In vitro PROLIFERATION ABILITY OF AXILLARY BUDS IN *Musa* spp

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ABSTRACT

Tissue culture method has always considered the apical bud as the initial explant for micropropagation of plantain until recently where it was shown with the variety Big Ebanga that axillary buds could equally serve as initial explant. As axillary buds have shown mass propagation abilities in Big Ebanga, this explant is tested in other banana varieties to confirm performances already observed. Axillary buds and shoot tips excised from : Pisang Mas (AA), Grande Naine (AAA), Batard and French Clair (AAB), CRBP 39 (AAAB) and Pelipita (ABB) varieties were used as explants. The proliferation rate of Axillary and apical buds and other growth parameters were measured. Results showed that no significant differences were observed between the two types of buds after four to five sub cultures in all the varieties except for CRBP 39 where the axillary bud exhibits a higher proliferation rate. Concerning the frequency of leaf emission, the diameter and the height of the pseudostems, no significant differences were obtained between plants derived from the two types of buds during the weaning and hardening phases. In conclusion, axillary buds proliferated for all varieties tested regardless of the genomic group. Plantlets obtained from the two types of buds showed similar performance during the weaning and hardening phases. The axillary bud can thus be used as initial explant for micro propagation in *Musa* sp.

Key words : *Musa* sp., micropropagation, axillary buds, shoot tips, genomic group.

RESUME

APTITUDE A LA PROLIFERATION in vitro *DES BOURGEONS AXILLAIRES CHEZ* Musa *spp*

Le bourgeon terminal a été longtemps considéré comme explant initial de micropropagation chez le bananier. Récemment, il a été montré chez le cultivar Big Ebanga que le bourgeon axillaire pouvait aussi servir d'explant primaire. Ce résultat peut-il être transposé à d'autres variétés ? Les bourgeons apicaux et axillaires des variétés : Pisang Mas et Grande Naine (AAA), Batard et French Clair (AAB), CRBP 39 (AAAB) et Pélipita (ABB) ont été utilisés comme explants. Le taux de prolifération, et certains paramètres quantitatifs ont été mesurés. Les résultats obtenus montrent qu'il n'y a pas de différences significatives entre les deux types de bourgeon après 4 à 5 subcultures chez la plupart des cultivars sauf chez l'hybride CRBP 39 qui présente un taux de prolifération supérieur à celui obtenu chez le bourgeon terminal. Quant aux autres paramètres de croissance : émission de feuilles, diamètre et hauteur des pseudo-tiges, des différences significatives ne sont pas observées entre les plants issus des deux types d'explants (bt, ba) pendant les phases de sevrage et d'élevage. Il y a une prolifération du bourgeon axillaire chez toutes les variétés étudiées et appartenant à différents groupes génomiques du bananier. Les plantules issues des deux types de bourgeons croissent de manière identique pendant les phases de sevrage et d'élevage. Le bourgeon axillaire peut donc être utilisé comme explant primaire de micropropagation chez Musa *sp.*

Mots clés : Musa *sp, micropropagation, bourgeons axillaires, bourgeons terminaux, groupe génomique.*

INTRODUCTION

The main constraint of banana and plantain cultivation is the lack of planting material. By this fact, there is a need to use *in vitro* culture to produce quantitatively the requested demand by diversifying explants sources. The apical bud or shoot tip of suckers in banana and plantain has usually been used as initial explant for micropropagation (Vuylsteke, 1985, 1998 ; Vuylsteke and De Lange, 1985). In this condition, only one explant can be excised from a sucker. It was found on one hand that this can be a handicap in the case of non availability of suckers and on the other hand, the percentage of somaclonal variants increases with the number of subcultures. Thereby, it would be interesting to have a large number of explants per sucker to produce the same number of plantlets with less subcultures. Previous results in Big Ebanga shown that the axillary buds of plantain suckers could not only proliferate *in vitro* as well as the apical bud, but respond more quickly in the early subcultures (Youmbi and Ngaha, 2004). Another experiment devoted to the study of the behavior of plants derived from apical buds, axillary buds and suckers of the Big Ebanga cultivar (plantain) showed similar agronomic characteristics between the three types of plants (Youmbi *et al.,* 2005).

In the former work using axillary buds as explant for plantain micropropagation, only one cultivar, Big Ebanga (AAB, plantain False Horn) has been tested. To verify the efficiency of this protocol and then assure its wide adoption, axillary buds have been excised from suckers of various cultivars belonging to other genomic groups in the present study.

