



MICROPROPAGATION OF BANANA (*Musa* spp) USING TEMPORARY IMMERSION BIOREACTOR SYSTEM

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ABSTRACT

Banana is an important crop in the tropics which possess the potential for commercial production in Nigeria. Large scale production requires large volume of planting materials which may be difficult to obtain using conventional methods of propagation. Temporary immersion bioreactor system (TIBs) is a cost effective method for micropropagation of plants. The present study was carried out to develop an efficient method for rapid multiplication of banana using temporary immersion bioreactor system (TIBs). Banana microshoots were regenerated from young suckers obtained from field grown plants using conventional plant tissue culture. Microshoots of 2cm length were used as explants for multiplication in temporary immersion bioreactor system. Ten (10) explants were cultured in bioreactor bottles containing Murashige and Skoog (MS) liquid media supplemented with different concentrations of 6-benzylaminopurine (BAP) with or without 250mg/L Activated Charcoal (AC). Results showed that explants cultured in media supplemented with 2 mg/L or 1mg/L BAP without AC gave the highest shoot multiplication rate of 900% and 800%, respectively compared to hormone free media. Production of competent plants (plants ready for ex vitro establishment) were however, influenced by the presence of AC and the highest percentage of competent plants (80%) were produced when media was fortified with 1mg/L BAP+ 250mg AC. Regenerated plants were successfully established in the field and were morphologically normal and fertile.

Key words: Cavendish Banana, Micropropagation, TIBs, BAP, Activated Charcoal

INTRODUCTION

Banana (*Musa* spp.) is one of the most important cultivated tropical fruits, with world production estimated at 113.9 million metric tons (FAOSTAT, 2017). The fruit is a good source of energy, vitamins A, B₆, C, minerals and dietary fiber (Mohapatra *et al.*, 2010). Banana is propagated by vegetative method using suckers that arises from the underground stems. This method of propagation is slow and allows for transfer of systemic diseases from generation to generation. Large scale production of banana requires large volume of planting materials, which are hardly obtained by conventional method of propagation, and as well, bulky to move. Plant tissue culture offers an opportunity for large scale production of disease-free, uniform planting materials in a short period of time. Rapid multiplication of banana using

conventional tissue culture has been reported (Karule *et al.*, 2016; Keshari and Pradhan, 2016). The use of conventional tissue culture for commercial production of banana is constrained by high cost of seedlings resulting from low multiplication rate of propagules, intensive labour requirement and low survival during acclimatization. Temporary immersion bioreactor system (TIBs) is a system that results in rapid multiplication of plants by intermittent immersion in liquid nutrients media under sterile condition. The system is based on periodic complete immersion of the propagules by pneumatic driven transfer of liquid medium. In temporary immersion bioreactor system, gelling agents are not required which in most cases are not inert components and do not allow for automation.

Temporary immersion bioreactor system is therefore, an automated system resulting in reduced cost, rapid multiplication rate and efficient growth of the cultured plants. An automated system for micropropagation of Cavendish Banana using temporary immersion is reported in this paper.

