Mass Propagation of Musa varieties in Odisha

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Abstract— Banana is the fourth largest produced food crop of the world and its demand is increasing day by day. It is available throw out the year and its cost is very less in comparison to other fruits. With the development in science new tissue culture protocols are standardized for mass propagation of Musa (Banana) on the basis of effects of plant growth regulators. BAP (6-Benzyl Amino Purine), KN (Kinetin) are most widely used cytokinins for shoot proliferation and IAA (Indole -3-acetic acid), NAA (Naphathalene acetic acid) are widely used auxins for root induction.

Key words: Musa, Mass Propagation

I. INTRODUCTION

Bananas and plantains are monocotyledonous plants in the genus Musa (Musaceae, Zingiberales). They are giant herbs, commonly up to 3-5 m in height, with no lignifications or secondary thickening of stems that is characteristic of trees[3]. Bananas and plantains are cultivated throughout the humid tropics and sub-tropics in the Americas, Africa and Asia, extending into Europe (Canary Islands) and Australia (Queensland). Bananas provide a starch staple across some of the poorest parts of the world in Africa and Asia, while dessert bananas are a major cash crop in many countries [1].

Banana and plantain are important cash and subsistence crops in most tropical and subtropical regions of the world, growing on production cycles of 12–18 months, essentially as perennial crops that can be harvested all year round [4]. Almost all banana and plantain cultivation falls within 30◦ latitude north and south of the equator [2]. They require an average temperature of about 30 ◦C and a minimal rainfall of 100 mm per month. These crops are cultivated worldwide with an annual production exceeding million metric tons, which are distributed among Africa, Asia, Latin America, and the Caribbean.

The major banana growing states in India are Maharashtra, Gujarat, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Odisha, Bihar, Madhya Pradesh, West Bengal, Assam, Tripura and Manipur. Banana is grown in the districts of Angul, Bolangir, Ganjam, Puri, Sundergarh, Nayagada, Mayurbhanj and Keonjhar.Bantal (Plantain), G9 (Banana) and Patkapura (Banana) are most commonly cultivated Musa species in Odisha.Fertility of soil is very important for successful cultivation, as banana is a heavy feeder. Banana is essentially tropical plant requiring a warm and humid climate that is available in the state.

The taxonomy of the approximately 50 species within thegenus Musa remains poorly resolved, not least because ofthe widespread vegetative reproduction and natural occurrenceof many hybrids. At the species level, the number of species andthe status of subspecies has been debated (TaxonomicAdvisory Group for Musa, 2007).The vast majority ofthe cultivated bananas (Pollefeys et al., 2004) are derivedfrom inter- and intraspecific crosses between two diploid(2n ¼ 2x ¼ 22) wild species, Musa acuminata and Musabalbisiana (Simmonds and Shepherd, 1955). In terms ofthe chromosome sets, these are designated as having the genome constitution AA (M. acuminata) or BB(M. balbisiana). These diploid Musa species have seededfruit with little starch and only a small amount of fleshypith, and are of no value as a crop. The cultivated bananas and plantains differ from theirwild relatives by being seedless and parthenocarpic – thefruit develops without seed development or pollinationand fertilization.

In vitro tissue culture propagation systems are very efficient in Musa. These can give high quality, uniform plants free of disease and nematodes, and much of the planting material used in commercial plantations, and increasingly in smallholder production, comes from mass micropropagation. Shoot tip cultures have been most widely used [5], but suspension cultures are also being developed [6]. In the present study a brief detail of the common method used for mass propagation of Musa in Odisha, India is given.

II. MASS PROPAGATION OF MUSA

A. Meristem Culture:

Apical meristems have been mostly used for mass multiplication in vitro. Meristematic tissues produce multiple shoots, depending on the varieties and the composition of the medium. Numerous factors were found to influence the induction of morphogenesis in plant cells and tissue cultures. Several culture media are commonly used, including formulations derived by Murashige and Skoog (1962); Gamborg et al. (1968); Nash and Davies (1972) and Smith and Murashige (1970)[7, 8, 9, 10]. The first report on in vitro shoot multiplication of Musa spp. appeared in the early 1960s [11, 12]. Micropropagation of banana was reported by various researchers using apical meristems, shoot tips, floral explants and immature fruits [13, 14, 15, 16, 17, 18].

