lanes 1-10: individuals from *P. wallichiana*. The amplified product was in range of the product size (149bp) reported with primer PCP26106





Gel image showing positive trans-specific amplification in *P. gerardiana* with primer PCP45071. Lane M: O'GeneRuler Ultra low range DNA ladder, Lanes 1-11: individuals from *P. gerardiana*. The amplified product was in range of the product size (149bp) reported with primer PCP 45071



list of primers 2003.xls

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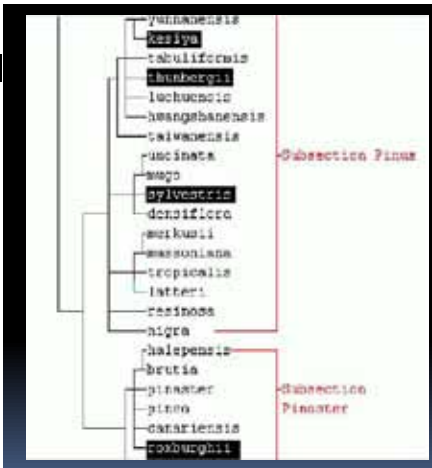

P. thunbergii, *P. sylvestris*, *P. kesiya* and *P. roxburghii* all belonged to the Section Pinus

The transference of nSSRs was more specific to the sections to which the source species of pine belonged.

P. resinosa, *P. densiflora* and *P. merkussi*
(Subgenus: Pinus; Section: Pinus; Subsection Pinus)

↓ No transfer

P. wallichiana
Subgenus : Strobus
Section:
Quinquefoliae
Subsection: Strobus

P. gerardiana
Subgenus : Strobus
Section:
Quinquefoliae
Subsection:
Gerardiana

P. taeda (section *Trifoliae*)

section Pinus
(*P. roxburghii* 25 % *P. kesiya*, 6.25 %)

distant section Quinquefoliae
(*P. wallichiana* and *P. gerardiana*, 3.12 % each)

rox.ppt

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IN VITRO REGENERATION OF *ACACIA MANGIUM WILLD.*

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ASPEE College of Horticulture and Forestry
Navsari Agriculture University, Navsari

INTRODUCTION

- Forestry products are the third most valuable commodity after oil and gas.
- essential that our forests managed sustainably for ourselves and for future generations
- *Acacia mangium* is of immense value for afforestation,
  - reclamation of wastelands
  - soil improvement,
  - for pulp, timber and fuel wood.
  - checking soil erosion
  - stabilization of sand dunes

- Seedling raised plantation exhibit a great deal of diversity in fibre content which is intolerable
- Regeneration through coppicing or pollarding is poor
- So there is urgent need of protocol for large scale production of quality planting material

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MATERIAL AND METHODS

EXPLANTS

↓

SURFACE STERILIZATION AND WASHING

↓

ESTABLISHMENT ON GROWTH MEDIUM

↓

PROLIFERATION OF SHOOTS

↓

IN VITRO ROOTING

↓

ACCLIMATIZATION

A) Phenotypically superior plus tree of *Acacia mangium*

B) Preparation of nodal explant for micropropagation

RESULTS

Table-1: Effect of types of cytokinin on shoot bud induction in *Acacia mangium*

Tr. No	Cytokinins		Establishment %	Number of shoots/explant	Length of longest shoot	Days taken for shoot bud initiation	Shoot vigor*
	BAP	TDZ					
T_1	1.0	0	64.4	1.3	0.9	26	++
T_2	1.5	0	65.5	2.7	1.4	21	++++
T_3	2.0	0	66.6	2.0	1.1	23	+++
T_4	0	1.0	50.0	1.3	0.4	25	++
T_5	0	1.5	54.4	2.3	0.8	26	++
T_6	0	2.0	58.9	1.3	0.9	32	++
SEm			3.27	0.30	0.10		
LSD 5%			10.07	0.93	0.33		

*shoot vigor was estimated on visual scale

+	++	+++	++++	++++
Very poor	poor	average	good	excellent

B) Malformed shoot (MS +1.5 mg/l TDZ)

C) Shoot bud induction (MS+1.5 mg/l BAP)

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Table-2: Effect of growth regulators combination on shoot bud induction in *Acacia mangium*

Tr. No	Growth Regulators			Response %	Number of shoots/explant	Length of longest shoot	Days taken for shoot bud initiation	Shoot vigor*
	BAP	Kin	NAA					
T_1	0	0	0	0.0	0.0	0.0	0	0
T_2	1.0	0	0	64.4	1.3	0.9	31	++
T_3	1.0	0	0.05	62.2	1.3	1.0	28	++
T_4	1.0	0.1	0	80.0	3.0	1.4	24	++++
T_5	1.0	0.1	0.05	75.6	2.0	1.2	25	+++
T_6	1.0	0.5	0	80.0	3.7	2.1	23	+++
T_7	1.0	0.5	0.05	70.0	1.7	0.8	29	++
T_8	1.5	0	0	65.6	2.7	1.4	26	++++
T_9	1.5	0	0.05	73.3	1.0	1.7	23	+++
T_{10}	**1.5**	**0.1**	**0**	**87.8**	**5.7**	**2.8**	**21**	++++
T_{11}	1.5	0.1	0.05	82.2	2.7	1.3	26	++++
T_{12}	1.5	0.5	0	70.0	4.0	2.5	29	+++
T_{13}	1.5	0.5	0.05	84.4	1.7	1.2	25	++
T_{14}	2.0	0	0	66.7	2.0	1.1	27	+++
T_{15}	2.0	0	0.05	68.9	1.0	1.1	27	+++
T_{16}	2.0	0.1	0	74.4	2.7	1.6	26	++
T_{17}	2.0	0.1	0.05	62.2	1.7	1.2	32	+++
T_{18}	2.0	0.5	0	70.0	2.0	1.2	31	++
T_{19}	2.0	0.5	0.05	65.6	1.7	1.2	32	++
SEm					2.82	0.38	0.15	
LSD 5%					8.08	1.08	0.45	

Cont.....

*Shoot vigor was estimated on visual scale

\+ Very poor ++ poor +++ average ++++ good ++++ excellent

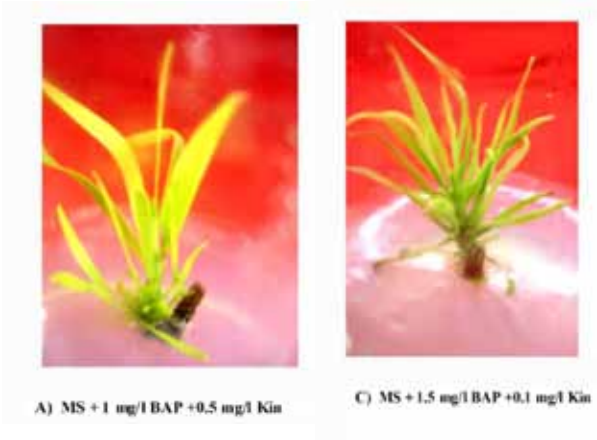
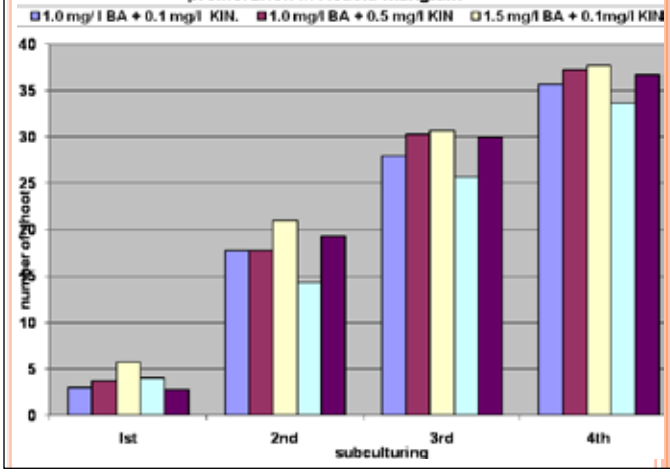


Fig-1a: Effect of subculturing on auxiliary shoot bud proliferarion in *Acacia mangium*



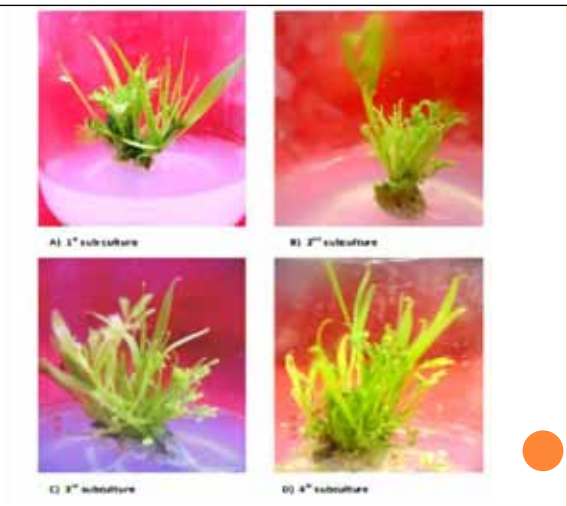


Table-3: Effect of types of auxin (IBA and IAA) on *in vitro* rooting in *Acacia mangium*

Tr. No	Treatment	Rooting (%)	No. of roots/ microshoots	Length of longest root
T_1	0.5 IBA	0.0	0.0	0.0
T_2	1.0 IBA	11.7	1.0	8.7
T_3	1.5 IBA	75.0	1.3	14.0
T_4	**2.0 IBA**	**88.3**	**2.7**	**18.0**
T_5	2.5 IBA	51.7	1.3	13.3
T_6	0.5 IAA	0.0	0.0	0.0
T_7	1.0 IAA	0.0	0.0	0.0
T_8	1.5 IAA	58.3	2.0	13.0
T_9	**2.0 IAA**	**70.0**	**2.3**	**17.7**
T_{10}	2.5 IAA	0.0	0.0	0.0
SEm		1.67	0.28	0.60
LSD 5%		4.91	0.82	1.79

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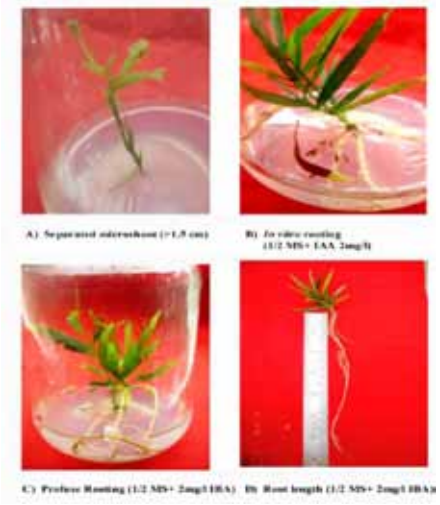

Table-4: Acclimatization of plantlets derived from micropropagation of *Acacia mangium*

Treatment	Per cent survival (mean± SE)
Sand	51.1 ± 1.9
Soilrite	**70 ± 3.3**
Cocpeat	38.9 ± 1.9

A) Hardening of micropropagated plantlets

b) Acclimatized plants ready to transfer in field.

CONCLUSION

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Biopiracy

The appropriation of the knowledge and genetic resources of indigenous communities by individuals or institutions seeking exclusive monopoly control (usually patents or plant breeders' rights) over these resources and knowledge

(Action Group on Erosion, Technology and Concentration (ETC Group),1993)

Biopiracy

Theft of :

- Biological and Genetic Resources
- Indigenous Knowledge Skills and Practices (IKSP)

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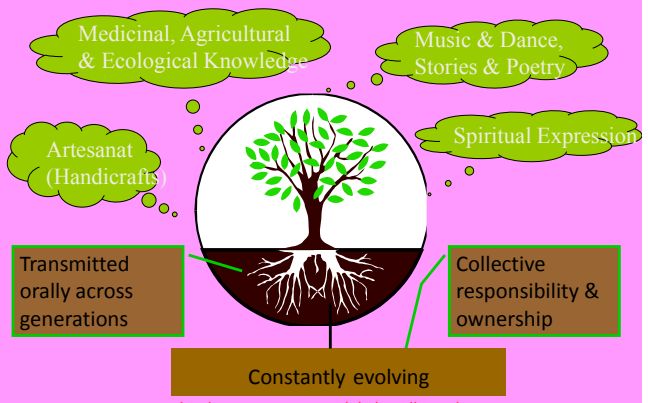
What is Traditional Knowledge?



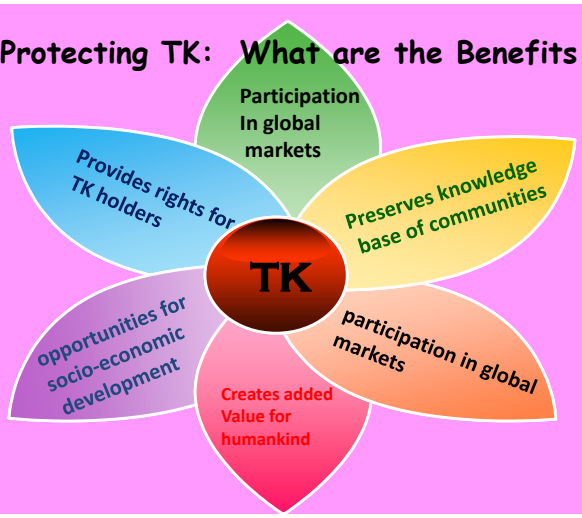
Source:- Richard Owens, Director, Global Intellectual Property IssuesDivision

Why is Traditional knowledge Important?

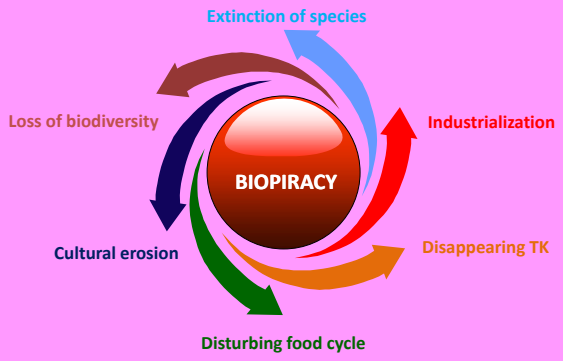
- ✓ Life depends on genetic resources
- ✓ TK is helping to preserve, maintain and increase biodiversity
- ✓ It is an important source of information for identifying new uses of FGR
- ✓ Useful for identifying the properties of FGR
- ✓ To develop new products
- ✓ It helping to scientists for understanding biodiversity

(Source-Protection of IK of Biodiversity, Gene campaign)

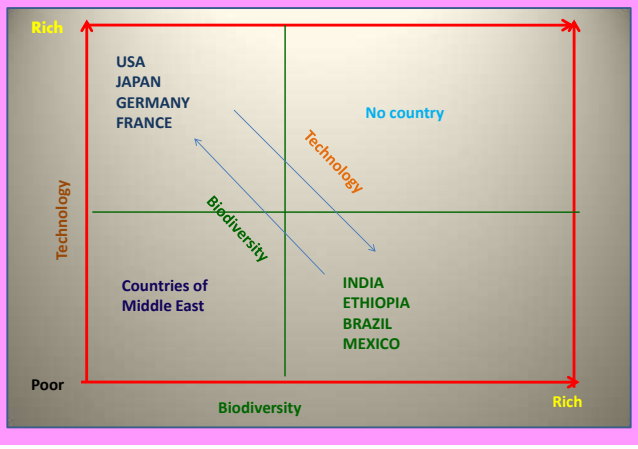
Protecting TK: What are the Benefits



Threats to Biodiversity



Relationship Between Biodiversity & Technology



Ways of Biopiracy

Bioprospecting

Scientific Exploration of biodiversity
- Chemical
- Genetic
- Bionic

IPR

- Patents
- Copyright
- Trademark
- Trade secret
- Geographical indications
- PBR's

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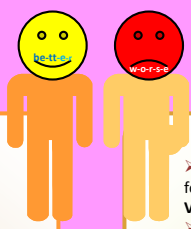
International agreements



be-tt-e-r

- ITPGRFA (International Treaty on Plant Genetic Resources for Food and Agriculture)
- CBD (Convention on Biological Diversity)
- WIPO (World Intellectual Property Organization)

w-o-r-s-e

- UPOV (International Union for the Protection of New Varieties of Plants)
- TRIPS
- DIRECTIVE 98/44/EC on the legal protection of biotechnological inventions

Piracy through Patents

The heart of GATT treaty & its patent is the treatment of biopiracy as a natural right of western corporations

Criteria for patent

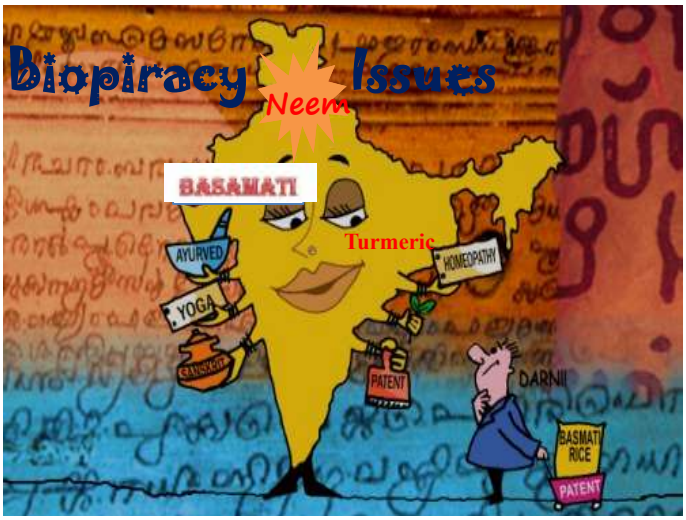
· Novelty

· Non obviousness

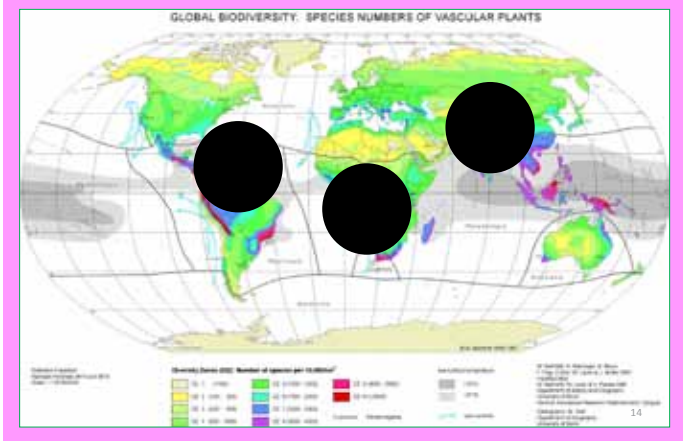
· Industrial application

Differences in TRIPS and CBD

Issue Area	TRIPS	CBD
Patentable subject matter	Circumscribes national sovereignty by mandating protection of biological and biotechnological innovations either through patents or sui generis protection	Principle of national sovereignty implies discretion in the drafting of IPR legislation, including the right to prohibit protection on biological resources
Benefit sharing	Strong private IPR with no corresponding rights for communities and farmers, and no mandated benefit sharing	Benefit sharing mandated, with the exact terms negotiated between government and interested parties
Protection of local knowledge	Narrow understanding of innovation associated only with commercial utility	Recognizes importance of indigenous knowledge
Role of the State	Role of the state to protect private intellectual property. **No role in maintaining, promoting or protecting biodiversity**	Access to biodiversity governed by principle of prior informed consent, including consultation with local communities



WORLD FAMOUS BIOPIRACY EXAMPLES



Patents on Indian Indigenous Medicinal Plants

Scientific Name	Indigenous Use	US Patent No Filed
Azadirachta indica	Biopesticide, medicine, biofungicide	65 Patents filed for antifungal property
Boswellia serrata	Astringent, skin diseases, piles	5494668
Curcuma longa	Wound healing	4 patents filed (5401504) Wound Healer
Melia azadirachta	Antifungal, antiviral	5478579 Inducing the absorption of Ca in bone tissue
Phyllanthus emblica	Fatigue, constipation, jaundice	5529778 Treatments of AIDS & TB
Sapium sebiferum	Promotes healing of wounds	5380894 Prodn. Of hydroxy fatty acids
Sterculia urens *Gun Karaya*	Astringent	192 patents filed between 1980-2001

Source- IPR & Conservation of Forest Product , Dr. Sudhanshu Gupta

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Role of CBD for preventing Biopiracy

Art 3 and 15	**States have sovereign rights over their biological and genetic resources**
Art 15.3 (MAT) 15.4 (PIC)	**Access to genetic resources can only occur on mutually agreed terms [MAT] and with the "prior and informed consent" [PIC] of States, unless States have otherwise determined**
Art 15.7	**Eequitable sharing of benefits**
Art 15.6	**User countries to promote the participation of provider countries in scientific research based on genetic resources provided by them**
Art 16.3	**User countries to allow participation of provider countries in scientific research based on genetic resources provided by them**

Neem Biopiracy

- Symbol of Indian Indigenous knowledge
- **Traditional use** – Building immunity, tooth powder, piles & urinary stone, Biopestiside
- **Patent Appeal**
- ✓ 1971-US Timber Importer Robert Larson Began to imorting of Neem seeds
- ✓ he extracted Margosan- O and received Clearence US EPA
- ✓ 1985- dozens of patents have been taken by W R Grage & Japanease Company

- 1994, a U.S. Department of Agriculture granted a patent for a fungicide made from Neem oil
- European Patent Office agreed to withdraw the patent in May 2000
- India won in 2005
- Key Petitioners- Vandana Shiva, Technology & Ecology in India; Dr. M D Nanjundaswamy

