CRUCIAL STAGES AND PROCESS FOR SUCCESSFUL BANANA MICROPROPAGATION THROUGH PLANT TISSUE CULTUTRE TECHNIQUES

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Banana is vegetatively propagated plant in which tissue culture is employed as a micropropagation technique. The apical shoot tip culture technique is capable to mass produce disease-free planting material for commercial propagation of banana seedlings. Healthy planting material serves as a major input for successful farming of year round crop. The success of tissue culture depends largely on the selection of mother suckers, in vitro culture and rooting and hardening of plants. The crucial stages for successful micropropagation are discussed in detail.

A. Stage I: Selection procedure

1. Selection of mother plant:

The selection of mother plants is a crucial step in plant breeding and propagation, whether for agricultural crops, horticultural plants, or ornamental species. Mother plants serve as initial material to represent genetic makeup for the production of respective offspring through plant tissue culture; ensuring desirable traits are passed on to the next generation. Here are the key considerations for the selection of mother plant for successful micropropagation:

a. Frequent Surveys:

- Conduct regular surveys in different geographic areas to identify potential mother plants.
- Assess the environmental conditions, climate, and soil types in these areas.

b. Health and Vigor:

- Choose mother plants that are healthy, vigorous, and show robust growth.
- Look for signs of nutrient deficiency, physical damage, or stress, as these can affect the quality of genetic material.

c. True-to-Type:

- Ensure that the selected mother plants are true to type, meaning they exhibit the expected characteristics of the desired variety.
- Avoid plants showing signs of genetic mutations or off-types.

d. Disease and Pest Resistance:

- Selecting healthy mother plants which aredevoid ofinfestations with diseases and pests, particularly viral diseases, which can have a significant impact on the offspring.
- Conduct regular screenings and tests for diseases, and eliminate any plants showing symptoms.

e. Vegetative and Fruiting Conditions:

- Evaluate the mother plants during both vegetative and fruiting stages to ensure consistency in trait expression.
- This helps to confirm that the desired traits are maintained throughout the plant's life cycle.

f. Management Conditions:

- Grow mother plants under optimal management conditions, including proper irrigation, fertilization, and pest control.
- Provide the necessary care to maximize plant health and productivity.

g. Yield and Productivity:

- Select mother plants with a history of better yield and productivity.
- High-yielding plants contribute to the propagation of offspring with similar performance.

h. Genetic Diversity:

- Consider the genetic diversity of the selected mother plants to maintain a healthy and adaptable population.
- Avoid excessive reliance on a limited number of individuals to prevent inbreeding depression.

i. Documentation:

- Maintain detailed records of the selected mother plants, including their origin, growth conditions, and any observed traits.
- Documentation aids in tracking the clonal performance and helps in making informed breeding decisions.

j. Continuous Monitoring:

- Regularly monitor the health and performance of mother plants even after selection.
- Replace or update mother plants if any decline in health or genetic purity is observed over time.

The meticulous selection of mother plants is fundamental to successful plant breeding programs. By prioritizing health, true-to-type characteristics, and environmental conditions, would assist the breeders for ensured production of high-quality offspring’s with desirable traits. Regular monitoring and management practices contribute to the sustained success of a breeding program.

2. Selection of plant material:

The selection of appropriate plant material is a crucial step in initiating in vitro culture, especially for crops like bananas where vegetative propagation is common. In banana cultivation, sword suckers are preferred over watery suckers for in vitro culture due to their superior vigor and potential for higher yield. Here's a detailed explanation of why sword suckers are chosen:

a. Definition of Sword Suckers: Sword suckers are a type of shoot or sucker emerging from the base of the banana plant. They are characterized by narrow, sword-shaped initial leaves.

b. Vigorous Growth: Sword suckers exhibit superior vigor compared to watery suckers.Vigorous growth is essential for successful in vitro culture, as it ensures the rapid multiplication of plant material in the laboratory.

c. Attachment to Healthy, Fruiting Mother Plant: Sword suckers are typically attached to a healthy, fruiting mother plant.Being connected to a fruiting plant indicates that the mother plant is mature and has been producing healthy offspring, suggesting good genetic traits.

d. Uniformity in Growth: Sword suckers tend to have uniform and consistent growth characteristics.Uniformity is important for in vitro culture as it simplifies the standardization of culture conditions and ensures consistent results.

e. Early Differentiation: Sword suckers often show early differentiation of shoot and root primordia.This early differentiation is advantageous for in vitro culture, where the goal is to induce the development of shoots and roots in a controlled environment.

