

Review article

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Improved Propagation Techniques to Enhance the Productivity of Banana (*Musa* spp.)

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Abstract: The objective of this article is to provide information on pertinent propagation techniques for increased banana productivity. Banana, a very important crop in many tropical and subtropical countries of the world, is propagated with extreme difficulties. Its ordinary propagation technique of using suckers directly detached from a mother plant is limited by low multiplication rates and propensity of disseminating pests and diseases, which culminates in reduced banana productivity. Improved propagation techniques such as mother plant stripping, decapitation and mini-corms that increase the number of suckers are also somewhat deficient for large scale seed production and quality. Consequently, tissue and cell culture methods have been developed to address some of the challenges of seed quantity and quality although they are yet to be widely adopted. In this detailed review that includes results from hard-to-find literature, we discuss the traditional and modern methods of banana propagation, their benefits and limitations. Specifically, tissue culture stands out as the most prolific method of delivering high quantity and quality seed in banana. Its applicability, however, is limited by high costs of production and a need for skilled personnel and specialized equipment. It is imperative that to build a sustainable and viable banana seed production system, a multiplication scheme that combines two or more multiplication methods including tissue culture for cleaning the seed stock is utilized. The information provided gives premise for interventions to alleviate the problems of low banana seed availability, quantity and quality.

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1 Introduction

The edible banana (bananas and plantains, *Musa* spp.) is an important perennial giant plant grown in the tropical and subtropical 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 (Heslop-Harrison and Schwarzacher 2007).

The genomes of banana are derived from crosses between the wild diploid species *Musa acuminata* and *M. balbisiana* that contribute the respective A and B genomes, resulting into the current AA, AB, AAB, ABB genomes (Simmonds 1962; Stover and Simmonds 1987; Ortiz and Swennen 2014) to which the diverse varieties belong. Banana domestication and its current breeding programmes have generated tetraploids of AAAA and AABB genome combinations that are directed towards seedless or parthenocarpic fruits (Uma et al. 2011). Accordingly, with the exception of breeding work where seeds are generated through controlled pollinations with a limited seed set and very low germination rates, edible bananas are conventionally vegetatively propagated. Banana suckers arise from axillary buds found between the bases of leaf sheaths on the plant's underground stem or corm (Figure 1).

Propagating banana by suckers from a disease and pest infested mother plant is the main channel through which banana pests and diseases are spread. Diseases and pests are among the main constraints that severely reduce banana yields and plantation longevity, often leading to total disappearance of plantations (Talwana et al. 2003; Blomme et al. 2011; Dubois and Coyne 2011; Tumuhimbise et al. 2016). The major pathogens that are spread via infected planting material include *Fusarium oxysporum* f.sp. *cubense* (Foc), which causes Fusarium

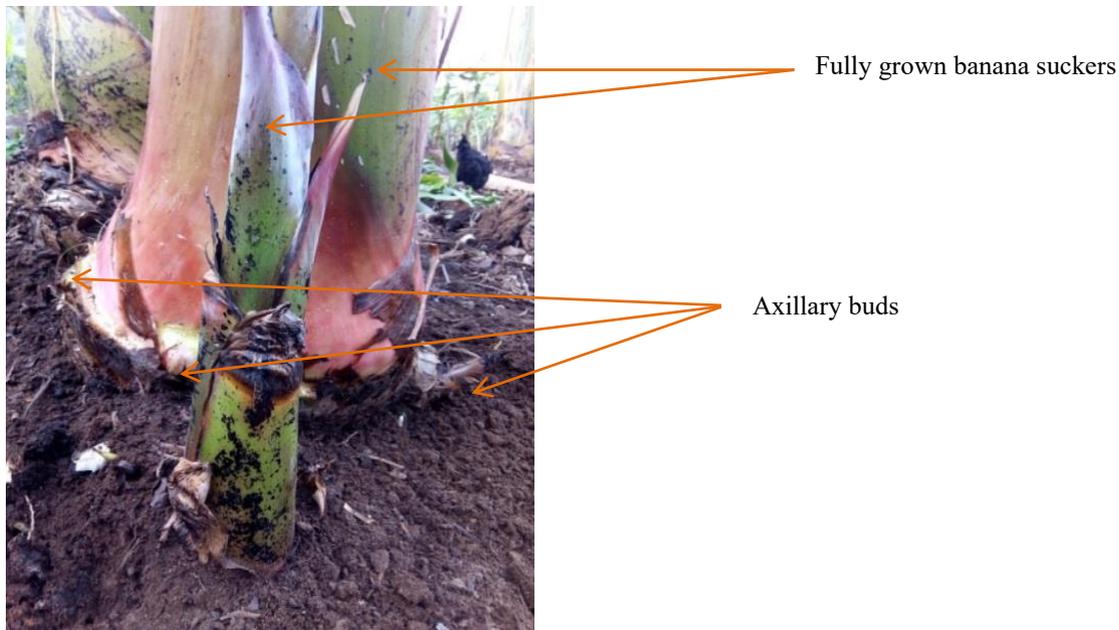


Figure 1: Axillary buds and a fully developed sucker emerging from the banana corm.

