MAURITIUS RESEARCH COUNCIL

REHABILITATION OF LOCAL BANANA CULTIVARS

Final Report

January 2007

MAURITIUS RESEARCH COUNCIL

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This report is based on work supported by the Mauritius Research Council under award number MRC/RUN-0108. Any opinions, findings, recommendations and conclusions expressed herein are the author's and do not necessarily reflect those of the Council.

End of project report

Mauritius Research Council –
Unsolicited Research Grant Scheme

Rehabilitation of local banana cultivars (MRC/RUN-0108)

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January 18

Project Title:

Rehabilitation of local banana cultivars

Funding agency: **Mauritius Research Council**

Contract number: MRC/RUN – 0108

Category: Unsolicited Research Grant Scheme

Project duration: Initially 2 years 2002-2004 but extended to December 2006 on grounds highlighted in the project.

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List of abbreviations

AC	-	Activated charcoal
AREU	-	The Agricultural Research and Extension Unit
AS	-	Adenine Sulphate
BAP or BA	-	6-benzylaminopurine or 6-benzyladenine
cm	-	centimetres
DC	-	Dwarf Cavendish
Foc race 1	-	*Fusarium oxysporum* f.sp.*cubense* (Race 1)
g, kg	-	Grams, kilograms
G.(Puchooa)	-	Gingeli (Puchooa)
g/L	-	grams per litre of water
IAA	-	Indole-3-acetic acid
IBA	-	Indole-3-butyric acid
INIBAP	-	International Network for the Improvement of Banana and Plantain
mg/L	-	Milligrams per litre of water
mm	-	millimetres
cm	-	centimetres
m	-	metres
MS	-	Murashige and Skoog (1962)
NAA	-	1-naphthaleneacetic acid
PC	-	Plant crop
PN	-	Petite Naine
R1,R2	-	First ratoon, second ratoon
SE	-	Standard Error of means
t/ha	-	Tonnes per hectare

1.0 Summary

Banana is one of the most appreciated local fruit in both green and ripe form with over 500ha under production. However, yield of the dual purpose banana (Ollier and Dwarf Cavendish) is low (18 – 22t/ha) (DAS, 2000) due to production under marginal conditions while the highly priced dessert-type Gingeli banana is becoming rare due to its susceptibility to Fusarium oxysporum f.sp. cubense race 1. In this new era of agricultural diversification, banana is one of the crop that is replacing sugarcane, however the availability of quality planting material for intensive plantation remains one of the major constraints. On the other hand, to prevent the wiping out of the Gingeli banana, there was a need to rehabilitate this and other local clones. The project was thus initiated to develop a protocol for rapid mass multiplication of selected clones to make larger population available for plantation. The first part of the project consisted of identification and horticultural characterisation of elite germplasm and the second part involved protocol development for in-vitro multiplication of the selected clones.

Horticultural characterisation enabled the local dessert-type banana to be classified into two groups (the "Gingeli" and the "Mamoul" type) based on peel coloration and pulp taste and texture. Preliminary observations also indicated that Gingeli banana was highly susceptible to weevil attack.

Protocol development for in-vitro multiplication was carried out through a series of trials based on Completely Randomised Designs. All micropropagation (shoot-tip) was carried out in modified Murashige & Skoog medium.

A fast and effective cleaning and disinfection protocol of explant was developed and explant browning was successfully controlled. The study also demonstrated that any plant part (bud, peeper, sucker) could be used as explant although suckers represented handling advantages. For culture initiation, excised explants (3 – 6mm with an average of 5mm) also represented handling advantages for routine shoot-tip culture. Although freshly extracted explants are recommended, storage of uprooted suckers or trimmed explants for 2–4 days did not significantly affect multiplication rates.

The effect of varying levels of 6-benzylamino purine (BAP) was studied in Petite Naine (Dwarf Cavendish), Gingeli and Mamoul clones to determine the optimum level of cytokinin for culture initiation and for subsequent rapid multiplication. To avoid the occurrence of somaclonal variation, modified MS supplemented with BAP at 5mg/L from initiation to 5th subculture and then using BAP at 2mg/L until 9th subculture was optimum for Petite Naine with 800 shoots produced per explant. For Mamoul clones, BAP at 5mg/L was also optimum while for the Gingeli banana BAP at 8 mg/L from initiation to 5th subculture followed by BAP of 2mg/L for further subcultures produced over 800 shoots from explant after 10 subcultures. For rooting, modified MS medium devoid of any plant growth regulator or containing 1mg/L IAA was significantly superior to other auxin sources.

Low cost options for weaning and hardening of plantlets was developed. This consisted of placing plantlets in trays containing appropriate low-cost medium and covering them with transparent plastic cover for 1 week in shadehouse, with manual watering. Appropriate low-cost medium for weaning of plants developed was flyash and scum mixtures (with at least 50% flyash) or sterile soil and manure (1:1) mix, which produced significantly superior plantlets at a faster rate than any other medium. For successful weaning and to avoid transplantation shocks plantlets should be at least 1.5cm tall (although 2.5 cm tall are best) with medium filled in the tray to at least 4cm depth.

The regenerated plants are currently being field evaluated and preliminary observations indicate normal plant development.

2.0 Introduction

Banana is among the most highly appreciated local fruit both by Mauritian and tourists. During period 2004-2005, over 500 hectares (ha) was under banana with a production of 11,000-12,000 tonne (DAS, 2005). The local banana varieties include Dwarf Cavendish and Ollier (dual-purpose banana, consumed in green or ripe form) and those that belong to the dessert-type banana category (consumed in ripe form only) such as Mamoul, Mamzelle and the Gingeli type's banana. The Gingeli banana is a highly prized dessert banana which has become very scarce probably owing to its susceptibility to Fusarium wilt, caused by Fusarium oxysporum f.sp. cubense (Foc) race 1. According to Rouillard & Guého (1999), the Gingeli cultivar in Mauritius would be close to 'Sucrier', also called Figue Sucrée.

In 2001, the Southern Planters Association expressed interest to rehabilitate and mass multiply such Gingeli clone (grown by the associated growers) and other local clones. Mass multiplication will not only help in preventing the wiping out of the highly prized, yet very susceptible Gingeli bananas but also make it available to larger section of banana growing community for commercial exploitation.

The Dwarf Cavendish and Ollier banana are the most consumed banana dual purpose banana. However, the local yield is very low (20-22 t/ha) (DAS, 2005) as over 90% of local banana is produced under marginal conditions. There is growing interest of growers for intensive banana production whereby yield improvement (40-60 t/ha) can be achieved. However, the high demand for quality planting material is one of the major constraints in the setting up of banana plantations. Development of protocol for in-vitro multiplication of these varieties will be required in the rapid multiplication of selected performing stools and make the plants available to growers for setting up of banana orchards.

Aim of study:

- To characterise and rehabilitate local Gingeli, Dwarf Cavendish and Ollier banana varieties by screening for good performing clones/stools and
- Develop protocol for rapid multiplication of selected local clones through in vitro techniques.

3.0 Research Objectives

a. To identify elite mother plants of the Gingeli & other promising local clones.
b. Develop protocol for mass production of selected clones
c. Mass produce indexed elite mother plants
d. Distribute the high quality planting material to growers of Southern Planters' Association (SPA) and other targeted growers for evaluation and commercial plantations.
e. Increase yield and quality of both Gingeli Cultivar and other promising clones for local and tourist market through advice to growers.

4.0 Programme of work

4.1. Survey to identify elite germplasm

In June 2002, a survey (Collaboration Mrs Ramburn and Mrs Bunwaree) was initiated in the South to identify elite stool. Thirty stools were randomly selected and growers were verbally asked on the performance of the clone and leaf samples were taken for screening against known viruses (Banana Streak Virus (BSV), Cucumber Mosaic Virus (CMV) and the Banana Bunchy Top Virus (BBTV)). However, following the passage of cyclone Dina (January 2002), there was 100% crop loss and the survey was based solely on the perception of the clone by the grower. In year 2004 and 2005, banana clones were surveyed in fields and backyards in Mont Blanc, New Grove, Beau Climat, Calebasses, Quatre Bornes, Vacoas, Rose Hill and Belle Rose. From 2002 to 2005, over 50 stools from backyard and fields were already identified for assessment and virus indexation. However, as the bunches of clones of any specific variety were not different, suckers from only 6 were brought back to AREU research station for further characterisation. For ease of follow-up, the selected clones were named either after the owner or after the institution/place from where it was selected.

4.2. Horticultural and genetic characterisation

Genetic characterisation requires DNA profiling and collaboration sought with INIBAP & Prof. Rubaihayo (Uganda) was in vain as DNA fingerprinting is expensive. Thus, characterisation was limited to horticultural/phenotypic descriptions.

Stools identified during the survey were characterised in either of these two ways:

- Re-visit to the stool once the bunch was ready for harvest or horticultural characterisation of bananas (few hands) provided by the owner or
- Selection of sword suckers from the stool and planting in AREU Research Stations at Richelieu (RLCRS), Reduit (RCRS) and Wooton (WCRS) for agronomic evaluation.

Activity 1: Horticultural characterisation of Gingeli Moodelly

The main Gingeli clone characterised was the one provided by the SPA (named Gingeli Moodelly). In November 2002, rhizomes of the Gingeli clone were uprooted from the South and brought to RCRS where they were split and propagated for field planting as from February 2003. Forty plants and four were planted at RCRS and RLCRS respectively. In 2005, four plants of were also planted at WCRS.

Yield performance & fruit assessment

At RCRS, the performance of the Gingeli Moodelly (G. Moodelly) (Plate 1) was evaluated from plant crop (PC), to third ratoon (R3) under rainfed conditions. Salient yield features are summarised in Table 1.

Table 1: Average agronomic performance of Gingeli Moodelly at Reduit

G. Plant characterisation

Crop Cycle	Plant ht (H) (cm)	Plant girth (G) (cm)	G/H	No of leaves at E	No of leaves at H	E-H* (days)	Wt of bunch (kg)	No of hands	No of fingers
PC	251	43.3	0.2	10.1	5.7	90	9.6	6.3	84.1
R1*	358	64.1	0.2	11.0	7.0	124	15.8	8.0	113.1
R2*				11.0	8.1	83	12.5	7.5	91.1

* R1, R2 = First and Second ratoon respectively

* E-H: Interval (days) between bunch emergence (E) and harvest (H)

(b) Fruit characterisation

Crop Cycle	Finger length (cm)	Finger width (mm)	Finger circ (cm)	Fruit wt (g)	Peel thickness (mm)	Brix	Acidity	Brix/ acidity	Pulp/ Fruit (%)
PC	14.9	33.7	11.3	115	1.9	14.5	0.5	34.0	72
R1*	15.3	34.9		143	1.8	17.0	0.5	31.6	70
R2*				140					

Gingeli Moodelly flowered 290 days after planting and all the plants were healthy. Like all Gingeli plants, G. Moodelly was relatively tall but not slender (G/H 0.2). However, they were prone to pseudostem blowdown during cyclone (Plate 2). Production in first cycle (plant crop) was low (bunch weight 9.6 kg) and all bunch parameters (bunch weight, bunch size) improved in first ratoon. (Table 1). The ripe fruits developed bright yellow peel with a dry, soft textured and slightly sweet pulp (Brix/acidity of 31-34) which was very appealing. Some informal taste panellists (constituting of neighbours and colleagues) were of opinion that G. Moodelly tasted similar to the Gingeli that existed in Mauritius decades back. After ripening (at 18^{o}C, under 80-95% relative humidity with 10-100 ppm ethylene), peel splitting of the clone often occurred. This probably occurred more in fruits that were harvested slightly past the ¾ maturity stage (stage recommended for commercial harvest of banana). Ripe fruits were very prone to fruit drop (up to 100%) whereby the banana fingers got detached from the hand at the pedicel level (Plate 3).

<u>Problems associated with G. Moodelly</u>

At RCRS, in first ratoon (R1), a corky pulp (either scattered as lumps in the pulp or persistent as a corky layer) (Plate 3) was noted in bunches harvested during October – December 2004. The fruit peel and respective bearing plants did not express any peculiar symptoms. This incidence was absent in bunches harvested as from January 2005 onwards. During the same period (late year 2004) leaf yellowing symptoms was noted in some stools and gradually more and more plants, along a definite band in the field, showed these symptoms. Leaf yellowing and death started with the outer leaves followed by the collapse of the inner leaves (Plate 4-5) and splitting of the outer sheaths of the pseudostem at collar level (Plate 6). When cut across, brownish circular rings starting from the base were noted. Any association with Foc race 1 and bacterial wilt was excluded as negative results were obtained from tissues taken from symptomatic plants.

However, banana weevil borer (*Cosmopolites sordidus*) (both larvae and adult) were noted on all affected plants but the number of adults trapped was below threshold level. The damaging effect was such that only 55% of plants reached the second ratoon (R2) stage and with only 40% plant left 3 ½ year after planting. Yield parameters also regressed as from R2 (Table 1).

In a neighbouring plot (just 2m away) with var. Petite Naine, weevil adults were also trapped but no symptoms of pest attack was noted. A field is considered to be infested when the infestation index exceeds 1 **(**ARMEFLHOR, 2001). Infestation index (using pseudostems as traps) was 0.34 in the Petite Naine plot and 0.66 in the Gingely Moodelly plot. Thus, it appears that this Gingeli clone is highly sensitive to another problem (most probably weevil borer) other than Fusarium wilt which resulted in the sudden death of so many plants in such a young plot (3-4 years old) even when infestation level was below threshold. This death, due to even a slightest attack, shows the sensitivity of the Dessert type banana and this may account for their rareness.

The resulting interruption in flow of assimilates along the affected pseudostem may have caused the appearance of corky lumps. It should be pointed out that some mother plants were false-decapitated (with a square opening made at ground level to destroy the inner meristem using a sharp knife) to maximize rapid production of suckers for in-vitro works. These openings may have probably been the first point of entry for the weevil.

Phenostages

On average the bunches took 90-120 days to mature depending on the period during which the bunch developed (Table 2).

Table 2: Average flower (E) to harvest (H) interval (E-H) (days) in G. Moodelly

Period	E-H (days)
End Aug – Mid Oct 03	106
Mid Oct 03 – Jan 04	85
End Mar – End May 04	150
Aug – Mid Oct	121
Mid Oct – Feb 05	113

Temperature influenced bunch development period, which is a common feature in banana (Robinson, 1998) like in other crops. Bunches that developed during cooler periods took longer time to mature as opposed to those that developed throughout peak summer periods.

Susceptibility to other diseases

G. Moodelly was susceptible to Mycosphaerella leaf disease complex but not as Dwarf.Cavendish (Petite Naine).

Plate 1: Gingeli Moodelly

Plate 2: Cyclone damage

Plate 3 : Corky pulp

Plate 4 : Yellowing and death of outer leaves

Plate 5 : Complete wilting in G.Moodelly

Plate 6 : Splitting of outer leaf sheaths

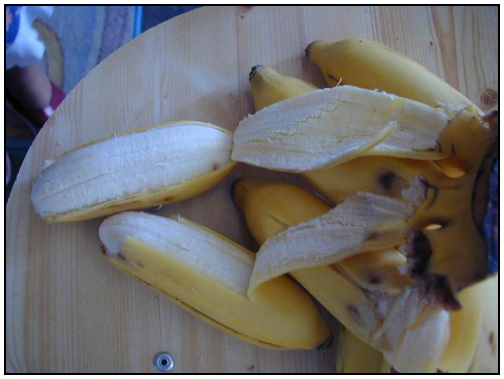


Plate 7 : Fruit drop

Performance of Gingeli Moodelly at Richelieu and Wooton CRS

Richelieu CRS (RLCRS) is a drier and hotter region (Temp: Mean max 30.0°C, Mean min 20.0°C, subhumid) than Reduit (RCRS). At RLCRS, all yield parameters were lower than at RCRS. On average, bunches were smaller (10kg v/s 9-16kg) with fewer hands and fewer fingers (71 v/s 90-113) Fruits were also smaller, less sweet (Brix/acidity ratio of 21 v/s over 30 at Reduit) but equally appealing and bunch developed within an average of 80 days after flowering. Crop performance improved in ratoon crops. No fruit corkiness or susceptibility to pest and disease was noted.
Due to late planting at Wooton CRS, only one harvest was possible. As Wooton is in a cool and superhumid zone, the plants took over 15 months to flower and the bunches were smaller (6.5 kg), due the presence of only 1-2 functional by harvest time.

Activity 2: Horticultural characterisation of other Gingeli / Dessert type clones

Other Dessert type clones characterised were either those already present in a germplasm collection at RLCRS or those that were planted after their selection for during the survey (see Section 3.0 (a)). As from 2004, suckers from some clones at RLCRS were also planted at WCRS for evaluation. There was on average 3 stools per clone and the bunches were characterised for at least 3 harvests.

Yield parameters

Gingeli Puchooa (G. Puchooa) (Plate 8a)
Suckers of this clone were uprooted from Mr Puchooa's backyard at Camp Fouquereaux and field planted at Reduit (RCRS) and Richelieu CRS (RLCRS). Out of the 2 plants planted at RCRS, one was eliminated due to presence of cucumber mosaic virus. At RLCRS, G. Puchooa performed well. On average, the plant was tall (3.0m) with narrow girth (49 cm), flowered 10 months after planting and bunches were ready after 99 to105 days at RLCRS and over 115 at RCRS (Table 3). Yield parameters improved in first ratoon with an average of 13.5kg bunches and 123 fruits compared to 11 kg bunch and 93 fruits in PC. Peel was of medium thickness (1.85mm) but peeled easily and the pulp

tasted similar to G. Moodelly. G.Puchooa was also very susceptible to fruit drop and fruit splitting (Plate 8b).

Plate 8a : Gingeli Puchooa

Plate 8b : Peel splitting in Gingeli

At WCRS, Gingeli Puchooa took longer time to develop (about 16 months to flower) and produced small bunch (6.5 kg) with only 85 fruits. On average fruits were 12 cm long with 27cm diameter and weighed 53g (Table 3). The peel developed slight sooty blotch but tasted equally nice.

Gingeli BES (G.BES)

Suckers from this clone was lifted from Barkly Experimental Station (BES) and planted at RLCRS. At RLCRS, adult plants were tall (2.8m tall) and slender (G/H = 0.15). Bunches matured faster (66 days) but produced smaller bunches (4-5 kg) than G. Moodelly and G. Puchooa (Table 3) with an average of 5-6 hands and 50-60 small fruits (75g fruit weight) like other Gingeli clones. Ripe fruits had a dry, soft texture mixed with an appreciated sweet/acid taste. Ripe G. BES was also susceptible to finger drop but with only 50% fruit drop as opposed to 100% drop in G. Puchooa and G.Moodelly.

Gingeli FUEL (G.FUEL)

Plants of this clone (taken from FUEL SE and planted at RLCRS), were also slender and tall and produced small bunches (4-6 kg) with few hands (5-6) and an average of 53 small fruits (90g). The pulp had a dry texture with a more sweet than acid taste. G. FUEL was also susceptible to fruit drop (30%). At WCRS also G. Fuel produced small bunches (4.5kg) with 5 hands and 60 small fruits.

Gingeli Savannah (G.Savannah)
Taken from Savannah SE, this clone was also tall like all Gingeli clones and produced appealing fruits with the typically soft, dry texture and sweet (acid taste). However, bunches were small (5-6 kg) with (6-7) hands and (65-90) small fruits (78g) in 2nd ratoon.

