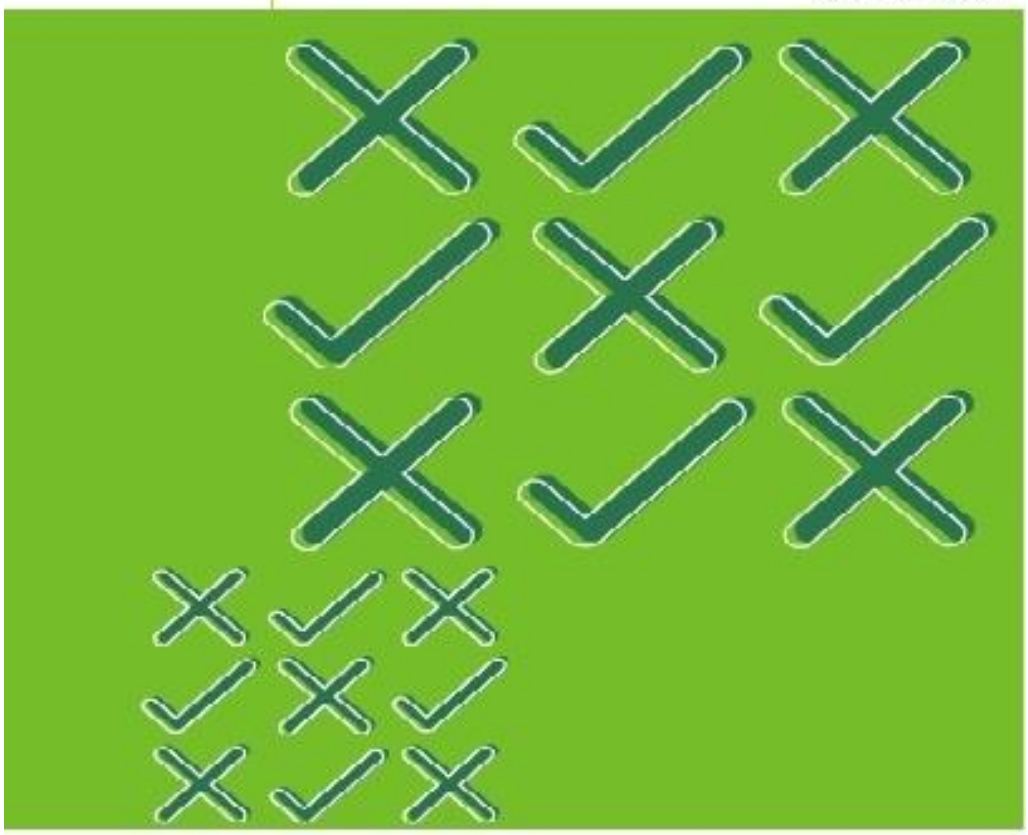
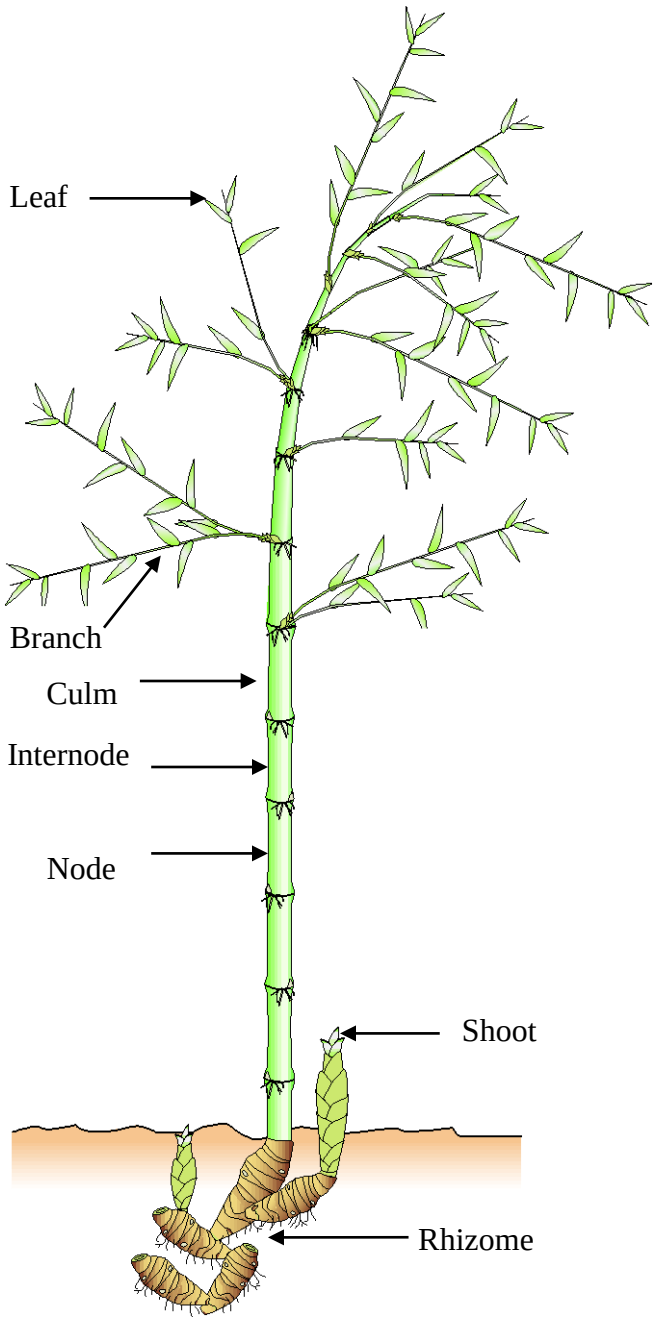
TRAINING MANUAL

Micropropagation of Bamboo



The Bamboo Plant and its Propagation



THE BAMBOO PLANT, unlike trees, has no central trunk or main axis; and it consists of an underground axes called rhizome and an over ground axes or terrestrial portion, called culm.

The rhizome consists of nodes and internodes which are covered with sheathes. From the nodes of the rhizome emerge roots to forage for nutrition, and buds to develop over the ground into culms. It also acts as a food reserve.

The culm consists of many jointed nodal and inter-nodal segments. The inter-nodal segments are usually hollow and are covered with culm sheaths at the initial stage of culm growth. Each internode bears a branch bud (or primordium). Buds are key growth elements in plant propagation and multiplication.

Branch buds of the culm produce branch and branchlets, and the number of branch buds are directly related to the number of potential branches at the node. The branch system, which may

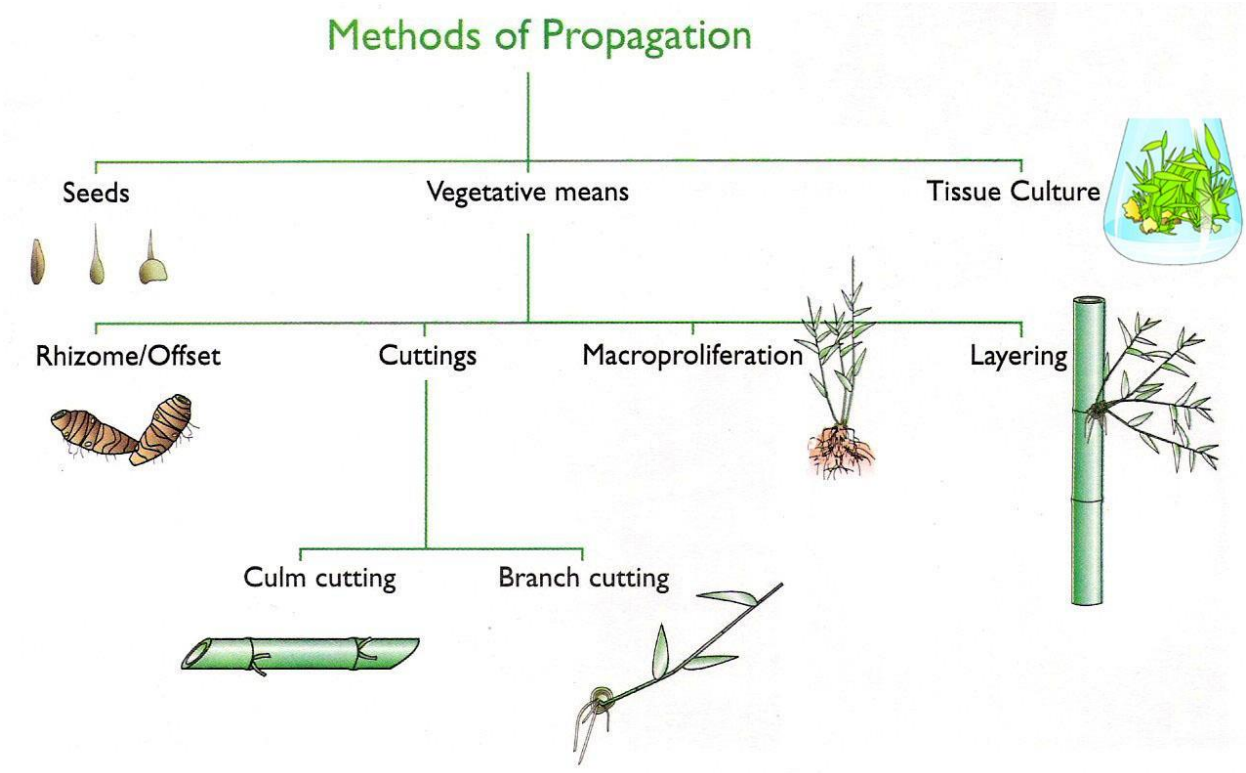
consist of one or many as well as equal, or sub-equal branches, is often very characteristic of a bamboo specie or genus. Branch bases may also develop rhizomatous swelling and root primordiums, which help in vegetative propagation through branch cuttings. Growth of rhizomatous swelling and root primordium can be induced by chopping the culm tops.

The branches/branchlets of the bamboo plant bear leaves which have distinct petioles as compared to most other members of the grass family.

Bamboo has traditionally been propagated through seed or through vegetative means. Each of these methods has their own advantages and disadvantages.

PROPAGATION BY SEED

Bamboos regenerate naturally by seeds, and this method of propagation is standardised, easy and inexpensive. However, long, erratic and unpredictable flowering cycle leads to uncertainty in availability of viable seeds. Under normal conditions, without any preservation facility, the viability of seed is very short. Plants produced through seeds also exhibit wide variation in growth and productivity.



