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APPROACHES OF IN VITRO CULTIVATION OF BAMBUSA VULGARIS

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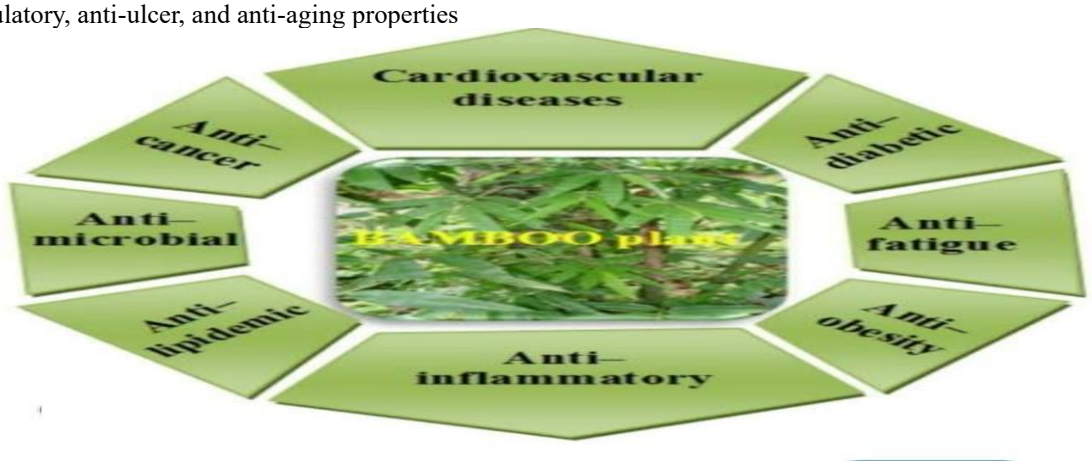
ABSTRACT
Bamboo species are a valuable source of renewable raw material, with Bambusa vulgaris being an economically significant species. However, there are certain constraints to large-scale cloning of adult-selected genotypes. This study aims to investigate the in vitro cloning of Bambusa vulgaris in several culture systems. Compared to in vitro approaches, vegetative multiplication through separation of culm, rhizome, and rooting of stem cuttings is rather slow. Bambusa vulgaris, or common bamboo, is grown in vitro, or in a controlled laboratory environment, using plant tissue or cells. This review discusses a few methods for cultivating Bambusa vulgaris in vitro.
KEY WORDS: *Bambusa Vulgaris, micropropagation, Somatic embryogenesis, in vitro seed germination, shoot tip culture, callus induction.*

INTRODUCTION

Common bamboo, or Bambusa vulgaris, is a natural species of bamboo found in South-east Asia. It is one of the bamboo species that is grown and distributed the most throughout the world. Because of its ability to outcompete local plants and its quick development, Bambusa vulgaris is regarded as an invasive species in some areas.

Bamboo is a stem, sometimes referred to as a culm, with the majority of its woody content found in the upper ground portion. It also features a cylindrical, hollow, and straight structure made up of internodes and nodes. The diameter decreases as the thickness of the culm wall increases from the bottom to the top.

Bamboo has been utilized in traditional medicine for ages, primarily in Asia. Several bamboo species possess pharmacological characteristics, including, Anti-inflammatory, anti-microbial, antioxidant, cardioprotective, neuroprotective, anti-diabetic, immunomodulatory, anti-ulcer, and anti-aging properties



[1]

Cultivation for Bambusa vulgaris demands careful consideration of environment and soil.
Maintenance includes temperature, suitable humidity, soil pH, and proper propagation method.
In vitro culture of Bambusa vulgaris includes cultivating bamboo tissues or cells in a controlled laboratory environment. In vitro cultivation procedures include seed sterilisation and germination, node culture, somatic embryogenesis, suspension culture, and so forth.