Philibert
This clone was selected from a previous germplasm collection at AREU. Philibert was equally tall (above 3m) (Plate 9) with a solid wide girth (70 cm). Bunches were relatively big (19kg) (Plate 10) with an average of 12 hands and 160 fruits at RLCRS. Ripe fruits were sweet (Table 3) and of similar texture as Mamoul. A sudden dieback of Philibert was noted at RLCRS (Plates 11) and a dissection revealed the presence of very few galleries and larva of *Cosmopolites sordidus*. This shows the sensitivity of the Dessert type banana.


Plate 9 : Philibert – bearing plant


Plate 10 : Philibert bunch


Plate 11 : Die back in Philibert

Mamoul

Clone Mamoul was selected from different zones and suckers planted at RLCRS for observations. The Mamoul 'type' clone is commonly seen in backyards. The plant is tall (above 3m) with a relatively solid girth (Table 3). At RLCRS Mamoul produced heavier bunches (18kg) and more hands (10) and fingers (120) than Gingeli clones. Fruits were straight & short as Gingeli clones (14cm long) but relatively larger (38mm wide). Ripe fruit had a firm texture (not dry/floury as Gingeli) and was sweeter than most Gingeli clones (Table 3).

Hybride Ducasse

This clone was present in the germplasm collection at RLCRS. Hybride Ducasse was also tall (above 2.7 m) with girth of 58 cm and had a high sucker producing tendency (up to 8-12-suckers/stool). Bunches looked like Mamoul and weighed on average 12 kg with 9 hands and 140 fruits (Table 3).

Table 3: Average plant and yield parameters of Gingeli clones at RLCRS (2004-2006)

	Plant height (H) (cm)	Plant girth (G) (cm)	G/H	Bunch wt (kg)	No of hands	No of fingers	Fruit wt (g)	E-H (days)	Peel thickness (mm)	Finger length (cm)	Finger diam (mm)	Fruit firmness	Pulp:fruit (%)	Brix	Acidity	Brix/ acidity
G Puchooa (PC to R2)	307.5	55.5	0.18	12.2	7.0	105.3	98.5	102	1.9	14.4	32.5	0.7	75	15.8	0.6	30.4
G BES (PC to R2)	297	49	0.16	5.1	6.1	60	75	66	1.6	13.5	31.5	0.4	78	16.8	0.50	33.6
G. Fuel (PC to R2)	300	50	0.17	5.5	6.0	53	90	-	2.1	15.4	35.1	0.5	80			28.7
G. Savannah (PC to R2)	275	42	0.15	5.7	6.0	65	78	-	2.0	11.5	31.0	0.3	78	15.6	0.61	25.6
Mamoul (PC to R2)	331	67	0.20	18.4	8.5	130	110	123	1.3	13.1	36.7	0.9	82	19.1	0.46	33.9
Philibert (PC to R2)	330	70	0.21	15.1	9.1	140	100	100	1.6	14.2	34.0		73	18.0	0.46	39.0
Hybride Ducasse	270	58	0.21	12.1	9.0	143	78	100		10.4	31.1		70	12.8	0.31	38.8
Ollier (PC to R2)	205	52	0.25	20	8.0	130	120	125	3.5	21.3	35.5		60	15.1	0.45	33.6
Local D.C	165	57	0.35	19.1	8.0	145	114	121	3.4	18.1	32.1	0.7	67	14.1	0.43	32.8
Introduced D.C (Petite Naine)	190	61	0.32	29.0	10.7	188	145	96	3.5	32.5	35.3	0.8	66	14.0	0.44	31.8

E= bunch emergence (flowering), H= Harvest; PC-plant crop, R1-first ratoon , R2-2nd ratoon, DC= Dwarf Cavendish

Bunch and fruit description

Characterisation of selected plant, bunch and fruit parameters were carried out using guidelines on 'Descriptors of banana – INIBAP' (1996). A brief outline on each parameter is as follows:

Bunch type	The bunch is fruiting part that contains the fruit. Arrangement of hands (whorl of banana fruits) along the bunch can be lax (each hands distinctly away from each other) to very compact (all hands tightly against each other)

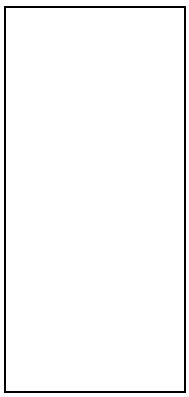
Lax bunch: One can easily place one's hand between hands of fruit

Very compact bunch: One cannot place one's finger between hands of fruit

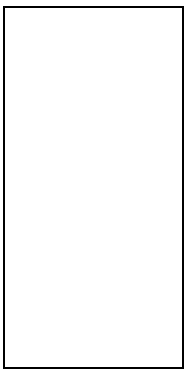
Rachis	.This consists of the stalk between the last hand (the one most below and the male bud . The rachis can be long or short. The rachis can also be :

Bare

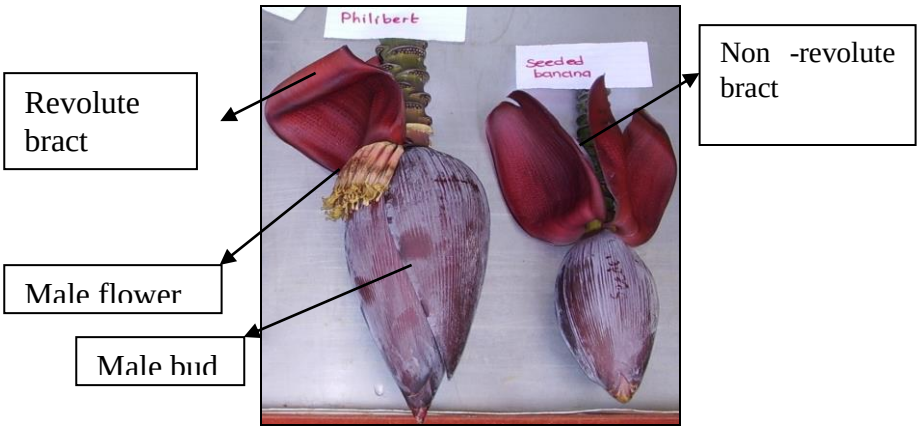
Contain only few hands of neutral flowers just below the last hand while the remaining part of the rachis is bare



Neutral flowers and presence of withered bracts (on whole stalk)



Contain only male flowers with persistent bracts in the lower part of the rachis (nearer to the male bud

Bell	This is consists of the male flowers. Each layer of male flower is enclosed with a bract. The bell dimension and shape varies with variety.  Right: A bell with non-revolute bracts and male flowers falling before bract Left: Revolute (rolling) bract
Floral relicts	These are the remains of the female flower on the apex (tip) of the banana fingers Floral relicts can fall as the fruit matures or may remain persistent (especially the style). The style may sometimes fall but its base may remain prominent.  No floral relicts at fruit apex  Persistent styles at fruit apex

Hybride Ducasse: (Table 4)

- Bunch type & position: Bunch lax to compact. Hanging at an angle to almost vertical (parallel) to the pseudostem.
- Rachis: Vertical and long (1m) and bare except for 2 small neutral hands.
- Male bud (bell): Lanceolate (20cm long, 27cm girth) with revolute bracts.
- Bracts: Moderately waxed and grooved. . Flowers: Some flowers fell after bracts. Flowers were partially persistent.
- Fingers: Silky and pale green
-

Mamoul: : (Table 4)

- Bunch type & position: Bunch compact to very compact. Vertical bunch hanging at an angle (45^{o}) to pseudostem,
- Rachis: Vertical, 30-60 cm long and bare except for 1-2 neutral hands in some
- Bell was lanceolate.
- Bracts: revolute and not persistent.
- Floral remnants: Base of style prominent.
-

Philibert: (Table 4) • Bunch type & position: Vertical bunch hanging at an angle (45^{o}) to pseudostem. Bunch lax to compact. • Rachis :Short to long (20-70 long) and bare or with 1-2 small neutral hands • Bell was medium to big and lanceolate (30cm long, 30cm wide) • Bracts were revolute and fell before male flowers • Floral remnants: Styles may remain persistent to absent; • Banana fruits silky, pale green. 	**Dwarf Cavendish/Petite Naine** (Table 4) • Bunch type & position: Vertical bunch hanging at an angle (45^{o}) to pseudostem. Bunch compact to very compact • Rachis: Vertical and short with neutral flowers and withered bracts persistent over the whole rachis. • Bell was medium to big and lanceolate • Bracts were revolute and fell before male flowers • Floral remnants: Persistent and hard styles • Banana fingers green and smooth 
Ollier (Table 4) • Bunch type & position: Compact bunch hanging at almost 45^{o} to pseudostem. . • Rachis : Long, bare and curved rachis • Bell : Lanceolate • Bracts : Rolling and not persistent • Floral remnants: Persistent styles (but not like PN) • Banana fingers :Green, smooth peel	**G. Savannah** (Table 4) • Bunch type & position: Lax to compact bunch hanging at almost 45^{o} to pseudostem. . • Rachis : Rachis bare and at an angle (a small bunch from neutral/hermaphrodite flowers was also noted in some bunches) • Bell & bract : Lanceolate bell with revolute bract • Floral remnants: Partially persistent • Banana fingers : Green , smooth peel with sweet with slight acid pulp

G. FUEL (Table 4)

- Bunch type & position: Lax bunch hanging vertically but at an angle.
- Rachis : Vertical and bare, and about 30cm long
- Bell : Lanceolate
- Bracts : Revolute and not persistent
- Floral remnants: Partially persistent styles
- Banana fingers: Green , smooth peel with sweet with slight acid pulp

G. Puchooa (Table 4)

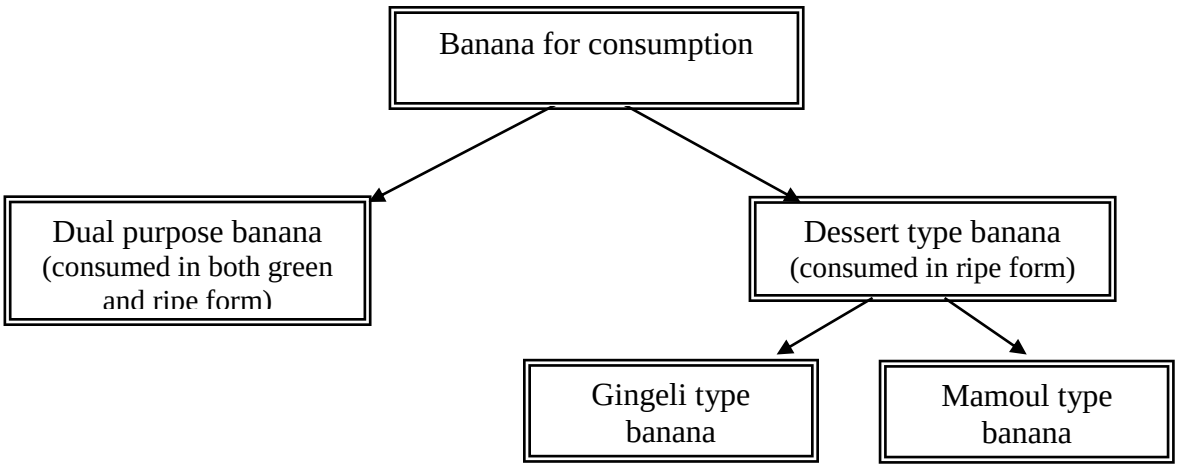
- Bunch type & position: Lax bunch hanging vertically but at an angle.
- Rachis : Long and bare except with 1-2 neutral hands just below the last hand
- Bell : Male bud lanceolate
- Bracts : Revolute and not persistent
- Floral remnants: Partially persistent
- Banana fingers: Green , smooth peel with sweet with slight acid pulp

Table 4: Bunch description of dessert-type clones.

	Bunch position	Bunch appearance	Rachis position	Rachis appearance	Bell shape	Average bell (Length, Circ), cm	Floral relicts	Rachis length (cm)	Other comments
Hybride Ducasse	Hanging at an angle	Lax to compact	Vertical	Bare rachis			Partially persistent	35	High sucker producing tendency (12 suckers at flowering)
Mamoul	Hanging at an angle	Compact	Vertical	1-2 neutral hands only to bare	Ovoid to lanceolate	(26, 27)	Base of style prominent	47	- 6 suckers at flowering stage - Fruit slightly ridged
Philibert	Hanging at an angle	Lax to compact	Vertical	1-2 neutral hands only to bare	Lanceolate	(25, 24)	Persistent	65	Bracts fall before flowers
Dwarf Cavendish	Hanging vertically	Very compact	Falling vertically	Neutral/ male flowers and withered bracts persistent along stalk	Lanceolate		Persistent style		
Ollier	Hanging vertically	Very compact	With a curve	Neutral hands present with few bracts in lower part of stalk	Lanceolate	(15, 17)	Partially persistent	53	
G. Savannah	Hanging at 45^{o}	Lax	Vertical to angled	Bare	Lanceolate	(22, 26)	Partially persistent	58	
G. FUEL	Hanging at an angle	Lax	Vertical	Bare	Lanceolate to avoid	(20, 26)	Persistent style	45	Apex lengthily pointed
G. Puchooa	Hanging at an angle	Lax	Vertical	Bare	Lanceolate	(23, 29)	Partially persistent to none	80	Had 5 suckers by flowering stage
G. BES	Hanging at 45^{o}	Lax	Vertical to slightly angled	Bare	Ovoid	(21, 26)	Persistent but drop easily after harvest	80	Bottle necked apex

Apart from Dwarf Cavendish (DC) (local) and Petite Naine (introduced), all other clones had hanging bunches with a bare and clean rachis except for 1-2 neutral hands just below the last hand. This implies that both male flowers and bracts were shed during bunch development and in most of them, the male flowers fell after the bracts. In general, all Gingeli clones (Moodelly, Puchooa etc) had fruits that were 11-15cm long and 35 mm wide (measured on the middle finger from the outer whorl of 2nd hand) and weighed 75-100g. From the above dessert-type clones a grouping was possible based on external appearance of fruit before and after ripening and pulp taste/texture (Figure 1).

Figure 1: Schematic classification of banana fruit based on external appearance and pulp taste/texture



Dessert-type Group 1-The Gingeli type's banana: Gingeli Moodelly, Puchooa, FUEL, Savannah and BES all had a green peel with no silky appearance (like D. Cavendish & Ollier) which developed into a bright yellow coloration (12C – 14C**)** (RHS –international reference) with an ivory/creamy pulp. The pulp had a typical soft, floury, dry texture with a sweet acid taste. However, the ripe Gingeli fruits were also very prone to finger drop with even a slightest jerk.

Dessert-type Group 2- The Mamoul type: This includes e.g. Mamoul, Philibert, Hybride Ducasse which were slightly bigger in size than Gingeli types, had a typical pale green peel with a silky appearance when green which became silky yellow in ripe fruits (13C or 14B) (RHS –international reference). The pulp was pale creamy 15A/155B (RHS – international reference) and had a relatively firm, texture (like Cavendish bananas) with a more sweet than acid taste. Unlike Gingeli bananas, the ripe fruits were not very susceptible to fruit drop.

Both Mamoul and Gingeli bananas had tart taste if eaten on the very day a uniform colouration was produced. They were normally more palatable after 2 days and this was maintained for an additional 4 days in winter and 2 days in summer.

The Local dual purpose bananas which include D Cavendish (the major variety grown over the island) and Ollier (commonly found in backyards, field border or in scattered plots with D. Cavendish fields) were longer (18-20cm) and slightly wider than the Gingeli and Mamoul clones (14-15cm). The performance of the introduced D. Cavendish (Petite

Naine) at RLCRS was also better (64 t/ha) (AREU, Annual Report 2005) than the average reported for island (constituting mainly of local Dwarf Cavendish, (DC)) (DAS, 2005). Apart from its shorter plant height, DC (or PN) can be differentiated from Ollier by its vertical rachis almost fully covered with neutral flowers and male flowers along with withered and persistent bracts. In Ollier, the rachis had a typical downward curve with only neutral hands on upper part and few persistent bracts (no male flowers) on the lower part of stalk.

Both D. Cavendish and Ollier had shiny green pseudostem with brown-black pigmentation. The petiole was slightly waxed pale green with extensive brown blotches at base. Similar pattern was noted in their suckers. Gingeli FUEL, G. Puchooa, G. BES and G. Savannah had shiny yellow green pseudostem with yellow-green, waxed petiole. The suckers had waxy yellow-green pseudostem and petiole. G. Moodelly had orange-yellow pseudostem and pale yellow petiole with similar wax pattern. Mamoul had a shiny pale green pseudostem with pinkish tinge. In all the clones, the bracts rolled (revolute) before falling.

Activity 3: Characterisation of local clones from backyards

Dessert-type varieties in the vicinity of Rose Hill, Quatre Bornes and Belle Rose were randomly selected and fruits from only 7 accessions were characterised with main objectives of finding clones resembling closer to the Gingeli types or which was highly appreciated by the owner. There was in general only one stool with 1-2 bearing plants and the owner provided only 1-2 hands for analysis, thus the values reported for each attribute is not an average. For ease of reference the clones were named after the area or owner.

- Dessert-clone Rose Hill (Hugnin)

Bunch size/type	-No measurement possible but looked like a Gingeli types. Owner provided one hand for analysis
Peel/pulp	-Green peel, with no silkiness, which developed into canary yellow peel and pale creamy pulp. Nice aroma with a typical dry texture mixed with sweet/acid taste.
Other attributes	-12.6cm long, 34.4 mm wide. Thin peel constituting, 25% of whole fruit. Ripe fruit not prone to fall. High Brix (18) with low Brix/acid ratio (28.6).

- Dessert-clone Sunassee (Hillcrest, Quatre Bornes)

Only one hand taken for characterisation. Fruit resembled Mamoul type bananas but had a mixed dry 'Gingeli' taste and texture of 'Mamoul'. Fruits were sweet and highly appreciated.

- Dessert -clone Badaurally (Rue Pasteur, Boundary Street, Quatre Bornes)

Bunch resembling Gingeli types banana. Peel of unripe fruit was green in colour but silkiness level very low. Only one hand provided for tasting. Ripe fruit had firm texture (like Mamoul types) with combined sweet (acid taste). (Brix/acidity of 24.6). Fruits were quite short 13cm long but wide 3.7mm wide with pulp constituting 64% of fruit.

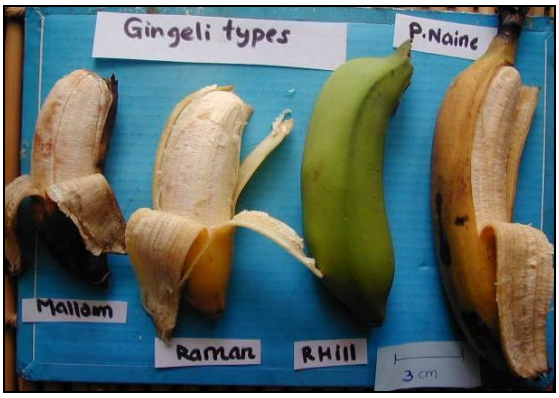


Plate 12: Comparison of Gingeli fruits form backyard and local P. Naine

- Dessert-clone Malam-Hassam (Rue Farquhar, Quatre Bornes)

Bunch appearance, peel coloration, pulp dry texture and sweet/acidulated taste all resembled to the Gingeli type bananas. The fruit was small (10cm long, 31mm wide) with a very thin peel, that peeled away easily.

- Dessert-clone Dr Raman (Morcellement Trianon, Quatre Bornes)

According to owner, this variety was brought from Rodrigues. Fruit ressembled Gingeli banana type and was small in size (10.5cm ling, 32mm wide). The peel was not very thin and underlying strands of peel remained adhered on the pulp. Fruit had nice aroma with sweet acid taste.

