The effect of bud splitting on suppression of apical dominance and inducing multiple buds development in banana shoot tip cultures of *cv.* 'Yangambi' (AAA) in Tanzania.

ABSTRACT:

Aims: The objective of this study was to investigate the effect of bud splitting technique on suppression of apical dominance and induction of multiple buds development in banana shoot tips of cv. Yangambi.
Study design: The experiment was conducted in a completely randomized design, with three treatments each replicated ten times.
Place and Duration of Study: The samples (explants sources) were collected from research farm at Chambezi near Bagamoyo Tanzania. The research was conducted at Mikocheni Agricultural Research laboratories at Dar es salaam. The duration of the study was three months.
Methodology: The buds containing the shoot tips were cut and longitudinally split into halves and quarter fourteen days after culture initiation. MS media supplemented with 5mg/l of BAP was used in buds proliferation stage.
Results: The results indicated that there was significant ($p \leq 0.001$) difference in number of shoots produced in each bud splitting technique; where by highest number of shoots were observed in quarter split~~ted~~ buds (8.37±1.48) shoots per bud. Significant ($p \leq 0.001$) increase in fresh weight was also observed in quarter split~~ed~~ buds (12.02±2.25) grams compared with the control.
Conclusion: Due to increased demand of banana planting material and increased costs of tissue culture materials and reagents, application of bud splitting technique stands a better chance of promoting buds proliferation and cut on the costs of sub culturing and time. This technique also minimizes physiological barriers that might require additional media formulations and the rate of somaclonal variation which results from continuous sub-culturing.

***Keywords**:* buds proliferation, longitudinal bud splitting, shoot tips culture

Introduction

The demand for banana planting material is a major constraint to the expansion of banana production. The long time required for conventional planting material to mature and the prevalence of pests and diseases contributes to these problems.

In the application of bud splitting technique, the banana plantlets of a younger physiological age (single leaf) are longitudinally split to increase the number of explants during tissue culture [1]. This is thought to promote buds proliferation by breaking the apical dominance.
It has been known for some time that lateral bud development in stems is under the control of the apical bud and that auxin application can sufficiently replace the stem apex in this function in as much as it has also been found that auxin stimulates C_2H_4 (ethyne) production in stems (Marcia et al., 2002). The development of auxiliary meristems is inhibited by apical dominance [2]. These auxiliary meristems are commonly the source of buds formation in nature especially when the apical shoots are damaged or injured [3].

Poor ratooning of plantains results from the strong apical dominance exhibited by the main plant and the competition between suckers [4, 5]. The apical dominance is controlled by growth substances released by the terminal bud, which inhibit growth of lateral buds. Thus, sword suckers of plantains remain small until after flowering of the mother plant because their demand for assimilates (sinks) is too weak under the influence of apical dominance [6].

Bud splitting is not a common practice in banana tissue culture in many public laboratories in developing countries particularly Tanzania. Some reports on the role of apical dominance in shoot production from explants of banana are conflicting. Ma and Shii (1970) confirmed that the arrest of apical dominance by removing the shoot tips was essential for the production of multiple shoot initials in cv. Cavendish. However, other studies reported that multiple initials were produced in presence of apical domes in cv. Robusta [7]. Abdullah et al. (1993) reported that removal of apical dome was not essential for multiple shoots formation. On different approach to the same treatment i.e ~~splitting~~splitting of the shoot tip; Swamy et al. (1983) reported a high increase in growth rate and shoot proliferation as compared with solid media on subculture of split shoot tips in liquid media. In another study Alvard *et. al* [8], found that temporary immersion of split shoot tips in liquid media for 20 minutes produced the highest multiplication rate after 24 hours.
On another study, Mateille and Foncelle [9] noticed that longitudinal cuts of buds induced a threefold increase in multiplication. The multiplication rate was also found to depend on the origin of sucker bud, where by lateral buds doubled in size within three weeks while the apical buds reached three times their size.
In general, different cultivars show variation in the degree of their shoot bud proliferation tendency and the type of multiple budding can be distinguished. This is because the multiple budding appears to be linked to genome configuration of a given cultivar [10] The effect of bud splitting on buds proliferation rate of specific cultivar is not known with precision. The aim of the present study was to investigate the effect of bud splitting technique on bud proliferation rate of (*Musa spp*.) var. Yangambi shoot tips cultured in vitro.

MATERIALS AND METHODS

Plant materials

Young suckers of Musa var. 'Yangambi' (AAA) were collected from a healthy true to type mother plants from a research farm at Chambezi near Bagamoyo Tanzania. A least one hundred suckers with an average height of 100cm were dug from the field and transported to the laboratory. The suckers were stored in the green house at room temperature (28°C) and they were initiated in culture after one day.