MATERIALS AND METHODS

The study was conducted at the National Sugar Development Council (NSDC) Sugarcane Biofactory, Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria. The media used in the study was modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3% sucrose. The pH of the media was adjusted to 5.8 using 0.5M NaOH and the media was autoclaved at 121°C for 15 minutes. Young suckers were obtained from matured field grown Banana plants and used as explants for *in vitro* establishment. Suckers were washed thoroughly under running tap water and trimmed to 4-5cm in length, then treated with commercial fungicide (Benlate) 2g/L for 20 minutes and rinsed in sterile distilled water before taken to the laminar flowhood. Suckers were surface sterilized by sequential treatment for 5 minutes in 70% ethanol and 20 minutes in 2.5% (w/v) sodium hypochlorite then rinsed three times in sterile distilled water. The sterilized suckers were peeled to 2-3cm in length then cultured on establishment media consisted of semi-solid MS media supplemented with 1mg/L BAP. Cultures were incubated under 16 hour photoperiod provided by white fluorescent lamps ($80\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for four weeks. The resulting plantlets with average length of 2cm were transferred to bioreactor bottles (950ml) containing 250ml of culture media. Ten explants were cultured in each bottle and two bottles were used per treatment replicated three times in a Completely Randomised Design. The TIBs culture media consisted of MS basal medium supplemented with 1mg/L or 2mg/L BAP and with or without 250mg/L AC. Cultures were maintained in the bioreactor room under temperatures of $25\pm 2^\circ\text{C}$ and 16 hours photoperiod for four weeks. Feeding cycle by immersion for 3 minutes every 4 hours was programmed using electronic timer. Data was collected after four weeks of culture on shoot number, shoot length, number of leaves per shoot, number of roots and root length. The data was subjected to analysis of variance (ANOVA) using SPSS version 23.0 and means compared using Turkey significant test.

RESULTS AND DISCUSSION

An automated rapid propagation system for banana based on the principle of temporary immersion has been achieved in this study. This micropropagation system enable a periodic immersion of the plant in the nutrient media which ensured both constant supply of nutrients and efficient aeration. Although hyperhydricity has been reported in liquid cultures of many plant species (Kevers *et al.*, 2004; Smith, 2013), such problem was not encountered in this study .

To evaluate the effect of BAP and AC on the *in vitro* morphogenesis in banana, different concentrations of BAP with or without AC were compared with the hormone free medium (Table 1). The media fortified with BAP alone had the highest multiplication rate. The multiplication rate increased by 800% and 900% in media fortified with 1mg/L or 2mg/L BAP respectively, compared to hormone free media. BAP increase shoot multiplication by inducing axillary buds proliferation via direct control on cell division and tissue differentiation. Shoot clusters were produced in the presences of BAP alone with banana shoots produced around a central region. These shoots were short and devoid of roots, therefore were not competent for *ex vitro* establishment. Earlier study on banana production using bioreactor (Lemos *et al.*, 2001) showed that when BAP alone was used for shoots proliferation, a rooting cycle on hormone free media is necessary.

The combination of 6-Benzylaminopurine (1mg/L or 2mg/L) with AC greatly increased both multiplication rate and number of competent plants (plants ready for *ex vitro* establishment). A strong interaction was observed between BAP and AC in the production of competent plants. While none of the regenerated plants obtained in media fortified with BAP alone was competent, the number of competent plants significantly ($P \leq 0.05$) increased to 300 and 120 in media fortified with 1mg/L BAP +AC and 2mg/LBAP + AC, respectively. The positive effect of AC in the production of competent plants in micropropagation of banana and other plants have earlier been reported (Dumas and Monteuis, 1995; Gübbük and Pekmezci, 2004; Nisyawati and Kariyana, 2013; Ahmed *et al.*, 2014). AC is used in tissue culture where phenolic secretion is a common problem. It acts by absorbing the toxic phenolic compounds which alters the pH of the media and block nutrient absorption by the explant. In addition to adsorption of the phenolic compounds, it also provides adsorption surfaces

for the nutrients, a process which greatly reduce the efficacy of the exogenous hormones. This could be the possible reason for low multiplication rate in media supplemented with BAP and AC when compared with media fortified with BAP alone. In the temporary immersion system, activated charcoal adsorb BAP and alters the hormonal balance in favour of root formation resulting in the increased number of competent plant.

The dynamics of root formation and BAP with and without AC is shown in Figure 1. The highest root morphogenesis was obtained in the hormone free media and media fortified with a

combination of BAP and AC. The presence of BAP alone in the media completely eliminates root formation. However when AC was added, significant ($P \leq 0.05$) increase in rooting was observed and the reason could be due to hormonal imbalance in favour of rooting. There were however, no differences in the number of leaves between media fortified with BAP alone and BAP + AC. This observation may be explained by reduction in leaves development in the temporary immersion system. The shoot clusters contained morphologically normal plants with differences only in the size, but the plants have almost equal number of leaves.