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[2]

An approximate estimate of 9.5 million tonnes of bamboo is produced in India each year, of which 4.5 million tonnes are used to make paper. The versatile species of bamboo, which has significant economic potential, is rapidly disappearing due to indiscriminate and unplanned harvesting. There is still much to learn about the unusual gregarious and monocarpic flowering habit that is followed by the death of the mother clump and all of its offspring. The traditional method of propagating seeds is unreliable because of the seeds' quick viability loss, infection, and damage from pests and diseases during storage in warm, humid climates, as well as the unpredictable flowering cycle that might potentially yield techniques by means of culm, rhizome, roots, or stem cuttings is rather low. Research has always been done on the propagation of bamboo using both traditional and tissue culture methods. Regeneration of plantlets through somatic embryogenesis A variety of explants were used to observe different species. Various stages of somatic embryo development have also been documented in vitro flowering in various bamboo species. Histological and biochemical investigations have been used to determine the time of embryogenic calli development, embryoid formation, gemination of somatic embryos, and flowering in in vitro grown shoots. This article provides an overview of micropropagation.



[3]

- ***Disinfection of Mature Explanted***

mature explants infested with disease Because of internal contaminations in bamboo, it can be challenging to establish aseptic cultures from mature explants; cultures may exhibit symptoms of contamination even after 8 weeks or after being cultured for extended periods of time. Fungal and bacterial contaminations could not be controlled by standard sterilisation techniques. Benlate and bavistin (50–500 ppm) were used with anti-fungal medicines such as tetracycline, chloromycetin, rifamycin, and streptopenicillin to treat the explants. About 50% of sterile cultures were obtained after treatment with benlate (100 ppm each)

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for 60 minutes, followed by HgC12 (0.1%). (Personal observation). After disinfection, contaminated cultures may occasionally be used in bavistin. For disinfection, several toxicides and fungicides were also employed.[4]

- ***Aseptic Culture Establishment from seed***

The majority of research on the micropropagation of different bamboo species comes from seed sources. Mature bamboo seeds were dehusked, rinsed in 0.1 % (v'.v) "Teepol" (Qualigen, India) detergent solution for IO minutes, and then rinsed with running tap water for 2O minutes. The samples were subjected to four thorough rinses in sterile double-distilled water after being surface sterilised for 25 milliseconds using a 0.2% (w/v) aqueous solution of mercuric chloride. The entire excised zootic seed was utilised as an experimental specimen._ Seeds were carefully inoculated into liquid MS medium supplemented with 2% (w/v) sucrose after being surface-stain-treated for ten minutes with either a 0.05% solution of mercuric chloride, chlorine water, or sodium hypochloride.Typically, a 0.1-0.5% (w/v) aqueous solution of mercuric chloride or 5- 10% sodium hypochloride could be used to disinfest the majority of explants from seeds and seedling sources for IO to 20 minutes. This washed down with many rinses (2–6) using sterile distilled water. Without the use of growth regulators, seeds were cultivated on Skoog 25 (MS) and basal Murashige medium. After gelling the medium with 0.8% (w/v) agar (BDH,England), 20 ml of the molten medium was poured into each 25 x 50 mm culture tube that had been filled with non-absorbent cotton and covered with a single layer of cheese cloth. For 15 minutes, the media were steam sterilised at 104 kPa. For germination, the cultures were maintained in the dark at 25 ± 2°C. 4

1. Micro propagation of Bambusa vulgaris using internode explant

The preservation of natural behaviours is necessary for the conservation of bamboo variety, and production through ex-situ conservation is currently not a feasible alternative [6-7]. Although the macro proliferation strategy for propagation is a significant advancement, the need for seeds remains a constraint. As a result, contemporary conservation techniques like micropropagation offer a substitute for the quick regrowth of new plants in species like bamboo.

Using a sterile blade, the inter-nodal area of the stem (Bambusa vulgaris Schrad. ex Wendl) was chopped up to three inches. To get rid of the wax and dust, the top layers of the explant were wiped off. After that, the internode explant was cleaned for ten minutes under running tap water. The explant was submerged in fungicide (Bavistin 1%) for 10 minutes before being rinsed with sterile distilled water twice or three times. The explant was further cleansed with distilled water containing 1% of detergent for five minutes. Following a one-minute surface disinfection with 70% ethanol, the explants were treated with 0.1% aqueous mercuric chloride (HgCl2) for five minutes, and they were then thoroughly washed four to five times with sterile distilled water under aseptic condition

- ***Prepration of MS Media***

Getting MS Ready Media Growth conditions and the medium of culture for this investigation, MS (Murashige and Skoog 1962) medium containing 2% (w/v) sucrose was utilized. BAP (0.3 mg/L) was added to the medium along with 3 mg/l of NAA, 2,4-D, and IAA, respectively. Before the medium gelled with 1% agar, the pH was brought to 5.6. The prepared media (Hi-media, Qualigens, and SD fine chemicals, India) were the chemicals employed in this investigation. Each of the 50 ml of Murashige and Skoog was poured into a 150 ml sterilized conical flask (Borosil) and sealed with a cotton plug that wasn't absorbent.

