

Advances In Propagation Techniques Of Banana

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INTRODUCTION:

The edible banana (banana and plantain, *Musa* spp.) is an important perennial herbaceous plant grown in the tropical and sub tropical countries of the world, mainly for its seedless fruits (Simmonds, 1962). It is the fourth most important food crop after rice, wheat and maize in many developing countries. Banana is a principal source of food, employment and income in its major production areas (Helslop-Harrison and Schwarzacher, 2007). The genus *Ensete* is composed of monocarpic herbs, none of which bears edible fruits but useful for fiber, starchy foodstuff, boiled vegetables and ornamental purpose (Stover and Simmonds, 1987). The Bananas were grown in Southern Asia even before the prehistoric periods and the world's largest diversity in Banana population is found in this area. The fruit is variable in size, colour and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which maybe green, yellow red, purple or brown when ripe. The fruits grow in clusters hanging from the top of the plant. Almost all modern edible parthenocarpic (seedless) bananas come from two wild species- *Musa acuminata* and *Musa balbisiana*. The scientific names of most cultivated bananas are *Musaacuminata*, *Musa balbisiana* and *Musa* × *paradisiaca* for the hybrid *Musa acuminata* × *Musa balbisiana* depending on their Genomic constitution. *Musa species* are native to tropical Indomalaya and Australia, and are likely to have been first domesticated in Papua New Guinea. They are grown in 135 countries, primarily for their fruit and to a lesser extend to make fiber, banana wine and banana beer and ornamental plants.



Propagating banana by suckers from a disease and pest infested mother plant is the main channel through which banana pest and diseases are spread. Diseases and pest are among the main constraints that severely reduce banana yields. The major pathogens that are spread via infected planting material include *Fusarium oxysporium* f.sp. *cubense* (Foc), which cause fusarium wilt of banana, *Xanthomonas campestris* pv. *Musacearum* causing Xanthomonas wilt of banana commonly known as BXW or BBW (for banana bacterial wilt), *Ralstonia solanacearum*, causing Moko disease, *Pseudocercospora fijiensis* or *Mycosphaerella fijiensis*, causing black sigatoka or black leaf streak disease and various viral disease. The pest of economic importance to banana is banana weevils (*Cosmopolites sordidus*) and a range of root nematode species. Weevils and nematodes inhabit the corm and roots, respectively, thus interfering with the plant nutrients uptake and anchorage. Their infection can cause > 30% reduction in fruit yield (Rukazambuga *et al.*,1998; Talwana *et al.*,2003). Propagation through suckers and other types of planting materials like bits, butts and peepers are easy to obtain but have poor suckering ability and serve as a carrier of pest and diseases. In order to address these problems rapid multiplication methods called micro and macro propagation for mass propagation techniques has been introduced. Micro propagation is a technique where Plants are grown under sterile condition on an artificial medium (i.e in a glass bottles in a lab). The meristem of the banana plant located on the top of the corm is cut out, sterilized and placed on agar in a glass bottle

Banana is a crop with dual propagation abilities, sexual through seeds and asexual through suckers. Seed propagation is common in wild species and the extend of seed set, germinability and dormancy depends on the species. All cultivated bananas are triploid and sterile except for a few parthenocarpic AA and AB which are diploids. Banana seedlings can be obtained through three methods namely: natural regeneration, tissue culture and macropropagation (Singh *et al.*, 2011).

BANANA PROPAGATION TECHNIQUE:

Different banana propagation techniques give different numbers of shoots (Kamura and Staver, 2010) That are correspondingly influenced by the banana's genotype (Vuylseteke *et al.*,1998: Singh *et al.*,2011). Nonetheless, there are two broad approaches to banana propagation, viz. traditional and modern techniques.

Traditional Method Of Banana Propagation Technique :

1. Suckers

Banana sprouts known as suckers, are detached for planting for planting from the mother plants growing in the field. Suckers carry different names depending on their stage of development. Their development consists of distinct physiological stages.

➤ **Sword sucker:** This is the best type for starting a new. Its leaves are cone shaped and developed. Since it is strongly connected to the mother plant, it receives all support and nutrients.

➤ **Water sucker:** This type of sucker is either not strongly attached to the mother plant or comes from an old deteriorating corm. It has broad leaves but lack vigor, therefore is not advisable to use for field planting.

➤ **Peeper sucker:** This is a very young sucker that starts to emerge from the mother plant without definite organs. This is only utilized for establishing nurseries.