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 diseases.

Fusarium wilt of banana is an important disease of dessert and juice banana varieties such as Sukali Ndiizi, Gros Michel, Lady Finger, Pisang Awak and Cavendish (Daniells 2009; Butler 2013; Dale et al. 2017). The disease has a high persistence in the soil of more than 35 years and has no effective control method, making it inadvisable to replant with the same susceptible cultivars. BXW and Moko disease affect all cultivars and lead to total yield loss as the infected fruits are unusable (Tripathi et al. 2009; Blomme et al. 2011). Viral pathogens that affect bananas include banana steak virus, banana bract mosaic virus, banana bunchy top virus and cucumber mosaic virus (Blomme et al. 2011).

The pests of economic importance to bananas are banana weevils (*Cosmopolites sordidus*) and a range of root nematode species (Dubois and Coyne 2011). Weevils and nematodes inhabit the corm and roots, respectively, thus interfering with the plant's nutrient uptake and anchorage. Their infection can cause > 30% reduction in fruit yield (Rukazambuga et al. 1998; Talwana et al. 2003). Where the application of chemical control methods for these pests may be advisable to save the crop, environment and human health hazards, as well as the affordability of the chemicals limit their use in

small-scale banana production systems. Since banana is mainly grown as a perennial crop, once the pests and pathogens are introduced in the plantations they will accumulate progressively during the plant growth cycles (Rukazambuga et al. 1998). The objective of this review therefore, is to provide information on pertinent banana propagation techniques for increasing the availability, quantity and quality of banana planting material for enhanced banana productivity.

2 Banana propagation techniques

Different banana propagation techniques give different numbers of shoots (Karamura and Staver 2010) that are correspondingly influenced by the banana's genotype (Vuylsteke et al. 1998; Singh et al. 2011). Nonetheless, there are two broad approaches of banana propagation, viz. traditional and modern techniques.

2.1 Traditional techniques of banana propagation

2.1.1 Suckers

Banana sprouts, known as suckers, are detached for planting from the mother plants growing in the field. Suckers carry different names depending on their stage of development (Purseglove 1985; Stover and Simmonds 1987). Their development consists of distinct physiological

stages (Figure 2): peeper sucker (small sucker appearing just above the ground and bearing scaly leaves only), sword sucker (large sucker with lanceolate leaves), and maiden sucker (large non-fruiting sucker with foliage leaves) (Swennen and Ortiz 1997). Propagation by suckers gives varying rates of planting materials depending on the banana genotype. Generally, 5-20 new suckers can be extracted for field planting from a banana mat in a year (Vuylsteke et al. 1998; Singh et al. 2011). Intact suckers are bulky to transport, hence peeper and sword suckers represent the smallest size of traditional planting material compared to the maiden suckers. However, peeper and sword suckers have fewer reserves in their corms, which can reduce plant vigor at establishment. Planting vigorous suckers reduces the length of the growing cycle and has been reported to give higher yields (Swennen et al. 1984; Swennen and De Langhe 1985; Ortiz and Vuylsteke 1994). Therefore, a large sucker at flowering and at harvest of the mother plant is desirable because it guarantees a fast succession of harvests and thus, increases the yield over time.

2.1.2 Improved traditional banana propagation techniques

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

Mother plant stripping: The outer leaf sheaths at the base of the mother plant's pseudostem 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 (Faturoti 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 (Figure 3). 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



Figure 2: Different sucker types: A = maiden sucker, B = sword sucker, C = peeper sucker.

meristem and adding growth regulators, achieving up to 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. 2013). Lateral buds 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. Up to 60 plantlets, depending on the banana cultivar used can be obtained from a single corm within four months (Figure 4). In plantain varieties, which have high bud dormancy, corms can be split, and - as with whole corms - these fragments can be planted in substrate, and their buds additionally scarified to stimulate the production of multiple buds from a single secondary buds - up to 1,000 plantlets can thus be regenerated over eight months (Faturoti et al. 2002). In a method called excised corm or



Killed growing point
of the mother plant

Figure 3: A decapitated banana plant with many suckers growing around it.



