Bamboo Tissue Culture and Micropropagation

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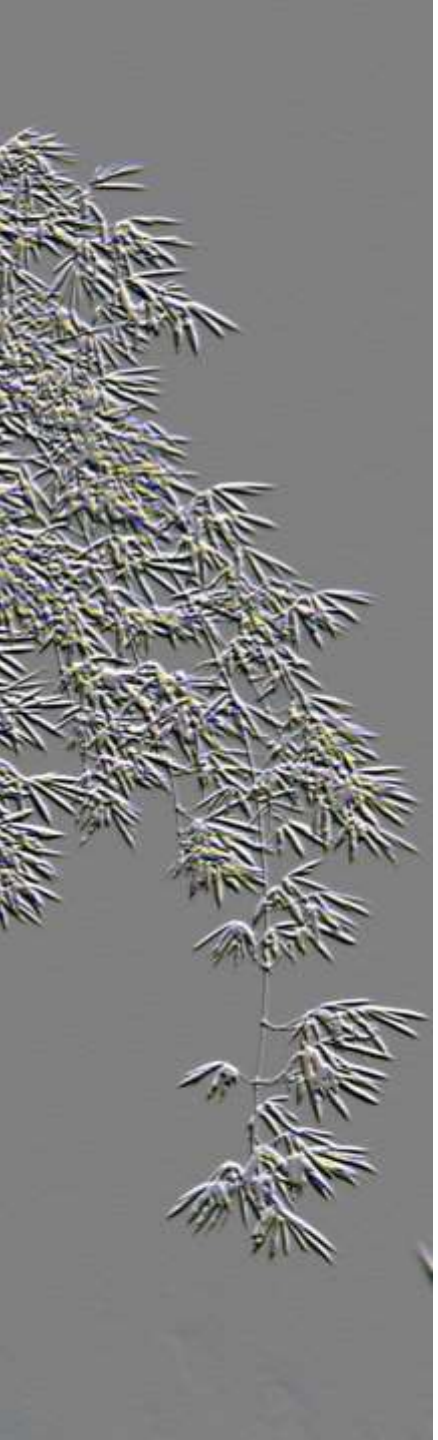


Large- scale production of Bamboo planting stock

- Increase in acreage and productivity of bamboo – A mandate of the National Bamboo Mission being implemented in all states

- The potential for increase in area of bamboo plantations is tremendous - Not just the native species but also exotics that have adapted well to different agroclimatic zones.

- A huge capability for production of bamboo planting material will have to be generated to enable the expansion of area under plantations across the country

- It is not just numbers that that needed but quality too

Streamlining of the mass propagation techniques

Technologies in use at present:

- Germination of seeds,
- Macro-proliferation of seedlings,
- Vegetative propagation methods : Rooted culm/branch cuttings, Rhizome offsets, Air layering
- Micropropagation through axillary bud proliferation

Poor multiplication rates and scale of production are still hurdles for mass multiplication in many of the important species

Plant Tissue Culture

The technique of growing plant cells, tissues or organs under sterile and controlled environment on a synthetic, defined nutrient medium

Micropropagation is the application of plant tissue culture techniques for large-scale propagation

Essentials for Tissue Culture:

- Sterile (Microbe free) environment
- Defined plant tissue culture medium
- Suitable tissues to initiate cultures

Tissue culture is a sterile procedure and therefore involves work in an environment free of fungi and bacteria

Sterility has to be ensured with

i. Plant material used to start the culture (explants)
ii. The tissue culture medium and the culture vessel
iii. The area in which the cultures are transferred between vessels

Plant Tissue Culture Medium

- A mixture of chemicals of defined composition essential for a particular type of plant tissue culture

- Provides for the nutrient requirement of the culture - Minerals, Vitamins, Carbon source (sugars)

- Plant Growth Regulators (hormones)

Applications of Plant Tissue Culture

1. Mass Clonal Propagation (Micropropagation):

- Large scale clonal propagation of superior varieties
- Propagation when conventional methods fail or are too slow.
- Production of disease free planting stock

Applications of Plant Tissue Culture

Advantages over conventional methods:

- Very high multiplication rates
- Disease free plants produced
- Production possible throughout the year
- Plant production done in a small area
- International transfer of plants without quarantine

Applications of Plant Tissue Culture

2. In Plant Improvement and Breeding

- Anther culture
- Production of somatic hybrids
- Somaclonal Variation Technology
- Production of triploids through culture of endosperm tissue
- Embryo rescue

Applications of Plant Tissue Culture

3. Genetic Engineering

- regeneration of plantlets from cells into which recombinant DNA has been introduced

***4*. Basic research**

- cell biology, molecular biology , gene expression studies etc

MICROPROPAGATION OF BAMBOO

Advantages of Bamboo Micropropagation

-- Large scale propagation possible where alternate methods are too slow to meet the demand

When seeds are not available, a few years after flowering

Very long flowering cycles in some species

Vegetative methods are insufficient

- Propagation of superior elite selections for genetic improvement

selected clumps with faster growth , higher yield, disease/pest resistance can be propagated.

