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A Review of Protocols for Macropropagation in *Musa* Species

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ABSTRACT

Bananas and plantains (*Musa* spp.) are the most important tropical fruit crops. They are staple food in most part of the humid tropics and important source of rural income for the smallholders who produce them in compound farms. A common limiting factor to large-scale production of *Musa* crops and expansion of existing plantations is the difficulty in obtaining planting materials. This is due to poor suckering ability accentuated by the strong hormone-mediated apical dominance exerted by the main plant. Rapid production of propagating materials could be achieved through various vegetative multiplication techniques, including micro-propagation, but micro-propagation is not an option for the traditional small-scale farmers in the humid tropics. Therefore, several macropropagation techniques have been developed, such as field decapitation, excised bud, and the detached corm techniques. These techniques are relatively simple and require minimum investment to set up, and plantlets obtained thereof, have the uniformity of tissue-culture plantlets. However, rootless explants obtained through macropropagation have lower survival rate during the acclimatization and stabilization stages in the nursery compared to tissue-culture plantlets. Several organic nursery substrates have been developed for optimum performance of *Musa* explants in the nursery. *Musa* plantlets require a warm, humid, and translucent nursery environment to allow the plantlets stabilize and escape desiccation. These conditions can be met by raising plantlets under green polyethylene chamber or under palm frond shade as commonly practiced in tropical sub-Saharan Africa. Above all, nursery substrates must be composted for at least eight weeks before use, and rooted explants should be preferred during nursery planting. Other valuable options discussed include nutrient, moisture and shade management.

Keywords: mass propagation, *Musa* planlets, nursery management, organic substrate

CONTENTS

INTRODUCTION

Bananas and plantains (*Musa* spp.) are the most important tropical fruit crops (Ortiz *et al*. 1998) and rank the fourth most important global food commodity after rice, wheat and maize in terms of gross value of production (INIBAP 1992; FAO 2001). They are staple foods for rural and urban consumers in the humid tropics and important source of rural income particularly in some locations where smallholders produce them in some compound or home gardens (Chandler 1995). Progressive decline in plantain and banana production over the years has been attributed to a high susceptibility to pathogens (Persley and de Langhe 1987), weeds (Ndubizu 1983), drought and organic matter status of the soil (Awodoyin 2003), poor suckering ability (Ndubizu 1985), pest and diseases, labour shortage, poor agronomic practices and post-harvest constraints (Robinson 1996). These factors combine to shorten the life-span of most plantations (Swennen *et al*. 1998).

A common limiting factor to large-scale production of bananas and plantain, and/or expansion of existing planta-

Fig. 1 Traditional *Musa* planting materials. From left to right: Maiden sucker; water sucker, early and late sword suckers; peeper; butt; bits.

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Table 1 Mean bunch weight (kg/plant) of the parent crop derived from five different types of plantain propagules.

	Plantain propagules					
Planting depth (cm)	**Peeper**	**Early sword sucker**	**Late sword sucker**	**Bit**	**Maiden**	**Mean**
10	8.50	10.10	10.10	9.70	9.90	9.64
22	7.50	11.00	11.03	9.40	8.80	9.60
30	11.10	10.30	11.33	10.20	8.80	10.34
Mean	9.03	10.47	10.87	9.77	9.17	

Source: Obiefuna (1983)
LSD at 5% for depth of planting means = 1.34
LSD at 5% for propagule means = 1.00

tions is the difficulty in obtaining planting materials (Tezenas du Montcel 1985; Schill *et al*. 1997; Baiyeri and Ajayi 2000; Schill *et al*. 2000), due to poor suckering ability (Robinson 1996). There are several types of propagating materials (maiden sucker, water sucker, sword suckers, butt, peeper and bits; see **Fig. 1** for illustration) used for the establishment of plantain plantations, but they vary in their degree of suitability (Ndubizu and Obiefuna 1982; Baiyeri and Ndubizu 1994; Baiyeri *et al*. 1994) and may be inadequate to meet the needs of medium to large-scale production at the recommended population of 1600-2500 plants ha^{-1} (Awodoyin 2003). A study (Obiefuna 1983) on plantain (*Musa* AAB cv. 'Agbagba') revealed that early- and late-sword suckers often flower earlier and yield better than the rest of the propagules (**Table 1**).