PROPAGATION BY VEGETATIVE METHODS

Bamboos can be propagated vegetatively by following means:

- *Rhizomes* - segments of the underground modified stems have traditionally been used for raising new plants.
- *Offsets* - the underground rhizome along with a portion of the overground stem is detached and planted as a separate plant.
- *Cuttings* - section of a culm or a branch that can be made to root are placed in soil to raise new plants.
- *Layering* - bringing a culm or a branch in contact with the rooting medium to induce root formation at nodes which are then separated into individual plants

Propagation by vegetative methods has the following advantages:

- Plants produced by vegetative methods of propagation are identical to the mother plant
- Since all the traits of the mother clump are passed on to the progenies, the growth performance of the progenies is predictable
- The processes and steps involved in vegetative propagation are time-tested and are easy to implement
- The commercial gestation period of vegetatively multiplied plants is shorter than those of seed or tissue culture origin.

Vegetative propagation has the following disadvantages:

- Offsets being bulky are difficult to excavate, stock and transport.
- The propagation method is labour - intensive
- Plant material can be produced only in limited numbers due to scarce availability of mother plants with desired traits.
- Success in vegetative propagation is restricted to thick - walled bamboos. Its extent also varies with species.
- Propagation is season - specific and is also influenced by the age of the culm.
- Uncertainty over the life span of the plant if the age of the mother clump from which the propagule has been derived is not known.

For vegetative propagation of bamboos, the propagules should be derived only from a superior clump (plus clump) of known age. This will not only ensure the quality of the propagules but also the expected life span of the progeny would be known.

Taking into account the constraints associated with conventional methods of propagation of bamboo, it is desirable to undertake mass multiplication of bamboos by micro-propagation or tissue culture.

What is Micropropagation?

MICROPROPAGATION IS A technique of producing large number of disease free plants by culturing plant cells, tissues or organs under controlled physico-chemical conditions. Maintenance of aseptic conditions in the lab is one of the most important considerations of micropropagation.

Micropropagation has the following advantages over conventional methods of propagation:

- A large number of plants can be produced within a short period of time
- Plants can be produced round the year in laboratory conditions without much seasonal constraints although, their field transfer is season- specific
- There is economy of space and a lab spread over less than a hectare is adequate to produce over a million plants annually
- Plants produced are genetically uniform and are true to the mother plant from where the initial tissue / explant is derived
- Plants produced are free of bacterial and fungal diseases; they would also be free of viruses if they have been multiplied after virus indexing (testing for the absence of viruses).

Tissue cultured plants are free from diseases but not disease-resistant; they are true to their mother plant and are not genetically modified.

The process of micropropagation is carried out in the following stages:

Stage 1 : Preparatory work
Stage 2 : Initiation of cultures
Stage 3 : Proliferation of shoots/callus
Stage 4 : Rooting of shoots
Stage 5 : Acclimatization/transplantation

STAGE 1: PREPARATORY WORK

The requirements to begin the process of micropropagation are arranged during this stage as described below.

Selection of Mother Plant and Explant Preparation

The mother plant is the plant from which young tissues/organs, called explants, are derived for the purpose of micropropagation. Explants should only be taken from superior and proven quality mother plants, also called "candidate plus clumps (CPC)". Explants should not be taken from seedlings as their quality is not yet established. Criteria for selection of CPC are given in Annexure – I.

Mother plants should be maintained in a hygienic environment, and under appropriate conditions of light, temperature and humidity that favour fresh growth. Maintaining the mother plants under hygienic conditions, reduces the need for harsh surface sterilization treatment to the explants, and also minimizes the incidence of contamination in subsequent stages.

Depending on the method of micro-propagation to be used, appropriate explants are selected from mother plant. For the axillary branching method, nodal explants containing buds are taken. Buds that are present at nodes or in the axils of leaves or branches are called axillary buds. In bamboo, axillary buds on primary branches (branches on the main culm) are used as explants. The required explants are excised from the mother plant with the help of secateurs (cutting instrument) and immediately submerged in water or placed inside polythene bags to avoid desiccation.

Preparation of Glassware

Glassware or the culture vessels in which the plants grow are a central part of micropropagation, as the environment of the growing plant becomes confined. The term '*in vitro'* often used for micropropagation has its genesis in 'in glass,' that is, confined inside the glass vessel. While recycling glass culture vessels, some of them may have fungal or bacterial contamination. It is extremely important to properly clean and steam sterilize (autoclave) these glassware without opening the closure so that all microbial (fungal/bacterial) contaminants are destroyed. After autoclaving, the culture vessels should be washed with sterile detergent and rinsed with de-ionized water to remove traces of detergents.

Preparation of Nutrient Medium

The nutrient medium for propagation consists of:

- Basal medium, which contains inorganic salts, vitamins and organic nutrients which are necessary for vital biochemical processes to support growth.
- Sucrose, as a source of carbon and energy for growth.
- Phytohormones, the compounds which modulate growth. Phytohormones are categorized into auxins, cytokinnins, gibberlins,

etc. based on differences in their chemistry and response they elicit in plants.

- Gelling agents, which provide physical support to the plant tissues. Gelling agents are high molecular weight substances which, upon solidification, develop a lattice structure that appears like a semisolid gel. Agar, agarose, phytagel are some of the gelling agents. The stiffness of the gel is controlled by varying the concentration of the gelling agent.

There are various formulations (recipes) of the basal media, developed by scientists. They differ from each other in the propagations in which different nutrient components are present. Murashige and Skoog's (MS) medium is one such formulation that is commonly used in bamboo tissue culture. The medium may be prepared using readymade nutrient available in the e market or from the scratch, using individual nutrient components depending upon their suitability and economic viability. Nutrient components of the basal medium or the phytohormones are stored in the form of concentrated stock solutions to be mixed during media preparation as per finalized composition. Stock solutions of groups of nutrients are prepared based on the concentration in which they are required and their chemical compatibility with each other. It is easier mix 3 to 5 stock solutions of nutrient combinations, than to mix 15 to 20 individual nutrients each time while preparing the nutrient medium. Moreover, in case of nutrients which are required in ultra small quantities, weighing is difficult; instead, it is more accurate to use a few mililitres of the 100ml stock solution.

Media Preparation

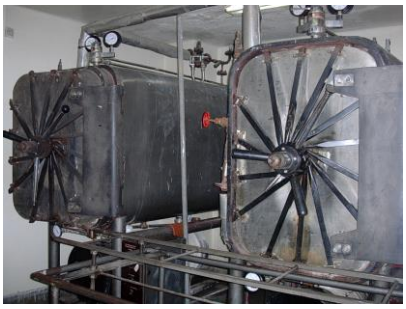
Steps in Media Preparation

- Calculated volumes of the stock solutions are taken in vessels of appropriate capacity containing a prescribed quantity of sucrose dissolved in a minimum amount of soft water, which may be de-ionized or obtained through reverse osmosis (RO). The final volume of the solution is made up by again adding soft water, and the contents are thoroughly mixed using a glass rod.

- Correct level of acidity of the nutrient medium is important to ensure stability of the nutrient components in the solution phase, and also to ensure their availability to the plants growing in it. Ph, a measure of the acidity of the nutrient medium, is adjusted to 5.8 using HCl (acid) or NaOH (base) with the help of Ph meter. While the coarse adjustment of the Ph is done using the1 N (concentrated) solution, fine adjustment is done using the 0.1 N (dilute) solution.

- To this, a measured amount of gelling agent is added, melted by heating and mixed thoroughly by swirling.

- Depending on the size of the culture vessel, a fixed quantity of nutrient medium solution is dispensed into suitable culture vessel (jar/flask/test tube) before it cools down and solidifies.



Autoclave- horizontal type

Sterilization of the Medium

The nutrient medium contained in the culture vessel (jar/flask/test tube) is rich in sucrose and other nutrients which also support growth of microorganisms such as bacteria and fungi. Since microbes tend to outgrow the plants leading to their death. It is essential to eliminate them. To ensure a completely aseptic/ sterile environment inside the culture vessels, these are placed in an autoclave, where they are subjected to steam at 1.06 kg cm^{-2} (15 psi) and 121 ^{0}C for about 15-20 minutes.

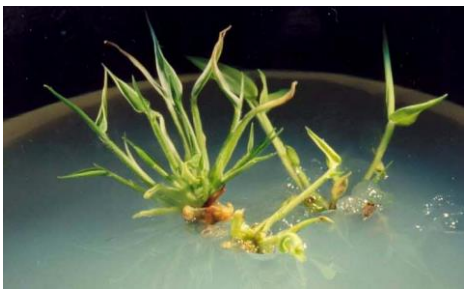
The sterilized vessels (jar/flask/test tube) with nutrient medium are stored in a separate media storage room located in the 'clean area', where a high level of hygiene is maintained through regular cleaning and fumigation with disinfectants.

The cultural vessels with nutrient medium are examined to confirm the absence of bacteria and fungus before these are used for initiation of cultures/sub-culturing.

STAGE 2: INITIATION OF CULTURES

A culture is live biological material growing on artificial nutrient medium in an aseptic environment confined inside a culture vessel. A newly initiated culture is the starting point of a series of processes that result in production of large number of plants. The process of initiation of cultures involves: (i) explant excision from the mother plant, (ii) surface sterilization, (iii) inoculating it in the nutrient media to induce desired growth pattern.

The process of surface sterilization comprises of the following:



A developing culture on nutrient medium

- Explants are washed in running tap water to wash off dirt.

- Explants are rinsed in 70% ethanol and washed with 2% Savlon (or similar germicide) along with a liquid surfactant such as Tween 20 (0.02% or a drop), for about 15 minutes.

- Final surface sterilization treatment is given in an aseptic environment in the laminar air flow cabinet with sodium hypochlorite (4% v/v for 10-20 minutes).

- Explants are then washed with sterile water 3-4 times.

- Explants are inoculated (placed) in the nutrient medium inside the sterilized vessels (jar/flask/test tube).

Culture initiation is influenced by explant selection, season, intensity of sterilization treatment, nutrient medium composition, and amenability of the chosen species to respond under *in vitro* condition.

