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Effect of Carbon Sources and Microelement Concentrations on *in vitro* Proliferation and Rooting of Pineapple (*Ananas comosus*)

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ABSTRACT

The regenerated shoots of Pineapple (*Ananas comosus* Cv. Smooth Coyenne) during the establishment stage were cultured individually on MS medium supplemented with BAP (2.0 mg/L) during the proliferation stage and IBA (2.0 mg) during the rooting stage. Various sugars (glucose, fructose and sucrose) and concentrations (0, 10, 20 and 30 mg/L) were tested. Copper and Boron microelements concentrations were also tested. Data indicated that all sucrose treatments enhanced the proliferation and rooting, however Glucose and fructose improved shoot length. Increasing copper sulfate to (0.05 mg/L) produced the best proliferation. Meanwhile, Boric acid at the rate of 12.4 mg/L was the most suitable concentration for improving rooting percentage.

Keywords: *Ananas cosus*, carbon source, microelements, proliferation.

INTRODUCTION

The Pineapple is a perennial monocot plant having a terminal inflorescence and fruit. The edible portion, that constitutes about 60% of the fresh fruit, has higher contents of proteins, sugars with low percentage of fats and fibers. The fruit is considered as a good source of vitamins A and B as well as bromelain which is a proteolytic enzyme. The world production of pineapple has shown a steady increase over the years. Much of this increase is due to the expansion of pineapple industry in the developing countries of the Far East, Africa, and Latin America. Therefore, the world production of Pineapple exceeds 15,889,647 MT annually as recorded by Faostat (2005). Sucrose is generally regarded as the best carbon source and is universally used as the principal energy source although in certain cases glucose and fructose may be substituted, but most other sugar are poor carbohydrate sources for the plant. The sugar concentration chosen is very dependent on the type and growth stage (Pierik, 1987). The ultimate goal of this investigation is to find out the best source and concentration of sugar and microelements for proliferation and rooting of Pineapple.

MATERIALS AND METHODS

The present study was conducted in the Tissue Culture Laboratory, Department of Horticulture, Faculty of Agriculture, Moshtohor, Zagazig University, during the period from 2005 to 2006.

The regenerated shoots of Pineapple (*Ananas comosus* Cv. Smooth Cayenne) from the establishment stage were cultured individually on Murashige and Skoog medium (1962) as a basal medium supplemented with 2.00 mg/L 6-benzylaminopurine (BAP) during the proliferation stage and 2.00 mg/L indole-3-butyric acid (IBA) during the rooting stage. The pH of the medium was adjusted to 5.7 and autoclaved at 121^{o}C at 15 Ib/in^{2} for 15 minutes. The cultured ex-plants were incubated under 16/8 photoperiod with artificial light (Fluorescent light at 30 UM/hz/Sec) at average temperature of 28-30^{o}C.

Effect of carbon source and concentrations

Different carbon sources at different concentrations were applied to Murashige and Skoog medium to formulate the requirement best shoot development of pineapple plant. The carbon sources glucose, fructose and sucrose were tested at the concentrations (10, 20, and 30 g/L) in comparing to basal medium without sugar (negative control).

Effect of some microelement concentrations

Copper in the form of copper sulphate and Boron in the form of Boric acid, were applied at different concentrations to test the best concentration for better *in vitro* growth of Pineaple plant. During the establishment stage, copper sulphate (0.0375 and 0.05 mg/L.) and Boric acid (9.3 and 12.4 mg/L) were added individually to MS medium and *in vitro* Pineapple growth were recorded under the same growth conditions.

Data and Statistical analysis

Scores were given for necrosis and vitality as follow: Negative results = 1, below average = 2, average = 3, above = 4 and excellent = 5 according to (Pottino, 1981). Also, shoot length (cm), proliferation percent and rooting percent were recorded.

Treatments were arranged in a complete randomized design, each treatment was replicated three times according to (Snedecor and Cochran 1980); each replicate involved 5 jars, each contained a single explant or two shoots developed *in vitro*. The obtained data were statistically analyzed and the means were differentiated according to Duncan Multiple Range Test with 1% level (Duncan, 1955).

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RESULTS

Effect of carbon sources and concentrations

Table 1 and photo 1 show that sucrose gave significantly the maximum proliferation percent and vitality compared to other sugars, however fructose at 30 g/L showed no significant difference. The significant highest shoot length was recorded with 30 g/L Glucose and 20 g/L fructose followed by 30 g/L fructose. However, all sucrose concentrations and fructose at 30 g/L succeeded in reducing necrosis significantly in comparison with other treatments and control.