f. Reduced Risk of Contamination: Sword suckers, being more compact and less watery, are less prone to contamination issues compared to watery suckers.Contamination can be a significant challenge in in vitro culture, and selecting less susceptible plant material helps in overcoming this issue.

g. Potential for Higher Yield: Sword suckers, when cultured in vitro and subsequently transferred to the field, have the potential for higher yield.The superior vigor and early differentiation observed in sword suckers contribute to their ability to produce healthy and productive plants.

h. Conservation of Desirable Traits: By selecting sword suckers from a healthy, fruiting mother plant, breeders aim to conserve and propagate desirable traits such as disease resistance, high yield, and uniformity.

i. Adaptability to In Vitro Conditions: Sword suckers are often more adaptable to in vitro conditions, facilitating successful initiation and proliferation in culture media.

The preference for sword suckers over watery suckers in banana in vitro culture is based on their superior vigor, attachment to healthy mother plants, uniform growth, reduced contamination risk, and the potential for higher yield. These characteristics make sword suckers a preferred choice for initiating successful in vitro propagation of bananas, ultimately contributing to the improvement and multiplication of desirable banana varieties.

3. Virus indexing:

Virus indexing is a critical aspect of plant propagation, especially in the context of micropropagation and the maintenance of mother plants. The primary purpose of virus indexing is to detect and eliminate plants infected with specific viruses. In the case of banana plants, four important viruses to be primarily monitored are Banana Bunchy Top Virus (BBTV), Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBrMV), and Cucumber Mosaic Virus (CMV). Here is a detailed explanation of the process and actions taken if infection is detected:

a.Indexing:

Definition: Virus indexing involves testing plant material for the presence of specific viruses.

Process: Samples from the mother plants and micropropagated plants are collected and subjected to laboratory tests, such as ELISA (Enzyme-Linked Immunosorbent Assay) or PCR (Polymerase Chain Reaction), to detect the presence of BBTV, BSV, BBrMV, and CMV.

b. Targeted Viruses:

- **BBTV (Banana Bunchy Top Virus):** Causes severe stunting and deformation of banana plants.
- **BSV (Banana Streak Virus):** Associated with streaking symptoms on banana leaves and affects fruit development.
- **BBrMV (Banana Bract Mosaic Virus):** Causes mosaic symptoms on bracts, affecting the appearance of the inflorescence.
- **CMV (Cucumber Mosaic Virus):** Affects a wide range of plants, including bananas, leading to mosaic symptoms.
- **Detection and Identification:** Laboratory tests help identify the presence of these viruses in the plant samples.Early detection is crucial to prevent the spread of viruses and the establishment of infected plants in the field.

c. Action if Infected:

- **Isolation:** If any of the tested plants are found to be infected, immediate isolation is essential to prevent further spread.
- **Removal of Entire Clump:** In the case of infection, it is recommended to remove the entire clump of the infected plant. This includes not only the above-ground suckers but also the underground mother corm.
- **Destruction:** The removed clump, comprising both above-ground and underground parts, should be destroyed to ensure the complete eradication of the infected plant material.
- **Biosecurity Measures:** Implement strict biosecurity measures to prevent the accidental spread of viruses to other plants or areas.

d. Preventive Measures:

- **Regular Monitoring:** Continuously monitor the health of mother plants and micropropagated plants for signs of viral infection.
- **Quarantine:** Introduce a quarantine period for newly introduced plants to observe and test for potential viral infections before integrating them into the production system.

- **Sanitization:** Practice proper sanitation measures in the greenhouse or propagation facility to minimize the risk of virus transmission.
- **Documentation:** Maintain detailed records of virus indexing results and actions taken for each plant.Documentation aids in tracking the health status of individual plants and helps in making informed decisions for future propagation.

Virus indexing is an essential component of plant health management, especially in the context of micropropagation and maintaining healthy mother plants. Rapid detection and strict actions, such as the removal and destruction of infected plants, are crucial to prevent the spread of viruses and ensure the production of disease-free plant material for further propagation and cultivation.

