



MICROPROPAGATION OF PINEAPPLE (*Ananas comosus* L. var. *Smooth cayenne*) IN TEMPORARY IMMERSION BIOREACTOR SYSTEM (TIPS)

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ABSTRACT

Pineapple is an important edible fruit in the family Bromeliaceae popularly grown in the tropical and subtropical countries. Commercial production of pineapple requires large volume of planting materials which could not easily be obtained using conventional method of propagation. A protocol for mass propagation of pineapple (*Ananas comosus* L. var. *smooth cayenne*) using temporary immersion bioreactor system has been developed. The protocol involves four immersion cycles in Murashige and Skoog (MS) media fortified with 1mg/L or 2mg/L 6-Benzylaminopurine (BAP) with or without 0.25g/L activated charcoal (AC). The highest multiplication rate (120 -130 plants/bottle) was obtained when media was fortified with 1mg/l or 2mg/L BAP alone. The presence of activated charcoal (AC) promoted root morphogenesis, resulting in significant increase in roots formation in BAP supplemented media. A combination of BAP with AC significantly increased the number of competent plants (20 – 30 plants/bottle) after four weeks of culture in temporary immersion system. The system is recommended for rapid and efficient micropropagation of pineapple.

Key words: Pineapple, Micropropagation, Temporary immersion system

INTRODUCTION

Pineapple is an important edible fruit among members of the family Bromeliaceae grown in the tropical and subtropical countries. Pineapple is ranked second most important tropical fruit after Mango in world production (FAO, 2017). The fruits are important source of energy, vitamins A, B₁ and contain an important phytochemical enzyme – bromelain. Global production of pineapple is estimated at 25.89 million tonnes (FAOSTAT, 2017). Nigeria is ranked number seven in production of pineapple with estimated annual production of 1,68 million tonnes. Since 1970, the demand of pineapple and other tropical fruits is increasing due to shifting of consumers' preference in favour of tropical fruits. In last decade, production of pineapple in Nigeria increased annually by an average of 1.87% largely by subsistent farmers who typically depend on conventional propagation methods (crown and suckers) for planting material to establish new plantations. This system is slow due to low multiplication rate and there is the danger of transfer of diseases from one generation to the next. Commercial level production of pineapple requires large volume of planting materials which could not easily be obtained using conventional

method of propagation. Propagation of pineapple using tissue culture technique offers a good opportunity for large scale production of planting materials. Several micropropagation protocols have been developed for pineapple (Escoban *et al.*, 1999; Soneji *et al.*, 2002b; Firoozabadi and Gutterson, 2004; Usman *et al.*, 2014, Buah *et al.*, 2015). However, the use of micropropagated plants in establishment of commercial plantations is constrained by high cost of seedlings resulting from high labour cost, low multiplication rate and poor survival rate during acclimatisation. This paper reports a cost-effective automated method of pineapple micropropagation using temporary immersion system.

MATERIALS AND METHODS

The experiment was conducted at the Sugarcane Biofactory of the National Sugar Development Council (NSDC), Ahmadu Bello University Zaria, Nigeria (11.1512° N, 7.6546° E). *In vitro* plants were regenerated using the procedure described by Usman *et al.* (2013). Young and healthy fruits were obtained from a local market in Zaria city, Kaduna State, Nigeria.

Crowns of the young fruits were used as a source of explants. Matured leaves were removed and crowns were thoroughly washed with detergent under running tap water. The crowns were further surface sterilized by sequential treatment for 2 minutes in 70% ethanol and 20 minutes in 10% commercial bleach (NaOCl - 3.5% w/v) plus 2 drops of Tween 20, after which they were rinsed thrice in sterile distilled water. Furthermore, the crowns were treated for 10 min in 5% commercial plus 2 drops of Tween 20 and rinsed three times in sterile distilled water. Axillary buds were excised from the crown and inoculated in test tubes over paper bridges on a liquid media consisting of MS basal media (Murashige and Skoog, 1962) supplemented with sucrose (3%), BA (2.5 μ M) and NAA (0.6 μ M). Cultures were kept in the growth room at a temperature of $27 \pm 2^\circ\text{C}$ and 16 hours photoperiod provided by cool white fluorescent tube ($80\mu\text{mol photons m}^{-2} \text{s}^{-1}$). After four weeks of inoculation, the resultant plantlets with average length of 2cm were transferred to bioreactor bottles (950ml) containing 250ml of culture media. Five explants were cultured in each bottle and six bottles were used per treatment in a Completely Randomised Design replicated three times. The TIBs culture media consisted of MS basal medium supplemented with 1mg/L or 2mg/L BAP with or without 0.25g/L activated charcoal. Cultures were maintained in the bioreactor room under temperatures of $25\pm 2^\circ\text{C}$ and 16 hours photoperiod ($80\mu\text{mol photons m}^{-2} \text{s}^{-1}$) 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, root length and number of competent plants (fully rooted plants ready for field). Data was subjected to analysis of variance (ANOVA) and means compared using least significant difference (LSD).