Figure 4: Macropropagation of bananas by planting whole corms in moist nursery substrate.

bud, individual buds along with some corm material are removed from large corms (forming so-called mini-sets) and planted individually (Faturoti et al. 2002). The corm of a harvested plant, also known as a bullhead, can be used for these methods and up to 150 suckers can be harvested per corm (Stover and Simmonds 1987; Tushemereirwe et al. 2001).

2.2 Modern techniques of banana propagation (Micropropagation)

2.2.1 Tissue culture

This is a laboratory based multiplication technique. It provides double advantages of high propagation rates and

elimination of pest and diseases (Pillay et al. 2011). The technique as applied to banana is also referred to as *shoot tip culture* based on the plant part used for multiplication, or *micropropagation* due to the small size of the starting material (2-20 mm). It is also known as *in vitro* propagation. The technique involves isolation of a growing tip from a sucker or male bud and disinfecting it to kill surface organisms (Yam and Arditti 2009). The cleaned tissue is planted in a vessel containing sterile nutrient growth medium (Madhulatha et al. 2004; Saraswathi et al. 2016). Growth regulators are added to induce bud proliferation and root development (Saraswathi et al. 2016). Growth is achieved under controlled light, temperature, and humidity conditions, while maintaining a meticulously clean laboratory environment to keep away microbes that would outgrow and kill the plants being multiplied. The tissue culture procedure of reducing the size of the growing point and sterilization process eliminates pests and diseases. Fungal and bacterial pathogens that can easily establish on the growth medium become detectable as contamination and are eliminated during the aseptic process. Once cleaned, the stock is multiplied through periodical transfer of the buds onto fresh medium. Growth regulators are adjusted to counter apical dominance and stimulate bud development. Multiplication rates vary with the banana variety and genome composition, with the B genome showing higher proliferation (Vuylsteke and Swennen 1996). Periodical

subdivisions and transfer of the culture to fresh medium lead to exponential multiplication of tissues. A record of up to 10,000 plantlets from a single sucker within eight months has been reported (Singh et al. 2013). This makes tissue culture the best and most prolific technique for banana multiplication. However, the multiplication cycles have to be controlled to limit the occurrence of off types (Singh et al. 2013). Due to the non-seasonal ability to produce these large numbers of propagules within a small laboratory area and relatively short time, Giles and Worfolk (1985) stated that *in vitro* propagation is the most efficient method of vegetative propagation. Tissue cultured plantlets (Figure 5) are lighter than suckers, especially if a light potting substrate is used. They grow more vigorous than the conventional suckers (Msogoya et al. 2006). Tissue cultured plantlets flower earlier than suckers and give a uniform harvest and have a fruit yield advantage of 20-50% (Robinson et al. 1993; Msogoya et al. 2006; Singh et al. 2013).

Although tissue culture is a powerful plant propagation method, it demands high investments, specialized equipment and skilled personnel (George and Manuel 2013). As a result, these factors raise the unit cost of a tissue-cultured plant. To circumvent this, attention has been placed on modifying the composition of the growth media, to accommodate the wide range of varieties (Talengera et al. 1994; Arinaitwe et al. 1999) and lower production costs (Ogero et al. 2012; Watt 2012).



Figure 5: Banana plantlets generated through tissue culture.

2.2.2 Cell culture

Instead of using intact shoot apices as starting material to induce micro-shoots, cell culture has been explored to increase the efficiency of the tissue culture procedure (Singh et al. 2013). Cells of a meristematic tissue are induced to profusely proliferate into a callus that is later dispensed into the liquid medium to start a cell suspension. A single ml of cells with a good regeneration capacity can yield up to 100,000 plantlets (Singh et al. 2013). Cell culture application is limited by low response of banana cultivars to generate callus as starter materials (Strosse et al. 2003). In addition, the system has a higher propensity to producing off-types due to strong auxins used to induce callus. Where this occurs, the regenerated plantlets might fail to meet the genetic integrity of the mother plant, leading to somaclonal variation. Optimization and field evaluation of this technology to a wide variety of cultivars is yet to be conducted for commercial applications. Since the tissue culture method results in the rapid multiplication of clean planting material of a small size, it is recommended for propagation of a variety of clonally propagated plants. Karamura and Staver (2010) analysed the risks of transmitting seed-borne pests and diseases by various banana propagation methods, and concluded that tissue culture was the most reliable technique to reduce transmission (Table 1).

