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Multiple Shoot Generation Media for *Musa sapientum* L. (False Horn, Intermediate French Plantain and Hybrid Tetraploid French Plantain) Cultivars in Ghana

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ABSTRACT

Plantains (*Musa sapientum*), a major staple in Ghana, encounter several production problems including availability of adequately healthy planting materials at the time the crop needs to be planted. In attempts to improve production, tissue culture methods were employed, using one medium. It was however realized that optimization of *in-vitro* rapid propagation protocol for mass production of different accessions of *Musa* was paramount. Excised buds from cultures with proliferating buds were used as explants in this experiment. The cultures of proliferating buds had been generated from excised apical meristem of four *Musa* varieties (False Horn; local names – Osoboaso and Apantu, intermediate French plantain; local name – Oniaba and FHIA 21, which is a hybrid tetraploid French plantain) which were cultured on Murashige and Skoog (MS) medium containing indole-3-acetic acid (IAA), citric acid, and 0-20 µM benzyl amino purine (BAP). The most popular local plantain variety, Apantu, only produced proliferating buds profusely when placed on routine medium (MS medium containing IAA, citric acid and 20 µM BAP). Reducing the concentration of BAP generated an average of more than 4 shoots/culture in 8 weeks. Medium not supplemented with any plant growth regulators also generated an average of less than 2 shoots/culture in 8 weeks. The other three *Musa* varieties generated 4-8 shoots/culture from proliferating buds, indicating that each cultivar has optimum concentrations at which rapid plantlet formation can be optimized to meet demands for planting material.

Keywords: buds, *in vitro*, medium with BAP, *Musa* sp., planting material, plantlet development, proliferating, shoot differentiation

INTRODUCTION

Musa species (plantains and bananas) are major food crops widely grown across the world's tropical and subtropical regions. The fruits are highly nutritious containing high amounts of carbohydrates, minerals such as phosphorus, calcium, and potassium as well as vitamins A and B. (INIBAP 2003). An estimated 20 million people eat banana and plantain as their major source of dietary carbohydrate (Bioversity International 2007). These crops serve as important source of revenue for many small-scale farmers. About 90% of the world's bananas and plantains are grown on small farms and consumed locally. World *Musa* production is around 85.5 million tonnes annually (FAO 2005), of which bananas cultivated for the export trade account for only 10%. Hence, fruit harvested from bananas and plantains are important components of food security in the tropical world, and provide income to the farming community through local and international trade (INIBAP 2003).

In Ghana plantains consumption was estimated at approximately 101.8 kg/cap/year (FAO 2005). This has increased over the years and to meet this demand, the main setback has been the availability of "clean" planting materials. Some efforts have been made to teach the farmers to produce clean planting materials by means of the split corm technique (Dzomeku *et al*. 2009). However other modern techniques such as tissue culture have to be employed to enhance the production.

Micropropagation of *Musa* is a widely utilized technique for the rapid production of clean clonal materials (Arias 1993). Around the world, there are several researchers producing clean planting materials using tissue culture protocols (Mateille and Foncelle 1988; Kalimuthu *et al*. 2007) and these can be adapted for different *Musa* species. There is however the need to optimize existing protocols to meet large-scale *in vitro* production targets especially, cultivars of local importance, since each genotype responds differently to the same culture medium (George 2008). Optimizing *in vitro* production of local cultivars will enhance the local industry and reduce importation. The culture media used for *in vitro* manipulations comprises of micro and macro salts, vitamins, plant growth regulators (PGRs) and a source of carbon. These components greatly affect production and the growth regulators used need to be critically analysed. Usually PGRs are included for organ development. The effect of PGRs is generally not absolute and specific, since responses of cultures vary based on culture conditions, type of explant and genotype (Dodds and Roberts 1995; Pierik 1998; George 2008). The cytokinin, 6-benzylaminopurine (BAP) is one of the PGRs commonly used in micropropagation of *Musa* species and it exhibits a typical effect of overcoming apical dominance and releasing lateral buds from dormancy as well as proliferation of adventitious buds (Dodds and Roberts 1995; Pierik 1998; George 2008) and this effect is manipulated in this study.

