

MACROPROPAGATION OF PLANTAIN (*MUSA SPP.*) CULTIVARS PITA 3, FHIA 21, ORISHELE AND CORNE 1: EFFECT OF BENZYLAMINOPURINE (BAP) CONCENTRATION

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Abstract: In Africa, plantain is one of the most important starchy food and cash crops. Nonetheless, one of the major constraints for its production was the unavailability of healthy planting materials at planting time. This constraint could be lifted using the cloning of planting materials via the *in vitro* micropropagation or *in vivo* macropropagation techniques. Shelled corms from four cultivars, known as PITA 3, FHIA 21, ORISHELE and CORNE 1, were used. Three treatments differing in three hormonal concentrations, especially 20.0, 30.0 and 40.0 mg L⁻¹ were tested. The control one was hormone free. Tested treatments were laid out in a split plot design. The decorticated banana corms were sprayed twofold at 2 weeks interval with BAP solution when placed in sterilized soil in high humidity plastic tunnel. It emerged from results, regarding BAP concentration effect, that BAP treatment with 40 mg L⁻¹ significantly reduced the emergence time of shoots at 20 days as against 25.1, 28.3 and 28.5 for the 2 tested other treatments as well as control, respectively. Likewise, the concentrations 40.0 mg L⁻¹ both recorded the largest number of sprouted buds per corm and number of shoots per corm. With respect to banana cultivar effect, PITA 3 showed the largest number of shoots per corm. Basing on such findings, it is concluded that MSD technique combined with BAP at 40.0 mg L⁻¹ is a suitable technique for improving of the *in vivo* macropropagation of plantain. This concentration increased at least 50 % of sucker production compared to control.

Keywords: Plantain, *in vivo* macropropagation, MSD, Benzylaminopurine (BAP)

Introduction

Plantain is a staple food for many people in Africa. Nearly 30 million tons of plantain is yearly produced in Africa, mostly by small holders and consumed locally [FAO, 2010]. The demand for this local product is very high in rural and urban markets. Plantain is ranked among the most preferred foodstuffs, highly valued and contributes in feeding more than 250 million people in countries of West and Central Africa [TOMEKPE & al.

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2011]. It is also a major source of income for many people and actors in the supply chain in the rural and urban sectors [JACOBSEN & al. 2004].

In spite of its great socioeconomic importance, the cultivation of the crop has however never satisfied the domestic demands. Low production is due to pest and disease constraints such as, among others, nematodes [FOGAIN, 2000]. Likewise, banana weevils, and foliar diseases such as black leaf streak caused by (*Mycosphaerella fijiensis*) are part of these constraints. Such a situation is aggravated by poor agronomic practices.

Plantain as parthenocarpic and seedless is traditionally propagated by planting corms and suckers. Suckers are traditionally used by farmers as planting materials coming from their own plantations. These suckers are most of time affected with pests (e.g., nematodes and weevils) and diseases (e.g. viruses such as banana bunchy top, banana streak). The suckering ability of plantain is very low with an average of about 3 suckers per year per stool depending on agro-climatic conditions and cropping practices [JOAB, 2004]. The lack and poor quality of planting materials are threatening plantain production and limit the expansion of plantations [DZOMEKU & al. 2014]. The quantity and quality of the planting material are major factors for successful crop production [TENKOUANO & al. 2006]. This could be achieved through clonal planting materials obtained via the *in vitro* micropropagation or *in vivo* macropropagation techniques. *In vivo* macropropagation is an alternative technique for mass production of banana planting materials under *in vivo* conditions [KWA, 2003]. Compared to the *in vitro* one, this technique is relatively simple, less expensive and provides in a short period pest-free and genetically identical plantlets [KINDIMBA & MSOGOYA, 2014].

