Comparative Studies of Callus Cell and Shoot Proliferation From Pineapple And Banana Culture in Vitro: Antioxidant, Carbohydrate, Pigment and Mineal Properties

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Abstract

The study was conducted to evaluate the comparison of the callus cell, shoot and stem proliferation from pineapple crown slice and banana inflorescence in vitro using NAA and BAP at different concentration. The pineapple shoot number and weight was higher in BAP at 2.0 mg/l than control and NAA 0.2 mg/l. However, the BAP + IAA (10mg/l + 1 mg/l) concentration produced higher callus cell weight, shoot number and stem length than MS medium (without hormone) and NAA 1mg/l concentrations in banana. Moreover, it has been shown that the sugar and chlorophyll content were higher in BAP treated hormone than MS medium and NAA 0.2mg/l concentration in pineapple. In addition to that the nutrient content (K+ and NO3--) was exhibited higher in the 2 mg/l BAP treated explants than MS (control) and 0.2 mg/l NAA treated explants in pineapple. Callus tissue weight was maximal in BAP (10mg/l) + IAA (1mg.l) treated explants compared to the NAA (1 mg/l) and MS treated explants in banana. Besides, shoot number and stem length were higher in the BAP (10mg/l) + IAA (1mg/l) treated explants than NAA (1 mg/l) and MS (control) media in banana. The highest sugar and chlorophyll content was recorded in BAP (10mg/l) + IAA (1mg/l) treated explants in banana. Meanwhile, the total phenol content was significantly increased by BAP treatment. K^+ and NO3-- content in explants were significantly increased by BAP and NAA treatments in banana. The result showed that the DPPH radical scavenging activity increased with hormone application. Finally it seemed that BAP was the best effective hormone for the both pineapple and banana explants regeneration. Therefore the highest shoot and stem was regenerated in BAP (2mg/l) in pineapple.

Keywords - *callus cell and tissue, proliferation, pineapple crown and banana bud, antioxidant*

I. INTRODUCTION

Cell and tissue culture from embryonic cell, embryo, bud, leaf and shoot tip, shoot, stem or crown, protoplast cell) has been successfully carried out in fruit, vegetable, ornamental plants and forest trees [1]. Millions of pineapple explant can be produced by cell or tissue culture from leaf, crown or stem per year in any plant production industry. However, the multiplication and total number of explants production were described by many plant tissue culture scientists. A total number of plantlets production were found 5000 [2]; 40000 [3]; 100000 [4] from single explant per year.

In vitro culture techniques have become most popular technique for producing high number of plantings material for banana [5, 6]. Explant regeneration of banana was successfully completed by meristem derived from corm tissue [7] and shoot tips [8], young zygotic embryos [9, 10] male bud inflorescence [11, 12, 13] female flowers [14], meristem inflorescences , and somatic embryo genesis [15] *in vitro*. The meristem inflorescences were used as materials for *in vitro* micro propagation techniques [16, 17] because these materials reduced the contamination rate compared to soil grown suckers.

Propagation of pineapple can be developed in vitro treated with BAP alone[17], mixture of hormones like BAP and naphthalene acetic acid (NAA) [18] indole butyric acid (IBA) [19], indole acetic acid (IAA) and 2,4-dichlorophenoxy acetic acid (2,4-D) [3] combination of BAP and two auxins as NAA and IAA [20] IAA and IBA [21] and NAA and IBA [22] However, the number of meristem tissue, shoots and explants production have yet limited information in related to hormone concentrations in the case of pineapple as well as Banana. The present study was performed with the aim to produce shoot and explant from pineapple crown and multiple meristem tissue as well as explant of banana from male inflorescence by using *in vitro* techniques.

II. MATERIALS AND METHODS

A. Pineapple experiment

B. Medium preparation

MS [23] medium was prepared (1 L) from stock solutions and supplemented with sucrose at 30 g/l. The medium was adjusted to pH 5.7 before adding agar at 7.0 g L^{-1}. The beaker containing the medium was placed over magnetic stirrer hot plate and heated to boiling to dissolve the agar and then dispensed equally (20 mL jar^{-1}) into 24 glass jars (5x15 cm) with screw rim and plastic lid which were autoclaveable. The medium was then autoclaved at 121°C and 1.5 kg cm^{-2} for 25 min. After that the autoclave was stopped and waited until it cooled down. The medium divided into 30 beakers (25 mL each). Hormone was not added (control) to the first 10 beakers and BAP at 2.0 mg/l was added to the 11-20 beakers and NAA 0.2 mg/l was added to the rest 21 -30 beakers, respectively.