This study was to optimize the routine rapid propagation medium to maximize *in-vitro* generation of shoots from cultures that profusely produce axillary buds. The information provided will immensely enhance micropropagation of local Ghanaian *Musa* cultivars and other introduced cultivars. The local industry adapting this protocol will boost production of clean planting materials for field establishment.

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MATERIALS AND METHODS

Suckers of Apantu (False horn) were obtained from the field, washed, trimmed and surface sterilized using 70% ethanol and 20% sodium hypochlorite solution with 6% active chlorine. Apical meristems were excised as explants and initiated *in vitro* on complete Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 3% sucrose, 2 mM IAA, 20 µM BAP, 10 mg/l citric acid, (all chemicals and reagents were obtained from Sigma) pH of 5.7 ± 1, and solidified with 0.6% agar. The quantity of media used was 15 ml per 25 × 150 mm culture tube. Cultures were incubated under a 16-h photoperiod (light intensity 40 µM m^{-2} s^{-1}) at 26 ± 1°C and transferred or subcultured every 4 weeks. The development of proliferating axillary buds initiated following three months of *in vitro* manipulation. Clusters of proliferating buds were excised (0.5 × 1 cm having 2-3 buds) from these *in vitro* maintained cultures and used as explants for the experiments. In a preliminary experiment, explants were separated from the proliferating axillary buds of Apantu cultures and cultured on media, basically as above, but with three levels of BAP (0, 10, 20 µM). Incubation conditions were the same as above and data was collected after 8 weeks: organ development in cultures as bud formation, shoot and root development. Following results obtained using Apantu, another experiment was carried out using the following levels of BAP: 0, 10, 15, 20 µM. *Musa* cultivars used were two cultivars of False Horn (local names Apantu, and Osobuaso), one variety of intermediate French (local name Oniaba), and one-hybrid tetraploid French plantain (FHIA 21). The number of cultures was 15-20 and the experiment was repeated three times between 2006 to 2008. The experimental design was a two-way ANOVA and the F-test was used to reject or accept the null hypothesis of equal means. Fisher's least significant difference (LSD) method for comparing treatment group means was applied. Furthermore, Duncan's Multiple Range Test was used to identify specific differences between pairs of treatment means. GENSTAT discovery version 3 was used to carry out all the statistical analyses. Hardening of *in vitro* generated 6-8 week old plantlets was carried out in steam sterilized loamy soil, at 60% relative humidity and temperature; 28-35°C in a screenhouse. The well developed plantlets were removed from culture vessels ensuring that no damage was caused to leaves and roots. Medium on the roots was thoroughly rinsed off in water, and plantlet was transplanted into the loamy soil in a black plastic bag (18 × 13 cm). Transplanted seedlings were watered daily.

RESULTS AND DISCUSSION

Bud clusters were excised from proliferating axillary buds as explants (0.5 × 1.0 cm), from the False horn (Apantu) cultures and cultured on three different media (**Fig. 1**). The cultures showed significant differences in the number of cultures developing proliferating axillary buds. All cultures on medium with 20 µM BAP had proliferating axillary buds (**Fig. 3A**) and < 97% was recorded for the 10 and 0 µM BAP-supplemented media. The shoots differentiating from proliferating buds per culture (**Fig. 1**) was significantly higher on the 10 µM BAP (4.6 shoots/culture) medium (**Fig. 3B**) when compared to medium without BAP (1.8 shoots/culture). At $P = 0.05$ there was no significant difference between the 10 and 20 µM BAP-supplemented media, although more shoots differentiated from the cultures in the 10 µM (4.6) than the 20 µM (3.3) BAP medium. In a follow-up experiment, where four different media of varied levels of BAP across four *Musa* varieties (**Fig. 2**) were used, the number of proliferating buds for all cultures was high. According to the ANOVA F-test for the buds, there were no significant differences among the varieties used, and this was confirmed by the *P*-value (0.559) at the 5% level of significance (**Table 1**).

