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The Effect of Some Natural Materials in the Development of Shoot and Root of Banana (*Musa spp.*) Using Tissue Culture Technology

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Abstract: Banana is one of the most important fruit crop worldwide, as its production reaches approximately 70 million tones per year. By tissue culture, triploid and seedless bananas can now be produced in a high quantity, in a less time and at any time of the year without being limited to a season. The aim of this study is to substitute the artificial hormones used in the tissue culture such as, cytokinins and auxins by natural materials such as, Pineapple, Coconut milk, Cacao, Coffee, and Charcoal. Five different media composed of these natural materials were prepared in different concentrations, Pineapple (5,10 and 20cm), Coconut milk (5,10 and 20cm), Cacao (1,2.5 and 5 g), Coffee (1,2.5 and 5 g) and Charcoal (1,2.5 and 5 g) to study their effect on the development and browning of the bananas' shoots and roots in vitro, with the aid of various statistical analyses. The results indicated that the pineapple and coconut milk produced the highest number and length of both shoots and roots, as well as the soluble sugars contents, while the cocoa and coconut milk showed the highest protein content. The charcoal reduced significantly the phenolic content. On the other hand, there was no significant difference between the coffee, cacao. This study has highlighted the benefits and advantages of using natural materials in producing high quality banana. [Irini Beshir, Sherif El Sharbasy, Gehan Safwat and Ayman Diab **The effect of some natural materials in the development of shoot and root of Banana (*Musa spp.*) using tissue.** New York Science Journal 2012;5(1):132-138]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 19.

Key words: Banana, Tissue culture, soluble sugars contents, Phenol content, Protein content

1. Introduction

Banana is regarded as the first fruit crop in the world, both in terms of production, and trade, as it produces around 98 millions tonnes and over US\$9506 million; respectively (FAO STAT database, 2010).

Banana is considered one of the most important fruits in the world, as a staple food, as well as, a major export commodity for many tropical and sub-tropical countries. Bananas are propagated vegetative through suckers. Since most of the edible bananas are triploid and are nearly sterile and parthenocarpic, the use of conventional breeding methods for their improvement are difficult and cumbersome. Mutation breeding and biotechnological methods can offer useful tools for banana improvement. The various experiments conducted for banana propagation have provided new opportunities for in vitro mutagenesis and selection in different banana cultivars (Banerjee and De Langhe, 1985; Wong, 1986; Novak et al., 1989; Suprasanna et al., 2002; Suprasanna et al., 2008).

Recently, Tissue culture technology has gained great attention and became very important field, as it enables the rapid production of a large quantity of uniform disease free plants from a single plant with a good genetic potential. There is a difference in the

growth and physiology of tissue culture plants compared to plants from suckers, especially at different developmental stages. Therefore, it was essential to make a critical evaluation of such plants under different field conditions, especially the leading commercial varieties like, Banana.

Research efforts have focused on the growth regulating compounds and salt mixes, and it seems that there is a good scope toward substituting the expensive chemical nutrient media by low cost natural extracts. Numerous researchers have investigated the effect of using yeast and plant extracts in different in vitro culture media. One of the earliest report addressing this point is that of Overbeek et al. (1941), who succeeded in growing immature *Datura* embryos by including the liquid endosperm of *Cocos nucifera* (coconut milk) in their culture medium.

In addition, coconut milk was found to stimulate cell division in other cultured tissues and it has been used as a supplement in many laboratories (Archibald, 1954; Wiggans, 1954). Other complex plant juices and liquid endosperms have been shown to possess stimulatory properties more or less similar to those of coconut milk. For example, the tomato juice (La Rue, 1954) and Pineapple juice; 100 g pineapple contain 47-52 calories, 85.3-87.0 g water, 0.4-0.7 g protein,

0.2-0.3 g fat, 11.6-13.7 g total carbohydrate, 0.4-0.5 g fibre, 0.3-0.4 g ash, 17-18 mg calcium, 8-12 mg phosphorus, 0.5 mg iron and 1-2 mg sodium (Patil et al. 2011). The analysis of raw coffee indicates: 12% moisture, more than 50% carbohydrates and 16% lipids, (Oliveria et al., 2006).