B. Mother Block:

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 plant should be healthy, true to type and free from diseases and pests, especially virus diseases. The mother plant should be checked for the presence of virus diseases (male flower buds exhibit symptoms of late infection of viruses like BBTV and BBrMV).Mother plants should be raised under roofless insect proof shade net with sufficient height. Mother plants should be grown under very good management conditions so as to facilitate the true expression of traits.

Fig. 1: Banana mother block.

C. Suckers Collection:

There are two types of suckers, sword suckers – with a well-developed base, pointed tip and narrow leaf blades, and water suckers, which are small, less vigorous, broad leaved and emerge in clumps. Sword suckers have a strong connection with the mother plant and therefore develop strong thick rhizomes of their own. For accelerating the propagation rate, suckers with growing buds or cut rhizomes called 'bits' and 'peepers' of sword suckers are used.

Fig. 2: (a) Sword suckerand (b) Water sucker.

D. Sterilization:

1) Inoculation Room:

Inoculation room is used for explant inoculation or subculture in Laminar Air Flow. It is sterilized by ultra-violate radiation emitted by U.V lights provided in laminar air flow and in the room. In banana tissue culture the inoculation room is U.V sterilized for 30 minutes before use.

2) Tools and Equipment:

Tools and equipment used in tissue culture are sterilized by both physical and chemical methods. In banana tissue culture physical method of sterilization includes autoclave, U.V and flaming whereas chemical method includes chromic acid, alcohol, and liquid detergent.

3) Culture Media:

The culture media provides all nutrients and minerals required by the explants for growth and development. If the media is not sterilized properly it will get frequently contaminated before or after inoculation of explants. The media is sterilized in autoclave at 121°C and 15 PSI for 15 – 20 minutes.

4) Sucker Sterilization:

Sucker collected from the mother block, green house and outer specimen contain many contaminations like bacteria and fungus that are present in soil. Before inoculation in media they are treated with different chemicals to make it sterilized. It is done by following steps:

- After processing suckers are washed in liquid detergent (Labolene) for 2-3minutes.
- Explants were then dipped in Bavistin solution (1 %) for 30 minutes.
- After 30 minutes the suckers are washed with autoclaved double distilled water and transferred to Mercuric chloride solution (0.5 %) for 30 minutes.
- The suckers are washed in 70 % alcohol solution for 1 minute.
- Finally the suckers are washed 3- 4 times with autoclaved double distilled water to remove excess chemicals from the sucker surface.

E. Preparation of Culture Media:

Fresh stocksolution of MS medium was prepared at every 1–2 month interval to avoid contamination. To prepare 1 liter medium, required volume of salts, vitamins and phytohormones from the respective stock solution were taken into conical flask (1000 ml) and to this 100 mg of myoinositol and 30 gm of sucrose were added. The volume was made up to 1000 ml with double distilled water.

The pH of the medium was adjusted 5.75 to 5.8 with 0.1N NaOH or 0.1N HCl. To one liter of semi-solid medium, 5.0gms of agar (Plant tissue Culture grade, Hi-Media, India) was added. All the media were autoclaved at 104 kPa and 121°C for 20 minutes. The autoclaved molten media were then dispensed into sterilized test-tubes and culture vessel inside a laminar air flow cabinet. Following inoculation the test-tubes and the culture vessel were capped.

F. Aseptic Transfer of Explants:

The working area of the laminar airflow cabinet was first wiped with cotton moistened with ethanol and then irradiated with ultraviolet light for 30 minutes before inoculation. The explants were surface sterilized as described earlier and cut aseptically at the middle by a sterile surgical scalpel. Then the explants were inoculated in the test-tubes/culture vessel containing induction medium. This sterilized tissue block was cut to obtain the apical or longitudinally into 4 equal half containing meristematic region. The second set of explants was prepared as above but had their apical domes together with subjacent leaf primodia removed so that the effect of apical dome on shoot initiation could be determined.

Fig. 3: (a) Explants and (b) Inoculated explants.

G. Subcultures of Explants:

The number of subcultures varies with species to species and techniques used for in vitro culture of Musa. During the subculture the explants develops and multiple shoot buds are produced which grow to form shoots with 2-5 leafs. After complete development of the shoot they are transferred to rooting medium for root formation.