Turmeric Biopiracy

- **Traditionally use- heal wounds and rashes**
- **1995, two Indian nationals at the University of Mississippi Medical Centre were granted US Patent 5,401,504 on 'use of turmeric in wound healing'**
- **CSIR requested the US Patent and Trademark Office (USPTO) to reexamine the patents arguing that turmeric has been used for thousands of years for healing wounds and rashes**
- **India won the fight in 1998**

Basmati Biopiracy

- 27 varieties grown in India
- Patent by RiceTec. Inc. in Alvin, Texas, USA with support by the IRRI (International Rice Research Institute)
- They produced, **'KASAMATI" & 'TEXAMATI'**
- India won the trial

20

Hoodia-Cactus Biopiracy

- Growing in the Kalahari desert
- Used as an appetite suppressant by the **San tribe**
- In 1996, the South African-based CSIR patented active compounds of Hoodia for selling diet pills
- licensing agreement between **CSIR** and **phytofarm** companies to develop and commercialize a Hoodia-based product
- Benefit Sharing: Pfizer and Phytopharm will pay 6 % of all royalties (only 0.003% of net to sales)

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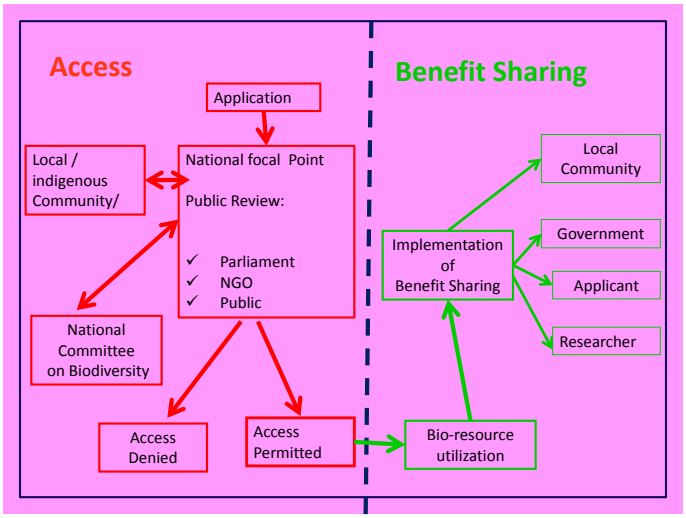

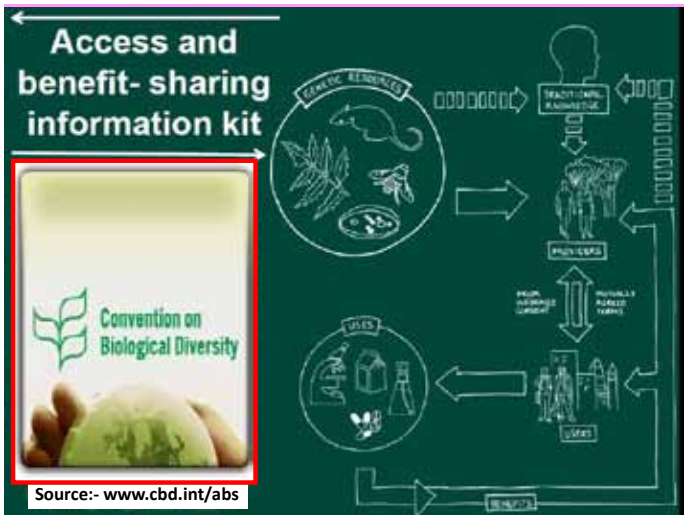

Source:- www.cbd.int/abs

Traditional knowledge Digital Library

- Collecting information on TK
- Information available in 5 international languages; **English, German, Spanish, French & Japanese**
- Tk Resource Classification (TKRC) based on International Patent Classification (IPC)
- Arurveda, Unani, Siddha & Yoga are converted in to a structured languages
- TKDL software used to convert local languages in to International languages

(**http://www.tkdl.res.in/tkdl/langdefault/common/Home**.)

People Biodiversity Register

The programme was started in a few Indian villages in 1995 by the Foundation for the Revitalisation of Local Health Traditions (FRLHT)

Public function to release the People's Biodiversity Register, in presence of the minister, 1995

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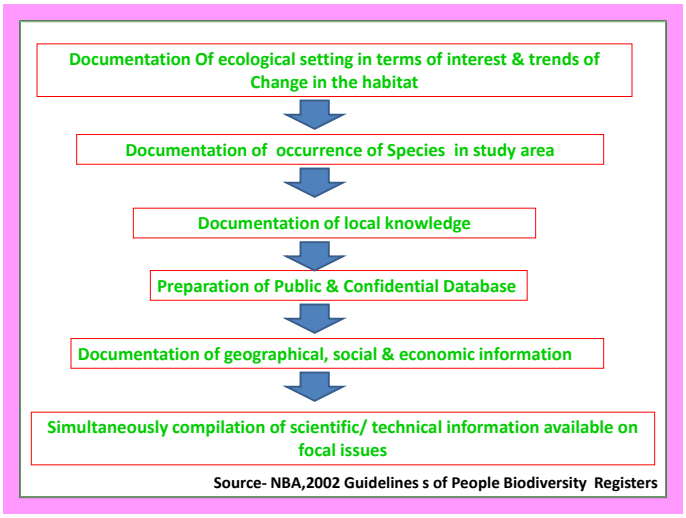
PEOPLE BIODIVERSITY REGISTER

- National Biodiversity Authority, Chennai Issued Guidelines For PBR
- Local level documentation of biodiversity &TK with management issues
- Through designed & led by local continually updated
- Organized to generate variety of product

P.L. Gautam, chairman, NBA; M.S. Swaminathan, chairman, MSSRF for issueing guideline of PBR's, 2010

Source- People's Biodiversity Register, Ghate Utkarsh, 1999



Source- NBA,2002 Guidelines s of People Biodiversity Registers

Benefit sharing with an indigenous community (tribe) – A Case Study

'Kani', a semi-nomadic tribal community inhabits in the 'Agasthyamalai' of the southern Western Ghat region of India.

Benefit sharing with an indigenous

The Kani experiment

Pushpangadan and co-workers (1987) came across an interesting use (anti-fatigue) of a lesser known wild plant while conducting the study on the forest dwelling Kani Tribe of South Western Ghat mountains.

Benefit sharing with an indigenous

Interaction with Kani Tribe

After a hard mountain trek, the author (Pushpangadan) and colleagues got exhausted and were taking rest. Then the Kani men accompanying them offered those dry fruits saying that when consumed they would reduce fatigue and provide energy.

Benefit sharing with an indigenous

Scientific Investigations

Collected adequate samples of this plant for detailed investigations at Regional Research Laboratory, (RRL), Jammu. Soon after reaching back at RRL, Jammu, Dr. Pushpangadan conducted the first scientific test to validate the Kani's claim on the anti-fatigue property of Arogyapacha.

Trichapus zeylanicus

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Benefit sharing with an indigenous

Filing of patents

❖ Three patents on the different pharmacological activities of the compounds isolated from this plant were made by RRL, Jammu.

❖ Drug "JIVANI " was prepared after 6 years during 1994

TBGRI, Kerala

Benefit sharing with an indigenous

Bottlenecks in implementation of the same

However, it took almost two years to transfer this benefit to be transferred to the Kani tribe due to inherent problems of the tribe.

Kani tribe is an unorganized semi-nomadic forest dwelling tribe. They later organized themselves and formed a trust with over 50% of adults from Kani Tribe as its members.

Benefit sharing with an indigenous

Actual transfer of money to Kani tribe

TBGRI transferred the money due to Kani tribe (Indian Rupees 650 thousand) in Feb 1999. They are now regularly getting 50% of royalty.

Kani tribal member identifies components of the *arogyapaacha plant*

Impact on Removing Poverty from this Initiative

DWELLING

Past

Present

Protect Community Against Biopiracy by:

1. *Documenting and Recording Community Biological Resources & Ethno-Botanical Knowledge*
2. *Creating Community Seed banks*
3. *Increasing Awareness of Biopiracy and Anti-Biopiracy Laws*
4. Confronting Bioprospecters or
5. Get PIC from the Community

CONCLUSION

The only way of preventing biopiracy is general awareness and make the legal policies strong

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Studies on Molecular Marker Development for Oleoresin Production in *Pinus roxburghii*

Santan Barthwal, Anita Rawat, H.S. Ginwal, D. K. Khurana* and Kuwant Rai Sharma*

Division of Genetics and Tree Propagation
Forest Research Institute, Dehradun
Dr Y. S. Parmar **University of Horticulture and Forestry**, Nauni , Solan

Details of the samples used for the study

S. No.	Genotype	Location	Resin yield	Class	Collection	Longitude	Latitude	Altitude
1	R-32/H-2	Nauni (Solan)	2.835 kg	High	20-Jun-07	77°10' 17.7"E	30°51' 21.1"N	1232
2	R-33/H-3	Nauni (Solan)	1.96 kg	High	20-Jun-07	77°10' 17.8"E	30°51' 21.2"N	1232
3	R-39	Nauni (Solan)	...		20-Jun-07		to be recorded	
4	L-2	Nauni (Solan)	...		20-Jun-07	77°10' 17.3"E	30°51' 17.9"N	1216
5	R-7/H-1	Nauni (Solan)	4.130 kg	High	20-Jun-07	77°10' 17.0"E	30°51' 19.6"N	1223
6	R-34/H-4	Nauni (Solan)	5.38	High	20-Jun-07	77°10' 19.1"E	30°51' 20.0"N	1226
7	R-18	Nauni (Solan)	...		20-Jun-07		to be recorded	
8	R-20/L-3	Nauni (Solan)	0.18 kg	Low	20-Jun-07	77°10' 18.6"E	30°51' 18.6"N	1232
9	I/2	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
10	I/3	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
11	I/5	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
12	I/6	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
13	I/7	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
14	II/4	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
15	II/5	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
16	II/7	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
17	III/6	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
18	III/7	Shilly(Solan)	...	High	20-Jun-07		to be recorded	
19	III/8	Shilly(Solan)	...	High	20-Jun-07		to be recorded	

41	R-26/L-4	Nauni (Solan)	0.390 kg	Low	18-Mar-08	77°10' 20.4"E	30°51' 18.0"N	1223
42	R-30/L-5	Nauni (Solan)	0.350 kg	Low	18-Mar-08	77°10' 20.4"E	30°51' 20.2"N	1220
43	R-68/L-7	Nauni (Solan)	0.610 kg	Low	18-Mar-08	77°9' 57.2"E	30°51' 26.6"N	1200
44	R-75/L-8	Nauni (Solan)	0.425 kg	Low	18-Mar-08	77°9' 56.6"E	30°51' 26.6"N	1192
45	R-86/L-9	Nauni (Solan)	0.510 kg	Low	18-Mar-08	77°9' 54.3"E	30°51' 25.5"N	1180
46	R-48/L-10	Nauni (Solan)	....		18-Mar-08		to be recorded	
47	R-108/L-11	Nauni (Solan)	0.370 kg	Low	18-Mar-08	77°9' 54.9"E	30°51' 23.6"N	1204
48	R-141/L-12	Nauni (Solan)	0.110kg	Low	18-Mar-08	77°9' 59.2"E	30°51' 16.3"N	1155
49	R-142 /L-13	Nauni (Solan)	0.555 kg	Low	18-Mar-08	77°9' 59.2"E	30°51' 16.6"N	1150
50	R-143/L-14	Nauni (Solan)	0.490 kg	Low	18-Mar-08	77°9' 59.2"E	30°51' 16.9"N	1153
51	R-9/H-5	Nauni (Solan)	2.430 kg	High	18-Mar-08	77°10' 17.4"E	30°51' 19.7"N	1220
52	R-11/H-6	Nauni (Solan)	2.730 kg	High	18-Mar-08	77°10' 17.1"E	30°51' 18.3"N	1216
53	R-5/H-8	Nauni (Solan)	2.035	High	18-Mar-08	77°10' 10.4"E	30°51' 25.2"N	1233
54	R-59/H-10	Nauni (Solan)	2.670 kg	High	18-Mar-08	77°9' 58.0"E	30°51' 26.4"N	1200
55	R-61/H-11	Nauni (Solan)	2.965 kg	High	18-Mar-08	77°9' 58.0"E	30°51' 26.2"N	1203
56	R-74/H-12	Nauni (Solan)	3.635 kg	High	18-Mar-08	77°9' 56.2"E	30°51' 26.7"N	1188
57	R-88/H-13	Nauni (Solan)	3.725 kg	High	18-Mar-08	77°9' 54.0"E	30°51' 24.7"N	1186
58	R-93/H-14	Nauni (Solan)	3.410 kg	High	18-Mar-08	77°9' 54.7"E	30°51' 24.7"N	1202
59	R-97/H-15	Nauni (Solan)	2.465 kg	High	18-Mar-08	77°9' 55.8"E	30°51' 24.9"N	1201
60	R-105/H-16	Nauni (Solan)	2.845 kg	High	18-Mar-08	77°9' 55.4"E	30°51' 24.4"N	1202
61	R-123/H-17	Nauni (Solan)	3.055 kg	High	18-Mar-08	77°9' 54.2"E	30°51' 21.3"N	1156
62	R-47	Nauni (Solan)	2.675 kg	High	18-Mar-08	77°10' 20.2"E	30°51' 22.2"N	1230

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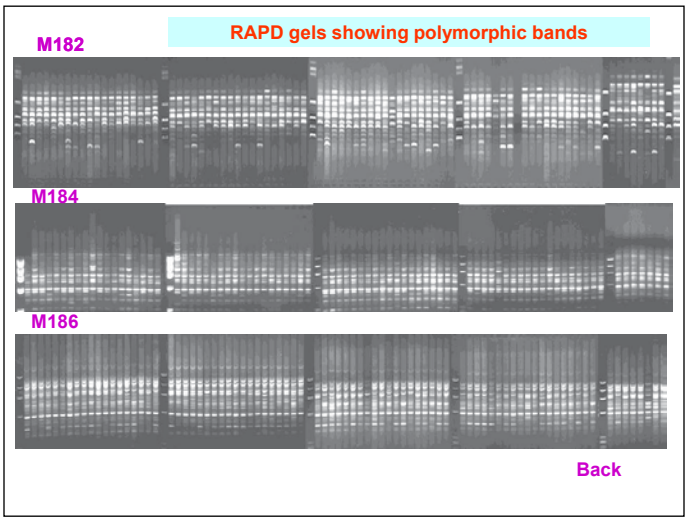
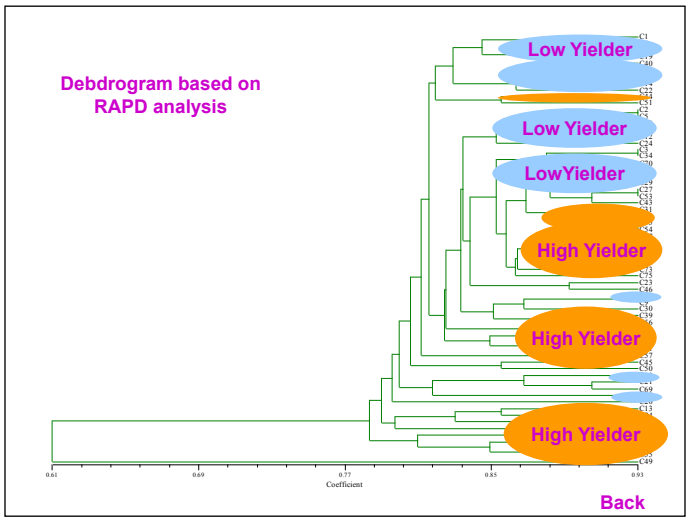
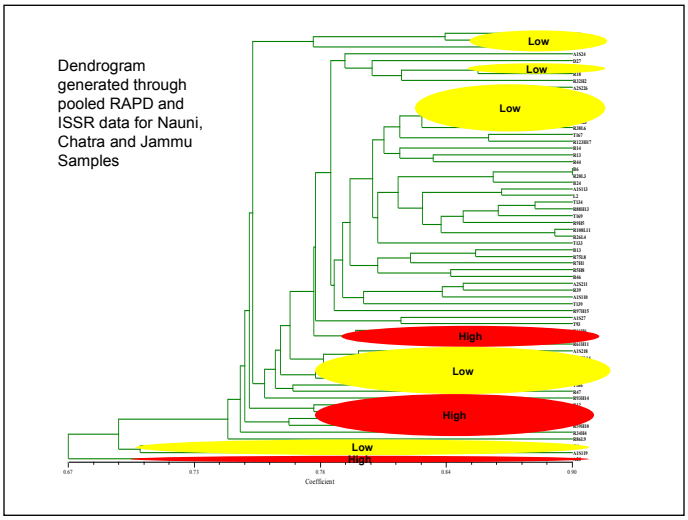






Summary of Genic Variation Statistics for All Loci Population wise

Population ID : 1
Population name : Chatra

Locus	Sample Size	na*	ne*	h*	I*
Mean	20	1.7103	1.5041	0.2852	0.4163
St. Dev		0.4552	0.3822	0.2006	0.2836

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

The number of polymorphic loci is : 103
The percentage of polymorphic loci is : 71.03 %

Population ID : 3
Population name : Nauni

Locus	Sample Size	na*	ne*	h*	I*
Mean	38	1.7448	1.4822	0.2775	0.4095
St. Dev		0.4375	0.3723	0.1947	0.2745

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

The number of polymorphic loci is : 108
The percentage of polymorphic loci is : 74.48 %

Population ID : 2
Population name : Jammu

Locus	Sample Size	na*	ne*	h*	I*
Mean	15	1.6276	1.4621	0.2576	0.3736
St. Dev		0.4851	0.4052	0.2143	0.3035

The number of polymorphic loci is : 91
The percentage of polymorphic loci is : 62.76 %

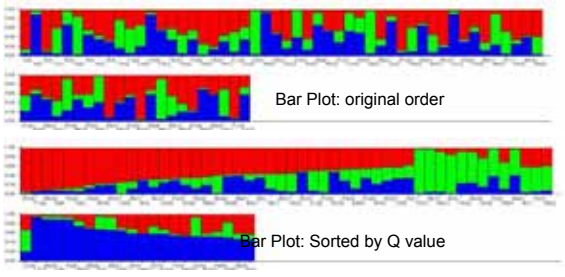
Summary of Genic Variation Statistics for All Loci

Locus	Sample Size	na*	ne*	h*	I*
Mean	72	1.8276	1.4964	0.2885	0.4309
St. Dev		0.3790	0.3619	0.1829	0.2506

* na = Observed number of alleles
* ne = Effective number of alleles [Kimura and Crow (1964)]
* h = Nei's (1973) gene diversity
* I = Shannon's Information index [Lewontin (1972)]

```
              +-------------------------pop1  Chatra (UK)
  +-----------1
--2           +-------------------------pop3  Nauni (HP)
  !
  +-------------------------------------pop2  Jammu (J&K)
```

Association analysis was performed for the entire locus generated through RAPD and ISSR markers and genotypes from Jammu, Chatra and Nauni. The population structure was worked out using STRUCTURE software and STRAT software was used for association analysis after analysis of population structure revealed that three RAPD loci (M-186-48, M- 186-49 and OPA-6-76) and one ISSR locus (ISSR – 7-108) have shown significant association with resin yield.