Preparation of explants

Shoot tip explants of banana were prepared by removing the outer layers of tissues from suckers and excess roots with clean knife. The suckers were washed with tap water and liquid soap to remove the extraneous materials, and then rinsed in running tap water.
The suckers were trimmed to size by removing layers of the developing leaves. Then the suckers were soaked in 1g/l ascorbic acid for one hour before transfer to laminar flow. The shoot apices explants were sequentially treated with 70% alcohol for 30 seconds, then treated with 100% (v/v) hypochlorite (the active ingredient was 3.85% sodium hypochlorite) mixed with few drops of Tween 20 for one hour in order to sterilize surface. This was then followed with treatment of 50% (v/v) hypochlorite of the same active ingredient for 30 minutes. The explants were further trimmed to remove the remaining hypochlorite and rinsed with sterile distilled water before initiation.

Culture media and experimental details

The basal media (BM) contained the inorganic salts of Murashige and Skoog (??) supplemented with glycine (2mg/l), Myo-inositol (100mg/l), thiamine (0.5mg/l), pyridoxine (0.5mg/l), nicotinic acid (5mg/l) and sucrose (20g/l). The pH was adjusted to 5.8 before addition of phytagel (4g/l).
The initiation media (IM) used for induction of shoot initials from shoot tip explants consisted of BM above supplemented with 5mg/l of 6 Benzyl aminopurine (BAP). The excised shoots were maintained in (IM) until at least one bud was observed from them. They were longitudinally split into two or four parts depending on the nature of the treatment.
They were then transferred to shoot proliferation media supplemented with 5mg/l of 6 Benzyl aminopurine (BAP). Five replicates were used for each treatment. This experiment was set to investigate the effect of bud splitting technique on buds proliferation under same concentration (5mg/l BAP) of cytokinins.
All explants were incubated at 25±1°C with a 16h photo period provided by cool fluorescent tubes at 60µEm-2S^{-2}.

Data collection and Analysis

Data on number of shoots per explants, shoot length and total fresh weight of shoots per explant were collected 28 days after transfer to shoot proliferation media. Data collected were analyzed for statistical significance using analysis of variance (ANOVA). These computations were done by using a statistical software program STATISTICA, version 2013 (StatSoft Inc., Tulsa, OK, USA). Fisher least significance was used to compare means at p=0.05 level of significance

RESULTS AND DISCUSSION

The effects of longitudinal bud splitting of explants on the rate of buds proliferation

In this experiment, longitudinal bud splitting technique had significant (p≤0.001) effect on the number of buds produced per explant (Table 1). The numbers of buds produced in half split~~ed~~ buds are significantly different to the number of buds produced in unsplit~~ed~~ buds.
Significant (p≤0.001) increase in number of buds per explant was observed on quarter split~~ed~~ buds where the number of buds increased two folds, from 3.88 in unsplitted buds to 8.34 in quarter split~~ted~~ buds. Quarter bud splitting technique ~~is not recognized practice~~ and its influence on the number of buds produced in this experiment may be attributed to large surface area to volume ratio in contact with the media which have facilitated high rate of nutrient uptake and consequently rapid growth and increase in number of buds (Figure 2).

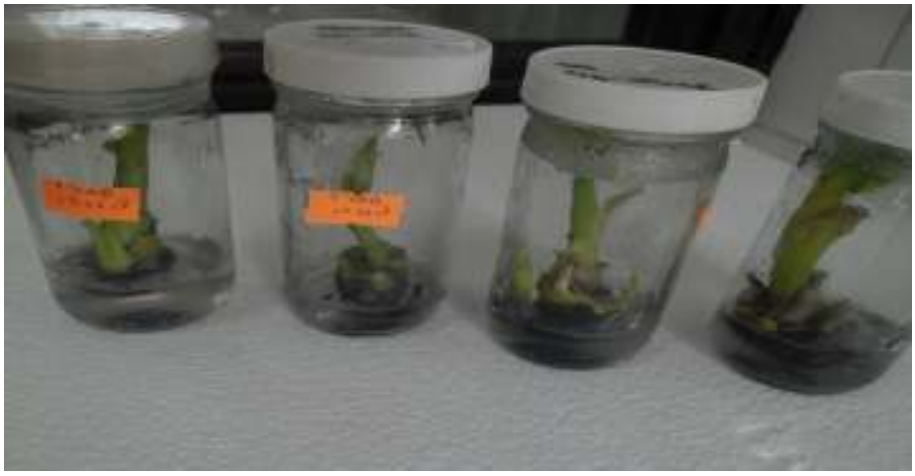

Figure 1. **Buds proliferation in unsplit~~ted~~ buds (Control) cultured for 28 days after initiation in buds proliferation media**

Figure 2. **Buds proliferation in quota splitted buds cultured for 28 days after initiation in buds proliferation media**

Decapitation of the bud tip before longitudinal splitting of the bud is known to arrest the apical dominance [11]. This coupled with appropriate concentration of cytokinin (5mg/l BAP used in our study) had the effect of increasing the number of shoots produced on longitudinally split~~ted~~ buds in this study.