The most shoots (7.8 shoots/culture) differentiating from proliferating buds was of the intermediate French plantain (Oniaba) cultures on 10 µM BAP medium. Also, the two false horn plantains had more shoots developing on this medium than on the other media used. It could therefore be deduced that the 10 µM medium is an optimum medium for shoot differentiation from proliferating axillary buds than local false horn and intermediate French plantain. One of the false horn plantain varieties (Apantu) had drastically few shoots differentiating on the 15 and 20 µM media. This is an indication that these concentrations are inhibitory for shoot differentiation from proliferating buds of that cultivar since only 0.75 and 1.1 shoots/culture, respectively developed. However, the other false horn (Osobo-aso) and tetraploid (Oniaba) hybrid had a high average number of shoots (4.9 to 6.7), developing on these media (15 and 20 µM BAP). FHIA 21, which is a hybrid, had comparatively few shoots developing (2.3 shoots/culture). As FHIA 21 had its highest mean number of shoots occurring on medium supplemented with 20 µM BAP, it is probable that higher shoot development can be attained for this particular cultivar at higher BAP concentrations. Similar

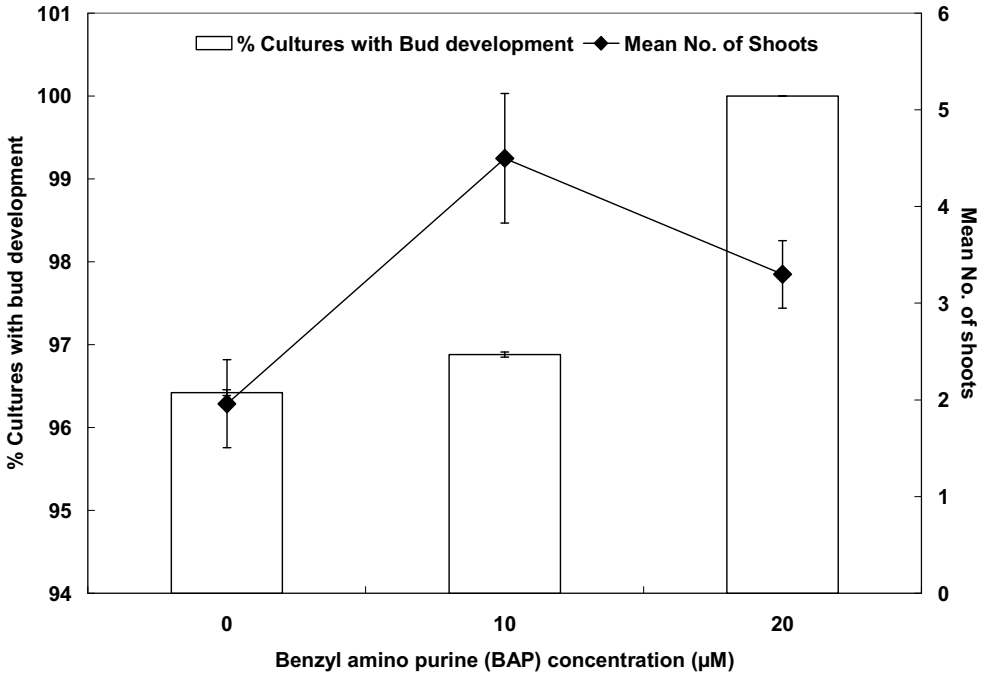

Fig. 1 Apantu cultures percentage bud development (bars graph) and mean number of leaves (line graph) following 8-week culture on media supplemented with three levels of benzyl amino purine (BAP). n = 15-20, bars = SE