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STRAT by Pritchard, Stephens, Rosenberg and Donnelly (AJHG, 2000)
Code by J.K. Pritchard
Version 1.1 June 2003

Input data file = project_data
Structure Results file = results
Number of populations = 4
Using phenotype column: 4
Number of simulated test stats per locus = 1000
EM stopping point = 1.000e-003
Alleles with fewer than 4 copies pooled

ASSOCIATION ANALYSIS

Band/locus — level of significance

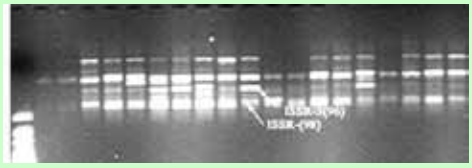
5: chisq= 8.111 1 df; TS = 3.74, p = 2.40000e-002 *
10: chisq= 0.409 1 df; TS = 3.48, p = 2.60000e-002 *
28: chisq= 6.337 1 df; TS = 5.67, p = 7.00000e-003 **
29: chisq= 1.024 1 df; TS = 3.83, p = 2.70000e-002 *
33: chisq= 6.334 1 df; TS = 7.63, p = 0.00000e+000 ***
35: chisq= 4.794 1 df; TS = 4.18, p = 3.90000e-002 *
36: chisq= 8.883 1 df; TS = 4.19, p = 2.70000e-002 *
48: chisq= 18.695 1 df; TS = 7.93, p = 2.00000e-003 **
49: chisq= 26.760 1 df; TS = 17.26, p = 0.00000e+000 ***
53: chisq= 9.266 1 df; TS = 5.90, p = 9.00000e-003 **
56: chisq= 3.201 1 df; TS = 4.97, p = 2.10000e-002 *
57: chisq= 3.495 1 df; TS = 4.84, p = 3.10000e-002 *
59: chisq= 4.391 1 df; TS = 5.22, p = 7.00000e-003 **
62: chisq= 0.016 1 df; TS = 3.16, p = 4.90000e-002 *
66: chisq= 0.807 1 df; TS = 5.49, p = 9.00000e-003 **
72: chisq= 4.969 1 df; TS = 8.36, p = 0.00000e+000 ***
76: chisq= 36.689 1 df; TS = 14.66, p = 0.00000e+000 ***
96: chisq= 0.021 1 df; TS = 4.89, p = 2.30000e-002 *
98: chisq= 2.985 1 df; TS = 4.86, p = 2.10000e-002 *
108: chisq= 34.592 1 df; TS = 16.41, p = 0.00000e+000 ***
115: chisq= 13.614 1 df; TS = 4.18, p = 4.40000e-002 *
117: chisq= 8.113 1 df; TS = 4.35, p = 4.10000e-002 *
120: chisq= 3.727 1 df; TS = 3.05, p = 2.90000e-002 *
121: chisq= 0.524 1 df; TS = 5.04, p = 2.80000e-002 *
126: chisq= 1.149 1 df; TS = 4.45, p = 1.00000e-002 *
139: chisq= 5.819 1 df; TS = 3.73, p = 2.50000e-002 *
145: chisq= 1.571 1 df; TS = 3.69, p = 4.10000e-002 *

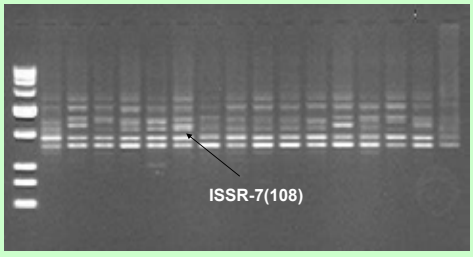
Markers showing significant positive association with resin yield

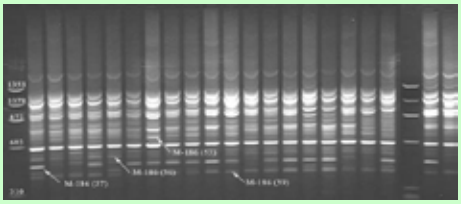
RAPD (M-186-48,M- 186-49, OPA-6-76)

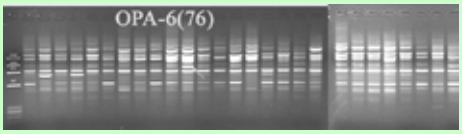
ISSR: ISSR – 7-108

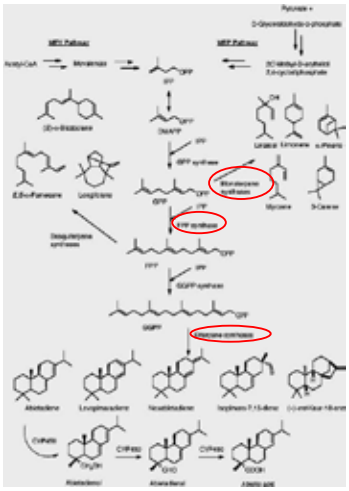
Band No.	Primer	Significance	% occurrence in low yielders	% occurrence in high yielders
28	OPA-17	**	38.09	61.29
33	**M-184**	***	**40.47**	**35.48**
48	M-186	**	54.76	19.35
49	**M-186**	***	**95.23**	**74.19**
53	M-186	**	83.33	70.96
59	M-186	**	95.23	90.32
66	OPE-8	**	92.85	93.54
72	**OPE-8**	***	**95.23**	**83.87**
76	**OPA-6**	***	**38.09**	**80.64**
96	ISSR-3	*	33.33	22.58
98	ISSR-3	*	45.23	45.16
108	**ISSR-7**	***	**100**	**74.19**
115	ISSR-8	*	80.95	67.74
117	ISSR-9	*	45.23	80.64
120	ISSR-9	*	90.47	100
121	ISSR-9	*	52.38	48.38
126	ISSR-9	*	66.66	54.83
139	ISSR-11	*	11.90	12.90
145	ISSR-12	*	71.42	80.64











Biosynthesis of terpenes. Prenyl transferases condense one or more isopentenyl diphosphates (IPPs) with dimethylallyl diphosphate (DMAPP) from the mevalonate (MEV) or methyl-erythritol 4-phosphate (MEP) pathways to produce geranyl diphosphate (GPP), farnesyl diphosphate (FPP), or geranylgeranyl diphosphate (GGPP). Terpene synthases then use these diphosphates as substrates to form the various terpenes. Additional enzymes, such as CYP450s, can further functionalize these terpenes. *New Phytologist* (2006) **170** : 657–675

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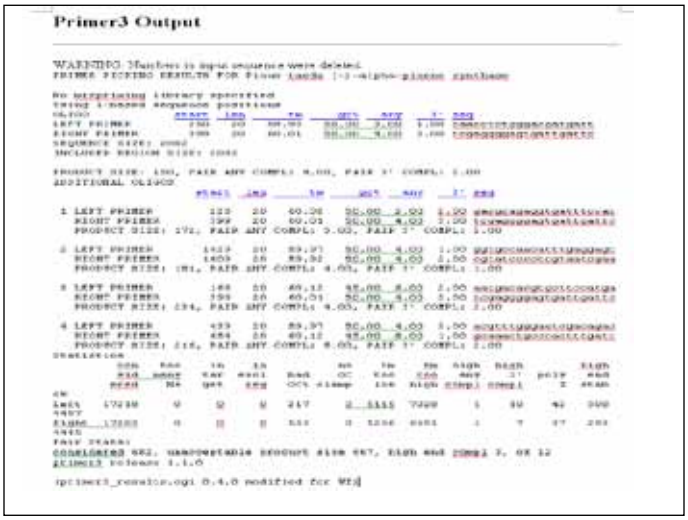
Enzyme specific primers designed using online Primer 3 software



Primers synthesized for three key enzymes

SSR Primers list for Pinus taeda diterpene synthase mRNA:

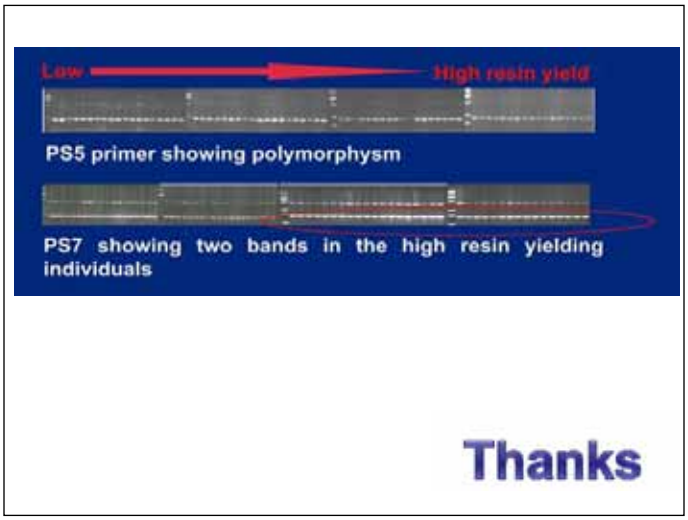
S.No.	Gene	Product size	Primer	Sequence
1	PTditer Syn 1		Left	CAAAAAGTTTGGGAGGTCCA
			Right	GCTGGAAGAGGCAGTTTTTG
2	PTditer Syn 2	555	Left	TTGGGTAGCAAGGATTCCAG
			Right	TTGTTCCCTGTACGCATGAA
3	PTditer Syn 3	556	Left	TTGGGTAGCAAGGATTCCAG
			Right	TTTGTTCCCTGTACGCATGA
4	PTditer Syn 3	587	Left	GGGGAAACTAATCCCTCTGC
			Right	TTGTTCCCTGTACGCATGAA
5	PTditer Syn 4	588	Left	GGGGAAACTAATCCCTCTGC
			Right	TTTGTTCCCTGTACGCATGA

SSR Primers list for Pinus taeda (-)-alpha-pinene synthase mRNA

S.No.	Gene	Product size	Primer	Sequence
1	PTpineSyn1	592	Left	ACGTTTGGGAATCGACAGAC
			Right	GCCTTTCCACCATCTAACGA
2	PTpineSyn2	515	Left	ACGAAGATGGCGAATCAATC
			Right	ATTCCTTGCTTCCAATCGTG
3	PTpineSyn3	520	Left	GTCAAACGAAGATGGCGAAT
			Right	ATTCCTTGCTTCCAATCGTG
4	PTpineSyn3	584	Left	CACGATTGGAAGCAAGGAAT
			Right	CTCCTCAAATGTTGGCACCT
5	PTpineSyn4	562	Left	ACAGGTCTGGTCGTGAGCTT
			Right	GCAAACTGCCCACTTTGATT

SSR Primers list for Pinus taeda alpha-farnesene synthase mRNA

S.No.	Gene	Product size	Primer	Sequence
1	PTfarSyn1	572	Left	ATGTATGGCGGTTGAAAAGC
			Right	GGAGAGTGGCTGCTCGATAC
2	PTfarSyn2	571	Left	ATGTATGGCGGTTGAAAAGC
			Right	GAGAGTGGCTGCTCGATACC
3	PTfarSyn3	566	Left	GTATCGAGCAGCCACTCTCC
			Right	CCTTAGGCTTCCATGTTCCA
4	PTfarSyn4	567	Left	GGTATCGAGCAGCCACTCTC
			Right	CCTTAGGCTTCCATGTTCCA
5	PTfarSyn5	547	Left	CTTCAACGCCTTTGGATGAT
			Right	GCTTTTCAACCGCCATACAT



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Identification of Sodium Transporter Gene Homologues from Salt Tolerant Tree Species

Sanu Mary Abraham, Selvakesavan, R. K., Thushara, P., Balasubramanian A., Aravinthakumar, V., Sowmiyarani, K.S., Sangeetha, M., Venkatachalam, R., Mathish Nambiar-Veetil

Plant Biotechnology Division,
Institute of Forest Genetics and Tree Breeding,
Coimbatore

Salt stress is an important environmental factor that adversely affects plant productivity.

- Salt affected soils are laden with toxic element sodium.
- Sodium transporter genes play a role in the uptake of salt from the soils, efflux of salt back to soil, transport of salt within the plant and compartmentalization into vacuoles thereby controlling the ionic and osmotic balance in the roots and shoots.

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The perennial tree species like *Prosopis juliflora, Pongamia pinnata, Acacia nilotica, Casuarina equisetifolia* and *Eucalyptus camaldulensis* are known to tolerate high levels of salinity and may harbour novel variants of genes conferring salt tolerance that could be used for development of climate resilient crops.

Objective of the Study

To Identify sodium transporter gene homologues from salt tolerant tree species like *P. juliflora, C. equisetifolia, E. camaldulensis and E. tereticornis*.

Target genes for the study

HKT1

- Involved in K^+ - Na^+ cotransport in *E. camaldulensis*.

Silencing the HKT1 gene

- In wheat, resulted in **improved Na^+ tolerance (Laurie, 2002)** .
- In Arabidopsis, rendered the plants Na^+ hypersensitive (Berthomieu et al, 2003) .

NHX1

- Involved in sequestering sodium into vacuoles.

Methodology

- Genomic DNA Isolation, RNA isolation, cDNA synthesis
- Design of Degenerate Primers
- PCR Amplification of Sodium transporter homologues
- Sequencing and annotation of the gene sequences

Primer designing for sodium transporter genes

Based on the sequences of the conserved regions of known *NHX1* genes from other plant sources, forward and reverse primers were designed using PriFi .

forward primer - 5´-GCCGCAACAGATTCTGTGTG-3´
reverse primer - 5´-GCCAAGTATAATGGGACATG-3´.

Eucalyptus tereticornis NHX1 antiporter

>gi|343786449|gb|JN157810.1| Eucalyptus tereticornis nhx1 antiporter (nhx1) gene, partial cds
CTGGAGTCCACTTTCCCCATCACCTGGTCAGAACGATAAAAGGAGAAAACGTAACCGATGTTCAATTACT
TGGTGCAGGTGCTTAATCAGGATGAGACACCTCTATTGTACAGTCTGGTCTTTGGGGAAGGCGTTGTGAA
TGATGCCGCATCAGTTGTTCTGTTCAATGCGATTAAGAGCTTTGACATTACTCACATCAACTCGAGGATT
GCCCTGGAGTTCATGGGCAACTTCTTATACTTGTTTATATTGAGCACGATGCTCGGAGTTTTGGTGAGAC
TCCCTCCTCCTTCCGCCCAACTTAAGAGGCTGTTCTTGATCTGTTTTGATATGTTTGGTTTGCATCCCTG
CAGGCGGGGCTGCTCAGTGCTTACATTGTCAAGAAACTCTACTTTGGCAGGTTGACAAATTGATCCTTAG
CACTGATGTCTGGTTTAATTGTGTATCAAATGAAAACAATTAACAATATCTGTTCAGTGCTCACATTTTA
TTTTGGTACTTTCTTTTTTGTGCTCTTATAGTTTATTTTACAATTGCAGGCACTCTACAGACCGTGAGGT
TGCGCTGATGATACTCATGGCATACCTTTCATATATGTTGGCCGAAGTAAGATA

The 614 bp partial sequence of *EtNHX1* codes for 82 amino acids in three different exons and showed 82 % identity with *Vitis vinifera NHX1* (AY634283.1) antiporter

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Eucalyptus camaldulensis NHX1 antiporter

>gi|343786457|gb|JN157814.1| Eucalyptus camaldulensis nhx1 antiporter (nhx1) gene, partial cds
TGGAGTCCACTTTCCCGATCACCTGGTCAGAACGATAAAAGGAGAAAACGTAACCGATGTTCAATTACTT
GGTGCAGGTGCTTAATCAGGATGAGACACCTCTATTGTACAGTCTCGTCTTTGGGGAAGGCGTTGTGAAT
GATGCCGCATCAGTTGTTCTGTTCAATGCGATTAAGAGCTTTGACATTACTCACATCAACTCGAGGATCG
CCCTGGAGTTCATGGGCAACTTCTTATACTTGTTTATATTGAGCACGATGCTCGGAGTTTGGTGAGACT
CCCTCCTCCTTCCACCCAACTTAAGAGGCTGTTCTTGATCTGTTTTGATATGTTTGGTTTGCATCCCTGC
AGGCGGGGCTGCTCAGTGCTTACATTGTCAAGAAACTCTACTTTGGCAGGTTGACAAATTGATCCTTAGC
ACTGATGTCTGGTTTAATTGTGTATCAAATGAAAACAGTTAACAATATCTGTTCAGTGCTCACATTTTAT TTGG

The **494 bp** partial sequence of ***EcNHX1*** codes for **80 amino acids** in **two** different exons and showed **81 %** identity with ***Vitis vinifera NHX1*** antiporter.

Casuarina equisetifolia NHX1 antiporter

>gi|353442076|gb|JN629033.1| Casuarina equisetifolia nhx1 antiporter gene, partial cds
TTCGTGCAGGTGCTCAATCAGGACGAGACACCTTTGCTATATAGTCTGGTTTTCGGGGAAGGGGTAGTAA
ATGATGCCACATCAGTGGTGCTTTTCAATGCAATCCAGAGCTTTGACTTGTCTCACTTCAACTCAAGCAT
TGCCTTGCAGTTTGTTGGAAACTTTTTCTATTTGTTTATCACAAGCACCGTGCTTGGAGTTTTTGTAAGT
CACTCTCCGGTGATCTGTGCTTTCTTGTGGTCGTACATCACTTTGATGACCTTTTGTTGATCTTTTGCAG
GCTGGACTGCTCAGTGCATACATGATCAAGAAGCTCTATTTTGGCAGGTT

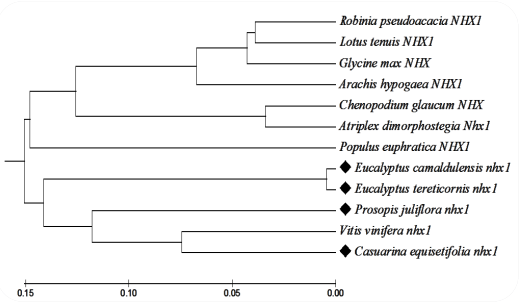
The **330 bp** partial sequence of ***CeNHX1*** codes for **80 amino acids** in **two** different exons and showed **87 % identity** with ***Vitis vinifera NHX1*** antiporter

Prosopis juliflora NHX1 antiporter gene

>gi|353442078|gb|JN629034.1| Prosopis juliflora nhx1 antiporter gene, partial cds
GACGAGACACCTTTATTGTACAGCCTGGTTTTTGGGGAAGGGGTCGTAAATGATGCGACATCTGTAGTGC
TCTTGAAAGCAATCCAGAACTTTGACCTTTCTCACATCGACTTGAATACTGGTTTCCACTTAATTGGAAA
TTTTTTCTACTTATTTACAGCAAGCACTGTGCTGGGAGTCTTGGTAAGTCTCTTCTTTTATCTACTTCGC
AGCGCTTAATTGGTTTCCTCAGATTTTGTTTTAACGTTTCAGGCTGGATTGCTCAGCGCATTCATCATAA
AAAAGCTTTATTTTGGCAGGTTGGTAAAGCCAGAGCGAACTGATTTTGTCACTGATTGCCATTAGTTCAA
TAGGGGAGAATTCAATTCCTG

The **371 bp** partial sequence of ***PjNHX1*** codes for **76 amino acids** in **two different exons** and showed **83 %** identity with ***Vitis vinifera NHX1*** antiporter.

Tree view produced using MEGA 5



Eucalyptus camaldulensis HKT1

>gi|343173302|gb|JF786711.1| Eucalyptus tereticornis sodium potassium symporter-like (HKT1) gene, partial sequence
ATACGTCTGCTACAGTACTTGATTTCTGCTTTCTTGCTGAATCTCCCCATGAGCCAAAGCGTGAACCGTAAG
GTCGATGGGAACAGCGTGTTCCCGAGAAGGACCTGAGGGATGAGAATCAGGAGGAGGCCGGAGTTTTTG
CTGAAGACAATCATGTTTTCATTCGTCGGGACAAACCCACAACTAGCGAAGGTCGACACGATGGTGAAGAC
GGAAAAGGTCACCATTTTGAGACCCTTCTTCTTTAGCACATCTCTGGCGCTAGAAACAAGTGTCATATAGAG
CGAAACTGCGGCAACCCCGAGAACTTGGACTCCCAGAAGGTAACCTAACACTACAAAACACAAAAACCTAA
CCGAACAATATTTCAAATAGTCAAGATCTGGGGACTCGTCTTGCGGACTGTACAATTGGAGGTCTACTTGTG
AGTTCAGGCAATCTAAATTCCCTACTACACCTAAACTCTATGTGGTCAACAATGTCGTTTGTTGGGTTCGAA
GGGGCAGAGATTGCTATTGACACAGGCGACCTTTTTCTTTCGGTTTGATGAGCCACCGGAGCTTTGTTTTC
CTAAATGAAGCCCAGCGAGAGAAATGAAGACCTCCCCTCCGATGAACATCAGGACGGTCA TCACGACA

Partial Gene sequences of Actin from *A.nilotica, C.equisetifolia* and *P.Pinnata*

>Partial gene sequence of Actin from cDNA of *Acacia nilotica*

GAAGACTTGTATGGTAACATTGTCCTCTCTGGAGGTTCAACCATGTTCCCTGGCATT
GCTGATAGAATGAGCAAAGAGATCTCTGCATTAGCCCCAAGTAGCATGAAGATCAA
GGTGGTTGCACCACCTGAGAGAAAGTACAGTGTCTGGATTGGTGGTTCTATTTGGC
ATCTCTCAGCACCTTCCAACAGATGTGGATTGCC

>Partial gene sequence of Actin from cDNA of *Casuarina equisetifolia*

CTATGGTAACATTGTTCTTAGTGGTGGATCTACCATGTTCCCTGGTATTGCTGACCGA
ATGAGCAAGGAAATCACTGCCCTTGCTCCCAGCAGCATGAAAATTAAGGTTGTGGC
CCCACCTGAGAGAAAGTACAGTGTCTGGATTGGAGGATCAATTCTCGCATCCCTCAG
CACCTTCCAGCAGATGTGGATTGCCAGGGCTGAG

>Partial gene sequence of Actin from cDNA of *Pongamia pinnata*

AAGGACCTTTACGGTAACATTGTCCTTTCAGGAGGAACAACCATGTTCCCTGGCATT
GCTGATAGAATGAGCAAGGAAATTTCTGCATTGGCCCCCAGCAGCATGAAGATCAA
GGTGGTAGCACCACCTGAGAGAAAGTACAGTGTCTGGATTGGTGGTTCTATCTTGGC
ATCTCTTAGCACCTTCCAGCAGATGTGGATTGCCAAGGCTGAGTAA

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Summary

Details of the sequences published in NCBI

S.no	Gene name	Plant species	NCBI Accession number	Published date
1	NHX1	Eucalyptus tereticornis	JN157810.1	22-AUG-2011
2	NHX1	Eucalyptus camaldulensis	JN157814.1	22-AUG-2011
3	NHX1	Casuarina equisetifolia	JN629033.1	25-OCT-2011
4	NHX1	Prosopis juliflora	JN629034.1	25-OCT-2011
5	*HKT1*	Eucalyptus tereticornis	JF786711.1	14-AUG-2011
6	*Actin*	Eucalyptus camaldulensis	JN157813.1	22-AUG-2011
7	*Actin*	*Pongamia pinnata*	JN157812.1	22-AUG-2011
8	*Actin*	*Acacia nilotica*	JN157811.1	22-AUG-2011

All these data are also available in the database that we are developing "*In silico* Gene bank for Plant Adaptation to Abiotic Stresses (IGPAAS)" www.igpaas.co.cc

Acknowledgements

The funding support from the Department of Biotechnology, GoI, for the project entitled "Web enabled database and analysis of gene sequences implicated in abiotic stress tolerance for screening gene homologues in salt tolerant tree species" is gratefully acknowledged.