C . Plant materials

Pineapple crown was collected from farmer garden and placed in a beaker, washed thoroughly having water and sterilized with Clorox (20% for 25 min). The cutting slice were then rinsed twice in distilled water for 5 min and cultured in cylindrical glass jar [24] with a rimmed neck and plastic cover containing 25 mL of hormone free MS medium (10 jars), medium with BAP at 2.0 mg/l (10 jars) and NAA 0.2 mg/l (10 jars), respectively. The cultures were transferred to incubation room and kept under the constant temperature of 25°C and photoperiod of 16 h of light provided by fluorescence lamp. Five replicates were used per treatment.

D. Data collection

After15 days callus started to initiate and after 60 days of incubation, data were collected. The number and length of shoots/explant and the total number of shoots produced were calculated and used for evaluation of the different treatments. The shoots removed from the cultures, weighed, separated into individual shoot for counting the number and measuring the length and weight of shoots.

E. Total sugar determination

Total soluble sugar was determined according to the phenol-sulphuric method of Dubois (1956).

F. Determination of Chlorophyll a and b

Total chlorophyll was determined according to the methods of Lichtenthaler and Wllburn [26]. The method consisted of repeated acetone extraction, until obtained colorless residue, with a pestle and mortar and filtered over filter paper (Whatman No.1 equivalent). The extracts were made up to 50 ml with acetone. The concentration of chlorophyll a at 666mm and chlorophyll b at 653 nm was measured in a Shimadzu UV 160A spectrophotometer. The amount of chlorophyll a and b was calculated according to the formula of Lichtenthaler and Wellburn(1983).

G. Nutrient content determination

Nutrient content (NO_3^- and K+) was determined by using Horiba Scientific NO_3 and K meters (Japan). 3 drops of juice sample were put on the disc sensor of the meter using small dropper and data were displayed and recorded.

I. Banana experiment

1. Materials and Methods

2. Plant materials

Inflorescence male bud of *Musa acuminata* cv. Pisang Mas were obtained from a farm Johore Bahru, Malaysia. Male inflorescences were collected when all the female flowers in a bunch were completely exposed and were cultured.

3. Media preparation

MS semi solid medium (MS control) were prepared and MS supplemented with 10 mg/l, of N6-benzylaminopurine (BA), 1 mg/l of Indole -3-Acetic Acid (IAA), 2.0 mg/l gylcine, 0.4 mg /l (ppm) thiamine, HCl , 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine, 10 mg/l ascorbic acid and 30 g /l sucrose (Ma 1991). Other media were supplemented accordingly with NAA 1 mg/l. The pH of the medium was adjusted to 5.8 prior to autoclaving and put into the autoclave having properties mentioned in Experiment 1. Thus media were prepared. Five replication were used per treatment.

4. Cultural procedure

The male inflorescence (bud) of *banana* were dissected and shortened to 6cm in length. The explants were then disinfested with 70% volume of alcohol for 10 min and rinsed with sterile distilled water three times. Then 50 explants were cut longitudinally to make it half and were placed onto a semi solid medium (Fig. 2). The cultures were transferred to incubation room and kept under under constant temperature of 25°C and photoperiod of 16 h of light provided by fluorescence lamp.

5. Data collection

After 21 days, explants were swelled up and turned green in color with the superficial bract curved outwards and exposing the rudimentary flowers. The white rudimentary flowers which appeared as white proliferating floral meristem were selected and cut into pieces and sub-cultured in the MS semi solid medium supplemented the same media and concentration of hormones mentioned previously. Finally, after 30 days of subculture, the individual callus and shoots were separated, washed and measured.

6. Total sugar determination

Total soluble sugar was determined according to the experiment 1 which was mentioned above.

7. Determination of Chlorophyll a and b

Total chlorophyll was determined according to the experiment 1 which was mentioned above.

8. Total phenols

The total phenolic content of explant was determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Folin-Ciocalteau (FC) colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. 1ml of leaf juice, gallic acid calibration standards, folin-Ciocalteau (FC) reagent stored in the dark and discarded if reagent becomes visibly green, Sodium carbonate solution, 100-ml were used in the volumetric flask. Spectrophotometer was set to 765 nm, with 1-cm, 2-ml plastic or glass cuvettes. 1ml of extract was added to 25 ml of volumetric flax, containing 9 ml of distilled water. A reagent blank also prepared. 1 ml of Folin –Ciacalteu's phenol reagent was also added to the mixture. The solution was diluted with distilled water and mix and incubated at room temperature. Absorbance against reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5 and expressed as mg gallic acid equivalent.GAE/ 100g fresh weight.