MATERIALS AND METHODS

PLANT MATERIAL

The axillary buds (AB) and shoot tip (ST) of cultivars : Grande Naine (GN : AAA) ; Pisang Mas (PM: AA) ; French Clair (FC : AAB) ; French Sombre (FS : AAB) ; Batard (B : AAB) an hybrid (CRBP 39 : AAAB) and Pelipita (ABB) were used as explants. These buds were excised from suckers collected from the *Musa* collection of Centre Africain de Recherche sur Bananiers et Plantains (CARBAP-Cameroon). They consisted of buds or «peeper suckers» and sword suckers.

METHODS

The culture medium consisted of basal medium of Murashige and Skoog (1962) supplemented with 2 ml of Morel vitamins (Morel, 1950), 2 mg / l BAP, 40 g / l sucrose and solidified with 2.2 g / l Phytagel. The pH was adjusted to 5.8 before autoclaving at 120 °C for 20 min under a pressure of one bar. Suckers were washed and trimmed and the apical and axillary buds were removed. They were then successively soaked in alcohol 70 % for 3 minutes, and in Mercryl lauryl solution for 15 minutes. Following this step and under the laminar flow hood, the buds have been immersed in a solution of sodium hypochlorite 8 % for 15 minutes and rinsed three times with sterile distilled water. After rinsing, axillary buds and the shoot tips reduced to a size of 2.5 to 3 cm were further immersed in a solution of sodium hypochlorite 6 % for 15 minutes and then rinsed three times with sterile distilled water. A final trimming is performed to obtain the explants of dimensions: 0.5 - 0.8 cm long and 0.8 - 1 cm in diameter for shoot tips and 0.2 - 0.5 cm long and 0.4 cm in diameter for axillary buds.

Seeding of shoot tips and axillary buds was done in jars containing 25 ml of the medium mentioned. Only one explant of each type of bud was put into a jar. These jars are sealed, labeled and transferred to a culture room where the average temperature was between 20 ± 2 °C, relative humidity ranging from 39.7 to 41.7 % and a photoperiod of 8/16 hours lighting was provided by fluorescent tubes 36 w.

The duration of introduction phase was two months during which there was an increase of the side of the explants in the first month after which the explants were removed and a cross section on the meristematic zone was done. They were then introduced in the same medium for buds formation at the end of the second month of the introduction step.

Multiplication phase : after the introduction step, the explants were removed and the buds initiated were separated and introduced in the proliferation medium on the basis of two per jar. Many (six) subcultures were done to produce plenty proliferated buds.

After the multiplication phase, the third step consisted in rooting and hardening of plantlets. The duration of this step was one month.

Acclimatization : the substrate used for acclimatization of plantlets consists of a mixture

of black soil- coffee husk (50 % - 50 %) sterilized for 12 hours using fire wood: The acclimatization consisted in weaning and hardening for four weeks of duration respectively.

Experimental design and measurement of parameters

The treatment consisted of seven varieties and two types of buds (axillary buds and shoot types). For each variety, 90 suckers (involving 90 axillary buds and 90 shoot tips) were divided in three lots of 30 suckers each and used as three replications. The experimental design was a complete randomization.

The comparison of the organogenic expression of these two types of buds was performed during the introduction and proliferation phases. Once the introduction was completed, observations were made every four weeks during trans-plantation and the Number of shoots issued and the number of leaves produced were evaluated.

After introduction and multiplication phases, the plantlets from these explants were transferred to rooting and hardening medium. This medium consisted in the multiplication medium without cytokinin (BAP). Some growth parameters: number of emerged leaves, height and diameter of the pseudostem were measured during weaning and hardening phases. The data obtained were subjected to a two-ways ANOVA to determine significant differences between buds and varieties, and the interaction between bud and variety. The Student New Man-Keuls test at the 5 % significance level was used to calculate Least Significant Differences. . These various data were processed with the software SPSS 10.1 for Windows

RESULTS

PROLIFERATION RATE OF SHOOT TIPS AND AXILLARY BUDS

In most of the varieties, axillary buds showed precocious proliferation compared to shoot tips (Figures 1 and 2). The number of buds produced varied among the cultivar and the genomic groups (Table 1). The rates of proliferation of both types of buds were not significantly different after five subcultures. This proliferation was very high in Grande Naine compared to other varieties. The varietal effect is significant at $p < 0.05$, for the first and third subculture but not significant in the second subculture. Beyond the third subculture, the varietal effect was significant $p < 0.001$. The varietal effect, as well as the bud-variety interaction was significant at $p < 0.001$.