- Disease and pest free planting material is ensured through tissue culture which is a sterile technique

- Centralised mass propagation facilities and easy transport of planting material to field

Small size of plantlets

Bamboo Micropropagation

1: Surface sterilization

Nodal explants of about 2-5 cms excised, cleaned of dead tissue, bracts. Washed with mild detergents

Surface sterilization with mercuric chloride or sodium hypochlorite solutions of varying concentrations and for varying durations

Rinses with sterile distilled water under sterile conditions

One of the requirements of micropropagation is the large scale regeneration of complete plantlets from cultured plant parts (explants)

This is achieved in bamboo through two **pathways**:

1. Induction of multiple shoots through enhanced proliferation of meristems followed by rooting of shoots
3. Induction of somatic embryos (adventitiously) followed by their germination or conversion to plantlets

Bamboo Micropropagation

2: Initiation of cultures

Axillary bud proliferation first obtained in sterile nodal explants on a simple nutrient medium with low levels of cytokinins especially Benzyl Amino Purine (BAP) or Kinetin (Kin)

Bamboo Micropropagation

3: Multiplication

Shoots are multiplied on media with varying levels of cytokinins with or without auxins (0.5 to 6 mg/l)

Liquid or agar solidified medium used for multiplication

Regular subcultures to fresh media every few weeks

4: Rooting

Shoots can be rooted *in vitro or ex vitro*

Media supplemented with auxins (NAA, IBA) used or shoots dipped in rooting solutions or powders

In vitro rhizome induction improves survival of plantlets

in vitro rooting

Somatic embryogenesis in bamboo

Somatic embryos

Plantlet regeneration

Hardened plantlets

Important Commercial Species with success in Tissue culture

1.	*Bambusa balcooa*	*Commercialised*
2.	*B. bambos*	*Commercialised*
3.	*B. nutans*	*Commercialised*
4.	*B. polymorpha*	
5.	*B. tulda*	*Commercialised*
6.	*B. vulgaris*	*Commercialised*
7.	*Dendrocalamus asper*	*Commercialised*
8.	*D. brandisii*	
9.	*D. giganteus*	
10.	*D. hamiltonii*	
11.	*D. longispathus*	
12.	*D. membranaceus*	
13.	*D. strictus*	*Commercialised*

14. Gigantochloa atroviolacea	
15. Guadua angustifolia	*Commercialised*
16. Melocanna baccifera	
17. Ochlandra travancorica	
18. Pseudoxytenanthera stocksii	*Commercialised*
19. Thyrsostachys oliverii	

Large-scale production of bamboo planting material

- **What are our priorities?**

Quality of planting stock

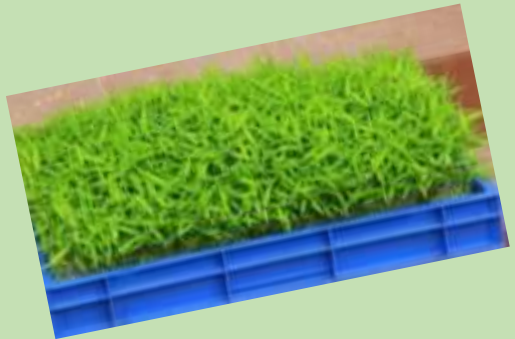
- **Precise Identification of species and clones**
- **Mass multiplication only of superior selections**
- **Quality of nursery planting stock**
- **Assurance of quality through certification**

Seeds for mass propagation – Why is it not desirable?

Pros : Maintains diversity in populations and enables selection at a later stage
Year of flowering can be estimated

Cons : Untested for superiority- years before performance can be evaluated
Short viability of seeds
Unpredictable flowering cycles

Except for *Bambusa bambos* and *Dendrocalamus strictus* availability of seeds in sufficient quantity is not assured



But what is to be propagated ?

The major issue however is the production of quality planting material *vis-a-vis* the status of genetic improvement programmes in bamboo.

The biology of bamboo makes it un amenable to conventional genetic improvement .

