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Tissue culture propagation of banana

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Banana is globally ranked fourth, next to rice, wheat and maize in terms of gross value of production. It is a major staple food crop for millions of people as well as provides income through local and international trade. Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha producing 95.6 million tonnes. Asia, Africa and Latin America are the major banana producing continents. Among the major producers, India alone accounts for 27.43 % (26.2 million tonnes) followed by Philippines, producing 9.01 million tonnes and China, Brazil and Ecuador, with production ranging from 7.19 to 8.21 million tonnes. Development of tissue culture technology has been the foundation of high quality, disease free planting material production at a mass scale, particularly in vegetatively propagated crops. Tissue culture is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants under aseptic conditions using *in vitro* techniques.

Selection of mother plants and establishment of mother block nursery: Mother plant should be healthy, true to type and free from diseases and pests, especially virus diseases. Mother plants should be raised under roofless insect proof shade net with sufficient height. Mother nursery must be located away from other banana plantations with an isolation distance of 500 m to maintain purity and to avoid spread of virus diseases. Mother plants should be grown under very good management conditions so as to facilitate the true expression of traits. Individual plants should be tagged with a master code number so that the plantlets developed could be traced back to the mother plant. Pedigree record and source of each mother plant should be maintained and catalogued. Once indexed, the mother suckers can be maintained in field or concrete rings with frequent decapitation to facilitate production of more axillary buds. They also serve as explants for culture initiation. Indexing should be carried out primarily for four viruses, namely, *BBTV, BSV, BBrMV* and *CMV,* and should be done twice during crop duration, at 6 months stage and at fruiting. If found infected, the entire clump comprising, suckers along with underground mother corm should be removed and destroyed.

Culture initiation: The sword suckers of 2-3 months are removed from healthy disease free mother plants for shoot tip culture (Fig. 1a). The plant material was thoroughly washed in running tap water followed by washing with a detergent solution to remove adhering soil particles. Later, rhizomes were kept immersed in a fungicide solution of 1% bavistin for half an hour, to further clean the planting material. The outer leaves, leaf base and corm tissue were trimmed using a sterilized stainless steel knife until the length of explant was 4-6 cm and the diameter, 3-4 cm. These trimmed suckers enclosing the shoot tip were washed with double distilled water. After trimming one more outer layer (Fig. 1b), they were soaked in a solution of 0.5% bavistin + 0.05% streptocycline for eight hours. After thoroughly washing with double distilled water, they were trimmed again, so that trimmed suckers were of 2-3 cm in length and 2-2.5 cm in diameter. These shoot tips were soaked in 0.05% cetrimide for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with 0.1% mercuric chloride in a closed container for 10 minutes. Further operations such as washing several times with sterile distilled water to remove all traces of chlorine, trimming of explants and inoculation were carried out under laminar air flow chamber.

Shoot tip explants were incubated in MS liquid medium containing 2 mg/l BAP and 75 mg/l adenine sulphate for two weeks maintaining standard culture conditions of 25 $\pm$ 2^0 C temperature, 70% RH and photoperiodic cycle of 16 hours light and 8 hours dark period (Fig. 1c-d).

After two weeks of incubation, all the explants (Fig. 1e) were evaluated for their ability to establish in liquid medium. Greening and swelling of the explants were utilized as important criteria for assessing the success in establishment. Shoot tips that had turned dark brown/black and which did not swell were considered as non-established. Healthy and contaminant free explants were excised by removing discoloured tissue and transferred to the semisolid medium supplemented with BAP (2 mg/l) and adenine sulphate (75 mg/l) and incubated for four weeks maintaining standard culture conditions. The explants were observed for their bulging in the tips and morphogenetic activity. Such explants were counted and expressed and in terms of per cent establishment. The successfully established cultures (Fig. 1f) were excised into 2-4 sections by giving vertical cuts through the tip. The excised sections were used for culture proliferation.

Decapitation and wounding of shoot tips are carried

out to overcome apical dominance and to encourage axillary bud proliferation. But injuring the apical bud through transverse sections, either four or eight cuts, is a much preferred method. Injuring the explant encourages more production of phenols, but it can be kept at minimum using antioxidants like ascorbic acid.