RESULTS AND DISCUSSION

The success of micropropagation of any given species depend largely on the ability to develop and optimize protocol for *in vitro* regeneration and multiplication of selected genotype. Using Modified MS media fortified with 1mg/L or 2mg/L 6-Benzylaminopurine, a multiplication rate of 24 to 26 plantlets per explant was achieved given an average of 120 to 130 plantlets per bioreactor bottle. This result revealed an improvement over what was reported using solid media (Usman *et al.*, 2013) and temporary immersion system (Escalona *et al.*, 1999). Shoot length was greatly reduced by the application of BAP, but a combination of BAP

and activated charcoal (AC) significantly increased shoot length (Table 1). Due to its potency in inducing adventitious shoot, BAP reduces shoot elongation as all available nutrients in the plants were deployed for lateral shoots formation. However, when AC was added shoot elongation was achieved. Although activated charcoal is used where phenolic secretion is a common problem, it also provides adhesion surfaces for other media constituents including exogenous hormones. The AC must have adsorbed the BAP molecules, reducing its availability in the media which subsequently resulted in the shift of hormonal balance in the plantlets in favour of shoot elongation. Ebert *et al.* (1993) reported that when 6-Benzylaminopurine was added to gelled medium in the presence of AC, less than 2% of the BAP was available to plant tissue after 3 days and the addition of the BAP to the medium influenced the availability of Auxin in the media. The role of AC in micropropagation has earlier been reported (Nisyawati and Kariyana, 2013; Ahmed *et al.*, 2014).

A combination of BAP with AC greatly increased root morphogenesis in pineapple under temporary immersion system (Table 1). Root morphogenesis was completely suppressed in media fortified with BAP alone and promoted by a combination of BAP and AC. The AC must have altered the hormonal balance in favour of root morphogenesis resulting in the development and elongation of roots. Firoozabady *et al.* (2006) reported that addition of AC considerably enhanced the rooting ability of transgenic shoots in pineapple and the role of AC on the *in vitro* rooting of many plant species has been reported (Thomas, 2008). The effect of BAP and AC on the number of leaves and competent plants (plant with well developed root ready for acclimatization) is shown in Figure 1. The highest number of competent plants was obtained when a combination of BAP with AC was used. Production of competent plants in this study most have been promoted by gradual release of the BAP adsorbed by AC. Activated Charcoal was reported to gradually release some of the adsorbed substances, such as nutrients and Plant Growth Regulators (PGRs) which promote plant growth (Johansson *et al.*, 1990; Thomas, 2008). There were no differences in number of leaves between media fortified with BAP alone, BAP with AC or hormone free media. This could be due to the general reduction in leaves development observed throughout the experiment. Similar observation was earlier reported in micropropagation of pineapple using temporary immersion system (Escalona *et al.*, 1999).

Table 1: Effect of BAP with or without activated charcoal on pineapple shoot and root morphogenesis in temporary immersion system

Treatment	Number of Shoots	Shoot Length	Number of Roots	Root Length
Hormone Free	22.00 ^c	4.50 ^a	5.00 ^a	4.27 ^a
Hormone Free + AC	25.00 ^c	3.76 ^{ab}	5.00 ^a	3.95 ^a
BAP (1mg/L)	120.00 ^a	1.75 ^{bc}	0.00 ^b	0.00 ^b
BAP (2mg/L)	130.00 ^a	1.43 ^c	0.00 ^b	0.00 ^b
BAP (1mg/L) + AC	40.00 ^b	3.62 ^{abc}	4.00 ^a	3.00 ^a
BAP (2mg/L) + AC	42.00 ^b	3.28 ^{abc}	4.00 ^a	2.78 ^a

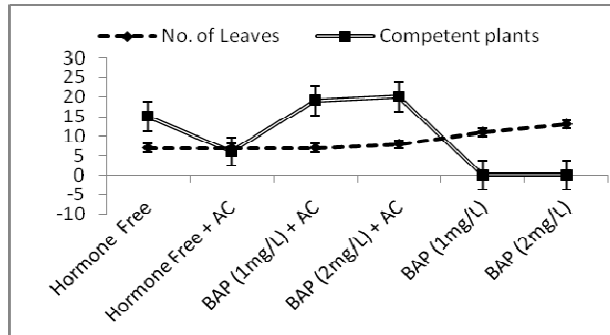


Figure 1: Effect of BAP with or without activated charcoal on number of leaves and number of competent plants. Each point represent the mean ±SE (n=30). Each treatment consist of six culture bottles containing five explants at the beginning of the culture in temporary immersion system

CONCLUSION

The key improvements in the micropropagation of pineapple reported in this paper are; (i) the used of four immersion cycles per day and low concentration of BAP (1 – 2mg/L) to significantly increase multiplication rate and (ii) the use of AC to increase the number of competent plants per

cycle which significantly reduced the cost of production. Majority of the problems associated with conventional micropropagation on gelled media has been overcome by deploying this system and is recommended for mass production of pineapple.

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