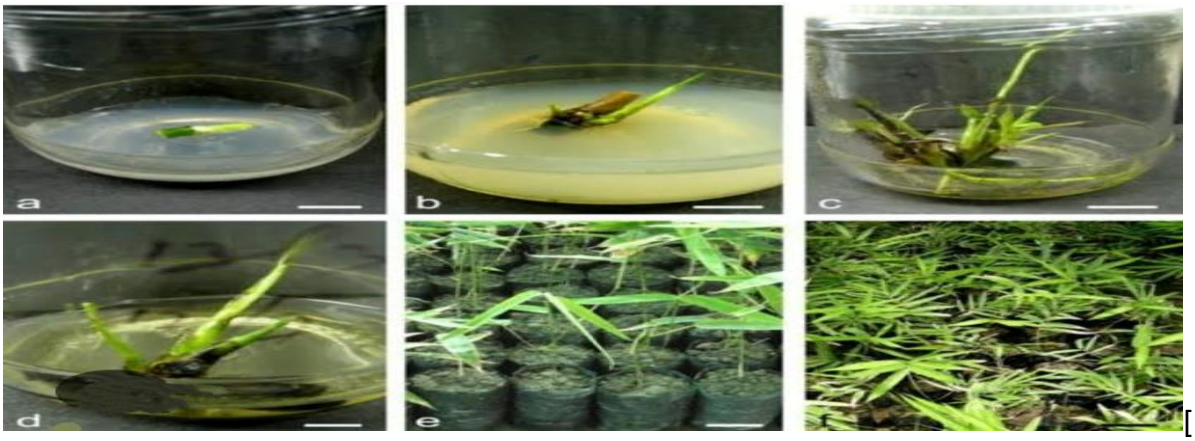
- ***Storage of Prepared Media***

Keeping Prepared Media Stored Following preparation, the media were autoclaved, allowed to come to room temperature, and then kept in a 6°C refrigerator. The quantity of cultural media utilized in the Culture Jar Each conical flask contained 20 cc of semi-solid culture media for the typical propagation plantlet regeneration experiment.

- ***Establishment of Shoot***

Culture MS media containing and without 0.1% activated charcoal was used to cultivate surface-sterilized immature and semi-hard wood shoots. The explants that survived were then moved to regeneration media. Over the course of four weeks, the percentages of browning and survivals, as well as the quantity of shoot buds begun, new leaves developed, and callus formation, were noted. After that, the cultivated explants were kept at 25 ± 20C in the plant tissue culture environment with cool white fluorescent lamps providing a 16-hour photoperiod. There was 50–55% relative humidity.[5]

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[6]

Table 1. Culture condition required for *in vitro* cultivation of *Bambusa vulgaris* Schrad. ex Wendl

Ex–plant	Temp.	Moisture	Light period	Time of regenretion
Inter node	25± 2	50-55	16 hours	3 Week

[5]

2.Somatic Embryogenesis

In callus cultures produced from nodal explants of in vitro-grown seedlings and excised mature zygotic embryos of bamboo species, plant regeneration by somatic embryogenesis was accomplished. Numerous research on in vitro shoot multiplication using various explants have been conducted. Plant regeneration and somatic embryogenesis from Bambusa zygotic embryo explants

- ***Material and Methods***

Plant matter and processing of explants. Mature Bambusa vulgaris seeds were gathered from trial crops. After the seeds were dehusked, they were rinsed for ten rains in a 0.1% (v/v) detergent solution called "Teepol" from Qualigen, India, and then for twenty rains under running water. The seeds were thoroughly cleaned four times in sterile double distilled water after being surface sterilized for 25 rains using a 0.2% (w/v) aqueous solution of mercuric chloride. Seeds were cultivated on basic MS (Murashige and Skoog 1962) medium without growth regulators.

