The African Journal of Plant Science and Biotechnology ©2011 Global Science Books

Effect of Substrate Type and Bulb Size on *in Vivo* Production of Seedlings in Three Cultivars of Plantain (*Musa* spp.)

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ABSTRACT

The expansion and improvement of plantain are limited by the lack of clean planting material free of pests and diseases. Several techniques have been developed to increase seed production. These techniques require a longer time to generate and they are laborious, carry vector diseases and sometimes, are too expensive for farmers. The present study aims to evaluate the effects of substrate type and bulb size on the production of healthy *vivo* plantain plants. The use of Furadan-Mancozeb significantly reduced the contamination rate. On average, the time necessary to observe the first appearance of buds was 24 days with small bulbs (weight < 150 g) and 14 days for large bulbs (weight > 600 g), independent of the cultivar. The number of buds produced varied with the cultivar, the substrate type and bulb size. In cv. 'Orishele', bulbs with sizes ranging from 450 to 600 g developed more buds (17.67) on sawdust. With cv. 'Corne 1' and 'French 2', forest soil was the best substrate (28.69 and 36.64 buds, respectively) with bulb size varying between 300 and 450 g. The time necessary for seed production ranged between 63 and 90 days.

Keywords: plantain, buds, substrate, mass propagation, suckers

INTRODUCTION

Plantain (*Musa* AAB group) is one of the main staple foods in Cote d'Ivoire. It is ranked as the fourth most important crop fruit in terms of consumption after rice, cassava and yam, with a yearly production of approximately 1.5 million tonnes (ANADER 2009). New markets developed in West Africa and Europe are so attractive for plantain export (Tabuna 1998) that local availability is dropping. For half the year, plantain supply is very low.

Despite the high value of plantain, growing pressures caused by pests and diseases have affected production, the most notable being the fungal disease, Black Sigatoka (*Mycosphaerella fijiensis*) (Koné 2008; Hermanto *et al.* 2010). Over the years farmers have been producing plantain in a traditional way using low-yielding poor quality cultivars and unhealthy planting materials, soil, disease and pest management practices. Yields have thus remained low and unsustainable (Hemeng 1991).

To face this shortage, increasing plantain production remains a challenge. Currently, banana plantations are established with various types of suckers and cultures performed on small plots (Traoré *et al.* 2009). Suckers produced naturally on farms are not sufficient to renew, extend or create a new plantain plantation. This situation then leads to a permanent need for plant material (Koulong 1999). In addition, infested suckers, transferred from field to field, contribute by spreading root pathogens, hence reducing farm productivity. The availability of suckers is also influenced by soil type, climate (soil quality, rain, sunshine, etc.) and cultural practices (eyeleting, fertilization, etc.).

To enhance the numbers of suckers used to initiate plantation and then allow plantain production to satisfy supply and demand, *in vitro* culture techniques (Youmbi and Ngaha 2004; Adaoha-Mbanaso *et al*. 2006; Uddin *et al.* 2006; Koné *et al*. 2010; Saha-Roy *et al*. 2010) and the rapid multiplication of horticultural methods (Auboiron 1997; Kwa 2003) have been applied. Horticultural methods are more efficient in terms of quantity of seed produced and are achieved in greenhouses or under shade. However, these propagation methods do not satisfy the important need for seed. Although accessible to farmers, many of these *in vivo* methods are limited by the presence of nematodes, soil fungi and weevils. In addition, the appropriate weight of bulbs is still unknown. On the other hand, many buds derived from the plant remain unexploited (Kwa 1993). Sawdust alone (Kwa 2003) or mixed with soil (Bakelana and Mpanda 2000) can be used as a culture substrate. However, sawdust is also used by women as a heating source for cooking in poor households. Therefore, the availability of an alternative substrate for poor farmer becomes crucial.

