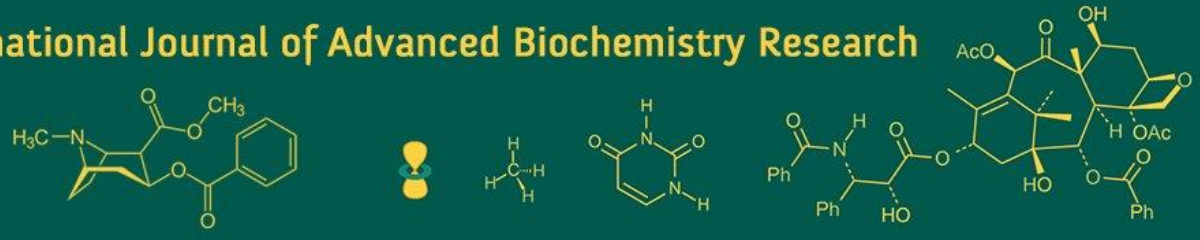
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 457-463
www.biochemjournal.com
Received: 28-10-2025
Accepted: 30-11-2025

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In-vitro propagation of bamboo species by *Reva flora culture*: A successful example of commercialization of bio-technological applications in forestry

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DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1Sf.6987

Abstract
Bamboo is a versatile crop. It can be used in 1, 500 different ways including as food, a substitute for wood, building and construction material, for handicrafts and paper. Around 80 per cent of bamboo forests lie in Asia with India, China and Myanmar having 19.8 million hectares of bamboo. India is the world's second largest cultivator of bamboo after China, with 136 species and 23 genera spread over 13.96 million hectares. According to the Union Ministry of Agriculture and Farmer Welfare, India's annual bamboo production is estimated at 3.23 million tonnes. However, despite all this, the country's share in the global bamboo trade and commerce is only 4 per cent. Bamboos are very diverse, arborescent, perennial and non-wood forest trees classified under grass family with very vast sociological, environmental and commercial importance. Propagation techniques are in practice for bamboo, such as seed propagation, clump division, rhizome and culm cuttings etc. But these traditional techniques coupled with many serious limitations or disadvantages for fulfill the large or mass scale demand of genuine quality planting materials propagation. At present for large scale production of quality planting material don't possible only through these classical methods, and in this situation, mass multiplication of genuine quality planting material of bamboo with fast pace is possible only through highly technical method or *in-vitro* tool of micro-propagation. Classical methods are largely in - efficient and also unsafe for the production of insect-pest free quality planting material. For mass scale propagation, largely insufficient and inefficient and micropropagation is the only viable bio-technological tool / method. Indeed, the order of magnitude of the demand for bamboo planting material indicates that micropropagation will largely inevitably be necessary for large scale propagation. According to very early history of plant tissue culture, attempts to induce the isolated cells into generating tissues masses by cell division were successful at achieving limited growth with root-explants of *Pisum, Zea and Gossypium*, and that was a bright spot during 1922 -1938 period by Kotte (1922a, b)) and Robbins (1922a). The potential of micro-propagation for production of insect-pest free good quality genuine planting material or mass scale propagation of bamboo has provided high hopes and a lot of research has been focused on the development of protocols for large and rapid scale propagation. These encompass optimization and establishment of *in vitro* culture techniques including micropropagation, somatic embryogenesis, *in vitro* flowering, macro proliferation, field performance and clonal fidelity. This research note briefly provides the state-of-the-art information on tissue culture mediated biotechnological interventions made in bamboo for large scale plant production through micropropagation, that being the need of the hour. Bio-technology has made very crucial contribution to the development of different forest and horticultural crops ranging from mass propagation of elite varieties, developing disease free stocks, in the directed breeding of new cultivars or varieties for sustainability in Agro-forestry. In October 2006, the Government of India (GOI) had launched the National Bamboo Mission (NBM) on the basis of the National Mission on Bamboo Technology and Trade Development Report, 2003. Reva flora Culture is a ISO 9001:2008 certified and recognized by NCS-TCP (Department of Bio-technology) tissue cultured lab situated at near Village-Borlai dist. Barwani (M.P.) and produced genuine good quality planting material of different species of green gold or bamboo as per demand of the different organizations including Govt. of M.P., Maharashtra, Telangana, Assam, U.P. and Bhutan and produced more than 35.00 lakhs of bamboo plants with the production stock of 49000 lakhs of different species of bamboo.