0.8% (w/v) agar (BDH, England) was used to gel the medium. Then, 20 ml of the molten media was poured into each 25 x 150 mm culture tube, which was then sealed with a single layer of cheesecloth and filled with non-absorbent cotton. The media were steam sterilized for 15 minutes at 104 kPa.7

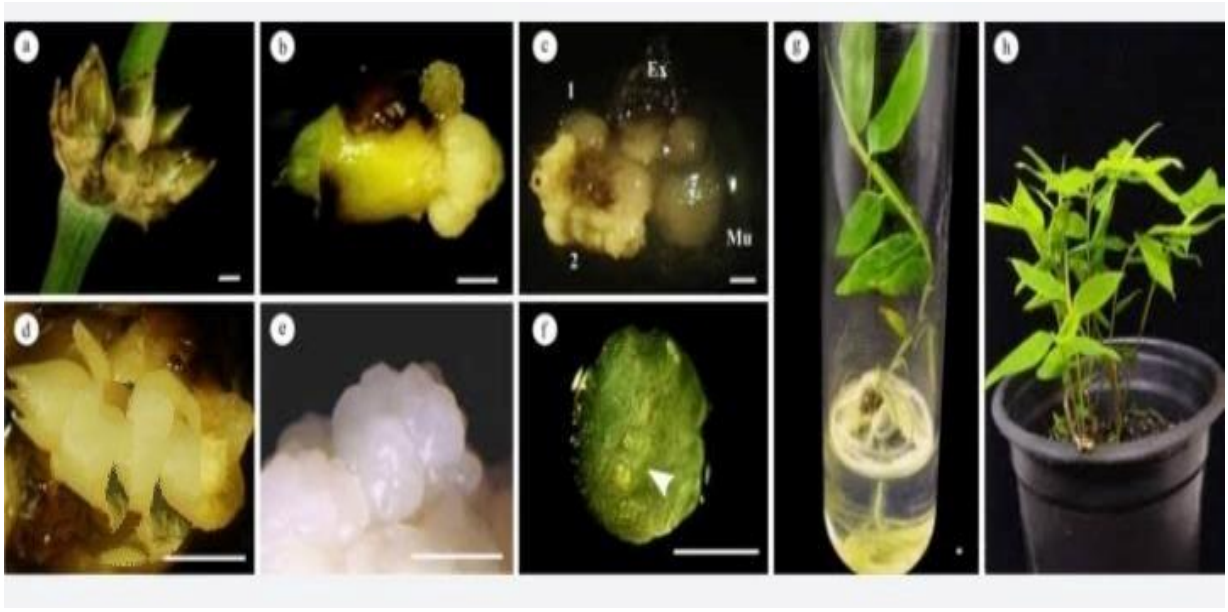
- ***Storage Condition***

Keeping prepared media stored following conditions, for germination, the cultures were stored in air-light containers under low or no light.7

- ***Establishment***

One node per nodal segment (1.0-1.5 cm) was measured. Semisolid MS media with different concentrations and combinations of Kn, BAP, NAA, IBA, and 2,4-D ranging from 0.0 - 3.0 mg/l was used to implant nodal tissue and excised zygotic embryo explants. Calli (about 200 mg + 20 mg) were routinely subculture every 4 weeks on fresh callusing medium or regeneration medium (1/2 MS + 0.5 mg/1Kn + 10 rag/1 Ads + 2.0 mg/l 2,4-D). Callus was routinely initiated from nodal explants and excised zygotic embryos on MS +0.25 mg/l Kn + 3.0 mg/12,4-D in species. Before being autoclaved and poured into culture tubes, all media were brought to a pH of 5.7 using either 0.1N NaOH or 0. I N HC1. 7

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[8]