The *multiplication sur souches décortiquées* (MSD) is one of methods of banana for *in vivo* macropropagation technique. This method exploits the entire potential of the corms to produce large quantities of healthy planting materials within a short period from secondary buds [KWA, 2003; NJUKWE & al. 2005; MSOGOYA & MWAKISITU, 2014]. Nevertheless, information about the response of plantain to MSD method in combination with BAP at different concentrations is hardly known. Benzylaminopurine is an adenine-based cytokinin popularly used for *in vitro* induction of axillary and adventitious shoots in banana [KALIMUTHA & al. 2007; BHOSALE & al. 2011; DEVENDRAKUMAR & al. 2013] and rarely used for *in vivo* macropropagation [KINDIMBA & MSOGOYA, 2014]. The testing of this technique in combination with BAP at different concentrations might allow the identifying of a concentration which may trigger strong production of suckers from corms as a function of cultivars. The objective of this study was to evaluate the effect of BAP concentration on *in vivo* proliferation technique (MSD) of 4 cultivars tested.

Materials and methods

Plant material, culture preparation and conditions

The study was carried out in 2012 and repeat in 2013 during 6 months at the Centre National de Recherche Agronomique (CNRA, Côte d'Ivoire), Azaguie Station, at 05°18'N and 04°09'W, 20 m above sea level. Four cultivars were used in this study. The CORNE 1 (False Horn) and ORISHELE (False Horn) are triploid plantains (*Musa spp.* AAB group) and are the most important varieties popularly grown in Côte d'Ivoire. The

FHIA 21 and PITA 3 are plantain-like hybrids belonging to the genome group AAAB with a ploidy level of 4x. FHIA 21 and PITA 3 hybrids are respectively crossed between AAB Plantain cv. AVP-67 x SH-3142 and AAB Plantain cv. Obino l'Ewaï x Calcutta 4. Ten corms of plant mother of each cultivar of 8 months were carefully removed from field grown banana plant. Corms weighing between 7 and 8 kg were used for the experiment. The corms were cleaned, pared to remove roots. The leaf sheathes of the corms were carefully stripped away one by one, to expose axillary buds nodes at the basis of each leaf (Fig. 1). The apical meristem of each corm was destroyed by decortication to overcome the apical dominance. The materials generated were planted 5cm deep in sterilized soil in high humidity plastic tunnel with temperature of 25–30 °C.

Experimental design

Two factors, namely cultivar and hormonal (BAP) concentration, each with 4 variants, were used. In all, 8 treatments, obtained from combination of the variants of 2 aforesaid factors, were repeated threefold and laid out in a split plot design. A replication consisted of 10 corms of each cultivar. Banana cultivars were the main plot factor while BAP concentrations (0.0, 20.0, 30.0 and 40.0 mg L⁻¹) were the sub-plot factor. The decorticated banana corms were pulverized by 10 ml of each BAP concentration twofold at 1 week interval. Irrigation was regularly carried out to maintain moist environment.

Data collection and statistical analysis

Seven variables were measured. These were : i) the number of days to first shoot or sucker emergence, ii) the number of sprouted buds per corm, iii) the number of shoots per corm, iv) the shoot height, v) the shoot collar girth, vi) the number of roots per shoot and vii) number of leaves per shoot. The collected data were analysed using STATISCA 6.0 software. The Bartlett's test for equality of variance of sub-populations analysed as well as that of Shapiro-Wilks for normality of the distributions of measured variables were used. These tests were performed prior to analysis of variance (ANOVA). Means were separated according to Student-Newman-Keuls' test at 0.05 probability.

