Original Research Article

Enhancing Micro-Propagation of Some Cameroonian Plantain (*Musa Paradisiacal*) Cultivars

.

ABSTRACT

Aims:
This study was conducted to enhance germplasm conservation of some indigenous plantain land races through tissue culture techniques in Cameroon.
Study design: The experiment was laid out in a completely randomized design (CRD) with three treatments in four replications.
Place and Duration of Study: The study was conducted in the tissue culture laboratory of the Institute of Agricultural Research for Development (IRAD), Bambui, Cameroon, in the first half of 2018.
Methodology:
Explant were gotten from three plantain land races (Kwah, Ngumbe and Sangmoh) gotten from Bambui. Shoot tips were excised and cultured on Murashige and Skoog (MS) medium supplemented with 30g of sucrose, 5ml of ascorbic acid, 4ml of 6- benzylaminopurine (BAP 1mg/l), 1ml indole-3- acetic acid (IAA 1mg/l) and 6g of agar at pH of 5.8±0.1 for shoot initiation and proliferation. Data was collected over a period of 12 weeks every 4^{th} week on the average number of buds, shoot length, number of leaves, number contaminated and number dead
Results:
All the land races responded positively to shoot tip culture, since they could adapt and regenerate by producing buds with Ngumbe giving the highest mean number (7.0) of buds and Sangmoh giving the lowest (3.0). Analysis of variance revealed significant differences (p = 0.05) in most of the parameters measured except for number contaminated. High dead rate was recorded in Kwah with dead mean of 3.0. Ngumbe was found to regenerate better than Kwah and Sangmoh.
Conclusion:
It is recommended that the three plantain land races, germplasm be conserved. The ramification of this finding vis-à-vis germplasm conservation and increase productivity is explained.

Keywords: Plantain, explant, shoot tip culture, germplasm conservation and IRAD Bambui.

1. INTRODUCTION

Plantain (hard and starchy), also broadly called banana (soft) is one of the oldest crops known to mankind [1]. According to [2,3,4] there is archaeological and linguistic evidence that plantain originated from Southeast Asia about 7000 years ago, and was subsequently

distributed all over the globe by migrants and travelers. Nowadays, the secondary loci with enormous genetic diversity are Africa, Latin America and the Pacific [5]. [3] reported that, there are more than one thousand landraces of domesticated plantain in the tropical and subtropical regions. *Musa* spp is ranked as the fourth most important food in the world after rice, wheat, and maize [5]. *Musa* spp are now a key staple for many people especially in the Tropics and Subtropics; consumed as raw edible banana (desserts) or cooked plantains (boiled, fried, brewed, powdered or roasted) [5][6]. *Musa* spp provide about 25% food energy requirement for about 70 million people. They are rich in carbohydrates, protein, and vitamins (A, B1, B2 and C) [7].

In Cameroon, plantain is predominantly cultivated in 7 regions: Center, East, Littoral, South West, North West, West and South [8][9] in small monocrop farms, or mixed with other crops such as cocoa, coffee and cocoyams.

It is the second most preferred staple after yam (*Dioscorea* spp.), with a huge potential for rural income generation [10]. It is important for food security in Cameroon [11], where consumption can reach 100 kg/capita/year and it is currently developing into a major export crop to Southern neighbours.

Plantain is susceptible to insect, nematode and disease attack, making monocrop on a large scale or backyard farming unattainable in many instances. Also given that plantain has a slow sucker production rate [12], and with an ever-increasing market demand [13], large, healthy and uniform production is required. This can only be attained from healthy, uniform, and large quantities of planting material.