In most plantain and banana cultivars, the emergence of new suckers follows a hierarchical pattern and natural regeneration is somewhat slow due to strong hormone-mediated apical dominance exerted by the main plant (de Langhe *et al*. 1983; Swennen *et al*. 1984). Besides, natural regeneration often produces materials that are usually contaminated by various soil-borne pathogens such as nematodes and banana-corm weevils (*Cosmopolites sordidus*). Rapid production of propagating materials can be achieved through various vegetative multiplication methods, including micropropagation (Vuylsteke 1998; Tripathi *et al*. 2007).

Micropropagation (i.e. meristem/tissue culture) assures more rapid production of healthy, vigorous, and disease-free planting materials (Swenmen 1990), but requires a more sophisticated technique, skill and care to handle (Vuylsteke and Talengera 1998). Thus, tissue culture as a method of generating planting materials is not an option for small-scale farmers who are the major stakeholders in *Musa* production in the humid tropics, so there is a need for cheap and simple techniques (Lopez 1994). Consequently, the Plantain and Banana Improvement Program (PBIP) of the International Institute of Tropical Agriculture (IITA), Nigeria, advanced the use of macro-propagation methods for increasing sucker multiplication at farm levels (Faturoti *et al*. 2002). It has been, however, observed that plantlets obtained through detached corm propagation have lower survival rate during the acclimatization and stabilization stages in the nursery compared to plantlets from tissue culture (Tenkouano *et al*. 2006). Intensive nursery management of macro-propagated plantlets becomes imperative.

This paper, therefore, summarizes methods of macropropagation in *Musa* species with farmer-friendly clues on the nursery management of excised plantlets for better survival in the nursery, and subsequent field establishment.

MACROPROPAGATION TECHNIQUES

These are methods that use whole suckers, large pieces of the parent corms, or sword-sucker-corms to produce planting materials (Faturoti *et al*. 2002). Repression of apical dominance to stimulate lateral bud development and increase suckering rate can be achieved through complete or partial decapitation on a field of growing plants or by detached corm techniques.

Field decapitation techniques

Field decapitation technique generally involves two methods *vis-à-vis* false decapitation and total decapitation. The two decapitation methods involve stimulating lateral bud production by destroying the active growing point (apical meristem) in the pseudostem (Wilson *et al*. 1985; Swennen 1990; Awodoyin 1997; Faturoti *et al*. 2002; Tenkouano *et al*. 2006). The rate of suckering per plant ranges from 9-14 suckers per annum.

In false decapitation, a window or small hole (5 cm × 10 cm) is made on the pseudostem slightly above the soil (corm) level, and the growing point physically removed, while the plant is left standing with foliage that remains photosynthetically active for approximately three months. In complete decapitation, the pseudostem is cut down at the soil (corm) level and the apical growing point destroyed by screwing with a metal blade. A study with plantain (*Musa* AAB) revealed that false decapitation when carried-out at five months after transplanting (MAT), performs superior to complete decapitation and natural suckering (Awodoyin 1997), in terms of net returns, cost-benefit ratio, quality and number of plantable suckers produced.

Plant growth regulator 6-benzylaminopurine (BAP) has been proved to enhance lateral bud production in field decapitation technique (Macias 2001). In his study with FHIA-20 banana (AAA) hybrid, Macias reported that a total of 156 plantlets could be obtained from one treated sucker, from the first to the third generation. Therefore, when five suckers are selected on each stool (mat) for mass propagation, 780 plantlets (which are very similar to *in vitro* plantlets) could be obtained per stool in 8 months.

