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Micropropagation in Bamboo - An Overview

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Abstract

The common term used for a diverse community (1250 species) of large woody grasses, ranging in height from 10 cm to 40 m, is bamboo. Bamboo may have potential as a bioenergy or fibre crop for niche markets, already in regular use by about 2.5 billion people, mainly for fibre and food in Asia, although some reports of its high productivity seem to be exaggerated. For bamboo, different propagation techniques are available, such as seed propagation, clump division, rhizome and culm cuttings. These are largely ineffective and inefficient for mass scale propagation and the only feasible approach is micropropagation. This study focuses on various Bamboo micropropagation techniques. Further research on propagation techniques, establishment and stand management is also required, and it is important to improve mechanised harvesting.

Introduction

Bamboos are versatile, non-wood forest and perennial grass with tremendous commercial and eco sociological importance. Bamboo is a fast-growing plant. Bamboo is a vernacular term for the members of subfamily Bambusoideae of the family Poaceae, the grasses. China and India together contribute more than half of the total bamboo resources of the world. In India it is known as 'Green gold' or 'Poor man's timber' and in Chinese, it is called as 'Friend of the people' and 'my brother' in Vietnamese. It is used as a source of food, fibre, building materials, beverages, medicines, biofuel and pesticides. The alternative sources of energy and helps to prevent soil erosion due to the closely woven mat of intertwining roots. Vegetative propagation is usually practised for cultivation but the plants developed will all be as old as their stock and will flower and die simultaneously as the actual age is the same in every part of the bamboo. Micropropagation of bamboos has to lead to the development of true to the type plants and new types of bamboos (ornamental) that can be year-round with great quality.

Micropropagation

Micropropagation is the process, using modern methods of plant tissue culture, of rapidly multiplying stock plant material to create several progeny plants. Micropropagation is an alternative technique to propagate plants to satisfy their rising demand for plants on a large scale. Various sections of a plant are used, such as embryos, pollen grains, and sections, such as roots, shoot tips, nodes, root tips, calluses, and single cells.

Basic Requirements of Micropropagation

- **Aseptic environments:** well-sterilized laboratory environments and cultures that are pathogen free.
- **Environmental conditions:** There should be air-conditioning

in the laboratory. 18-27 °C is the most widely used temperature. The strength of light that is normally followed is 300-400 K lux.

- **Culture media:** The nutrient requirement is fulfilled by culture media for different types of tissues. MS media (Murashige and Skoog) and B₅ are the most widely used.
- **Subculturing:** The transition of callus from old cultural media to new media is known as subculturing until the nutrients are fully used to ensure the good health of the callus.

Steps Involved in Micropropagation

- **Selection of Explants:** The tissue used for regeneration should be true to the type, healthy, virus free and vigorous. The explants should be sterilized.
- **Establishment of Explants Plant:** The explants should be sterilized and placed in the culture media for multiplication.
- **Tissue Proliferation:** The tissue regenerates on the culture media and give rise to a cell mass known as a callus. Repeated sub culturing is required for more proliferation.
- **Embryogenesis:** The process of formation of somatic embryos from the callus is called embryogenesis.
- **Organogenesis:** The process of differentiation of shoot and root from the somatic embryos is called organogenesis.
- **Acclimatization or Hardening:** The delicate laboratory plants should be hardened by modifying the laboratory environment and then are transferred to the main field.

Advantages of Micropropagation

- Large scale multiplication in limited time and space.
- Production of virus free plants.
- Plants can be produced throughout the year.
- It helps in reducing the breeding programme cycle.
- Production of true to the type plants.

Disadvantages of Micropropagation

- The infrastructure and materials required are very expensive.
- Technical skills are required to carry out the procedure.
- Rapid multiplication of pathogens once appeared.
- Raising laboratory grown plants in the main field is difficult.

Micropropagation in Bamboo

Micro-propagation is one of the alternatives to traditional vegetative propagation methods to increase a huge number of true-to-type propagules in a very short period of time in a small space. It guarantees the availability of better planting materials. This assists in the preservation of bamboo germplasm. It is the only reliable method for the propagation of safe plant types on a mass scale. It helps to grow disease and pest-free planting materials during the year.

Micropropagation techniques followed in Bamboo

- Juvenile (Zygotic embryo, seed or seedling).
- Mature (nodal buds) tissues.
- Somatic embryogenesis.

Juvenile (Zygotic Embryo, Seed or Seedling)

As a new generation, micro-propagating seedlings are necessary and easier to multiply in vitro, but their drawbacks are inadequate genetic background information, reduced seed availability and lack of germination potential, etc.

Micropropagation from Juvenile Explants

In vitro germination and direct shoot organogenesis from seeds

- Seeds are harvested from flowering spikes.
- The husk of the seeds should be removed and washed thoroughly with 0.1% w/v Exalin (Merck, India) detergent solution for 10 min.
- Surface sterilization of seed with 20% (v/v) of sodium hypochlorite (NaOCl) for 20 min.
- The seeds should be placed in test tubes containing MS media.
- The pH of the medium was adjusted with 1 N NaOH and 1 N HCl to 5.7±0 before the addition of 0.8% agar (Hi-Media).
- All culture tubes containing media were autoclaved at 121 °C for 20 min.
- A temperature of 25±2 °C should be maintained with a 16 h photoperiod at 90-95 µmol mG secG provided by cool 2 1 white fluorescent lamps.
- All media were supplemented with different concentrations of nutrients, and PGRs (BAP, Kn and GA, 0.1, 0.2, 0.3, 0.5, 1.5 and 3.0 mg LG).
- Filled endosperm and dark colored seeds without wrinkles fully matured aseptic seeds should be selected subcultured for callus induction.
- *In vitro* raised shoots (1.5-2.5 cm) should be harvested for rooting experiments and be cultured on half-strength MS medium supplemented with IBA or NAA.
- The rooted plantlets should be washed with sterilized water.
- The cleaned plantlets should be transferred to a bottle containing sand and soil at a ratio of 1:1 for acclimatization or hardening.
- The plantlets should be transferred to pots after one month

and left open in greenhouse (Barpanda *et al.*, 2017).