- Dessert-clone Ragavoodoo (Jean Le-Brun Street, Ollier)

Tall plant resembling Mamoul type clone with respect to bunch appearance, green peel coloration and silky appearance. On average fruits were 14.5cm long and 3.9mm wide and weighed 122g. Fruits were straight with intermediate thick peel (2.2mm) but which peeled easily. Pulp constituted 75% of fruit. Fruit was straight with yellow peel and pale cream pulp when ripe. Fruit was sweet (Brix/acidity of 44) with firm texture. Relicts were absent.

- Dessert-clone Somoo (Sodnac, Quatre Bornes)

Located in a field (non-irrigated plant), it was a tall plant (3.4m) with girth 57cm producing bunch (5.3kg) with 7 hands and 102 small finger. In appearance, the clone looked closer to Mamoul type.

Of all the above, clone Malam-Hassam and clone Dr Raman were more of Gingeli subgroup while clone Ragavoodoo, was distinctly of Mamoul type. Clones Somoo, Sunassee and Badaurally were somewhere between the two subgroups. None were taken for micropropagation as they were not distinctly different from the ones already in AREU-germplasm collection.

4.3. Indexation of planting material

i) Against Virus

Aim was to ascertain that stool from which any material was taken for in-vitro multiplication were healthy and virus-free. This activity was undertaken by Plant Pathology Division (AREU) (Mrs Bunwaree and Mr Lobin) for indexation of mother plants against Banana streak virus (BSV), cucumber mosaic virus (CMV) and banana bunchy top virus (BBTV) using ELISA technique. This activity was repeated randomly on plantlets mass-produced from the indexed mother plants to ensure that propagated plants were equally virus-free.

ii) *Fusarium oxysporum*

Gingeli banana is very susceptible to Foc race 1 and this activity was to test the sensitivity of all selected clones to this fungus. Although Foc race 1 is found in our soil, tissues from plants with wilting symptoms and soil around the plant did not contain any such fungus. Moreover, due to lack of technical expertise on fungal taxonomy, the isolation of the fungus was very difficult. Collaboration with the Taiwan Banana Research Institute (TBRI) was sought to provide the Foc race 1 isolates for double-tray study but was not realised due to local quarantine restrictions.

This activity will thus be undertaken under research activities within AREU programme of work whereby tissue-cultured plants of selected Gingeli clones and dessert-type bananas will be planted in open-field in different agro-climatic zones for observations.

4.4. Repository for mother plants

Indexed mother plants from performing stools were supposed to be kept in a repository (insect-proof conditions) to ensure the plants are not infected by vector for BSV or CMV during the course of selection of suckers for multiplication. Due to financial constraints, this repository was not created and the germplasm of mother plants was in the open field at Richelieu CRS. However, the plants were regularly virus indexed.

4.5. Protocol development for in-vitro propagation of local clones

Brief on problems faced during initial stages

This part of the project faced several hurdles. Preliminary works was initiated by ARS (Miss Gooriah) on the premises of University of Mauritius but as Miss Gooriah had to do everything (sucker cleaning, glassware washing and decontamination) by herself, research activities were delayed as only 2 days per week were available for in-vitro works. About 4 months were spent controlling in-vitro browning and contamination of explant. Varying levels of 6-Benzylaminopurine (BAP) (2-12mg/L) was used on modified Murashige and Skoog (MS) medium (Appendix 1) using 7mm shoot-tips. Even 3-4 months after culture

of 7mm shoot tips, no response was noted. Other medium tried were varying BAP or Kinetin levels in ½ MS and MS, MS with coconut water and Knudson medium but within the one year that Miss Gooriah worked no multiplication was noted. Contamination level was often noted (>50%) with the regular traffic in the manipulation and inoculation area. Following a correspondence with Dr Bekheet (Personal communication) large shoot sizes (8-12mm) were tried for better bud development but risks of browning and contamination was increased. Further studies were stopped between February 2004 and August 2004 when Miss Gooriah left for a permanent job.

As from August 2004, a new ARS, Miss Rogbeer provided her assistance to the project and the facilities at the Tissue Culture lab at Barkly Experimental Station (BES) were extended. After Miss Rogbeer, who left in November 2004, Miss Kallydin took over all activities as from December 2004. Thus, after almost 2 years of constraints, the protocol development aspect was reinitiated as from August 2004 and the support of laboratory attendants (posted at BES) was provided.

A number of experiments were initiated but were concentrated mainly on var. Petite Naine/D. Cavendish (dual purpose) and Gingeli Moodelly (for dessert type banana) as these clones had enough mother plants to meet the regular demand of explants (8-12 suckers/week).

<u>Preparation of medium and growth of cultures</u>
For all the experiments carried out:

- The pH of the medium was adjusted to 5.8 before autoclaving at 121^{o} C and 103 kPa for 20-30 minutes.
- Cultures were either set in test-tubes (15 cm tall) (with 20 ml medium) (at least for initiation part) or in glass jars (9.5 cm tall, 6 cm wide mouth) with metal caps (screw type). Use of transparent, polypropylene caps is recommended as this allows light penetration into the cultures, but due to lack of facilities the metal caps were used.
- The cultures were maintained under a 16h:8h light/dark cycle with light from two 36 Watt fluorescent light-tubes at 25±2^{o} C .

Culture room

Causes for loss of shoots during culture

Contamination in tissue culture may be caused by endogenous bacteria that escape initial disinfection or by microorganisms that entered into culture during tissue-culture manipulation (Strosse *et al*., 2004). Both bacteria and fungi can be sources of contamination.

Throughout the experiment, loss of explants due to contamination was very low. Loss of explants, irrespective of culture number, was mainly due to necrosis (Plate 13) (death of explants) (35-64%) (Figure 2). Bacterial and fungal contamination (Plate 14) accounted for less than 18% contamination. Medium of necrosed explants was often brown with no contamination. Bacterial contamination was often charaterised with slimy spots almost embedded in the medium and blackened explants. Fungal contamination consisted often of grey, black or white thick cover on the medium with thread-like structures (spores) on their top.

Conclusion

Explants were successfully manipulated to allow minimum contamination.

Figure 2: Causes of loss of shoots during in-vitro culture of banana

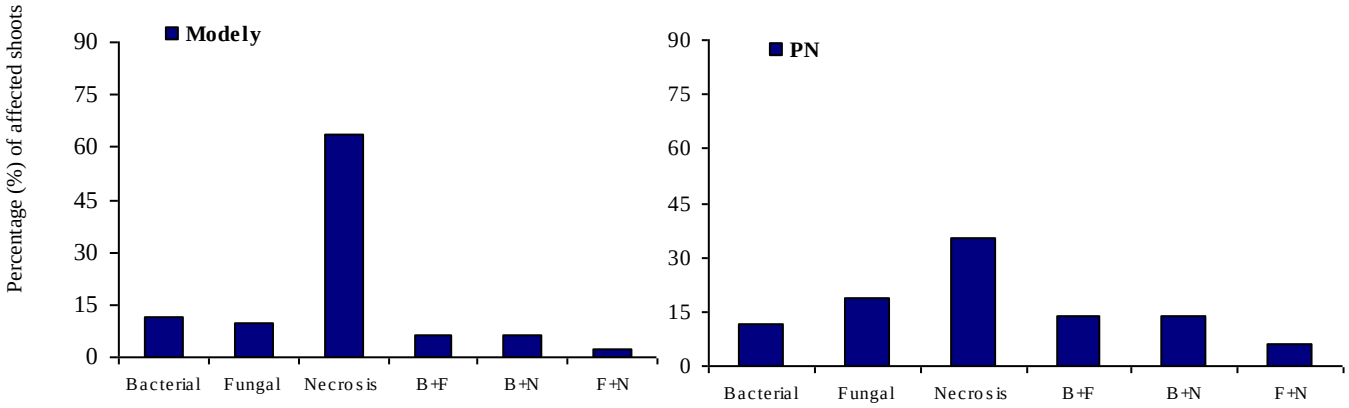


Plate 13: Necrosis

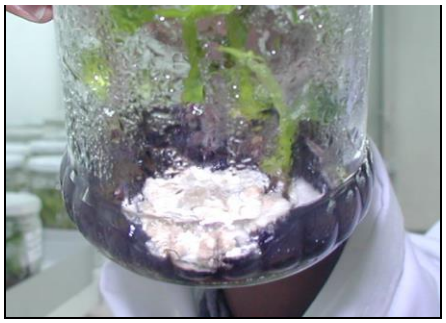


Plate 14: Fungal contamination

Activity 1: Control of shoot-tip browning/ blackening

Background

Browning or blackening of inoculated explants is a common problem in banana. This is mostly due to oxidation of phenolic compounds released from wounded tissues around the basal cut surfaces (Strosse *et al*., 2004). If left in the medium, these exudates may hinder explant development by forming a barrier and preventing nutrient uptake (Strosse *et al*.,, 2004). Antioxidants (ascorbic acid, citric acid) for washing or in the medium (10-150mg/L), dip in cysteine (50mg/L) (Strosse *et al*.,, 2004), or use of activated charcoal and polyvinyl pyrrolidone (PVP) may alternatively be used to control browning. However, a slight brown coloration around the injured surface of explant is quite normal and explant development can still occur provided that the black tissues are trimmed away during subculture (Bekheet, Pers. Comm)..

Aim

Aim of this activity was to find solutions to the browning of the explants.

Methodology

This activity was not carried out using any Experimental Design. Several random attempts were tried to control initial problems of browning of the trimmed shoot-tips, as follows:

a. Explants were dipped in ascorbic/acetic acid solution and then kept in dark at 28^{o}C for one week until the pale white shoot-tip became green.

b. The ascorbic/acetic dip was replaced by regular transfer (weekly/biweekly) of freshly initiated explant in fresh medium to prevent accumulation of the exudates. According to Vuylsteke (1998), this is the best alternative to control browning but as experience was gained over the months, the brown coloration was found not to hinder plant development and was taken as a normal phenomenon.

c. The last method tried and adopted throughout the whole experiment was by including ascorbic acid (10 mg/L) in the medium before autoclaving and using Gelrite/Phytagel (instead of agar), and keeping freshly inoculated explants in darkness for 3-4 days, until explant turned pale green.

This option saved on labour and chemicals required for the weekly transfer for the first one month following initiation of freshly extracted explant.

Activity 2: Determination of the effect of different plant parts for explant extraction

Background

All plant parts that contain shoot meristem can be used for shoot-tip culture (Vuylsteke, 1998) and these include terminal inflorescences apices (Ma *et al*., 1978) such as peeper, buds or eyes (Vuylsteke, 1998), rhizomatous off shoots (Jarret *et al*., 1985). According to Vuylsteke (1998), the physiological and ontogenic age of shoot-tips did not affect their *in-vitro* performance but buds, peepers and small sword suckers are preferred due to their ease in handling.

Aim

Aim of this study was to investigate the effect of different origin and age of rhizomatous off shoots on *in-vitro* performance of both P Naine (or Dwarf Cavendish) (PN or DC) and Gingeli Moodelly (G. Moodelly).

Methodology

Eyes, buds, peepers, sword suckers of varying sizes were used as follows for each clone:
Petite Naine (PN): Eyes or buds; small suckers (30-35 cm tall) and suckers (50-55 cm tall)
G. Moodelly: Eyes, peepers (5-10 cm), small suckers (20-35 cm), suckers (40-60 cm) and tall suckers (70-80 cm) were used, with 4 replicates per group.

The explants were cleaned and trimmed down to 5-7 mm sized shoot-tip for inoculation. Based on preliminary observations the shoot-tips were inoculated in the following medium.

Accession	Medium
PN	Modified MS + 2 mg/L BAP + 0.2 mg/L IAA
G. Moodelly	Initiation (S0) and first subculture (S1) in modified MS + 5 mg/L BAP then S2 was carried out in MS supplemented with 2-3 mg/L BAP and S3 onwards in modified MS + 1 mg/L BAP.

Observations were carried out on explants up to the 6th subculture (S6).
Parameters recorded:

- -Size of planting material for use as explant.
- -Number of necrosed explants
- -Time for shoot-tip to green.
- -Number of buds at each subculture (for computation of multiplication rates).

The experiment was laid down using a Complete Randomised Design with 4 replicates per treatment and the Analysis of variance was based on the multiplication rates of each explant after each subculture.

Results & Discussions

Irrespective of variety, there was no significant effect of type and age of rhizomatous part, used for shoot-tip extraction on multiplication rate across each subculture (Table 5). These observations agree with previous observations on other clones (Jarret *et al.,* 1985).

There was also no significant effect of source of shoot-tip on explant survival although mortality or loss of shoot tips during culture was more associated with eyes.

Due to time constraint, subculture at required time was not always possible. However, it was noted that eyes took longer time to green (more than 7 days) and the interval between first to second subcultures was relatively longer than other explants. Locating the meristematic area in the flat eyes or buds was also relatively difficult compared to those in relatively grown out structures like peepers or suckers.

Conclusion

Any rhizomatous plant part at any ontogenic age can be used for shoot tip culture. Nevertheless lifting of above ground structures such as peepers or suckers is easy as they can be easily uprooted and separated from the mother plant without causing damage to the latter. However, the possibility of putting all eyes into culture following the lifting of whole banana rhizome further increases the possible plant parts that could be used to micro-propagate a desired or rare clone.

Table 5: Effect of plant part on rate of *in-vitro* multiplication of PN and G Moodelly.

		Multiplication rate at each subculture interval				
Clone	Type of Explant	$S_0 - S_1$	$S_1 - S_2$	$S_2 - S_3$	$S_3 - S_4$	$S_4 - S_5$
PN	Eyes/Buds	0.8	9.0	2.5	1.3	1.5
	Small Sucker (30-35 cm)	1.0	6.5	1.9	2.3	1.4
	Sucker (50-55 cm)	3.0	4.0	2.0	2.5	1.2
	SE (±)	1.01	0.52	0.45	0.86	0.33
	(P≤0.05)	ns	ns	ns	ns	ns
G.Moodelly	Eyes/Buds	1.3	4.3	1.6	1.0	1.2
	Peeper (<10 cm tall)	1.5	1.0	3.0	1.1	1.4
	Small Sucker (20-35 cm)	2.0	1.0	1.6	0.8	1.8
	Sucker (40-60 cm)	1.5	1.3	1.0	2.3	1.1
	Tall Sucker (>70 cm)	1.0	1.2	2.5	1.0	1.0
	SE (±)	0.33	0.76	1.02	0.34	0.45
	(P≤0.05)	ns	ns	ns	ns	ns

n.s: non-significant at 5% level of significance
SE: Standard error of mean

Activity 3: Effect of explant (shoot-tip) size used for inoculation

Background

After surface sterilisation of blocks of tissue (consisting of shoot apical meristem enclosed by leaf primordial (6-10 overlapping leaf sheaths with axillary buds)) and a basal corm (submeristamic tissue) further trimming is usually carried out in steps to finally to reduce the tissue to size ready for inoculation. This final size of meristematic zone with basal corm can vary from 0.5 to 10 mm. The 0.5 – 1.0 mm long explants (meristem tip culture) with 1-2 leaf sheaths is recommended for elimination of virus and bacteria although they have very slow growth rate (Strosse *et al*., 2004). Larger shoot tips (3-10 mm) are used for routine micropropagation works although risks for blackening or contamination are higher. However, 5 mm long shoot tips are routinely used in banana micropropagation by Taiwan Banana Research Centre (TBRI), Indian Institute of Horticultural Research, INIBAP Transit Centre and as reported by Vuylsteke (1998) and Bekheet and Saker (1999). During the initial stages of the experiment 5-7 mm sized explants did not proliferate even 2-3 months after initiation and thus larger explants (8-12 mm), with more overlapping leaf sheaths and enclosed axillary buds were used. The larger explants developed faster but blackening and contamination was increased. It was thus important to know the optimum size of explant for *in-vitro* multiplication of local banana clones.

Aim

To determine the optimal explant size for commercial shoot tip micropropagation of PN (or DC) and G. Moodelly.

Methodology

Sword suckers (30-80 cm) were selected from field and explants (shoot-tips) of 3 sizes (1-2.9 mm, 3-6 mm and 7-10 mm tall) were inoculated in modified MS medium supplemented with BAP, IAA and myoinositol as in Activity 2. Observations were made up till the 3rd (S_3) or 4th (S_4) subcultures.

The experiment was laid down using a Complete Randomised Design with 5 replicates per treatment. Multiplication rates and other parameters for each size of explant were compared after each subculture (irrespective of clone).

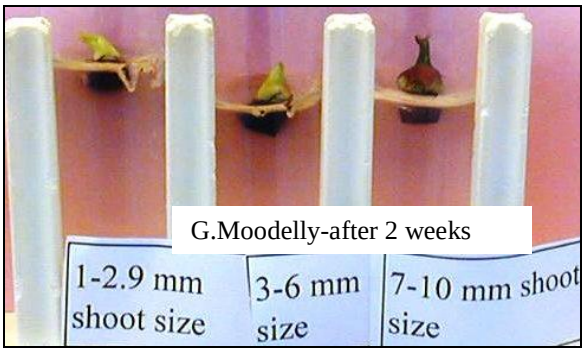
Parameters recorded:

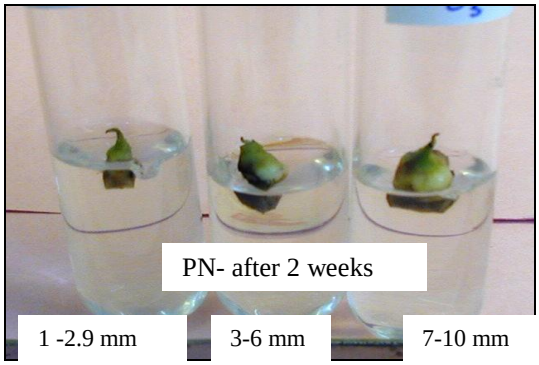
- Size of explant prior to inoculation.
- Days for pale white shoot to turn green.
- Days for explant to blacken/contaminate.
- Number of buds/shoots at each subculture.
- Number of buds per explant, number of roots.
- Length of shoots at end of subculture (measured randomly on 7-10 shoots)

Results and Discussions

All the white explants, kept in darkness turned green and proliferated after subculturing. The smallest explants (1-2.9 mm) took longer time to green (1½ weeks) and this slow growth confirms findings of Vuylsteke and De Langhe (1985). Larger explants consisting of 6-8 overlapping leaf sheaths can potentially produce multiple shoots at a faster rate (Dore Swammy *et al*.., 1983) but these are often associated with elevated blackening and

contamination risks. But, in this study, contamination was not noted and blackening did not affect shoot tip development.





Irrespective of variety, multiplication rate at each subculture was not significantly affected by size of explant used at initiation (Table 6). At end of each subculture, there was no significant effect of size of explant on number of buds per proliferating shoot (hence multiplication rate) and shoot length of subcultured explant (Table 7). The effect on the number of roots is not reported here as in all treatments roots were initiated and was not influenced by treatment.