The procedure for this *in-situ* mass propagation as described by Macias (2001) is as follows: Sword suckers of height 20-30 cm from a preflowering mother-plant are used. The pseudostem of each sucker is cut transversally 2 cm above the collar of the rhizome and the apical meristem removed at a depth of 4 cm, leaving a 2 cm-diameter cavity in the rhizome. The pseudostem fragment is then cut with crosswise incisions, boring down to the rhizome collar. Four ml of BAP at 40 mg/l distilled water is deposited in the cavity left by the removal of the apical meristem. The rhizomes are then covered with well-composted organic substrate up to 5 cm above ground level. The so-called second-generation suckers (G2Ss) appear after 3 months on each treated sucker. When these G2Ss have differentiated and reached a height of 20 to 30 cm, they are dissected and treated following the same routine described above to obtain the third-generation suckers (G3Ss). Sixty-days later, the G3Ss are treated in the same way as the preceding generations to obtain fourth-generation suckers (G4Ss) which are excised and raised in nursery bags for subsequent field planting.

It should be noted that this technique, when carefully applied on the mother-plant (without damaging the root systems), could still bear its bunch normally. The technique also makes it possible to obtain in 8 months, propagules that are practically free of pests and diseases when healthy plants are selected for field multiplication.

Excised bud technique (EBT)

Following the evident disruption of the mother-plants using

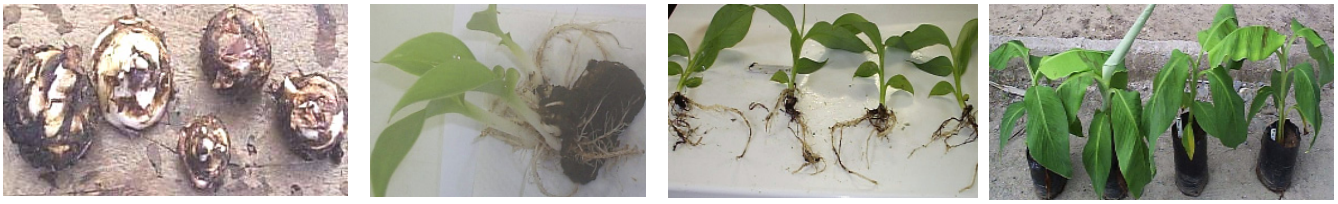

Fig. 2 Macropropagation via excised bud technique. From left to right: Excised buds; bud forms multiple shoots; multiple shoots separated; separated shoots grown into independent suckers.

Table 2 The main effect of genotype on number of days to plantlet emergence and total of plantable explants produced per corm.

	Days to specific emergence					
Genotype	**1st**	**2nd**	**3rd**	**4th**	**5th**	**Plantable explants/corm**
PITA 22 (AAAB)	33.7	39.6	53.0	62.4	85.4	3.0
PITA 25 (AAAB)	52.2	66.1	74.4	118.0	151.4	3.5
Nsukka Local (AAA)	45.8	55.3	65.7	73.6	84.6	2.9
Agbagba (AAB)	36.3	45.8	55.6	83.0	96.1	4.5
FHIA 17 (AAAA)	31.3	41.4	49.9	62.9	69.8	3.1
LSD $_{(0.05)}$	7.1	9.6	12.2	18.8	26.8	NS

Adapted from Baiyeri and Aba (2005).

field decapitation techniques, an alternative method, EBT was introduced (Lopez 1994) to spare the mother-plants. Upper bud development is stimulated in the field of healthy, vigorous plants by exposure and earthing up of the corms. Peepers (side shoots) which consequently develop to a height of 3-5 cm are carefully excised (separated from the mother-corms) and raised in nursery bags in fertile topsoil or well-composted organic substrate. These are kept under the canopy of banana crop or in a half-shade environment until they are ready for field planting (see **Fig. 2**).

Detached corm multiplication techniques

A well-developed banana or plantain corm contains several axillary buds, which essentially host meristems of different ages and stages of development (Kwa 2003). Sword-sucker-corms, as well as corms from preflowering and harvested plants could be used in detached corm multiplication techniques (Faturoti *et al*. 2002; Tenkouano *et al*. 2006). Corm techniques can either be in form of whole-corm or split-corm. In split-corm technique, the dug-out corm is cleaned of roots and outer leaf sheaths to expose the lateral buds, then washed and split into two or several fragments (bits). These are planted face down and raised in a well-composted organic nursery substrate.