Results and discussion

Under field conditions, suckers production by plantains is very low. Indeed, in spite of the presence of several axillary buds, they produced only about 10 suckers during the crop cycle due to apical dominance. Even if, the apical dominance is removed at flowering, often it is only few of the primary buds that develop into daughter suckers. However, with this MSD technique, several of axillary buds could be activated to sprout as healthy seedlings for planting. The same result could be obtained with the PIBS (Plants Issus de Bourgeons Secondaires) technique [KWA, 2003; DZOMEKU & al. 2014]. The results of our study revealed a significant effect of BAP concentration on axillary buds activation. The number of axillary buds activated significantly ($P \leq 0.05$) increased as BAP concentration increased from 0.0 to 40.0 mg L⁻¹ (Tab. 1). BAP at 40.0 mg L⁻¹ resulted in the largest number of sprouted buds of 12.4 buds per corm followed by BAP at 30.0, 20.0 and 0.0 mg L⁻¹ with 9.9, 8.5, and 7.0 sprouted buds per corm, respectively.

Results also indicated that BAP concentration had a significant ($P \leq 0.05$) influence on the number of days from corm sowing to first shoot emergence and number of shoot per corm (Tab. 1). Banana corms treated with BAP at 40.0 mg L⁻¹ produced the first shoot earlier at 20.0 days followed by corms treated with BAP at 30.0, 20.0 and 0.0 mg L⁻¹ with 25.1, 28.5 and 28.3 days, respectively (Tab. 1). This result quite agrees with the study of KINDIMBA & MSOGOYA (2014) who shows the effect of BAP on first shoot emergence. In another study, MSOGOYA & MWAKISITU (2014) reported the action of another cytokinin (Thidiazuron, TDZ) on the first shoot emergence and the number of shoot per corm. Cytokinins such as benzyl amino purine (BAP) and Kinetin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana [DEVENDRAKUMAR & al. 2013]. The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of banana *in vitro* micropropagation and *in vivo* macropropagation [BUAH & al. 2010; AZAM & al. 2010; JAFARI & al. 2011; BHOSALE & al. 2011; KINDIMBA & MSOGOYA, 2014]. Sometime, the BAP is combined with additives like bio-fertilizers such as *Bacillus subtilis* to induce more sprouting of axillary buds in banana [SAJITH & al. 2014].

With respect to BAP concentration effect, in relation to the number of shoots per corm, the number of shoots significantly ($P \leq 0.05$) increased with BAP concentration from 0.0 to 40.0 mg L⁻¹ (Tab. 1). Four statistically different groups of means were evidenced. First, represented by BAP concentration of 0.0 mg L⁻¹ was characterised by very low number of shoots per corm (165.0 shoots). Second, illustrated by BAP concentration of 20.0 mg L⁻¹ differed from the first by low number of shoots per corm (181.2 shoots). Third, consisting of BAP concentration of 30.0 mg L⁻¹ was marked by fairly high number of shoots per corm (204.8 shoots). Fourth, comprising BAP concentration of 40.0 mg L⁻¹ stood out from the first 3 groups by the highest number of shoots per corm (280.8 shoots).

Number of leaves per sucker, sucker height and sucker collar girth significantly ($P \leq 0.05$) increased as BAP concentration increased from 0.0 to 40.0 mg L⁻¹ (Tab. 2). Moreover, number of roots per sucker did not influenced by BAP concentration. Corms treated with BAP at 40.0 mg L⁻¹ produced suckers with the largest number of leaves of 4.4 per sucker followed by corms treated with BAP at 30.0, 20.0 and 0.0 mg L⁻¹ with 4.0, 3.4 and 2.9 leaves per sucker, respectively. Conversely, corms treated with BAP at 40.0 mg L⁻¹ had largest collar diameter and tallest sucker followed by corms treated with BAP at 30.0, 20.0 and 0.0 mg L⁻¹. Similar result was obtained by KINDIMBA & MSOGOYA (2014) with the positive action of BAP at 1.5 and 3.0 mg L⁻¹ on banana growth parameters and shoots production. Contrary to KINDIMBA & MSOGOYA (2014) work, positive responses of banana were obtained in our study with high BAP concentration (40.0 mg L⁻¹) where corms from mother plant were used compared to corms from suckers used by KINDIMBA & MSOGOYA (2014). In a similar experiment, MANZUR MACIAS (2001) increased suckers proliferation by injecting 4.0 ml of BAP at 40.0 mg L⁻¹ in the cavity left by the removal of the apical meristem of the corms. Under *in situ* conditions where BAP at 40.0 mg L⁻¹ treated sucker produced an average of 4 suckers at both G1s and G2s stages and the same technique applied to G3s produced an average of 13 plantlets, which are very similar to those obtained *in vitro*.