➤ **Maiden sucker:** This a full grown sucker bearing typical leaves of banana plant.

Selection of suckers

The criteria for selection of suckers are as follows:

- a) The suckers should have broad corm.
- b) The suckers must have narrow sword-like leaves.
- c) The suckers from viral, fungal and bacterial infection free mother plants must be selected.

Some other criteria which must also be considered are

- a. Suckers of 2-4 months age are selected.
- b. Suckers should weight about 500-1500 g.
- c. They should have a stem diameter of 2-6 inches.
- d. The suckers should be selected from plant which are ,
 - ✓ Healthy,
 - ✓ With desirable bunch qualities and disease resistance
 - ✓ High yielding ability.
- e. The plant must produce bunches with atleast 10 hands/ bunch.
- f. Suckers used should be freely dug.
- g. Suckers may be selected from healthy banana field.

- h. Suckers should not be damaged during digging and transport.
- i. The leaves are commonly cut off in nursery trade.

Advantages and disadvantages of suckers as planting material:

Advantages:

- a) Easy to obtain and cheap.
- b) Easy to transport and manage.
- c) Little field care is required.

Disadvantages:

- a) Carriers of pest and diseases.
- b) Low multiplication rate.
- c) Risk of variety mix up in the field.

1. Rhizomes: whole or split rhizomes can also be used when suckers are not available. Bits of rhizomes of 2 kg or more may be planted in the nursery for sprouting or directly sown in the main field. For quick multiplication of a variety rhizome bits may be used though the plant will require a little time to fruit.

2. Bits are corms with a matured bud or eye.

3. Seed: seed propagation is common in wild species which are diploid and undergo normal meiosis, fertilization and seed set. The extent of seed set, germination and dormancy depends on the species. Fruits of wild species are inedible, being full of seeds that are enveloped in thin mucilaginous pulp. In India this propagation system is not followed because all cultivated commercial banana are parthenocarpic.

Table 1. Growth characteristics of in vitro-propagated and sucker-derived banana plants under field conditions.

Planting material	Leaf number	Leaf area	Leaf dry weight	SLA
(cm ²)	(g)	(cm g ⁻¹)		
Sucker-derived	5.4±0.3	6613.1±7.8	48.8±6.2	135.1±6.1
In vitro-propagated	7.5±1.0	8001.5±6.4	80.6±6.7	99.0±6.7
t-test	**	**	**	*

*** significantly different at P<0.01 and 0.05, respectively.

n=20 for each planting material.

Table 2. Yield characteristics of in vitro-propagated and sucker-derived banana plants under field conditions.

Planting material	Days to flowering	Days for fruit filling	Days to harvest	Bunch weight	Fruit weight	No. of weight	No. of weight	No. of hands
fingers	Suckers							
Sucker-derived	379.6±2.5 84.2±0.4	80.8±4.4	487.4±5.1	9.0±0.3	136.4±5.0	8.4±0.4		
In vitro-propagated	305.2±3.0 94.6±3.0	70.6±5.6	375.8±5.5	15.2±0.3	200.0±4.2	8.6±0.4		
t-test	***	*	***	***	***	ns	ns	**

Bunch weight is in kg. Fruit weight (g) is the average weight for one finger of banana.

* ** *** significantly different at $P < 0.05$, 0.01, 0.001, respectively (n=20)

Improved Traditional Banana Propagation Technique:

Different propagation techniques have been devised to enhance rapid sucker production from the corms of banana plants – both in the field or in humidity chamber, the later are generally referred to as macropropagation techniques.

➤ **Mother plant stripping :**

The outer leaf sheath at the base of the mother plant's pseudo-stem are stripped off to expose the buds followed by mounding up soil around the base of the plant to allow the buds to grow into suckers. This method can double the sucker production per mat in a year to as high as 40 suckers per plant (Baker 1959; Buah, 2000).

➤ **Decapitation :**

Suckering can be stimulated in banana mats by reducing the apical dominance posed by the mother plant. This is achieved by killing the growing point of the mother plant through a window made at the base of the pseudostem (Faturotiet *et al.*, 2002; Pillay *et al.*, 2011). This method is also known as false decapitation, as the foliage of the mother plant remains physiologically active to feed the suckers. Complete decapitation involves stimulating suckering by cutting back the whole pseudostem of the mother plant. Modification of this method has been experimented by scoping out the meristem and adding growth regulators, achieving upto 780 suckers on a mat per year (Manzur, 2001; Singh *et al.*, 2011; Mintah, 2013).