In whole-corm technique, the apical meristems of the pared corms are scarified, either by making two cross-wise incisions on the buds (Kwa 2003; Tenkouano *et al*. 2006) or by mechanical removal by screwing with sharp knife (Baiyeri and Aba 2005). These methods are illustrated in **Fig. 3**. Scarification of side buds on the corm has the potential to further increase plantlet production by a factor of 2-10 (Tenkouano *et al*. 2006). In nematode or weevil infested soils, it is recommended (Baiyeri and Aba 2005) that the pared corms are sterilized in 10% solution of household bleach, 'JIK' (3.5% a.i., sodium hypochlorite, NaOCl) and allowed to air-dry and cured for three days before planting to avoid pest dissemination. Alternatively, hot-water treatment, for 20 minutes at a temperature of 53-55°C practically frees planting material from nematodes (Speijer *et al*. 1995, 1999). Lateral bud growth is activated by planting the corms in rich composted organic substrate (Faturoti *et al*. 2002) in nursery bags or more recently, in humidity chamber conditions resulting in the high production of planting material (Kwa 2003; Tenkouano *et al*. 2006).

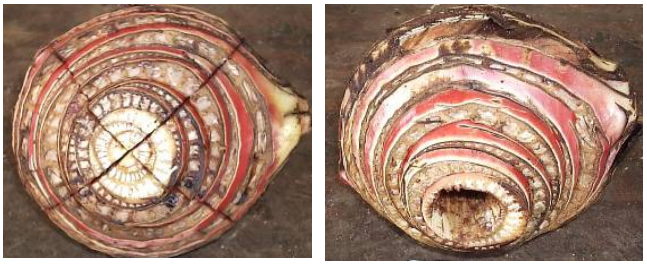

Fig. 3 Two methods of physically damaging apical dominance. Cross-wise incision (left); screwing (right).

The humidity chamber consists of a 2 m × 5 m × 1.5 m framework mounted on a block-wall base of height 0.6 m, with a hinge door (0.5 m × 1 m) for accessibility. The sides and roof are made of thick transparent polyethylene material to allow for light penetration, moisture conservation and heat build-up within the chamber. During planting, the 0.6 m block plat-form is nearly filled with well-composted sawdust, wherein the corms are planted completely-buried to a depth of 3-5 cm. Watering in the chamber is done by sprinkling only twice a week. The whole structure is best sited under a half-shade environment (translucent roof). At three-leaf stage, the plantlets are ready for nursery planting and are carefully excised and raised in the nursery for subsequent field planting. A well-composted ricehull could also be used as an alternative plantlet initiation medium (Baiyeri and Aba 2005) to the recommended sawdust. This detached-corm technique is relatively simple and requires minimum investment to set up. Moreover, plantlets obtained thereof, have the uniformity of tissue-cultured ones.

An *ex-vitro* multiplication study (Baiyeri and Aba 2005) revealed that there were significant variations in genetic response of *Musa* species to sucker plantlets initiation (**Table 2**); the study revealed that genotypes with the 'B' genome had more plantlets than the AAA group. However, Hirimburegama and Gamage (1997) reported that in an *in-vitro* study the multiplication rate was found to be variable among cultivars, and it appeared that genome B had the lowest multiplication rate compared to the AAA group. The variance in results of the two studies might be due to inherent genotype by environment interaction.

NURSERY MANAGEMENT OF MACROPROPAGATED *Musa* PLANTLETS

The nursery phase is an important part of the planting operation in the cultivation of many tropical fruit trees. Keeping the seedlings to grow in the nursery until they are larger, tougher and more vigorous, makes it possible to give maximum care to weak seedlings, saves seeds, space and water (Baiyeri 2003), and reduces the risk of damage to, or loss of the plant. It also allows the grower to select the most vigorous seedlings for transplanting into the permanent field (Aiyelaagbe 1989), as the quality of nursery seedlings influences re-establishment and the future productivity of the orchard (Baiyeri and Ndubizu 1994).