Similar to our study, best proliferation rate was obtained at relatively low concentration of 3.0mg/l BA on split~~ted~~ buds compared to high concentration of 7.0mg/l BA on complete buds [1]. This indicates that at low concentration of BA split~~ted~~ buds responded better to buds proliferation treatment than unsplit~~ted~~ buds.

Table 1. Effect of bud splitting technique on the number of buds formed, fresh weight and shoot length~~.~~

Treatment	Growth Parameters		
	Number of Buds	**Total Fresh Weight**	**Average Shoot Length**
Bud Splitting technique			
Complete Buds	3.88±0.69b	6.36±0.89b	5.93±1.11a
Half Split~~ed~~ Buds	4.44±0.53b	8.69±0.99ab	4.59±0.45ab
Quarter Split ~~ed~~ Buds	8.37±1.48a	12.02±2.25a	3.54±0.39b
1-Way ANOVA(F – Statistic)			
Main Effect			
Bud Splitting technique	5.72**	3.58*	2.73*

*, P≤0.05. **, P≤0.001. Values (Mean ± SE) Followed by dissimilar letter(s) in a column are significantly different by Least Significant Difference test at P=0.05.

It is obvious that increasing the surface area at the point of contact between the explant and the medium created through longitudinal bud splitting had the positive influence on explants' response to the media. In another study it was found that bud cuts caused a faster sprouting of the bud fragments; it was further suggested that concentrically arranged leaves, according to the phyllotaxy of the banana pseudostem was responsible for inhibition of growth of meristems [9]. This necessitates buds splitting and cuts during tissue culture.
The effect of longitudinal bud splitting on shoot multiplication is also reported by other studies where by threefold increase in multiplication rate was obtained in banana cv. Poyo [9].

The effects of longitudinal splitting of explants on the fresh weight of buds produced

Similar to the number of buds produced, longitudinal splitting of explants had the effect on the fresh weight of shoots produced. The highest increase in fresh weight was observed on quarter split~~ted~~ buds. The analysis indicated that there were significant (p≤0.001) differences in total fresh weight of shoots produced on quarter split~~ted~~ buds compared with the rest of

other treatments (Table 1). The fresh weight increased about two times higher in the quarter split~~ted~~ buds compared to the unsplit~~ted~~ buds.

The increase in total fresh weight of explants in this study was again attributed to increase in number of shoots produced per bud and the increase in surface area at contact point between the media and explant which facilitated nutrient absorption and good response to media contents. This is because the same media was used in all treatments but the response of the explants to the media was different, this was reflected in this growth parameter. *In vitro* buds initiation and development in banana is reported to be cultivar dependent [12], therefore for this variety bud splitting has enhanced its response by increased total fresh weight.

The effect of longitudinal bud splitting on the length of shoots produced

In this experiment, good response of shoot length was observed on unsplit~~ted~~ buds. In this treatment, the average shoot length was higher compared with the rest of other treatments (Figure. 1) and (Table 1).The rate of shoots elongation was also observed to be higher in this treatment. The lowest shoot length was observed in quarter split~~ted~~ buds. This was contrary to the other growth parameters assessed in this experiment, which are growth and buds proliferation. This can be attributed to the fact that, apical meristem dominance encouraged shoot elongation rather than buds proliferation. This was observed in a control treatment. In this study it was evident that apical dominance can play a critical role in determining the rate buds proliferation *in vitro* irrespective of cytokinin concentration applied. Therefore there is a need to arrest apical dominance *in vitro* through buds cut and longitudinal splitting to encourage buds proliferation.

CONCLUSION

In conclusion this study had shown that bud splitting technique in tissue culture fourteen days after culture initiation encouraged increase in fresh weight and the number of buds development during first cycle of tissue culture. Since high number of shoots formation can be achieved in first cycle of tissue culture through this technique, it is evident that this technique is economically viable option for a variety which responds positively like 'Yangambi'.

Due to increased demand of banana planting material and increased costs of tissue culture materials and reagents, application of bud splitting technique stands a better chance of promoting buds proliferation and cut on the costs of sub culturing and time. This technique also minimizes physiological barriers that might require additional media formulations and the rate of somaclonal variation which results from continuous subculturing.

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