In this study, the growth promoting activity of various natural materials, as well as, their effect on banana are being characterized and identified.

2. Materials and Methods

These experiments were carried out in the Central Laboratory for date palm research and development 2011/2012.

Plant materials

Banana (*Musa* spp.), Grand Nain cultivar was utilized as a source for plant materials for all experiments in this study. Shoot-tips containing several sheathing leaf bases enclosing the axillary buds were taken from small suckers of about 45-75 cm in length. The isolated shoot-tips were soaked in an antioxidant solution (100 mg/l. citric acid and 150 mg/l. ascorbic acid) for 1 hour and then soaked under aseptic condition in 4% sodium hydrochloride for 20 minutes. Tween 20 was also used as a surfactant. The explants were then rinsed several times using sterilized distilled water for 5 minutes to remove all traces of sodium hydrochloride.

Tissue culture experiment

Shoot meristems with 1-2 leaf primordial were excised and used as explant materials. Explants were germinated in 150 and 250 ml glass jars. Media contained 4.4 g/l MS (Murashige and Skoog, 1962) basal medium, 30g/l sucrose, and 9 g/l agar supplemented with various concentrations of natural materials such as, Pineapple juice (5,10 and 20 cm), Coconut milk(5,10 and 20 cm), Coffee(1, 2.5 and 5g), Cocoa Liquor (1, 2.5 and 5g) and Charcoal (1, 2.5 and 5g). Prior to medium sterilization, pH was adjusted to 5.7 in all media with 1M NaOH or 1M HCL. Media were autoclaved for 15 minutes at 121°C and a pressure of 1.05 Kg/cm². All cultures were incubated in a growth chamber at temperature of 25±2°C provided with light using white fluorescent lamps. At the end of the experiment, the data in every treatment were calculated. Data were taken as the average of each medium; shoot length, root length, and fresh weight. Data of browning value of culture were calculated visually according to Pottino, 1981.

Chemical analysis

The chemical parameters included concentration of soluble sugars i.e fructose, sucrose, glucose of different treatment of natural materials were measured using HPLC (Lopez-Hernandez et al, 1996)

Total phenol content

The total phenol content of extracts was determined by the Folin-Ciocalteu colorimetric method according to Singleton et al. (1999). Briefly, 1 ml of the extract solution was mixed with the Folin-Ciocalteu reagent (1 ml) and 7.5% Na₂CO₃ (3 ml). After 1 h of incubation at room temperature, the absorbance was measured against water.

Measurements of Protein Concentration;

Protein concentration was determined spectrophotometrically at 280 nm using UV-visible spectrophotometer by the method of Plummer (1978)

Antioxidant activity;

Banana shoots extracts were measured as scavenging free radical potential in methanolic solutions of DPPH (1,1-diphenyl-2-picrylhydrazyl), as described by Burda and Oleszek (2001). The antiradical activity was calculated as percentage of DPPH decoloration compared to the control.

Statistical Analysis

The experiments were carried out using completely randomized blocks design and with three replicates. The results were analyzed using analysis of variance and the means compared using L.S.D. at the 5% level, all obtained data were subjected analysis of variance completely randomized blocks according to (Snedecor and Cochran, 1980).

3. Results and Discussion

Effects of the natural materials on in vitro propagation were evaluated in terms of regeneration. The results showed significant differences among the five sources of . The pineapple juice on MS medium gave the best result with respect to direct regeneration parameters (Fig1).The pineapple juice contains a number of organic acids such as malic acid and citric acid, which are readily neutralized by strong bases and can be titrated against standard bases such as sodium hydroxide (Swati et al 2011)

On Pineapple juice and Coconut milk containing medium, The shoots produced healthy while the roots produced were thick (Table 1)