The quality of nursery potting medium is important to the successful growing of plants in containers (Bunt 1988). The physical composition has a profound effect on the supply of water and air to the growing plants (Beardsell and Nichols 1982), as well as affects anchorage, and nutrient and water-holding capacity. It is often common to think of soil as a good medium, but most soils when used alone are very poor growing medium. Soil has been indicated as the easiest way through which seedlings become infected by diseases such as root knot nematode and seedling root rots (Egunjobi and Ekundare 1981). Besides, soils have the attribute of heavy weight when large volumes are used to raise containerized plants, and nursery men may have the problem of bulkiness in transporting them (Bunt 1988). However, Baiyeri (1997) recommended a 100 kg N/ha combined with 9-day watering interval as nursery practice for upgrading plantain peepers to certified planting materials in 12 kg of topsoil. The soil should also be amended with P_2O_5 (using single superphosphate), K_2O (using muriate of potash) and a systemic insecticide-Furadan 5G at the rates of 60, 480 and 172 kg/ha respectively.

The use of organic substrate (compost) offers a great advantage over the conventional topsoil (Akanbi *et al*. 2002; Adams *et al*. 2003). Organic substrates provide better root-substrate relation than conventional soil mix, adequate nutrients for the seedlings, less pre-dispose the seedlings to soil borne pests and diseases, assures better moisture and nutrient management (by minimizing leaching losses) and as well maintain optimum pH. The nutrient value of nursery mixtures could be further improved by incorporating inorganic salts such as rock phosphates, lime and nitrogenous fertilizers during composting (Matthew and Karikari 1990).

Several organic nursery mixtures had been developed (Baiyeri and Aba 2005; Baiyeri 2005) for raising *Musa* plantlets to vigorous suckers for field planting (in 8 to 12 weeks). Among the so far selected elite mixes, include:

- Ricehull + poultry manure (3:1, v/v)
- Sawdust + ricehull + poultry manure (1.5:1.5:1 v/v/v)
- Plantain fruit pulp + sawdust + poultry manure (2:2:2, v/v/v).
- Plantain fruit pulp + sawdust + poultry manure + topsoil (1.5:1.5:1.5:1.5, v/v/v/v).
- Topsoil + poultry manure + sawdust (3:2:1, v/v/v).

Nursery substrates must be composted for at least 8 weeks before use. Composting ensures that compounds of high molecular weights are broken down into smaller molecules to allow for readily availability of nutrients previously tied-up (Bunt 1988). Decomposition of sawdust, for example, causes nitrogen deficiency as microflora deplete available nitrogen in the decomposition process (Wootton *et al*. 1981). Consequently, well-composted sawdust is preferred in any nursery mix.

As media development continues world over, a wide range of crop residues, organic wastes and other industrial by-products could be used as nursery potting medium; preference of any should largely be determined by considerations of availability, economics, physical and chemical characteristics (Akanbi *et al*. 2002).

Other factors that influence the performance of vegetative propagules in the nursery include choice of nursery site, intense wind, excessive tropical heat, and the relative humidity of the nursery environment (Hartmann and Kester 1975). An ideal nursery site for *Musa* plantlets must be free of flooding, close to constant and good quality water, and should be sited under a natural shelter against wind and intense tropical heat.

Management of shade in the nursery can be very critical since lack of shade or excess of it could lead to poor seedling growth. Palm frond is probably the oldest and most common nursery shade used in Nigeria (Baiyeri 2006), and had proved satisfactory for the young delicate *Musa* plantlets (Baiyeri and Aba 2005). Different polyethylene colours used as shade reflect different spectra of the visible light and transmit some spectra of the visible light with consequent effect on physiological behaviour of plants (Hart 1988). Consequently, green polyethylene nursery shade enhanced the highest percentage emergence and the best seedling quality of paw-paw (*Carica papaya*) in the nursery and hence, better field re-establishment (Baiyeri 2006). A similar result was observed in *Musa* plantlets initiation (Baiyeri, unpublished data) using different colour shades in a plantlet initiation chamber. *Musa* plantlets generally require a warm, humid and half-shade/translucent environment to allow the plantlets stabilize and escape desiccation immediately after excision from mother-corms. The plantlets should be excised at 2-3 leaf stage and planted immediately after excision from the mother-corm. With over-grown plantlets, it is better to cut-back to a maximum height of 10 cm. This would help to maintain fairly uniform plantlets. Furthermore, the floor of the nursery shade should be lined with moist sawdust, and mist spraying practiced intermittently for the first 2 weeks after transplanting (especially in hot weather) to abate desiccation of the young plantlets.