9. Antioxidant as DPPH activity

The DPPH free-radical scavenging activity was determined as described in Yang et al. (2008).

10. Nutrient content determination

Nutrient content (NO_3^{--} and K+) was determined according to the experiment 1 which was mentioned above.

II.I.K. Experimental design

The experiments designed as Complete Randomize Block Design (CRBD) and the means significance tested (LSD) at p = 0.05.

III. RESULTS

A. Pineapple experiment

The maximum pineapple shoot number was obtained in BAP at 2.0 mg/l than control and NAA 0.2 mg/l (Table 1). The total pineapple shoot number was found 23 per explants in BAP at 2.0 mg/l followed by 17 and 12 per explants in control (MS medium with hormone free) and NAA at 0.2 mg/l. There was a significantly difference between MS medium and MS medium treated with BAP and NAA.

In Table 1, it has been shown that the pineapple shoot and stem length were higher in BAP at 2 mg/l concentration than in MS medium and NAA at 0.2mg/l. However, it was the highest shoot length (9.1cm) in BAP at 2 mg/ for pineapple. There was a significantly difference of shoot length between explants in MS medium and the BAP and NAA treated explants. The results exhibited the better effect of BAP treated pineapple explants than the explants produced in MS medium and NAA treated medium. But there was no significant difference between the MS medium (control) and NAA treated explants. The higher pineapple stem length was exhibited in the BAP treated explants than the NAA concentration and MS medium (Table 1).

In addition to that, the maximum total shoot weight was found in the BAP treated explants compared to the NAA concentration and MS medium (Table 1). There was no significant difference between the BAP treated explants and MS medium as well as NAA treated explants. Photograph shows the cultural results from crown of pineapple at different growth hormones in Fig. 1 . It has been shown the total sugar, chlorophyll content and nutrient content (K+ and NO3-) in Table 2 for pineapple.

The absorbance properties of photosynthetic pigments allowed the amount of total chlorophyll (a, b) in pineapple determined by spectrometer (Table 2). As shown in Table 2, the amount of chlorophyll was higher in BAP treated hormone than MS medium and NAA concentration in pineapple. Moreover, the highest sugar content of 6.66 g was recorded in BAP 2mg/l treated explants followed by MS medium and NAA 0.2mg/l treatments which recorded a sugar content of 4.41 and 5.2g respectively (Table 2) in pineapple. It has been found that nutrient content (K+ and NO3-) was higher in the 2 mg/l BAP treated explants than MS (control) and 0.2 mg/l NAA treated pineapple explants.

B. Banana experiment

The BA + IAA (10mg/l + 1 mg/l) concentration increased callus cell weight, stem number and stem length than MS dedium (without hormone) and NAA 1mg/l concentrations (Table 3) in banana. Callus tissue weight was maximal in BAP (10mg/l) + IAA (1mg.l) treated banana explants than NAA (1 mg/l) and MS treated explants. Shoot number and shoot length were higher in the BA (10mg/l) + IAA (1mg.l) treated banana explants than NAA (1 mg/l) and MS (control) media for banana.

The highest sugar content of 3.2 g/100g was recorded in BAP 2mg/l treated banana explants (Table 4). The amount of chlorophyll was higher in BAP+IAA treated hormone than MS medium and NAA concentration in Banana. The total phenolic compound was significantly increased by BA treatment (Table 4). K^+ content in explants was

significantly increased by BA and NAA treatments. The NO3- content was higher in the 2 mg/l BAP treated banana explants than MS (control) and 0.2 mg/l NAA treated banana explants. The DPPH radical scavenging activity measured in extracts was affected by different growth regulators (Table 4). The result showed that the DPPH radical scavenging activity increased with hormone application. In addition, the results exhibited that the DPPH radical scavenging activity increased up to 40.8% and 32.9% in extracts from the BA and NAA treatments, while activity in the control was only 26% (Table 4) for banana. According to the comparative study, the number of shoot and stem was regenerated higher in pineapple (BAP) than in Banana. The chlorophyll content, total sugar, nutrient (minerals) like K+ and NO3-- were higher in pineapple (BAP) explants than in Banana (BAP) explants. Fig 1. Photograph shows the culture from crown of pineapple and subculture from explants at different growth hormones. Fig. 2 shows the proliferation of shoots derived from floral meristems cell and tissue by using BAP+IAA. Fig. 3. exhibits the comparative studies of correlation between treatments (MS media, NAA, BAP) and total sugar content in pineapple and banana explants. The highest correlation was found in BAP in the case of both pineapple and banana (Fig.3). However the strong correlation was observed in pineapple ($R^{\surd}$=0.794) where it was $R^{\surd}$=0.470 in banana.