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Presented By

Shivani Dobhal

Division of Genetics & Tree Propagation

Forest Research Institute

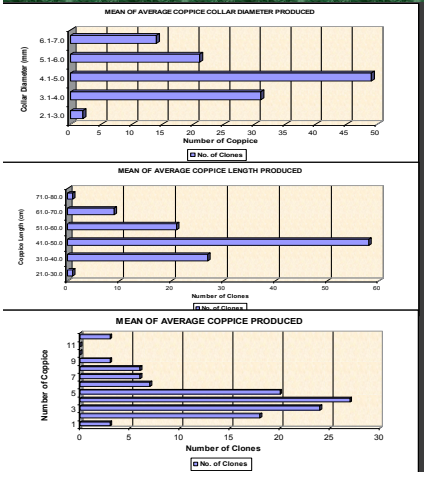
Dehradun, Uttarakhand

METHODOLOGY

Screening of promising clones of *Dalbergia sissoo (Roxb.)* through coppicing ability for vegetative propagation

Selection of clones on the basis of high coppicing ability and high index value based on index method of selection (Cotterill and Dean, 1990)

SCREENING OF THE CLONES



A total of 48 genotypes

Uttarakhand	17
Uttar Pradesh	14
Punjab	08
Rajasthan	07
Haryana	01
Nepal	01

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CLONAL TRIAL ESTABLISHMENT

- All selected clones of *Dalbergia sissoo* Roxb. were planted following lattice design with minimum of six replications and 2 ramets each in a uniform spacing of 3X3m in an area of 0.51 ha

LOCATION	STATE	LATTITUDE & LONGITUDE
BITHMERA	HARYANA	N 29^0 31′′59.3′′and E 75^0 55′′11.5′
HOSHIARPUR	PUNJAB	N 31^0 33′′31.7′′and E 75^0 49′ 0.5′′

- Periodic observation on the field performance all clones were taken and data on the following traits recorded
  - Height
  - Collar Diameter

EXPEREMENTAL DESIGN FOR *DALBERGIA SISSOO* : HOSHIARPUR,PUNJAB (Location 1)

Forest Area

CSO *Dalbergia sissoo* | Eucalyptus Plantation

REPLICATION- I								REPLICATION- II							REPLICATION- III						
5027	5012	5029	5025	5024	5026	5017	44	5017	5004	5053	94	1004	6	5024	80	5004	7006	18	1003	218
5027	5012	5029	5025	5024	5026	5017	44	5017	5004	5053	94	1004	6	5024	80	5004	7006	18	1003	218
20	5	23	16	15	18	6	46	5024	5006	7002	99	1009	15	46	5009	6	103	1013	5045	5029
20	5	23	16	15	18	6	46	5024	5006	7002	99	1009	15	46	5009	6	103	1013	5045	5029
138	90	218	103	99	107	94	31	5012	5003	5045	90	1003	5	1009	5001	94	5007	5026	31	23
138	90	218	103	99	107	94	31	5012	5003	5045	90	1003	5	1009	5001	94	5007	5026	31	23
5001	1003	5002	1010	1009	1013	1004	86	5029	5011	C	218	5002	23	7002	138	5017	16	59	5003	5002
5001	1003	5002	1010	1009	1013	1004	86	5029	5011	C	218	5002	23	7002	138	5017	16	59	5003	5002
5009	5003	5011	5007	5006	5008	5004	47	5025	5007	7006	103	1010	16	15	86	5053	47	107	5012	5011
5009	5003	5011	5007	5006	5008	5004	47	5025	5007	7006	103	1010	16	15	86	5053	47	107	5012	5011
80	31	86	47	46	59	44	80	5027	5009	9064	138	5001	20	99	5027	44	1010	5008	5	C
80	31	86	47	46	59	44	80	5027	5009	9064	138	5001	20	99	5027	44	1010	5008	5	C
9064	5045	C	7006	7002	9058	5053	59	5026	5008	9058	107	1013	18	5006	20	1004	5025	9058	90	9064
9064	5045	C	7006	7002	9058	5053	59	5026	5008	9058	107	1013	18	5006	20	1004	5025	9058	90	9064
REPLICATION- IV							REPLICATION- V							REPLICATION- VI							
9058	5017	47	1009	5009	218	5	7006	20	5011	5017	31	1013	99	5002	94	5008	20	5024	47	5045
9058	5017	47	1009	5009	218	5	7006	20	5011	5017	31	1013	99	5002	94	5008	20	5024	47	5045
1013	94	5025	15	80	C	5003	1010	5009	86	94	5012	18	7002	218	44	1013	9064	5006	16	5012
1013	94	5025	15	80	C	5003	1010	5009	86	94	5012	18	7002	218	44	1013	9064	5006	16	5012
18	5053	103	5006	5027	5002	31	103	5001	23	44	5003	9058	5024	5011	1004	5026	80	7002	103	5
18	5053	103	5006	5027	5002	31	103	5001	23	44	5003	9058	5024	5011	1004	5026	80	7002	103	5
5008	1004	7006	46	138	23	5012	5007	5027	218	1004	5045	59	15	C	5017	18	5001	46	5007	90
5008	1004	7006	46	138	23	5012	5007	5027	218	1004	5045	59	15	C	5017	18	5001	46	5007	90
107	44	5007	7002	20	5029	1003	47	138	C	6	1003	5026	5006	86	6	107	5027	1009	7006	5003
107	44	5007	7002	20	5029	1003	47	138	C	6	1003	5026	5006	86	6	107	5027	1009	7006	5003
5026	5004	16	99	5001	86	5045	5025	9064	5002	5004	5	107	46	5029	5004	9058	138	15	1010	31
5026	5004	16	99	5001	86	5045	5025	9064	5002	5004	5	107	46	5029	5004	9058	138	15	1010	31
59	6	1010	5024	9064	5011	90	16	80	5029	5053	90	5008	1009	23	5053	59	5009	99	5025	1003
59	6	1010	5024	9064	5011	90	16	80	5029	5053	90	5008	1009	23	5053	59	5009	99	5025	1003

Forest Path

1	No. of clones	49	4	Design	Lattice design	REPLICATIONS DETAILS		
2	No. of replications	6	5	Spacing	3 x 3 m	REPL - I	REPL - II	REPL - III
3	No. of ramets / repl	2	6	Area	0.51 ha	REPL - IV	REPL - V	REPL - VI

DATA COLLECTIONS

MEAN PERFORMANC OF THE CLONES

	HEIGHT (cm)	COLLAR DIA. (cm)
Average	68.15	1.10
Maximum	112.52 (C 5003)	2.02 (C 5006)
Minimum	41.17 (C 94)	0.59 (C 31)
Std. Dev.	22.05	0.47

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ANOVA TABLE FOR GROWTH TRAITS

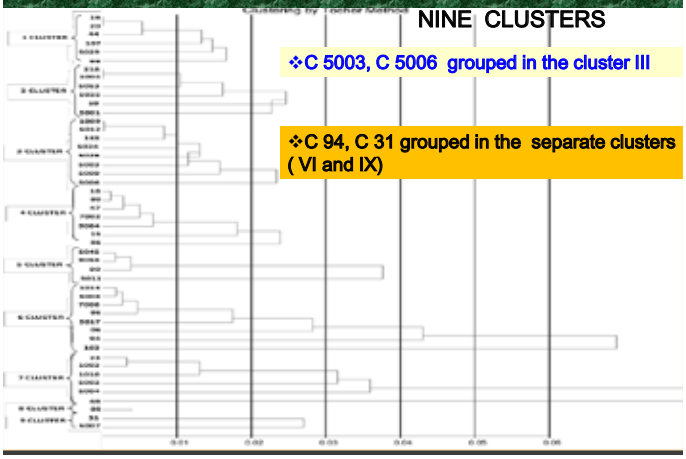
Source of Variations	Degree of freedom	Sum of squares		Mean sum of squares		F Ratio	
		Height	Collar diameter	Height	Collar diameter	Height	Collar diameter
Clones	47	274148.46	125.98	5832.94	2.68	13.89***	26.15***
Location	1	12041.96	2.09	12041.96	2.09	8.66**	12.32***
Replication	5	12041.96	2.09	2408.39	0.41	5.73***	4.09**
Interaction	5	12041.96	2.09	2408.39	0.41	5.73***	4.09**
Total	11	12041.96	2.09	1094.72	0.19	2.60**	1.86*
Error	517	216981.89	53.10	419.69	0.10		

Significance level: *P< 0.05, **P< 0.01, ***P< 0.001

VARIANCE ANALYSIS

Genetic parameter	Height	Collar Dia.
Variance Genotypic	451.10	0.22
Variance Phenotypic	870.79	0.32
Variance environmental	419.69	0.10
GCV	31.16	42.03
PCV	43.30	51.08
ECV	30.06	29.03
Heritability (broad sense)	0.518	0.68
Genetic Advance (5%)	31.49	0.79
Genetic Advance (1%)	40.35	1.00
Genetic advance as % of mean (5%)	46.20	71.23
Genetic advance as % of mean (1%)	59.22	91.28

CLUSTERING OF THE CLONES



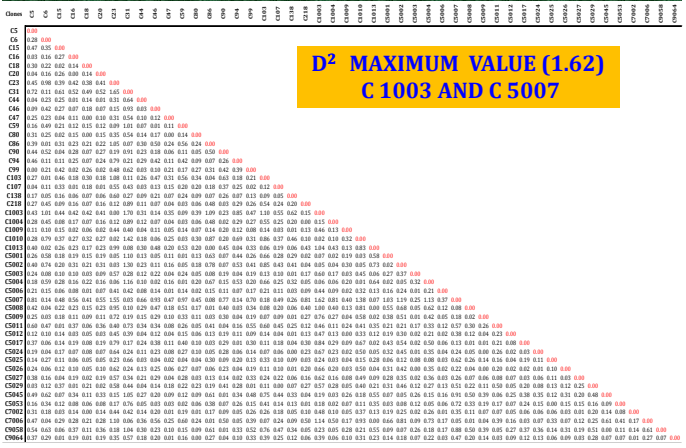
CLUSTERING OF THE CLONES

CLUSTERS	No. OF CLONES	GEOGRAPHIC LOCATION
I	6 (SIX)	HARYANA: 1, NEPA L: 1, PUNJAB: 1, UK: 2, UP : 1
II	6 (SIX)	PUNJAB: 2, UK: 5, UP : 1
III	8 (EIGHT)	PUNJAB: 2, UK: 5, UP : 1
IV	7 (SEVEN)	RAJASTHAN: 1, UK: 2 , UP : 4
V	4 (FOUR)	PUNJAB: 1, RAJASTHA N: 1, UK: 2
VI	8 (EIGHT)	PUNJAB: 1, RAJASTHA N: 3, UK: 2, UP : 2
VII	6 (SIX)	UK: 2, UP : 4
VIII	1(One)	UP : (CLONE 05)
IX	2 (TWO)	UK: (CLONE, 5007) UP : 1 (CLONE 31)

CLUSTERING OF THE CLONES

S.No.	Clusters	No. of clones	Mean value	
			Height (cm)	Collar diameter (cm)
1	Cluster I	6	50.04	0.74
2	Cluster II	6	53.17	0.69
3	Cluster III	8	45.84	0.65
4	Cluster IV	7	47.08	0.61
5	Cluster V	4	50.69	0.60
6	Cluster VI	8	41.09	0.61
7	Cluster VII	6	57.83	0.73
8	Cluster VIII	1	52.50	0.79
9	Cluster IX	2	35.13	0.56

D^2 Matrix Table



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Optimization of DNA extraction protocol of *Pongamia pinnata* Linn.

Shruti Sharma

Division of Genetics and Tree Propagation
Forest Research Institute
Dehradun

(shrutiddn@gmail.com)

INTRODUCTION

***Pongamia pinnata* (L.) Pierre**

- Commonly known as Karanja
- Belongs to the family Fabaceae
- Indigenous to the Indian sub-continent and South-East Asia
- Medium sized tree ranging from 12-15 m in height, drought resistant and semi deciduous
- Fuel, control soil erosion, highly tolerant to salinity, nitrogen fixing species

Importance

- Seeds are used for the extraction of "Karanja oil"
- Using advanced biotechnological tools:
  - Can understand genetic diversity of the species
  - Can analyze high oil yielding genotypes

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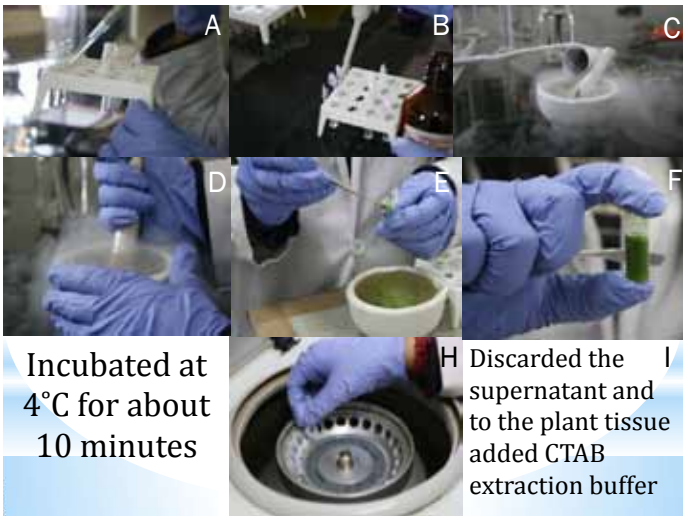
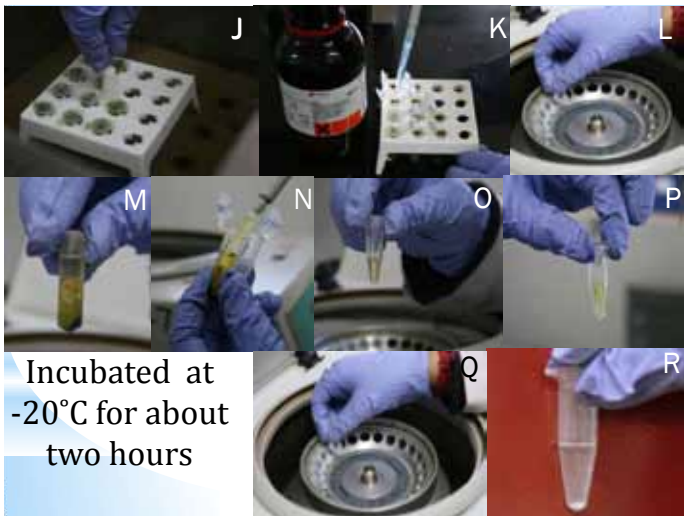
*METHODOLOGY

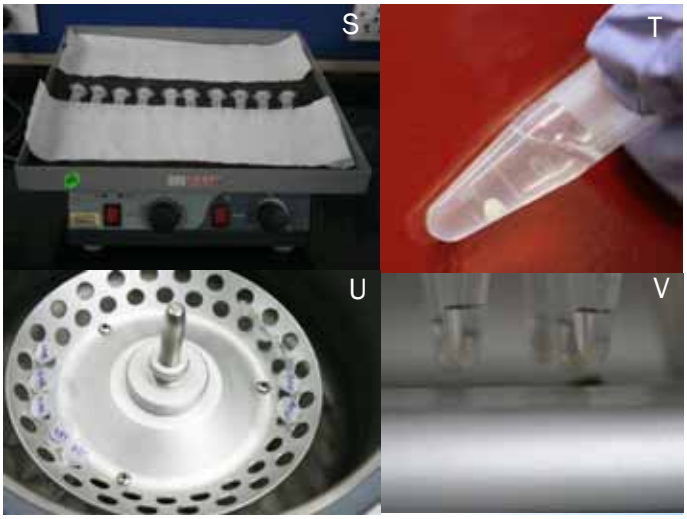


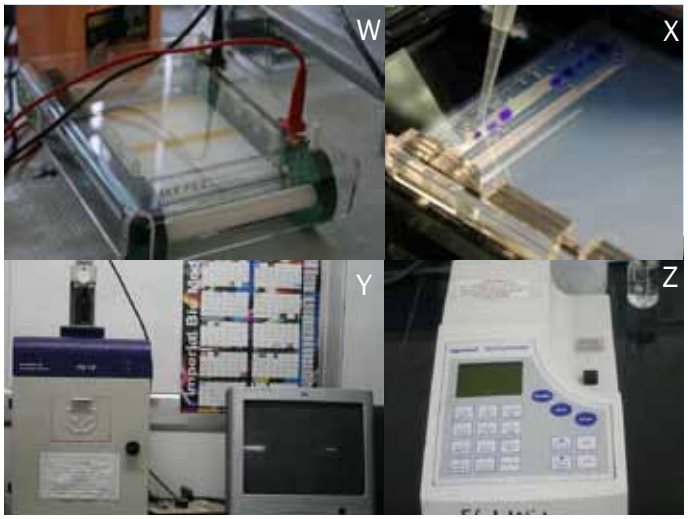
Incubated at 4°C for about 10 minutes

Discarded the supernatant and to the plant tissue added CTAB extraction buffer



Incubated at -20°C for about two hours





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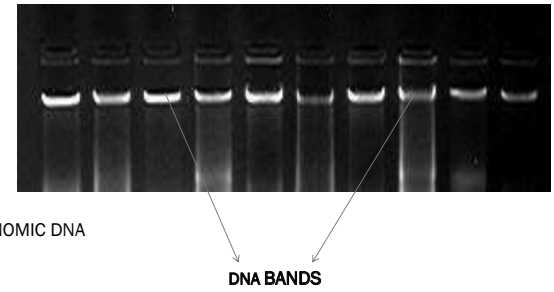
RESULTS



Protocol based on modification of CTAB method given by Doyle and Doyle (1990) and Stange *et al*.(1998) was optimized.

- Modified protocol helped in:
  - Removal of polysaccharides
  - Removal of polyphenols
- Extended time:
  - For incubation with extraction buffer in water bath
  - Of washing of DNA pellet with alcohol

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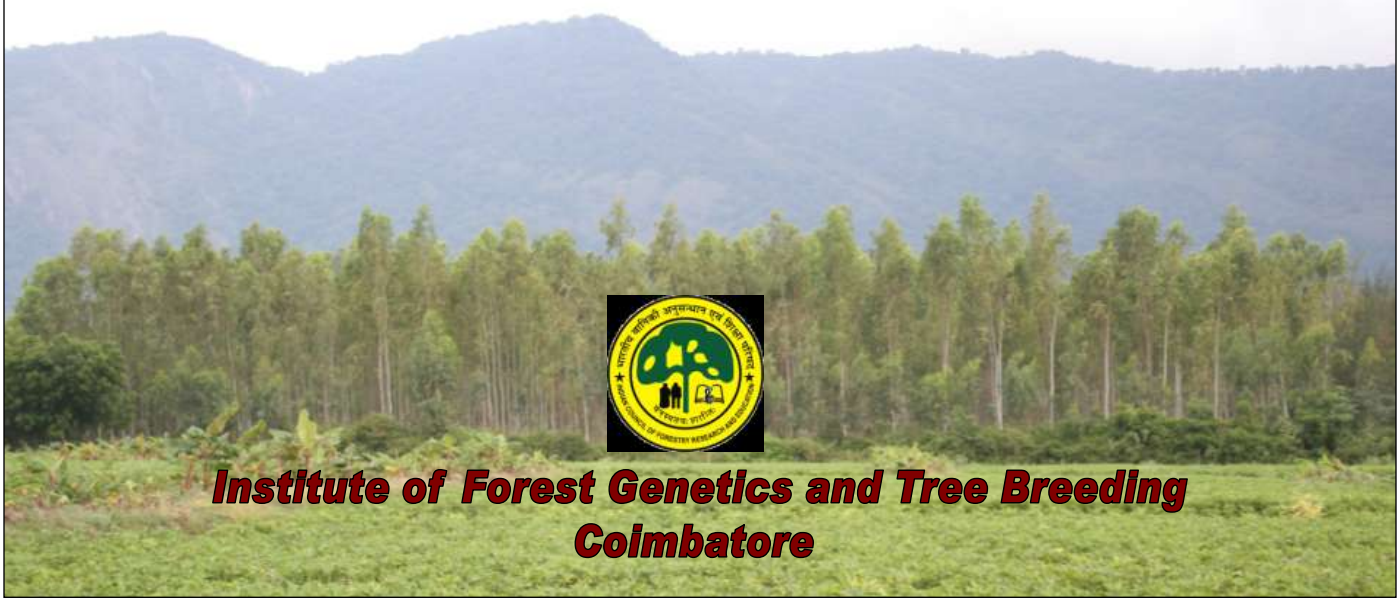
Eucalyptus improvement in Southern India

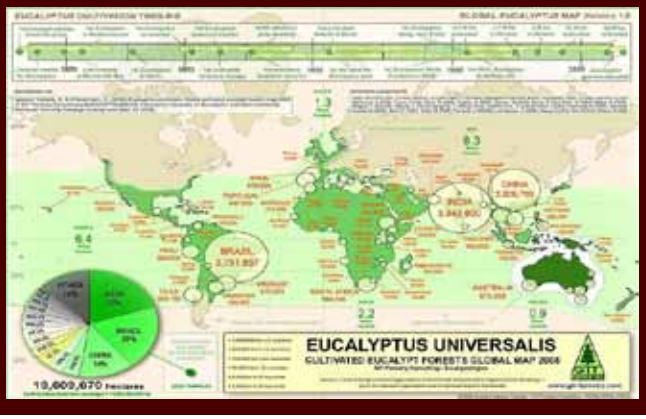
Sivakumar V., Nicodemus A., Gurudev Singh B. and Krishnakumar N.