B. Stage II: Initiation

1. Culture initiation and proliferation

The process described involves the careful collection, preparation, and initiation of sword suckers for in vitro culture. This method aims to ensure the cleanliness and sterility of the explants, providing an ideal environment for the growth and development of shoots in a controlled laboratory setting. Here's a detailed explanation of each step:

a. Collection of Sword Suckers:

- **Careful Harvesting:** Collect sword suckers from the field with care to avoid injury to the growing meristem part, which is crucial for subsequent successful culture.

b. Cleaning the Suckers:

- **Rinsing with Running Water:** Wash the collected sword suckers in running tap water to remove soil debris and other contaminants. This step helps in ensuring a clean starting material for the in vitro culture.

c. Preparation of Explants:

- **Exposing Shoot Tip Region:** Cut the sword suckers to expose the shoot tip region. This is typically the actively growing part of the plant.

d. Surface Sterilization:

- **Fungicide Treatment:** Treat the explants with a fungicide to eliminate any fungal contaminants that may be present on the surface.
- **Sodium Hypochlorite Solution:** Submerge the explants in a 4% sodium hypochlorite solution. Sodium hypochlorite acts as a broad-spectrum disinfectant, effectively eliminating bacteria, fungi, and other surface contaminants.

e. Washing and Outer Surface Removal:

- **Wash with Water:** After the sodium hypochlorite treatment, wash the explants thoroughly with sterile water to remove any residual disinfectant.
- **Remove Outer Surface:** Carefully remove the outer surface of the explants, which may still harbor contaminants. This outer layer is often removed to expose cleaner tissue for initiation.

f. Inoculation into Medium:

Sterile Conditions: Carry out all these steps under sterile conditions to avoid contamination.

- **Initiation Medium:** Inoculate the shoot tips into a sterile initiation medium. This medium typically contains nutrients, vitamins, and growth regulators to support the initiation and development of shoots.
- **Container:** Place the explants in a 250 ml glass jar container containing 30 ml of the initiation medium.

g. Incubation Conditions:

- **Optimal Temperature:** Maintain an optimum incubation temperature in the range of 24-26 °C. This temperature range is suitable for the initiation and growth of shoots in vitro.
- **Dark Conditions:** Culture the explants under dark conditions during the initiation phase. This is often done to encourage shoot development before exposure to light.

h. Monitoring and Subsequent Steps:

- **Observation:** Regularly monitor the explants for signs of shoot initiation and growth.
- **Transfer to Growth Conditions:** Once shoots have developed, the culture can be transferred to conditions with a photoperiod (light and dark cycles) suitable for further growth and development.

This detailed process ensures the careful collection, cleaning, and initiation of sword suckers for in vitro culture. The use of sterile conditions, surface sterilization, and controlled incubation parameters are critical for the success of the culture, minimizing the risk of contamination and promoting the healthy growth of shoots in a laboratory setting.

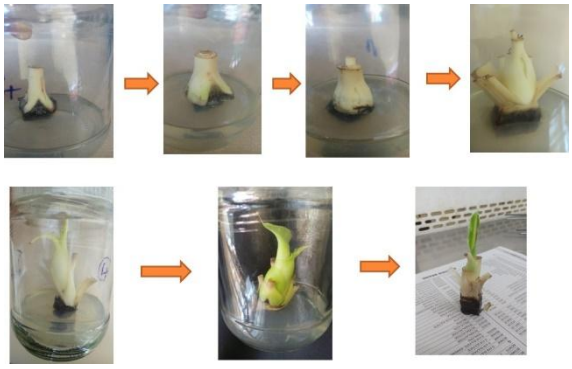

Fig 1: Steps in culture initiation

C. Stage III: Multiplication:

After the initial subculture, conducted 20-25 days post-initiation, cultures are meticulously examined for contamination. Bacterial contamination symptoms typically manifest within 3 to 6 days, while fungal contamination may become apparent after one week. Observable signs include alterations in medium color, texture, or the emergence of visible colonies within a week or one month.

The subculturing process involves the elimination of dead tissue from the base of the explant. Few leaf bases are carefully peeled until the meristematic tip is exposed. The apical meristem is excisedinto two parts and inoculated into fresh regeneration medium.The number of axillary buds developed during the first and second subcultures varies from 1 to 5, depending on the genome of the variety. Clusters of buds begin to develop after the third to fourth subculture. In subsequent subcultures, explants are divided into four pieces and inoculated into individual culture bottles. The cycle is repeated at 25 days intervals to enhance the rateof proliferation. At the end of fifth subcultures, a single clump typically encompasses 15-25 proliferating shoots.