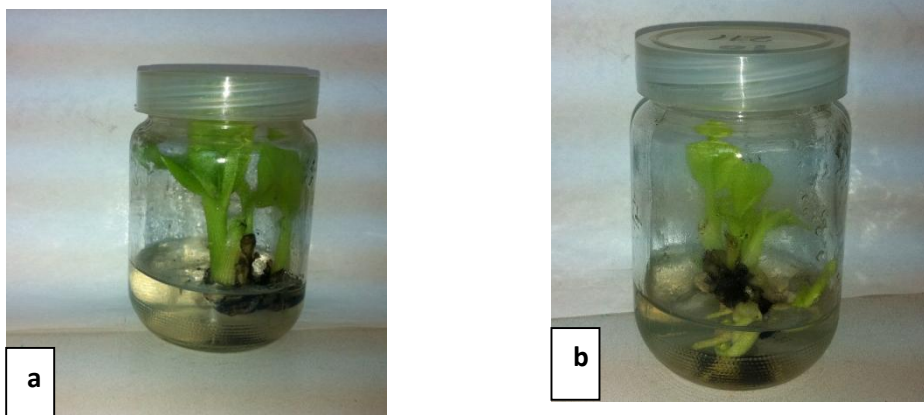


Fig (1): Shoot and roots generation after six weeks using pineapple juice with MS medium
a) shoot elongation b) roots elongation

Soluble Sugars contents

Starch is converted into sugar, through enzymatic breakdown process (Yang and Hoffman, 1984). Starch content declines from 20-30% to 1-2%, but starch amount could be as high as 11% depending on variety. Carbohydrate type in banana is resistant starch and non- starch polysaccharides (Lehmann and Robin, 2007).

Sugar content of the five different treatments was analyzed in Table (2) and the data obtained pointed out that the three types of soluble sugars were influenced by pineapple and coconut milk. Fructose was the major sugar in the five types of treatments, followed by sucrose.

Table (1) shows the effect of various natural materials on Banana cultivar (*Musa spp*) regarding shoot length, shoot number, root number, root length and browning.

Treatments	Con.	Shoot Length	Shoot Number	Root Length	Root Number	Browning
Pineapple juice	5cm	0.667D	2.33BC	4.45A	4.455A	2.00C
	10cm	1.00D	3.00AB	5.67CD	5.500BC	3.33A
	20cm	2.333C	3.667D	7.000B	6.76ABC	2.33BC
Coconut milk	5cm	1.167D	4.333B	3.489AD	5.00ABC	0.000E
	10cm	3.33A	5.333A	4.56D	4.989D	0.000E
	20cm	1.333D	2.333C	6.67BC	6.061B	0.000E
Coffee	1g	2.33AB	3.33A	1.667CDE	3.667A	0.667CD
	2.5g	2.5AB	2.00B	1.33DE	2.00CDE	0.333D
	5g	2.00B	1.667BC	1.00E	1.00E	0.000D
Cocoa Liquor	1g	1.67AB	2.33A	2.33B	1.1667BC	0.667B
	2.5g	1.00B	1.667AB	1.167C	1.00C	1.33AB
	5g	1.33AB	1.00B	0.00D	0.00D	2.33A
Charcoal	1g	2.33AB	1.667A	1.33D	4.00AB	0.667CD
	2.5g	2.00B	2.00B	3.33BC	4.667A	0.333D
	5g	2.5AB	3.33A	2.667C	3.667ABC	0.00D