Institute of Forest Genetics and Tree Breeding
Coimbatore

Eucalyptus cultivation in India

India is the largest planter in the world -3.9 million ha



Utility

- Major source of pulp for paper and rayon industry
- Minor uses – firewood, scaffolding, ply and furniture

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Yield

Avg height	: 12 m
Avg GBH	: 35 cm
Volume/tree	: 0.075 m^3
Present yield	: 40 MT/ha (dry)
(7 years)	75 MT/ha (wet)

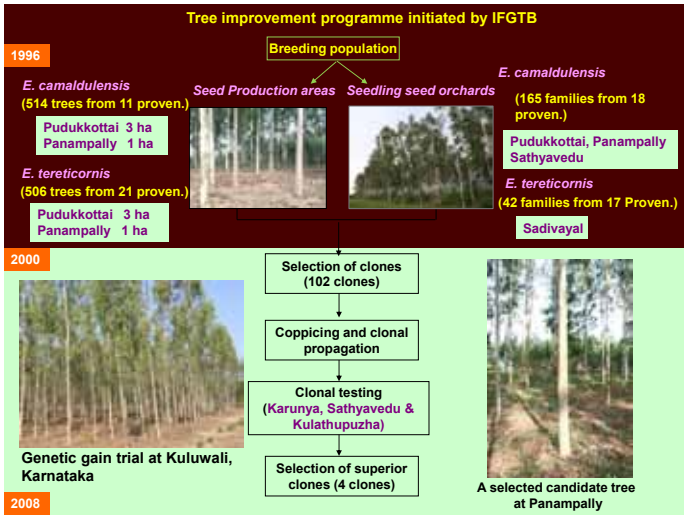
Eucalypts introduction

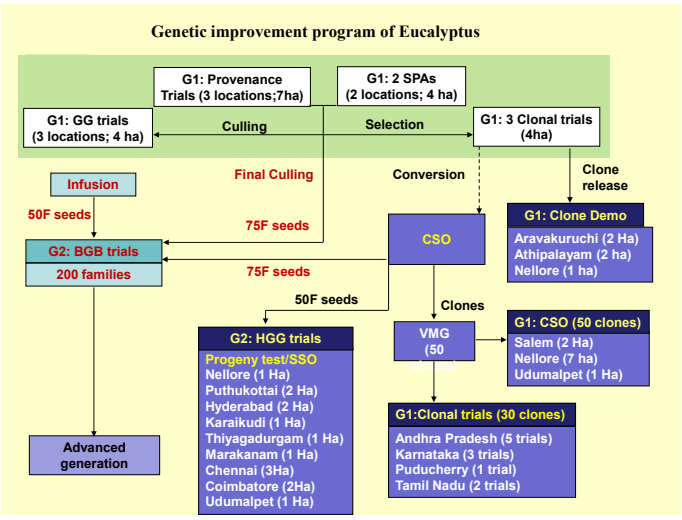
Tippu sultan	1790	16 species
TNFD	1910-1915	8 species introduced
TNFD, APFD	1950-1960	Mysore gum trials
F.Depts (A.P, T.N)	1960-1975	94 Species introduced
ERC	1975-1985	29 species introduced
FRC, TAFCORN, ERC	1975- 1985	15 provenances of *E.camaldulensis* and *E.tereticornis* (each)
IFGTB & TAFCORN	1996	11 provenances (514 trees) of *E. camaldulensis* (2 trials) & 21provenances (506 trees)of *E. tereticornis* for SPA (2 trials)
IFGTB, TAFCORN & APFDC	1996	18 provenances (165 families) of *E. camaldulensis (Total 3 trials)*
IFGTB	1996	17 provenances (42 families) of *E. tereticornis* (1 trial)
IFGTB & TAFCORN	1996	Progeny testing of 50 trees of 4 provenances (2 trials)

Eucalyptus seed sources for improvement programme

Australia

IFGTB	1999	-	Selection of plus trees from 4 SPAs and 4 Provenance trials and 2 progeny trials
IFGTB	2000	-	Establishment of Clonal trials in three locations
IFGTB	2003	-	Genetic gain trials
IFGTB	2008	-	Establishment of VMG for mass multiplication of selected clones
IFGTB	2009	-	Conversion of clonal trials into Clonal seed orchards
IFGTB	2009	-	Model clonal plantations -20 Ha Progeny testing of selected 50 clones Clonal trials 18 Ha. in AP, Karnataka & TN Clonal Seed Orchard (10 Ha.) in TN
IFGTB	2010	-	Release of 4 clones of Eucalyptus for commercial cultivation





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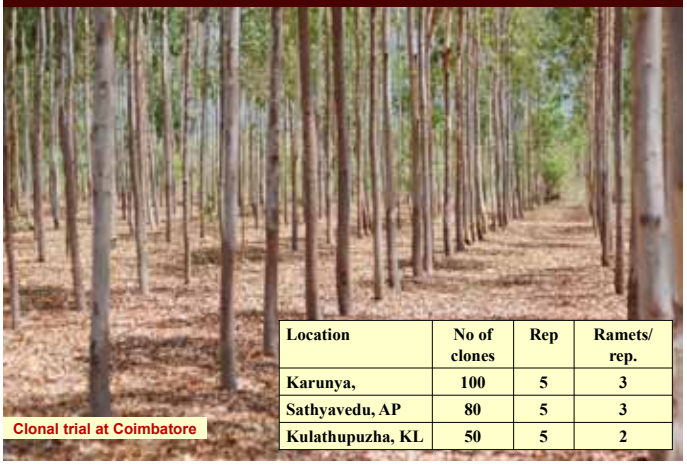
Clone selection

Year of selection : 1999

Satyavedu SSO	Ec
Pondy SSO	Et
Pudukkottai SPA	Et
Kennedy R- PRS	Ec
Pudukkottai SSO	Ec
Panampally SSO	Ec
Panampally SPA	Ec
Panampally SPA	Et
Karunya SSO	Et

Clones: 106

Clonal trials



Clonal trial at Coimbatore

Location	No of clones	Rep	Ramets/ rep.
Karunya,	100	5	3
Sathyavedu, AP	80	5	3
Kulathupuzha, KL	50	5	2

Superiority of the selected clones

Clone no. - IFGTB-EC-1 Clone ID in the test = **C-53**

Characters	Performance trial (Age wise)						% of superiority
	2	3	4	5	6	7	
Survival %	92.2	92.2	92.2	92.2	92.2	92.2	22.1
Height (m)	5.93	8.62	9.93	12.21	13.26	15.17	33.82
DBH (cm)	4.82	6.35	8.38	10.14	12.22	13.53	42.03
S. tree volume (m^3)	0.005	0.015	0.030	0.054	0.085	0.120	169.8
CBH (m)	2.27	4.55	6.20	8.10	9.20	10.55	68.0
St'ness	3.18	3.18	3.18	3.18	3.18	3.18	-2.05
Pruning Ability	No	Yes	Yes	Yes	Yes	Yes	-
Disease	Nil	Nil	Nil	Nil	Nil	Nil	-
Insects	Nil	Nil	Nil	Nil	Nil	Nil	-
Others	Rooting = 30-40%						

Clone no. IFGTB-EC-2 Clone ID in the test = **C-69**

Characters	Performance Trials (Age wise)						% of superiority
	2	3	4	5	6	7	
Survival %	97.8	97.8	97.8	97.8	97.8	97.8	29.4
Height (m)	6.29	8.85	10.26	12.42	13.5	15.6	38.32
DBH (cm)	4.84	6.39	8.22	9.81	11.7	12.9	35.61
S. tree volume (m^3)	0.006	0.015	0.029	0.051	0.08	0.11	154.4
CBH (m)	3.35	6.10	8.30	9.65	11.1	12.5	100.0
St'ness	3.53	3.53	3.53	3.53	3.53	3.53	8.90
Pruning Ability	No	Yes	Yes	Yes	Yes	Yes	
Disease	Nil	Nil	Nil	Nil	Nil	Nil	
Insects	Nil	Nil	Nil	Nil	Nil	Nil	
Others	Rooting = 40-50%						

Clone no. – IFGTB-EC-3 Clone ID in the test = C-111

Characters	Performance Trials (Age wise)						% of superiority
	2	3	4	5	6	7	
Survival %	97.8	97.8	97.8	97.8	97.8	97.8	29.4
Height(m)	5.12	7.59	8.82	10.7	11.9	14.2	25.3
DBH (cm)	4.19	5.69	7.33	8.84	10.7	12.5	32.0
S. tree volume (m^3)	0.00	0.01	0.02	0.03	0.05	0.09	118.4
CBH (m)	2.10	3.35	4.65	6.20	7.15	8.75	36.00
St'ness	3.59	3.59	3.59	3.59	3.59	3.59	10.62
Pruning Ability	No	Yes	Yes	Yes	Yes	Yes	-
Disease	Nil	Nil	Nil	Nil	Nil	Nil	-
Insects	Nil	Nil	Nil	Nil	Nil	Nil	-
Others	Rooting = 50-60%						

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Clone no. – IFGTB-EC-4 Clone ID in the test = c-19

Characters	Performance Trials (Age wise)						% of superiority
	2	3	4	5	6	7	
Survival %	92.2	92.2	92.2	92.2	92.2	92.2	22.1
Height (m)	6.04	8.65	10.0	12.2	13.4	15.2	34.51
DBH (cm)	4.99	6.46	7.96	9.33	10.9	11.7	23.67
S. tree volume (m^3)	0.006	0.015	0.02	0.046	0.069	0.09	105.72
CH (m)	2.15	4.45	5.85	8.65	9.45	11.5	84.00
St'ness	3.60	3.60	3.60	3.60	3.60	3.60	10.96
Pruning Ability	No	Yes	Yes	Yes	Yes	Yes	-
Disease	Nil	Nil	Nil	Nil	Nil	Nil	-
Insects	Gall formation only in coppice shoots						
Others	Rooting = 70-80%						

Stability Analysis

(Height after 7th year)

Clone	Mean Ht (m)	*bi*	Rank	S^2_d	Rank
19	15.243	1.014	1	-0.302	10
53	15.167	1.36	23	0.099	3
69	15.678	0.969	3	0.42	19
111	14.201	0.748	18	0.431	20
113	14.357	0.486	27	2.199	26
121	14.214	0.833	12	0.659	22
276	14.689	1.218	15	-0.202	7

(DBH after 7th year)

Clone	MEAN DBH (cm)	*bi*	Rank	S^2_d	Rank
19	11.785	1.262	22	0.294	13
53	13.533	1.272	24	0.719	20
69	12.857	0.692	26	1.903	29
111	12.584	0.937	6	1.435	28
113	11.934	0.531	31	-0.339	18
121	11.157	1.056	5	0.008	1
276	11.929	1.139	13	-0.216	9

Ranking of entries (Height in m)

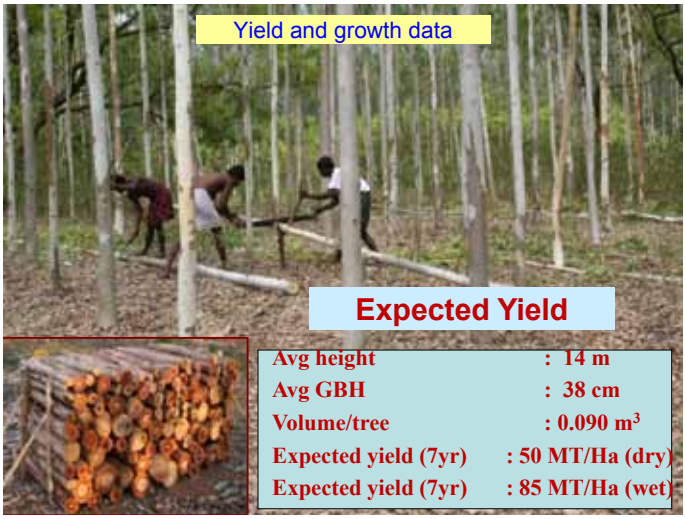
Clone ID	Coimbatore	Rank	Sathyavedu	Rank	Kulathupuzha	Rank	Over all	Rank
19	16.88	6	16.8	1	12.05	5	15.24	2
23	14.42	20	14.05	22	8.09	30	12.19	25
26	17.27	3	14.33	20	9.47	18	13.69	12
53	18.05	1	16.5	2	10.95	9	15.17	3
69	18	2	16.33	3	12.7	3	15.68	1
75	15.67	9	14.65	15	10.35	16	13.56	13
109	15.45	11	14.9	14	8.87	22	13.07	18
111	14.93	17	15.87	7	11.8	6	14.20	7
113	16.3	7	13.83	26	12.94	1	14.36	5
121	15.02	13	16.08	4	11.54	7	14.21	6
Comm.1	17.03	5	14.92	13	8.25	26	13.40	14
Comm.2	13.87	25	15.27	12	8.14	29	12.43	24
Comm.3	17.13	4	16.03	6	10.9	11	14.69	4
277	15.22	12	15.55	10	10.37	15	13.71	11
278	15	15	12.15	31	7.9	31	11.68	27
279	12.07	32	14.55	16	11.35	8	12.66	21
282 (seed)	12.08	31	14.38	19	10.92	10	12.46	23
283 (Seed)	13.03	28	15.43	11	12.84	2	13.77	9
284 (seed)	11.73	33	14.02	23	8.25	27	11.33	30

Ranking of entries (DBH in cm)

Clone ID	Coimbatore	Rank	Sathyavedu	Rank	Kulathupuzha	Rank	Over all	Rank
19	10.55	7	16.74	2	8.06	11	11.78	6
23	10.42	9	13.48	24	5.83	25	9.91	22
26	10.32	10	14.38	15	6.07	20	10.26	16
53	12.11	1	18.6	1	9.89	5	13.53	1
69	11.52	4	15.92	5	11.33	2	12.92	2
75	8.79	28	14.89	9	5.7	26	9.79	24
109	8.95	27	13.58	23	4.68	32	9.07	28
111	11.07	5	16.5	3	10.18	3	12.58	3
113	11.75	2	13.88	21	10.17	4	11.93	4
121	10.19	14	15.28	6	8.01	13	11.16	8
271 (ITC 3)	10.08	18	13.16	25	6.6	19	9.95	21
272 (ITC 7)	9.52	21	15.07	7	4.86	31	9.82	23
276 (ITC10)	11.69	3	16.05	4	8.04	12	11.93	5
277	10.19	15	14.6	13	7.41	14	10.73	10
278	8.59	31	12	30	5.43	28	8.67	31
279	8.23	32	14.42	14	8.35	7	10.33	15
282 (seed)	9.12	23	14.26	17	8.16	9	10.51	12
283 (Seed)	9.02	26	14.62	12	11.36	1	11.67	7
284 (seed)	8.09	33	5.85	33	5.85	24	8.78	30

Ranking of entries (Single tree volume m^3)

Clone ID	Coimbatore	Rank	Sathyavedu	Rank	Kulathupuzha	Rank	Over all	Rank
19	0.081	5	0.203	2	0.034	8	0.091	4
23	0.068	13	0.110	26	0.012	28	0.052	25
26	0.079	7	0.128	18	0.015	21	0.062	13
53	0.114	1	0.247	1	0.046	5	0.120	1
69	0.103	2	0.179	4	0.070	2	0.113	2
75	0.052	23	0.140	11	0.015	23	0.056	21
109	0.053	22	0.119	21	0.008	31	0.046	26
111	0.079	8	0.187	3	0.053	4	0.097	3
113	0.097	4	0.115	23	0.058	3	0.088	6
121	0.067	15	0.162	6	0.032	10	0.076	8
271 (ITC 3)	0.075	9	0.112	25	0.016	20	0.057	20
272 (ITC 7)	0.054	21	0.150	8	0.008	32	0.052	24
276(ITC10)	0.101	3	0.178	5	0.030	12	0.090	5
277	0.068	10	0.143	9	0.025	14	0.068	10
278	0.048	26	0.076	31	0.010	29	0.038	31
279	0.035	32	0.131	15	0.034	7	0.058	18
282 (seed)	0.043	29	0.126	19	0.031	11	0.060	15
283 (Seed)	0.046	28	0.143	10	0.072	1	0.081	7
284 (seed)	0.033	33	0.130	16	0.012	27	0.044	27



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Development of genetic linkage map in *Eucalyptus camaldulensis* X *E. tereticornis* using microsatellite markers

Subashini V*,Shanmugapriya A, Shobana S, Mayavel A, Sivakumar V, Bachpai V K W, Ganesan M, Modhumita Dasgupta, Nagarajan B, Krishna Kumar N and Yasodha R.

Division of Plant Biotechnology
Institute of Forest Genetics and Tree Breeding
Coimbatoe-641002

Conventional breeding

- Traditional breeding methods constrained by the long reproductive cycle and the difficulty in achieving significant improvements to complex traits like wood property traits, disease resistance, salt tolerance.
- Simple sequence repeats (SSRs) are used in genetic improvement of many crop and tree species

Linkage maps

- Position of DNA markers/genes/QTLs on the chromosome is called the linkage map or genetic map which is the basis for marker assisted selection and Marker Assisted Breeding
- Linkage maps are useful in physical mapping of specific gene clusters and for map based positional cloning to isolate complete gene.
- Populations for Linkage map construction
  - F2
  - Back cross
  - RILs (Recombinant Inbreed Lines)
  - NILs (Near isogenic Lines)
  - DHs (Double Haploids)

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Double Pseudo-testcross mapping strategy

- Forest trees (out breeding species) - non availability of inbred lines and self pollination not possible
- Pseudo-testcross - Testcross mating configuration is not known a priori
- Configuration is inferred a posteriori genetic segregation of the marker in the progeny of a cross between highly heterogenous parents

P1 X P2
Aa aa

Aa Aa aa aa 1 : 1 segregation

Objective

- To generate genetic Linkage map for the Interspecific cross *Eucalyptus camaldulensis* X *E. tereticornis* targeting the QTL for salinity tolerance trait

Materials and Methods

Selection of Parents

- Highly productive clones were screened with salt 14 clones of *E. camaldulensis* and 2 clones of *E. tereticornis* were used for screening salinity tolerance
- Highly salt tolerant clone of *E. camaldulensis* and salt susceptible clone of *E. tereticornis* were selected
- Interspecific hybridization for the DNA polymorphism and hybrid vigor
- Controlled Hybridization
  - *E. camaldulensis* - seed parent
  - *E. tereticornis* - pollen parent

Controlled Hybridization

- Clonal Seed Orchard (CSO) at Karunya, Coimbatore
- One month old seedlings were transferred to the polybags
- Six months old plants were field planted.

F1 individuals raised in the nursery

Grounded plants

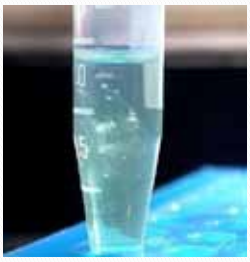
six months old seedlings

One month old seedlings

Seedlings in sand bed

DNA isolation

- Two hundred F1 individuals randomly selected for genotyping.
- Fresh leaves taken for DNA isolation using Qiagen DNeasy Plant Mini Kit
- The isolated DNA is quantified using picodrop spectrophotometer and used for genotyping



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Screening of primers

- 212 primers from different species were used for cross amplification.
  - ❖185 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)

Genotyping of parents

- Screening of parents for polymorphism is done with 5% ployacrylamide gel electrophoresis.
- Polymorphic Information Content, heterozygocity were generated using Power marker
- Further genotyping of hybrids in ABI 3500 Genetic Analyzer

PCR reaction mixture (Three primer strategy, Schuelke, 2000)

- 10 µl volume contains the following constituents:
- 1 x buffer
- dNTP mix - 125µM
- Forward primer with M13 tail at 5' end - 0.1 pmol
- Reverse primer - 0.4 pmol
- Fluorescently labeled universal M13 primer - 0.2 pmol
- Template DNA - 10 ng
- Taq DNA polymerase - 1 U

PCR condition

- First sequence of reaction
  - ❖94°C for 5 min
  - ❖30 cycles of
    - ➢ 94°C for 45sec,
    - ➢ Ta for 30 sec
    - ➢ 72°C for 1 min
  - ❖72°C for 15 min(elongation step)
- Second sequence of reaction
  - ❖20 cycles of
    - ➢ 94°C for 30sec
    - ➢ Ta of labelled M13 at for 45sec
    - ➢ 72°C for 45sec
  - ❖72°C for 30 min (final extension)
- Amplification carried out in Veriti PCR of Applied Biosystems, USA
- The PCR products were electroinjected ABI 3500 Genetic Analyser and sized using Genemapper version 4.1 software.