To our knowledge, no study has been reported on the influence of substrate type and bulb size on plantain propagation. The objective of the present work was to: (1) evaluate the effects of sterilizing agents on the macroscopic health of seed, (2) estimate the influence of culture substrates (forest soil, sawdust, sand sea) and (3) bulb size on *in vivo* mass propagation in three cultivars ('Corne 1', 'French 2' and 'Orishele') of plantain (*Musa* spp. AAB group) commonly grown in Côte d'Ivoire.

MATERIALS AND METHODS

Plant material

Plantain suckers of three cultivars namely, 'Orishele', 'French 2' and 'Corne 1' (*Musa* AAB) were used in this study. Suckers were excised from plants growing in the experimental field of the University of Abobo-Adjamé located in the south part of Côte d'Ivoire (5°23 North latitude and 4°11 West longitude, 100 m altitude). The temperature fluctuates between 25 and 32°C and relative humidity varies from 70 to 90%. Plantains grown in Côte d'Ivoire are dominated by Horn (Corne) and French types. They represent over 90% of the country's annual production (N'da Adopo *et al.* 1998). The preference for these types compared to disease-resistant hybrids reflects culinary and socio-cultural practices (Koffi 2004; Dzo-

Received: 30 December, 2010. Accepted: 7 June, 2011.

Original Research Paper

meku *et al*. 2006, 2007). The major obstacle to adopt elite hybrids is their inadequacy to traditional dishes (N'Guessan *et al.* 2000).

Explant preparation

Suckers 5-50 cm long and the mother plant from which they were derived were excised in the early morning and transferred to the laboratory. After excising roots and necrotic parts, suckers were then thoroughly rinsed with tap water to remove all soil particles. The pseudostem was reduced to 1 cm above the stem's last visible node.

Influence of sterilizing agents on the macroscopic sanitary quality of *in vivo* shoots

Bulbs were soaked for 15 min each in sodium hypochlorite (NaOCl: commercial bleach containing 0.25% active chlorine; Colgate Palmolive, Abidjan, Côte d'Ivoire), warm water at 45-50°C and warm water (45-50°C) containing NaOCl solution + Furadan 5 g/l (STEPC, Abidjan, Côte d'Ivoire) + Mancozeb 15 g/l (Callivoire, Abidjan, Côte d'Ivoire). Tap water was used as the control. After the different treatments had been applied, bulbs were let to dry for 48 h in the shade outdoors.

The bulbs were considered to be infected when fungal mycelium was visible on the top of substrates. After emergence and growth, aphids observed on buds were also considered to be a symptom of contamination. 60 days after sowing, the bulbs' buds were not dug up and their physiological state was established. The contamination rate was evaluated by visual observation of the substrate and by the appearance of the plants during the growth stage.

Effect of substrate type on bud production

After the drying period, the pseudostem was reduced by removing a thickness of 3 to 5 mm of the outer layer. Two incisions were made in the central part of the bulbs to destroy the apical meristem. The bulbs were labeled and transferred onto the culture substrate previously sterilized with an autoclave at 121°C for 30 min under 1 bar pressure (sterilization can also be achieved over wood fires in barrels for about 6 h). The culture substrates used were sea sand (sand), forest soil containing topsoil and humus (soil) and sawdust.

Influence of bulbs size on bud production

Bulbs were calibrated using a scale of weights. The weight (P) range used after calibration was: P < 150 g; 150 < P < 300 g; 300 < P < 450 g; 450 < P < 600 g and 600 g < P. The bulbs were arranged randomly in the blocks. The distance between any two bulbs was 10 cm to prevent the development of infection among bulbs.

Culture of bulbs in incubator

The incubator, 20 cm deep, was crafted in the open ground with bricks. The experimental design consisted of three blocks and four sub-blocks (1 m × 1 m). Bulbs were placed in a vertical orientation in the incubator containing the substrate. The excised parts of suckers faced up and were covered with 2 to 3 cm of the incubator substrate. The incubator was sealed with a transparent plastic film (completely covered). The bulbs were thoroughly watered 24 h after incubation and watered weekly depending on the condition of incubator moisture.