Table 1. Shoot weight and stem length at different media. Means followed by the common letters are not significantly different at the 5% of significance level by Least Significant different test (LSDT). Mean ± SE (n= 5).

Medium	Shoot weight (g)	Shoot number	Shoot length (cm)	Stem length (cm)
BAP 2.0	1.66±0.05	22.2±0.1	9.1 ±0.07	6.1±0.05
MS	1.41±0.05	16.4±0.2	6.9±0.05	5.2±0.05
NAA 0.2	1.2±0.05	12.1±0.1	5.8±0.06	5.5±0.03

Table 2 . Total sugar, Chlorophyll and nutrient content determination. Means followed by the common letters are not significantly different at the 5% level of significance by Least Significant different test (LSDT). Mean ± SE (n= 5).

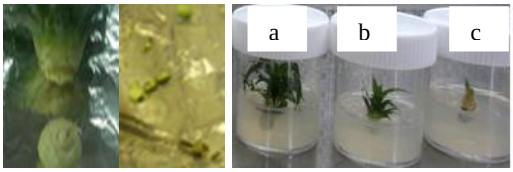
Medium	Total sugar (%)	Chlorophyll content	K+ content(ppm)	NO 3- content (PPM)
BAP 2.0	6.66a	4.2a	269a	385a
MS	4.41b	3.4b	231c	344c
NAA 0.2	5.2b	3.1b	245b	359b

Table 3. Callus weight, shoot number and length determination. Means followed by the common letters are not significantly different at the 5% level of significance by Least Significant different test (LSDT). Mean ± SE (n= 5).

Medium	Callus cell weight (g)	Shoot number	Stem length (cm)
BAP+IAA	3.2±0.04	12.2±0.06	2.1 ±0.02
MS	1.8±0.01	5.4±0.07	1.3±0.02
NAA	2.3±0.02	8.1±0.05	1.7±0.01

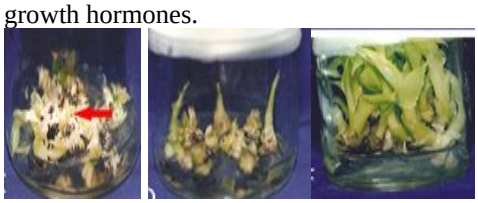
Table 4. Total sugar, chlorophyll, antioxidant and nutrient content determination. Means followed by the common letters are not significantly different at the 5% of significance level by Least Significant different test (LSDT). Mean ± SE (n= 5).

Hormone and Media	Total sugar (%)	Chlorophyll (µg/100g)	Total Phenol GAE/100g	K+ content	NO 3-- content	DPPH activity mg/100g
BAP+IAA	3.2a	5.2a	421a	259a	355.1a	15.4a
MS	1.8b	3.4c	375cb	236b	334.5b	10.0b
NAA 10	2.3b	4.1b	388b	244b	335.2b	12.6ab



Pin. crown Crown slice, Culture in the plastic jar after 30 days, a=BAP, b=NAA, c=MS

Fig 1. Photograph shows the culture from crown of pineapple and subculture from explants at different growth hormones.



a b c

Fig. 2 (a): The proliferation of white floral meristems cell and tissue. (b): Formation of shoots derived from floral meristems cell and tissue. (c): vigorous shoots derived from green competent meristem by using BAP+IAA.

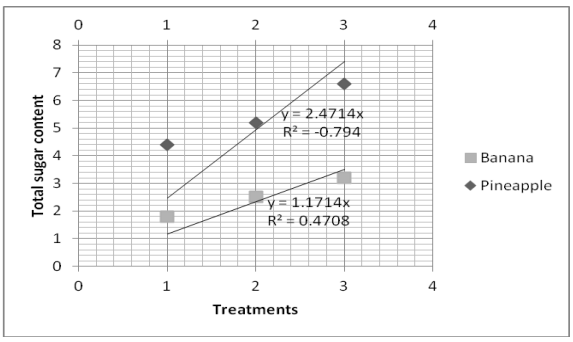


Fig. 3. Comparative studies of correlation between treatments and total sugar content in pineapple and banana explants. 1= MS media, 2= NAA, 3= BAP.