Micro-propagation has gained prominence in banana and plantain breeding programs worldwide [14][15] especially for commercial purposes [12]. The benefits of *in vitro* micro-propagation are enormous: plantlets from this method establish faster, healthier, stronger, shorter production cycle and higher yield than those produced via conventional methods [16]. In addition, uniform-aged plantlets can be guaranteed, suitable for mass production for domestic and export markets. More critically, the ease of travel in recent decades has facilitated rapid distribution of genetic material all over the world. [17] argues that such movement of genetic material have implication for biodiversity in the new areas, in many cases competing with locally adapted varieties, poor resistance to pest and disease leading to a social and an economic cost, thus the need to preserve local valuable germplasm. Rapid propagation can also serve as an easy method of preserving the valuable germplasm.

In this study, the performance of Tubah plantain (local) landraces in growth media as a means of germplasm preservation and rapid multiplication was evaluated. We selected the local and indigenous cultivars since they are generally likely to be more useful and tolerant to local conditions, and can attain peak yield with minimal inputs.

2. MATERIAL AND METHODS

2.1. Source of plant material

The plant materials (cultivars) were obtained from Tubah subdivision of the North West Region of Cameroon. Tubah Sub-division is located between latitudes 4°50' to 5°20'N and longitudes 10°35' to 11°59'E of the Green Wish Meridian [18]. Tubah is one of the five Sub-divisions making up Mezam Division in the North West Region of Cameroon. It is bordered by Belo in Boyo Division (North), Bafut (North West), to the South and South East by Ndop and Balikumbat in Ngoketungia Division and Nkwen in Bamenda III Sub-division (West). Tubah has a considerable surface area of 365 km^2 giving a population density of 145 persons/km^2. Tubah is made up of four main villages; Bambili, Bambui, Kedjo-Ketinguh and Kedjom-Keku. More than 80.0% of the inhabitants are farmers. Here, plantain, cocoyam, rice, maize, and beans play a huge role in their every diet and energy sources.

2.2. Plant material

Three plantain land races namely; Sangmoh, Ngumbe and Kwah (figure 1) were identified from a smallholder plantain farm in Bambui of the Tubah sub-division in the Mezam division of the North-West Region of Cameroon and sword sucks and peepers collected. They all take an average of 2 years from planting to harvesting. Sangmoh produces 2-3 hands with 3-4 fingers each. Most farmers prefer this because of the large sizes of its fingers and taste. It grows to a height of about 3.5-4m. Ngumbe produces 4-6 hands with 10-12 fingers each. This land race is preferred because of its economic value. It grows to a height of about 3m. Kwah produces an average of 6 hands with 10-12 small fingers per bunch and they mature within a short interval. It is preferred because of the big bunch size and the height (1.8-2m) of the plant.

Figure 1. Plantain land races used in the study. (a) - Sangmoh, (b) – Ngumbe, (c) – Kwah

2.3. Medium preparation and Culture condition

The explants were cultured on the Murashige and Skoog (MS) medium [19] with cytokinnin and auxin solutions at a concentration of 1 mg/ml. the entire process is summarized in figure 2.

3.3.1. Shoot initiation

For shoot initiation, MS basal salts were supplemented with BAP, sugar and ascorbic acid. A weighing balance was used to weigh the solid chemicals (table sugar ~ 30g, agar ~ 6g), while a pipette and measuring cylinder was used to measure the liquid chemicals (macro ~ 100ml, micro ~ 100ml, iron stock ~ 5ml, vitamins ~ 5ml, ascorbic acid ~ 5ml, IAA ~ 5ml and BAP1mg/l ~ 2ml). The pH (using some drops of NaOH 1M and HCl 1M) and temperature were maintained at 5.8 ± 0.1 and 25 ± 2, respectively. After preparation of the medium, 50ml each were dispensed in sterile baby food jars while still hot and fast enough to avoid the medium from solidifying in the beaker. The jars were corked and placed in the autoclave for sterilization at a temperature of 121^{o}C and at a pressure of 103.4Kpa for 15minutes. After sterilization, the medium was allowed to cool.