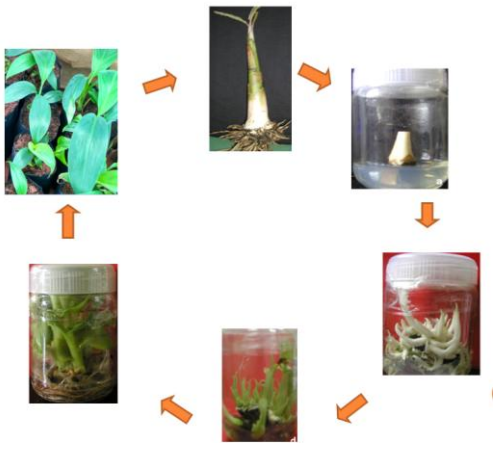

Fig. 2: Micropropagation of banana using apical meristem as explants: (a) Sword sucker (b) Inoculated banana shoot tip (c) multiplication of shoot tip after inoculation (d) Axillary shoot tips proliferated on regeneration medium. (e) Plantlets on rooting medium (f) hardened plants

D. Stage IV: Rooting:

After seven subcultures, the proliferated buds are transferred to a rooting medium with reduced concentrations of IAA or BAP. These plantlets are then maintained under a 16-hour light/8-hour dark cycle. Approximately 45 days later, the rooted plantlets are prepared for the hardening process. To minimize somaclonal variation, subculturing is limited to a maximum of seven cycles.

E. Stage V: Hardening

1. Primary hardening

The well rooted plants are exposed to acclimatization process to facilitate adaptation to the greenhouse and subsequently to field conditions. During the hardening phase, the plantlets undergo physiological changes to cope with varying external factors such as temperature, water, relative humidity and nutrient availability.

In the initial stage of hardening, well-rooted plants are carefully selected, and their roots are gently washed with water to remove adhered agar, which can encourage microorganism growth due to the presence of sucrose in agar. The media for primary hardening is cocopeat and/orSoilrite with fine sand in equal proportions. NPK (nitrogen, phosphorus, and potassium) is supplied in liquid form on a weekly basis.

To ensure successful hardening, the hardened plants should be placed in optimal conditions, maintaining a temperature of 24-26 °C and humidity levels exceeding 80% for a duration of 4-6 weeks.

2. Secondary hardening

Following 5-6 weeks of primary hardening, plantlets undergo a transition from portrays to polybags. The base substrate typically comprises soil and sand, supplemented with cost-effective materials like coir pith, sawdust, or rice husk. Prior to the transfer to polybags, plantlets are immersed in a fungicide solution (0.1% bavistin) and then planted in polybags filled with a suitable substrate.

Initially, these polybag-contained plants are kept under low light intensity shade nets with a relative humidity of 70%. The hardening process involves a gradual increase in light intensity and a reduction in relative humidity to 40%. After 5-6 weeks, the plants are deemed ready for field planting, possessing 3-5 well-developed leaves and a substantial mass of fibrous roots.

Throughout both primary and secondary hardening phases, regular monitoring is essential, with the removal of variants at weekly intervals. Variants may exhibit vegetative deformities such as dwarfism, leaf variegation, or rosette foliage.

3. Testing for genetic fidelity and virus indexing of tissue culture plants

Ensuring the production of high-quality, disease-free planting material through tissue culture technology requires the implementation of vital processes such as indexing of virus and fidelity testing, particularly crucial for crops like banana that are susceptible to various viruses. Among the threats to banana crops are viruses like banana streak virus, banana bunchy top virus, cucumber mosaic virus, and banana bract mosaic virus. These viral infections can significantly diminish crop quality, making it imperative to assess tissue culture plants for the presence or absence of these viruses and obtain certification confirming their virus-free status, a procedure known as virus indexing.

Virus indexing involves the systematic screening of tissue culture plants to identify any viral contamination. This is typically carried out through various diagnostic techniques, such as polymerase chain reaction (PCR) assays, enzyme-linked immunosorbent assays (ELISA), or other molecular biology methods specific to the targeted viruses. By conducting comprehensive virus indexing, the aim is to certify that the tissue culture plants are devoid of harmful viruses, ensuring the subsequent production of healthy and disease-resistant banana crops.

In addition to virus indexing, genetic fidelity testing is another critical step in tissue culture technology. This testing is performed to confirm that the plantlets generated through tissue culture are genetically identical to the mother plant, ensuring that the desirable traits of the original plant are preserved. This process is crucial for maintaining the genetic purity and uniformity of the propagated plants.

Genetic fidelity testing involves the use of molecular markers and DNA-based techniques to compare the genetic profile of the tissue culture-derived plants with that of the mother plant. Techniques such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), or Amplified Fragment Length Polymorphism (AFLP) are commonly employed for this purpose. The goal is to identify any genetic variations or mutations that may have occurred during the tissue culture process, thereby ensuring the true-to-type nature of the propagated plantlets.

By combining virus indexing and genetic fidelity testing, producers can confidently certify the tissue culture-derived banana plants as both virus-free and genetically identical to the mother plant. This comprehensive approach not only safeguards the crops against viral infections but also maintains the integrity of desirable traits, ensuring the production of high-quality, consistent, and true-to-type planting material for banana cultivation.

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