Weaning (acclimatization) of shoot buds

Shoot buds with 2-3 leaves were excised using a blade sterilized with 80° alcohol. Plantlets without any roots were excised by maintaining a piece of the bulb. Plantlets 6 cm in height were transferred to pots (125 ml) containing a mixture of soil and sand (1:2, v/v). The potted plants were placed in a micro-greenhouse (shelters of 2 m × 2 m completely covered with a transparent plastic film) (80 to 90% relative humidity and temperature varying from 29 to 33°C) for hardening. Three weeks after the hardening phase, plantlets > 6 cm in height were transferred into bags containing a mixture of sand-soil-well decomposed poultry manure (1: 2: 1, v/v/v). In the micro-glasshouse, plants were watered daily with a fine spray of water (1 l/m^3). Plants in the shade were watered 2-3 times a week depending on the weather.

Experimental design and statistical analysis

The trials were laid out in a completely randomized block design with three replicates of 30 bulbs per treatment. Data were expressed as the means of three replicates. All statistical analyses were carried out using STATISTICA (version 7.0). The results were analyzed using two-way ANOVA followed by Newman-Keul's test at 5%. For proportions and percentages, arcsin transformation (p = proportion) was performed before any analysis.

RESULTS

Influence of sterilizing agents on the health and rate of buds produced

Contamination observed on the surface of bulbs consisted of white or black mold and bulb rot. During the nursery phase, contamination induced a decline in growth followed by the death of seedlings. The different estimated contamination rates are illustrated in **Fig. 1**. Statistical analysis showed that the rate of contamination was highly influenced by the type of sterilizing agent (P = 0.002371). A higher rate of contamination was observed on bulbs in the control treatment (tap water). In all cultivars, a significant reduction in the contamination rate was exhibited by all the sterilizing agents tested. The lowest rate of contamination was expressed in the presence of Furadan/Mancozeb.

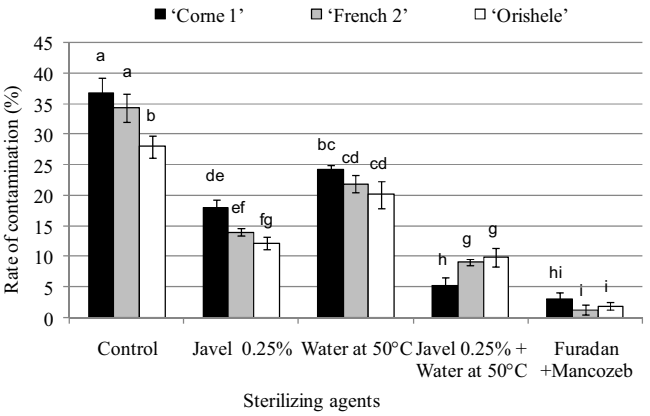

Fig. 1 Effect of sterilizing agents on buds contamination rate in three cultivars of plantains (*Musa* AAB). Average values (mean ± standard deviation) followed by the same letter are statistically identical at P = 0.05 (Newman–Keul's).

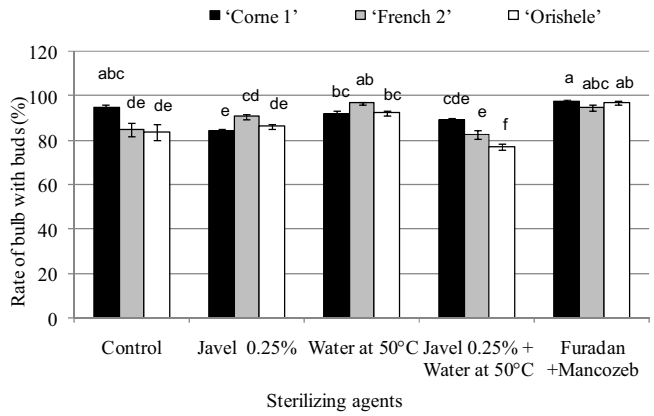

Fig. 2 Effect of sterilizing agents on the rate of bulbs with buds in three cultivars of plantains (*Musa* AAB), 45 days after sowing. Average (mean ± standard deviation) values followed by the same letter are statistically identical at P = 0.05 (Newman–Keul's).