3.3.2. Explant preparation and sterilization

The suckers were washed thoroughly under running tap water and roots cut off. The corm and the pseudospem of the suckers were reduced and submerged in a well labelled beaker filled with tap water to prevent dehydration of the explants. The explants were washed thoroughly under running tap water using a brush to remove all the debris before taking into the laboratory. Two solutions were used to sterilize the explants inside the laminar flow hood: the shoot tips were submerged in 96% ethanol (1minute), later into30% sodium hypochlorite with 20 drops of tween 80 (10 minutes). Finally, they were rinsed 2-3 times with sterile distilled water.

3.3.3. Shoot tip excision

This was done under a laminar flow hood previously swapped with 70% alcohol. Using a blade mounted on a blade holder and forceps, the shoot tip of 0.5cm long with 3-4 leaf

ultivars were obtained. The shoot tips were placed on the medium in an upright position. The jars were labelled and incubated in the growth room at a temperature of 25^{o}C ± 2 with a photoperiod of 16hr/day. The cultures were transferred to a fresh medium every 2 weeks after initiation twice for one month under a laminar flow hood. During each transfer, each culture was removed carefully from the medium and the blackening basal tissues were cleaned off as well as dead leaf sheaths before placing in a fresh medium. Four weeks after, the cultures were transferred on to a multiplication medium.

3.3.4. Shoot proliferation

For shoot multiplication, medium, MS basal salts (Murashige and Skoog, 1962) were supplemented with sugar (~30g), macro (~100ml), micro (~100ml), iron stock (~5ml), vitamins (~5ml), ascorbic acid (~5ml), IAA (~1ml) BAP1mg/l (~2ml) and agar (~6g).The stocks were used to prepare medium. A 1000ml graduated beaker was placed on an electronic stirrer which helped in dissolving and homogenizing the media. Sterilized distilled water was used in the medium preparation and for rinsing equipment which were used, to avoid mix up and contamination of different chemicals. Each stock was poured in to the beaker. After making up the volume with sterile distilled water to 1000ml, the pH of the medium was adjusted to 5.8 ± 0.1 using a pH meter. After preparation of the medium, 50ml each were dispensed in sterile baby food jars while still hot and fast enough to avoid the medium from solidifying in the beaker. The jars were corked and placed in the autoclave for sterilization at a temperature of 121^{o}C and 103.4Kpa for 15minutes. After sterilization, the medium was allowed to cool.

4.3.5. Transfer and maintenance of culture

This was done in the laminar flow hood previously swapped with 70% alcohol. Each culture was removed carefully from the initiation medium. This was followed by cleaning of the blackening basal tissues, reducing the corm size as well as dead leaves and subdividing the bud cluster into small pieces, to induce the formation of new buds. They were all subculture inside the proliferation medium in an upright position and the jars were labelled and incubated in the growth room at a temperature of 25^{o}C ± 2 with a photoperiod of 16hrs/day. Sub culturing was done every 4 weeks and at each sub-culture the corm size was reduced, blackening cleaned, dead leaf sheaths removed, and the bud clumps divided.

There were four replications per treatment organize in a randomized complete design (RCD).

3.6. Data analysis

The data was analyzed using, the Statistical Package for Social Science (SPSS) (vers. 23.0) One-way analysis of variance (ANOVA) was used to compare if there were significantly different means. Means significantly different were separated using the Duncan's Multiple Range Test (DMRT) at probability level 0.05. Excel 2016 was used to plot the graphs.

Figure 2. Summary of plantain cultivar proliferation: a – paring of cultures; b – shoot tip submerged in solution for sterilization; c – excised shoot tips ready to be placed on culture medium; d – cultures 1 week after subculturing; e – cultures 2 weeks after subculturing; f – cultures 4 weeks after subculturing

3. RESULTS

4.1. Number of buds

The average number of buds 12 weeks after culturing varied with the plantain land races. The mean number of bud ranged from 3.0 buds in Sangmoh to 7.0 buds in Ngumbe. The Analysis Of Variance revealed significant differences ($F = 1.857$, $df = 2, 21$, $p < 0.05$) in the treatments. The land race Ngumbe had the highest average number of buds (7.0) (figure 3).