H. Effect of Cytokinins in Shoot Proliferation:

Cytokinins play an important role in buds formation in vitro. Howeverbuds proliferation in vitro is influenced by apical dominance which is controlled by various growth regulators[19, 20].Cytokinins such as benzyl aminopurine (BAP) and kinetin are known to reduce the apical meristem dominanceand induce both auxiliary and adventitious shoot formation from meristematic explants in banana [23].However, the application of higher BAP concentrations inhibits elongation of adventitious meristems and theconversion into complete plants [24].Adenine-based cytokinins are used in several Musa spp. for in-vitro propagation [21]. N6-benzylaminopurine(BAP) is the most commonly preferred cytokinin [25]. The others are isopentyladenine (2-ip), zeatin and kinetin[22]. The concentration of exogenous cytokinin appears to be the main factor affecting multiplication.Gubbuk and Pekmzci (2004), reportedthat moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrationsdecreased multiplication and especially depressed shoot elongation. Also they reported higher shootproliferation and elongation with Thidiazuron (TDZ) than with BAP. However, BAP above 20 µM and TDZover 2 µM decreased shoot elongation.

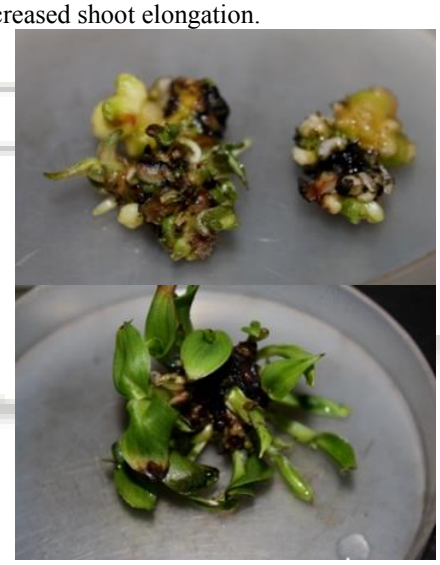

Fig. 4: (a) Shoot buds and (b) Developed shoots.

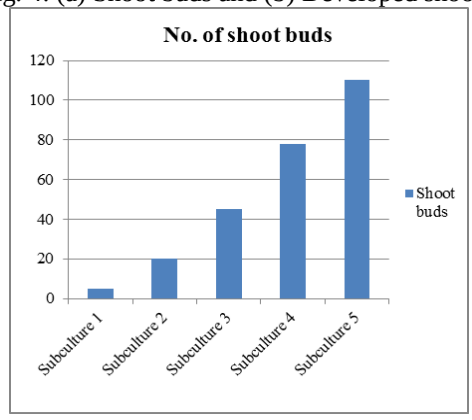

Fig. 5: Numbers of shoot buds obtained after 5 subcultures of single explant using the in vitro meristematic culture process.

I. Effect of Auxins in Root Induction:

Although rooting of microshoots is generally done ex vitro, there are many reports on in vitro rooting. The induction of roots in micropropagated shoots depends on the composition of mineral nutrients and growth regulators in the medium. Ma and Shii (1972) reported that the cultured shoots were easily rooted on sphagnum moss. Berg and Bustamante (1974) indicated that the inclusion of 1.0 mg/l NAA in the culture medium helped in better rooting than IAA or indole-3-butyric acid (IBA). Auxins such as Naphtalene acetic acid (NAA) have been reported to promote plant rooting in vitro [25, 26].

Fig. 6: Banana shoots with roots.

III. Conclusion

Beside starch banana is also an excellent source of Vitamin C (which boost immune system and promote wound healing and collagen formation), Vitamin B6 (which help in cellular metabolism and repair DNA), Manganese (which promotes bone density and healing), Fiber (which helps lower bad cholesterol and promote digestion), Magnesium (which promotes bone health), and Potassium (which plays an important role in controlling your blood pressure). It possesses efficient medicinal values such as stem juice is also used in nerve affectations like epilepsy, hysteria, and in dysentery and diarrhea. Several oligosaccharides comprising fructose, xylose, galactose, glucose and mannose occur naturally in banana making it an excellent prebiotic for the selective growth of beneficial bacteria in the intestine.

The success in producing banana plantlets depends on the tissue culture technique used for its mass propagation which over comes all the limitations including contamination, lethal brown and optimum concentration of auxins and cytokinin used in the medium. To eliminate these limitations new and advance mass propagation methods should be developed for future benefits of both farmers and consumers of Banana.

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