3. In Vitro Seed Germination of Bamboo

There is a lot of variances in bamboo's ability to stay viable and dormant in soil, and its demographic traits are still mostly understood when it comes to seed regeneration. Due to the lengthy intermast time, it would be challenging to store a lot of seeds for large-scale planting until the following flowering cycle is repeated. Since endogenous levels of auxins and abscisic acid (ABA) in seeds have been discovered to be one of the key variables related to the reduction in seed viability in stored bamboo seeds, seed viability is often quite poor in bamboos. Bamboo seeds have a brief lifespan; they can germinate in three to seven days, lose viability in one to two months, and their ability to do so varies with the season.[9]

- ***Material and Methods***

Procedures vegetation Caryopses, which are single-seeded propagation units of Poaceae, are formed when the test is united with a thin pericarp. For this experiment, seeds were purchased. The seeds were ageing; they were more than six months old. The seeds are 5-7.5 mm long, broadly oval, dark brown in hue, and rounded at the base with a pointy end. The seeds were stored in the Institute's seed bank in addition to being germinated.[9]

- ***Culture Establishment***

The glumed seeds, or explants, were meticulously prepared by floating them in water. This allowed for the separation of debris and empty seeds. After the dehusked seeds were gently shaken for 45 minutes, they were treated with a broad-spectrum antibiotic, streptomycin sulphate, 0.25% (w/v), and bavin 0.25% (w/v) as an antifungal treatment. The seeds were rinsed in 0.01% (v/v) Tween 20 for 10 minutes. The last stages of surface sterilization were carried out in a laminar flow using 0.1% (w/v) HgCl2 and 15% sodium hypochlorite for ten minutes each. After every treatment, the items were repeatedly washed in autoclaved distilled water for a minimum of three times.[9]

- ***Germination***

Sterile MS media medium (pH 5.6–5.8) was used to inoculate the transplants. Different quantities and combinations of plant growth regulators, such as 6-benzylaminopurine (BAP) and kinetin (KN), were added to the medium. Depending upon the necessity for initiation media or proliferation media 2.0-3.0% (w/v) sucrose was used as a carbon source with 0.75% (w/v) agar. For every treatment, three duplicates containing ten seeds each were obtained.[9]

- ***Transplantation and Acclimatization***

To lessen the likelihood of fungal contamination, plantlets were treated with an aqueous solution of bavistin after being carefully cleaned with lukewarm water and a sable hair brush to remove any remnants of agar adhering to the roots. Plantlets were then moved to a potting mix consisting of sand, soil, and FYM: :(1:1:1), and they were housed for a week in a greenhouse in a polytunnel with low light intensity and high humidity. Watering them frequently and misting them with Hogland's solution every seven to ten days was considered proper care. Following each week, data on growth performance and survival % were recorded.[9]

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Fig. 2—(a) Morphology of seed showing rounded base with a pointed end, dark brown in colour about 5 mm long, (b) initiation of germination by emergence of radicle (R) about 2 mm long, (c) micropylar end showing emergence of radicle and plumule (P), (d) radicle showing numerous root hairs.

[9]

4. Shoot tip Culture of Bambusa Vulgaris

Small-scale production can benefit from the enormous amount of material required for propagation by culm cuttings (single, double, triple, and whole node cuttings), as the right stage of material is only available for a brief period of time. Furthermore, this approach works well for producing clonal planting material on a modest scale. Every bamboo plant has a node on its segmented axis that contains a bud or a branch, and the branches themselves have a bud in their axil. The goal of the little research on its vegetative propagation has been to develop as many of these buds as possible into plant material (Banik, 1994). Numerous bamboo species have been effectively vegetatively propagated by the use of adventitious rooting in culm/branch cuttings (Agnihotri et al.,2009) Clonal propagation through shoot proliferation from field grown mature nodal shoot explants have been described with different effectiveness in many bamboo species viz; 54 species from 15 genera of bamboo.

- M***aterials and Procedures***

Plants from the greenhouse were taken.