Table 6: Effect on explant size on multiplication rate of PN & G. Moodelly

Clone	Size of Explant	$S_0 - S_1$	$S_1 - S_2$	$S_2 - S_3$	$S_3 - S_4$
PN	1-2.9 mm	0.9	2.1	1.6	0.6
	3-6 mm	1.5	1.1	2.1	1.5
	7-10 mm	0.6	1.8	2.5	1.6
	SE (±)	0.30	0.51	0.71	0.36
	(P≤0.05)	*ns*	*ns*	*ns*	*ns*
G.Moodelly	1-2.9 mm	1.5	2.3	1.6	2.5
	3-6 mm	0.8	2.5	1.5	2.5
	7-10 mm	0.8	3.8	3.6	1.1
	SE (±)	0.35	0.71	0.52	0.42
	(P≤0.05)	*ns*	*ns*	*ns*	*ns*

n.s: non-significant at 5% level of significance
SE: Standard error of mean

Table 7: Effect on explant size on the number of buds per clump and shoot length after each subculture

Clone	Size of Explant used at initiation	At 2nd Subculture		At 3rd Subculture		At 4th Subculture	
		Number of buds/clump	Shoot length (cm)	Number of buds/dump	Shoot length (cm)	Number of buds/clump	Shoot length (cm)
PN	1-2.9 mm	2.2	3.4	0.0	6.5	0.7	3.3
	3-6 mm	1.5	3.8	n.a	n.a	2.7	4.3
	7-10 mm	1.2	3.2	0.8	6.3	0.5	1.6
	SE (±)	0.71	0.71			0.72	0.71
	(P≤0.05)	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
G.Moodelly	1-2.9 mm	0.5	1.4	0.5	1.6		
	3-6 mm	1.3	2.4	n.a	n.a		
	7-10 mm	0.8	2.2	0.3	1.3		
	SE (±)	0.37	0.67				
	(P≤0.05)	*ns*	*ns*				

n.a: not applicable
n.s: non-significant at 5% level of significance
SE: Standard error of mean

Conclusion

Shoot-tip (explant) of as small as less than 3 mm and as big as 10 mm can be successfully put into culture initiation. However, slow growth associated with the smallest shoot tip (1-2.9 mm) may be unnecessary for routine rapid *in-vitro* multiplication and for practical purposes 3-6 mm sized explants can be recommended.

Activity 4: Effect of plant growth regulators on shoot/bud proliferation

Background
Plant growth regulators (PGRs) are essential media components for *in-vitro* development of all explants (Vuylsteke, 1998) but their types and concentrators depends on species, variety and ploidy level. In banana culture, cytokinin supplemented to growth medium promotes buds and shoots while auxin is used during regeneration (rooting) step. The concentration and ratio of PGRs determine growth and morphogenesis of banana tissues (Strosse *et al*., 2004) also the pattern of shoot multiplication (Patil and Singh, 1996). Of all the cytokinins, 6-benzyl aminopurine (BAP) or (benzyl adenine) is preferred (Patil and Singh, 1996; Vuylsteke, 1998; Strosse *et al*., 2004).

Trials were set up to investigate into the effect of source and concentration of cytokinin and auxin on *in-vitro* response of banana shoot-tip cultures.

Activity 4a: Effect of type and concentration of cytokinin on shoot/bud proliferation

Activity 4a(i): Effect of BAP levels on in-vitro response of Musa clones.

Aim
To determine the most appropriate concentration of the most commonly used cytokinin (BAP) (6-benzylamino purine) (also referred to as BA) for culture initiation and *in-vitro* multiplication of banana in PN, G. Moodelly and other dessert-type clones.

Methodology
Explants (shoot-tips 4-8 mm long with an average of 5 mm) freshly harvested from rhizomatous plant parts of 10 clones were initiated and later subcultured (for at least 4 subcultures) in modified MS-medium with 0.2 mg/L IAA, supplemented with different BAP levels (1, 2,4,5,6 & 8 mg/L). BAP treatments were carried out in the following clones: Petite Naine (PN), G. Moodelly, G. Puchooa, G. Savannah, G. FUEL, G. BES, Philibert, Mamoul and Hybride Ducasse.
For each clone a Completely Randomised Design was used with 3-4 replicates per BAP treatment (for G.Moodelly and PN) with at least 2 explants per replicate. Due to insufficient explants (buds/peepers or suckers) in the field, there was in general 2-4 replicates for the other clones. Any particular replicate at each level of BAP was followed throughout the series of subcultures as the same replicate. However, due to contamination in any replicate at some point resulted in the discontinuation of data collection for that replicate and this resulted in missing data. Thus it was not possible to analyse the effect of BAP on the multiplication rate for each subculture using a Completely Randomised Design. The average multiplication rate or each BAP level at each subculture was analysed using a two-way analysis of variance.

Parameters recorded:
- Time for inoculated explant to turn green.
- Number of buds/shoots after each subculture

- Shoot length and number of roots (from randomly selected 5 shoots) after second and fourth subculture.

Results and Discussions

Different cultivars respond differently to the BAP levels (Le Thi *et al.,* 1997) and also have different optimum BAP level for maximum proliferation. Although 5 mg/L (22.2 µM) of BAP is the standard for multiplication of most Musa cultivars (Vuylsteke, 1998), 0.1- 20 mg/L BAP is usually used (Strosse *et al*., 2004) as some cultivars exhibit low proliferation rates and respond only when BAP is raised above this standard level (Vuylsteke, 1998).

Varying response to BAP has been reported. Zamora *et al.*, (1985) observed a decrease in multiplication with BAP above 10 mg/L while Patil and Singh (1996) found 2.5 mg/L appropriate for a range of Musa cultivars and 2.3 mg/L BA in combination with IAA is routinely used at INIBAP transit centre (Strosse *et al*., 2004). This reflects the variability that exists among cultivars. Increasing BAP above the optimum may not only reduce proliferation rate but may cause adverse effect on morphology of culture and thus increase risks of somaclonal variation (SV) (Strosse *et al* ., 2004). To avoid occurrence of SV, high levels of BAP may be used for initial stages, this allows the explant to accumulate enough cytokinin to release it from apical dominance. Further subcultures are then carried out using lower cytokine levels. Robinson (1998) also pointed out that SV can be kept within commercial limit if a maximum of 1000 shoots are produced from one single explant.

In this trial, *in-vitro* multiplication of banana followed an exponential growth and the difference among varying BAP concentrations was more apparent as from 3rd subculture onwards.

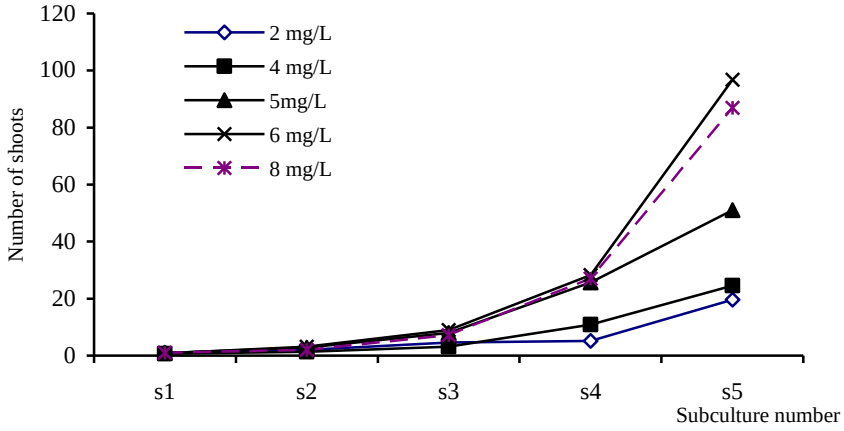
Petite Naine

Irrespective of BAP level, shoot elongation started as early as 1½ weeks subculture. Shoot development was significantly affected by BAP levels, for example 4 weeks after the 4th subculture, longest shoots were associated with 2 mg/L BAP (6cm), while in the other BAP levels, average shoot length varied between 3-4 cm.

PN produced profuse roots even in the multiplication. Medium supplemented with BAP at 2mg/L produced significantly more roots (average of 9 roots) than in the other treatments where an average of 1.8-3.0 roots was produced 4 weeks after subculture.

Shoot bud proliferation in PN was influenced by BAP although, due to insufficient replicates, this was not significant. Except for initiation to 1st subculture which took about 4 weeks the duration of subsequent subcultures was 3-4 weeks. A trend was derived based on the rates recorded at end each subculture and using one explant as starting point (Figure 3). PN responded well to the modified MS medium even at BAP 2mg/L (Plates 15a-15c), however, multiplication rate increased with increase in BAP from 2-6 mg/L while further increase in BAP above 6 mg/L did not improve bud proliferation. (Figure 3).

Figure 3 : Effect of BAP levels on number of shoots in PN (based on multiplication rates)



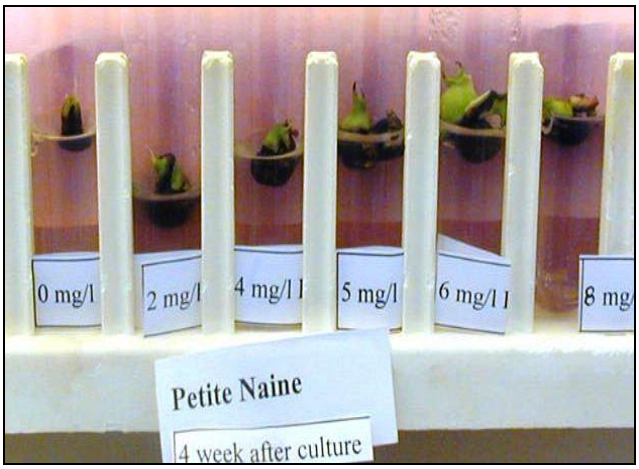


Plate 15a: Explants of var PN four weeks after inoculation in modified MS with varying BAP levels

Plate 15b: Explants of var PN after 4th subculture in modified MS with varying BAP levels

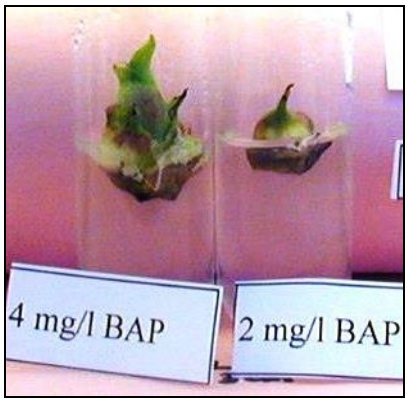


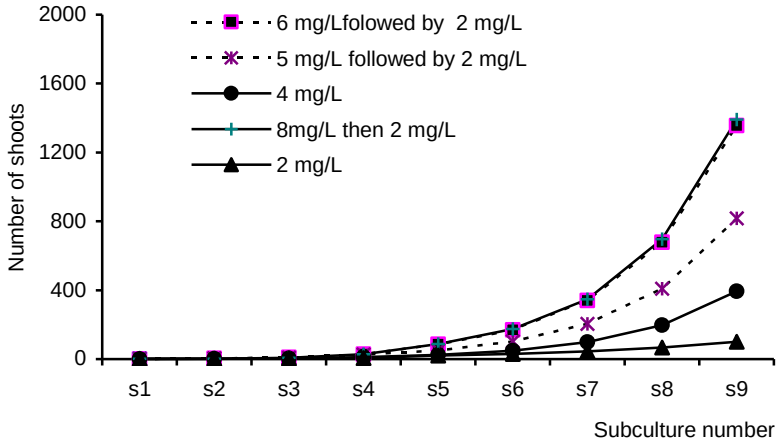
Plate 15c: Comparison of explant development in BAP at 2 mg/L v/s 4 mg/L

From Figure 3, it is evident that improved proliferation rates were associated with BAP 5 mg/L and above until 4th subculture after which BAP at concentrations 6 to 8 mg/L produced more shoots. Due to time constraint, further subcultures were not carried out and thus a 2nd trend of expected output of number of shoots per explant was made based on these observations and those from other similar trials carried out throughout this project (Figure 4).

The trend was based on the following assumptions:

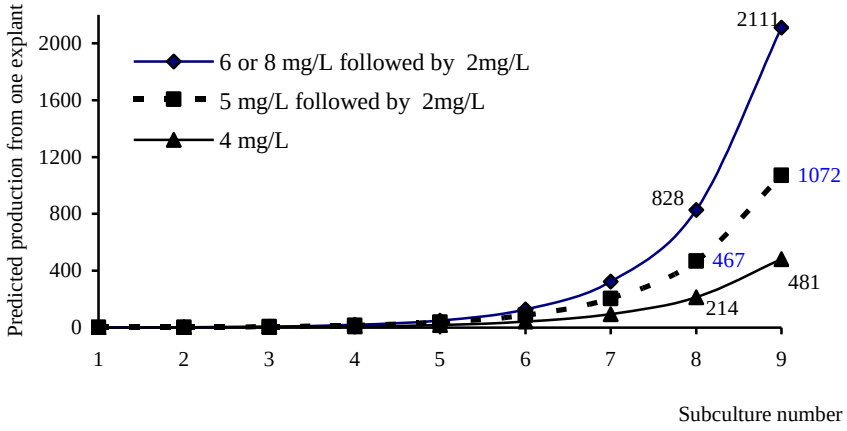
- Shoot-tip culture was initiated and multiplied at specific BAP level till 5th subculture and thereafter BAP level was dropped to 2 m/l from S6 onwards
- To avoid occurrence somaclonal variation, a maximum of 1000 shoots to be produced from one explant and number of subcultures to be less than 12.

Figure 4 : Expected shoot production in PN at varying levels of BAP



From this trend (Figure 4) an exponential model was generated (Figure 5) for BAP levels 4-8 mg/L.

Figure 5 : Model on shoot production in PN at varying levels of BAP



Note : (Fit : For 8+6 mg/L: $Y = 0.4646e^{0.9357X}$ For 5 mg/L: $Y = 0.6091e^{0.8303X}$ For 4 mg/L : $Y = 0.3291e^{0.8098X}$ with $R^2 > 0.98$ for all fits).)

Interpretation of the model:

- Supplementing modified MS medium with either 6 or 8 mg/L of BAP for 5 subcultures and thereafter using 2 mg/L, resulted in the same number of shoots. Both produced over 2000 shoots by the 9th subculture which is to be avoided.
- Using BAP at 5 mg/L for 5 subcultures then dropping it to 2mg/L, produced around 1000 shoots per explant by the 9th subculture. This BAP level appears to make the production economically viable and at the same time to cause less disturbance at the tissue level.
- With BAP at 4 mg/L the explant multiplied but this rate is not economically viable.

Conclusions

BAP at 5 mg/L is commonly used for micropropagation of banana (Vuylsteke, 19980) to promote bud proliferation. In this study with BAP at 5mg/L up to 1000 shoots could be safely produced from one explant of var. PN after 9 subcultures (within 30-37 weeks). However, in case 6 mg/L BAP is available it can be used for multiplication of PN only before the 4th or 5th subculture. Moreover, 6 mg/L BAP can be used provided that a maximum of 8 subcultures is carried out to avoid all risks of somaclonal variation

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Gingeli Moodelly

The response of G. Moodelly (probably of AAB genome due to its resemblance to Silk banana) to BAP levels was different from that of PN (AAA genome). While PN multiplied in medium supplemented with even 2mg/L BAP, G.Moodelly responded better to high BAP levels (although not significant for same reasons as in PN) (Plate 16). Moreover, while on average PN took 3-4 weeks for each subculture, G.Moodelly took 6 weeks to respond.

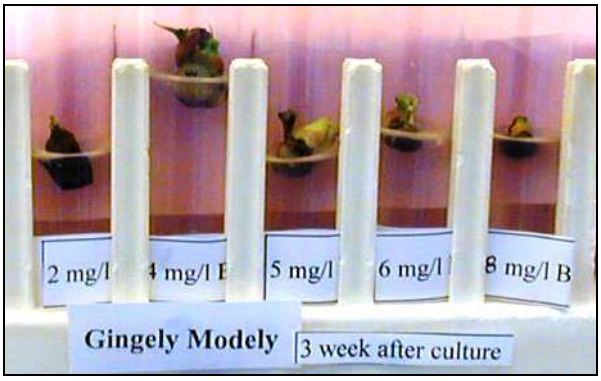


Plate 16a: Response of G.Moodelly under varying BAP levels 3 weeks after culture

Plate 16b: Response of G.Moodelly under varying BAP levels-6 weeks after culture

Shoot length was, in general not significantly affected by varying BAP levels and in general shoot length was on average 2 cm and 5 cm, four weeks and ten weeks after subculture respectively. Unlike PN, G.Moodelly did produce profuse roots and also there was no significant effect of BAP on root formation which on average ranged from 0 to 3 within 7 weeks after subculture.

Like in PN, due to the exponential relation between shoot production and time, the response to BAP became more evident as from S3 (3rd subculture) onwards (Figure 6). From S3 onwards there was linear trend in multiplication rate and BAP levels (Figure 6).

Figure 6: Evolution in shoot production in G.Moodelly with respect to number of subcultures (S0-S5) at varying BAP levels

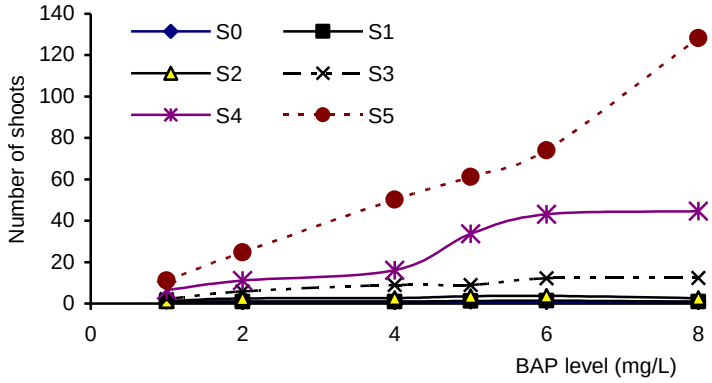
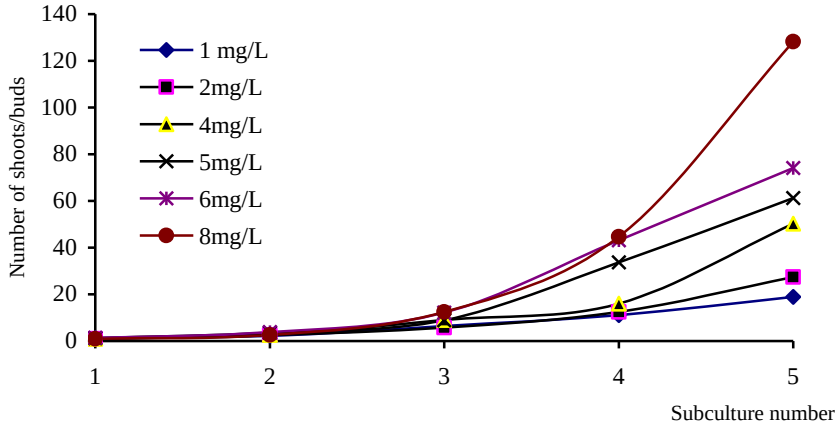
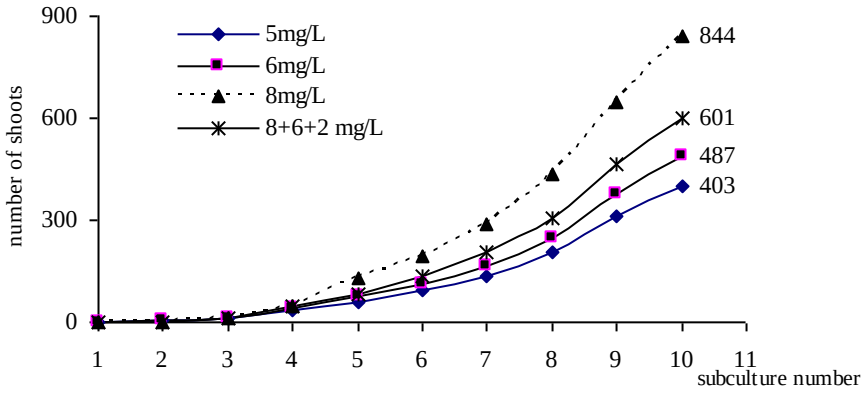


Figure 7: Expected shoot production in G. Moodelly at varying levels of BAP



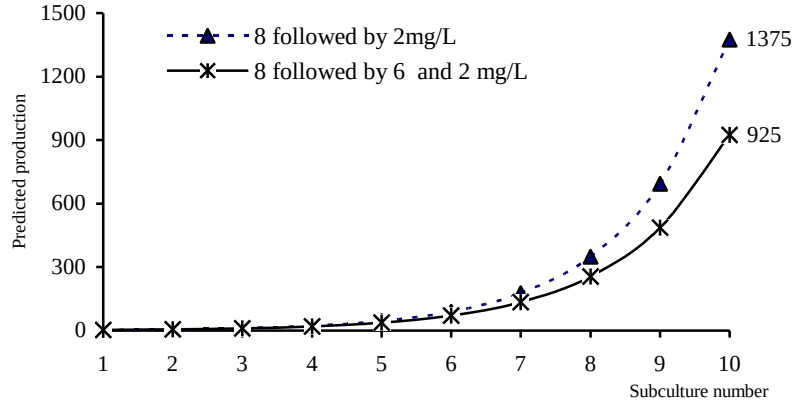
Based on multiplication rates a trend was extrapolated with single explant as starting point (Figure 7). Although non-significant it is clear that G.Moodelly responded better to BAP above 5mg/L. A further trend was extrapolated based on results from 5,6 or 8 mg/L BAP for 5 subcultures and then using 2 mg/L BAP for next subcultures for multiplication purpose. A combination of 8 mg/L for 3 subcultures followed by 6 mg/L for next two subcultures and then using only 2mg/L was also extrapolated (Figure 8). From Figure 8, it is clear that BAP 8 mg/L lead to more proliferation than even 6 mg/L alone and a combination of 8 and 6 mg/L slightly improved the performance of 6 mg/L BAP alone.