Bamboo explants, upon cutting and if injured during handling, often releases phenolic substances. These phenolic substances get oxidized into growth-inhibiting substances which are brown in colour and can be easily observed. To protect explants form such a phenomenon, antioxidant chemicals such as polyvinyl pyrrolidone (PVP), citric acid or ascorbic acid can be added to nutrient medium. This problem can also be aoided without incorporation of the above chemicals, by frequently transferring planting material to fresh nutrient medium until exudation of the phenolic substances diminishes. .

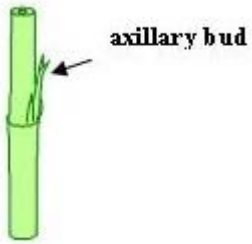
The physiological state of a plant varies from plant to plant, and also with its location on the plant due to differences in its phytohormonal profile. Thus, the source of explants influences the initiation of the culture by virtue of differences in their physiological state.

Season has a strong influence on the physiological state of the mother plant at the time of plant excision, which in turn influences the response of the buds in culture. Bamboo explants collected and cultures initiated during the spring season and monsoons generally give much better results as compared to other months of the year. In those areas where the relative humidity (RH) remains high all round the year, cultures could be initiated during other months also with a fair amount of success. However, in highly humid conditions explants harbour many more microbial organisms and, due to this, need extra attention in sterilization.

It is extremely essential that all precautions are taken to prevent contamination of cultures while inoculating the explants. To ensure this, inoculation is carried out under strict aseptic conditions inside a laminar air flow cabinet. The instruments used for inoculation, such as forceps, scalpel, scissors, etc., are sterilized by inserting them into a glass bead sterilizer (steripot) maintained at 250^0C for about a minute. The sterilized instruments are placed on a steel tray to allow them to cool down before they are used.

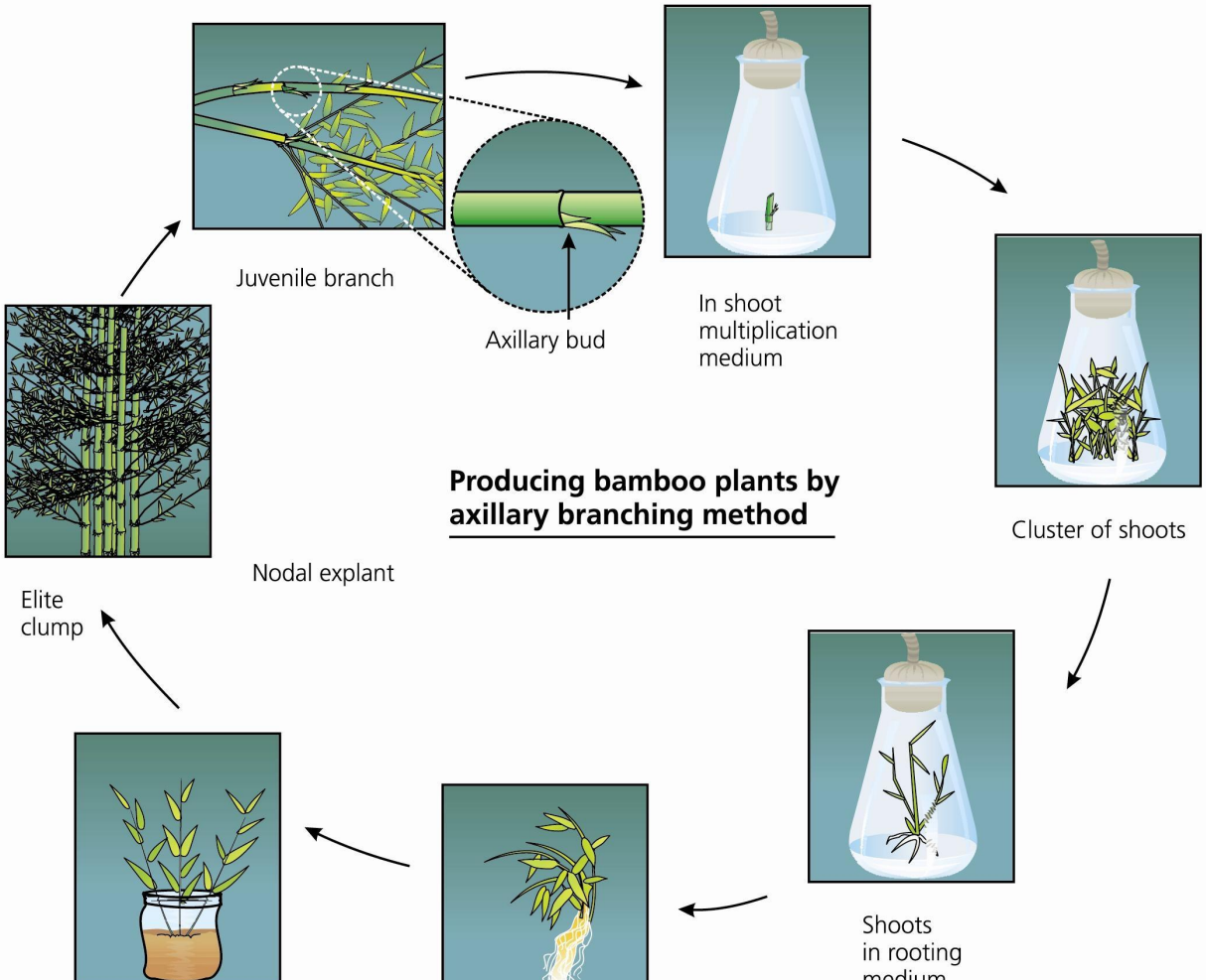
STAGE 3: PROLIFERATION OF PLANTING MATERIAL

Initiation of the cultures is followed by the stage of shoot/callus proliferation in which the bamboo cultures are made to grow and increase in quantity. Proliferation or multiplication can be brought about by three methods: axillary

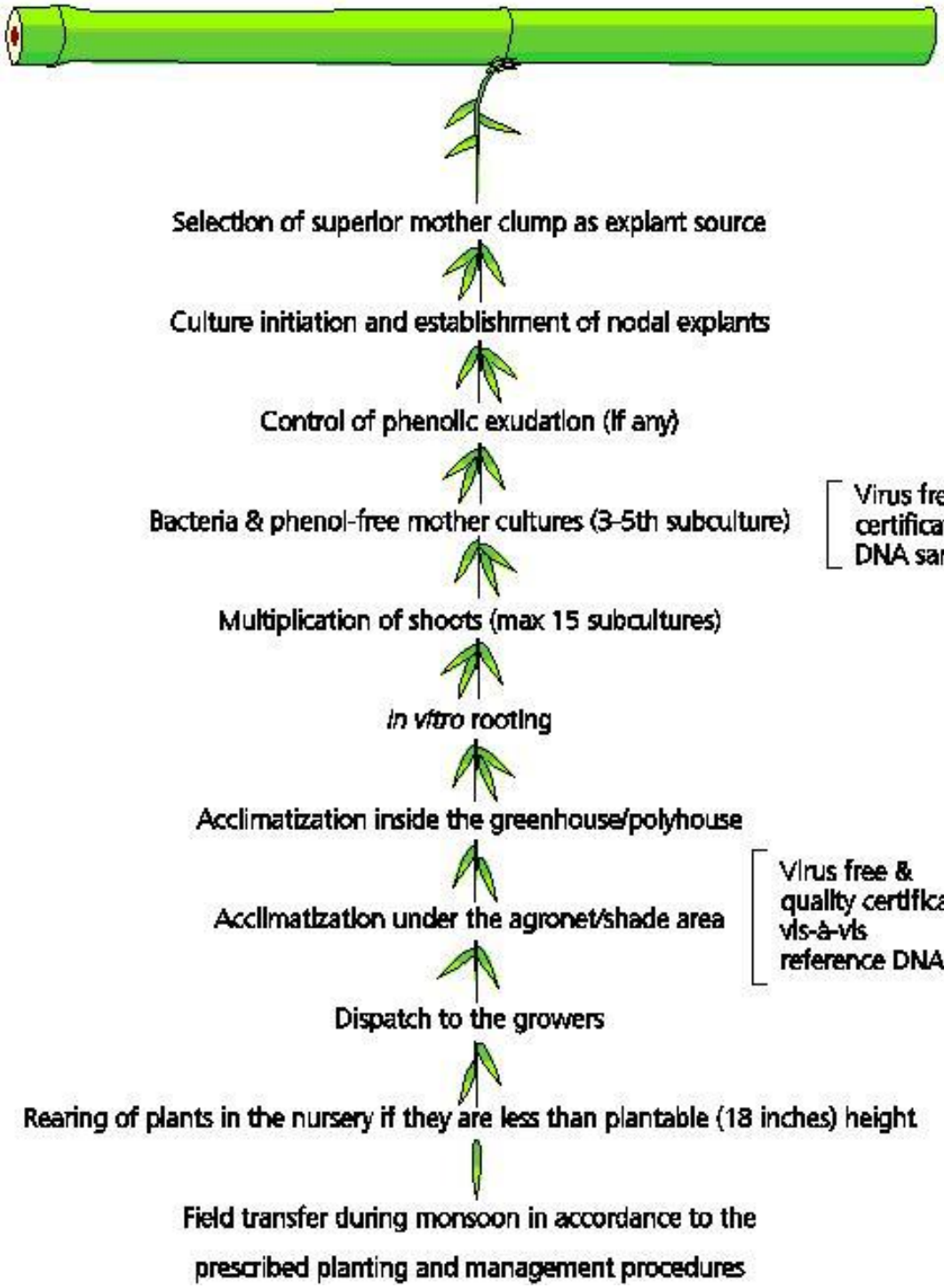


Nodal explant

Micropropagation through axillary branching method



Production of bamboo by axillary branching method: Key steps



Cluster of shoots develop after 3-4 weeks. These clusters are again divided into 2-4 smaller clusters each containing at least three shoots. This process is called sub-culturing and is repeated to multiply the shoots exponentially every 4 weeks.