The solution:

- Establishment of clonal /rhizome bank of precisely identified and genetically superior plants and
- A certification system that ensures quality control of planting material produced through accredited bamboo nurseries.

The best option available for quality bamboo planting material for large scale plantation programmes is:

Efficient micropropagation of superior clones selected early in the flowering cycle

What is a superior bamboo clone?

- Productivity in biomass

- Number of culms per clump formed /surviving

- Quality in straightness and taper

- Lack of congestion in clump in clumping species (length of rhizome neck)

- Long/Short internodes

- Absence of flowering or resumption of vegetative growth after flowering

- Known year of flowering

Selections to be done from natural and planted forests followed by multilocational field performance testing

Certification of bamboo planting material

- Planting material produced through all the methods are available and often plants offered for sale are of dubious origin.

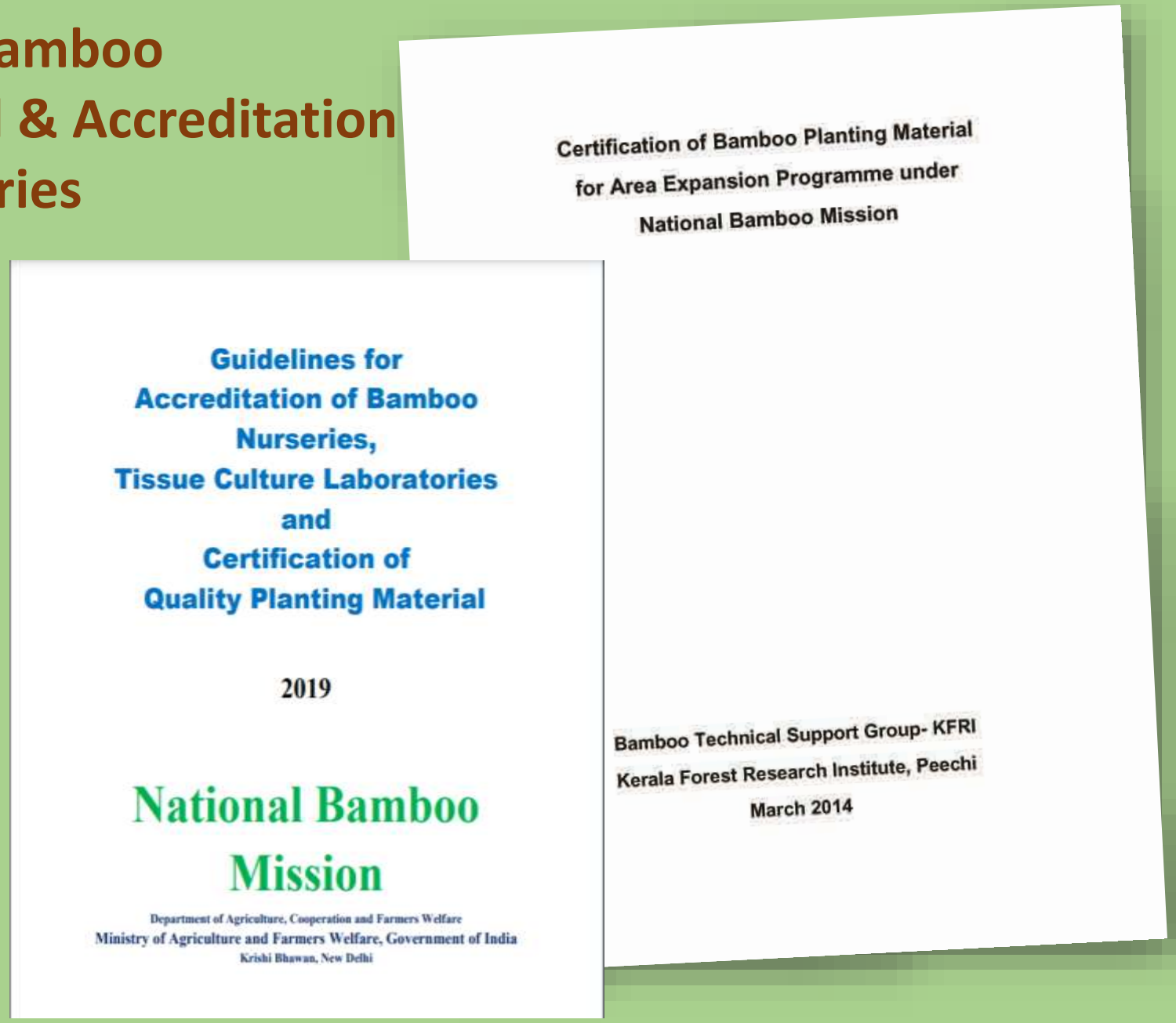
- To ensure that only quality planting stock is used NBM has embarked on a programme of certification of bamboo planting material which will be eventually mandatory for future NBM plantations