Figure 1 : Single plantlet regeneration from shoot tip.

Régénération d'une seule plantule à partir du bourgeon apical.

Figure 2 : Precocious proliferation from axillary bud.

Prolifération précoce à partir su bourgeon axillaire.

Table 1 : Comparison of the average number of shoots produced per variety and genomic group as a function of subcultures.

Comparaison de pousses émises par variété et par groupe génomique en fonction des subcultures.

Treatment		Subcultures					
		1	2	3	4	5	6
GN	ST	1.93±0.16f	9.55 ef	11.55 e	12.75 e	13.7 de	14.75 def
(AAA)	AB	1.44±0.08e	9.7 f	12.35 f	13.5 f	14.5 e	15.66 f
PTa	ST	0.57±0.13b	8.45 cd	10.55 d	12.15 de	13.65 de	14.95 ef
(ABB)	AB	0.03±0.03a	7.5 b	9.1 b	11.6 cd	13.2 cd	14.9 ef
FC	ST	1.30±0.15cde	7.35 b	9.25 bc	11.25 bc	12.4 bc	14 cd
(AAB)	AB	0.99±0.08cd	7.85 bc	10 cd	11.55 cd	12.65 c	13.75 bc
CRBP 39	ST	0.90±0.09b	6.05 a	7.2 a	8.6 a	9.65 a	11.75 a
(AAAB)	AB	0.03±0.02a	7.25 b	9 b	12.4 de	13.4 cd	14.4 cde
PM	ST	0.03±0.06a	8.95 de	9.95 cd	12.35 de	14.1 de	15.35 ef
(AA)	AB	0.03±0.09a	9.4 ef	10.4 d	13 ef	14 de	15 ef
B	ST	1.43±0.17e	8.2 c	9.3 bc	10.6 b	11.65 b	13.05 b
(AAB)	AB	1.09±0.08cd	7.4 b	8.8 b	10.6 b	11.85 b	13.7 bc
Bud		*	Ns	*	***	***	***
Variety		***	***	***	***	***	***
Interaction		***	***	***	***	***	***

Values followed by the same letters in the same column are not significantly different
*, **, *** Significant at p <0.05, 0.01, 0.001
NS: not significant () a
AB : Axillary Buds and ST : Shoot Tip

LEAF EMISSION OF PLANTLETS DERIVED FROM AXILLARY BUDS AND SHOOT TIPS

Foliar emission varied from one variety to another and between the two types of buds. If the differences are not noticeable between the two types of bud, the plantlets from axillary bud of CRBP 39 produce more leaves than those from the apical bud. (Table 2) The varietal effect was significant at $p < 0.001$ and 0.05 respectively at the end of weaning and hardening for foliar emission. The interaction bud - variety is significant at $p < 0.001$

HEIGHT OF PLANTLETS DERIVED FROM AXILLARY BUDS AND SHOOT TIPS

Growth in height was evaluated. The results (Table 3) show that the plantlets from apical and axillary buds did not show significant differences during the seventh and eighth week (hardening phase). This is very important because at this stage the seedlings are ready for planting. The bud effect is significant at $p < 0.5$ during late weaning and not significant at the end of hardening. The varietal effect and the interaction between variety and bud is significant at $p < 0.001$ and at the end of weaning and hardening.

DIAMETER OF PLANTLETS DERIVED FROM AXILLARY BUDS AND SHOOT TIPS

The diameter evolved with an increase in the size of the plantlets. Mean comparison (Table 4) showed that the bud effect was significant at $p < 0.001$ in late weaning and not significant at the end of hardening. The varietal effect is significant at $p < 0.001$ in late weaning and significant at $p < 0.5$ in late hardening while the interaction between variety and buds is significant at $p < 0.001$ in late weaning and late hardening.

Generally, these results show that plantlets from tissue culture of axillary buds do not show overall significant differences compared to plantlets from apical buds

Table 2 : Average number of leaves produced during weaning and hardening.

Nombre moyen de feuilles émises au cours du sevrage et de l'élevage.