Advantages

- Seedlings are a new generation.
- An easy technique for in vitro multiplication.

Disadvantages

- Poor seed availability.
- No proper knowledge of the genetic background of the explants.
- Poor germination capacity.

Micropropagation from Mature Tissue Explants

A desirable alternative for large-scale bamboo propagation would be the *in vitro* method of propagation using explants from selected mature plants. The use of nodal explants resulted in a plant true to form. Because of reused food materials, the nodal segments are regarded as more efficient explants for *in vitro* culture.

Steps Involved in Nodal Propagation (Nodes Bear Axillary Buds):

- The collection of nodal explants usually February to March is a good period for obtaining axillary buds for cultural development.
- Any part of the nodal segment can be selected other than top and base nodes.
- The explants should be surface sterilized with 70% ethanol for the 30s to 1 min.
- Washing in 4-6 drops of detergent (Tween 20/ Tween 80) for 30 mins to reduce the rate of contamination.
- 0.1% Mercuric Chloride is the best surface sterilant.
- Explants should be cultured in MS media specifically liquid media for faster results and facilitates less time for hardening.
- Natural and synthetics phytohormones (6-Benzyl aminopurine (BAP), 6-Benzyl adenine (BA), should be used in tissue culture for shoot proliferation and IBA (Indole 3-Butyric Acid) is mostly preferred for root proliferation.
- The transfer of in vitro propagated plantlets from Lab to land is the most important step where a ratio of 1:1:1 Sand: Farmyard manure: Soil respectively should be used for hardening or Acclimatization.
- Splitting of rooted tillers to enhance the rate of multiplication of in vitro raised plants is known as macroproliferation.

Advantages

- True to type plant production.
- Food materials present in mature parts makes them more effective.
- Presence of highly active meristematic tissue.

- Reduces the chance of somaclonal variation.

Disadvantages

- Endogenous contamination.
- Instability of multiplication rates.
- Requirement of high skills.

Somatic Embryogenesis

Somatic embryogenesis is a mechanism where a single somatic cell is produced from a plant or embryo. Without any seed coat and endosperm, embryos are formed from ordinary plant tissue. Explant selection is the most critical aspect of somatic embryogenesis (Rout and Khare, 2018).

Steps Involved in Somatic Embryogenesis (Mature Plant)

- The equipment and the laboratory should sterilize thoroughly before cultivation.
- Three months before aseptic culture, the mother plant is selected and pruned regularly. Newly sprouted axillary shoots of 4–6 in should be selected (Cheah *et al.*, 2011).
- Soak the plant materials in 70% alcohol for 5 minutes and wash them 3 times with deionized water.
- The planting material should be placed in MS media with vitamins, phytohormones, etc.
- Culture the explants under 16/8-h light/dark photoperiod (cool white 40W fluorescent lamp) with an average temperature of 25-27 °C.
- Subculturing should be done three months before embryo induction seems to increase the ability of the cells to induce embryogenic calli.
- The elongated roots should be cut transversely and subcultured on the media which leads to the formation of white callus.
- The embryogenic calli should be separated from the stem every week and should be subcultured until proliferating embryogenic calli were produced which ultimately leads to the development of somatic embryo.
- The somatic embryos should be further converted to artificial seeds which are further germinated into complete plantlets.
- Plants are transferred into the greenhouse for acclimatization.

Problems

- A high rate of contamination during the initial establishment stage that is browning due to the exudation of phenols in controlled conditions is a major problem observed in bamboo.
- High rate of plant loss when transferred to natural or *ex vitro* conditions.
- The use of the technology is limited until better juvenile-mature correlation is established or molecular markers can be used to carry out selection for useful traits at the seedling stage. Proper knowledge and skill.

- High cost of production.

Future Prospects

Bamboo is a crop that is versatile. Because of its growing demand in the global market for its low cost sophisticated goods, which eventually began a million dollar market, historically used as low-cost building material (poor man's timber). A 100 percent Centrally Funded Scheme called the Mission for Integrated Horticulture Production (MIDH) is being implemented by the Department of Agriculture & Cooperation (DAC), Ministry of Agriculture & Farmers Welfare, under which the National Bamboo Mission (NBM) is being implemented as a sub-scheme in the North Eastern region and states like Madhya Pradesh, Maharashtra, Chhattisgarh, Odisha, Karnataka. In multiplying the commodity, tissue culture or micropropagation plays an important role in meeting its growing demand. Biotic and abiotic stresses can be regulated by genetically modified plants.

Conclusion

The study concluded that there is a strong bamboo tradition in Asian countries. Recent work in the tissue culture of bamboos has enhanced future development.

Two essential aspects of micro-propagated bamboo species are hardening and acclimatisation. In order to characterise *in vitro* plant quality and to estimate the establishment of plants accurately and economically, it is necessary to define the plant quality level. In addition, to ensure the output of the bamboo plants and the genetic fidelity of the clonal planting stocks, an effective quality control policy is required. Further research on bamboo should be carried out to establish training centres and programmes for the benefit of farmers and growers, in order to enhance their skills.

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