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Banana (Musa spp.)- It's Multiplication Through Tissue Culture

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Introduction

Tissue culture means cloning and micro-propagation of tissues of the selected Elite plants and daughter suckers. The process consists of five crucial steps: Initiation, Multiplication, Shooting and rooting, Primary Hardening in greenhouses and Secondary Hardening in shade houses. Strict adherence to aseptic standards and micro-climatic conditions and care during the hardening process alone can ensure success. Banana (Musa spp.) is India's major fruit crop and production of different commercial varieties and it is the most popular crop being multiplied through tissue culture, accounting for more than 45% of the total tissue culture production in the country. A large number of varieties are grown in India, of which only a few are commercially multiplied under *in vitro* conditions.

Varieties of Banana

Dwarf Cavendish, Robusta, Rasthali, Poovan, Monthan, Elakkibale, G-9 would be the major variety followed by Elakki and then Nanjanagudu Rasabale. The rationale behind this is based on the demand for these varieties in the market. Currently, G-9 has the highest demand, especially in Maharashtra and Karnataka.

Preparation of explants

The sword suckers of 2-3 months are removed from healthy disease-free mother plants for shoot tip culture. The suckers are cut to expose the shoot tip of 10 cm^3, washed in running tap water for 10 min., and cut further to about 3 cm diameter and 5 cm length. The explant should be carefully cut to avoid injury to the growing meristem. They are then soaked in Bavistin solution (4%) for 18 hours to remove all fungal contaminants and washed with tap water for 5 min. explants are then soaked in a

mild detergent solution (Tween 20/ Active 80) for six hours. After washing them in tap water, they are taken to LAF for further surface sterilization.

Surface sterilization

In the LAF (Laminar Air Flow) chamber, the prepared explants are sterilized using mercuric chloride (0.01%) for 15 to 20 minutes with continuous agitation. Surface sterilized shoot tips are washed three times (10 min. each) using sterile water. The outer surface of the explant exposed to a sterilizing agent is removed and the explants are trimmed using a surgical blade.

Culture Initiation

The block of tissue measuring 1.5 x 1.5 x 1.0 cm is excised using a sterilized scalpel and forceps and inoculated onto initiation medium (MS media supplemented with 30 g/l sugar, 4.5 g/l agar, 1.5 mg/l BAP 1.5 and 20 mg/l Adenine Sulphate). Bottles, after initiation, are transferred to the incubation room maintained at 24 ± 2°C temperature and 16: 8 hours light: dark cycle

Banana tissue cultures often suffer from excessive blackening caused by the oxidation of polyphenolic compounds released from wounded tissues. These undesirable exudates form a barrier around the tissue, preventing nutrient uptake and hindering growth. Therefore, during the first 4-6 weeks, fresh shoot tips are transferred to a new medium every 1-2 weeks. Alternatively, freshly initiated cultures can be kept in complete darkness for one-week Antioxidants, such as ascorbic acid or citric acid in concentrations ranging from 10-50 mg/l, are added to the growth medium to reduce blackening.

Shoot multiplication

As a response to the growth regulators treatment, the explants start turning green, two weeks after culture. Further, explants will start swelling forming a light callus at the base. At this stage, the first subculture is done by removing one or two outer layers of explants and giving two to four vertical cuts to the explants. During subsequent cultures (at 4 weeks intervals), multiple shoots arise from the base of the explant. These shoots are cut at the base, separated and placed in a fresh MS medium supplemented with sugar (30 g/l), agar (4.5 g/l), BAP (2 mg/l), NAA (0.5 mg/l) and Adenine Sulphate (20 mg/l). The multiplication rate depends on the cultivar, the concentration of cytokinin and the number of subcultures. The number of subcultures should be limited to six or seven, in order to reduce the incidence of off-types arising through somaclonal variation. Meanwhile, a set of well-grown healthy shoots are taken for rooting

Rooting

Individual micro shoots are separated from the clumps and inoculated onto half-strength MS

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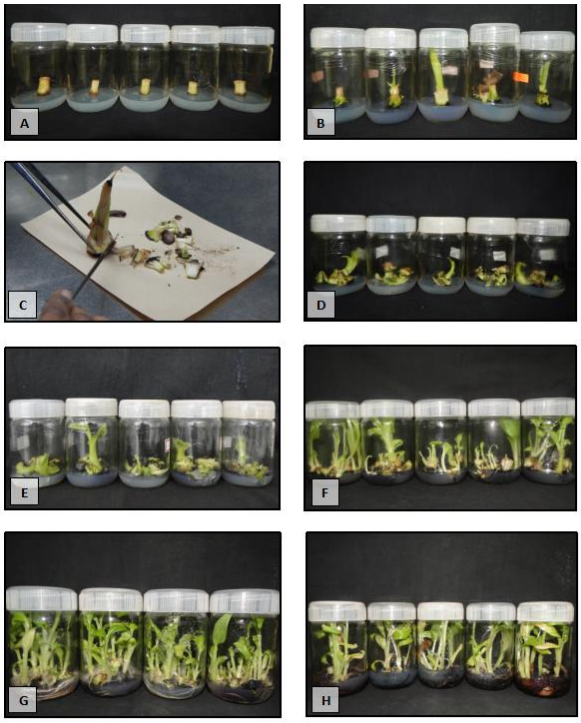
medium containing IBA (1 mg/l), NAA (05. mg/l) and Activated Charcoal (2 g/l). The rooted plantlets are taken out of bottles and washed carefully with water to remove the adhering agar.

Acclimatization

The rooted plantlets are sorted according to size (large: >5 cm tall, medium: 3-5 cm tall and small: < 3 cm tall). For primary hardening, rooted plantlets are planted into the pro-trays containing coco peat. These trays are maintained in the mist house for three to four weeks. For secondary hardening, plantlets are transferred to plastic bags filled with red soil: sand: and cocopeat in a 1:1:2 proportion. These plants are then kept in the greenhouse for three to four weeks. At this stage, the plants attain a height of around 25-30 cm and are ready for sale to the growers.



Explants at the time of initiation and after 4 weeks of initiation (A) and (B), operations carried out during first subculture (center cut stage, TC_1) (C), explants after 2 weeks and 4 weeks of first subculture (D) and (E), multiple shoots arising from the base of the explants after second subculture (TC_2) and third subculture (TC_3) (F) and (G), rooted plants (F)

Fig.1 Different Stages of Banana in Tissue Culture

Conclusion

Banana (*Musa spp*.) is affected by *Fusarium* wilt, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). The clone 'Nanjanagud Rasabale' (NRB), once a leading variety of Mysore, is falling apart because of Panama wilt and is on the verge of extinction. The use of conventional breeding for developing banana clones resistant to *Foc* is hindered due to its sterile, parthenocarpic and polyploidy nature. Under such circumstances, the use of *in vitro* culture technique appears promising to achieve in the production of large-scale and disease-free plantlets.