A[illegible] method for micropropagation of b[illegible] genetic variation in the progeny. T[illegible] tent of sub-culturing should be limited to 15 passages only, and fresh inoculations from mother plants growing in soil should be done regularly.

shoot cluster in aseptic t

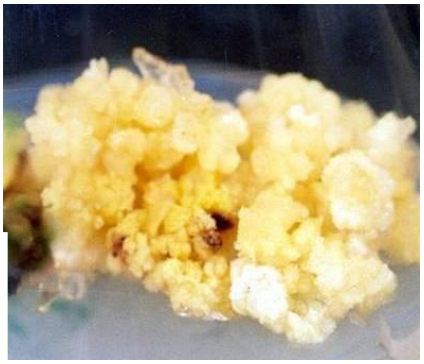
Splitting of shoot cluster during sub-culture

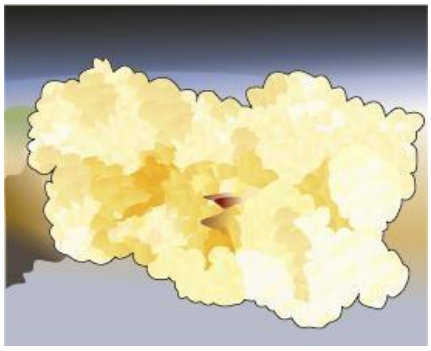
Somatic embryogenesis

Under natural conditions, a sexual process involving fusion of pollen (contributed by the male sex organ) with egg (contributed by the female sex organ) results in formation of a zygote (fusion product). The zygote grows and develops into an embryo. This process of embryo development is called zygotic embryogenesis and the embryo so formed is called zygotic embryo. Eventually, this embryo germinates and gives rise to a complete plantlet.

Cells other than the zygote may also produce embryos. Such cells are called vegetative or somatic cells (cells not involved with sexual reproduction); the embryos formed by these cells are called somatic embryos, and the process of embryo development is called somatic embryogenesis. Each somatic embryo

is a potential plantlet. It is a bipolar structure, with both shoot and the root axes. Upon its germination, shoot as well as root formation occurs in the same med ium.





Callus: mass of cells formed by disorganized division of cells

A germinating somatic embryo

Somatic embryogenesis is an extremely rapid process and it consists of the following stages:

Induction of embryogenic callus

Induction of embryogenic callus is a stage that precedes the formation of somatic embryos. During this stage, culturing of selected explants such as internodes and petioles or *in-vitro* formed axillary shoots (in case of *Dendrocalamus hamiltonii*) in a semi-solid MS medium (sucrose 2-3%) in the dark produces callus, which is an unorganised mass of cells arising due to irregular cell division. Callus formation is induced by applying single or a combination of auxins especially 2,4-dichlorophenoxy acetic acid (2,4-D) in the nutrient medium.

Callus formed during the process may be friable (loose) type or compact nodular type, however; only the latter can be stimulated to regenerate somatic embryos and may be called embryogenic callus. Identification of appropriate state of callus is very essential for successful somatic embryogenesis.

In the induction medium, 2,4-D is widely used at a concentration of 2-10 mg/l depending on the species. Reduction in 2,4-D level from the callus induction medium results in formation of somatic embryos from the callus.

Mature somatic embryos just before germination

Multiplication of somatic embryos

Somatic embryos may give rise to more somatic embryos either directly or indirectly. While in direct multiplication, the new embryos are directly formed from the pre-existing embryos, in indirect multiplication, first the callus is formed and then somatic embryos develop from it. Factors such as genotype, media composition and growth conditions influence the multiplication process.

Maturation of somatic embryos

Maturation of somatic embryos (attainment of certain physiological state) is important for them to develop capacity to germinate. It occurs in the same media as used for multiplication. However, transferring to a separate auxin-free medium, application of low concentrations of abscisic acid (ABA), high concentrations of sucrose or agar and desiccation of embryos may help in maturation of embryos.

Germination of somatic embryos into plantlets

Germination

Mature embryos on being transferred to germination medium, form both roots and shoots. The process of germination can be facilitated by reducing the concentration of auxins and/or adding cytokinins (BAP or kinetin) to the culture medium. In some cases, a separate rooting stage may be necessary to improve the rooting frequency and/or the quality of rooting.

Germination is a crucial stage of somatic embryogenesis and close monitoring of the cultures should be ensured. There have been instances in past where good embryo multiplication was achieved but, thereafter, either there was no germination or the germination frequency was extremely poor.

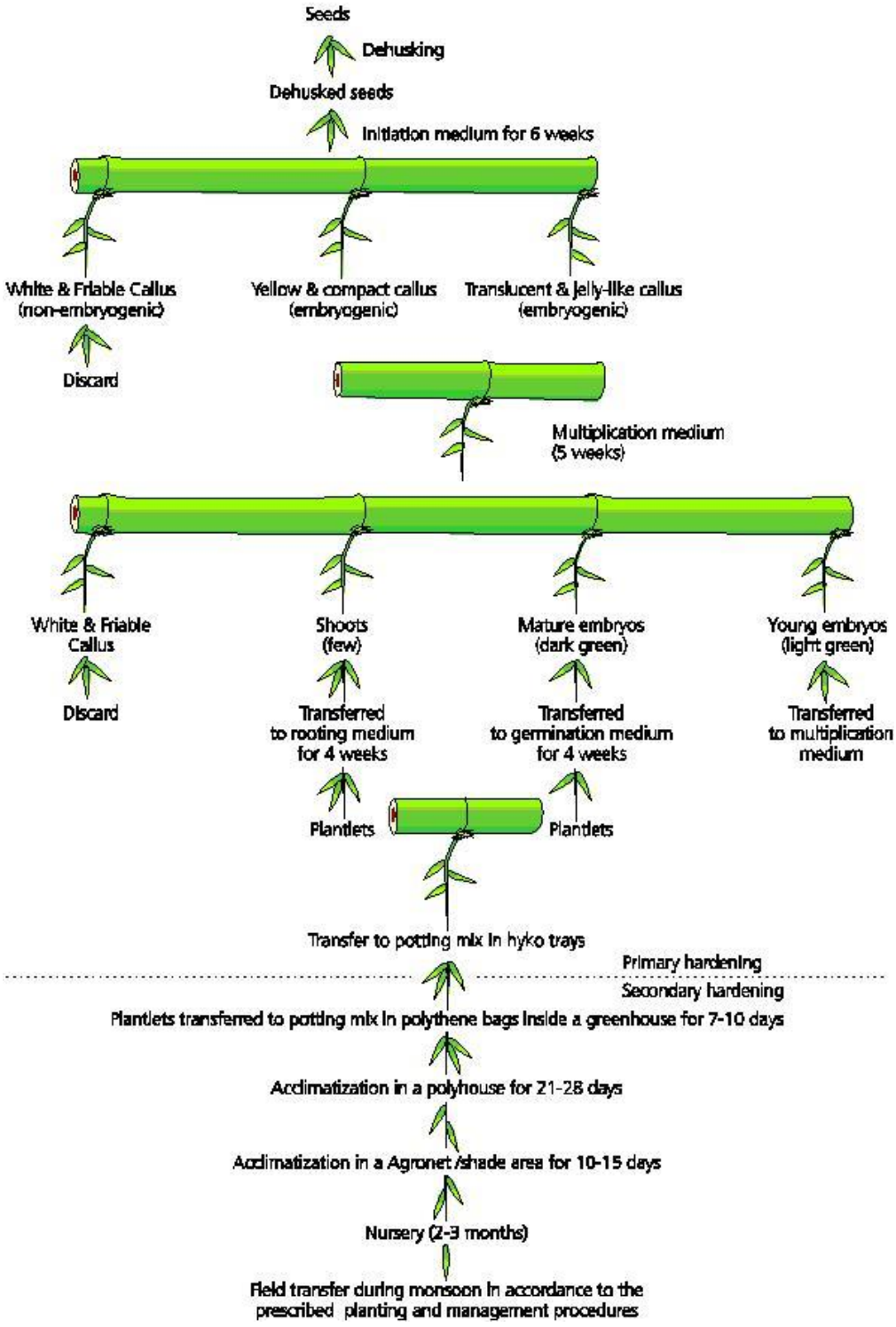
In India, somatic embryogenesis has been found to be successful for mass multiplication of *Dendrocalamus strictus, Dendrocalamus hamiltonii* and *Bambusa bambos*.

Irregularities in cell division at the callus stage brings in genetic variations in somatic embryogenesis. For this reason, somatic embryogenesis is less preferred than the axillary branching method.

Organogenesis

In this method, differentiated tissues such as parts of stem, leaf and petioles are induced to form buds directly or indirectly (through an intervening callus phase) under the influence phytohormones. In the case of callus there is *de novo* formation of buds that develop into shoots over a period of time. At a later stage, these shoots can either be rooted to produce a plantlet or multiplied, as in the case of axillary branching method, to produce additional shoots. Multiplication response is dependent on the nature of explant, the species and the phytohormones applied.

Production of bamboo by somatic embryogenesis method: Key steps



The process of organogenesis differs from somatic embryogenesis in the following respects:

- While the somatic embryo originates from a single cell, the origin of the bud in organogenesis is multi-cellular.
- While in organogenesis there is a pre-existing vascular connection between the newly formed bud and the cultured explant, there is no such vascular connection between the embryo and the explant tissue.
- Whereas in somatic embryogenesis both shoot and root are formed on the same media (single-step process), in organogenesis shoots and roots are generated in a sequential manner (two-step process).

Organogenesis is least favored approach in micropropagation because of the high risk of genetic variation associated with it. The process of conversion of differentiated tissue into un-differentiated tissue is considered to be the major cause for genetic variations.

STAGE 4: ROOTING OF SHOOTS

In this process, clusters of bamboo shoots are split into smaller clusters with at least three shoots each, and these are placed on the rooting medium containing growth regulator substances that stimulate development of roots. Single shoots are not preferred for this purpose, as it affects further growth and root initiation.

The culture medium for rooting generally contains high auxin, low or no cytokinin, low sugar and low salts. Various auxins such as IAA, IBA or NAA can be tried either individually or in combination, at various concentrations (ranging from 0.1 ppm to 5 ppm). The rooting process generally takes 2-3 weeks.

STAGE 5: ACCLIMATIZATION OR HARDENING OF THE PLANTS

During culture, the bamboo plants are in an artificial environment with high humidity and synthetic nutrient supply. Such an environment results not only in impairment of their photosynthetic ability, but also in poor development of structural features such as roots, vascular system and protective waxy layer on

Incubation/ growth room with cultures

Rooted shoot cluster

leaves. Such plants cannot survive the harsh and dry outside environment; therefore, they need to be hardened (made to strengthen their features) by gradually exposing them to the natural environment. This process of hardening is called 'acclimatization'.