Keywords: Bamboo, micropropagation, somatic embryogenesis, *in vitro* flowering, macroproliferation, field performance, tissue culture

Introduction
Bamboo is a versatile crop. It can be used in 1, 500 different ways including as food, a substitute for wood, building and construction material, for handicrafts and paper. Around 80 per cent of bamboo forests lie in Asia with India, China and Myanmar having 19.8 million

hectares of bamboo. India is the world's second largest cultivator of bamboo after China, with 136 species and 23 genera spread over 13.96 million hectares. According to the Union Ministry of Agriculture and Farmer Welfare, India's annual bamboo production is estimated at 3.23 million tonnes. However, despite all this, the country's share in the global bamboo trade and commerce is only 4 per cent.

The NBM's key objective was to address issues relating to the development of the bamboo industry in the country, provide a new impetus and direction and enable the realisation of India's considerable potential in bamboo production. Multi-disciplinary and multi-dimensional in its approach, major interventions planned under it were to focus on research and development, plantation on forest and non-forest lands through Joint Forest Management Committees (JFMCs) or Village Development Committee (VDCs) and to ensure the supply of quality planting materials by establishing centralised and kisan/mahila nurseries. NBM was started as a Centrally Sponsored Scheme in 2006-07. The Agriculture Finance Corporation (AFC) India Limited, a consultancy organisation, prepared the mid-term evaluation study report of NBM in 2012-13. For the study, the organisation covered 9 states and 18 districts out of 27 states that had implemented NBM during the 10th and 11th Plan period. The report made some positive revelations. To popularise various bamboo handicrafts and other products as well as bamboo food items like processed shoots, six retail outlets were created. The report further noted that a beginning had been made in using tissue cultured planting material in some states, with encouraging results. Bamboo Development Agencies (BDAs) in all states have done excellent work with regard to bamboo plantation on forest land through JFMCs and VDCs. Of course, there are some drawbrakes too. The plantation on non-forest land involving farmers and private land owners has not taken full momentum. Transfer of technology through training and demonstrations form an integral part of the NBM. But the quality of the training needs to be further upgraded to improve the practical knowledge and skills of the stakeholders.

Earlier batanists considered as to how the complexity in a plant could be dissected out into individual cell and tissue types, so that these could be subjected to direct experimental control, free from the complex interactions occurring in the intact plant. Such an experimental tool which became available in the middle of the present century came to be known as 'tissue-culture'. Schwann's (1839) cell theory which proposed that each living cell of an organism, if provided with proper environment, would be capable of independent development gave birth to the concept of 'totipotency'. The theory of 'totipotency provides that it should be possible to reproduce an organism from anyone of its nucleated cells, since all the information needed to specify an organism is contained in its DNA. However Vochting inspired by Schwann's Cell theory performed some basic experiments during 1878, aimed at demonstrating the totipotency of the cells within certain limitations. However according to very early history of plant tissue culture, in Bio-technological science between 1902 and 1938 all attempts to induce the isolated cells into generating tissues masses by cell division failed.