IV. DISCUSSION

A. Pineapple experiment

In the results, it has been found the highest shoot number and length in BAP at 2 g/l treated explant. Hammad and Taha [23] reported that shoot number was higher in BAP at 1.5 mg/l. Our result showed similar compared to their result. It might be due to the shoot/explant reflects the proliferation potentiality or BAP activation than other growth regulators and MS medium. The highest shoot weight was found in 2mg/l BAP concentration. This result showed the better performance than previously reported data by Hammad and Taha [23]. According to their report, the tallest (25 mm) and heaviest (0.67 g) shoots obtained in hormone free and at 0.25 mg/l and the shortest (7 mm) and lightest (0.13 g) shoots at 1.25 mg/l. The prime aim of tissue culture is to optimize the multiplication. In the present results, it has been obtained higher shoot number (22.2) per explant. Hammad and Taha (2009) reported that 12 shoots per explant of pineapple obtained in agar solidified MS enriched with BAP at 2.25 mg/l. However, the previously reported 10 shoot per explant (Sripaoraya *et al*., 2003) obtained in response to BAP at 2.0 mg /l and 7 (Aydieh *et al*., 2000) and 3 shoots per explant (Zepeda and Sagawa, 1981) obtained by 1.0 mg/l BAP and the 10 shoots (Firoozabady and Gutterson, 2003) found by 3.0 mg/l BAP.

The total sugar and chlorophyll content were higher in BAP treated explant than other treatment. Basically these total sugars are fructose, glucose and sucrose. Sucrose has been synthesized from triose phosphates made in the Calvin cycle and exported from the chloroplasts then It was converted fructose 6-phospate which combined with UDP-glucose to form sucrose phosphate, catalyzed by sucrose phosphate synthase. It might be due to the more photosynthetic yield produced by the acceleration of BAP hormone. Moreover, due to the same cause, it has been found that K+ and NO3- was higher in the 2 mg/l BAP treated explants than MS and NAA treated explants.

B. Banana experiment

In the present study, it has been shown that the callus tissue weight, shoot number and stem length were higher in 10 mg/l BAP + 1mg/l IAA treated explants than 1 mg/l NAA and MS medium (Table 3). It has been found in previously reported data that shoot number was higher in BAP at 1.5 mg/l reported by Hammad and Taha [23]. Our result showed similar trend compared to their result. It might be explained that the shoot per explant influenced the proliferation potentiality or BAP activation than other growth regulators and MS medium. The highest shoot weight was exhibited in 10mg/l BAP + 1 mg/l IAA concentration. Hammad and Taha [23] observed that 12 shoots per explant of pineapple found in MS with BAP at 2.25 mg/l. In our results, it has been showed that 12.2 shoots are produced per explant in 10mg/l BAP + IAA 1mg/l that is a little higher than their report. In this experiment NAA has induced stimulating effect on compact floral meristems cell or callus cell. The compact floral meristems cell or tissue exhibited higher weight than MS medium. This implies that NAA has induced active meristem . This is supported by finding of Cronauer and Krikorian [9] and reported that only the active meristems of the inflorescence apices was induced to form multiple cell, tissue and shoots in the culture.

The total sugar, chlorophyll, phenol content, antioxidant (DPPH activity) were higher in 10 g/l BAP + IAA 1mg/l treated explants than 1 mg/l NAA and MS medium. It may be due to the higher photosynthetic yield produced by the acceleration of BAP + IAA hormone. However, it has been observed that K+ and NO3-- content were higher in the 10 mg/l BAP + 1 mg/l IAA treated explants than MS and

0.2mg/l NAA treated explants. In the comparison of the regenerated shoot and stem, chlorophyll content, total sugar, nutrient (K+ and NO3--) in pineapple and banana, were found higher in pineapple (BAP) explants than in Banana (BAP) explants. It might be due to the more effectiveness of BAP (2mg/l) in pineapple casing higher cell division and differentiation, light penetration in the explants of pineapple than in banana. The combination of the concentration of 10 mg/l BAP + 1 mg/lIAA may be less effective in banana.

V. CONCLUSION

It is concluded that the shoot number and nutrient content (K^+ and NO3--) produced from pineapple crown was the highest in BAP (2.0 mg/l) compared to the NAA and MS media.. In addition to that callus cell, shoot number, total sugar, antioxidant and total phenol content produced from banana meristem showed significantly higher in 10 mg/l BAP + 1 mg/lIAA than NAA and MS medium. In addition to that the regenerated shoot and stem, chlorophyll content, total sugar, nutrient (K+ and NO3--) were found higher in pineapple (BAP) explants than in Banana (BAP) explants.

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