It is clear from Table 2 and photo 2 that higher concentrations of sucrose (30 g/L) had significant effect on growth parameters under study followed by 20 g/L then 10 g/L as compared with other treatments and control. However, necrosis was not recorded when fructose treatments were used. Meanwhile both sucrose and fructose at the highest concentration (30 g/L) as well as sucrose at the rate of 20 g/L gave the maximum rooting percentage. The lowest growth & rooting % and vitality were obtained from the lower concentration of glucose and the control. Concerning vitality, the maximum result was recorded at 20 and 30 g/L fructose followed by 20 and 30 g/L sucrose as well as 30 g/L glucose and 10 g/L fructose.

Effect of some microelement concentrations

The results (Table 3 and Photo 3) showed significant decrease in necrosis, and increase in shoot length with increasing Boric acid concentration and copper sulphate to 2 folds (12.4 mg/L and 0.05 mg/L) respectively. Meanwhile, both shoot length and proliferation were significantly increased when copper sulfate (0.05 mg/L) was used followed by copper sulfate (0.0375 mg/L) then

Table (1): Effect of carbon source concentrations on growth and proliferation of Pine apple.

Treatments	Necrosis	Shoot length	Proliferation %	vitality
Control	3.67 ^{A}	1.60 ^{E}	40.67 ^{F}	2.67 ^{D}
Glucose **(a)**	3.00 ^{B}	1.93 ^{E}	53.63 ^{E}	3.00 ^{C}
Glucose **(b)**	2.00 ^{C}	2.00 ^{D}	66.67 ^{D}	3.00 ^{C}
Glucose **(c)**	2.00 ^{C}	3.67 ^{A}	80.00 ^{BC}	3.67 ^{B}
Fructose **(a)**	3.00 ^{B}	2.00 ^{D}	73.36 ^{CD}	3.33 ^{B}
Fructose **(b)**	2.00 ^{C}	3.60 ^{A}	88.67 ^{B}	3.67 ^{B}
Fructose **(c)**	1.00 ^{D}	3.30 ^{B}	96.00 ^{A}	4.82 ^{A}
Sucrose **(a)**	1.67 ^{CD}	2.36 ^{C}	98.00 ^{A}	4.63 ^{A}
Sucrose **(b)**	1.00 ^{D}	2.73 ^{C}	99.00 ^{A}	4.67 ^{A}
Sucrose **(c)**	1.00 ^{D}	2.42 ^{C}	100.00 ^{A}	5.00 ^{A}

Means followed by the same letter are not significantly different from each other at 1% level. **(a):** 10 g/L, **(b):** 20 g/L, and **(c):** 30 g/L.

Table (2): Effect of carbon source concentrations on growth and root formation of Pineapple.

Treatments	Necrosis	Growth %	Rooting %	vitality
Control	3.67 ^{A}	29.30 ^{F}	53.33 ^{E}	2.82 ^{D}
Glucose **(a)**	2.00 ^{C}	33.63 ^{D}	66.76 ^{D}	3.00 ^{C}
Glucose **(b)**	2.33 ^{B}	43.33 ^{D}	76.33 ^{CD}	3.00 ^{C}
Glucose **(c)**	2.33 ^{B}	53.33 ^{C}	88.67 ^{B}	3.33 ^{B}
Fructose **(a)**	3.00 ^{B}	33.33 ^{E}	80.00 ^{BC}	3.67 ^{B}
Fructose **(b)**	2.00 ^{C}	43.33 ^{D}	88.00 ^{B}	4.33 ^{A}
Fructose **(c)**	1.00 ^{F}	53.33 ^{C}	100.00 ^{A}	4.67 ^{A}
Sucrose **(a)**	1.33 ^{F}	66.67 ^{B}	90.00 ^{B}	3.00 ^{C}
Sucrose **(b)**	1.33 ^{E}	73.33 ^{AB}	100.00 ^{A}	3.33 ^{B}
Sucrose **(c)**	1.67 ^{D}	80.00 ^{A}	96.00 ^{A}	3.67 ^{B}

Means followed by the same letter are not significantly different from each other at 1 % level. **(a):** 10 g/L, **(b):** 20 g/L, and **(c):** 30 g/L.