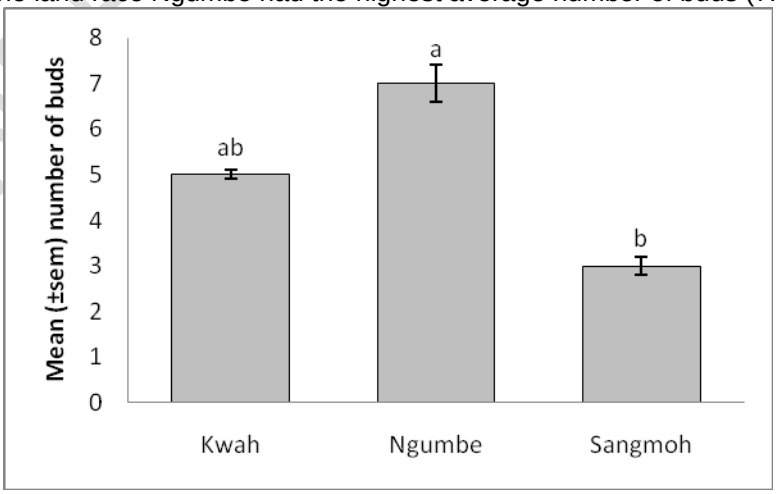

Figure 3. Mean number of buds of plantain land races twelve weeks after culturing. Means bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4.2. Length of shoots

The shoot length 12 weeks after culturing differed in the plantain land races. The mean shoot length ranged from 0.3 cm in Sangmoh to 0.8 cm in Kwah. Analysis of variance revealed significant difference (F = 0.424, df = 2, 21, $p < 0.05$) in the shoot length for the plantain land races. The plantain land race Kwah had the longest shoot length (0.8) (figure 4).

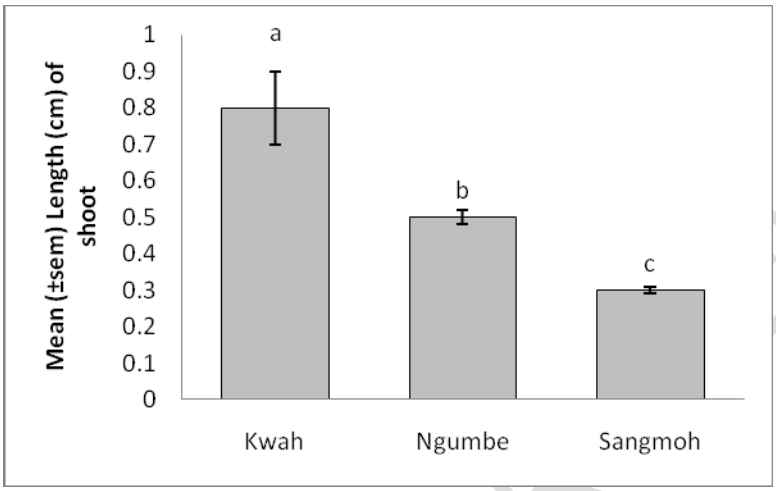

Figure 4: Mean of shoot length of plantain land races twelve weeks after culturing.
Means bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4.3. Number of leaves

The number of leaves 12 weeks after culturing varied with the plantainland races. The mean number of leaves ranged from 0.1 in Ngumbe to 0.3 in Kwah. The analysis of variance indicated significant differences (F = 0.139, df = 2, 21, $p < 0.05$) in the treatments. The land race Kwah had the highest number of leaves (0.3) (figure 5).