Chi square test and purity index

- Chi-square test was performed to check the segregation distortion.
- Purity index (%) = Number of true hybrids (containing alleles of both the parents)/Total number of hybrid seeds tested ×100

Results and Discussion

Transferability of microsatellites

- 212 loci developed from other species were used for screening
- 139 primers (65%) found to be cross amplified in parents
- 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).
- Heterozygocity was found to be 0.65
- Polymorphic Information Content was found to be 0.5 which has been reported between 0.2 to 0.6 (Faria et al. 2010)

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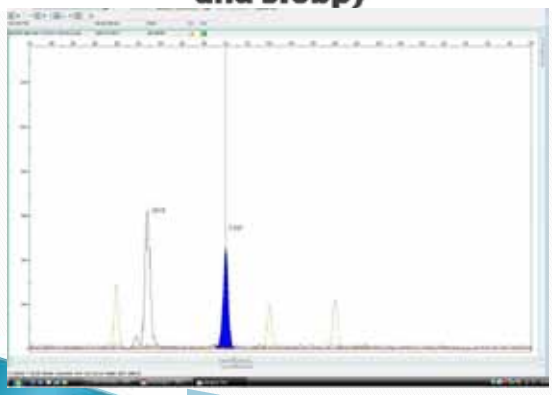
Table showing hybrid segregation pattern and individuals with non hybrid alleles

	E12		E36		E147	
Locus	Size 1	Size 2	Size 1	Size 2	Size 1	Size 2
E. camaldulensis	145	147	152	164	196	208
E. tereticornis	135	145	131	158	184	184
Hybrid	135	147	131	152	184	208
Hybrid contamination	128	135	150	162	200	200

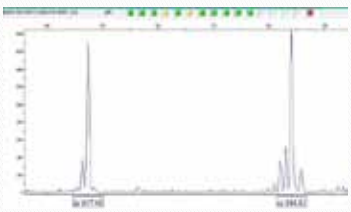
- **120 primers finally were chosen for the amplification of 200 F1 hybrids**

EMBRA89 locus with two allele (303bp and 310bp)

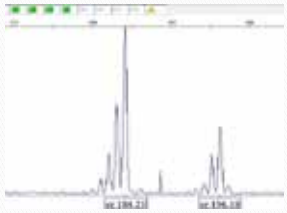


Genotyping pattern

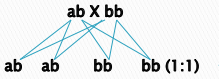
Alleles of *E. camaldulensis* with loci EMBRA 50



Alleles of *E. tereticornis* with loci EMBRA 50



Segregation pattern



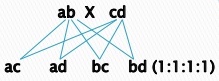


Table showing segregation pattern for six markers

Segregation type	Markers
MI(ab x bb / ab x cc)	EMBRA101, EMBRA 147, EMBRA 122,EMBRA50
FI(ab x cd / ab x bc)	EMBRA12 AND EMBRA 36

MI- Maternally Informative, FI- Fully Informative

Hybrid Purity

Loci	EMBRA12	EMBRA36	EMBRA122	EMBRA147	EMBRA50	EMBRA101
Purity Index in %	86	78	78	61	80	96

Hydrid purity of the pedegree varies between the markers. Purity Index ranges from 61% to 96%

Chi square test

Loci	χ^2 value
EMBRA12	0.243
EMBRA36	8.529
EMBRA122	2.7
EMBRA147	0.113
EMBRA101	11.17
EMBRA50	0.666

χ^2 SIGNIFICANT VALUE= 3.84 (df = 1)

The Chi-square value is less than the test value 3.84, which means that there is not statistically significant deviations from a 1:1 segregation ratio on the 5% level.

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 markers can be used for linkage map construction.

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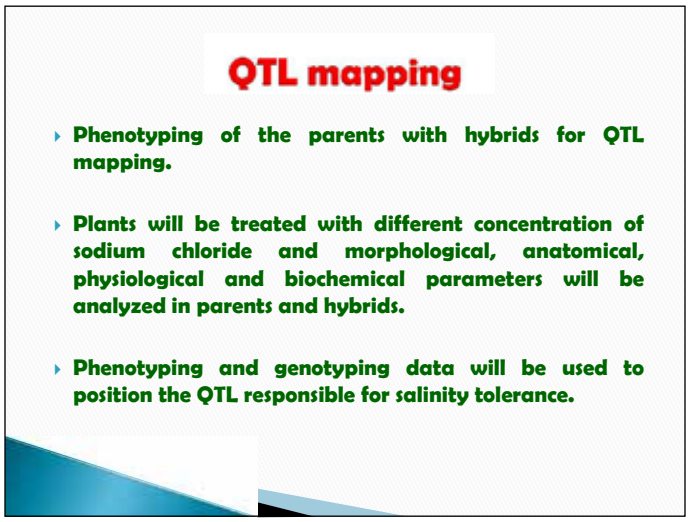

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INFLUENCE OF TIME OF CONE COLLECTION ON CONE CHARACTERISTICS IN BLUE PINE

S. K. LAVANIA, VIRENDRA SINGH AND BIRENDRA PRASAD

College of Forestry & Hill Agriculture, G.B.P.U.A.&T., Ranichauri, Tehri Garhwal, Uttarakhand, India -249 199

INTRODUCTION

Blue pine (*Pinus wallichiana*, A.B. Jacks) is evergreen tree with bluish foliage. It is distributed throughout temperate Himalaya at an altitude ranging between 2000-3000m and prefers cool and moist places for growth. It frequently occurs mixed with other species such as *Abies pindrow, Cedrus deodara, Picea smithiana*, *Quercus* species.

USES

Wood is used in packing cases, furniture, planking, doors and window frames, paper and pulp industry.

Wood is also used as a fuel and for manufacturing of charcoal.

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STUDY SITE

- Site: Harsil (Gangotri Range, Uttarkashi Forest Division)
- Altitude: 2700 m
- Latitude: 31° 1‘ 0"
- Longitude: 78° 45‘ 06"
- Cone collection was started on the 15th September, second on 30th September, third on 15th October and fourth on 30th October.

SEED EXTRACTION FROM CONES

RESULTS

Mid diameter, fresh weight and specific gravity of cones as influenced by time of cone collection

Time of collection	Cone mid diameter (cm.)		Cone fresh weight (g)		Cone specific gravity	
	I st Year	II nd Year	I st Year	II nd Year	I st Year	II nd Year
15th September	2.74	2.81	116.39	136.19	1.03	1.05
30th September	2.83	2.90	131.82	133.06	0.99	1.02
15th October	2.92	2.98	112.20	109.56	0.90	0.98
30th October	3.01	3.12	94.63	88.32	0.83	0.85
C.D. (5%)	0.17	0.13	7.35	12.40	0.13	0.10

- The cone diameter increased from first cone collection date to fourth (last) cone collection date during both collection years.
- The lowest cone fresh weight was recorded from the last collection date of 30th October during both the years.
- Cone specific gravity declined from first cone collection date to fourth cone collection date during both the collection years.

Total number of scales/cone and number of fertile scales/cone as influenced by time of cone collection

Time of collection	Total number of scales/cone		Number of fertile scales/cone		Percentage of fertile scales/cone	
	I st Year	II nd Year	I st Year	II nd Year	I st Year	II nd Year
15th September	71.45	75.68	58.10	59.23	81.32	78.26
30th September	73.79	79.44	62.29	63.63	84.42	80.10
15th October	74.64	79.56	63.69	59.14	85.33	74.33
30th October	77.50	81.71	62.99	64.75	81.28	79.24
C.D. (5%)	NS	NS	NS	NS	NS	NS

TOTAL NUMBER OF SCALES AND FERTILE SCALES PER CONE

Total number of scales and number of fertile scales per cone were statistically at par for different dates of cone collection during both the collection years.

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Per cent empty, non viable and viable seeds in cones as influenced by time of cone collection

Time of collection	Empty seeds (%)		Non viable seeds (%)		Viable seeds (%)	
	Ist Year	IInd Year	Ist Year	IInd Year	Ist Year	IInd Year
15th September	44.00	50.40	27.80	26.80	28.20	22.80
30th September	37.40	39.60	16.20	13.20	46.40	47.20
15th October	24.00	27.40	7.20	6.20	68.80	66.40
30th October	19.60	24.20	4.80	3.20	76.60	72.60
C.D. (5%)	3.32	3.76	3.58	5.04	3.79	4.08

PERCENT EMPTY, VIABLE AND NON VIABLE SEEDS/CONE

Percentage of empty and non viable seeds per cone decreased while percentage of viable seeds increased from first cone collection date to fourth cone collection date and recorded minimum for the cone collection date 30th October during both the collection years.

Moisture content, germination per cent and germination value of seeds as influenced by time of cone collection

Time of collection	Moisture content (%)		Germination (%)		Germination value	
	Ist Year	IInd Year	Ist Year	IInd Year	Ist Year	IInd Year
15th September	23.47	24.47	16.20	15.40	0.84	0.80
30th September	17.68	15.86	35.40	38.40	2.47	2.81
15th October	14.19	13.39	49.80	52.60	4.71	4.78
30th October	12.76	11.85	60.40	58.20	5.32	5.29
C.D. (5%)	1.57	1.28	4.08	3.99	0.15	0.08

MOISTURE CONTENT, GERMINATION PERCENT AND GERMINATION VALUE OF SEEDS

- **Seed moisture content tended to decrease from the first cone collection date to fourth/last cone collection date during both the collection years.**
- **Germination percentage and germination value improved significantly from first cone collection date to fourth cone collection date and maximum values recorded from the 30th October collected seeds during both the collection years.**

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Effect of salt stress on growth and proline levels in tolerant and susceptible clones of *Casuarina equisetifolia*

Thushara Parameshwaran, Sanu Mary Abraham, Selvakesavan, R.K., Aravinthakumar,V., Balasubramanian, A., Sangeetha, M., Sowmiyarani, K.S., Venkatachalam, R., Mathish Nambiar-Veetil

Institute of Forest Genetics and Tree Breeding Coimbatore

Plants response to high salt concentrations

- ❑Cellular Ion Homeostasis
- ❑Osmotic Homeostasis
- ❑Stress damage control and repair under salt stress

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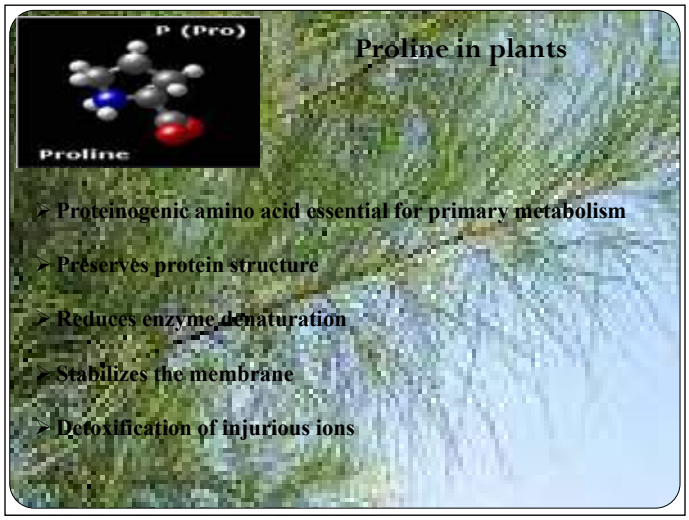
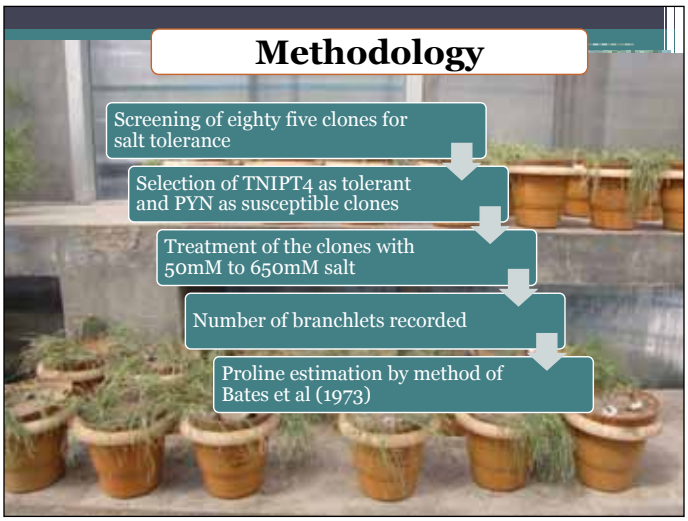


Studies in *Casuarina equisetifolia*

- Casuarinas are grown widely as wind shields in coastal areas.
- Tomar and Gupta (1984-94) categorized *C. equisetifolia* as moderately tolerant (EC 25-35 dS/m).
- It was reported that *C. equisetifolia* plants primarily synthesize proline as a major compatible solute to adjust the osmotic pressure under salt-stress conditions (Tani and Sasakawa, 2006).
- Studies on different Casuarina species clearly showed that the proline levels elevated till 150 mM salt concentration, and *C. junghuhniana* accumulated more proline than C. *cunninghamiana* and *C.equisetifolia* under salt stress (Reddy, 2001).

OBJECTIVE OF THE STUDY

- To study differences in proline accumulation during salt stress in tolerant and susceptible clones of *C. equisetifolia*



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RESULTS

The tolerant clone– TNIPT4 The susceptible clone– PYN

Drooping of Branchlets

- Drooping and yellowing of branchlets was observed at 250mM/ 4th week for PYN and 300 mM/ 5th week for TNIPT4.

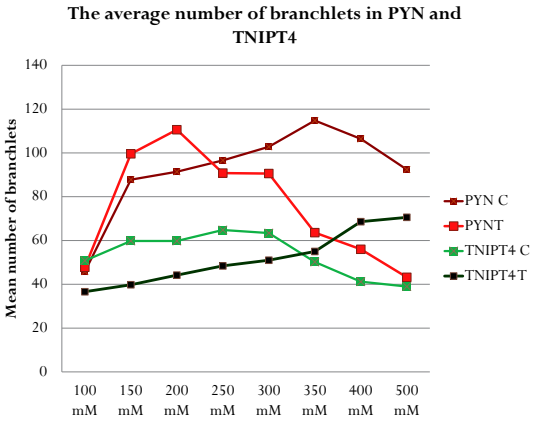
Tip Drying of branchlets

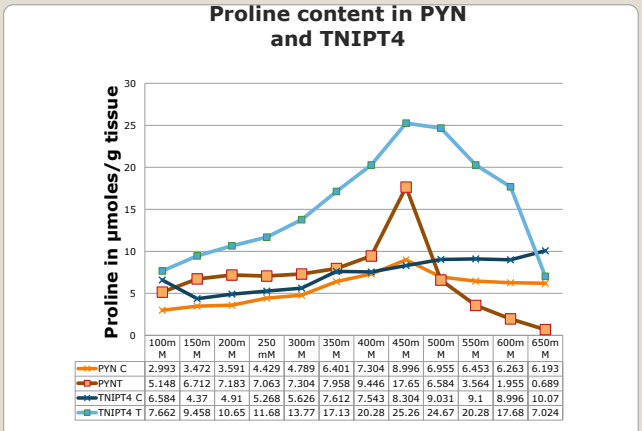
Drying of Branchlets

- The branchlets fell off the plants at 500 mM/9th week for PYN and 550 mM/ 10th week for TNIPT4.

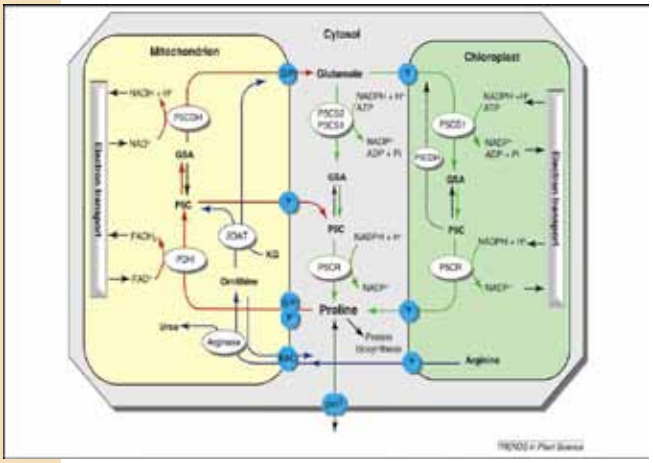
- It was also observed that PYN could not withstand NaCl concentration above 550 mM while TNIPT4 was able to survive till 650mM salt concentrations.

Death of the plants





	100m M	150m M	200m M	250 mM	300m M	350m M	400m M	450m M	500m M	550m M	600m M	650m M
PYN C	2.993	3.472	3.591	4.429	4.789	6.401	7.304	8.996	6.955	6.453	6.263	6.193
PYNT	5.148	6.712	7.183	7.063	7.304	7.958	9.446	17.65	6.584	3.564	1.955	0.689
TNIPT4 C	6.584	4.37	4.91	5.268	5.626	7.612	7.543	8.304	9.031	9.1	8.996	10.07
TNIPT4 T	7.662	9.458	10.65	11.68	13.77	17.13	20.28	25.26	24.67	20.28	17.68	7.024



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"Reproductive biology of *Aquilaria malaccensis* Lamk. a critically endangered species of North East India"

TN MANOHARA, MITALI DEVI, PALLABI BORA, GYAN PROTIM GOGOI

(Nov. 22-25, 2011, 1st Indian Forest Congress)

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Rain Forest Research Institute, Jorhat, Assam

(Indian Council of Forestry Research and Education)

16-01-2013

Class :MAGNOLIOPSIDA

Order: MYRTALES

Family: THYMELAEACEAE

Scientific Name: *Aquilaria malaccensis* Lamk.

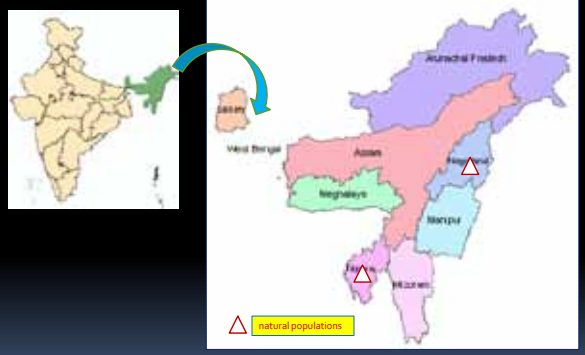
Common Name/s: Agarwood, Aloewood, Eaglewood, Lign-aloes; "*lequid gold*"

Native to - Bangladesh, Bhutan, India, Indonesia Iran, Malaysia, Myanmar; Philippines, Singapore, Thailand.

World wide - 15 sps; 02 in India - *A. malaccensis* and *A. khasiana.*

Tree, 20-40m tall, 60cm dia, found in wet-evergreen forests, rarely in semi-evergreen forests, 200-700m (up to 750m) altitude, on sandy loamy soil. India- endemic to NE states. At 5-6 years age it starts flowering.

Aquilaria malaccensis –distribution in India



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Significance

For centuries - traded internationally for the wood infected with fungi, called agar or amongst other things; it is used as incense, perfume and in traditional medicine etc. (borer: Zeozera conferta – Zigzag tunnel; Fungus: *Phialophora parasitica*; *Botryodiploidia theobromae*)

Red List Category & criteria: (IUCN Red List Version 2009.2) Vulnerable A1cd. Year Assessed: 1998.

In India it is considered as critically endangered, included in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and export has been prohibited.

16-01-2013

Methodology

✓Through regular field visit in different *Aquilaria* growing area - information on phenology, pollinators, pollination biology, natural recruitment from soil seed bank etc gathered.

✓Pollen viability - FDA test, *In vitro* pollen germination test - Brewbackers and Kwacks (1963) - Hanging drop culture; and pollen fertility - Aceto carmine method.

✓Pollen-pistil interaction: Stigma receptivity- determined by esterase activity and peroxidase test.

✓Microsporogenesis and male gametophyte development - customary method of Microtomy.

✓For other test standard methodology involved in reproductive biology was followed.

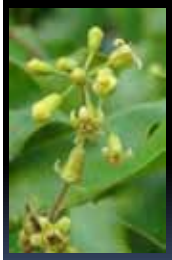
GPS Location of study sites

Salna (Salbari), Nagaon district, Assam	Longitude: N 26^{0}26′36.3″ Latitude: E 093^{0}59′49.9″ Altitude: 65m
Narengre (Darugiri), Tura, Meghalaya	Longitude: N 25^{0}36′36.1″ Latitude: E 090^{0}44′32.0″ Altitude: 301m
Dimapur(Tahykhu Village), Nagaland	Longitude: 25^0 32′ 19.3″ Latitude: E 093^0 32′ 39.4″ Altitude: 355m
Old Beisumpui, near Jalukae, Nagaland	Longitude: 25^{0}31′33.0″ Latitude: E 093^{0}35′44.8″ Altitude: 355m
New Beisumpui (Itangkam village), near Jalukae, Nagaland	Longitude: 25^{0}32′19.3″ Latitude: E 090^{0}44′32.0″ Altitude: 335m
Trishna WLS, Tripura	Longitude: 23^0 16′ 57.8″ Latitude: E 091^{0}23′ 22.6″ Altitude: 223m
Moreh, Imphal, Manipur	Longitude: 24^0 15′ 0.36″ Latitude: E: 094^0 17′ 57.2″ Altitude: 223m

Results

Phenology: Flowering- March – May; Fruiting: April -July.