Hardening is done in two stages: primary hardening and secondary hardening. The functionality of the roots developed *in vitro* is poor and these roots can not support the growth of plants under *ex vitro* conditions. Primary hardening facilitates development of functional roots, while secondary hardening brings about further growth and rhizome development, making the plant robust.

Primary hardening

Micropropagated bamboo plants with well developed roots are taken out of culture vessels and the adhering agar is washed off under running tap water. These plants are graded according to their size (identical size plants have identical growth characteristics). They are then transferred to suitable potting mix in hyko trays (plastic trays with 20 or 25 cavities, 4 or 6 inches deep) or in polythene bags (2.5" x 4"). A good rooting substratum should have high waterholding capacity, yet provide good aeration. Generally, a mixture of soil and agropeat (3:1) is preferred; however, materials like peat, perlite, vermiculite, pumice, rockwool, sand, soil etc., can also be used. Use of FYM (Farm Yard Manure) or vermicompost at the greenhouse stage should be avoided as it may cause microbial contamination. Before transplantation, a portion of the *in vitro* root can be removed which helps in development of new roots. The *in vitro* roots do not have root hairs and die in due course of time. While taking the plants to the greenhouse for transplanting, they should be protected from drying by covering them with a moist cloth or wet paper.

Tissue cultured plants in hyko trays

Tissue cultured plants being transferred to polybags

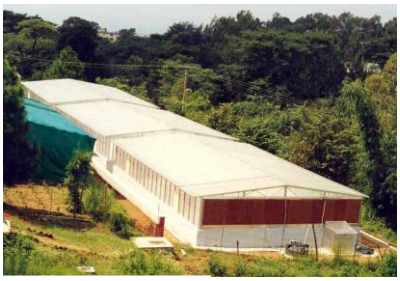
After transfer to hyko trays, the plants are reared inside a greenhouse under controlled temperature and relative humidity, and are irrigated with ¼ MS (inorganic salts only) solution for 2 weeks.

Initially, in the greenhouse, the bamboo plant are kept close to the cooling pads where the RH is above 85%, and then gradually shifted towards the exhaust fan where the RH is comparatively low (around 60%). The plants are retained inside the greenhouse for about 2-3 weeks.

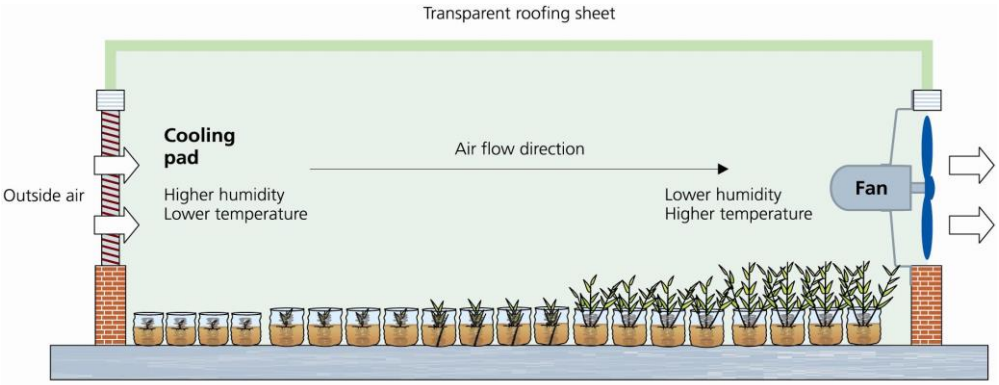
From the greenhouse, the plants are shifted to a polyhouse where relatively less stringent control over growing conditions of temperature and humidity are maintained. Inside the greenhouse and polyhouse, fresh growth occurs in the plants and they get increasingly adapted to the external environment.

With the development of new roots the plants acquire the ability to absorb nutrients. To start with, NPK with higher nitrogen proportion is applied to the plants either as a foliar spray or as drenching at the root zone. With proper hardening, the roots grow and form a ball around the substratum. This takes around 4-6 weeks from the date of transplanting and is called primary hardening.

Hyko trays and polybags in the greenhouse



Greenhouse



Typical design of a green house with evaporative cooling

Plants inside greenhouse

Polyhouse

Polytunnels inside polyhouse

Net-house for secondary hardening of plants

Secondary hardening

The plants from the polyhouse are shifted to a shade-house made up of Agronet shade (50% cut off) where there is no control on the humidity and the plants are merely protected from direct sunlight. The plants are kept in shade for about 2-3 weeks depending upon the weather conditions. Later, they are transferred to an open nursery where they are retained till they reach to a height of 1-1.5 ft.

Based on market demands, the plants can be delivered either after primary hardening or after secondary hardening. The attributes of primary and secondary hardened plants are given below.

S.No.	*Trait*	*Primary hardened tissue cultured plant*	*Secondary hardened tissue cultured plant*
1.	Minimum height of the shoot	6 inches	18 inches
2.	Minimum number of shoots	> 3	> 4
3.	Minimum number of leaves per shoot	> 4	> 8
4.	Root system	Well developed	Well developed with adequate number of secondary and tertiary roots with root hair.
5.	Rhizomes	Small rhizomes in the initial stages of development	Proper rhizomes
6.	Minimum age of the plant	1 month	> 3 months

Packaging, Transportation and Handling

A fully hardened plant

TISSUE CULTURED PLANTS should preferably be transported at the primary hardened stage with minimum 6-8 inch height and with well developed rhizomes. Transportation in bare-rooted condition further saves transportation cost.

To transport plants in bare-rooted condition, they are taken out of the polybags and packed in bunch of 10 or more. To avoid dryness, the roots are covered with agro-peat and wrapped in newspaper. The bunches are then placed in cartons (with small holes in side walls to provide aeration) and transported by air/road/ train in as short a time as possible, not exceeding 48 hours. Spray of anti-transpirants on plant leaves, and mixing of water absorbing gel 'ABSORB' in the agro-peat minimizes mortality, if the transportation time exceeds more than 2-3 days.

Bare-rooted plants should be transplanted in polybags at the earliest after reaching their destination. Polybags should be at least 5 x 8 inches in size and filled with mixture of soil, sand and compost in the ratio of 2:1:1. Survival of bare rooted plants varies with the season; it is low during harsh seasons and high during warm and wet seasons. Irrespective of whether

Fully hardened plants in polybags

plants are transported in polybags or under bare-root condition, plants should be kept in a nursery till they have recovered from transportation shock. The nursery should be located near the plantation site and should have facilities for proper watering and shade management.

While secondary hardened plants may be planted in the field after keeping in the nursery for 10-15 days, primary hardened plants need to be kept for longer duration of 2-3 months till they attain height of about 18 inches.

Truck with multiple shelves in its carriage for transporting plants

Quality Management

QUALITY IS THE hallmark of tissue culture raised plants. To ensure this, it is important that laboratories follow certain quality norms. Although there are no prescribed quality standards that are mandatory, DBT (Department of Biotechnology) guidelines exist for quality parameters. It is advisable for the producing units to maintain quality parameters as mentioned below.

Culture Initiation

- Cultures must be initiated from mother material of confirmed identity collected from an authentic source. For initiating the cultures only 'superior' bamboo clumps should be used.

- While collecting the explants, all necessary passport data must be collected. These include: clump location and age, number of culms, culm height and diameter, time of collection, contact person and a photograph of the clump. Details of the edaphic and climatic characteristics of the site should also be recorded.

- An accession number should be allocated to the clump. This number should be marked on the culture vessels throughout the production process, to avoid any mixing of cultures.

- To do away with the risk of monoculture and to keep the genetic base broad, cultures should be initiated from several 'candidate plus clumps', and each group of cultures should have separate identity coding.

Shoot multiplication

- The facilities should use recognized aseptic initiation and propagation procedures, which would maintain sterile conditions.

- During the second or third cycle of shoot multiplication, samples of the shoots should be sent to an accredited laboratory for **virus indexing**. Details of such labs are in Annexure-III, and are also available on DBT website (http://www.dbtmicropropagation.nic.in). Only if the cultures are free of known viruses such as bamboo mosaic potex virus, and sugarcane mosaic virus, they should be inducted into production for mass multiplication.

- At this very stage a **DNA fingerprint profile** using suitable molecular marker should be developed. This profile can be used later as a reference to ascertain the clonal uniformity of tissue cultured plants prior to dispatch. This is particularly important when the planting material has undergone several multiplication cycles. The clonal uniformity of tissue cultured plants can be tested at any of the centers (Annexure-III) recognized by the DBT for this purpose.

- Ideally, the shoots should be multiplied only by axillary branching method without any involvement of callus. This will minimize the risk of genetic variation during the *in vitro* regeneration process.

- Only aseptic cultures should be multiplied in production. Any contaminated culture should be summarily discarded.

- Strong quality checks should be exercised on the health of the culture. Shoots showing symptoms of vitrification (hyper-hydration) for instance glassiness or any other visible abnormality should be removed.

- Random samples of shoots should be tested periodically for their clonal fidelity. Multiplication of shoots should be stopped as soon as any genetic variation is detected. However, to be on the safer side, it is desirable to discontinue multiplication of shoots beyond 15-16 sub-cultures.

Rooting of shoots

- The rooting quality (both in terms of number and length) should be visibly good to avoid any losses at a later stage.

Hardening of plants

- Inside the hardening facilities (greenhouse and polyhouse), efficient sanitation practices including insect and disease monitoring and their prevention, must be adhered to.

- The accessions must be properly labeled and separated by physical barriers to prevent any inadvertent mixing of clones.

- Before despatch to the farmers, the tissue cultured plants growing in the nursery should be tested through random sampling for absence of viruses and clonal uniformity.

Limitations of Micropropagation

Although bamboo tissue culture has good potential to provide bulk quantities of quality plant material, there are several constraints associated with it. By adopting a careful approach, these limitations can be taken care of to a large extent. Some of the constraints and suggested approaches to attend to them are as mentioned below:

.	*Drawback/constraints*	*Suggested approach*
1.	Monoculture	- The risk could be minimized by multiplying multiple clones instead of a single one
2.	Potential risk of early flowering	- Initiate cultures from clumps of known age - Plant multiple clones of a particular species - Grow a mixture of three or more species instead of just one species
3.	Genetic variability in tissue cultured plants on account of tissue culture process (somaclonal variation)	- Produce tissue culture plants only in certain accredited labs which have the necessary infrastructure as well as technical expertise to produce good quality plants - While raising plants through tissue culture, emphasis should be placed on axillary branching method instead of somatic embryogenesis or organogenesis as both these methods are more prone to give rise to genetic variants.