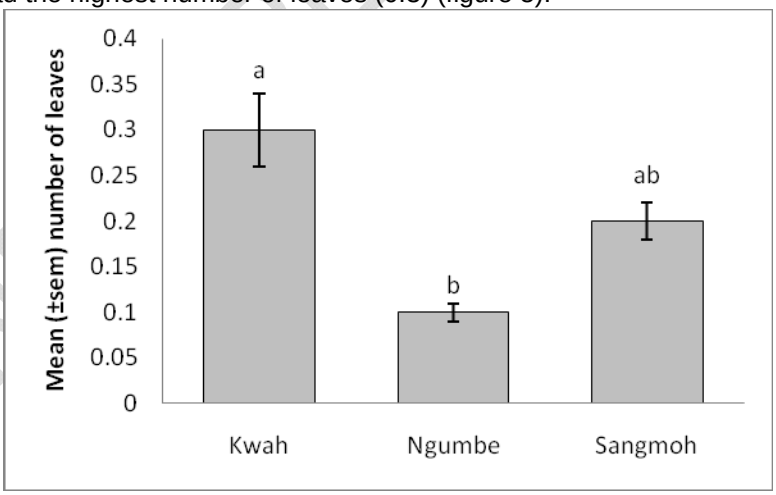

Figure 5: Mean number of leaves of plantain land races twelve weeks after culturing
Means bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4.4. Number contaminated

The number contaminated 12 weeks after culturing differed in the plantain land races. The variation in the mean number contaminated ranged from 0.4 in Sangmoh to 0.5 in Kwah and Ngumbe. The analysis of variance revealed no significant differences (F = 67.11, df = 2, 33,

$p > 0.05$) in the number contaminated for the plantain land races. The land race Sangmoh had least number contaminated (0.4) (figure 6).

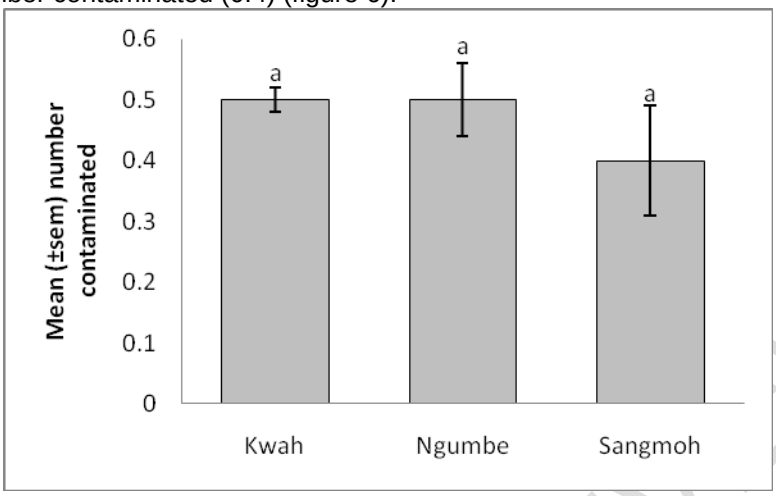

Figure 6: Mean number contaminated of plantain landraces twelve weeks after culturing
Means bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4.5. Number dead

The number dead 12 weeks after culturing varied with the plantain land races. The mean number dead ranged from 2.0 in Ngumbe and Sangmoh to 3.0 in Kwah. The analysis of variance revealed significant differences ($F = 3.361$, $df = 2, 33$, $p < 0.05$) in the treatments. The least number of dead occurred in Ngumbe and Sangmoh (2.0) (figure 7). In other words, the number of dead was same for Ngumbe and Sangmoh.