Flowers: bisexual, entamophilous



A flowering branch

Pollen Count/ pollen to ovule ratio
Flower examined- from RFRI GARDEN

Date	No. of ovule per Flower	No of Pollen per flower	Pollen to ovule ratio
15.05.2011	02	7799	1: 3899

Pollination efficiency

Flowers collected from Dimapur (NL) on April 14th 2011
(Flowers at senescence stage selected)

Total No. Of flowers observed	Flowers with Pollen on Stigma	Flowers with Normal Ovary/ovule	Damaged Ovary/ovule
68	63	54	14
Pollination efficiency 63/68 pollinated 93%		14 ovule found damaged-by larvae of psylidae- sap succer- present on stigma/ovary 21% -infestation	

A fruiting branch

Fruit at dehiscence

Seed

Seed caruncle

Seed dispersal by Wasp

Seeds are recalcitrant with 27-30% moisture content

Fresh Weight (10 seeds)	Dry Weight (10 seeds)	% moisture content per seed
0.88gm	0.616gm	27

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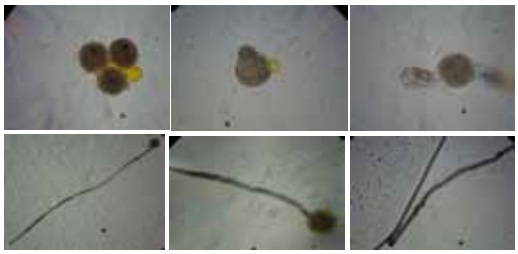
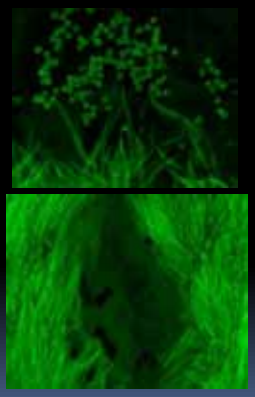
In vitro pollen germination studies



Brewbaker & Kwacks, 1963, medium with 15% Sucrose gives optimal result, Pollen germinate after 1 hr of Incubation. Pollen freshly collected shows 80% viability, at room temperature storage after 5 days- complete loss of viability, at-4°C storage viability extended to 7 days and at -20°C to 12 days.

16-01-2013

Aniline blue florescent micrographs of stigma and style



Stigma Receptivity by Esterace activity test

Stage - 0	Stage -1	Stage -2	Stage -3
Unopened flower bud	1 Day After opening	2 Day After Opening	3 Day after opening
Less intensive stain	Reddish stain	Reddish stain	Staining intensity less

Stigma receptivity starts at Anthesis stage and max. on one Day After Opening and lost for 3 Day after Opening of flower.

Esterace Test for Stigma receptivity

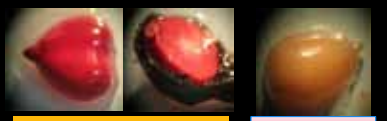
16-01-2013

Studies on seed germination

Germination is Epigial type and starts after 15 days

Seeds – room temp (±30°C) up to - 10 days; -4°C up to 20 days; and -20°C up to 30 days retain viability – however germination vigour declines as day proceeds in all the cases.

TTC – Test for seed viability



Seeds Darkly Stained
Viable

Seed Lightly STAINED
Non viable

Protura
It feeds on caruncle and seeds of *Aquilaria*
Class- Insecta Three pairs of legs), Super Order/ Group- Apterygota(Wingless, microscopic insects, < 10-15mm in size).

16-01-2013

Seed germination studies

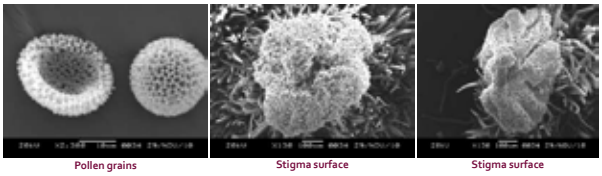
Room Temp. (Soil bed germination)

Date of Collection	Date of Sowing	Date of Observation	% Germination
19/08/10	20/08/10	11/20	55
19/08/10	25/08/10	7/20	35
19/08/10	27/08/10	3/20	15
19/08/10	31/08/10	0/20	0

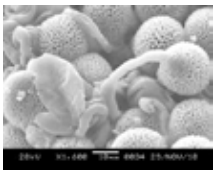
Refrigerated (Soil bed germination)

Date of Collection	Date of Refrigeration	Date of Sowing	Date of Observation	No. of Germination	% Germination
30/07/10	02/08/10	09/08/10	14/09/10	2/25	8%
30/07/10	02/08/10	16/08/10	14/09/10	1/25	4%
30/07/10	02/08/10	23/08/10	14/09/10	0/25	0
30/07/10	02/08/10	30/08/10	14/09/10	0/25	0

16-01-2013

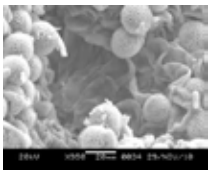


Pollen grains | Stigma surface | Stigma surface



Germinating Pollen grains on stigma surface

SEM Photo MICROGRAPHS



Germinating Pollen grains on stigma surface central receptive surface

Pollen grains shows the presence of viscin threads

16-01-2013

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Breeding system

- Bagging experiments were conducted to test Apomixis, auto gamy, self pollination, cross pollination .
- It is revealed that it is an obligate out breeder. No apomixis.
- Open pollination (1/5 of flowers) fruit sets; autogamy and selfing no fruit sets – incompatibility barrier on stigma surface.
- Insect visited flower (nectar is the reward; 5-10µl per flower present)shown fruit set – pollinators plays crucial role.

New recruitment from soil seed bank

Seeds can disburse max. 20 meter. Seed disburse – wind and gravity, to some extent wasps which feed on caruncle also help in this process. Maximum density of seedlings found 2 m to 5 m radius below canopy (240-350/sq m). Up to 2 months 30 % mortality, 6 months old 50-60% mortality observed- competition by weeds and feeding by larvae - hampers the growth only 10-20% reach sapling stage.

Young yatching moth

Young flower infested by larva

Adult moth

Infestation by larvae of *Heortia vitessoides* (Pyralidae: Lepidoptera)

16-01-2013

Ladybird beetle

Pollinators

Dorsal view

Ventral view

16-01-2013

Thrips

Pollinators

Lateral view

Thorasic region ventral view

Ventral view

Un identified

16-01-2013

Pollinators

Butter fly

Apis dorsata
Honey bea

16-01-2013

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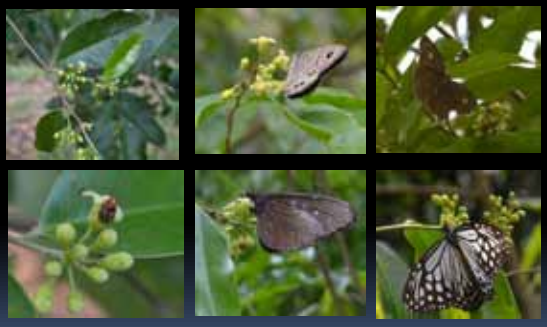
Pollinators



20 year old plant at TRISHNA WLS

Infested by fungus

16-01-2013

Conclusion

- Considering endemisity, economic importance and present status of conservation - confined to NE India, almost become rare in natural forests; found only in home stead proper protection required-reintroducing to RF / Protected areas.
- overexploitation and habitat fragmentation are apparent factors – population decline, from studies on breeding system- obligate out-breeder – pollen from other population is required for pollination, Maintaining different viable population (only can attract insects) essential. Insect plays a pivotal role in pollination. Protecting pollinators in the fragiling ecosystem, maintaining biodiversity as alternate food source to pollinators is very essential.
- Seeds are recalcitrant loses viability shortly, difficult to store high moisture content – fungus infection – collecting and subject to germination at nursery and field transfering of established seedling needed.
- Further understanding of reproductive biology of other RET species very essential to device strategy for conservation.

Acknowledgement

- Financial assistance from ICFRE is thankfully acknowledged.
- I am thankful to Director, RFRI, for the keen interest and encouragement.
- Thanks are due to Prof. K.R. Shivanna, INSA senior Scientist, ATREE, Bangalore.

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A Complete Protocol for The Native Biodiesel Plant - *Pongamia Pinnata* Using Low Cost Alternatives For Development Of High Frequency Micropropagation

Presented by:
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INTRODUCTION

- India is sixth in the world in energy demand accounting for 3.5% of world commercial energy consumption.
- A large part of the population has no access to commercial energy from hydrocarbons at all.
- India's import of crude oil is expected to go up from 85 million t to 147 million t by 2007.
- Bio-energy, as a replacement for transport fuel can be alcohol, bio-oil or bio-diesel.
- Bio-diesel, considered an equal replacement of petro-diesel (with 5% less efficiency), can be made after transesterification from virgin or used vegetable oils (both edible or non-edible).

PLANT OILS USED FOR BIO-DIESEL

- A variety of biolipids can be used to produce biodiesel. The main plants whose oils have been considered as feedstock for bio-fuel are mentioned are artichoke, canola, castor, coconut, cottonseed, flax, hemp, rapeseed, safflower, *Jatropha, Pongamia* etc.
- Among the important biodiesel plant *Pongamia pinnata* had been taken up for this study.

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A. Tree of *Pongamia pinnata* in nature

B. Flowering stage

C. Fruting stage

- *Pongamia pinnata* (L.) Pierre is commonly called Pongam, Karanja.
- Its belong to the family *Legumenaceae*.
- It is often planted as an ornamental in garden and along avenues and roadsides for its fragrant wisteria-like flowers.
- *Pongamia pinnata* is one of the nitrogen fixing tree species.
- This plant native to India, appears to have good potential for biodiesel production from its seed oil.
- The seeds of this tree contain about 30-40% oil.
- Seed oil contain two flavonoids, Pongamol and Karanjin, which makes it unsuitable for edible purpose.
- Pongam oil has been recognised as "Biodiesel" as several parameters of diesel.
- Biodiesel from these seeds is fast emerning as a viable alternative to fossil fuel.

Uses of plant parts

Pongamia also possess valuable medicinal properties. The reference literatures related to different systems of medicine in India specially related to Ayurveda are full of miraculous therapeutic properties of *Pongamia*.

- Leaves juice is used for cold, cough, diarrhea and leprosy.
- Roots are used for cleaning gums, teeth and ulcers.
- Bark is used internally for bleeding piles.
- The leaves are used as a fodder.
- Dried leaves are used in stored grains to repel insects.
- The seedcakes is used as cattle and poultry feed and biogas.
- The waste pulp is used as an organic fertilizer, which provides a good income to the rural poor.
- The oil is used as a lubricant, water paint binder, pesticide and in soap and tanning industries.
- The use of wood is limited to cabinetmaking, cartwheels, posts and fuel.
- The ash of the wood is used in dyeing.

Low Cost Alternative

- Low cost technology means an advanced generation technology in which cost reduction is achieved by improving process efficiency and better utilization of resources.
- Low cost option should lower the cost of production without compromising the quality of the micropropagation and plants .
- 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.
- Out of all component used in a media, gelling agents such as agar contribute 70% to the total cost of media .
- *Pongamia* utilizing low cost alternatives aimed at cost reduction. We describe a reproducible protocol for micropropagation of *P. pinnata* through mature nodal segments.

Methodology

Selection of sample trees: Healthy sample trees were selected from local population in around Jodhpur region and from 10-15 years old mature trees growing locally in AFRI campus, Jodhpur.

Explant selection and sterilization: Twigs of *Pongamia pinnata* were collected from mature trees. Nodal explants of approximately 3-4 cm in length. Explants were treated with few drops of tween 80 detergent solution and rinsed with distilled water. These were then treated with alcohol for 3-5 minutes, then explants dipped in solution of Bavistin and Streptomycin for 15-20 minutes. Surface sterilized with 5% NaOCl for 5 minutes.

Tissue culture media: Various types of predefined synthetic media (like MS, Gamborg, Anderson's etc.) had been prepared and tested for response. Various levels of basal salt concentration had been tried out and the effect had been studied. Full strength MS medium supplemented with 3% sucrose, 0.8 % agar and different gelling agents was used during the study.

Alternatives of the gelling agents: This had been studied on various stages of tissue cultures with the aim of standardization of plantlet regeneration protocol with cost efficiency. For induction, multiplication and rooting of shoots, the explants were cultured on MS medium supplemented with BAP along with gelling agants like sago powder, isabgol and guargum.

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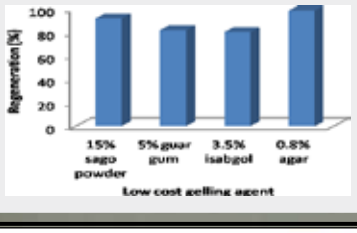
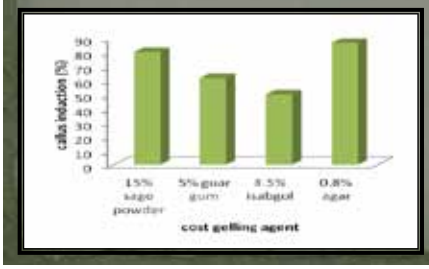
Bud break response in *Pongamia pinnata* with low cost alternatives

➢ Bud break response was observed in mature nodal segments when cultured on MS medium supplemented with BAP. Best response (100% bud break vis-à-vis micro-shoot proliferation) was observed on media supplemented with BAP and agar.



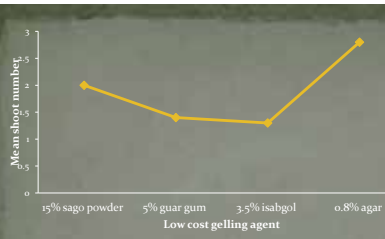
Effect of low cost gelling agents on regeneration in *P. pinnata*



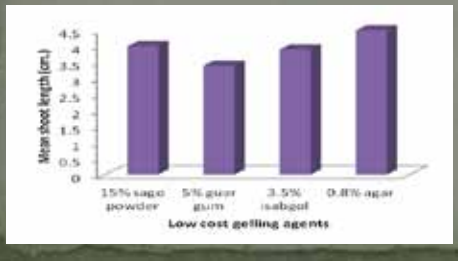
Effect of low cost gelling agents on callus induction in *P. pinnata*

Multiplication in *Pongamia* with low cost alternative from mature stem nodal segments

- Full strength MS medium supplemented with BAP for *in vitro* shoot multiplication responses.
- Sucrose was used as the carbon source in all the combination.
- The low cost media were solidified with sago powder, guar gum and isabgol in place of agar.
- The frequency of explants producing shoots, number of shoots per explants and shoot length were observed after 8 week of culture



Effect of low cost gelling agents on number of shoot produced /explants in *P. pinnata*



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

Rooting experiment in *Pongamia pinnata* with low cost alternative

- Rooting experiment were successfully done on various gelling agents of MS medium along with different concentration of IBA.
- 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
- Coir in liquid medium as alternative to the agar

- Micro-shoot proliferation and *in vitro* rooting experiment were completed.
- In *Pongamia pinnata* complete protocol will be achieved.

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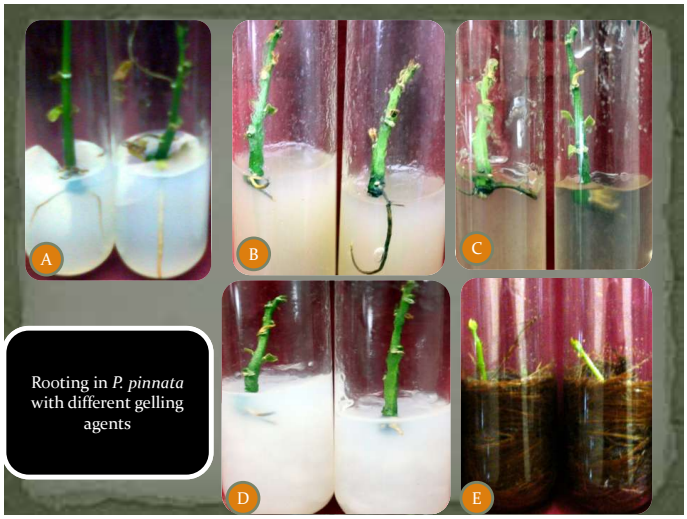
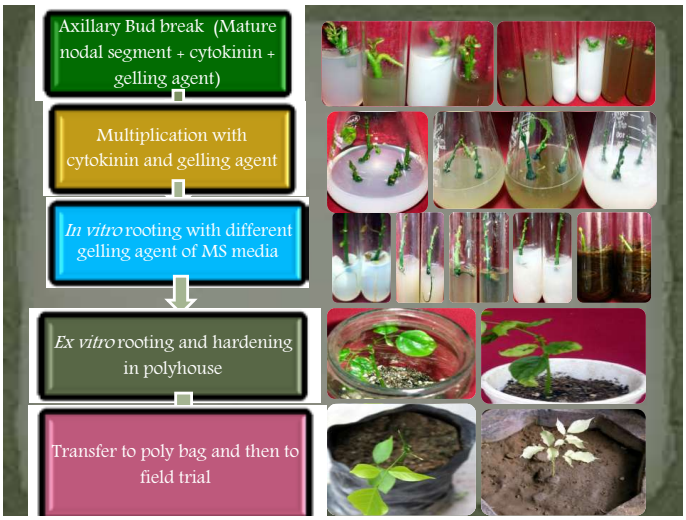


Effect of gelling agents on rooting of micro shoots of *Pongamia pinnata*

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±6.9^{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}
0.8% Agar (C)	100.00±0.0^{c}	2.5±0.2^{c}	1.5±0.0^{d}	76.67±7.8^{c}

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



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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

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College of Forestry & Hill Agriculture,
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Hill Campus, Ranichauri – 249 199

INTRODUCTION

- **Botanical name :** *Cedrus deodara*
- **Common name :** Deodar
- **Family:** Pinaceae
- **Distribution:** Western Himalayas chiefly between 1700 m – 2500 m, rarely up to 3000 m elevation
- **Uses:** Timber, Cedar oil, urban landscape

Deodar tree bearing cones

Deodar cones

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Deodar mature cone

Deodar seeds with wings

MATERIALS AND METHODS

- **Place of cone collection:** Buranskhanda, Compartment 4b, Dhanolty Range, Mussoorie Forest Division
- **Time of cone collection:** 1st October, 2009
- **Altitude:** 2385 m
- **Aspect:** 30˚ 26' N Latitude 78˚ 14' E Longitude

- **Position of crown:** Upper 1/3 crown, Middle 1/3 crown, lower 1/3 crown
- **Number of trees:** 10 trees
- **Number of cones:** 40 from each position
- **Seed weight & moisture content:** I.S.T.A.
- **Replication:** 4 each containing 100 seeds
- **Temperature:** 20ºC ± 0.5

- **Sowing of seeds in nursery:** March, 2010
- **Depth of sowing:** 10 mm
- **No. of seed/m²:** 400 in 4 lines
- **Germination Value:** Czabator (1962)
- **Speed of germination:** Maguire (1962)
- **Germination index:** Kendrick & Frankland(1969)
- **ANOVA:** Snedecor & Cochran (1989)

RESULTS

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	NS	12.84	13.27	24.18	3.50

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Table 2: Seed length, width, thickness, 100 seed weight and moisture contents influenced by crown position

Crown position	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	100 seed fresh weight (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

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

Table 4: Seed germination, germination value, germination index and speed of germination as influenced by crown position under nursery condition

Crown position	Germination per cent	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

Conclusion:

1 The results of the present study clearly insinuated:

- that the seeds extracted from the cones collected from upper crown exhibited superiority in different attributes over the seeds extracted either from middle or lower crown. Therefore, the preference should be given to the collection of cones from upper crown to get the superior quality seeds in Himalayan Cedar.

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Use of most common alleles for species discrimination in *Eucalyptus camaldulensis* and *Eucalyptus tereticornis*

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Eucalypts

- India is the major planter of *Eucalyptus* with an area of 3.943 M ha
- *Eucalyptus tereticornis* and *E.camaldulensis* are widely planted for pulp wood.
- Both belonging to the section *Exsertaria* of subgenus *Symphyomyrtus*.
- They are diploids (n=11) with a genome size of 1.23 and 1.20 pg/2C and about 590 Mbp/C and 580 Mbp/C for *E. camaldulensis* and *E. tereticornis* respectively.

- Domestication program of these species was systematically implemented in India and provenance cum progeny trials, SPAs, half pedigreed SSOs and clonal plantations were established.
- Both the species are closely related and form hybrids naturally

Differentiation of *E.camaldulensis* and *E.tereticornis*

- Discrimination of these species and its hybrids based on morphological features is very difficult.
- Species identity is highly essential during the establishment of seed orchards and progeny trials. Confirmation of genetic purity of the species facilitates estimation of genetic worth in progeny trial as well as assures seed purity.
- Use of mixture of species or hybrids would deter genetic quality of seeds and consequently productivity.
- Development of microsatellite markers (SSRs) with high discrimination power are possible.
- Hence, efforts were made to use microsatellite markers and discriminate the species and their putative hybrids.

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Plant Materials

- 40 *E. camaldulensis*
- 35 *E. tereticornis*
- 7 landraces (putative hybrids between EC and ET)
- The pure species samples were collected in the provenance trial cum seed orchard raised during 1995 from seeds belonging to Australia and Papua New Guinea provenances supplied by CSIRO, Australia.
- Landraces were selected from the seed raised plantations

Microsatellite Markers

- 109 microsatellite loci developed for *E. grandis*, *E. urophylla*, *E. nitens* and *Corymbia* were cross amplified in *E.camaldulensis* and *E.terticornis*
  - EMBRA -SSRs (Brondani et al. 2006)
  - CSIRO -SSRs (Thamarus et al. 2002)
  - EST-SSRs (Yasodha et al. 2008)
  - EMCRC -SSR (Sheperd et al. 2008)

Methods

- Microsatilite amplification was carried out using the genomic DNA and resolved on 5% denaturing polyacrylamide gels
- Bands were detected with Silver staining
- Analysis of most common alleles was carried out using the GDA 1.1 software
- Population structure was estimated using STRUCTURE software
- Dendrogram was generated using the Power Marker software.