Banana cultivar had a significant ($P \leq 0.05$) effect on the number of sprouted buds per corm, number of days to first shoot emergence and number of shoots per corm (Tab. 3).

Cultivar PITA 3 (plantain-like hybrid) produced the first shoot earlier at 26.3 days and the largest number of sprouted buds with 8.0 buds per corm against 5.8, 6.3 and 7.8 buds sprouted per corm in ORISHELE, CORNE 1 and FHIA 21, respectively. PITA 3 also produced the largest number of shoots of 198.2 per corm compared with banana cv. CORNE 1, ORISHELE and FHIA 21 with 137.3, 147.8 and 178.6 shoots per corm, respectively. However, banana cultivar had no significant ($P \leq 0.05$) effect on sucker height, collar girth, number of leaves and roots per sucker. The results of the investigation revealed an influence of the banana variety to MSD technique on shoot proliferation. Indeed, varieties behavior is not the same *in vivo* macropropagation [DZOMEKU & al. 2014, MSOGOYA & MWAKISITU, 2014]. PITA 3 and FHIA 21 belonging to the same subgroup of plantain (French's group) produced the largest number of sucker compared to CORNE 1 and ORISHELE belonging to False Horn group. This result concurs with those of KWA (2003), who found that the average number of suckers was significantly higher for French clair (French) and French sombre (French) with approximately 18 and 17 suckers, respectively than Bâtard (False Horn) and Mbouroukou N° 1 (False Horn) with approximately 14 and 16 suckers, respectively. The best performance of FHIA 21 and PITA 3 seems to be linked to their ploidy level. FHIA 21 and PITA 3 are hybrids belonging to the genome group AAAB with a ploidy level of 4x, whereas, CORNE 1 and ORISHELE are triploid plantains belonging to the genome group AAB with a ploidy level of 3x. The superiority of tetraploid hybrids would be partly due to gene dosage effects at polyploid level [ORTIZ, 1995; TOMEKPE & al. 1995].

The interaction of banana cultivars and BAP concentrations had a significant ($P \leq 0.05$) effect on number of sprouted buds per corm (Fig. 2) and number of shoots per corm (Fig. 3). Banana cv. PITA 3 produced the largest number of sprouted buds of 13.3, 10.9, 9.5 and 8.0 buds per corm with BAP at 40.0, 30.0, 20.0 and 0.0 mg L⁻¹ respectively, followed by FHIA 21, CORNE 1 and ORISHELE (Fig. 2). Shoot production by PITA 3 was the highest among the cultivars whatever BAP concentrations. Indeed, PITA 3 produced the largest number of shoot of 344.1, 240.5, 213.1 and 198.1 shoots per corm with BAP at 40.0, 30.0, 20.0 and 0.0 mg L⁻¹ respectively followed by FHIA 21, ORISHELE and CORNE 1 (Fig. 3). The interaction of banana cultivars and BAP concentrations had no significant ($P \leq 0.05$) effect on sucker height, sucker collar girth and number of leaves per sucker.

Conclusion

We postulated that there may be a BAP concentration able to increase effectiveness of the *in vivo* macropropagation of plantain. After the testing, only the BAP concentration equal to 40.0 mg L⁻¹ was the most effective. The *multiplication sur souches décortiquées* (MSD) technique is an effective method that could generate large quantities of healthy planting materials from any type of corms. The technique was more efficient when combined with BAP at 40.0 mg L⁻¹. This dose increased at least 50 % of shoots production. The response of banana to BAP depends on cultivars where cv. PITA 3 provides the highest *in vivo* multiplication rate with BAP at 40.0 mg L⁻¹. Further studies are required to test the responses of other plantain cultivars to *in vivo* macropropagation in combination with different BAP concentrations or other cytokinin growth regulators.