Bio-technological Applications /approach useful in plant Science: During the 60 years plant tissue culture techniques have been a research tool and of economic advantages in agriculture practice. Their role in supplementing the existing methods of agriculture, horticulture, forestry and plant breeding will be quiet evident from the following applications:

Table 1: Different important Bio-technological approaches Useful if Plant science

S No.	Name of Bio-technological approach/Applications	Brief description
1	Clonal Propagation	Clonal propagation is a method by which identical plants of superior specimens can be obtained within a short span of time or period. This application is principally based on the fact that every cell of an organism has all the information necessary to reproduce the whole organism if favourable environment are provided. The most commonly used tissues for culture are the apicalk- or the axillary -growing points. Morel (1960) on culturing the orchid meristems observed a large number or shoots which colud be rooted to form a complete plants. In this sequence, methods for obtaining plantlets from meristems were developed for several herbaceous plants (Murashige, 1974). This technique has now become an important tool for producing vegetatively a large number of genetically identical progeny from selected parental specimens.
2	Recovery of Disease-free Plants	Virus infection is a major problem with vegetatively-propagated species sugarcane, potato, etc. The recovery of pathogen free plants through shoot-tip culture is based on the assumption that pathogen concentration is not uniform throughout the infected plant. Morel and Martin (1952) were the first to obtain virus free dahlias (ornamental flowering plant) from infected plants.
3	Isolation of Haploids	Haploids were first successfully isolated from anther of Datura innoxia and cultured *in-vitro* (Guha and Maheshwari, 1966). Haploids callus cuilture was obtained from the cultured anthers of *Solanum indicum* but the shoot bud differtentiated from them were found to be diploid (David and Chinchanikar, 1980). Recently haploids plantlets have been obtained from Custard apple at NCL (Nair *et al.*, 1983).
4	Isolation of Triploids	Triploids plants are usually seed-strile and non-importannt for plants where seeds are of commercial importance. AS compared to diploids the triploids *Populus tremuloids* has more desirable pulpwood characteristics. Triploidy can also be exploited for plants improvement. Economically important plants whose triploids are presently in commercial use4 include several varieties of apple, banana, sugarbeet, tea and watermelon (Johri and Bhojwani 1977)).
5.	Isolation of Mutants	Cell culture technique has great potential in the isolation of Variants.Nickell and His goup (Nickel and Heiz, 1973) were the first to observe that many of the plants regenerated from a sugarcane cell suspension culture of a single parental clone differed both from the parent clone as also from each other. At the NCL, variants of sugarcane resistant to mosaic virus and turmeric plants of the of the variety 'Tekurpeta' containing about twice the curcumin concentration in the rhizome have been isolated and planted in the field (Nadgauda and Mascarenhas, 1986).

Growth of immature embryos, Protoplasts and somatic hybridization, Uptake of macromolecules and transfer of genetic information and most important Germplasm preservation cum conservation are the others important technological applications are very mostly useful for different purposes (for growing interspecific hybrid embryos, development of numbers of hybrids through embryo culture, protooplasts and somatic hybridization is a process known as "Parasexual hybridization" holds immense possibilities in the area of genetic improvement of plants). An outstanding example of interspecific fusion of protoplasts is the work of Melchers *et al.* (1978)) who have reported regeneration of somatic hybrids between potato and tomato. in plant science. Hess *et al.* (1973) reported uptake of exogenous DNA in protoplasts of Petunia hybrid (an ornamental plants species). I india studies on protoplasts are in progress at the Boss Institute on Rice at IARI, New Delhi, on tomato at Calcutta University, on tobacco, *Vigna sinensis*, Recently plantlets regeneration through somatic embryogenesis has been obtained from Sandawood protoplasts at the BARC and from mothbean and economically important legume, at NCL.A combination of *in-vitro* techniques and cryopreservation is now being employed as complementary for the preservation og germplasm potato, cassava, sweet-potato and Yam.