		- Verify the clonal uniformity of tissue cultured plants at different stages, by analyzing a certain percentage of plants using molecular markers such as AFLP and ISSR. This can be got done at any of the laboratories listed in Annexure –III.
4.	Absence of efficient micropropagation protocols for many important bamboo species	- Intensify research efforts both in the public as well as private institutions. - Researchers must share information (even failures) so that all those involved in bamboo research are benefited and duplication of research is avoided

Establishing a Commercial Tissue Culture Laboratory

PRODUCING PLANTS by tissue culture is a highly professional activity. There are several laboratories in India researching on tissue culture of bamboo but only a few of them are engaged in mass production. A list of such labs has been provided in Annexure III.

The requirements for commercial laboratories are different in terms of scale and activity from a research laboratory. It is important to consider the following factors that have a critical impact on setting up and commercial viability of a tissue culture laboratory.

Location

Factors that should be considered for locating a commercial tissue culture lab are: moderate climate, cleaner environment, availability of good quality water, skilled manpower, proper connectivity (road, rail or air), and proximity to the target market. Dry areas are preferred over humid and rainy places, due to ease in maintaining asepsis. Locating laboratories near sea-shore area would reduce the life of air filters in the laminar air flow benches of the lab.

Capital Cost

Tissue culture laboratories can be established with different scale of investment, depending on the level of sophistication and the size of operation. Most of the equipment required for the such laboratories are available indigenously with good service back-up. Usually, the level of sophistication and the capital costs increases with the size of operation, but it reduces the investment on a per plant basis. The establishment cost of a facility with an annual capacity of five lakhs plants at current rates is approximately Rs.55 lakhs.

Importance of aseptic conditions

Hygiene is one of the most important requirements of a micropropagation lab. Poor standards of cleanliness lead to loss of cultures on account of bacterial and fungal contamination, and hence periodic screening of cultures for bacteria and fungus is recommended. Contamination losses for a viable lab should not exceed 5%.

Specifications of Lab Building

The building and rooms should be designed in such a way that dust free environment is maintained. This can be achieved by avoiding wall projections and the wall having curved corners at the ceiling and the floor, smooth and light floor, and using plastic paint on the walls. The outer walls should not permit water seepage into the building and should be painted white to minimize heat absorption. The roof of the lab should be insulated or covered in order to reduce the impact of outside direct heat to the air conditioned rooms.

INFRASTRUCTURE

Lab Building

The lab building of a tissue culture lab comprises of the following:

- *Office***:** For the staff involved in planning, monitoring and executing various activities related to the laboratory.
- *Control Panel/Room:* For regulating electricity supply, back- up power supply.
- *Maintenance / Tool Room***:** A large lab requires continuous backup of maintenance services.
- *Store:* For storage of stocks of consumables and equipment
- *Washing room*: Washing of glassware

- *Entry room*: Washing and disinfecting legs/hands, wearing autoclaved coat and cap
- *Media preparation room*: For media preparation related activities.
- *Autoclave room*: Houses autoclave for sterilizing the medium. This room also has a rack with shelves to temporarily hold the culture vessels with media prior to autoclaving.
- *Media storage room*: For stocking autoclaved media and other items required for micropropagation, prior to their usage.
- *Inoculation room*: It houses laminar flow hoods, in which all the inoculations (aseptic manipulations) are carried out in an aseptic environment.
- *Growth rooms*: Rooms with regulated temperature and illumination, meant for incubation of cultures.
- *Transfer & dispatch room*: Plants to be transferred (to the greenhouse or for dispatch) are washed, sorted and packaged in this room.

Equipments and facility

The equipments and facilities required for a tissue culture production facility can be broadly classified into two categories: laboratory area and hardening area.

Facility	*Items*
Laboratory Area	
Washing area	• regular water supply, • a glassware washing machine with brushes mounted on a motor, • brushes of various sizes and shapes, • sinks of stainless steel, • plastic tubs to dip the glassware,

	• an industrial oven to dry the washed glassware, and • a cupboard to store the dried glassware
Media preparation room	The media preparation room is equipped with: • supply of de-ionized water or a reverse osmosis unit, • benches at a suitable height to work while standing, • a deep freeze for storing the stock solutions and other phytohormones, • a refrigerator to store chemicals, plant material and stock solutions, • weighing balances, • hot plate cum magnetic stirrer for dissolving chemicals, • a pH meter, • a gas stove or a microwave oven for melting of agar, • all kinds of glassware ,plasticware and other consumable items.
Autoclave room	It houses : • autoclaves required to sterilize the media, • uninterrupted water and electricity supply, • exhaust fan, • a steel trolley to load and unload the media, • a shelving unit to keep the autoclaved items till the medium has solidified/gelled.
Media storage room	• several steel racks on which sterilized media is stored prior to its use. • also used for storage of other sterilized material such as empty jars, forceps and other tools, petri-plates or papers
Inoculation room	• laminar air flow cabinets required to carry out all types of aseptic manipulations. • glass bead sterilizers (placed inside the laminar air-flow cabinet) to sterilize the tools. • mask and aprons for workers. • steel trolleys to carry cultures.

Growth rooms	• racks with shelves fitted with artificial lights for incubating cultures (these days movable trolleys are preferred for better space utilization) • photoperiodic simulator for controlling the duration of illumination/darkness. • temperature controller for maintaining constant temperature inside the growth room • airconditioning.
Hardening Area	
Transfer area	• stainless steel sinks for washing-off the gelling agent • benches at a suitable height to transfer the plantlets from the culture vessel to polybags / portrays / hykotrays prior to hardening
Greenhouses	• transparent walls and roofs that separate the inside environment from the outer environment. • cooling pads on one end and exhaust fans on the other. The two help to create a gradient of relative humidity (RH) and temperature inside the greenhouse • movable benches on which the plants are kept • protection from sunlight by placing agronet, for hardening (gradual acclimatization) of tissue cultured plants
Polyhouses	• a tunnel-like structure made of transparent polythene sheet with coolers/cooling pads at one end and exhaust fans at the other. • the top and the sides of the structure are covered with agronet (50-75% cut-off) to protect plants from direct sunlight. (The function of polyhouse is same as that of a greenhouse, except the conditions with respect to RH and temperature inside the polyhouse are relatively less stringent as compared to a greenhouse.)
Shade area	• made up of black agronet (usually 50% cut-off) where there is no control on the humidity and the plants are merely protected from direct sunlight
Nursery	• an open stretch of land with facilities for irrigation where the plants are retained untill they are dispatched

The requirements of equipments and facilities may vary with the climate of the site location. Though the basic equipments required for commercial lab such as, air conditioners, laminar air flow benches, autoclave, pH meters, weighing balances, are all same, these differ according to the requirements of an R&D lab in design and capacity. It is important that the capacity of all equipment matches and is balanced with the production capacity of the lab with no bottle-neck at any point in the process flow. Stand-by arrangements for critical equipments are essential, and the capacity should be worked out with 20% buffer.

Skilled manpower

	Manpower	Role
1.	Manager/Scientist	Managing the production; technical backing
2.	Supervisor	Supervising the work of operators; maintaining quality
3.	Operators	Media preparation; aseptic manipulations
4.	Field workers	Hardening and other field operations

Technical know how

There should be technical know how for sourcing tested micropropagation protocols for large- scale multiplication of tissue cultured plants for the selected bamboo species. The protocol should preferably be with adult tissue so that both quantitative and qualitative gains can be accrued.

Sourcing of a certified/authentic mother material
Criteria for selection of mother material has been listed in Annexure-I.

It is advantageous to have a research lab attached to the production facility to cater to research needs that may arise during the course of production. The facilities can also be utilized to improve the efficiency of the existing micropropagation protocol and to develop cost- effective measures to reduce the cost of plantlet production. To minimize the risks due to fluctuations in market requirement, it is better to base the tissue culture lab on a mix of different species.

Investment
The approximate cost of setting up of a tissue culture lab including infrastructure, equipments and hardening facility for a production capacity of 4-5 lakh plants per annum would be Rs.55 lakhs. Details of the same are provided in Annexure II.

OPERATIONAL REQUIREMETS

Capacity utilization
Conducting operations in more than one shift helps in a higher capacity utilization, thereby increasing production without incurring any extra expenditure on infrastructure.

Meeting the hygiene standards
The cleanliness of the lab is measured in terms of count of air-borne particle of at least 0.5 micron size per cubic foot of air, with the help of particle counter. The growth room, media store room and clean area corridor should be between 'Class 1,000' and 'Class 10,000' while inoculation room should be 'Class 1000' and the inoculation chamber should be less than 'Class 100'. Since the particle counting equipment is expensive, exposing petriplates with Potato Dextrose Agar (PDA) media for 5 minutes in all the

rooms in the clean area, along with a control plate exposed to outside room, is taken as measure to check the level of bacterial and fungal count.

Clean Area Entry Procedure

The heart of a tissue culture operation in a lab is sub-culturing of plants in the inoculation room and growing of cultures in the growth room. These areas are termed as clean rooms, which are maintained at very high levels of sterility. A good production lab should be cleaner than an operation theatre of a hospital. Entry into such clean area is not only restricted but also requires a stringent protocol to be adopted, because a high percentage of microbial contaminants are brought in the clean area by the human beings. Personnel entering the lab must first

- Remove all source of contaminants such as footwear, rings, bangles, watches, hand-kerchiefs, hand bags, etc. and wash feet and hands with aseptic soap using a scrubber.
- Enter the changing room after dipping his/her legs in aseptic solution and put on a clean and autoclaved apron and headwear. Women must tie their hair under the headwear.
- Dip hands in an aseptic solution without wiping to avoid any contamination.
- Periodically change the sterile garments and the solutions for disinfection.

Aseptic Manipulations

Shoot multiplication

Using a sharp sterilized blade, carefully remove the damaged and discolored tissues from the bamboo cultures.

Divide the cultures at the base using their natural demarcations and split them into clumps each containing 3 or more shoots.