Treatment		Time (weeks)					
		Weaning		Hardening			
		3	4	5	6	7	8
GN	ST	7.6 ef	9.55 ef	11.55 e	12.75 e	13.7 de	14.75 def
(AAA)	AB	8.15 fg	9.7 f	12.35 f	13.5 f	14.5 e	15.66 f
Pta	ST	7.51 def	9. 62 ef	11.54 e	12.81 e	13.81 de	14.65 de
(ABB)	AB	8.41 g	9.71 f	12.41 f	13 ef	14.12 de	14. 93 ef
FS	ST	7.45 def	8.45 cd	10.55 d	12.15 de	13.65 de	14.95 ef
(AAB)	AB	6.5 bc	7.5 b	9.1 b	11.6 cd	13.2 cd	14.9 ef
FC	ST	6.35 b	7.35 b	9.25 bc	11.25 bc	12.4 bc	14 cd
(AAB)	AB	6.85 bcd	7.85 bc	10 cd	11.55 cd	12.65 c	13.75 bc
CRBP 39	ST	5:00 AM	6.05 a	7.2 a	8.6 a	9.65 a	11.75 a
(AAAB)	AB	6.65 bc	7.25 b	9 b	12.4 de	13.4 cd	14.4 cde
PM	ST	7.8 efg	8.95 de	9.95 cd	12.35 de	14.1 de	15.35 ef
(AA)	AB	8.4 g	9.4 ef	10.4 d	13 ef	14 de	15 ef
B	ST	7.2 cde	8.2 c	9.3 bc	10.6 b	11.65 b	13.05 b
(AAB)	AB	6.45 bc	7.4 b	8.8 b	10.6 b	11.85 b	13.7 bc
Bud		*	Ns	*	***	***	***
Variety		***	***	***	***	***	***
Interaction		***	***	***	***	***	***

Values followed by the same letters in the same column are not significantly different / *, **, *** Significant at p < 0.05, 0.01, 0.001 / NS : not significant.
The time (1, 2) weeks are not included in the tables because observations began two weeks after the transfer of plants into pots
AB : Axillary Buds and ST : Shoot Tip.

Table 3 : Change in average height (cm) of the pseudostem during weaning and hardening.

Variation de la taille moyenne (cm) des pseudotiges au cours du sevrage et de l'élevage.

Treatment		Time (weeks)					
		Weaning		Hardening			
		3	4	5	6	7	8
GN	ST	4.48 bcd	8.27 d	10.04 e	13.49 d	15.46 bcd	17.73 c
(AAA)	AB	4.77 cde	7.7 d	11.2 f	15.22 e	16.96 cd	18.31 c
Pta	ST	5.40 de	5.91 b	8.92 d	10.12 c	13.50 abc	16.65 c
(ABB)	AB	5.15 de	5.79 b	8.90 d	10.15 c	13.95 abc	17.12 c
FS	ST	7.665 g	8.28 d	10.18 e	11.34 c	19.92 d	21.38 d
(AAB)	AB	4.93 de	5.79 b	7.88 c	10.15 c	13.51 abc	17.65 c
FC	ST	3.98 abc	5.33 b	6.41 b	7.86 ab	11.06 abc	18.77 c
(AAB)	AB	5.41 de	6.68 c	7.51 c	11.14 c	13.95 abc	18.05 c
CRBP 39	ST	3.71 ab	3.92 a	4.66 a	6.53 a	7.88 a	11.9 a
(AAAB)	AB	6.39 f	6.86 c	9.22 c	7.88 ab	11.29 abc	13.95 ab
PM	ST	5.15 de	5.94 b	7 b	8.57 b	12.78 abc	15.08 b
(AA)	AB	5.5 e	8.02 d	8.9 d	11.03 c	12.55 abc	14.95 b
B	ST	4.79 cde	5.33 b	6.15 b	7.15 ab	10.18 ab	14.29 b
(AAB)	AB	3.19 a	3.94 a	5.09 a	6.92 a	13.52 abc	13.79 ab
Bud		Ns	*	***	***	ns	ns
Variety		***	***	***	***	**	***
Interaction		***	***	***	***	***	***

Values followed by the same letters in the same column are not significantly different
*, **, *** Significant at p < 0.05, 0.01, 0.001 / NS : not significant
The time (1, 2) weeks are not included in the tables because observations began two weeks after the transfer of plants into pots / AB : Axillary Buds and ST : Shoot Tip

Table 4 : Change in average diameter (cm) of the pseudostem during weaning and hardening.

Variation du diamètre moyen (cm) des pseudotiges au cours du sevrage et de l'élevage.