Technology Availability and its application in India

The details of technological availability related with Bio-technology in different crops/plants are as follows:

Table 2: Availability of Biotechnological Technologies and Their Applications in Agricultural Crops and Forest Tree Species in India

S No.	Plant Species	Institution/Centres	References
1	Agricultural Crops *Allium cepa*, *Brassica oleracea,* *Triticum aestivum*	IIHR, Banglore BARC, Bombay BARC, Bombay	Srinivasa Rao (Personal Communuication) George and Rao (1980) Eapen and Rao (1982)
2	Forest Trees *Bambusa arundinaceae* *Dalbergia sisso* *Dalbergia latifolia* *Eucalyptus citriodora* *Eu. Tereticornis* *Eucalptus globus* *Santalum album* *Pinus gertardiana* Biota Sp. *Sapium sebiferum* *Tamarindus indica*	Delhi University, Delhi Delhi University NCL, Pune IISC, Banglore RRI, Jammu NCL, Pune BARC Bombay NCL, Pune NCL, Pune NCL, Pune	Goyal *et al*. (1980) Mukhopadhyay and Mohan Ram (1981) Mascarenhas *et al*.(1981) Lakshmi Sita and Vidyanathan (1979) Grewal *et al., (1980)* Gupta *et al., (*1981, 1982, 1983) Rao and Bapat (1978) Konar (1974, 1975) Kotwal *et al.* (1983) Kulkarni *et al.* (1981)

Reva Flora Culture: A Successful Bio-technology Tissue-Culture Laboratory

Fig 1: View of Reva Flora Culture Laboratory at near Village-Borlai dist. Barwani (M.P.)

Reva flora culture is Tissue culture Plants production laboratory devoted for mass production of quality genuine planting materials of different species of Bamboo including *Bambusa balcooa, B. Tulda, Dendrocalamus strictus etc* and Banana variety-Grand Naine (G-9) through scientific manner for maintaining the standard protocol and produced quality planting material for strengthening reliability of farmers and other agencies. Mr. Vijay Jadhav Bio-technologist is a Executive Head of the Reva Flora Culture, the brief biodata of are as follows:

Table 3: Professional Profile, Qualifications, Experience, and Expertise of Mr. Vijay Jadhav

S No	Heading/Particular	Detailed infomation
1	Name	Mr. Vijay Jadhav
	Educational Qualification	University of Pune Bachelour of Science (B.Sc.) Plant Tissue Culture (1997-2014), SRTMU, Nanded Master of Science -Biotechnology (1999-2001)
2	Summary	1. Research and development in Plant Tissue Culture of banana, pomegranate and potato. Production protocol designing andhardening. 2. Consultant in setting up of Plant tissue culture lab, Mediapreparation, Production planning, Primary & secondary hardening of ex-agar plants, Plantation. 3. Molecular Biology, Genetics, Plant Transformation. 4. Mushroom technology. 5. Immunological Techniques such as ELISA, Immunoelectrophoresis, Agglutination, Precipitation, Coagulation, Aproduction, Ag-Ab interactions etc. 6. Nanotechnology, Nano particle synthesis (Biological & Chemical), Nano particle characterization, Applications of nano particle in ananti cancerous activity study.
3.	Experience	Reva Flora Culture Manager/Lab -Incharge April 2015-Present (More than 5 years)
4	Publication	1. 'Influence of physical parameters, such as pH and temperature on biodegradation of dimethoate by Actinomycetes sp isolated from pesticide contaminated grape field soil from Nashik 9. Text book of Plant Diversity-II, F.Y. B.Sc. Paper-I, Term-II, Botany 5. "Effect of wrapping materials onthe storage of cut flowers" 8."Microbial degradation of Dimethoate by Gram Negative Soil Microorganism isolated from cottonfield" 7. "Preparation of Soil Fertility Map of Experimental Farm, Nashik, Maharashtra, India.
	Top Skills	Plant Tissue Culture, SOP Preparation, PTC lab setup, Media preparation, Production planning & Hardening ofex-agar pla.