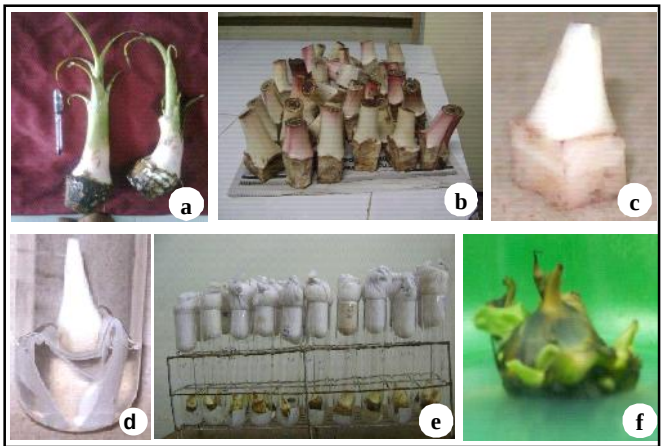

Fig. 1 : Stages in *Culture initiation* of shoot-tip cultures: (a) Sword suckers: (b) Dissected sucker ready surface sterilization with 0.05 per cent cetrimide treatment: (c) Final size of explant from dissected suckers ready for inoculation: (d) - (e) Shoot-tip inoculated in liquid medium: (f) Established aseptic shoot-tip culture

Culture proliferation: First subculture is done after 20-25 days of initiation when the explants turn green in colour. The cultures are first checked for contamination, in general symptoms of fungal contamination appear within one week and bacterial contamination symptoms like change of medium colour and texture or visible colonies appear within one week to one month. For subculturing, the outer dead tissue from the base of explant is removed and one or two leaf bases are peeled till the fresh meristematic tip gets exposed. The apical meristem is cut with two gentle cross incisions and the explant is transferred to subculture medium. During 20-25 days after the first subculture, the central meristem produces clusters of proliferating buds and one to three axillary buds get regenerated from the basal parts of explants around the central apical meristem (Fig. 2a). The number of axiliary buds developed during first and second subculture range from 1 to 5 depending on genomic constitution of the variety. In general, diploids like Matti, Anaikomban and Senna Chenkadali produce more buds than commercial cultivars. Among the latter, the number of buds produced during subculture is high in Cavendish (Robusta, Grand Naine – AAA genome) group followed by Plantain (Nendran – AAB genome) and Monthan (ABB genome) types.

Subsequent subculture is done by trimming the tip of emerging axillary buds and removal of dead tissue at the base of explant by gentle scratching. Clusters of proliferating buds develop during third and fourth subculture (Fig. 2b-c). For further subculturing, the explant is cut into three to four pieces and each slice with two to three proliferating clusters is inoculated to individual culture bottles. This subculture cycle is repeated at 3-4 weeks interval to increase the proliferation rate. During fourth and fifth subcultures, a single clump contains about 15-25 proliferating shoots. After 5-6 subculture cycles, the proliferated buds are transferred to rooting medium containing IBA and activated charcoal. After a month, the rooted plantlets are ready for hardening (Fig. 2d). To minimize somatic variation, the subculturing is restricted to a maximum of seven cycles when each bottle contains 25-30 plantlets with well developed shoots and roots.

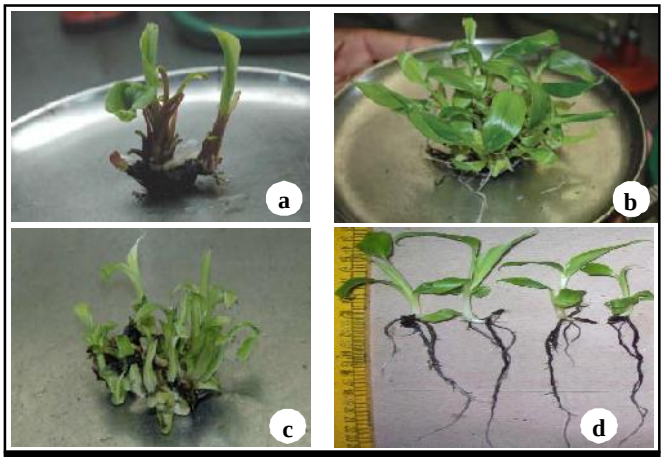

Fig. 2 : Culture proliferation: (a) shooting after apical disabling; (b) proliferation; (c) multiple shooting;(d) rooting

Hardening : Once the plantlets are ready for shifting outside the laboratory, they are carefully acclimatized to adapt to the green house and later to least protected field conditions. During hardening, the plantlets undergo physiological adaptation to changing external factors like water, temperature, relative humidity and nutrient supply.