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Tab. 1: Effect of BAP concentrations on *in vivo* multiplication of plantain cultivars

BAP concentration (mg L ⁻¹)	Number of sprouted buds per corm	Number of days to first shoot emergence	Number of shoots per corm
0.0	7.0 ^a	28.5 ^a	165.0 ^a
20.0	8.5 ^b	28.3 ^a	181.2 ^{ab}
30.0	9.9 ^c	25.1 ^b	204.8 ^b
40.0	12.4 ^d	20.0 ^c	280.8 ^c
CV (%)	15.1	12.3	17.4

Means followed by the same letters within the column are not significant different at 5% level after Student Newman-Keuls' test.

Tab. 2: Effect of BAP concentrations on growth parameters of plantain suckers

BAP concentration (mg L ⁻¹)	Number of leaves per suckers	Sucker height (cm)	Sucker collar girth (cm)	Number of roots per sucker
0.0	2.9 ^a	10.2 ^a	0.9 ^a	8.8 ^a
20.0	3.4 ^b	11.1 ^b	1.1 ^b	9.1 ^a
30.0	4.0 ^c	12.5 ^c	1.3 ^c	9.2 ^a
40.0	4.4 ^d	14.2 ^d	1.9 ^d	9.3 ^a
CV (%)	16.2	12.7	20.1	9.7

Means followed by the same letters within the column are not significant different at 5% level after Student Newman-Keuls' test.

Tab. 3: Response of plantain cultivar to MSD technique on *in vivo* shoot proliferation

Cultivar	Number of sprouted buds per corm	Number of days to first shoot emergence	Number of shoots per corm
CORNE 1(False Horn)	6.3 ^a	28.3 ^a	137.3 ^a
ORISHELE (False Horn)	5.8 ^a	28.1 ^a	147.8 ^a
PITA 3 (French)	8.0 ^b	26.3 ^b	198.2 ^b
FHIA 21 (French)	7.8 ^b	28.0 ^a	176.6 ^b
CV (%)	18.3	10.4	15.8

Means followed by the same letters within the column are not significant different at 5% level after Student Newman-Keuls' test.

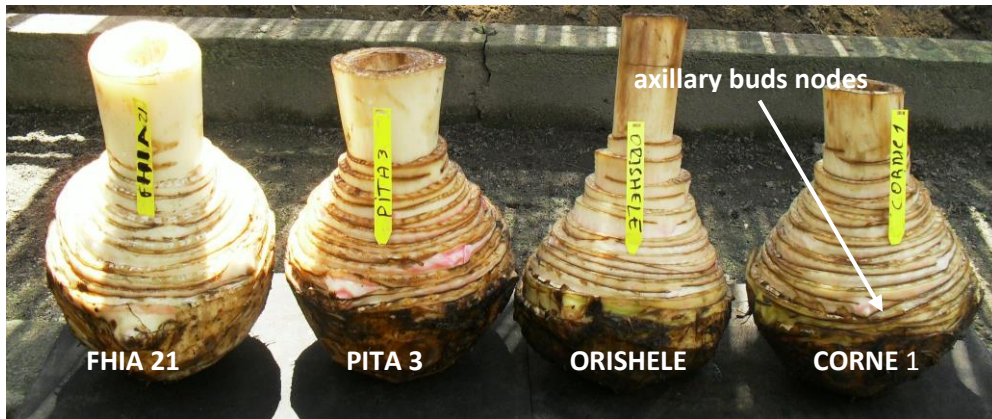


Fig. 1. Desheathed and decorticated corms of plantain cultivars FHIA 21, PITA 3, ORISHELE and CORNE 1