Table 1 Influence of culture substrate on buds production in three cultivars of plantain (*Musa* AAB), 45 days after sowing.

Cultivars	Substrate culture	Rate of bulbs (%) with buds	Average lag time (days)	Average number of buds per bulbs
'Corne 1'	Soil	98.33 ± 2.08 a	27.02 ± 5.05 a	19.49 ± 8.12 a
	Sawdust	98.67 ± 1.15 a	27.55 ± 3.71 a	6.96 ± 3.56 b
	Sand	96.00 ± 0.00 a	27.98 ± 2.33 a	5.89 ± 3.89 b
	P	0.226	0.425279	< 0.0001
'French 2'	Soil	96.94 ± 0.93 a	24.93 ± 4.05 a	22.16 ± 10.15 a
	Sawdust	97.39 ± 0.73 a	24.09 ± 4.71 a	9.90 ± 4.64 b
	Sand	98.08 ± 1.88 a	25.73 ± 3.31 a	7.47 ± 5.11 b
	P	0.476765	0.110823	< 0.0001
'Orishele'	Soil	96.94 ± 0.93 a	23.13 ± 4.05 b	11.49 ± 6.21 a
	Sawdust	97.39 ± 0.73 a	25.84 ± 4.29 a	8.67 ± 5.1 b
	Sand	98.08 ± 1.88 a	25.55 ± 3.08 a	8.04 ± 5.17 b
	P	0.109935	0.000385	0.002819

Average values (mean ± standard deviation) followed by the same letter are statistically identical at $P = 0.05$ (Newman–Keul's)

Table 2 Influence of substrate and bulbs size on lag time (days after sowing) in three cultivars of plantains (*Musa* AAB).

Substrates	Bulbs size (P)	'Corne 1'	'French 2'	'Orishele'
Soil	P < 150 g	22.9 ± 5.14 c	25.34 ± 3.98 b	24.99 ± 4.36 c
	150 < P < 300 g	22.25 ± 4.02 c	24.49 ± 4.42 bc	22.92 ± 3.89 d
	300 < P < 450 g	21.16 ± 2.98 d	20.99 ± 3.03 de	21.08 ±1.87 e
	450 < P < 600 g	20.98 ± 2.49 d	20.6 ± 2.36 e	20.69 ± 2.35 e
	600 g < P	14.94 ± 2.24 e	14.67 ± 2.27 f	14.71 ± 2.26 fg
Sawdust	P < 150 g	28.20 ± 3.48 b	25.25 ± 4.36 b	25.21 ± 3.23 bc
	150 < P < 300 g	27.14 ± 3.95 b	24.39 ± 2.98 bc	26.23 ± 4.30 abc
	30 0 < P < 450 g	21.06 ± 3.01 d	22.06 ± 3.49 d	25.40 ± 4.83 abc
	450 < P < 600 g	20.87 ± 2.35 d	21.26 ± 2.29 de	21.54 ± 3.05 e
	600 g < P	13.86 ± 2.99 f	13.83 ± 2.28 f	13.79 ± 2.98 g
Sand	P < 150 g	30.23 ± 0.94 a	25.48 ± 3.38 b	26.51 ± 2.99 ab
	150 < P < 300 g	27.31 ± 2.10 b	27.31 ± 2.12 a	25.91 ± 2.65 abc
	300 < P < 450 g	20.61 ± 2.46 d	23.69 ± 4.15 c	26.70 ± 2.49 a
	450 < P < 600 g	20.87 ± 2.35 d	24.99 ± 3.69 b	22.70 ± 3.52 d
	600 g < P	13.22 ± 2.66 f	14.90 ± 3.90 f	15.60 ± 3.88 f
	P	< 0.0001	< 0.0001	< 0.0001