Treatment		Time (weeks)					
		Weaning		Hardening			
		3	4	7	8	9	10
GN	ST	0.675 e	0.83 g	1:00 AM	1.02 d	1.07 cde	1.26 c
(AAA)	AB	0.6 cde	0.81 fg	1:00 AM	1.06 d	1.15 e	1.29 c
Pta	ST	0.65de	0.76def	0.86 a	0.99 d	1.12 de	1.26 c
(ABB)	AB	0.59cd	0.65 cd	0.79 a	0.87 c	1.02 bcd	1.23 bc
FS	ST	0.69 e	0.73 def	0.92 a	1 d	1.16 e	1.22 bc
(AAB)	AB	0.55 c	0.63 c	0.79 a	0.99 d	1.01 bcd	1.16 abc
FC	ST	0.45 b	0.56 b	0.61 a	0.87 c	1.08 cde	1.41 d
(AAB)	AB	0.53 c	0.65 cd	0.73 a	0.99 d	1.17 e	1.41 d
CRBP 39	ST	0.56 bc	0.74 defg	0.76 a	0.88 c	1 bc	1.195 abc
(AAAB)	AB	0.62 cde	0.71 cde	2.74 b	1 d	1.12 de	1.26 c
PM	ST	0.59 cd	0.68 cde	0.83 a	0.88 c	1.02 bcd	1.16 abc
(AA)	AB	0.65 de	0.77 efg	0.86 a	0.90 c	1.02 bcd	1.11 ab
B	ST	0.41 b	0.42 a	0.51 a	0.78 b	0.95 b	1.23 bc
(AAB)	AB	0.35 a	0.41 a	0.51 a	0.62 a	0.81 a	1.07 a
Bud		**	***	**	Ns	Ns	Ns
Variety		***	***	***	***	***	*
Interaction		***	***	***	***	***	***

Values followed by the same letters in the same column are not significantly different

*, **, *** Significant at p <0.05, 0.01, 0.001. NS : not significant

The time (1, 2) weeks are not included in the tables because observations began two weeks after the transfer of plants into pots. AB : Axillary Buds and ST : Shoot Tip

DISCUSSION

The axillary bud proliferated *in vitro*. If at the beginning of transplanting, the apical bud had a proliferation rate higher than that obtained with the axillary bud and, these two types of explant gave from the fifth or sixth subculture, an almost identical proliferation rate. The variety in which the axillary bud had a different rate than that obtained with the apical bud during the sixth subculture was CRBP 39. Earliness was absent in the first subculture in both the French Clair and Batard as observed in the Big Ebanga (Youmbi and Ngaha, 2004). The lack of earliness may be related to the variety or quality influenced by its environment

Despite the small differences in the rates of proliferation of both types of explant and that the behavior of the two types of bud is the same as from the sixth subculture shows that axillary and apical buds have the same aptitude to proliferate in vitro. This result is similar to that recently observed with the cultivar Big Ebanga (Youmbi and Ngaha, 2004). The use of axillary bud independently to the cultivar, has led to the doubling in the number of plantlets obtained after the sixth subculture. This has a great advantage over the classical protocol described by many authors in the micropropagation of *Musa* sp (Lee, 1992 ; Okole and Schulz, 1996 ; Kalimuthu *et al.,* 2007 ; Amomwat and Kamnoon, 2007 ; Kone *et al.,* 2010 ; Govindaraju *et al.,* 2012). Another advantage in the use of axillary bud would limit the number of subcultures for a large number of tissue culture plantlets free from somaclonal variants (Müller and.Sandoval, 1986 ; 1987)

Plantlets from the two types of buds were hardened before acclimatization. During acclimatization, quantifiable parameters (diameter and height of the pseudostem, number of leaves produced) were evaluated. The fact that varietal effect was significant, confirmed the observation that vegetative growth is a function of the species, the variety or cultivar (Gübbük

and Pekmezci, 2004). The results obtained showed that, whatever the cultivar, height, as well as the diameter are identical in plantlets from the two types of buds. This showed that these plantlets when well conducted in the nursery can be transferred at the same time in the field. This result also confirmed those obtained on the weaning of plantlets from apical and axillary buds in Big Ebanga, plantlets that are consistent with no somaclonal variants (Youmbi *et al.,* 2005).

CONCLUSION

The observations obtained on plantlets from axillary and apical buds in the Big Ebanga are comparable to those obtained on the same types of explants from cultivars belonging to different genomic groups. If the varietal effect was at times significant at the beginning of the proliferation, the multiplication rate did not indicate any significant difference as from the fourth subculture. The rate of proliferation of axillary buds remained high in the CRBP 39. During acclimatization, the plantlets from two types of explant showed no change, indicating that these plantlets are identical. These results confirm that axillary bud can be considered as initial explant in the micropropagation of *Musa* sp.

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