- Complementing the certification of planting material NBM also has a scheme for Certification of Bamboo Nurseries

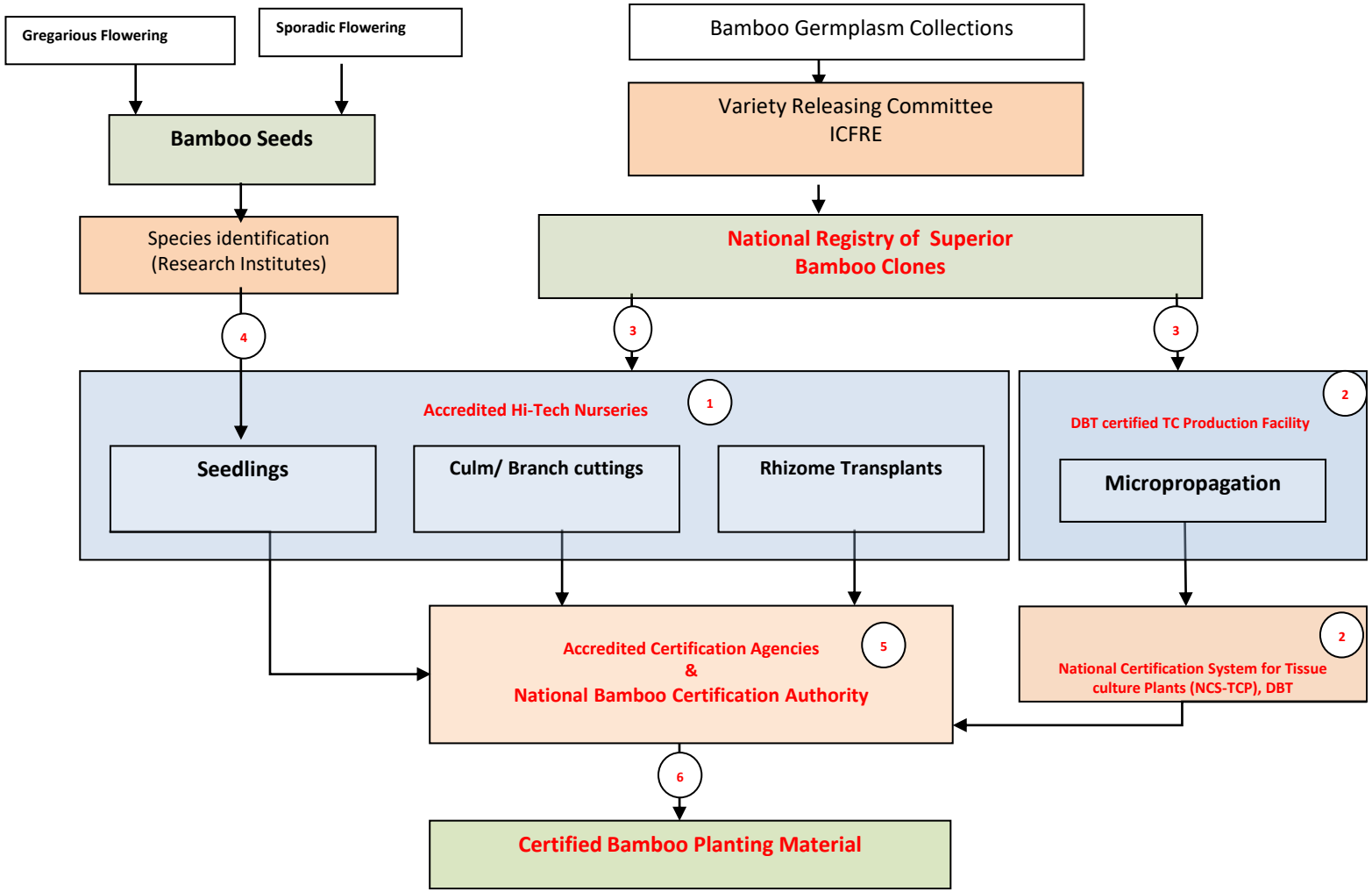
- Only mother clumps identified by experts will be used for large scale propagation. All nurseries will maintain or have access to Rhizome bank /Clonal Garden

- All parameters of plant health and quality will have to met in a certified Bamboo planting material

Certification of Bamboo Planting Material & Accreditation of Bamboo Nurseries



Framework of the National Certification Process for Quality Planting Material in Bamboo



(X) Requires Certificate for ensuring traceability through chain of custody

Certification of bamboo planting material

- Requires establishing the taxonomic and clonal identity of the planting material available in the nurseries.