Average values (mean ± standard deviation) followed by the same letter are statistically identical at $P = 0.05$ (Newman–Keul's)

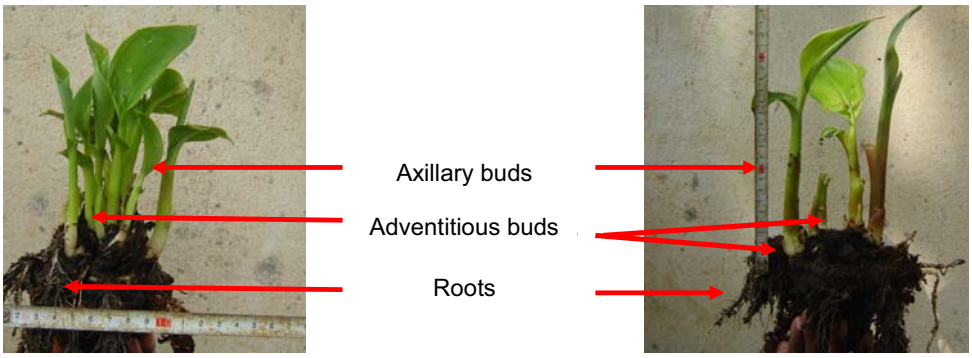

Fig. 3 Bulbs size between 300 and 450 g with 16 buds (6 axillary buds and 10 adventitious buds).

Fig. 4 Bulbs size higher than 600 g with 8 buds (5 axillary buds and 3 adventitious buds).

In this experiment the frequency of bulbs inducing buds was also recorded: > 80% of bulbs with buds formed in all treatments, including the control (**Fig. 2**). Bud production was best in the Furadan/Mancozeb treatment.

Effects of culture substrate on bud production

The frequency of bulbs with buds was statistically the same, irrespective of the cultivar and culture substrate (**Table 1**). Generally, time necessary to observe the first appearance of a bud by a bulb varied between 24 and 27 days. However, bulbs of cv. 'Orischele' produced buds more rapidly (23 days) than the other cultivars when they were planted into forest soil.

No statistical differences were observed in the number of buds produced by the cultivars on substrates composed of sand and sawdust. Irrespective of the cultivar, a higher number of buds per bulb was observed on the forest soil substrate.

Influence of culture substrate and bulbs size on bud production

Two types of bubs have been simultaneously induced from the mother bulbs in the culture substrates used in this experiment. The first one is an axillary bulb while the second type is an adventitious bulb (**Figs. 3, 4**, respectively). Independent of the substrate used, the shortest period (13-15 days) required to induce buds in all cultivars tested was observed when bulbs > 600 g were used to initiate cultures (**Table 2**). The smaller the bulb size, the greater the time required to induce buds.

On the culture substrates tested, the average number of buds per bulb obtained in 'Corne 1' and 'French 2' increased and reached a maximum when the bulb size was between 300 and 450 g (**Table 3**). Above this size range, there was a significant reduction in the number of buds. In 'Orischele', the highest number of buds per bulb was observed when bulb size varied from 450 to 600 g. In all cases, bulbs size inferior to 150 g exhibited the lowest average

Table 3 Influence of substrate and bulbs size on Number of buds produced in three cultivars of plantains (*Musa* AAB), 45 days after sowing.