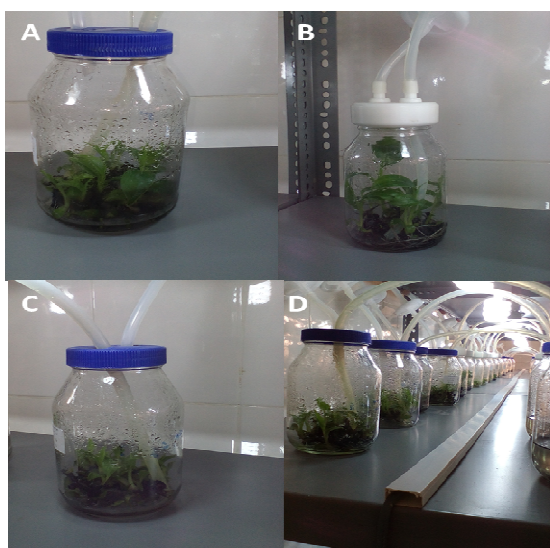


Plate 1: Micropropagation of Cavendish Banana Using Temporary Immersion Bioreactor System (TIBs). (A) multiplication on MS + 1mg BAP, (B) hormone free MS, (C) multiplication on MS + 1mg BAP without activated charcoal, (D) experimental layout

Table 1: Effect of BAP ± AC on multiplication rate and number of competent plants in Banana under temporary immersion system

Treatment	Initial number of explant	Multiplication rate	Total number of Plant ± SE	Number of competent Plants ± SE	competent plants (%)
Hormone Free	60	1	60.00±2.90 ^e	58.00±3.07 ^c	96.7
BAP (1mg/L)	60	8	480.00±3.65 ^b	0.00±0.00 ^d	0
BAP (2mg/L)	60	9	540.00±5.76 ^a	0.00±0.00 ^d	0
BAP (1mg/L) +AC	60	6	360.00±2.89 ^c	300.00±28.87 ^a	80
BAP (2mg/L) +AC	60	5	300.00±2.78 ^d	120.00±5.77 ^b	40

Means with same letter along the column are not significantly different using Turkey significant test at 5% probability level.

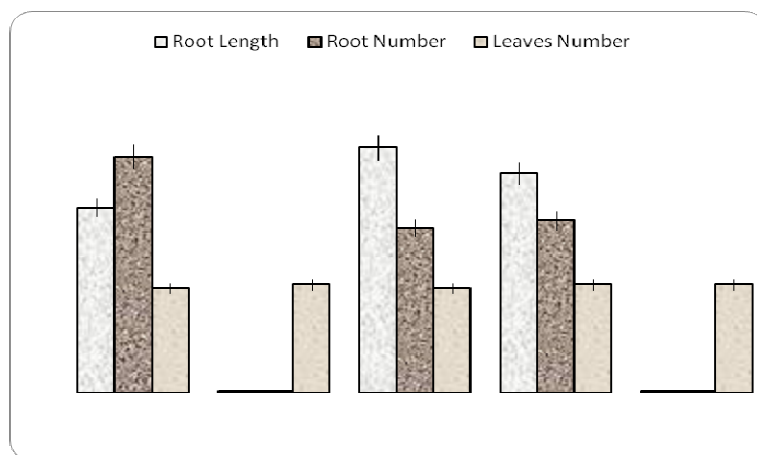


Figure: Effect of BAP±AC on root morphogenesis and number of leaves in Banana under temporary immersion system.

CONCLUSION

An improved method for banana multiplication using temporary immersion system has been developed to reduce the cost of micropropagated plants. The improvement achieved in this study is the elimination of rooting cycle which greatly reduced the

micropropagation time. Using this system it is possible to obtain 50 competent plants from 10 plantlets in 4 weeks and further extension of the production scheme to 6 months will result in the production of 156,250 competent plants ready of *ex vitro* establishment.

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