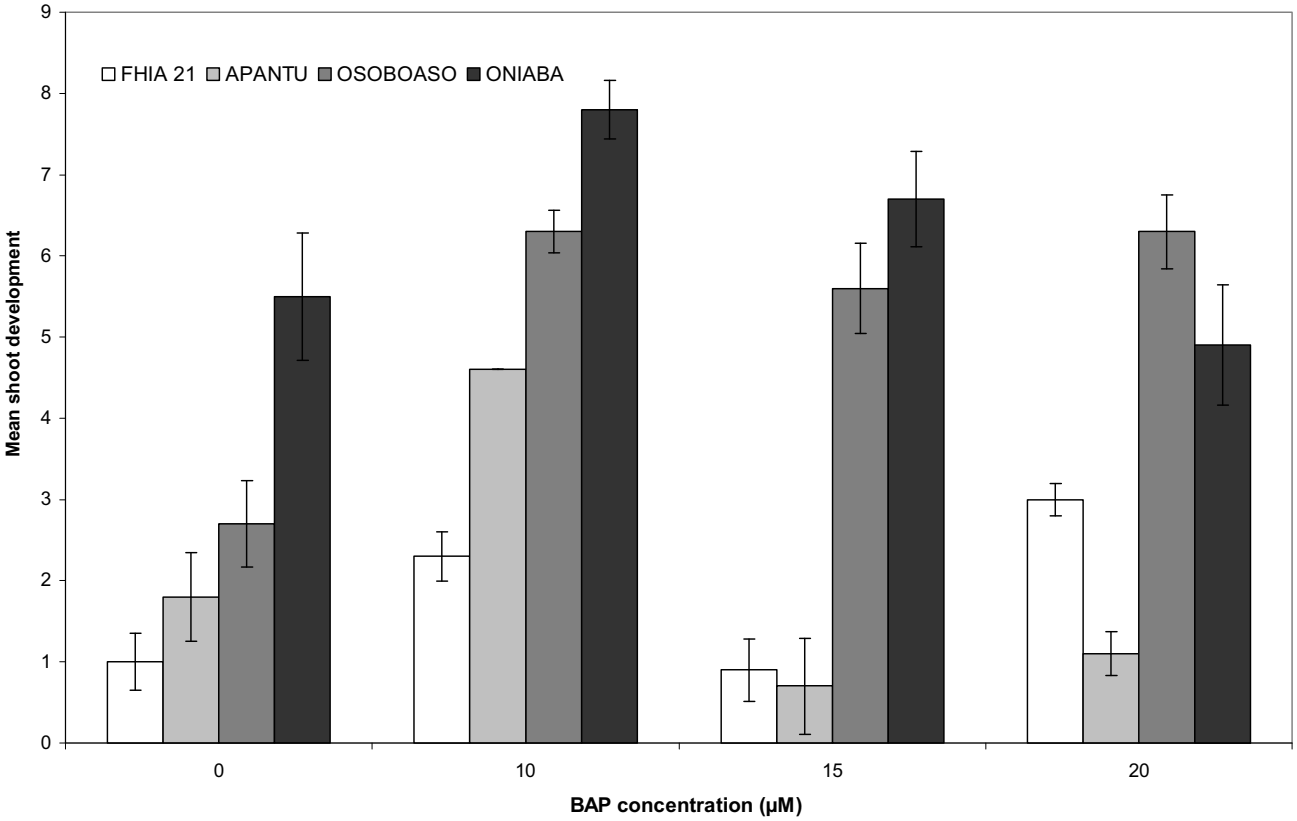

Fig. 2 Shoot development of various *Musa* varieties on media supplemented with four BAP concentrations. n = 15-20, bars = SE