3 Relative efficiency of the different banana propagation techniques

Irrespective of the propagation method used, it is important to note that the attributes of the products generated in terms of quantity, quality, cost of multiplication and multiplication ratio per unit time are important when

deciding on the technique to adopt. As such, banana being a vegetatively propagated crop, the key determinant of its planting material is the presence/absence of the pest and /or pathogen. Table 2 summarises some attributes of different banana seed propagation methods. The data presented indicate that propagation methods differ greatly in the quantity of planting material they are able to generate from various starting materials, be it a mother plant in the field, a whole or a split corm, or even a single meristem. Traditional methods of banana propagation generally show a significantly lower rate of multiplication compared to modern methods. Tissue and cell culture are the only propagation techniques that can produce large quantities of clean planting material within a relatively short period of time.

4 Integrated benefits of all banana propagation techniques

Banana production can be improved by timely access and use of clean planting material of desirable banana varieties in adequate quantities. The propagation methods discussed above have applications at different levels of production. Traditional field grown suckers have the lowest multiplication ratios and pre-planting treatments are inadequate to eliminate some pests and diseases. Of the improved propagation methods described above, tissue culture remains superior over others as it enables the elimination of systemic pathogens and allows for subsequent faster multiplication of the clean material. Tissue-cultured plants, however, are always tender and require greater care during the first two months after planting. Once established, the tissue-cultured plants have a higher ability to produce suckers, attributed not to residue growth regulators from *in vitro* culture, but

Table 1: Relationship between risk of pest and/or disease transmission and banana propagation method (Karamura and Staver 2010)

Pest/disease	Suckers from field in production	Suckers in a multiplication plot	Micro-corms	Tissue culture
Bacterial diseases	2.0	1.5	1.0	0.5
BBTV	2.0	1.5	1.0	0.0
BSV	2.0	1.0	1.0	2.0
Other viruses	2.0	1.5	1.0	0.5
Fusarium wilt	2.0	1.5	1.0	0.5
Nematodes	3.0	1.0	0.0	0.0
Weevils	3.0	1.0	0.0	0.0

BBTV= banana bunchy top virus; BSV = banana streak virus; 0 = zero risk; 1 = low risk; 2 = moderate risk; 3 = high risk.

Table 2: Some attributes of the different banana propagation methods and their respective advantages of quantity and quality of plantlets they generate in a single year

Method of propagation	Ratios of suckers from a single plant year ¹	Rate of multiplication	Quality of seed [§]	Source
Suckers	1:5-20	Low	Low	Purseglove (1985), Stover and Simmonds (1998), Vuylsteke et al. (1985), Singh et al. (2011), Baker (1959), Buah et al. (2000)
Mother plant stripping	1: 30-40	Medium	Low	
Decapitation	1:9-40	Medium	Low	Faturoti et al. (2002), Pillay et al (2011), Manzur (2001) Singh et al. (2011), Mintah (2013)
Corm	1:100	High	Medium	Stover and Simmonds (1987), Manzur (2001), Faturoti et al. (2002) Baiyeri and Aba (2005), Mintah (2013)
Tissue culture	1:10,000	Very high	Very high	Vuylsteke (1989), Manzur (2001), Singh et al. (2011)
Cell culture	1:100,000	Very high	Very high	Singh et al. (2013), Strosse et al. (2003).

[§]The quality of seed produced largely depends on the starting planting material

rather to ones synthesized by the vigorous root system of plants grown in containers prior to field planting (Smith et al. 2001). However, the suckers are small and cannot be easily separated from the mother plants without injuring them or the mother plant. Daniells and Smith (1991) indeed recommend de-suckering the first flush of suckers from field planted tissue culture plants. Suckers that emerge later will have relatively bigger corms and will be distanced from the mother plant. These suckers can then be used as clean starter planting material for the conventional multiplication techniques and can also be integrated into low agro-input farming systems. The banana cropping system is perennial and peace meal replanting or gap filling with tissue cultured plantlets cannot give the benefit of using clean plantlets. This is due to the risk of infection and infestation from neighbouring old banana mats. Therefore a virgin field is recommended for clean planting materials.

5 Conclusion

All techniques of banana propagation have limitations ranging from low multiplication rates, genetic fidelity, disease elimination and affordability of the generated suckers. If the central objective of the seed system is to generate high quality planting materials, tissue culture technique offers the highest seed quantity and quality and therefore, is highly recommended. The technique can be integrated in the downstream propagation and dissemination of cleaned suckers to address the problem of seed quantity and quality.

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