Figure 8: Extrapolation of results to estimate of shoot production in G. Moodelly



Like for PN, a model was fitted on trend observed in Figure 8 and is represented in Figure 9. Since 0.1- 20 mg/L BAP is usually used (Strosse *et al*., 2004), using 8 mg/L for 5 subcultures appeared within range and thus from the model (Figure 9) it can be concluded with 8 mg/L up to 690 and 1300 shoots can be safely produced by the 9th and 10th subculture respectively.

Figure 9: Model on shoot production in G. Moodelly at varying levels of BAP



Note: (Formula for 8 mg/L: $Y = 1.4525e^{0.6853X}$; for 8 then 6 mg/L: $Y = 1.4897e^{0.6431X}$ with $R^2>0.9$ for both)

It was noted that at high BAP levels the proliferating shoots produced clumps initially which then developed into shoots with pointed leaflets but they became normal once the BAP level was reduced. At this stage, since these plants have still not been field planted, it can not be said if these plants are true-to-type but since the BAP used was just slightly above the standard it appears that the response was quite normal.

Other Clones*: *Banane carré, G. Puchooa, G. Savannah, G. FUEL, G. BES, Philibert, Mamoul and Hybride Ducasse.

Note: Due to time constraint, readings were taken on number of buds only after initiation, 1st and 2nd subculture only. However, based on observations on PN and G.Moodelly, the effect on multiplication rate became more evident as from S3 onwards. Thus, observations are still being maintained although this project is over.

Results

Banane carré was successfully multiplied in MS with 4 mg/L BAP with 87 plantlets produced from 7 explants after 3 subcultures. No further trials were done (Plate 17a) as this clone was received during late stages of project and there were not enough suckers to represent treatments of this trial.

For the other clones:

Based on observations made after 3 subcultures, there was no significant effect of BAP level on the multiplication rate of the above dessert types clones except in G.Puchooa after 2nd subculture (Table 8). However, despite an overall non-significant effect of BAP on multiplication rate of the several clones, there is a trend for the multiplication rate to increase with increase in BAP (Figure 10). There was no significant difference of BAP levels on bud length.

Table 8: Effect of BAP levels on average multiplication rate from 2nd to 3rd subculture

Clone	BAP level (mg/L)					SE (±)	($p \leq 0.05$)
	2	4	5	6	8		
G. Puchooa	1.7^{c}	2.1^{b}	3.5^{ab}	3.3^{a}	**4.8^{a}**	0.51	*
G.BES	3.1	3.1	1.3	3.3	3.5	0.76	ns
G. Savannah	2.1	3.3	1.3	3.0	2.4	0.64	ns
G. FUEL	4.2	4.5	2.3	4.0	4.9	1.10	ns
Hybride Ducasse	2.8	4.1	2.4	1.8	2.0	0.65	ns
Philibert	1.5	1.5	2.0	1.8	2.0	0.42	ns
Mamoul	1.8	3.0	1.0	1.9	1.7	0.49	ns

Figures with same superscript along the row not significantly different at 5% level
n.s: non-significant at 5% level of significance
SE: Standard error of mean

Clones that belonged to the 'Gingeli group' (G.Savannah, G.Puchooa, G.BES, G.FUEL) responded better to varying BAP levels with multiplication rate after 2nd subculture ranging from 1.3 to 4.9 while accessions belonging to the 'Mamoul group' (Philibert, Mamoul, H.Ducasse) responded poorly to culture conditions and also there was a decrease in multiplication rate with BAP above 5 mg/L (Figures 10 &11).

Figure 10: Multiplication rate of Gingeli and Mamoul group after initiation and 2nd subculture.

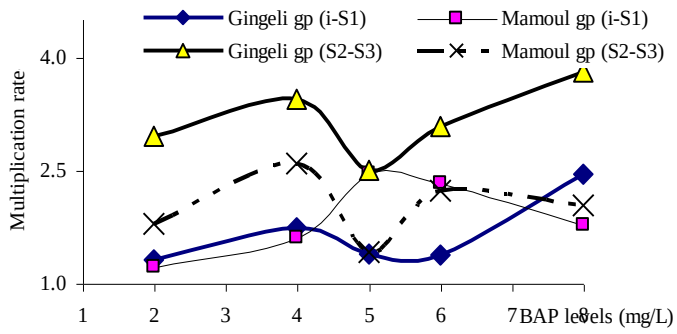
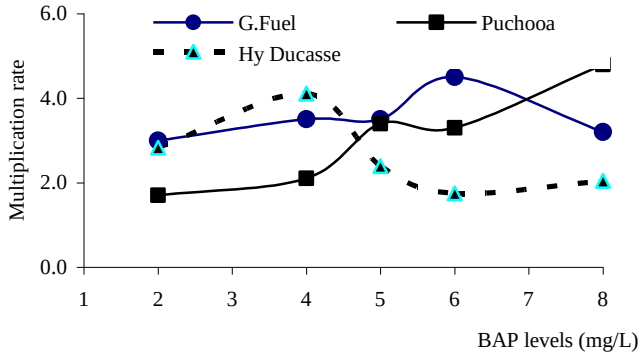
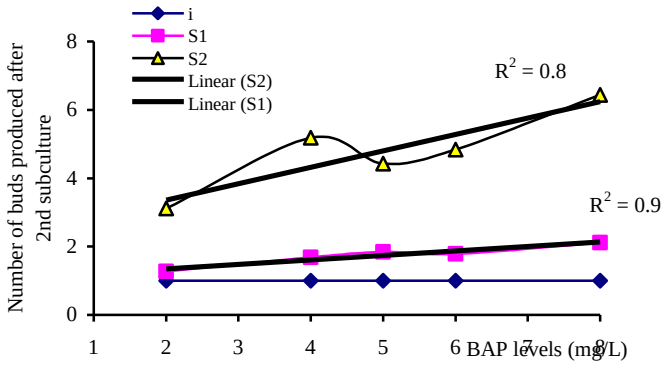


Figure 11: Effect of BAP on multiplication rate of selected Gingeli and Mamoul types banana



An expected bud production computed on multiplication rates and with one explant as the starting point is represented in Figure 12. In the Gingeli group there is a linear relation of number of buds and increasing BAP levels during the first 3 subcultures.

Figure 12: Expected shoot production in Gingeli clones at varying levels of BAP



Conclusion:

Although the Gingeli and Mamoul banana took longer time to react to the medium (Plate 17b), multiplication rate in accessions belonging to the Gingeli group increased with increase in BAP level. Thus like G. Moodelly these clones should preferably be initiated and multiplied for about 5 subcultures in modified MS supplemented with 8 mg/L BAP. During multiplication phase the Gingeli banana had a tendency to produce clumps of cormlets (Plate 17b) but once the BAP was lowered the plantlets were normal. Clones belonging to the Mamoul group should preferably be cultured in MS with 4 or 5 mg/L.

Plate 17a: Banane carré in 4 mg/L BAP

Plate 17a: Comparison of Mamoul, Gingeli, banane carre and PN in MS with 4 mg/L BAP

Activity 4(a)(ii): Effect of adenine sulphate on shoot multiplication

Background

In banana culture, adenine sulphate (AS) is not essential but may be added to culture medium to supplement other sources of cytokinin (especially kinetin). In the presence of BAP, AS have either not contributed significantly to shoot-bud proliferation (reported by Vuylsteke, 1998) or provided synergistic effect in other Musa spp. (Mante & Tepper, 1993).

Aim

To investigate into effect of different concentrations of AS supplemented to modified MS medium containing 2mg/L BAP, on shoot proliferation and development.

Methodology

Proliferated tissues of var PN from the same multiplication stage and acclimatised in modified MS medium supplemented with 2 mg/L BAP for 1 subculture were subcultured in same medium supplemented with varying levels of AS (0,40,60,80 & 160 mg/L). Readings were taken after 2 subcultures. The experiment was laid out in a Completely Randomised Design with 4 replicates per treatment.

Parameters recorded:

Number of buds/shoots and shoot length before and after treatment.

Results and discussions

PN which normally responded well to modified MS supplemented with even 2 mg/L BAP failed to multiply in the presence of AS. Thus, AS did not contribute to shoot-bud proliferation when added to medium containing BAP (2mg/L).

Vuylsteke (1989) also found the non-significant effect of AS on shoot-bud multiplication in the presence of BAP while Ma and Shii (1972) found AS to be conducive to proliferation only in the presence of kinetin.

Conclusion

In the presence of BAP there was no conducive effect of adding adenine sulphate to the multiplication medium.

Activity 4(b): Effect of auxin on rooting of banana shoots

Activity 4(b) (i): Use of varying sources of auxin

Background

Individual shoot or shoot clumps need to be put in a medium that stimulates root formation without shoot proliferation. Rhizogenesis is promoted by auxin which is normally included in the medium as IAA (indole-3-acetic acid), NAA (1 - naphthalene acetic acid) or IBA (indole-3- butyric acid) at the rate of 0.1 to 2 mg/L (Strosse *et al*., 2004). The auxin included should allow shoot elongation and rooting only.

Aim

To find the best alternative for shoot elongation, leaf formation and root formation to produce healthy rooted plantlets

Methodology

Shoots of both PN and G. Moodelly from the same multiplication stage (MS + 2 mg/L BAP) were randomly selected, roots were removed and shoots were trimmed to a produce 10-12 mm tall explants. Four treatments (modified MS devoid of plant growth regulators (PGR), modified MS medium supplemented with NAA, IAA and IBA, each at 1 mg/L of water) were carried with 5 replicates per treatment and 5 explants per replicate. Experiment was laid in a Completely Randomised Design

Four weeks after subculture, the following parameters were recorded:

- Shoot length, number of leaves and number of roots per explant
- Length of longest root (on 5 randomly chosen roots) was also measured at hardening time.

Results and discussions

An ideal banana plantlet should have a well developed shoot (pseudostem) and enough leaves and well-developed roots that will enable the plantlet to undergo weaning successfully once deflasked and transferred into trays for hardening.

Shoots elongation and leaf formation was noted in all medium. The roots started to develop 1½-2 weeks after subculture and the production and development of roots increased with time in culture. However, there was significant effect of type of auxin or even absence of auxin on all parameters (Table 10 &11).

In G. Moodelly, the number roots was significantly more (>10) in MS + NAA or IBA than in MS medium devoid of any hormone (3 roots) but in the latter they were well developed and relatively long (26 cm) compared to the compact, thin roots (4-6 cm long) noted plantlets regenerated in NAA or IBA. Moreover, with NAA and IBA, the shoots were significantly shorter (3-4 cm), with 2-3 leaves compared to hormone-free medium or MS supplemented with IAA (6-7 cm tall and 4 leaves).

Vuylsteke (1998) found that NAA (0.2-1.0 mg/L) was more effective and reported that it was preferred than auxin for shoot elongation and rooting. However, in PN and G. Moodelly shoot elongation was lowest in basal medium supplement with NAA.

Similarly, the type of roots, shoot length and number of leaves were significantly superior in MS with either no auxin or with IAA (Table 9 &10) (Plates 18a-c). Although the number of roots in the zero-hormone was half than those in NAA, they were individually more developed and 5 roots were enough for the plantlet to be hardened.

IAA, IBA and NAA are commonly used to initiate rhizogenesis (Strosse *et al*., 2004 but INIBAP uses IAA while Bekheet and Saker (1999) found that NAA and hormone-free medium produced significantly better rooted plantlets. In this trial, the positive response of both PN and G. Moodelly to MS medium devoid of any plant growth regulator thus indicates that, although IAA was found to be the best auxin for regeneration of rooted banana plantlets, rooting without the need to purchase the expensive plant growth regulators is a low-cost measure.

Table 9: Effect of varying source of auxin on rooting of PN and G.Moodelly

(a) Clone G.Moodelly

Rooting treatment	Average number of roots	Average shoot length (cm)	Average number of leaves	Description of roots
MS + IAA	8.8^{a}	7.4^{b}	3.8^{a}	All individual and well developed roots.
MS + NAA	10.0^{a}	3.9^{a}	3.4^{a}	Thin roots clustered into a mass.
MS + IBA	10.4^{a}	3.0^{a}	2.4^{b}	Thin roots clustered into a mass.
MS–no PGR	3.4^{b}	6.6^{b}	4.4^{a}	All individual and well developed roots
SE (±)	0.94	0.43	0.39	
(P≤0.05)	*	*	*	

(b) PN

Rooting treatment	Number of roots	Shoot length (cm)	Number of leaves
MS + IAA	10.4^{a}	9.5^{a}	5.0^{a}
MS + NAA	6.2^{b}	4.2^{c}	3.4^{c}
MS + IBA	10.0^{a}	6.9^{b}	4.6^{b}
MS–no PGR	5.8^{b}	9.3^{a}	5.8^{a}
SE (±)	1.02	0.49	0.25
(P≤0.05)	*	*	*

*: significant at 5% level of significance
Figures with same superscript along the row not significantly different at 5% level.
SE: Standard error of mean

It should however, be pointed out that banana cv PN produced normal looking shoots, leaves and individual roots even during multiplication phase. Moreover, although shoot

elongation in multiplication phase is less pronounced (3-6 cm) than those promoted in rooting medium (4-8 cm) the shoots can be successfully hardened. Hence, for PN the rooting step can even be by-passed and complete rooted plantlets from late multiplication phases can be selected and hardened straight away. During on-going of works it was noted that rooted plantlets with for example 3 well developed roots underwent hardening as successfully as plantlets with more than 10 roots. This can probably be due to inherent ability of plantlet to produce further roots once the aerial parts (pseudostem & leaves) are healthy and fully functional.

Table 10: Average plantlet parameters of rooted plantlets just prior to hardening.

	G. Moodelly			PN	
Rooting treatment	Number of roots	Shoot length (cm)	Length of Longest root (cm)	Shoot length (cm)	Length of Longest root (cm)
IAA	8.1	7.8^{a}	15.5^{b}	10.0^{a}	7.3^{bc}
NAA	12.0	3.5^{b}	4.0^{c}	7.3^{b}	5.8^{c}
IBA	12.1	4.0^{b}	6.2^{c}	11.0^{a}	8.8^{b}
MS + no PGR	6.1	8.0^{a}	26.5^{a}	9.5^{a}	20.0^{a}
SE (±)	0.50	0.28	5.26	0.84	0.74
(P≤0.05)	*	*	*	*	*

*: significant at 5% level of significance

Figures with same superscript along the row not significantly different at 5% level.

G. Moodelly, unlike PN, did not often produce roots during multiplication phase and the leaves were also more elongated. However, once exposed to either MS with lower BAP levels (1 mg/L or less), in rooting medium supplement or even hormone–free medium, the regenerated plantlets looked healthier.

Thus, in both Gingeli and PN the rooting step can be by-passed.

Plate 18a: Effect of source of auxin on rhizogenesis

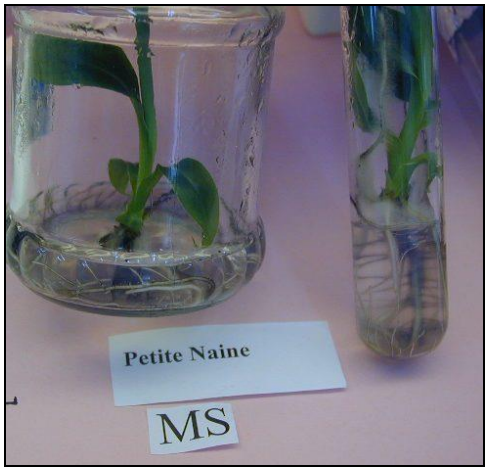


Plate 18 b: normal rooted plantlet in modified MS with no auxin

Plate 18c: Effect of source of auxin on rhizogenesis in G. Moodelly

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Activity 4(b) (i): Use of activated charcoal and varying strength of MS

Background

As IAA was found to be the best source of auxin for rooting, its performance was compared with activated charcoal (AC) and MS at half concentrations (½ MS).

Aim

To find other rooting alternatives for the banana shoots.

Methodology

Explants of 10-12 mm tall (of PN and G. Moodelly) were inoculated in modified MS devoid of PGR, on modified MS medium supplemented with IAA (at 1 mg/L of water), ½ modified MS (½ MS) and modified MS supplemented with AC (5g/L). Trial was laid down in a Completely Randomised Design with 5 replicates per treatment and 5 explants per replicate.

Parameters recorded

After 1 month, readings were taken on shoot length, number of leaves, number of roots and length of longest root (on 5 randomly chosen roots)

Results and discussions

In PN, there was no significant effect of treatment on shoot length (4-6 cm) and number of leaves (4-6) produced per rooted plantlet, but both the number of roots and root length was significantly reduced in AC (Table 11). In G. Moodelly, shoot development was affected but this was not significantly different from other treatments. Similarly, root development was affected in MS supplemented with AC. Although there was no significant difference between MS and ½ MS, it should be noted that shoots regenerated in the latter medium were thinner (no readings taken).

Table 11: Effect of auxin and activated charcoal on average rooting and shooting parameters of banana plantlets

	Treatment	Shoot length (cm)	Number of leaves	Number of roots	Root length (cm)
Petite Naine	MS + IAA	4.3	6.0	12.3[a]	8.9[a]
	MS (no PGR)	4.4	5.7	11.3[a]	5.5[a]
	Activated charcoal	4.3	4.3	3.7[b]	1.3[b]
	½ MS	6.1	5.0	9.7[a]	3.0[b]
	SE (±)	0.90	1.07	2.47	0.76
	(P≤0.05)	ns	ns	*	*
G. Moodelly	MS + IAA	3.7	3.5	3.0	7.4[b]
	MS (no PGR)	6.5	4.5	4.0	3.1[a]
	Activated charcoal	1.6	2.0	2.0	7.9[b]
	½ MS	1.9	2.0	2.0	
	SE (±)	0.57	0.35	0.71	0.63
	(P≤0.05)	ns	ns	ns	*

*: significant at 5% level of significance;
ns: not significant at 5% level of significance
SE: Standard error of mean
Figures with same superscript along the row not significantly different at 5% level.