- Identification in bamboo is based on the morphological features (culm sheath and flowers)

- Culm sheaths an important feature does not persist beyond a few months

- The unique growth pattern and semelparous (monocarpic) flowering imposes a hurdle in easy identification

- Other features like culm colour, diameter etc. is not dependable since it is influenced by the environment and age of the culm

Major Constraints:

Species Identification:

- Bamboo identification in all species is difficult at all stages of growth but especially at the juvenile stage

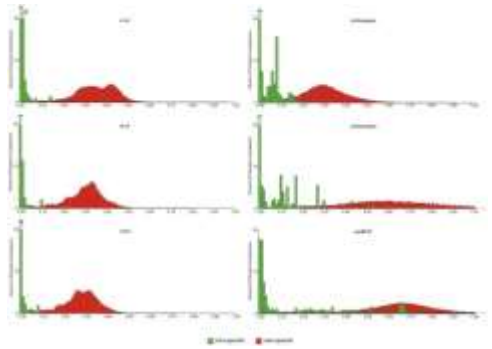
- In planting stock derived through seeds or clonal propagation in the nursery simple identification procedure will help mixing up of species

Solutions:

Species identity established by taxonomists from mother clumps + meticulous documentation (Paper trail) up to sales outlet.

Identification based on DNA: Precise identification is feasible when DNA barcoding technology is standardized.

DNA BARCODES



Short stretches of DNA from a prescribed region which show unique pattern within the species but differ between species

Any tissue collected at any stage of growth regardless of the type of propagation can be used or even from herbarium specimens .

Identification of clones:

- The use of DNA fingerprinting techniques will provide precise identification of clones within species

- Precise identification of clones derived and traceable to superior selections of known origin will help ensure quality of planting material and

- Avoid the risk of unpredictable gregarious flowering and death of planted bamboo

- Clonal identity is precisely established through well established DNA fingerprinting techniques

- Use of molecular makers - ISSR and SSR

Scaling up of micropropagation

- Laboratory protocols are available for a number of species
- Commercial micropropagation is restricted to less than a dozen
- Institutional facilities and priorities not suitable to scale up protocols
- Academia – Industry collaboration in R& D needed

Constraints in Tissue culture of Bamboo

Intitiation Phase:

Choice of Mother plant, explant , season of collection
Juvenile vs. Mature phases
Microbial contamination

Multiplication Phase:

Latent contamination
Low Multiplication rates
in vitro flowering and death of shoots

Plantlet regeneration:

Low rooting frequencies
Poor acclimatization

Cost effectiveness:

Low efficiency of micropropagation

Overcoming the constraints

Mechanization – Use of liquid media and Multiplication in Bioreactors

- Shoots maintained upto 2 ½ months without subculture simple bioreactors
- Very high multiplication rates
- Considerable reduction in labour intensive tissue culture steps
- Scaling up is needed for commercial applications

Shoot multiplication in Modified Airlift Bioreactor

Photoautotrophic Tissue culture

- High CO_2 levels
- High light intensities in the PAR range
- Production costs can be upto 40 % lower when compared to conventional micropropagation

CO_2 Growth Chamber

Cost reduction in Micropropagation

Use of liquid stationary media

Use of cheaper media components and containers

Photoautotrophic micropropagation

Use of ambient temperature and light

In vitro Rhizome Induction

In vitro Rhizome formation

- Improved hardening of micropropagated plants
- Improved survival rates in nursery and field

Hardened plants

Control of microbial contamination

- Improved prophylactic treatments in plants under greenhouse to obtain clean explants
- Standardisation of novel surface sterilization treatments
- Suppression of latent microbial contaminants through biostasis

Control of *in vitro* flowering

- Understanding of the factors influencing in vitro flowering
- Control over plant growth regulators and culture protocol
- Reduction of stress factors in tissue culture

Conclusions

- Technical expertise, research capability in bamboo tissue culture adequate in India
- Installed capacity for bamboo micropropagation sufficient for the country is not difficult to achieve
- Industry – Academia collaboration for scaling-up of lab procedures will help commercialization of additional bamboo species
- Adoption of latest developments in techniques will be of great benefit

Thank You