The methodology and protocol followed by Reva flora for Bamboo plants production are as follows:

Fig 2: Selection of high quality mother plants for selection of Single Nodal segments and Initiation of culture

Selection of Superior Quality Mother plants

Healthy single nodal segments (1.5 to 2.0 cm in length) with internodal portion were excised from young lateral branches of main culm of 04 years old plants of *Bambusa tulda* and *B. balcooa* from a natural bamboo stands. Leaf sheath of nodal segment were removed, sized and were surface sterilized by using cotton swab dipped in 70 percent ethanol. They were then disinfected with 0.1per cent HgCl2 solution for 5 minutes. After that disinfected explants were dipped in 5 percent (v/v) Tween 20 solution for 2 hour and then washed thoroughly under running tape water for 30 min. The explants were then rinsed with sterile distilled water. Pre-treatment of the explants were carried out with aqueous solution of 0.5 per cent of Bavistin a systemiccarbendazim fungicide (BASF India Ltd., Mumbai, India) and gentamycine for 15 minutes.

Fig 3: Explants Selection and Sterilization

Nodal segments were aseptically cultured on culture tubes containing 15 ml of semi solid Murashige and Skoog's medium 20in 2.5cm X15 cm culture tubes (Borosil, India). The MS medium was prepared using 100 mg/ lmyoinositol, sucrose 3 per cent (Commercial grade, DCM Sri ram Industries, Meerut, India), Gelrite 2.5 per cent (Sigma chemicals Pvt. Ltd.) with concentrations of plant growth regulators like 6-Benzyle Adenine (BA), Indole-3-Butyric Acid (IBA), Indole-3-Acetic Acid (IAA), kinetin (Kn) and naphthalene acetic acid (NAA) in combination. Observations were recorded after 4 weeks when axillaries bud break occurred and shoots were proliferated. The proliferated shoots were excised from mother plant and cultured on MS medium supplemented with cytokinin in which concentration bud break and multiplication rate was more.

Commercial Production of Green Gold

Fig 4: Images of laboratory displayed Autoclave, Inspection of and Shoot proliferation with standard protocol

Multiplication of shoots

The entire clusters of proliferated shoots were transferred to 250 ml glass culture bottles with polypropylene cap containing 25 ml of media for 4 weeks. By this time shoots were elongated and developed into a number of multiple shoots. When sufficient shoots were obtained the proliferating shoots were cut into 3 shoot cultures and sub cultured in same semi solid MS medium.

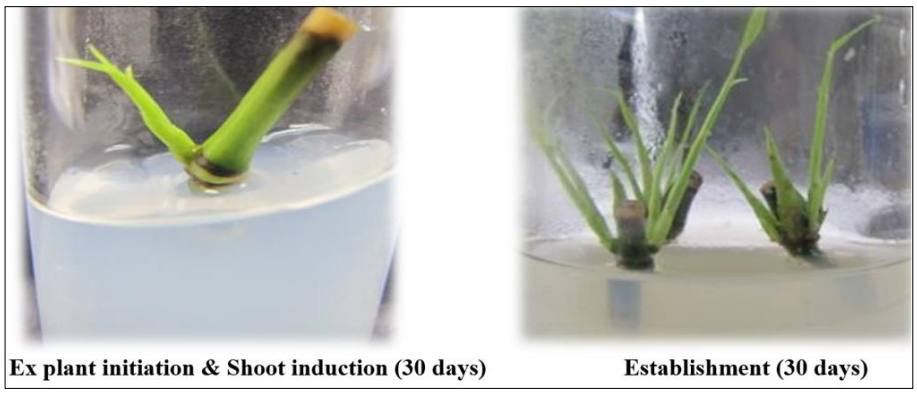

Fig 5: Initiation & Culture Establishment

Rooting

For *in vitro* rooting, the propagules bearing 3 to 5 shoots were transferred to MS semi solid media with Auxins like- IAA, IBA and NAA.