Conclusion:
Rooting can be successfully carried out in MS medium devoid of any PGR.

Activity 5: Effect of wounding on shoot proliferation

Background
Throughout the project, during initiation or subculture, explants were trimmed to remove shoots, superfluous corm tissues (decapitation) to produce individual (5 mm) shoot tips. It has been reported that decapitation is not essential for multiple shoot initiation and that vertical cuts or splitting of explants is preferred (as reported by Vuylsteke, 1998), to promote lateral bud development.

Aim
Aim was to assess the effect of wounding of the excised shoot-tip on shoot/bud proliferation

Methodology

Proliferating shoots were randomly selected, trimmed to remove shoots and superfluous corm tissues to produce individual 5-7 mm shoot tips. Trial was laid down in a Completely Randomised Design with 3 treatments and 4 replicates per treatment.

Treatments were:

- Shoot-tip wounded in a single longitudinal slit leaving base intact (vertical incision).
- Shoot-tip split into halves by a vertical cut through the apex down the base (fragmentation).
- Intact shoot-tip (i.e. only trimmed on all 4 sides and on top) (trimming).

The shoot-tips were transferred in modified MS-medium supplemented with 2 mg/L BAP and 0.2 mg/L IAA. Observations were taken after only one subculture.

Parameters noted were: Explant survival, time for explant to green and number of buds produced per explant after each subculture

Results and discussions

Proliferation in banana can be promoted by including cytokinin in the growing medium. However, Vuylsteke (1998) pointed out that effect of apex incision (wounding) had almost same the effect on proliferation rate as increasing cytokinin levels. Wounding reduces apical dominance (Vuylsteke, 1998) and this can be a solution to poor shoot proliferation.

In this study, there was no significant effect wounding on *in-vitro* response of the explants. Explants split longitudinally did not produce significantly more bud than trimmed explants. Moreover, blackening was more associated with fragmented explants.

Although the longitudinal split (shoot-tip fragmentation) is the most effective technique (Jarret *et al*., 1985*),* in this study the outer leaf sheaths grew in size quite fast and overarched without producing more buds. Dore Swamy *et al*., (1983) also found no significant difference between intact or quartered shoot-tips. Adaoha Mbanaso *et al*., (2006) also found that although fragmentation may be used to inexpensively increase proliferation, it impaired the ability of the explants to survive. It could be probable that bud proliferation from the intact shoots was the maximum the clones could produce.

Conclusion:

There was no advantage of wounding or fragmenting excised shoot-tips on multiple shoot formation.

Activity 6: Effect of storage of mother plant and explant on response to in-vitro culture

Background

It is preferable that all tissue-culture works are carried out on freshly extracted explants. However, due to some constraints, immediate inoculation may not be possible and storage of mother plant or extracted explants may be an option.

Aim

To investigate into effect of storage of either the mother plant (suckers) or explant (extracted from mother plant) on multiplication rate of banana.

Activity 6 (i):Storage of suckers

Methodology

Three intact uprooted suckers of var. G. Moodelly and P. Naine were kept at room temperature for 2 and 4 days prior to initiation on modified MS + high levels of cytokinin (4-8 mg/L). Three subcultures were carried out. Trial was laid down in a Completely Randomised Design with the 3 treatments (0, 2, 4 days of storage).

Parameters noted were:

- Explants survival
- Time for explants to green
- Occurrence of browning of explants,
- Number of buds/shoots produced per explant at each subculture.

Results and discussions

Explants excised from stored suckers underwent more browning and took longer time to green (extra 4-5 days) than those from freshly uprooted suckers. However, after the first subculture (S_1) the intensity of browning was significantly reduced. As from S_1, all the explants from fresh suckers and stored suckers responded similarly and had similar multiplication rate (Table 12).

Table 12: Multiplication rate in PN following storage of uprooted suckers

	Multiplication rate across each subculture				
Storage (days)	$S_0 - S_1$	$S_1 - S_2$	$S_2 - S_3$	$S_3 - S_4$	$S_4 - S_5$
Day 0	0.9	3.0	2.8	3.2	2.0
Day 2	0.8	4.3	1.9	3.8	-
Day 4	1.0	3.0	3.0	5.0	2.5
($P \leq 0.05$)	ns	ns	ns	ns	-

*: significant at 5% level of significance; ns: not significant at 5% level of significance
Figures with same superscript along the row not significantly different at 5% level.

With subculture confounded, there was also no significant effect of explant storage on multiplication rate, which ranged from 2.5 -3.0 (SE ± 0.3). In all the 3 treatments multiplication rate increase significantly from 1.0 to 4.0 by the 3rd subculture.

Activity 6 (i):Storage of trimmed suckers

Aim

Aim was to investigate whether cool stored (18^{o}C) trimmed suckers could be subjected to *in-vitro* culture.

Methodology

Freshly uprooted suckers were chopped and outer leaves were removed until 10 cm tall explants were obtained. These explants were washed in running water and detergent for 30 minutes then trimmed to produce 5 cm tall explants. These trimmed explants were stored at 18^{o}C (refrigerator) for 2 days prior to further surface sterilisation and inoculation in MS with BAP (4 mg/L) and 0.2 mg/L IAA. There were 3 replicates per treatment and observations were carried during 3 subcultures.

Parameters noted were:

Explant survival, time for explants to green, intensity of browning and number of buds after each subculture

Results and discussions

Further trimming of stored explant was relatively difficult as the explants lost turgidity during the 2 days storage. Stored explants underwent more browning and took longer time to green than freshly inoculated ones. However, the proliferation rate was not significantly better in fresh explants (Table 13).

Table 13: Multiplication rate of fresh and stored trimmed explants

Type of Explants	**$S_0 - S_1$**	**$S_1 - S_2$**	**$S_2 - S_3$**
Fresh	0.8	2.9^{a}	2.7
Stored (2 days)	2.3	1.2^{b}	3.5
SE (±)	0.47	0.38	0.22
(P≤0.05)	ns	*	ns

*: significant at 5% level of significance; ns: not significant at 5% level of significance
Figures with same superscript along the row not significantly different at 5% level,
SE: Standard error of mean

Conclusion

Once trimmed explants should preferably be immediately cultured. However, in event that this is not possible, they can be stored provided. Storage of explants (at least 5cm tall) is an alternative possibility. Storage of explants render them soft (due to loss of turgidity) making further trimming difficult.

Activity 7: Cleaning – Sterilisation Step

Background

The first step to achieve successful *in-vitro* propagation of banana is the establishment of aseptic culture of explants. This involves cleaning of explant (e.g. sucker) careful removal of block of tissue containing apical meristem, surface disinfection (in Benlate and bleach) following aseptic excision of explant. Variants of the above sterilisation procedure prior to excision of explant exist depending on origin of field material used for TC works. The type of sterilisation procedures directly influence duration for preparation of explant and gain in time implies less time spent on unnecessary sterilisation procedures.

Aim

To establish a fast and effective cleaning and disinfection of explants for shoot-tip culture of banana.

Methodology

Trial was set in a Completely Randomised Design with 4 sterilisation treatments (Table 14) and 4 replicates per treatment. All treatments were performed on same day and explants were inoculated and left in same medium (MS + 5 mg/L/BAP) for 4 weeks.

Parameters noted were:

- Explant size prior to inoculation and 4 weeks after inoculation.
- Explant coloration score based on the following arbitrary scale at weekly interval
  Black (score 1), Brown (score 2), White Brown (score 3), White (score 4), Light green tinge (score 5), Light green (score 6), or Green (score 7);
- Browning of medium, score based on the following scale
  No browning (original) colour of medium (score 1)
  Browning limited to 5 mm radius of explant (score 2)
  Browning within 5-10 mm radius (score 3)
  Browning >10 mm radius of explant (score 4)
- Number of shoots, shoot length, number of leaves after first subculture.
- Number of necrosed explant and contaminated medium.

Table 14: Description of the four sterilisation treatments

Control	Treatment A	Treatment B	Treatment C
Uprooting of suckers	Uprooting of suckers	Uprooting of suckers	Uprooting of suckers
Quick washing of suckers to remove soil.	as in control	as in control	as in control
Chopping of sucker and removal of outer leaves to produce 5-7 cm tall and 3 cm wide block of tissue on 3 cm tall corn (done outside wash room).			
Bringing sucker to wash room.			
Washing of excised explant in running water and few drops of detergent (30 mins).	Washing of explant in running water and detergent (10 mins)	Washing of explant in running water (10 mins)	Washing of explant in running water and detergent (10 mins)
Trimming of explant to excise smaller sized explant (3 cm tall and 0.7 cm wide block of tissue on 1 cm tall corm.	Trimming as in control.	Trimming as in control.	Trimming as control
Disinfection in Ben late and Tween (15 mins).	Disinfection in Ben late and Tween (5 mins).	Disinfection in Ben late & Tween (15 mins).	Dip in ethanol (70%) for 10 sec).
Aseptic transfer and surface sterilisation in Bleach (2%) and tween for 15 mins.	Surface sterilisation in Bleach (15 mins).	Surface sterilisation in Bleach (5 mins).	Surface Sterilisation in Bleach (15 mins).
Trimming to produce 1.5 cm tall explant.	Trimming to produce 1.5 cm tall explant	Trimming to produce 1.5 cm tall explant	Trimming to produce 1.5 cm tall explant
3 rinses for 15 mins each in sterile deionised water under aseptic conditions	3 rinses in sterile water for (5,10 15 mins respectively)	3 rinses in sterile (5,10, 10 mins respectively)	3 rinses in sterile water (5,10,15 mins respectively)
Further trimming to produce 5 mm tall explant with corm and meristematic tissue for inoculation.	Trimming as in control prior to inoculation.	Trimming as in control prior to inoculation.)	Trimming as in control prior to inoculation
Total fixed time for treatment: **1hr 45** mins (excluding handling time)	Total fixed time for treatment **1 hr** (excluding handling time)	Total fixed time for treatment **55 mins** (excluding handling time	Total fixed time for treatment **55 mins** (excluding handling time)

Results and discussions

Each step in explant cleaning disinfection has its importance as described below.

Washing of suckers	Washing to remove soil reduces risks of contaminating sucker during handling.
Cleaning to remove top part of sucker and outer leaf sheaths and corm tissue outside the laboratory	This first step in trimming allows removal of superfluous tissue and leaving them outside laboratory. Only the inner meristem protected by leaf sheaths and corm tissue is taken inside the laboratory.
Washing of extracted tissue in running water	This is an important step in explant cleaning especially if it involves leaves. However, in banana the shoot apex is already enclosed within many tightly overlapping leaf initials giving this part a typical conical shape (Vuylsteke, 1998). Thus, as this block of tissue extracted is relatively less affected by surface contaminants, the washing step for banana should normally not be as lengthy as for leaves (i.e. 1 hr). In this project, initially the washing was carried out for 1 hr but with experience 30 mins was found to be enough..
Washing in Benlate with Tween	Benlate is a fungicide that allows disinfection and ensures that fungal contaminates are removed. Alternatively, the block of tissue can be dipped in ethanol (70%) for 10 sec, (Strosse *et al*., 2004) or 95% for 15-30 sec (Vuylsteke, 1998).
Surface disinfection in Bleach (2%)	Sodium hypochlorite (NaOCl) is commonly used in the concentration range between 0.5-1.0% (Vuylsteke,1998). However laundry bleach (0.5% NaOCI) is equally effective (Vuylsteke, 1998).
Excision of explant	This involves further reduction of superfluous tissue (leaf and corm) that have been disinfected. Bleach can damage the tissue and thus trimming also removes the bleached tissues. This allows the extraction of explant which contains minimum of superfluous tissue.
3 rinses in sterile water	Transfer of explant from bleach to sterile water (all 3 rinses) is done under aseptic conditions to minimize surface contamination. In routine *in-vitro* works, the duration of rinses is 15, 30, 45 minutes from first to third rinse. The first one is short to allow quick extraction of any remnant bleach from the tissue. The 3rd one is longest to ensure maximum cleaning of explant prior to final excision.
Excision of shoot-tip	This final trimming after completion surface sterilization and rinsing of explant ensures that maximum care and aseptic conditions are maintained to avoid the loss of explant due to contamination.

The control in this trial was the method adopted throughout this project and it should be pointed out that even this procedure was slightly improved from the one initially adopted (i.e changing washing time in detergent/water from 1 hour to 30 minutes (mins), changing the 3 rinsing duration from 15,30,45 minutes to 15 minutes each). However, as pointed out banana shoot apex is an already much protected explant, hence further reduction in duration of unnecessarily lengthy treatment permits a faster and more handling of tissues at a time.

Results and Discussion

Fungal or bacterial contaminations usually appear within 5-10 days after initiation (Vuylsteke, 1998). Ten days after inoculation no contamination, no intense browning and no explant mortality was noted. Moreover, there was no significant difference of cleaning treatment on explant development. The explant size was all approximately 7 mm and their coloration which ranged from white with greenish tinge (Treatment B) to green (in other treatments and control).

Conclusion and Implication:

Thus, the explant preparation and surface sterilisation steps of banana can be successfully reduced from 1hr 45 minutes to only 55 minutes. With less time spent in explant preparation, there is gain in time for other activities

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Activity 8: Investigation into gelling agent

Background

Gelling agents contribute to 70% of costs of media (Prakash *et al.*, 2004) and agar, a seaweed derivative (Khanna, 2002) is most commonly used (Prakash *et al.*,. 2004). Agar substitutes 'Gellan gum' marketed under trade names 'Phytagel' and Gelrite are often used to replace agar, especially in culture of explants that one prone to blackening due to production of phenolics. Gellan gum products are usually added at low rate of (2 g/L) compared to that of agar (5-8 L/) and they produce clear gels and allow better shoot proliferation. Gelrite (2-4 g/L) is preferred for banana micropropagation because it allows earlier detection of contaminants and because it is relatively free of contaminants (Vuylsteke, 1998). However, they are more expensive.

During the early stages of this project when explant browning was considered a major hurdle, substituting agar with a more purified form of gelling agent (Gelrite) was one of the steps taken to control browning. Phytagel was also noted to give similar successful results as Gelrite and was used throughout the experiment. However, as these agar substitutes are more expensive than agar, it was important to evaluate their importance for banana micropropagation.

Aim

To find an appropriate, economic and effective gelling agent for medium preparation

Methodology

(i) At culture initiation

The following gelling agents were tried in modified MS medium supplemented with 5 mg/L BAP:

Agar (12-14 g/L)

Gelrite: Phytagel mixture (1:1 g/g basis) at 1g/L each.

Gelrite (1g/L): agar (7 g/L) mixture (each at 50% normal rate)

Gelrite (2mg/L)

Trial was set in Completely Randomised Design with 5 explants (replicates) initiated in each medium and the following observations were made:

- Description of medium based on the following arbitrary scale:
  1-Clear or translucent; 2- opaque; 3 – opaque/red tinge
- Medium browning (as described in Activity 7)
- Explant browning (as described in Activity 7)
- Medium texture (hard soft, watery), explant size, number of buds and roots (if any) at one month and 2 months after initiation.

(ii) During multiplication stage

Shoots from 5th subculture were randomly selected for this trial.

The following gelling agents were tried in modified MS medium supplemented with 5 mg/L BAP:

- Agar (12-14 g/L)
- Gelrite: Phytagel mixture (1:1 g/g basis) at 1g/L each.
- Gelrite (1g/L): agar (7 g/L) mixture (each at 50% normal rate)
- Gelrite (2mg/L)

Result and discussions

Table 15: Summary of medium and explant parameter 2 months after initiation

Source of gelling agent	Medium original coloration	Average medium browning score	Explant colour	Average explant size (cm)	Average number of buds
Agar	Opaque with red tinge	2.3^{a}	Green	14.6^{a}	0.2^{b}
Agar:Gelrite	Opaque and whitish	1.4^{a}	Green	7.8^{b}	2.8^{a}
Phytagel:Gelrite	Translucent	0.2^{b}	Green	11.0^{a}	4.2^{a}
Gelrite	Translucent	0.2^{b}	Green	17.2^{a}	0.8^{b}
SE (±)		0.33		2.08	0.68
(P≤0.05)		*		*	*

*: significant at 5% level of significance; ns: not significant at 5% level of significance
Figures with same superscript along the row not significantly different at 5% level

All the medium were soft and allowed easy inoculation of explants. Medium constituting of Phytagel or Gelrite only were translucent while that of agar was opaque with red tinge.

Addition Gelrite to agar improved medium coloration (opaque with white tinge) (Table 15) thus making any contamination more visible than in agar.

Medium browning is caused by accumulation of exudates from wounded corms of the inoculated explant. In this experiment, there was a significant effect of gelling agent on medium browning (discoloration), explant development and proliferation rate. In agar, freshly inoculated explants produced exudates which were trapped in the medium and gave the latter a brown/black coloration (score of 2.4). This was not visible in medium with Gelrite or Phytagel (score 0.2). This indicates that probably in medium composed of purified form of gelling agent there is less stress on the excised explants.

Explant development was also significantly affected with more buds production in Phytagel: Gelrite (4.2) or agar: Gelrite mixture (2.8) than in agar (0.2) or Gelrite (0.8) alone.

The cost of agar, Gelrite and Phytagel was respectively Rs.3/, 60/ and 11 per g (as at mid-2006). However, based on amount used per litre of medium, the cost was Rs. 21/,120/ and 22/ per litre respectively for agar, Gelrite and Phytagel. Thus, in the initial stages when explant browning (and medium discoloration) is intense, the use of Phytagel can be used and then ultimately replaced by agar. However, based on actual price indications, use of Phytagel throughout all cultures can be recommended. In event of an increase in price of Phytagel then a combination of Phytagel and agar can help to overcome disadvantages associated with agar only.

(ii)During Multiplication Stage

Results and Discussions

Multiplication was better and more in Phytagel, Gelrite or mixture of Gerlrite/agar followed by agar only. According to Khanna (2002), some synthetic gels can cause vitrification and a mixture of Gelrite (or Phytagel) (1 g/L) and agar (4 g/L) (as in one of the treatment) can offer best attribute of products and also reduce vitrification.

Conclusion

High quality of synthetic gel (Gelrite/Phytagel) promotes *in-vitro* response of shoots. However based on an overall performance of the explants in this trial use of Phytagel is recommended, if its cost remains as described above. In event of an increase in price of Phytagel then a combination of Phytagel and agar can help to overcome problems of explant browning and promote shoot bud proliferation.

Activity 9: Lost cost options for banana micropropagation

Activity 9(i): Alternative to distilled water

Background

Ninety five percent of nutrient medium consists of water (Khanna, 2002). Distilled or double-distilled and de-ionised water are routinely used in tissue-culture research (Prakash *et al.*, 2004). There sources of water are quite expensive as they require special expensive equipment that also need regular maintenance. Low cost options for alternative water sources such as untreated rainwater (Khanna, 2002), tap water (Ganapathi *et al.*, 1995) and bottled water (Prakash *et al.*, 2004) have been reported. However, these options provide less control over the inorganic and organic content of the medium (Khanna, 2002).

Aim

To investigate into the possibility of finding alternative to distilled/deionised water.