L.S.D at 0.05% A= 0.4491 B= 0.3889 AB= 0.7778

Table (2) Soluble Sugars contents

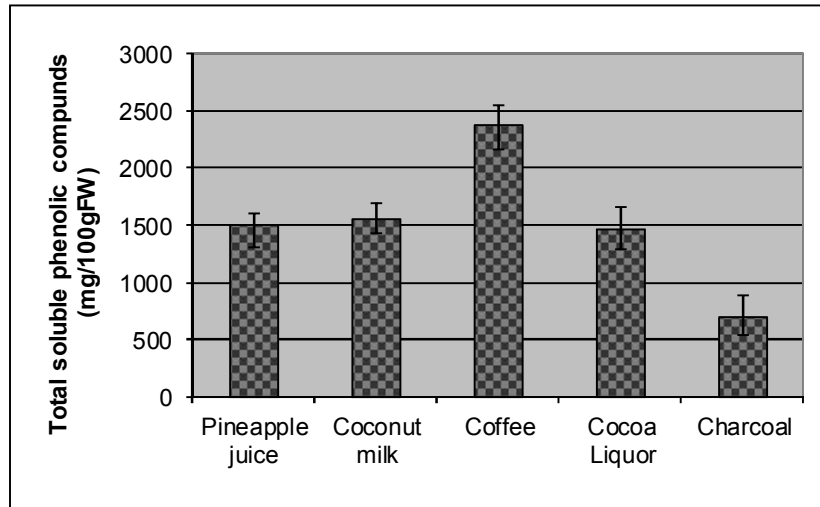
Soluble Sugar (mg/gD.W)	Pineapple juice			Coconut milk			Coffee			Cocoa Liquor			Charcoal		
	5cm	10cm	20cm	5cm	10cm	20cm	1g	2.5g	5g	1g	2.5g	5g	1g	2.5g	5g
Glucose	43.4	43.3	47.5	35.4	37.8	39	19.3	17.8	22	19.8	21.3	23.2	18.8	18.8	20
Fructose	65	68	71.7	60.2	65.3	66.6	44.2	40	47.7	54	54.6	57.2	33	30	31.7
Sucrose	20	20.2	26.8	24.5	27	27.9	18.9	18	23	19.8	22	23.5	10.	11.8	12

Determination of Total Phenolics

The extracted yield of these banana shoots are showed in Figure (2). The phenolic content was grouped into three groups: high (> 2000 mg /100g (fw), moderate (>1400mg/100 g (fw) and low (< 1000 mg /100g (fw).

As it is known, phenolics are synthesized in leaves and then carried to other tissues and organs. Therefore, amounts of total phenolic compounds in leaves are more than the other tissues and organs of the plants (Ozyigit, 2008). Activated charcoal is commonly used in tissue culture media. Its addition to culture medium may promote or inhibit in vitro growth, depending on species and tissues used. The

effects of activated charcoal may be attributed to establishing a darkened environment; adsorption of undesirable/inhibitory substances; adsorption of growth regulators and other organic compounds, or the release of growth promoting substances present in or adsorbed by activated charcoal (Pan and Van, 1998). Generally, the browning and necrosis activities are correlated with the accumulation of excessive phenolics. Interference of phenolics with the process of growth and differentiation is a common phenomenon. Therefore, this study was undertaken to determine the precise role of phenolic compound and to develop a possible link between browning and accumulation of phenolics.

**Fig (2) Total soluble phenolic compounds of banana shoots using different types of natural materials in vitro.****Antioxidant activity**

Bananas should be considered as a good source of natural antioxidants for foods. The antioxidant compounds from bananas were studied and the results showed strong antioxidant activity with cocoa

and coconut milk (Figure3). Therefore, this fact explains the increasing content of antioxidant in the shoots of banana that germinated in MS medium containing the cocoa and coconut milk.

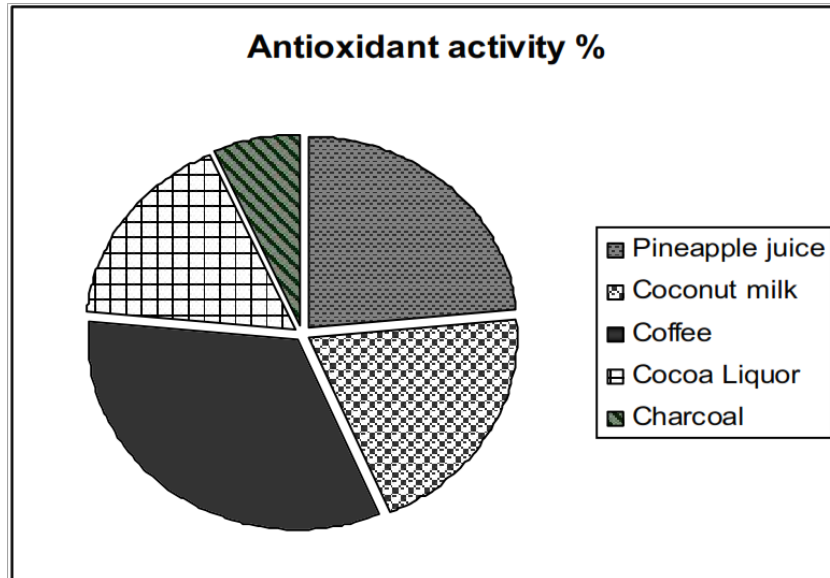


Fig (3) Antioxidant activity% of banana shoots using different types of natural materials in vitro