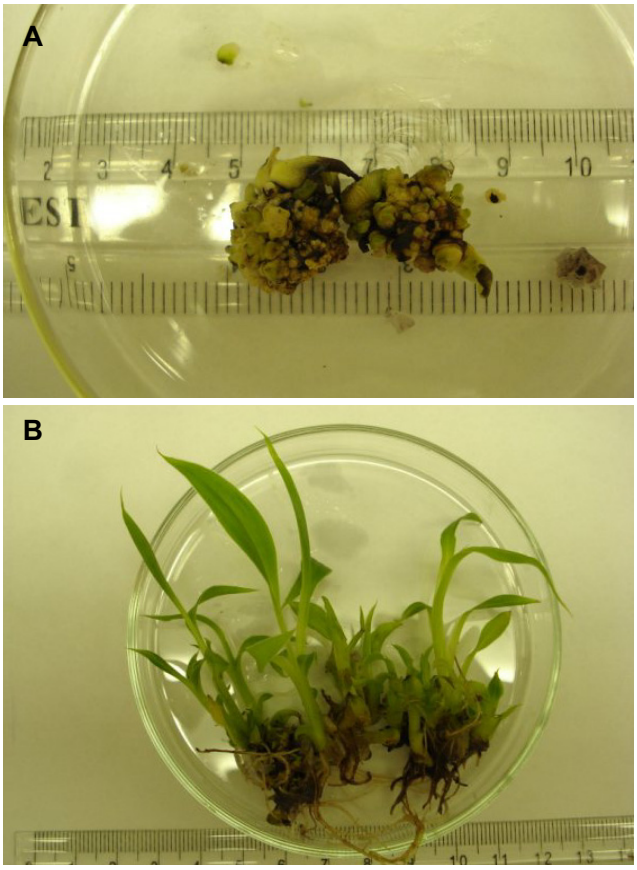

Fig. 3 Development of Apantu cultures following 8 weeks of growth on media supplemented with 20 µM (A) and 10 µM (B).

findings have been made by others (Gubbuk and Pekmezci 2004; Rahman *et al*. 2005). It could be deduced that the banana/plantain characteristics, namely tetraploid, may be influencing its low performance on the culture media used, compared to the triploid cultivars used in the study. The statistical analysis indicated that on the basis of both variety and concentration of BAP used, the differences recorded in the numbers obtained are very significant since a very low *P*-value (<0.001) was recorded and this was confirmed by Duncan's multiple range test. **Tables 1**, **2** and **3** indicate some of the statistical analyses.

The mean number of shoots recorded in this study is comparable with reports by Rahman *et al*. (2002, 2005). It

Table 1 Two-way ANOVA for buds.

Treatment	**Buds***
BAP (µM)	
0.0	0.925 b
1.0	0.975 ab
1.5	1.000 a
2.0	1.000 a
L.S.D (0.05)	0.0613
P-value (0.05)	0.071
VARIETY	
Apantu	1.000 a
Fhia 21	0.980 a
Oniaba	0.960 a
Osoboaso	0.980 a
L.S.D (0.05)	0.0549
P-value (0.05)	0.559
Interaction (BAP x VARIETY)	
BAP vs VARIETY	Ns

	VARIETY			
BAP µM	Apantu	Fhia 21	Oniaba	Osoboaso
0.0	1.000	1.000	0.800	0.900
1.0	1.000	0.900	1.000	1.000
1.5	1.000	1.000	1.000	1.000
2.0	1.000	1.000	1.000	1.000

L.S.D (0.05)	0.1227
P-value (0.05)	0.201
Grand mean	0.980

Ns: not significant; * Means followed by a different letter are significantly different, Duncan's Multiple range test (P ≤ 0.05)

Table 2 Two-way ANOVA for shoots.

Treatment					Shoots*
BAP (µM)					
0.0					2.75 b
1.0					5.24 a
1.5					3.46 b
2.0					3.83 b
L.S.D (0.05)					1.235
P-value (0.05)					<0.001
VARIETY					
Apantu					1.63 b
Fhia 21					1.66 b
Oniaba					5.27 a
Osoboaso					4.40 a
L.S.D (0.05)					1.104
P-value (0.05)					<0.001
Interaction (BAP x VARIETY)					
BAP vs VARIETY					Ns
		VARIETY			
BAP µM	Apantu	Fhia 21	Oniaba	Osoboaso	
0.0	1.80	1.00	5.00	so 2.70	
1.0	4.56	2.30	7.80	6.30	
1.5	0.70	0.90	6.70	5.56	
2.0	1.10	3.00	4.90	6.33	
L.S.D (0.05)					2.469
P-value (0.05)					0.010
Grand mean					3.24

** - significant at 5%; * Means followed by a different letter are significantly different, Duncan's Multiple range test (P ≤ 0.05)

Table 3 Two-way ANOVA for roots.