➤ **Corm technique (macropropagation):**

This is a faster technique of inducing plantlets (suckers) production from banana corms removed from the field plants. One approach involves destroying the apical meristem of a relatively large corm and planting it whole in moist nursery substrate under warm and humid conditions in a humidity chamber (Pillay *et al.*, 2011; Singh *et al.*, 2003). Lateral buds are thus stimulated to sprout, and the shoots produced are thus stimulated to sprout, and the shoots produced are removed and hardened in the nursery to obtain small plants. Cutting off these shoots reduces apical dominance further and stimulates more sprouting.

Steps required preparing the planting material and harvest clean banana plantlets in macropropagation:

Preparing The Planting Media.

1. Select clean and healthy banana suckers from desired mats. Using a knife remove all soil and roots from the corm of selected disease-free suckers.
2. Boil water in a large pot. When water has reached a rolling boil, submerge clean banana corms into boiling water for 30 seconds. Remove treated corms and place on a clean polythene plastic sheeting. This step will help to sanitize corms and reduce the chance of spreading nematodes and soil borne diseases.
3. Wash and then sanitize knife and remove each leaf sheaths at the base. After each sheath is removed, use a knife to cross out each bud. After majority of the leaf sheaths have been removed cut the remaining leaf sheath at the base.
4. After removing all the leaf sheaths, use knife to terminate the apical meristem.
5. The corm is treated with fungicide(Bavistin @ 4g/L water) and insecticide for 30 minutes and dry the treated corms in shade.
6. Treatment of corms by growth regulators viz. BAP @ 40 mg and 60 mg and IBA @ 0.1% and 0.2% for 20 minutes.
7. Place the prepared corm into the growth chamber and burry with saw dust.

Managing Shoots And Harvesting Propagules:

1. After about 10-15 weeks, 10-50 secondary shoots will have emerged, each with 2-3 small leaves. These plantlets are detached with a sterilized knife.
2. Those with roots are planted in a prepared potting mixture using one plant per bag.
3. Those without roots are replanted in sawdust for 10 days prior to their movement in the potting mixture, a little portion of corm should be remained attached to provide the plants with nutrient reserve.
4. Allow shoots to acclimatize and develop a strong root system for at least one month.
5. Once plants have hardened off plant out into the field.

Advantages and disadvantages of macropropagation.**Advantages:**

- a. Macropropagation technique is user-friendly technique that require little technical skill and equipments.
- b. It is simple and cheap, suitable for small-medium enterprise.
- c. It is cost effective and affordable which can be adopted by poor farmers.
- d. Acceptable multiplication rate.
- e. Plantlets obtained through this method have the uniformity of micropropagated seedlings while being less prone to adverse post establishment factor.

Disadvantages

- a. Risk of diseases.
- b. Requires clean substrate.
- c. Initial capital investment is high.
- d. Lack of technical skills.
- e. Moderate care required in the field.

Modern Techniques Of Banana Propagation (Micropropagation):

Micropropagation has been defined as in vitro regeneration of plants from cells or protoplasts, tissues or organs on specially formulated nutrient media. Under correct conditions, an entire plants can be regenerated from a single cell. It produces multiple copies of a single plant using tissue culture techniques. It increase the number of planting materials to facilitate distribution and large scale planting. In this way thousands of copies of a plant can be produced in a short time. Micropropagated plants are observed to established more quickly, grow more vigorously, are taller, have a shorter and more uniform production cycle and produces higher than conventional propagules (Vuylsteke and Ortiz, 1996).

Advantages and disadvantages of micropropagation.**Advantages :**

Micropropagation has has a number of advantages over traditional plant propagation techniques:

- a. The main advantages are the production of many plants that are clones of each other.
- b. It can be used to produce disease-free plants.
- c. It can have an extraordinary high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
- d. It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- e. It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seeds cannot be stored.
- f. It often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods like seeds or cuttings.
- g. A greater number of plants can be produced per square meter and the propagules can be stored longer and in smaller area.

Disadvantages.

Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:

- a. It is very expensive and can have a labour cost of more than 70%.
- b. A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- c. An infected plant sample can produce infected progeny.
- d. Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explants.
- e. Sometimes plant or cultivars do not come true to the type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- f. Some plants are very difficult to disinfect of fungal organisms.