Methodology

Three sources of water (deionised distilled (control), table bottled water and tap water) were used to prepare propagation medium using Gelrite as gelling agent. Proliferating tissues of var PN from the 7th subculture were randomly selected, trimmed then subcultured in MS + 2 mg/L BAP made from the above water sources. Trial was set in a completely randomised Design with 5 replicates per treatment.

Observations/parameters noted were:

- pH of medium prior to pH adjustment to 5.8;
- Coloration score of shoots using the following scale:
  Black (1), Brown (2), White Brown (3), White (4), Light green tinge (5), Light green (6), Green (7);
- Coloration of medium, score using the following scale:
  No browning (original colour) of medium (score 1)
  Browning limited to 5 mm radius of explant (score 2)
  Browning within 5-10 mm radius (score 3)
  Browning >10 mm radius of explant (score 4)
- Texture of medium
- Number of shoots, shoot length, number of leaves and number of roots after first subculture

Observations

- Bottle water and tapwater were both alkaline (pH > 7) but pH adjustment for tapwater was more difficult.
- Medium made from tapwater had a yellowish tinge and did not set well and the explants also had a brown coloration.

- No shoot proliferation took place 1½ months after subculture even in control. In all the medium, the individual shoots subcultured elongated and produced leaves. But in the control, both shoot length (19 cm) was superior to those in medium constituted with tap or bottled water (9 and 10 cm respectively) (Table 16). Roots, an average of 3, were also formed only in the control.

- Inorganic and organic content of tap water is very high and this could have been responsible for the yellowish, matt coloration of medium

Table 16: Average shoot development parameters in medium made from varying water sources

Water source	Shoot Length (cm)	Number of Leaves
Tap water	9.3[b]	3.5
Bottled water	10.0[b]	4.0
Deionised/distilled water control	19.5[a]	4.3
SE (±)	1.05	0.46
(P≤0.05)	*	*ns*

*: significant at 5% level of significance; ns: not significant at 5% level of significance
Figures with same superscript along the row not significantly different at 5% level

Conclusion:
No effective alternative was found to deionise d/distilled water. However, bottled water can be used if three is no other alternative.

Activity 9(i): Alternative to agar/Phytagel

Background
Agar is commonly used as gelling agent for solid and semi-solid media preparation (Prakash *et al.*,.2004). However, cheaper sources of gelling agent such as white flour, corn starch, rice powder, laundry starch have been reported (Prakash *et al.*, 2004). An observational trial was set to see if locally available commercial grade cornstarch could be used instead of agar.

Aim
To find cheaper sources of gelling agent as alternative to agar or synthetic gelling agents

Methodology and findings
MS medium was prepared as usual and agar (12 g/l) and corn starch was added in last. The cornstarch rate ranged from 10-40 g/L but the starch failed to set well and thus the trial was stopped. Gelrite and cornstarch have been successfully used in propagation of apple, pear, banana, ginger (as quoted by Khanna *et al.*, 2004). Due to time constraint, other brands of cornstarch was not tried but this an alternative to try especially during the late multiplication stages, once the browning has stopped

Activity 10: Optimisation for a low cost post-flask management (weaning and hardening) of the rooted plantlets

Activity 10a: Preliminary observations to find low-cost structure for weaning of banana plantlets.

Background

The last step after regeneration of rooted plantlets is to wean the plantlets by transferring them into trays containing light medium that will allow proper plantlet development prior to field planting. TC-derived banana plantlets are extremely tender and fragile (Vuylsteke, 1998) and thus require special post-flask management conditions during weaning. When removed from jars, the plantlets are suddenly exposed to outside environment where light levels and temperature are varying, humidity is reduced and pathogen load is relatively high (Vuylsteke, 1998). To ensure proper adaptation to the outside environment, the plantlets should be acclimatized through a hardening phase.

Controlled humidity, controlled temperature, a free draining medium in an appropriate container and supplementary nutrition are the basic requirements to ensure successful weaning and hardening. Nurseries can vary from simple sheds made from locally available material to sophisticated and automated weaning house (Vuylsteke, 1998).

Aim

To find low-cost alternative (structure and system) for weaning of banana plantlets.

Methodology

This part was not carried out as a trial but was carried out in almost all the plantlets that were taken out for weaning from 2004-2006.

(i) Preparation of plantlets

Step 1 Gentle removal of plantlets from agar

Step 2 Gentle washing of all agar under running water

Step 3 Trimming to reduce root length and remove brown roots

Step 4 Dipping of plantlets in Benlate (1g/L) for 10 minutes

After grading into sizes, the plantlets were then either individually transferred into soaked jiffy pellets (Plate 19a) or plugged into seedling trays containing autoclaved soil manure mix at a ratio of 1:1.(Plate 19b).

Plate 19a: Plantlet in jiffy pellet

Plate 19b: Plantlet in sterile soil mix

Control of humidity, light and temperature

In order to preserve high humidity around the plantlets for at least 7 to 10 days (TBRI, Bulletin 81-2), and to reduce rate of leaf drying, humidity should be kept to 70-80%. In specialised greenhouses, this can be achieved by automated misting system. However, placing planlets inside a humidicrib for 4-5 days with regular watering with hosepipe (no coarse covering plantlets with a plastic sheet sprays) can also ensure successful establishment (Vuylsteke, 1998). Thus, throughout this project the plantlets freshly taken out of the jars were kept under plastic sheet or inside a plastic structure (Plate 20) for 4-6 days. The plantlets were sprayed with a hand-held fine spray to keep the leaves moist and also to maintain humidity around the plantlets. This also controlled the temperature.

Too much light can cause burning. For the first 3 weeks, this is theoretically controlled by placing trays under 80% shade (this may be used in combination with regular misting to maintain high humidity). After 3 weeks, the plants are normally transferred into a 40-50% shade for another 4 weeks (Branch and Robinson, no date). In this project, changes were adapted with facilities available at Barkly Experimental Station (BES).

After one week under the plastic sheet (usually left within the wash room), the plantlets were then transferred into a shade house (70% light) with an automated misting system. After 4-5 weeks, the plants were individually potted into polythene bags (1.5-2L) containing non-sterile soil and manure mixture and maintained under 70% shade for about 3 weeks after which they were transferred into nursery with 50% shade. They were left there until they reached 25 cm tall and ready for field planting (Plate 21a-d).

Plate 20: Plantlets kept under high humidity

Plate 21a : Potted plantlets under 70% shade

Plate 21b: Plants under 50% shade (BES)

Plate 21c : Plants hardened under full sunlight (AREU)

Plate 21d: Plants under 20% shade (AREU)

Activity 10b: Determination of a low cost medium for weaning and hardening

Background

Micro-propagated plants are produced in a closed, sterile environment and grown on nutrient-rich medium under high humidity and low temperature. If the transfer of the rooted plantlets from closed environment into external environment is not done well this can result in significant loss of plants. As highlighted above several precautions are needed and one of them is the need for a free draining medium. Sterile potting mix containing vermiculite and peat are often used in commercial nurseries but these need to be imported and are very costly. Based on the observations from the above preliminary trial, a study was carried out to evaluate the performance of several easily available local and imported materials as potting mix for transplantation of banana plantlets.

Aim

To determine a low cost option for medium (potting mix) to be used for weaning of micro-propagated plantlets.

Methodology

Plantlets of var. PN (at least 2.5cm tall) were randomly selected and planted in trays (dimension 26 * 31 cm and 6 cm deep). A trial with 11 different potting medium (Plate 22) and 20 rooted plantlets per medium was set up using a Completely Randomised Design. Potting media used were:

- 100% Scum
- 100% Flyash
- 40% Flyash + 60% Scum
- 50% Bagasse + 50% Flyash
- 50% Flyash + 50% Scum
- 60% Flyash + 40% Scum
- 70% Flyash + 30% Scum
- Cocopeat
- Jiffy pellets
- Non-sterile soil+ decomposed cow manure (1:1) and
- Autoclaved (sterile) soil+ decomposed cow manure (1:1)

In each tray, the medium was filled to a depth of 3.5-4 cm and the plantlets were transferred at a density of 3.3 plants per $100cm^2$ (25 plantlets per tray).

The trays were placed in the pathway of an anthurium shed with 70%shade from the roof and 50% shade from the sides. All the trays were covered with plastic sheet for one week with watering using a fine hose twice daily (Plates 23a-b). After one week the plastic cover was removed and the plantlets were allowed to develop for further 2-4 weeks prior to potting into individual bags.

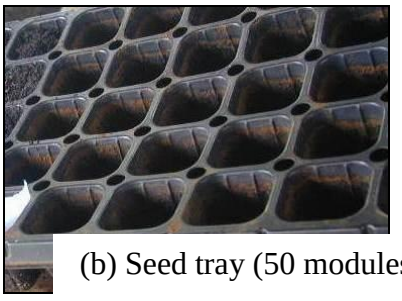
Plate 22 : Potting mixes used

Plate23a :Fixing of plastic sheet

Plate 23b: Watering of plantlets

(a) Jiffy tray

(c) Solid tray perforated only in the



(b) Seed tray (50 modules)

(d) Perforated tray

Plate 24a-d : Comparison of plastic containers

Side observations:
(i) Very small (0.5 cm tall), small plantlets (1.5 cm to 2 cm tall) and rooted plantlets in clumps (2 plantlets attached together) were also transferred to assess rate of successful transplantation.
(ii) The following plastic containers (trays) were used, to observe the development of plantlets:

- Jiffy tray with cups (designed to hold jiffy pellets) of depth up to 4cm and holding medium to a depth of 1.5 cm (Plate 24a).
- Seed tray (50 modules) with cups 6 cm deep and holding medium to a depth of 3.5-4 cm (Plate 24b).
- Solid tray (6-7 cm deep, holding medium at 4 cm deep) with perforations only in the base (Plate 24c)
- Plastic tray (6-7 cm deep, holding medium at 4 cm deep) perforated from all the sides but a perforated plastic film placed on the base (Plate 24d)

Parameters noted were:

- Plantlet height (from base to 'V" area formed by the 2 youngest fully developed leaf), number of fully developed leaves and roots prior to transplantation
- Weekly leaf emergence (recorded randomly)
- Number of died plantlets
- Plantlet height, number of leaves, number of roots (on 5 random plantlets), length and width of youngest fully developed leaf at time of potting of weaned plants in individual potting bags (not recorded for all medium)

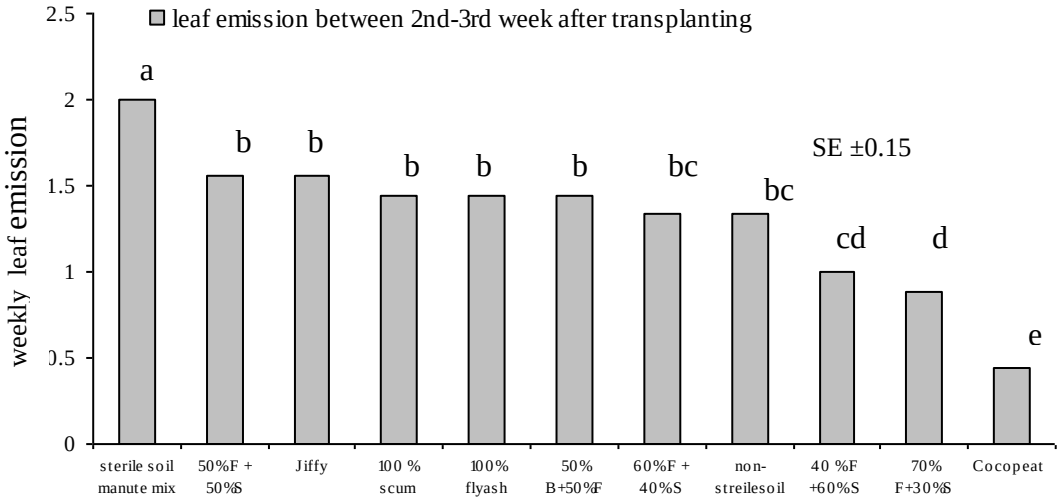
Results and Discussions
After the first week, the older leaves became yellow in all the medium but was more intense in cocopeat. During the first week, all medium with at least 50% scum had ants and this was probably due to the presence of sugar. The ants were controlled using Karate.
0-10% mortality was noted among the plantlets in the various potting media used (Plate 25a-b). However, there was significant difference between the potting mixes with respect to the rate of shoot and leaf development.

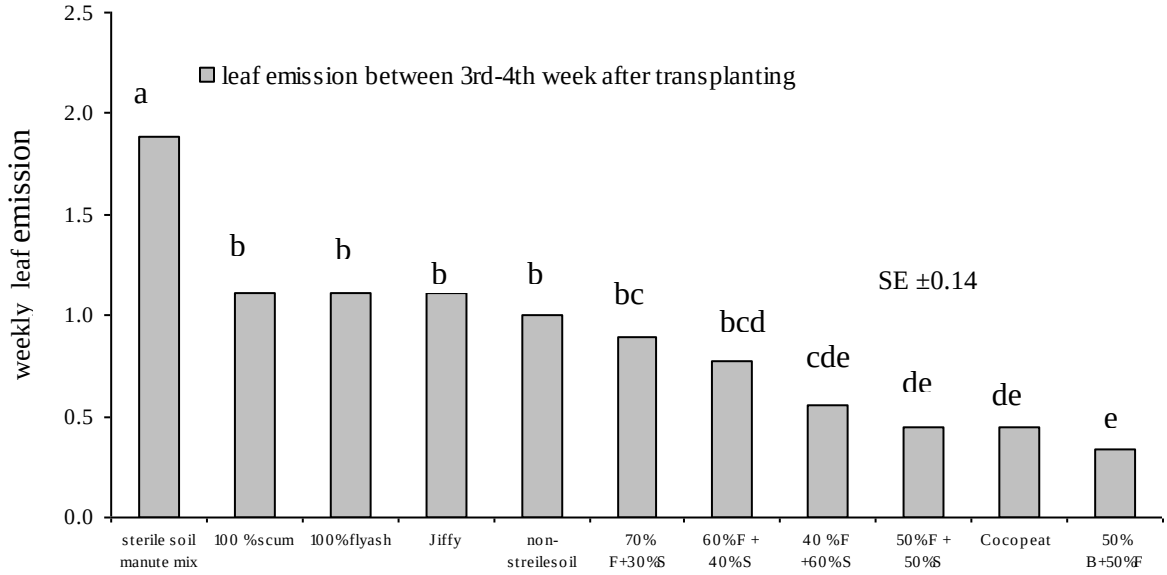
Leaf emission between the 2nd and 3rd week (Figure 13a):
Leaf emission was significantly faster in sterile soil+manure mix (2.0) followed by 100%scum, jiffy, non-sterile soil and medium with at least 50%flyash (1.3-1.6). Performance in 60% flyash+40% scum and non-sterile soil mix (1.3) was not significantly different from 40% flyash+60% scum. Leaf emission was least in cocopeat (0.4).

Leaf emission between the 3rd and 4th week (Figure 13a):
Like in previous week, leaf emission was significantly fastest in sterile soil mix (1.9). An average of one leaf was emitted in medium with at least 60% flyash (with scum), 100% scum, jiffy pellets and non-sterile soil. Medium with 40-50% flyash mixed with scum produced 0.4-0.6 leaves but these were not significantly different from those in cocopeat (0.4). Leaf emission in cocopeat in the 2nd week did not improve in the 2nd week while that of plants in 50% bagasse +50% flyash dropped to 0.3. Plantlets in bagasse looked normal within the first two weeks but after that the leaves became pale green with

yellowish tinge and this could account for drop in leaf emission, which did not improve over the weeks.

Figure 13a: Effect of potting medium on average leaf emission between 2nd to 3rd and 3rd to 4th week



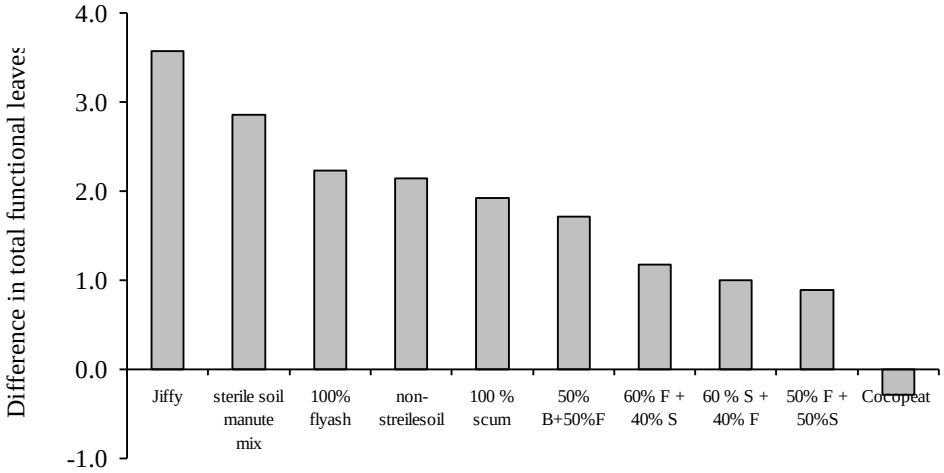


Shoot development

It was also noted that all plantlets in cocopeat became pale green shortly after transfer and remained so until for further 3 weeks, after which the leaves became very dark green and looked healthier. The leaf yellowing in cocopeat is probably due to the high electric conductivity associated with cocopeat shortly after wetting and this could account for the poor leaf emission. As pointed out before, shortly after transplantation 1-2 older leaves in all plants became yellow and senesced, however, in cocopeat as the production of new leaves was severely impeded. This accounts for the negative value in difference in total leaves between time of transplantation to one month later (Figure 13b). Plantlets in

bagasse looked normal within the first two weeks but after that the leaves became pale green with yellowish tinge and leaf emission of 0.3 per week did not improve over the weeks. Plant development in flyash and scum mix with at least 50% scum was relatively slower than those with less scum.

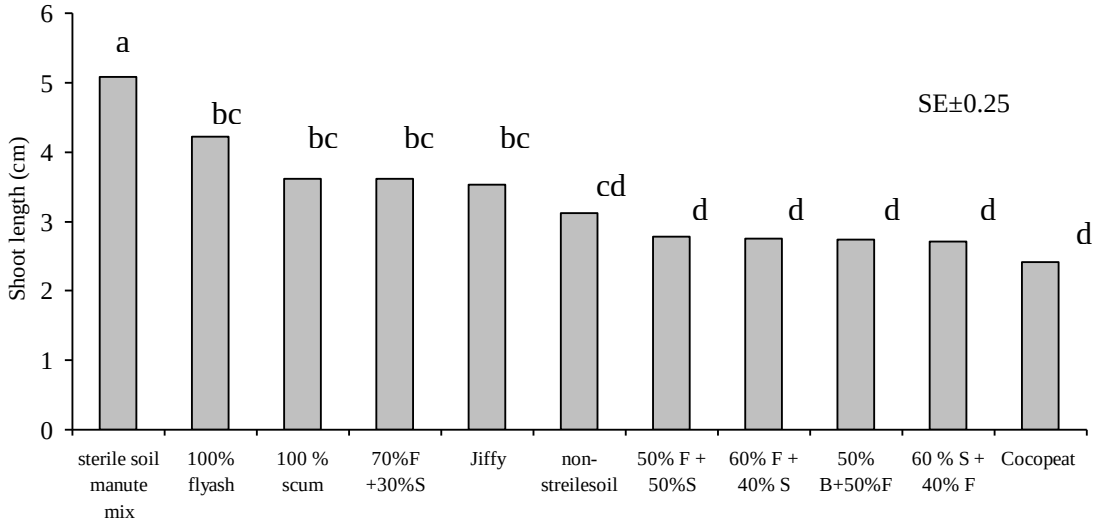
Figure 13b: Effect of medium on difference in total green leaves at and one month after transplantation



Shoot length, one month after transplantation, was also significantly affected by potting medium (Figure 14). Plantlets in sterile soil mix was distinctly superior (5cm tall), followed by those in 100%flyash, 100% scum 70% flyash+30%scum and jiffy pellets 93.5-4.2 cm). Plant development in non-sterile soil and manure mix (3.1 cm) was intermediate.