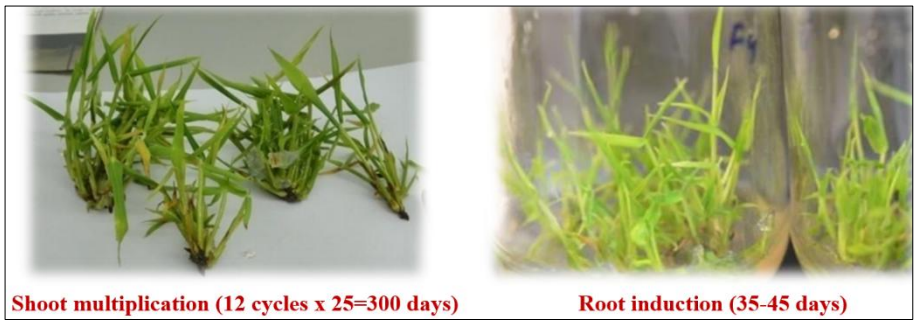

Fig 6: Shoot Multiplication & Rooting in Bamboo

Fig 7: Reva Flora Family: Total numbers of Staff: 530

Hardening

The transfer of *in vitro* propagated plantlets form Lab to land is another big nutshell of micro-propagation. The plant developed *in vitro* is unable to survive *in vivo* directly due to lack of adaptation and proper hardening however it has well-developed roots. To overcome the bottleneck of hardening, researchers have followed various hardening procedures. In general, the healthy and well-rooted plantlets are washed to free from the rooting medium and transferred to the pot containing growth supporting composition such as soil, sand, soil rite, perlite, cocopeat, agro peat, vermiculite, compost, farmyard manure, etc either alone or in various ratios. Most researchers have used mention substrate in 1:1:1 ratio or modified. Some researchers have described the primary hardening and secondary hardening to obtained maximum numbers of plantlets. Like, *In vitro* plantlets were transferred to ½ strength MS liquid medium without plant growth regulators and vitamins for hardening in *D. asper*, *B. nutans*, and *D. hamiltonii*.

Fig 8: Hardening and acclimatization

Fig 9: Storage and dispose/supply of Bamboo tissue-cultured plants

Table 4: Name of 13 Economically Important Bamboo Species conserved and maintained at Reva Flora Culture, Village-Borlai Dist.-Barwani

S No.	Name of Bamboo Species	Source Destination
1	*Bambusa balcooa-*	Jablpur, RFRI Jorhat
2	*Bambusa tulda*	-
3	*Bambusa nutans-*	RFRI Jorhat
4	*Dendrocalamus hamiltonii-*	RFRI Jorhat
5	*Dendrocalamus strictus-*	Narsingpur MP
6	*Dendrocalamus asper-*	IWST Bangaluru
7	*Dendrocalamus brandisii-*	IWST Bangaluru
8	*Dendrocalamus stocksii-*	IWST Bangaluru
9	*Bambusa vulgaris (green)-*	Ukai Gujarat
10	*Thyrsostachys oliveri-*	Reva Bamboo Setum
11	*Bambusa multiplex-*	Reva Bamboo Setum
12	*Bambusa balcooa-*	Jablpur, RFRI Jorhat
13	*Bambusa bambos-*	Chandrakesar Dewas

Table 5: Bamboo Production Stock at Reva Flora Culture

S No.	Name of Species	Stock details
1	*B. balcooa*	35, 96, 000
2	*B. tulda*	9, 40, 000
3	*B. nutans*	2, 30, 000
4	*B. vulgaris green*	7000
5	*B. polymarpha*	1, 20, 000
6	*D. asper*	58, 000
7	*D. brandisii*	45, 000
	Total	4996000.00

Bamboo supply Tenders received by Reva Flora Culture

- Madhya Pradesh Bamboo Mission
- Maharashtra Bamboo Mission
- Chhattisgarh Bamboo Mission
- Telangana Bamboo Mission

Table 6: Status of Bamboo plants business (sale) from Reva Flora Culture are as follows

S. No.	Name of State/Country	Stock Business details
1	Madhya Pradesh	11, 98, 987
2	Maharashtra	16, 96, 085
3	Telangana	4, 31, 300
4	Asam	15, 00, 00
5	Uttar Pradesh	45, 000
6	Bhutan	40, 000
	Total	3561372

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