Results

- 62 microsatellite loci - (55 loci belong to 11 linkage groups and 7 loci are unmapped) – gave proper amplification.
- Three populations were considered –
- *E.tereticonis*, *E.camaldulenis* & Landraces
  - 59 loci were polymorphic
  - 3 loci were monomorphic (across all 3 groups)
  - 24 loci were monomorphic between the 2 species
  - 38 loci polymorphic across all 3 groups

Identification of Most common alleles

- 38 microsatellite loci
  - Polymorphic across all the 3 groups
  - Analysed for most common alleles

Species specific alleles

- *E. tereticornis* – 23 loci
- *E. camaldulensis* - 14 loci
- Landrace – 38 loci
- 13 SSR loci of landraces shared with either of the species

Most common alleles(bp)

Locus name	LG	*E. camaldulensis*	*E. tereticornis*	Landraces
Embra11	1	138	136	138
Embra56	1	160	148	148*
Embra6	1	140	148	140
Embra70	1	158	162	154
Embra12	1	134	134/142	134
Embra35	1	232/254	240	262/230
Embra100	1	238	250	246
En10	1	144	140	150
Embra172	2	296	294	292
Embra43	2	102	114	102
Embra207	2	236	228	220
Embra227	3	312	292	318
Embra122	3	136	144	124
Embra77	3	318	308	286/318
Embra24	5	152	148	148*
Embra5	5	130	126	124

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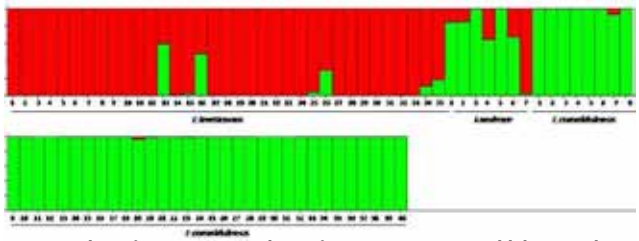
Population Structure

- Prior population, no admixture model
- Evanno's ΔK = 2
- Two genetic clusters represent *E.camaldulensis* and *E.tereticornis*

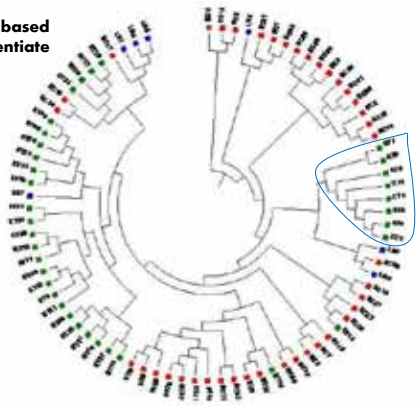


Barplots of STRUCTURE analysis of *E.tereticornis*, *E.camaldulensis* and Indian landraces. Each bar represents a single individual.

Genetic diversity and clustering

Roger's genetic distance based dendrogram could differentiate the species

- Landraces did not group in specific cluster
- Landraces are the composite mixture of ET and EC
- Orobay provenance of ET formed a separate cluster indicating its isolation in PNG - Owen Stanley Range



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EFFECT OF POTENTIAL ISOLATES OF ECTOMYCORRHIZAL FUNGI ON GROWTH IMPROVEMENT OF COMMERCIALLY IMPORTANT PLANTATION SPECIES, *CASUARINA EQUISETIFOLIA* AND *C. JUNGHUHNIANA* SEEDLINGS

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Forest protection Division
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SIGNIFICANCE OF ECM FUNGI

- Improved nutrient uptake.
- Adaptation and Survival of land plants.
- Longevity of its feeder roots.
- Increased rooting of woody plant cuttings.
- Increased tolerance of
  1. Drought
  2. Salts
  3. Heavy Metals
  4. Pathogens

WHY CHOOSE CASUARINAS?

- Multipurpose farmer friendly tree species.
- Root nodules contain *Frankia.* They fix atomospheric Nitrogen and enhances nitrogen nutrition in the soil.
- Casuarina has wide ecological adaptation.
- Grows well in coastal and salt affected areas (El-Lakany *et al.*, 1990; Marcar, 1996).

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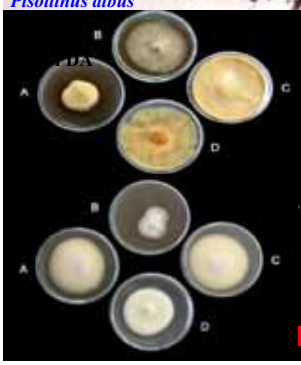
METHODOLOGY

Pisolithus albus

Laccaria fraterna



Pure cultures of *Laccaria fraterna*

Pure cultures of different isolates of *Pisolithus albus*

ECM fungal fruit bodies of *Pisolithus albus*

ECM fungal fruit bodies of *Laccaria fraterna*

Basidiospore inoculum of *L. fraterna*

Basidiospore inoculum of *Pisolithus albus* (4 isolates)

P. albus

Types of ECM inocula

Basidiospore inoclum

L. fraterna

Vermiculite based Mycelial inoculum

Alginate bead inoculum

RESULTS

Data on growth parameters of both ECM (*L. fraterna* and *P. albus*) inoculated and uninoculated (control) seedlings of *Casuarina equisetifolia* and *C. junghuhniana* was collected at periodical intervals.

Among different isolates of *P. albus* tested, vegetative mycelial (vermiculite based) inoculum of Isolate 3 was found to be the most efficient inoculum which gave 2-3 fold growth increment in Casuarinas in nursery.

It was observed that number of root nodules was found to be more and highly significant in *Casuarina* seedlings inoculated with vegetative mycelial inoculum of *P. albus*.

It was also found that ECM inoculated plant samples of both *C. equisetifolia* and *C. junghuhniana* revealed appreciable amount of biochemical parameters such as protein, carbohydrate, phenol and lipids.

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Effect of different types of inocula of ECM fungi on the shoot length (cms) of *Casuarina equisetifolia* seedlings in different soil types in the nursery

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	23.00cde	27.94b	35.82b	28.59g	32.69b	39.33b
T2	22.80cde	34.44cd	41.89c	23.23def	39.44c	44.37c
T3	21.53bcd	32.64c	38.96c	16.86ab	34.72b	40.98b
T4	20.73bc	36.74de	41.24c	18.68bc	41.38cd	46.05c
T5	24.66e	40.28fg	47.65def	28.10g	45.46ef	50.60d
T6	21.26bcd	35.48d	40.90c	21.93def	43.33de	46.09c
T7	18.53b	38.53ef	45.06d	20.20cd	47.09f	52.93de
T8	29.00f	44.66h	50.62f	24.06f	54.24hi	59.28g
T9	24.33de	40.40fg	47.80def	20.86cde	50.32g	55.09ef
T10	21.46bcd	42.47gh	49.24ef	20.06cd	51.97gh	53.92ef
T11	28.93f	48.98i	57.53h	22.44def	55.40i	63.97h
T12	20.10bc	43.49h	53.98g	23.93f	53.83hi	59.30g
T13	22.66cde	34.82cd	46.84de	20.66cd	38.72c	50.79d
T14	25.00e	44.59h	49.06ef	24.33f	43.09de	56.51fg
T15	22.13cde	39.05ef	48.40def	21.33de	40.83cd	52.46de
T16	15.30a	20.41a	25.12a	15.83a	26.00a	27.36a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found to be better, followed by basidiospore and alginate bead inocula of *P. albus*.

Effect of different types of inocula of ECM fungi on the total dry weight (gm) of *Casuarina equisetifolia* seedlings in different soil types

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	0.71bcdefg	7.42b	14.62b	0.81efg	8.76b	15.57b
T2	0.74cdefg	9.37d	17.62d	0.69cde	11.78d	20.22e
T3	0.58bc	8.46c	15.70c	0.55b	9.81c	17.44c
T4	0.57bc	10.62e	18.91e	0.72cde	12.25e	20.89f
T5	0.65bcde	12.51g	21.82g	0.81efg	15.88i	24.50h
T6	0.57b	8.96d	17.85d	0.60bc	13.77f	19.25d
T7	0.59bcd	12.56g	20.78e	0.75def	16.24i	23.85g
T8	1.02h	15.92k	26.22j	1.05h	20.92m	30.46m
T9	0.65bcdef	13.89i	22.84h	0.65bcd	18.24k	27.30k
T10	0.78efg	15.08j	25.22i	0.81efg	17.39j	26.30j
T11	0.83g	18.37l	32.90l	1.15h	22.82n	36.73n
T12	0.64bcdef	16.09k	28.17k	0.87fg	19.18l	30.17m
T13	0.75defg	10.81e	22.39gh	0.66bcd	14.43g	25.36i
T14	2.06i	12.94h	26.92j	0.93g	16.04i	28.76l
T15	0.80fg	11.71f	25.24i	0.77def	14.96h	26.98k
T16	0.418a	4.06a	8.34a	0.40a	5.73a	9.85a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found to be better, followed by basidiospore and alginate bead inocula of *P. albus*.

Effect of ECM inoculation on growth improvement of *Casuarina equisetifolia* seedlings

Sterilized soil

Unsterilized soil

T1-Basidiospore (*L. fraterna*)
T2-Vegetative mycelial (*L. fraterna*)
T3-Alginate bead (*L. fraterna*)
T4-Basidiospore (*P.albus*-1)
T5-Vegetative mycelial (*P.albus*-1)
T6-Alginate bead (*P.albus*-1)
T7-Basidiospore (*P.albus*-2)
T8-Vegetative mycelial (*P.albus*-2)
T9-Alginate bead (*P.albus*-2)
T10-Basidiospore (*P.albus*-3)
T11-Vegetative mycelial (*P.albus*-3)
T12-Alginate bead (*P.albus*-3)
T13-Basidiospore (*P.albus*-4)
T14-Vegetative mycelial (*P.albus*-4)
T15-Alginate bead (*P.albus*-4)
T16-Control

Effect of different types of inocula of ECM fungi on the shoot length (cms) of *Casuarina junghuhniana* seedlings in different soil types

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	34.33fg	32.18b	37.60a	30.99def	35.25b	40.30b
T2	37.60g	35.15b	42.30e	32.93f	39.11cd	45.30d
T3	29.39cde	34.18b	39.50a	29.86cdef	37.27bc	42.70c
T4	25.20bc	38.69c	43.70f	23.93ab	42.34de	47.50e
T5	27.64bcd	42.32def	48.40g	31.60ef	47.07fgh	44.70b
T6	27.33bcd	40.15cde	40.40d	25.46ab	44.38ef	53.60f
T7	26.46bcd	42.01def	49.30h	24.07ab	47.92ghi	54.80g
T8	33.73efg	45.62gh	53.30l	28.66bcdef	51.46ij	60.08k
T9	26.93bcd	43.05efg	50.60i	26.26bcd	49.66hi	57.00h
T10	30.60def	44.96fgh	52.60k	25.76bc	54.33jk	58.20i
T11	32.83ef	51.98i	60.60o	27.20bcde	58.11l	64.63l
T12	30.06def	46.85h	55.80n	24.19ab	55.04kl	60.60k
T13	26.60bcd	38.34c	48.20g	25.66bc	45.91fg	54.60g
T14	30.73def	46.96h	54.60m	26.61bcd	49.64hi	59.30j
T15	24.80b	39.53cd	51.70j	24.00ab	48.74ghi	57.40h
T16	18.33a	21.91a	27.20a	20.06a	28.68a	29.40a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found to be better, followed by basidiospore and alginate bead inocula of *P. albus*.

Effect of different types of inocula of ECM fungi on the total dry weight (gm) of *Cassuarina junghuhniana* seedlings in different soil types

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	1.00g	8.27b	15.20b	1.44e	10.00b	17.00b
T2	1.22h	10.02c	18.60d	2.13f	11.60c	20.20d
T3	0.67bcde	8.73b	16.50c	0.85cd	10.50b	18.40c
T4	0.53abcd	11.37d	20.50e	0.79cd	12.80d	22.10e
T5	0.74ef	14.05g	23.20f	0.94d	16.20g	25.40g
T6	0.62bcde	11.85d	19.40d	0.58abc	13.40d	23.00f
T7	0.73def	13.36f	24.00f	0.68bcd	16.70g	27.50h
T8	1.06gh	17.84j	28.20i	0.89d	22.70j	33.40l
T9	0.65bcde	15.98i	25.20g	0.75cd	19.40i	29.90j
T10	0.71cdef	17.99j	29.00i	0.67bcd	23.30j	32.40k
T11	0.94fg	23.91l	37.30k	0.83cd	28.40l	40.80n
T12	0.64bcde	20.18k	32.40j	0.66bcd	24.20k	36.10m
T13	0.51abc	12.47e	24.00f	0.71bcd	14.10e	25.80g
T14	0.70cdef	14.74h	28.90i	1.21e	18.10h	32.60kl
T15	0.48ab	13.75fg	27.00h	0.46ab	14.90f	28.50i
T16	0.39a	4.53a	9.00a	0.37a	6.29a	10.20a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found to be better, followed by basidiospore and alginate bead inocula of *P. albus*.

Effect of ECM inoculation on growth improvement of *Casuarina junghuhniana* seedlings

Sterilized soil

Unsterilized soil

T1-Basidiospore (*L. fraterna*)
T2-Vegetative mycelial (*L. fraterna*)
T3-Alginate bead (*L. fraterna*)
T4-Basidiospore (*P.albus*-1)
T5-Vegetative mycelial (*P.albus*-1)
T6-Alginate bead (*P.albus*-1)
T7-Basidiospore (*P.albus*-2)
T8-Vegetative mycelial (*P.albus*-2)
T9-Alginate bead (*P.albus*-2)
T10-Basidiospore (*P.albus*-3)
T11-Vegetative mycelial (*P.albus*-3)
T12-Alginate bead (*P.albus*-3)
T13-Basidiospore (*P.albus*-4)
T14-Vegetative mycelial (*P.albus*-4)
T15-Alginate bead (*P.albus*-4)
T16-Control

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Effect of different types of inocula of ECM fungi on the total number of mycorrhizal root tips of *Casuarina euisetifolia* seedlings in different soil types

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	23.00b	169.00bc	341.00b	19.00b	161.00b	327.00b
T2	37.00d	206.00cde	422.00d	34.00de	198.00d	389.00d
T3	30.00c	194.00bcd	376.00c	27.00c	186.00c	360.00c
T4	36.00d	214.00cdef	437.00e	30.00cd	205.00de	427.00e
T5	56.00f	252.00efghi	553.00k	49.00f	243.00i	501.00i
T6	44.00e	224.00defg	467.00g	37.00e	213.00fg	446.00g
T7	42.00e	152.33b	452.00f	36.00e	210.00ef	436.00f
T8	65.00g	263.00fghij	541.00j	58.00g	253.00j	516.00j
T9	53.00f	240.00defghi	481.00h	46.00f	226.00h	459.00h
T10	64.00g	275.00ghij	556.00k	57.00g	261.00k	523.00k
T11	80.00i	309.00j	602.00m	73.00i	289.00m	572.00m
T12	71.00h	292.00ij	570.00l	66.00h	276.00l	531.00l
T13	46.00e	231.00defgh	446.00ef	39.00e	218.00g	429.00e
T14	68.00gh	281.00hij	535.00j	64.00h	263.00k	520.00jk
T15	53.00f	271.00ghij	518.00i	51.00f	252.00j	503.00i
T16	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found better, followed by alginate bead inoculum of *P. albus*

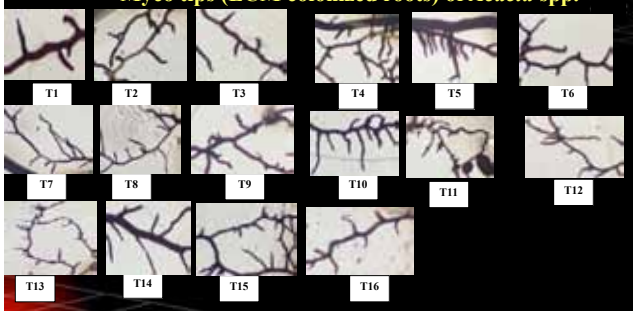
Effect of different types of inocula of ECM fungi on the total number of mycorrhizal root tips of *Casuarina junghuhniana* seedlings in different soil types

Treatment	Sterilized Soil			Unsterilized Soil		
	Age of the seedlings (Months)					
	3	6	9	3	6	9
T1	20.00b	158.00b	327.00b	17.00b	149.00b	305.00b
T2	34.00d	195.00d	402.00d	32.00de	176.00d	358.00d
T3	26.00bc	182.00c	363.00c	24.00bc	164.00c	338.00c
T4	32.00cd	199.00de	419.00e	28.00cd	185.00de	407.00e
T5	53.00gh	236.00f	527.00j	44.00fg	204.00g	478.00gh
T6	40.00de	209.00e	432.00ef	34.00de	197.00fg	429.00f
T7	38.00de	204.00de	437.00f	33.00de	188.00ef	426.00f
T8	60.00hi	249.00g	508.00i	52.00hi	233.00i	489.00hi
T9	49.00fg	228.00f	464.00g	43.00fg	214.00h	438.00f
T10	60.00hi	253.00gh	531.00j	55.00ij	241.00ij	509.00jk
T11	72.00j	288.00i	584.00j	68.60k	269.00l	541.00l
T12	66.00ij	263.00h	543.00j	60.00j	255.00k	521.00k
T13	43.00ef	208.00de	423.00ef	37.00ef	199.00g	408.00e
T14	62.00i	263.00h	509.00i	58.00ij	245.00jk	495.00ij
T15	51.00g	258.00gh	486.00h	47.00gh	231.00i	472.00g
T16	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

Means sharing a common letter in the same column with soil types are not significantly different at P = 0.05% level

Vegetative mycelial inoculum was found better, followed by alginate bead inoculum of *P. albus*

Myco tips (ECM colonized roots) of *Acacia* spp.



T1-Basidiospore (*L. fraterna*)
T2-Vegetative mycelial (*L. fraterna*)
T3-Alginate bead (*L. fraterna*)
T4-Basidiospore (*P.albus*-1)
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T13-Basidiospore (*P.albus*-4)
T14-Vegetative mycelial (*P.albus*-4)
T15-Alginate bead (*P.albus*-4)
T16-Control

Morphological and anatomical features of ECM colonized roots of *Casuarina equisetifolia* and *C. junghuhniana*

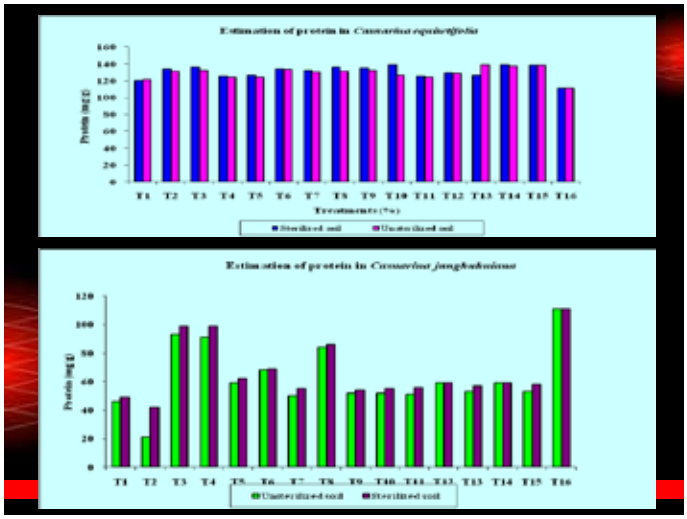
ECM colonized root tips

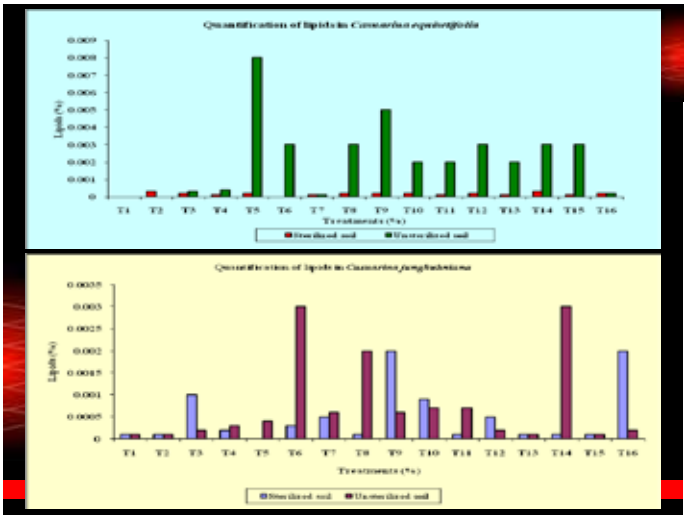
Extramatrical hyphae with clamp connections (x 200)

T.S. of root (myco tip) (x 200)

Mantle with radiating hyphae in root (x 400)

Extramatrical hyphae with clamp connections (x 400)





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SUMMARY

- Mycorrhization of seedlings with different forms of inocula of ECM fungi exhibited their potential in improving planting stock of *Casuarina* species.
- Seedling health in terms of height, biomass, volume and quality indices, shoot root ratio was comparatively higher in all treatments over control.
- Vegetative mycelial inoculum of *P. albus* was found to be the most efficient inoculum which gave maximum per cent of Mycorrhizal Inoculation Effect (MIE).
- Morphological and anatomical studies revealed that ECM fungus, *P. albus* colonizes the roots of both ECM fungi inoculated plants of all the tree species.
- Number of myco tips is more in ECM inoculated seedlings of all the tree species grown in sterilized potting medium than those grown in unsterilized potting medium at all age levels.
- ECM inoculated plant samples of all the tree species revealed appreciable amount of biochemical parameters during the period of observation.

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