Depending on the culture vessel size & volume, inoculate a fixed number of plants.

Rooting

Remove discoloured leaves and tissues from the culture, divide the cultures into clumps containing at least 3 shoots.

If the shoots are not big enough they are transferred again to shoot multiplication media, to allow them to grow further.
During inoculation, care should be taken to see that the cultures are not touching the rim of the culture container.

Managing of Laboratory under Large-Scale Operation

MANAGEMENT AND INFORMATION SYSTEM (MIS)

Large-scale production of tissue culture plants involves keeping records of several species, clones as well as thousands of cultures for précise production and handling at different stages in the growth room and in the green house. This leads to enormous data generation and management. Data also needs to be collected on stock of consumables, scheduled delivery of plants, activities in greenhouse, such as spraying insecticide, fungicide, nutrient, etc. Management Information System (MIS) is required in a tissue culture lab for timely and efficient recording, classification, analysis and access of the data.

QUALITY PARAMETERS

- The lab should be swabbed with an antiseptic solution, and fumigated at regular intervals. Similarly, the steripots, air conditioners and Laminar Air Flow machines should be inspected at regular intervals to avoid breakdowns. The frequency of these activities varies from lab to lab depending upon local weather and capacity utilization.

- The lab aprons and headwear should be washed regularly.

- The feet and hand dip solutions should be replaced every alternate day.

- The 70% alcohol solution should be produced every day for the day's requirement.

- All the consumables for the plant propagation should be collected in a bag after work and disposed in an appropriate manner.

PRODUCTION PLANNING AND DELIVERY SCHEDULE

To ensure production and delivery of large number of quality plants, it is essential to work out an annual production plan of the lab, keeping in view the following:

- Availability of explants to initiate culture
- Shoot multiplication rate
- Incubation period between two sub-cultures
- Rooting frequency and time taken for hardening
- Percentage of losses due to various reasons
- Post hardening survival

Once the master production plan is made out, regular production is undertaken with specific segmental targets based on the master plan. The work target is divided into months and further into weeks. The actual work done is verified against the target on a weekly basis. Any difference in achievement is adjusted in the subsequent week and the production plan is continued further. The production plan is better done by computation through software rather than manually.

SKILL REQUIREMENT OF PLANT INOCULATION TECHNICIANS (PITS)

The training and skill development of a Plant Inoculation Technicians (PITs) of a commercial TC lab plays an extremely critical role in meeting the higher plant production targets in terms of quality and quantity. The technician must be trained to follow the procedure given below:

- The PIT, after entering the lab, must see that the steripot and laminar air flow bench are switched on at least half-an-hour before the work.

- The PIT must change the blades in an aseptic manner, and sterilize the forceps and blades holders in steripots for at least 30 seconds.

- The technician must swab his hands from fingertip to elbow, and the work place with 70% alcohol using a cotton pad.

- Before taking out the culture, the mother culture container should be inspected for the presence of minute bacteria and fungi. Only good grade culture containers should be used for propagation.

- After every dissection cycle, the set of forceps and blades are re-sterilised in steripots. Three sets of instruments can be used to maintain a constant cycle.

- The PITs must swab their hands every time they move them away from the laminar air flow and after every inoculation cycle.

- The culture vessels should be immediately labelled with clone code, date, number of cultures, name of the PIT, stage of the plant and cycle of multiplication.

- Under no circumstances should the PIT touch or let his hands move over the cardboard while working. If there is a break in the electricity supply and LAF not working for more than 15 seconds, all the materials such as cardboard, culture, etc., should be replaced and the instruments resterilized.

Conduct of a Good PIT

The qualities and habits of a PIT play an important part in avoiding contamination. The following qualities must be acquired by a PIT during training and must be practiced at work.

- Wear clean tidy clothes, and have neatly cut nails, devoid of nail polish
- The PIT must not be ill and coughing & sneezing, or have skin disease.
- Men must be cleanshaven and women must plait and tie hair inside the headwear.
- Edibles, smoking and chewing of any kind (including tobacco) must not be permitted in the work area.
- Take a bath before coming to the lab.
- Fill the Daily Operator Sheet for the work done in the day.

- Maintain a balance between the number of cultures handled and their quality.
- Talking should be to a minimum while working in the lab.

Measures to Reduce Costs of Production

The major components of the cost of production of tissue cultured plants are manpower, electricity and the nutrient medium. Therefore, efforts should be made to cut down costs on these components, by properly organizing following segments of a tissue culture lab.

PROTOCOL PARAMETERS

- The micropropagation protocol should be highly efficient, i.e., the multiplication rate, rooting frequency, and survival rate during hardening of plants should be high.
- As far as possible, multiplication and elongation of shoots should be achieved in the same medium.
- The number of propagules to be cultured in a vessel should be adjusted in such a manner that there is no overcrowding, yet there is optimal utilization of space and medium.
- Sub-culturing time should be optimized so that each passage is of shortest possible duration.
- *Ex vitro* rooting is preferred over *in vitro* rooting, as it helps in reducing the cost of media, electricity and manpower. It also reduces the time taken to produce a plantlet.
- Wherever possible, expensive chemicals should be substituted by commercial-grade inexpensive chemicals. For example, AR-grade sucrose can be replaced by pharma grade sugar, instead of AR-grade agar, food grade agar can be used, etc. Care should be taken that cost reduction is achieved without compromising on the quality of the plants.

- Ordinary glassware such as jam bottles can be used as the culture vessels, instead of expensive glassware (Borosil).

Inoculation and Production Room

- Dissection of plants can be done on disposable paper instead of on glass plate or petri dish or porcelain plate.

- The person handling the culture should be encouraged to give more output without compromising on the quality of the plants. Introduction of incentive system for quality production would increase productivity.

Media Preparation Room

- Steam generation by oil-fired boiler is a better choice compared to an electrically operated boiler, and it should be placed nearer to the autoclave to reduce the waste of the steam by condensation.

- Deionized water is more cost-effective than distilled water for media preparation.

Plant Growth Room

- Use of mobile racks for better handling.

- The height of the growth room should not be more than 10 feet to reduce the airconditioner tonnage.

- The airconditioning system should be in multiple units so that it can be operated at partial load to reduce the cost of electricity. This also provides self stand-by in the event of a breakdown.

- Since airconditioning is expensive, the lighting systems chosen (tubelights with electronic ballasts) should generate the least amount of heat. The growth room itself should be positioned in that part of the building where natural heat buildup is minimal (such as basements).

Some labs have reduced electricity consumption for lighting by designing the growth room for efficient use of sunlight.

- In the growth room the walls, racks, trays, floors and ceiling should be white in colour. This would maximize the reflection of light and thus its absorption by the plants.

GENERAL MAINTENANCE

- Regular preventive maintenance of all the equipment should be carried out to avoid breakdown and consequent disruptions in routine work.

The measures listed above are of general nature. Depending on the location and the design of the lab, several other initiatives could be undertaken to cut down the cost of production. However, such cost-cutting measures should be first tested on a small scale and then introduced gradually to cover the entire production process.

The tissue culture lab should be run by professional and experienced managers. This is extremely important because most of the cost-cutting measures are dependent on the location and design of the building, and other local factors/conditions. Professionals should be included right from the designing stage because later on making alterations in the design, air conditioning etc., would become difficult and expensive.

Annexures

ANNEXURE – I: SELECTION OF PLUS CLUMPS

All the plants produced by micropropagation technique are identical to the parent plants; therefore, selection of mother clumps assumes a lot of significance. Any error in the selection of the mother clump can be catastrophic because several thousand copies of the mother plant would be produced. Since bamboo is a perennial, the negative impact of a wrong selection would be of recurring nature, causing enormous economic damage to the grower.

Features of Plus Clumps

• Healthy and not infected by any disease	*(for all end uses)*
• Producing high number of culms	*(for all end uses)*
• Producing solid culms or culms with narrow lumen	*(for construction, and laminated board)*
• Producing culms with comparatively elongated internodes	*(for mat making, and weaving)*
• Branching mostly at the top, and with none or less at the bottom	*(for easy harvesting)*
• Ability of grow in waterlogged/ flooded areas	*(for ecological considerations)*
• Ability of grow in dry and/or saline areas	*(for ecological considerations)*
• Capacity to survive even after flowering (if the clump is in flowering stage)	*(for ecological considerations)*
• Observes sexual (seeding) & vegetative growth, simultaneously	*(for ecological considerations)*
• Somewhat open (not congested) clump	*(for easy harvesting)*
• High capacity of viable seed production (if the clump has flowered)	*(for ecological considerations)*
• More succulent and palatable young shoots	*(for shoot edibility)*
• Any other desirable trait depending on the end-use, such as more thorny nature (for perimeter fencing)	*Bambusa bambos*

ANNEXURE – II: REQUIREMENT SPECIFICATIONS FOR A RESEARCH CUM PRODUCTION FACILITY WITH CAPACITY TO GENERATE 4-5 LAKH TISSUE-CULTURED PLANTS PER ANNUM

Lab Area

	Description	Area (Sq. ft) (approx.)
1.	Growth room	500
2.	Inoculation room	250
3.	Media preparation	250
4.	Glassware washing	150
5.	Autoclave room	150
6.	Store	150
7.	Office	150
8.	Place for keeping DG Set, UPS, etc	100
9.	Observation room	100
10.	Transfer area	200
	Total lab area	2000

In addition to lab area additional about 12,000 sq feet will be needed for hardening of tissue- cultured plants (inclusive of nursery space)

Equipment required

	Item	Number
1.	De-ionized water unit	1
2.	Ultrapure water unit or distillation unit	1
3.	Air conditioners of 2 tonnes	8
4.	Fixed racks with electrical fittings	200
5.	Photoperiod simulator	1
6.	Temperature controller	1
7.	Laminar-air flow unit	5
8.	Glass bead sterilizers	10
9.	Autoclaves	1
10.	Refrigerators (360/400L)	1
11.	Electronic balances	2
12.	pH meter	1

13.	Microwave oven	1
14.	Oven	1
15.	Magnetic stirrers	1
16.	Heat convector	2
17.	Glassware washing machine	2
18.	Chairs	12
19.	Other furniture	As per need
20.	UPS	1
21.	Computer	1
22.	Generators (100kv)	1

Cost (approximate)