Stages Of Micropropagation :

Selection of mother plant(0 day)

(healthy, disease free)



Preparation of explants (0 day)



Fresh inoculation (0 day)

(inoculated explants show bulging within 7 days)



Multiplication (90 days)

(separating, culturing, transferring (7-8 cycles))



Rooting (6 months)

(plants 2-3 cm length to HB(half basal) media for generating roots)



Planting out (7 months)(fully rooted plants)



Primary hardening (7 months)(mist chamber>2-3 weeks (greenhouse)>2-3 month (field)



Secondary hardening (8 months)



Field planting (9 months)

Methods:

Tissue culture:

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant (totipotency). Single cells, plant cells without cell walls (protoplast), pieces of leaves, syems or roots can be often used to generate a new plant on culture media given the required nutrients and plant hormones.

In vitro shoot tip:

Shoot tip culture may be described as the culture of terminal (0.1-1.0mm) portion of shoot comprising the meristem (0.05-0.1 mm) together with primordial and developing leaves and adjacent stem tissue.

Meristem culture :

In meristem culture the meristem and a few subtending leaf primordial are placed into a suitable growing media. An elongated rooted plantlet is produced after some week and is transferred to the soil when it has attained a considerable height. A disease free plant can be produced by this method.

Callus culture:

A callus is a mass of undifferentiated parenchyma cells. When a living plant tissue is placed in an artificial growing medium with other conditions favorable, callus is formed. The growth of callus varies with the homogenous levels of auxin and cytokinin and can be manipulated by endogenous supply of these growth regulators in the culture medium.

Suspension culture :

A cell suspension culture refers to cells and or group of cells dispersed and growing in an aerated liquid culture medium is placed in a liquid medium and shake vigorously and balanced dose of hormones (Street 1997, Thorpe 1981).

Embryo culture:

In embryo culture, the embryo is excised and placed into aculture medium with proper nutrient in aseptic condition. To obtain a quick and optimum growth into plantlets, it is transferred to soil. It is particularly important for the production of interspecific and intergeneric hybrids and to overcome embryo.

Protoplast culture:

In protoplast culture the plant cell can be isolated with the help of wall degrading enzymes and growth in a suitable culture medium in a controlled condition for regeneration of plantlets. Under suitable conditions the protoplast develops a cell wall followed by an increase in cell division and differentiation and grows into a new plant. The protoplast are cultured first in liquid medium at 25-28° C with a light intensity of 100-500 lux or in dark and after undergoing substantial cell division, they are transferred into solid medium congenial or morphogenesis in many horticultural crops respond well to protoplast culture.

Somatic embryogenesis :

It is an artificial process in which a plant or embryo is derived from a single somatic cell. Somatic embryos are formed from the plant cells that are not normally involved in the development of embryos i.e ordinary plant tissue. Somatic embryos are mainly produced in vitro and for laboratory purposes, using either solid or liquid nutrient media which contain plant growth regulators. Somatic embryogenesis (SE) through embryo cell suspension (ECS) cultures is an important milestone method for serves as powerful cellular tool for its non-conventional improvement (Gracia Medrano *et al.*, 2016)

Organogenesis :

In plant tissue culture, organogenesis is the process of differentiation by which plant organs like roots, shoots, buds etc are formed from the unusual points of origin of organized explants where a preformed meristem is lacking.

Conclusion:

Major constraints to the expansion of banana cultivation is the scarcity of healthy planting material and farmers in developing countries like India depends on natural regeneration of suckers for the supply of planting material because it is easily available and affordable compared with other sources of planting materials. Although suckers are easily obtained, field regeneration is a very slow process that often produces small amount of planting material that is usually contaminated by various soil-borne pathogens such as nematodes.

To overcome these constraints different techniques have been developed for rapid multiplication of banana and plantain such as micro propagation under aseptic conditions in the laboratory. While micro propagation can provide large numbers of healthy plantlets, it is



usually expensive and most of our farmers are unable to afford it. In addition, tissue cultured plants cannot withstand dry weather condition if established towards the end of rainy season and require extra care and management for successful field establishment.

Hence macro propagation that is cost effective and affordable has been promoted by International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria as an alternative method for producing and rapidly multiplying healthy planting material. Macro propagation technique, although genotype dependant, can produce 8-15 new plants/corm within 15 days, while secondary scarification of newly emerging buds has the potential to further increase the number of plantlets by a factor of 2-3 within the same time frame. It is simple and cheap which can be adopted by small-medium enterprise. Furthermore, plantlets obtained by macro propagation have the uniformity of tissue cultured plants while being less prone to adverse post establishment factor in the field.

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