Substrate	Bulbs size	'Corne 1'	'French 2'	'Orishele'
Soil	P < 150 g	13.44 ± 4.89 f	12.02 ± 5.67 gh	8.01 ± 4.42 f
	150 < P < 300 g	22.34 ± 3.47 b	16.14 ± 5.43 e	12.2 ± 4.66 d
	300 < P < 450 g	28.69 ± 5.59 a	36.64 ± 6.99 a	14.51 ± 2.38 c
	450 < P < 600 g	22.71 ± 4.13 b	27.2 ± 7.24 c	14.77 ± 2.83 c
	600 g < P	16.22 ± 4.19cde	19.14 ± 4.24 d	12.91 ± 3.81 d
Sawdust	P < 150 g	7.55 ± 2.98 h	11.48 ± 4.65 gh	6.23 ± 2.54 g
	150 < P < 300 g	9.51 ± 3.25 g	12.67 ± 4.23 g	10.73 ± 5.49 e
	300 < P < 450 g	17.49 ± 7.8 c	28.66 ± 6.14 b	12.27 ± 5.67 d
	450 < P < 600 g	15.35 ± 5.03 de	17.69 ± 4.51 e	17.67 ± 5.93 a
	600 g < P	13.12 ± 2.84 f	16.89 ± 3.08 e	12.87 ± 2.64 d
Sand	P < 150 g	6.21 ± 3.12 i	6.92 ± 3.01 i	6.35 ± 2.55 g
	150 < P < 300 g	10.36 ± 3.16 g	10.38 ± 3.17 h	9.90 ± 3.21 e
	300 < P < 450 g	16.47 ± 4.59 cd	14.88 ± 4.50 ef	12.92 ± 3.17 d
	450 < P < 600 g	14.68 ± 5.22 ef	14.26 ± 3.60 f	16.41 ± 4.39 b
	600 g < P	10.02 ± 3.83 g	11.87 ± 2.88 gh	12.57 ± 2.58 d
	P	< 0.0001	< 0.0001	< 0.0001

Average values (mean ± standard deviation) followed by the same letter are statistically identical at $P = 0.05$ (Newman–Keul's)

number of buds per bulb on the culture substrates tested.

Effects of bulb size on seedling survival rate and on the average time for a seedling to be transferred from forest soil substrate to the field

Independent of the cultivar and bulb size, healthy hardened plantlets were obtained with a survival rate raging from 79 to 100%, without statistical differences (**Table 4**). The average time necessary for transferring a seedling to the field was greatly affected by the cultivar and bulb size. The shortest period (i.e., 63-65 days) was observed in plantlets derived from bulbs whose size ranged from 450 to 600 g followed by bulbs superior to 600 g. In all cultivars, the longest period (85-88 days) required to transfer a seedling to the field was exhibited by bulbs < 150 g.

DISCUSSION

The presence of contamination during *in vivo* multiplication may arise from the possibility of pathogen spread from plants produced from seed. In this study, to minimize and control the presence of contaminants, bulbs were treated with several chemical solutions to reduce contamination. The best treatment was Furadan+Mancozeb (**Fig. 1**). The effectiveness of chemical treatments could be attributed to the systemic nature of these molecules. Mateille *et al.* (1988) reported that the action of chemicals (carbofuran; isazophos and phenamiphos) can persist for 3-4 months. These products act directly on the culture substrate, on the corm tissue layers, or after absorption by roots. The high rate of infection observed in the control treatment could be due to excess water when watering bulbs. Quénéhervé and Cadet (1985) and Mateille *et al*. (1988) indicated that during the rainy period, the rate of infestation caused by nematodes (*Radopholus similis*, *Hoplolaimus pararobustus* and *Cephalenchu emarginatus*) decreased on heavily-infested banana (*Musa* AAA). The use of a warm bath only was recommended for seed pest control (Prasad and Reddy 1994; Bridge *et al.* 1997; Mbwana *et al.* 1998 cited by Luc *et al*. 2005). As in our experiment, the warm bath reduced contamination by more than 75%, although the remaining contamination is due to the non-persistent nature of the treatments, which prevent the development of the predominant pest populations.