SE±0.25

Figure 14: Shoot length one month after transplantation



Transfer to individual bags

By one month, all plantlets in sterile soil, 100% flyash, jiffy pellets, non-sterile soil and 70% flyash were ready for transplantation in individual bags (Plate 26a-d), as they were relatively taller and had 5-6 leaves per plantlet. The others had 3-4 leaves by that time and transfer to individual bags was possible about 2 -3 weeks later (Plates 26e-g). Plantlets in sterile soil were not only significantly taller and had the highest number of leaves (Plate 26 h), but also the internode length (not measured) were distinctly longer and the youngest leaf was significantly longer and wider than leaves in other medium transferred into soil at the same time (Table 17).

Table 17: Plant parameters at time to transfer in individual bags

Medium	Shoot length (cm)	Leaf length (cm)	Leaf width (cm)	Root number	Root length (cm)
Sterile soil	5.4[a]	10.1[a]	5.6[a]	4.5	10.9
100% flyash	3.8[b]	6.6[b]	3.3[b]	3.8	11.4
Jiffy	3.4[b]	6.5[b]	3.2[b]	4.0	8.3
Non-sterile soil	3.1[c]	6.5[b]	3.3[b]	4.8	10.4
SE(±)	0.22	0.48	0.36		
(P< 0.05)	*	*	*	n.s	n.s

There were 4-5 well-developed roots per shoot and there was no significant difference between medium on the number of roots. There was also no significant difference between the four media on root length (8-11 cm).

The cost and availability of a medium are also important point to consider. Sterile soil and manure mix gave the best results but autoclaving volume of soil is not always feasible. Non-sterile soil gave satisfactory results in this trial as the var. used was PN, but in the hardening of Gingeli clones, this should be avoided as our soil may contain Foc race 1 to which Gingeli banana is sensitive. Jiffy pellets are available in ready-to-use units, they are easily stored and are not cumbersome to use. However, one pellet costs Rs 2.40 and this may increase cost of production.

Bagasse, flyash and scum are by-products of sugarcane, relatively easily available and they can be used without prior sterilisation. The banana plantlets performed better in scum+flyash mix when there was at least 50% flyash. Per tonne (transport cost excluded) flyash cost only Rs 100 while scum and bagasse cost Rs 2000 and 700 respectively. The use of flyash and flyash mixture with scum can highly assist in reduction in cost of production of banana TC materials.

Conclusions

With very low-cost facilities using trays, available potting mixes and plastic cover to maintain humidity within a shed covered with Sarlon (at least 50% shade), the sensitive banana plantlets can be successfully weaned.

As there was negligible mortality associated with any of the mixes tried, any of the above mixes can be used for the post-flask transfer of banana plantlets, if there is no other alternative. However, with respect to rate and degree of plantlet development (shoot and leaf development), sterile soil+ manure mix is the best medium followed by 100% flyash, 100% scum, non-sterile soil and flyash + scum mixture with at least 50% flyash. After the post-flask transfer the plantlets can be transplanted into individual bags as early as 3 -4 weeks after transplantation.

Side observations:

Side observation	Results	Conclusion
Use of plastic containers	3 weeks after transplantation : - 90-100% success in all trays except jiffy trays. - In jiffy trays: 30% died, 23% developed well, 37% suffered initially but started to recover gradually after 2-3 weeks	Adequate depth of medium is important for root development, hence plant development. Trays should be at least 6 cm deep and holding medium to a depth of 3.5- 4 cm
Transfer of small plantlets	One month later: - There 15% mortality in plantlets less than 0.5 cm tall. -Plantlets 1.5-2 cm developed much faster than the 0.5 cm tall ones (Plates 26 i-k) -Plantlets in clumps can also develop but with a dominance of the taller ones.	.Plantlets taller than 2.5 adapt better to transplantation shock. Plantlets 1.5-2.5 also respond well but after a slight delay One month after transfer plantlets less s than 0.5 still very small. **Thus**: Plantlets should be sorted prior to transfer into trays as small ones lag behind the taller ones and this may hinder development of the latter if they are placed in the same tray.
Other observations	One batch transplanted in the anthurium shed at Reduit suffered from sunburn within the first few days. It was noted that given the plantlets were placed at soil level, light was coming more from the side (Sarlon cloth at 50% shade). Thus a sarlon cloth of 50% shade was placed on top of the plastic structure (placed to maintain humidity).4 days later the Sarlon cloth was removed when the plants had recovered. At WCRS a super-humid zone, plantlets semi-hardened in a misting room for 1 week was transferred in an anthurium shed with similar shade cloth covers as at RCRS. There was no need for irrigation because WCRS is a super-humid zone.	We have to be careful and take into consideration several factors.

Plate 25a-b: Banana plantlets at transplantation

Plate 26a: Plantlets one month after transplantation

Plate 26b : Outperformance of sterile soil/manure mix (one month after transplantation)

Plate 26c : Plantlets in jiffy pellets (one month after transplantation

Plate 26d : Plantlets in non- sterile soil/manure mix (one month after transplantation)

Cocopeat Jiffy pellets

Plate 26e : Comparison of plantlets in jiffy pellets and cocopeat (one month after transplantation)

Plate26f : Comparison of plantlets in sterile soil mix and flyash+scum or flyash +bagasse mix (one month after transplantation)

Plate 26g : Comparison of plantlets in sterile soil mix v/s 50%bagasse+50%flyash mix (one month after transplantation)

a: Sterile soil
b: 100% flyash
c: Jiffy pellets
d : Non-sterile soil

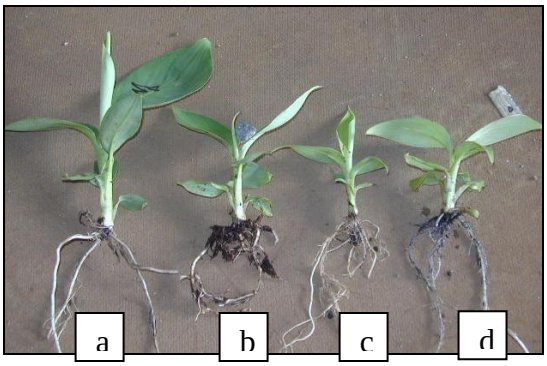


Plate 26h : Whole plantlets one month after transplantation

Plate 26i : Transfer of one month old weaned plantlets for hardening

Plate26j : Small and very small plantlets at time of transplantation

Plate26k : Response of small and very small plantlets one month after transplantation

Activity 11: Field evaluation of tissue-cultured plantlets.

Banana plantlets produced during the early stages of this project were field planted on-station and on-farm. Over 4000 PN and 1500 G.Moodelly shoots were produced. Over 500 G.Moodelly were hardened and delivery to the President of Southern Planters' Association (SPA) is on-going. However, the SPA is looking for an appropriate planting site prior to taking of all the G.Moodelly plantlets. Weaned semi-hardened plantlets were received from BES and they were potted and hardened at WCRS, prior to field evaluation.

On-station evaluation:

Petite Naine/ D.Cavendish:

15-20 plants (15-25 cm tall) were planted on each of the 3 stations under different agro-climatic zones between January to August 2006. Those planted in January 06 at RLCRS produced 2-4 leaves per month and flowering started 11 months after planting. By bearing time the plants had 5-7 suckers. The first harvest was carried out 90 days after flowering and the plants were on average 160 cm tall with girth of 51 cm. Bunches weighed 17 kg (37 t/ha) and had 11 hands and 185 fingers (Plates 27a-d).

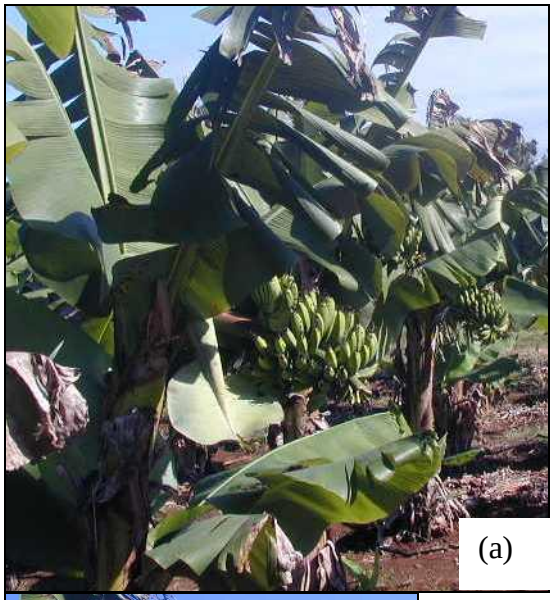
Plate 27a: One month old PN

Plate27b: 5 months old PN

Plate 27c : PN bunch

Plate 27d : PN bunch

It should be pointed out that at the initial stages of the project at least 15 suckers, not necessarily from elite mother plants, were initiated per week just to get hands on the extraction of shoot-tip, control of browning and also to understand the response of PN to the various medium tried. As the randomly carried out ELISA was negative, the resultant shoots were not discarded and they were also later field planted. Some of these plants have also flowered but since the mother plants were not elite ones the performance of the micropropagated plants was not improved (Plate 28a-b), some even has CMV and were rogued out (Plate 28c). This confirms the need for a micropropagation of suckers from testified elite mother plants and also to grow mother plants in protected structures (something not possible for this project)



(a)

Plate 28c: Infected plant

(b)

Plates 28a-b: Micropropagated PN from non-elite mother plants

At other stations the plants are still during vegetative stage. With respect to plant development, production and susceptibility to cyclone, the plants appeared true-to-type and not affected by the micropropagation techniques.

Gingeli and other clones

During year 2006, Gingeli Moodelly, Puchooa and Ollier were also planted on the stations. At Reduit they are all healthy looking (Plate 29a-c).

At WCRS, G.Moodelly planted in February 2006 started to flower in end Mar 07 (13 months after planting) but the plants were broken in cyclone Gamede (24-25 Feb 07). At bearing the plants were 221 cm tall (Figure 15) with a girth of 44 cm with bunches carrying 5 hands and 55 fingers. The other clones are still during the active vegetative stage (Figure 15). At WCRS, the other clones have produced 2-3 suckers per stool.

Figure 15 : Plant height over time in TC plants at WCRS

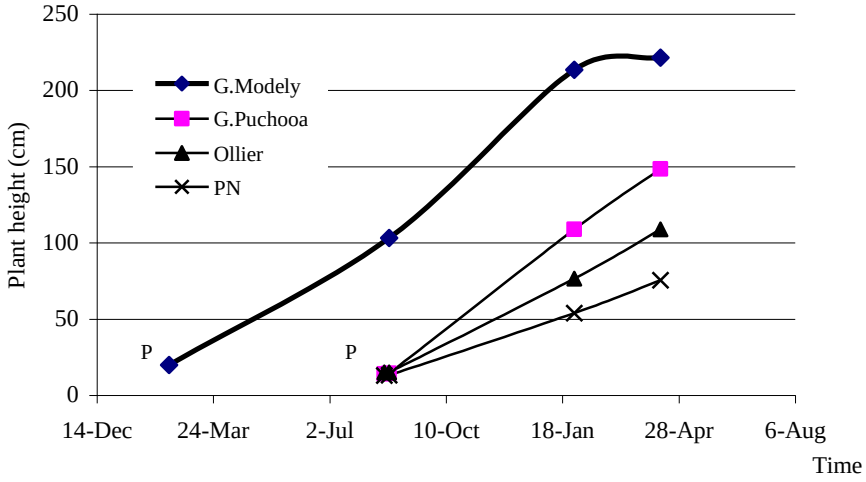


Plate 29a: Other clones at vegetative stage

Plate 29b: Ollier

Plate 29c: Gingeli Puchooa

On-farm evaluation of DC /PN plants:

Growers were provided with plants of PN/DC as from year 2006 and some have been earmarked for further distribution (As per list below)

Grower	No. of plants (Var DC)
Mr Ramphul	400
Mr Prayag	20
Mr Jhanmukhia	130
Mr Proag	100
Mr Jeewon	130
Mr Bhoojawon	130
Mr Poonith (from Bassin)	
Mr Nobeen	40
Mr Seechurn of Riv. Du Rempart	150
Ashram at Dubreuil	200
Mr Bheenick	50
Soeur Cowen	25
RicheLieu Open prison	100
Mr Malabar	250
Ashram –Valetta	50

Field visits were made to most of the places and grower was advised on cultural practices. At R.du Rempart (Mr Seechurn), plantation was effected in June 06 and a visit was effected in September 06. Except for 2 plants found CMV infected, all others were healthy and normal. The plants reached bearing stage about 9-10 months after planting and the bunches are still developing. However, yield is very low as cyclone Gamede affected these plants during late vegetative stage and the bearing plants have leaves below the optimum for bunch filling.

CMV infected plants

PN -on-farm evaluation

PN plants –vegetative stage

5.0 Guidelines (Protocol) for micro-propagation of local banana clones

Stage 1: Explant preparation

Selection of suckers from elite mother plants
(Buds, peepers, suckers of varying heights)

↓

Uprooting of suckers (avoid damage to base)

↓

Clean suckers outside laboratory (to remove soil)

↓

Chopping of sucker and removal of outer leaves to produce 5-7 cm tall and 3 cm wide block of tissue on 3 cm tall corn (done outside wash room).

↓

Bringing sucker to wash room.

↓

Washing of explant in running water and detergent (10 mins)

↓

Trimming of explant to excise smaller sized explant (3 cm tall and 0.7 cm wide block of tissue on 1 cm tall corm.

↓

Disinfection in Benlate and Tween (5 mins).

↓

Surface sterilisation in Bleach (15 mins).

↓

Further reduction in size to 1.5 cm tall explant

↓

3 rinses in sterile water for (5,10 15 mins respectively)

↓

Further trimming to produce 5 mm tall explant with corm and meristematic tissue for inoculation.

Stage 2: Initiation of shoot cultures

For Petite Naine/ Williams/ Ollier and Mamoul group banana

Place explant directly in modified MS-based medium supplemented with 5 mg/L BAP+ IAA (0.175 mg/L). The modified MS also contains myo-inositol (2 mg/L), ascorbic acid (10 mg/L) (added to medium prior to autoclaving) and Phytagel (2g/l).

For Gingeli banana

Place explant directly in modified MS-based medium supplemented with 8 mg/L BAP+ IAA (0.175 mg/L), myo-inositol (2 mg/L), ascorbic acid (10 mg/L) (added to medium prior to autoclaving) and Phytagel (2g/l).

All cultures to be covered with black cover for 3-4 days until explant turn green. Cultures to be maintained at an optimal temperature of 27± 2^{o}C under a 16h/:8h light/dark cycle with light delivered by white fluorescent tubes with an irradiance of an average of 50µmol$m^{-2}s^{-1}$.light.

Stage 3: Multiplication of shoot-tip cultures

After 3-4 and 5-6 for weeks Petite Naine and Gingeli group respectively, the explants can be transferred into fresh medium and further subcultures carried out as follows in modified MS supplemented with myo-inositol (2 mg/L), ascorbic acid (10 mg/L) and IAA (0.175 mg/l.

Subculture	Petite Naine/ Williams/ Mamoul group	Gingeli group	
		Either	or
S1	BAP 5mg/L in medium until 5th subculture	8 mg/l BAP	6 mg/l BAP
S2			
S3			
S4			8 mg/l BAP
S5			
S6	BAP 2mg/L in medium until 5th subculture	2mg/l BAP	2 mg/l BAP
S7			
S8			
S9			
S10			
Subculture interval	3-4 weeks	5-6 weeks	5-6 weeks

Should avoid producing more than 1000 shoots from one explant.

Stage 3: Regeneration of plantlets

Banana shoots during regeneration phase produce roots at the same time. Thus if the roots are well developed the plantlets can be weaned directly. Otherwise rooting can be initiated by using modified MS medium with either 1 mg/L IAA or in modified MS medium with no auxin. Rooting takes about 3 weeks.

Stage 4: Weaning of plantlets

Important point to consider:

- Avoid transfer shock to the sensitive plantlets during transfer from jars to open environment
- Too much light will cause burning of leaves
- High relative humidity to be maintained during at least one week
- Frequent watering (as leaves should remain moist at all times)
- Avoid wind movement as this will remove boundary layer of high humidity for the leaf surface.

Steps

1. Open jars and remove plantlets gently.
2. Wash away gel from plant under running water and trim yellow/dead leaves, dead roots and extra long roots.
3. Dip plantlets in Benlate (0.5g/L) for about 10 mins
4. Plug plantlets in trays (5x5 or 5x6 cm spacing) (one plant per 30 cm^2).
5. Either

- Place trays on a bench under 80% shade for 3 weeks and under a permanent automatic misting room to maintain leaves and soil moist and to keep relative humidity to 70-80%

Or

- Within an existing shed (70% shade) construct a minitunnel (semi-circular metal frame) covered with clear polyethylene sheeting.

- If there is no shed, then both the polyethylene and shade netting (70%) should be placed on top of the frames to cover the trays from all the sides.

6. Plantlets can be watered using watering can with fine hose
7. Remove polythene sheeting after 7 days and leave plantlets in trays under the same shade until they reach 4-5 cm tall with at least 5 leaves (after 4 -5 weeks).

Low cost potting mix can be used. Scum+flyash mix (with at least 50% flyash) (without prior sterilisation) or sterile soil+manure (1:1) mix can be safely used.

Stage 5: Hardening of plantlets

1. Remove plantlets from trays and pot individually in black polyethylene bags (1.5-2 L) filled with potting mix of soil+manure (1:1) (non-sterile). Keep plantlets under same shade for one week.
2. Transfer plantlets to 50% shade for 1 month then to 20-30% shade for another 3weeks until plants reach at least 20 cm for field plantation.
3. Any offtype (discoloured leaf or plantlets with striking small leaves and short internode should be rogued out (This part requires expertise)
4. Regular watering, and spaying of pesticide or fungicide possible, if needed
5. Foliar feeding with e.g (Mairol at 1g/L) every 3 weeks

Appendix 1

Composition per litre of the modified Murashige and Skoog (1962) medium (Personal communication, INIBAP)

		mg/L
Macronutrients	KNO_3	1900
	NH_4NO_3	1650
	$CaCl_2.2H_2O$	440
	$MgSO_4.7H_2O$	370
	KH_2PO_4	400
Iron	$FeSO_4.7H_2O$	27.8
	$Na_2EDTA.2H_2O$	37.3
Micronutrient	$MnSO_4.4H_2O$	22.3
	H_3BO_3	6.2
	$ZnSO_4.4H_2O$	8.6
	KI	0.83
	$Na_2MoO_4.2H_2O$	0.25
	$CuSO_4.5H_2O$	0.025
	$CoCI_2.6H_2O$	0.024
Vitamins	Myo-inositol	2.0
	Thiamine chloride	0.1
	Nicotin acid	0.5
	Pyridoxine hydrochloride	0.5
Carbon source	Sucrose (Table sugar used in this project	40,000

6.0 References

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Final statement of accounts

The project was allocated a budget of Rs 600, 000 which, as indicated in progress reports was already spent and the project was later funded with AREU's Budget.

Please find annexed copies of vouchers and final statement of accounts (excel file attached)..