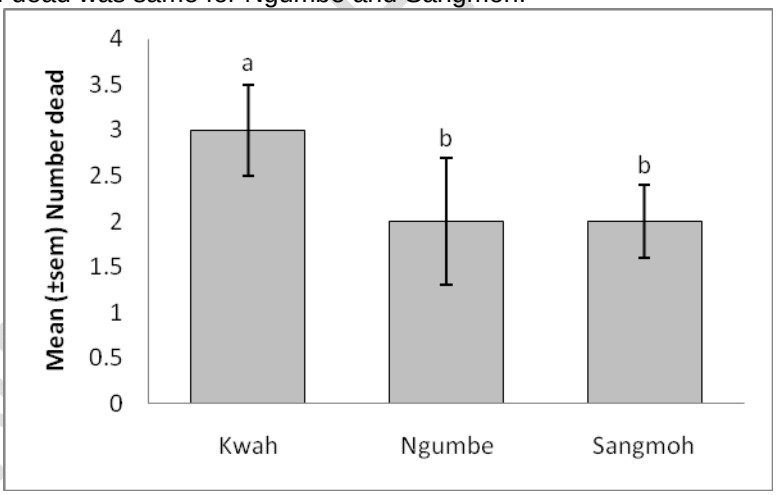

Figure 7: Mean number dead of plantain land races 12 weeks after culturing
Means bars with the same letter(s) are not significantly different (DMRT, $p < .05$)

4. DISCUSSION

Success in tissue culture of banana/plantain from various genome groups has been reported by several researchers and it was well-documented that the regenerative capacity *in vitro* is highly genotype specific and greatly influenced by growth regulator amended in shoot-inducing medium [20-22]. Different genome groups, especially with balbisiana (AAB, ABB or BBB groups) in their ploidy appeared to be more difficult to propagate than all ultivars (AAA) groups [20][23][24]. Evidently, the present study indicates that the different varieties differ in the regenerative capacity *in vitro*.

The proliferation of buds in Sangmoh was lower than that of Kwah and Ngumbe. The highest number of buds produced was observed in Ngumbe followed by Kwah. Comparing the three land races, Ngumbe can be considered to have a high regenerative capacity since it produced a high number of buds which can be attributed to the genotypic difference among the land races. Thus, it further confirms that the rate of bud proliferation is cultivar dependent [25][26]

Ngumbe and Sangmoh recorded the shortest shoot length while Kwah had the longest shoot length. This difference in shoot length could be influenced by concentration and combination of cytokinin and auxin in the culture media. This finding agrees with [27] who observed longest shoot length between two banana cultivars in MS medium supplemented with BAP having concentration 5.0 mg/l.

The difference in the number of leaves in the plantain land races varied from 0.1 leaves in Ngumbe to 0.3 leaves in Kwah. This could be because of their genotypic difference and concentration of cytokinin in the culture media. It should be noted that [27] reported a maximum number of leaves of 5.0; produced in two banana cultivars with 5.0 mg/l BAP, which was identical with our concentration of 4.0 mg/l BAP. [28] also found that 5.0 mg/l BAP concentration was the best for number of leaves per plantlets. Perhaps the concentration of BAP in this study could be adjusted for optimization.

Contamination and blackening of the cultivar tissues' can be considered as constraints in this technique. In fact, blackening of the cultivar tissues was the main difficulty. Although contamination was controlled with proper handling and surface sterilization technique, Sangmoh was the least contaminated while Kwah and Ngumbe were the most contaminated. This concurs with [29] who said that contamination has been reported as a constant problem in tissue culture, which can compromise development of all *in vitro* techniques. It should be noted that the contamination level in this study is within acceptable limits.

Blackening or browning observed especially in cut surfaces of explants in tissue culture is widely known as the oxidation of phenolic compounds resulting in the formation of quinines which are highly reactive and toxic to plant tissue [30-32]

The high level of dead amongst the plantain land races could be attributed to the high level of blackening observed on the tissues. This agrees with [33], who observed that at higher levels of blackening, death of tissues could be expected as browning is due to oxidation of polyphenols which are highly toxic and prevents the tissues from taking up nutrients.

5. CONCLUSION

In conclusion, *in vitro* introduction of the three plantain land races to enhance conservation were efficient given that all the plantain land races adapted and regenerated. From the result obtained Ngumbe had the highest respond in the MS media used followed Kwah and Sangmohbeing the least regarding the proliferation rate. All land races showed that tissue technique can be used both for proliferation and for germplasm preservation. Further studies on different media and ingredient concentrations should be investigated.

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