Treatment					Roots*
BAP (µM)					
0.0					0.150 a
1.0					0.050 b
1.5					0.000 b
2.0					0.028 b
L.S.D (0.05)					0.0847
P-value (0.05)					0.003
VARIETY					
Apantu					0.000 b
Fhia 21					0.000 b
Oniaba					0.080 a
Osoboaso					0.102 a
L.S.D (0.05)					0.0758
P-value (0.05)					0.010
Interaction (BAP x VARIETY)					
BAP vs VARIETY					Ns
		VARIETY			
BAP µM	Apantu	Fhia 21	Oniaba	Osoboaso	
0.0	0.00	0.00	0.40	0.20	
1.0	0.00	0.00	0.00	0.20	
1.5	0.00	0.00	0.00	0.00	
2.0	0.00	0.00	0.00	0.11	
L.S.D (0.05)					0.1695
P-value (0.05)					0.007
Grand mean					0.046

* - significant at 1%; * Means followed by a different letter are significantly different, Duncan's Multiple range test (P ≤ 0.05)

can be deduced that above 20 µM, BAP suppresses shoot differentiation and elongation from proliferating axillary buds, though the same concentration ensures the prolific production of axillary buds. This result confirms the report that above 20 µM BAP suppressed shoot elongation in three selected superior banana types namely "Alanya 5", "Anamur 10" and "Bozyazõ 14" (Gubbuk and Pekmezch 2004). However, other researchers used BAP concentrations ranging from 4.4 to 30 µM to screen for the most efficient medium for some banana cultivars (Dhumali *et al*. 1997; Josekutty *et al*. 2003).

The shoots that differentiated were transferred to fresh medium without any PGRs to enhance root development since on the 20 µM BAP-supplemented medium, root development was inhibited, and this can be associated with the use of a high cytokinin concentration that suppresses root development (Dodds and Roberts 1995; Pierik 1998; George 2008). Transferring shoots to medium without PGRs is also a means of initiating weaning off of cultures for field establishment (Dodds and Roberts 1995; Pierik 1998; George 2008). Plantlets developed within 4 weeks and were successfully hardened in the screenhouse. Screenhouse survival was 95-100% (**Fig. 4**), the seedlings were then transferred to the field for establishment after 6 weeks.

Fig. 4 ***Musa*** **seedlings surviving in the screenhouse.**

In this present study, false horn (Apantu) was initiated and proliferating axillary buds were established on medium with 20 µM BAP, and the proliferating buds were transferred to 10 µM BAP to enhance shoot differentiation from proliferating buds. The developed shoots were then transferred to medium without PGRs for plantlet development. This procedure could be adapted for other local varieties to ensure optimum production to meet demands for planting materials. However, a report by Kalimuthu *et al*. (2007) used one medium for shoot initiation, multiplication and plantlet development in a Cavensish dwarf variety.

CONCLUSION

It can be concluded that the local *Musa* cultivars have their peculiar responses to the media used. Shoot differentiation and development from proliferating bud cultures can best be attained in local varieties (Apantu, Osoboaso, and Oniaba) on 10 µM BAP medium. The tetraploid hybrid cultures have best shoot development on 20 µM BAP, however this treatment suppresses shoot development in Apantu. It is therefore recommended that for an effective rapid multiplication of these cultivars *in-vitro*, the response of each cultivar needs to be investigated and a protocol developed to maximize the generation of planting material. Differences in the number of shoots that develop could be associated with differences in response to the same treatment by different crop varieties (George 2008).

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