Relationship between Phenolic Contents and Antioxidant Activity

The results of this study demonstrate a positive correlation between the free radical scavenging activity (antioxidant activity) and the amount of total phenolics in Banana samples. Miller et al.(2000) have measured the total antioxidant activity of 15 fresh fruits and 20 fresh vegetables using DPPH assay. The ranking order of six plant species was as follows: Red Grapes > Garlic > White Grapes > Banana > Red Onion > Cucumber. Many factors could contribute to this variation, such as the plant variety, growing condition, maturity, season, geographic location, fertilizer used, soil type, storage

conditions and amount of sunlight received. Other contributing factor for this difference may be also due to sample preparation and analytical procedures (Al-Farsi, 2000)

Protein content

Belewu and Azeez (2008) recorded that the highest crude protein was found in cocoa powder followed by coconut milk which support the results of this study, where the high percentage of protein (95%) was found in the banana samples germinated in MS medium supplemented with pineapple medium followed by the samples that extracted after adding the coconut milk (76%), as illustrated in Figure 4.

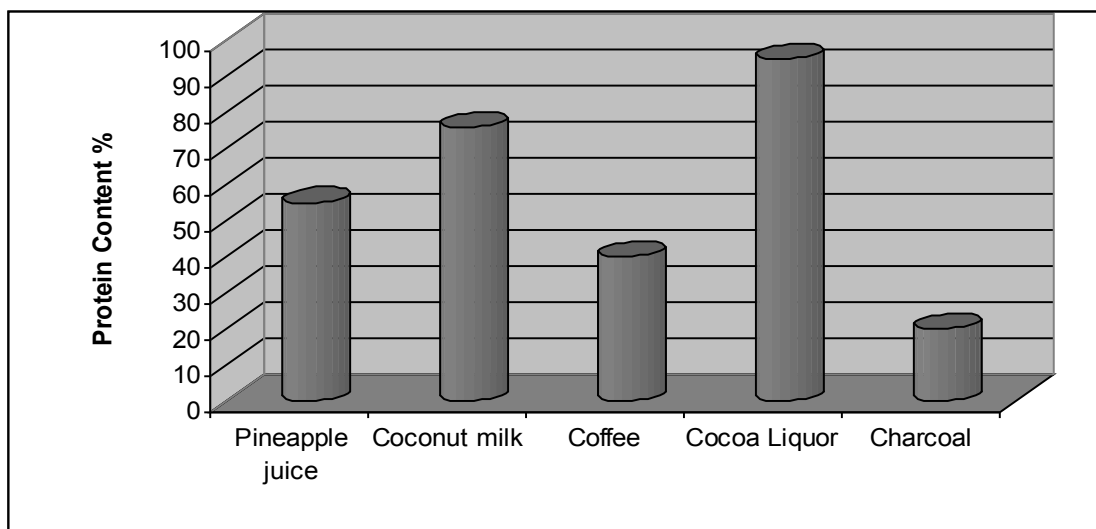


Fig (4) Protein content of banana shoots using different types of natural materials in vitro

Conclusion

Nowadays, Scientists from all over the world tend to use the natural alternative to the chemical plant hormones, it may enhance the plant growth, but its effects on the human health are still unknown, and the excessive use of plant hormones may also harm the plant, for example, according to the North Carolina State University, use of auxin inhibitors near greenhouses should be avoided because the chemical can damage greenhouse plants, and so, using natural materials like used in the study would be no problem for either the plant, as it came from a plant source, or for the human when used as it is totally natural and there is no fear from inducing any kind of allergies or immune response.

This study shows that the use of natural materials such as, Pineapple juice, Coconut milk, Charcoal, Coffee, and Cocoa have effect on tissue culture of banana.

Based on the data acquired from our study; it was remarkable to show how to reach the maximum regeneration of plant with adequate concentrations of Pineapple and coconut milk. Furthermore, we have developed a promising method for an effective plant regeneration system from shoot tips with fewer amounts with phenol content with using charcoal in the Medium

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