The high rate of bulbs with buds observed with the different treatments showed no disturbance during the resumption of the bulbs (**Fig. 2**). Indeed, the lowest rate was observed in the presence of NaOCl, which would act on axillary buds by destroying the striped cell surface. Unlike warm water, which is a surface disinfectant, NaOCl solution would penetrate and destroy bud tissue (Dychdala 2001).

Forest soil substrate exhibited the shortest lag time. This substrate retains more moisture and improves the conditions

Table 4 Evaluation of bulbs size effect on success rate of seedlings and average time to have transferable seedlings on field, in three cultivars of plantains (*Musa* AAB).

Cultivars	Bulbs size (g)	Success rate (%) 45 days after weaning	Average time to transfer a seedling to the field (days)
'Corne 1'	P < 150 g	79.72 ± 1.09 a	86.56 ± 8.31 a
	150 < P < 300 g	92.33 ± 6.23 a	79.06 ± 5.89 b
	300 < P < 450 g	98.78 ± 2.12 a	71.95 ± 2.81 c
	450 < P < 600 g	100.00 ± 0.00 a	64.39 ± 6.30 e
	600 g < P	100.00 ± 0.00 a	67.80 ± 9.07 d
	P	0.279493	< 0.0001
'French 2'	P < 150 g	96.57 ± 3.37 a	88.41 ± 4.91 a
	150 < P < 300 g	96.56 ± 5.95 a	78.23 ± 5.93 b
	300 < P < 450 g	98.20 ± 3.12 a	72.58 ± 3.69 c
	450 < P < 600 g	97.85 ± 2.16 a	63.09 ± 7.94 d
	600 g < P	96.62 ± 5.85 a	65.72 ± 10.29 e
	P	0.279493	< 0.0001
'Orishele'	P < 150 g	82.74 ± 5.68 a	85.16 ± 7.22 a
	150 < P < 300 g	93.29 ± 9.46 a	77.81 ± 6.38 b
	300 < P < 450 g	90.21 ± 10.60 a	73.73 ± 5.39 c
	450 < P < 600 g	86.82 ± 6.33 a	65.39 ± 5.99 d
	600 g < P	96.67 ± 5.77 a	64.94 ± 10.66 d
	P	0.279493	< 0.0001

Average values (mean ± standard deviation) followed by the same letter are statistically identical at $P = 0.05$ (Newman–Keul's)

to allow the bulb to recovery from quiescence and early induction of buds (**Table 1**). Larger bulbs, which have more mature axillary buds, were also the first to produce buds (**Table 2**). Therefore, maintaining the latter in favor conditions activity causes a gradual entry into the buds. Bud activity starts with oldest and most mature buds, which are located in the basal part of the corm. Thus, Aillaud *et al.* (1989) reported that bud dormancy is different within the same plant. These authors suggested that the state of dormancy varies depending on the branch, the position and the level of insertion of the buds on the same branch. On the bulb that could be explained by the fact that the basal part containing meristematic points will mature early development of the latter. A correlative inhibition (Scribd 2009) could be suggested in our case since the activity of axillaries buds is limited by the apical meristem. Any bulb with healthy buds at the end of the experiment would be assumed to have undergone a delayed reaction. Similar results have previously been observed by Kwa (1993) and more recently by Boyé *et al.* (2008), who argued that sucker growth is inhibited in a natural way when they are separated from the mother bulb.

The average number of buds induced varied depending on the culture substrate tested. Forest soil yielded significantly more bud shoots (**Tables 1, 3**). This substrate would retain humidity more easily and would be rich in mineral

and organic elements necessary for buds. One week after incubation, all bulbs induced roots. Therefore, they were able to absorb water and salts from the substrate in order to mobilize the various mechanisms leading to bud production. The cultivar 'Orishele' responded best on the culture substrate composed of sawdust (**Tables 1, 3**). Probably, the quantity of nutrients present in the substrate would be sufficient to produce many buds. On the other hand, sawdust would be richer in nitrogen, whose decomposition could release mineral elements (Akanbi *et al.* 2002) taken up easily by 'Orishele'.