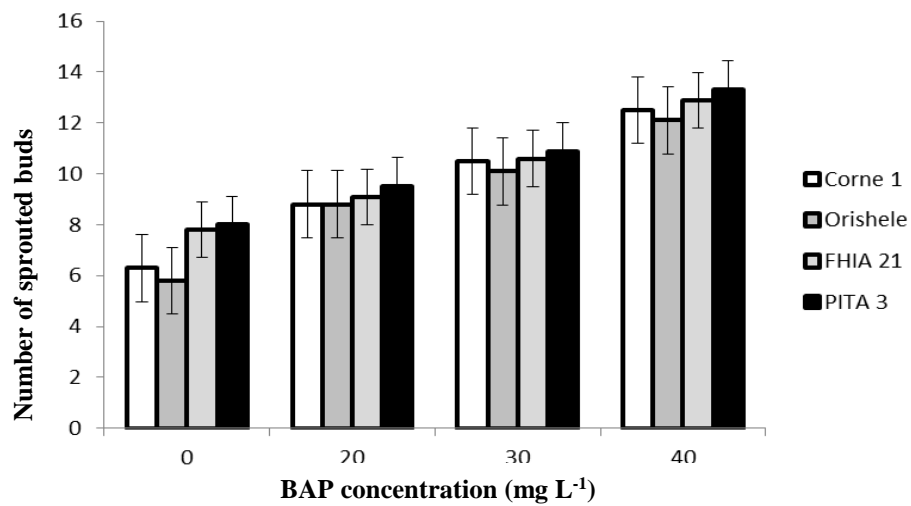


Fig. 2. The effect of different BAP concentrations on number of sprouted buds per corm of plantain cultivars CORNE 1, ORISHELE, FHIA 21 and PITA 3. Bar indicates the standard error of mean

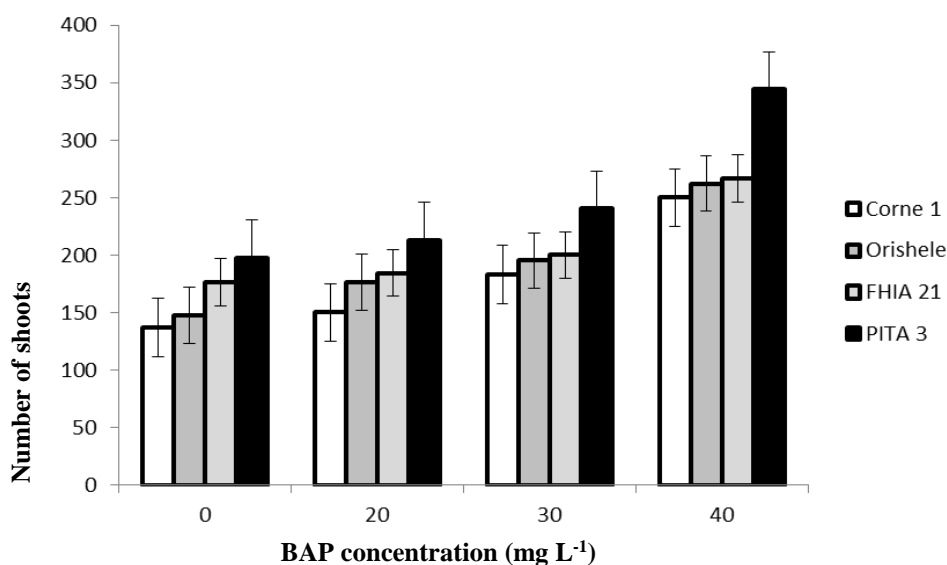


Fig. 3. The effect of different BAP concentrations on number of shoots per corm of plantain cultivars CORNE 1, ORISHELE, FHIA 21 and PITA 3. Bar indicates the standard error of mean

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