- Civil construction @ Rs.1000/- per sq.ft : 20.0 lakhs
- Green house (100 -125 sq m) @ Rs.10 lakhs :10.0 lakhs
- Cost of 4 polyhouses (16m x 6m) and a Net House (15m x 15m) : 4.0 lakhs
- Equipment (excluding consumables) :21.0 lakhs

Rs. 55 lakhs

The cost of setting-up the facility would be determined by:

- *the location of the facility*
- *level of sophistication and state-of-the-art specifications*
- *make and quantity of the equipment*
- *choice of the building material*

ANNEXURE – III: INSTITUTIONS

Indian institutions involved in production of tissue cultured plants

Aditya Biotech Lab and Research Pvt. Ltd., Raipur
Marketing Manager,
Aditya Biotech Lab & Research Pvt. Ltd.,
Near Nalghar Chowk, Chhotapara, Raipur, Chhatisgarh.
Tel : 0771-2534854/5036053/3090850 9826640555
Fax : 0771-2537634
E-mail: abdindia@rediffmail.com

Century Laminating Co. Limited, Hapur, Ghaziabad, UP
Manager, Agro Division, Century Laminating Co. Ltd.,
Village Achheja, P.O. Hapur, Hapur – 245 101. Distt. Ghaziabad (UP)
Tel : 0122-2308601-09
Fax: 0122-2308611
E-mail: merinohr@ndf.vsnl.net.in, merinohapur@merinoindia.com
Website: www.merinoindia.com

Forest Research Institute, Dehradun, Uttaranchal
Tissue Culture Laboratory, Forest Research Institute,
P. O. New Forest, Dehradun, Uttaranchal – 248 006.
E-mail: aryaid@icfre.org

Global Greens, Bangalore
Managing Director, Global Greens,
439, Kaggalipura, Kanakapura Road, Bangalore – 560 062.
Tel: 080-28432811 9448324069
E-mail: suma@globalgreens.in

Growmore Biotech, Limited, Hosur, Tamil Nadu
Director, Growmore Biotech Ltd.,
41-B, Sipcot Phase-II, Hosur, Tamil Nadu- 635 109
Tel. 04344-260564, 260565, 09443351957
Fax. 04344-260560
E-mail: growmore@vsnl.com
Website : www.growmorebiotech.com

Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh
Head, Division of Biotechnology,
Institute of Himalayan Bioresource Technology (CSIR),
PO Box 6, Palampur, Himachal Pradesh - 176 061.
Tel : 01894-233337
Fax: 01894-230433

Institute of Wood Science and Technology, Bangalore, Karnataka
Director, Institute of Wood Science & Technology,
18th Cross, Malleswaram, Bangalore, Karnataka - 560 003.
Tel: 080-23346811
Fax: 080-23340529

Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu Director, Institute of Forest Genetics and Tree Breeding,
Indian Council of Forestry Research and Education (ICFRE),
P.B.No.1061, Coimbatore, Tamil Nadu - 641 002.
Tel : 0422-2431540, 2435541,2450302
Fax: 0422-2430549

Kerala Forest Research Institute, Peechi, Thrissur, Kerala
Director, KFRI,
Thrissur, Peechi, Kerala – 680 653.
Tel : 0487-2699061-63
Fax : 0487-2699249
Website : www.kfri.org

National Chemical Laboratory, Pune
Head, Tissue-Culture Pilot Plant, National Chemical Laboratory,
2nd Floor, Bio-chemical plant, Pashan Road, Pune, Maharashtra - 411 008.
Tel: 020-25893338, 24682100, 51504900
Fax: 020-25893761, 24682145

Phulwari Biotech Limited, Manimajra, Chandigarh
Director, Phulwari Bio-Tech Limited,
Chandigarh-Kalka Road, Manimajra, Chandigarh – 160 101.
Tel : 0172-2734337
Fax: 0172-2584648
E-mail : phulwari@rediffmail.com

Sheel Biotech Limited, New Delhi
President, Sheel Bio-Tech Ltd,
S-255, Greater Kailash-II, New Delhi – 110 048.
Tel: 011-51637730, 51637731, 98113344555
Fax: 011-51637732
E-mail: sheel@ndf.vsnl.net.in
Website: www.sheelbiotech.com

Sunglow Biotech, Coimbatore
Managing Director, Sunglow Biotech,
No. 3, Krishnaraja Nagar, 12th Cross, New Thillai Nagar, 1st Avenue,
Vadavatli (P.O.), Coimbatore – 641 041.
Tel: 0422-2431912, 5538546, 9894044566
E-mail: sunglowbiotech@sify.com, glowsun@rediff.com
Web: www.sunglowbiotech.com

The Energy and Resources Institute (TERI), Habitat Place, Lodhi Road, New Delhi
Fellow & Area Convener,
Plant Tissue Culture & Molecular Biology, TERI,
Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi-110 003.
Tel: 011-24682100, 51504900 (ext.2525), 9811392248
Fax: 011-24682144/45
E-mail: sanjays@teri.res.in
Website: www.teriin.org

Whitefield Agrotech Pvt. Ltd., Bangalore
CEO,
Whitefield Agrotech Pvt. Ltd. Alembic Glass Industries Compound
Whitefield, Bangalore – 560 066.
Tel: 080-28452204/2673
Fax: 28452207
E-mail: wapl@vsnl.net

Institutions recognized by the Department of Biotechnology, as National Virus Testing Facilities

Main Centre

Virology Department, IARI, New Delhi

Satellite Centres

Indian Institute of Horticultural Research, Bangalore
Dept. of Biotechnology, Indian Institute of Horticultural Research,
Hessaraghatta Lake PO, Bangalore-560089.
Tel: 080-28466353 / 28466370
Fax: 080-28466291
E-mail: root@iihr.kar.nic.in

Institute of Himalayan Bioresource Technology, Palampur
Director, Institute of Himalayan Bioresource Technology (CSIR),
P.O. Box 6, Palampur, Himachal Pradesh – 176061.
Fax: 01894-230433

Institutions recognizes by Department of Biotechnology for testing for genetic uniformity in tissue-cultured plants through molecular diagnostic tools

Golden Jubilee Biotech Park for Women Society, Kanchipuram
Golden Jubilee Biotech Park for Women Society,
Siruseri Village, Inside SIPCOT –IT Park, Old Mahabalipuram Road,
P.O.- Navalur, Distt – Kanchipuram, Tamil Nadu – 603103
Tel: 044-24482830,30906313,
E-mail:tsloki@yahoo.com, biotechpark@vsnl.net

National Chemical Laboratory, Pune
Head, Tissue Culture Pilot Plant, National Chemical Laboratory,
Pune – 411008.
Tel: 020-25893338, 24682100,51504900
Fax: 020-25893761, 24682145

The Energy and Resources Institute (TERI), New Delhi
Fellow & Area Convener, Plant Tissue Culture & Molecular Biology,
TERI, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi-110 003.
Tel: 011-24682100, 51504900 (ext. 2525), 9811392248
Fax: 011-24682144/45
E-mail: sanjays@teri.res.in
Website: www.teriin.org

GLOSSARY

a

acclimatization a process of making the plants gradually accustomed to a field environment with different conditions such as temperature, humidity, photoperiod and light intensity.
asepsis without infection or contaminating microorganisms
axil upper angle formed by a leaf/twig etc. and the stem from which it grows. **axillary:** Location in the angle formed between a leaf/twig etc. and the stem from which it grows

c

callus an unorganised, mass of proliferative living cells; a wound reaction
cell a very small, complex unit of protoplasm, usually with a nucleus, cytoplasm, and an enclosing membrane: all plants and animals are made up of one or more cells that usually combine to form various tissues
clump a cluster of bamboo culms arising from a single rhizome system, and are therefore, joint to each other under the soil.
culm the jointed stem of various grasses; usually hollow.

d

de-novo afresh, anew
desiccation prolonged period of drying/dehydration.

e

ELISA (Enzyme Linked Immuno Sorbent Acid), a technique used to detect viruses in plant tissue
embryo the rudimentary plant contained in a seed, usually made up of hypocotyl, radicle, plumule, and cotyledons, that later grows up into a complete plant **explant** a segment of living tissue taken from its original plant and transferred to an artificial nutrient medium for growth or maintenance.

g

greenhouse a building made mainly of glass (or a substitute material), in which the temperature and humidity can be regulated for the cultivation of delicate or out-of-season plants.

h

hardening the process of making the plants accustom to a new environment with different conditions such as temperature, humidity, photoperiod and light intensity.

i

in vitro confined inside test tube (glass) conditions.

j

juvenile a young and growing plant or part of it differing variously in form, features, etc. from the adult. It has a higher capacity for growth and differentiation.

l

layering a method of vegetative propagation, whereby, roots are developed on aerial parts of a plant by special treatments, followed by their detachment from the parent plant, hence creating an independent plant.

m

micropropagation a technique of producing large number of plants under influence of phytohormones, on synthetic nutrient medium in aseptic environment, confined inside an enclosed glass vessel.

o

offset a side shoot (underground rhizome branch) that develops roots and starts a new plant. In bamboo, basal part, 3-5 nodes of a 1-2 year old culm along with an underground rhizome system. It is used as a propagule in conventional propagation of bamboos.

p

phenol exudation cut ends (injured parts) of some plants release phenols that get oxidized to produce brown coloured toxic substances, and hamper growth **phytohormones** the broad category of chemical substances that are capable of modulating growth of plants and plant parts.
polyhouse a structure consisting of transparent, UV stabilised plastic sheet supported usually by a metal frame, inside which, the temperature and humidity can be regulated for the cultivation of delicate or out-of-season plants. **propagule** a unit of plant that can potentially develop into a complete plant.

r

recalcitrance the characteristic of being non-responsive to growth promoting treatments
rhizome a creeping stem lying, usually horizontally, at or under the surface of the soil and differing from a root in having scale leaves, bearing leaves or aerial shoots near its tips, and producing roots from its undersurface.

s

somaclonal variations multiplication of plants/cells without involvement of sexual/meiotic processes normally results in derivatives identical to each other. However aberrations in this process result in variations in characteristics. Such variations are known as somaclonal variations.
somatic: cells/parts of plant other than those involved in sexual reproduction.

succulent having thick, fleshy tissues for storing water, as in cactus.

t
tissue the substance of an organic body or organ, consisting of cells and intercellular material.