The bulb with small size had a high proportion of adventitious buds compared to the axillaries. However, the total number of buds remained low, probably because of their small size. Bulbs with high weight induced significant production of axillaries buds compared to the adventitious ones. The cross incision in the central meristem stress the plant material and help stimulate the proliferation (production of adventitious buds) of callus at the heart of the bulb set incubator (Kwa 2003). Indeed, the cross cut of the bulb central meristem constituted an important factor to induce the first proliferation observed with *in vivo* culture. Along these proliferations, axillaries buds are not visible and grow rapidly and converted into leaf shoots. Similar results were observed *in vitro* by Koné *et al.* (2010). Medium bulb allowed the development of both types of buds with a very high proportion of adventitious buds. This weight range would be efficient to appropriate production of hormones related to bud production in plantain. This production with favorable hormonal balance auxin/GA_3, was already suggested by Devos (1985) and Noupadja (2000). The setting of bulb in incubator was an awakening phase of the buds, comparable to the recovery of seeds during germination. This recovery requires the mobilization of reserves for bud growth. This mobilization would depend on the activation of hydrolytic enzymes (Kwa 1993) present in corm. The rhizome is essentially rich in starch. Starch is hydrolyzed by α-amylase in soluble sugars, necessary for the resumption of bud growth. Smith (1972) and Fischer *et al.* (1995), suggested degradation of the amino acids, some proteins and the starch whose metabolism would give energy necessary for development and buds survival.

The destruction of the apical meristem is a physical stress that may promote the removal of inhibition. This type of inhibition was called endo-dormancy by Chao *et al.* (2007). This stress would act in the direction of the inactivation of the apical meristem of the shell bulb promoting differentiation (formation of adventitious buds) and the development of buds (axillaries) through the activation of basal metabolic functions (Osonubi and Davies 1978; Kwa 1993). Thereby, promoting the action of hormonal signal that affect the genetic information making contribution to the growth and development of the latter (Chao *et al*. 2007).

The significant effects of the weight of bulbs on the rate of withdrawal and duration of plants production are linked to differences in the physical characteristic of buds produced. The low rate of seed buds weaned compared to revenues was mainly due to losses during weaning. Buds produced by the bulbs with small weights presented more adventitious buds strongly linked to each other. So, their separation is difficult and could not be done without loss. These bulbs with small weight generally produced small buds in a relatively longer compared to those of higher weights. The small size and fragility of shoots produced require a cure time (acclimatization) before they advance in shade (farming) as opposed to large-sized buds. The latter were directly put in shade. The bulbs of big size would present mature but inhibited buds. During the preparation of bulbs, inhibition of suckers (Boyé *et al.* 2008) is lifted. This lifting of inhibition would promote the rapid development of existing mature buds. The average time to obtain a seed increased inversely with the size of bulb cultured. Bulbs with great weight weaned with high rate in all cultivars. The average time for seedlings transferred to the field was a function on the weight of bulb source, whatever the cultivar.

CONCLUSION

In conclusion, Furadan+Mancozeb formulation is the sterilizing agent of choice allowing a significant reduction of contamination rate during plantain bulbs culture. This treatment is also favorable for buds production with all cultivars of plantain tested. The substrate composed with forest soil induces a great number of buds with the cultivars used and the time necessary to observe the first appearance of bud varied between 13 and 15 days. The forest soil is easily available and reusable. While sawdust is used by women for cooking and the sea sand is used for houses construction. The average number of buds per bulbs varied significantly with the cultivar and the bulb size. Bud shoots are successfully hardened and plantlets with high survival rate are transferred to field where they attained the maturity and set fruits. Compared to *in vitro* techniques, this protocol reduced significantly the time (6 weeks to 2 months) required for getting plantlets ready to be transferred to the field.

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