The plantlets from culture vessels/bottles are moved from the laboratory to a room at ambient temperature and kept open for 4-6 days. Later they are shifted to green house for primary hardening (Fig. 3a-b) where they are first gently washed free of agar medium. This is important as sucrose in agar encourages microorganisms. 8 cm shoots with 3-4 ramified roots are planted in individual micropots in a protray. In places where weather is conducive (24-26^{0}C temperature and more than 80 % humidity), the plantlets are hardened for 4-6 weeks under polytunnel. During this period, 90-95 % humidity is maintained for the initial 6-8 days under diffused light.

The humidity is slowly reduced to 70 %, light intensity raised to normal and temperatures brought to 26^0 C by the end of 6 weeks.

Structures used for primary hardening vary with the climatic conditions. These can be highly sophisticated with UV-stabilized polysheet covering, multiple misting options, thermal shade net and auto-monitoring of light intensity, temperature and humidity. On the other hand, the structures can be simple with polycarbonate roofing, shade net on all sides with fogger facilities. Temperature, RH and light intensities are monitored manually using thermometer, hygrometer and lux meter, respectively.

Planting media for primary hardening range from sieved sand augmented with nutritions to mixtures of cocopeat and Soilrite with fine sand in equal proportions. NPK is provided in liquid form on weekly basis.

After primary hardening for 5-6 weeks, the plantlets are transferred from protrays to polybags for secondary hardening (Fig. 3c-d). Base substrate is generally soil and sand along with low cost materials like coir pith, sawdust or rice husk. Organic manure is either in the form of farm yard manure or poultry manure. Plantlets from protrays are, dipped in fungicide solution (0.1% bavistin) and planted in polybags containing suitable substrate. Initially, these are maintained in low light intensity shade nets and 70 % RH. The plants are hardened by gradually increasing the light intensity and reducing RH (40 %). After 5-6 weeks, the plants become ready for field planting having 3-5 well developed leaves and a good mass of fibrous roots.

During both primary and secondary hardening, the stocks should be rouged for variants at weekly intervals. These could include vegetative deformities like dwarfism, leaf variegation, rosette foliage and leaf crinkiness. Other precautions to be followed are the rooting media should be completely free from pathogens, water used for irrigating the plants should be free from pests and pathogens. Sample plants from each batch should be randomly virus indexed (at least 10 plants from each batch/explant).

Manuring and plant protection in nursery: Plantlets should be 2-3 weeks old before any fertilizer is applied. 100 ml water containing 0.5 g urea, 2 g superphosphate and 1 g muriate of potash can be applied per plant. The manuring is repeated by doubling the dosage after three weeks. Spraying of commercially available micronutrient mixtures during sixth week helps in better establishment both in nursery and field. Strict sanitary measures are adopted in the nursery to avoid the risk of damage by pests and diseases either through substrate or irrigation water.

Fig. 3 : (a) - (b) Primary hardening; (c) - (d) Secondary hardening

Ideal tissue culture raised plant: An ideal tissue culture raised plant should:

– be 30 cm in height and have a pseudostem circumference of 5.0-6.0 cm after 60 days of total hardening

– have 4-5 photosynthetically active leaves and inter-foliar space must be not less than 5.0 cm

– have approximately 25-30 more than 15 cm active roots at the end of secondary hardening

– be free from any visual symptoms of leaf spot, pseudostem rot and physical deformations

– be free from root pathogens like *Erwinia*, nematode lesions and root knots. Random checking of roots is essential to ensure health of plantation.

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