Plant Materials and Culture Medium

Young, healthy B. vulgeris nodes were surface-sterilized for one minute using 70% ethanol, then treated for fifteen minutes with a 0.2% sodium hypochlorite solution (v/v), and then rinsed five times with sterile distilled water. The seeds were solidified with 0.8% agar and cultivated in MS (Murashige and Skoog 1962) with vitamins supplied with 3% sucrose as a carbon source.[10]

Culture Conditions for the growth of roots and shoot regeneration:

Similar to this, about one week old nodes were positioned vertically on MS medium supplemented with varying doses of BAP (0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) + Kinetin for shoot induction. Healthy node explants were sectioned into 1 cm in length and inoculated into shoot induction medium. Cool white fluorescent light with a 16-hour photoperiod and an intensity of 40 εmol m-2 s-1 were used to incubate the cultures at 26 ± 0.5 °C. Every experiment was run in triplicate, with roughly 50 explants per treatment. An appropriate nutritional medium was standardized in order to compare the morphogenic response of internode explants towards varying concentrations of benzyl aminopurine (BAP) and kinetin. Following a 4-week incubation period under the same conditions,

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each explant was graded according to the quantity of responsive explants displaying shoot regeneration. Extended shoots measuring 2-4 cm were placed in MS medium that contained varying amounts of indole-3-butyric acid (IBA; 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) as well as 2.5 mg/l of charcoal to aid in rooting.[10]

Growth and Elongation of node

In synthetic media, node development and elongation are mostly dependent on the external supply of nutrients and plant growth regulators. The establishment of a precise tissue culture technique for effective in vitro organogenesis in bamboo made it possible to study the critical function that phytohormones play in plant regeneration.

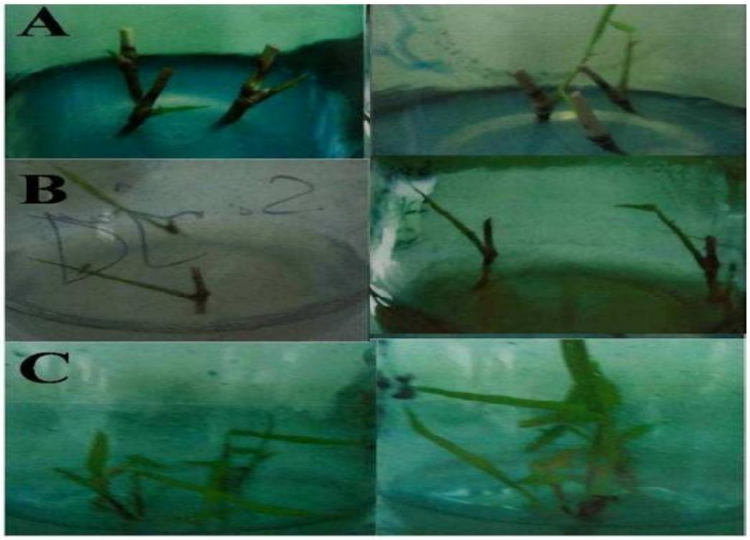


Fig. 1. *In vitro* propagation of bamboo shoot: [A] Shoot initiation in MS+BAP; [B] and [C] Shoot induction in MS+kinetin+BAP.

[10]

5. Callus Induction

- **Initiation of callus from seed source**

Callus initiation from the seed source There have been reports of callus induction indifferent ex plants produced from several different species of bamboo. After two weeks of inoculation, callus was started from excised mature zygotic embryos and nodal segments formed from 10-day-old in vitro-grown seedlings on MS basal media supplemented with different combinations of kinetin and 2,4-dichlorophenoxyacetic acid (2,4-D). Incubating cultures in the dark resulted in a higher callus proliferation rate. The ideal concentrations of 2, 4-D and Kn for inducing callus varied little between species. The callus was first creamy white, friable, and nodular. The rate of callus growth slowed down and the callus turned brownish as the auxin concentration increased.[11]

- ***Report and Conclusion***

As the country's economy grows swiftly and has a high potential, the demand for bamboo has accelerated the depletion of its rootstock. Bamboo has a high capacity for carbon sequestration and is used to mitigate climate change and environmental issues. Similarly, it provides an alternative source of forest. As a result, it plays a significant role in conservation biology and has emerged as a top priority. With understanding of conservation biology and the environment, individuals must meet massive market demands and utilize finite resources. Harvesting from resources necessitates the production of a significant number of bamboo plantlets via micropropagation in order to cover plant stock gaps. Different methods of production are explained in this review

This protocol's excellent rates of shoot multiplication, rooting, and plant survival in the field indicate its potential to fulfil the expanding demand for disease-free, high-quality plant material

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