Watering of the potted plantlets should be done at most twice a week to avoid water-logging with the consequent root asphyxiation, and/or leaching losses. It is rather a good practice to moisten the floor of the nursery shade daily to maintain fairly high air humidity within the nursery environment. The nursery bags should, as well, be adequately perforated underneath to ease drainage and improve root aeration. With time, the plantlets require more light for further growth and development. Thus, at about 6-8 weeks after transplanting, only light shading is required until about five days prior to field planting when the young suckers are allowed to acclimatize to the ambient environment. Nursery practices are best done in the dry seasons to get plants ready for wet season planting.

CONCLUSION AND RECOMMENDATIONS

Macropropagation provides cheap, simple, and relatively rapid techniques for vegetative multiplication of *Musa* species that could be amenable to the low-income, unskilled, small- and medium-scale farmers who are the major growers of bananas and plantains in the humid tropics. These *ex-vitro* multiplication techniques including on-farm decapitation, involve the stimulation of lateral shoot development on the sucker corms.

More than 30% of the plantlets obtained through detached corm propagation are usually without roots (**Table 3**), and these rootless plantlets have lower survival rate during the acclimatization and stabilization stages (**Table 4**) in the nursery (Baiyeri 2005). The root system is the link between the plant and the soil. It is responsible for the absorption of water and nutrients, anchorage, synthesis of some plant hormones and storage (Blomme *et al*. 2000). These indispensa-

Table 3 Percentage of rooted and rootless plantlets at the time of excision as influenced by initiation media and genotype.

Genotypes	Ricehull		Sawdust	
	Rooted (%)	Rootless (%)	Rooted (%)	Rootless (%)
PITA 22 (AAAB)	44.4	55.6	55.9	44.1
PITA 25 (AAAB)	28.6	71.4	63.2	36.8
Nsukka Local (AAA)	76.5	23.5	88.0	12.0
Agbagba (AAB)	83.0	17.0	85.7	14.3
FHIA 17 (AAAA)	68.0	32.0	71.4	28.6
Mean	60.1	39.9	72.8	27.2

Adapted from Baiyeri and Aba (2005).

Table 4 The effects of plantlet initiation media and rooting status of plantlets on percentage survival of plantlets in three weaning/rooting media at the nursery stage. Data shown are the mean performance of five genotypes (PITA 22, PITA 25, FHIA 17, Agbagba, Nsukka Local).

Physiological status	Plantlet Initiation Media						
	Sawdust			Ricehull			Mean
	SD+PM	RH+PM	RH+SD+PM	SD+PM	RH+PM	RH+SD+PM	
RD	29.9	33.1	21.3	44.5	21.0	45.9	32.6
RS	70.1	66.9	78.7	55.5	79.0	54.1	67.4
RLD	57.5	93.8	20.0	85.0	43.8	55.2	59.2
RLS	42.5	6.2	80.0	15.0	56.2	44.8	40.8

Adapted from Baiyeri (2005)
RD: Explants had roots but died in the nursery;
RS: Explants had roots and survived in the nursery;
RLD: Explants had no roots and died in the nursery;
RLS: Explants had no roots but survived in the nursery.

ble roles of the root explain the non-stability of the rootless plantlets. Therefore, cultural practices that could enhance rooting at the plantlet initiation stage should be pursued.

Conventionally, the sucker corm is a nutrient reserve, which could support growth for sometimes prior to foliage development (Butler 1960). The inclusion of nutrient source (organic, inorganic or a combination, thereof) to the sucker plantlet initiation media to supplement the nutrient reserve of the corms, may enhance the shooting ability of most corms, as well as plantlet quality. More research, however, should be carried-out to decry the efficacy of (in)organic fertilizers and plant growth regulatory hormones on the development of plantlets in the multiplication chamber.

The physical composition of the growing media have a profound effect on the supply of water and air to the growing plant (Beardsell and Nichols 1982), as well as affect anchorage, and nutrient and water holding capacity of the medium. These physical characteristics of the growth medium affect the emergence and vigour of seedling with consequent effect on quality of seedlings produced. Inasmuch as, rooted explants should be preferred during nursery planting, nursery substrates must be well composted before use.

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