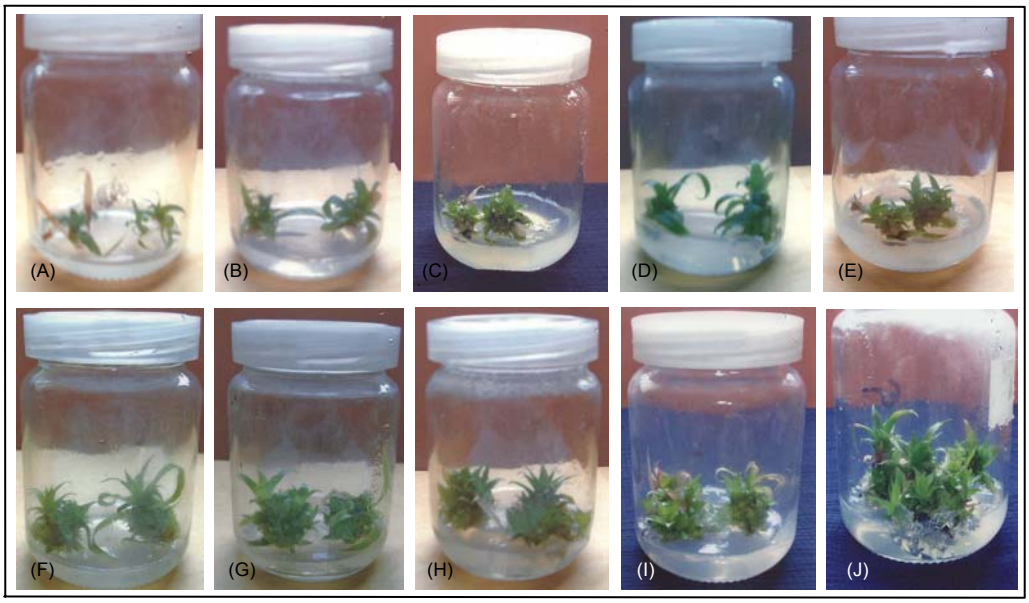

Photo (1): Effect of carbon source concentrations on growth and proliferation of Pineapple; **(A)** Control (No carbon source was added), **(B)** 10 g/L glucose, (**C**) 20 g/L glucose, (**D**) 30 g/L glucose, **(E)** 10 g/L fructose, (**F**) 20 g/L fructose, (**G**) 30 g/L fructose, (**H**) 10 g/L sucrose, (**I**) 20 g/L sucrose, and (**J**) 30 g/L sucrose**.**

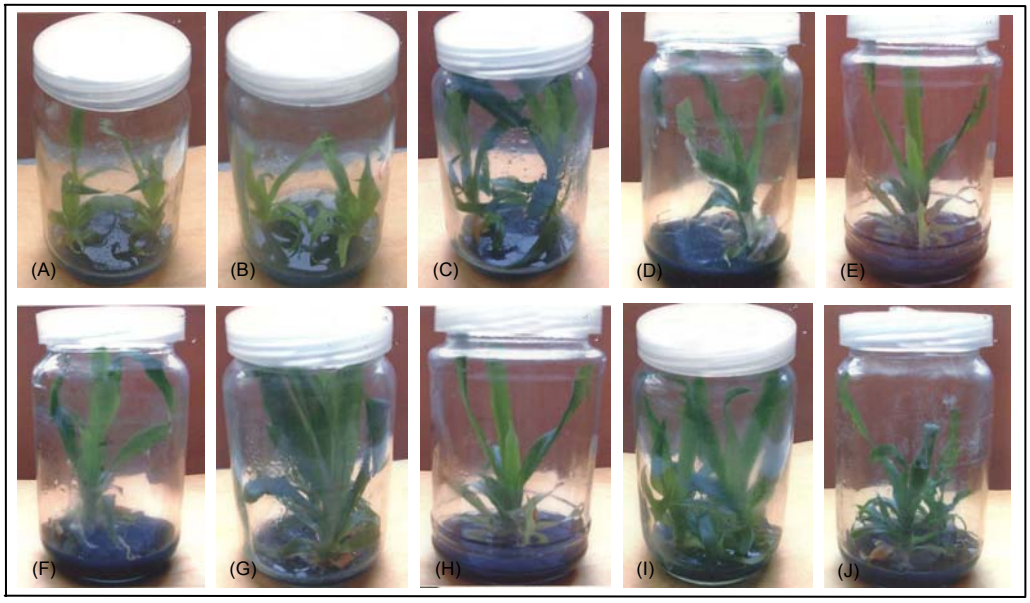

Photo (2): Effect of carbon source concentrations on growth stage and root formation of Pineapple; **(A)** Control (No carbon source), **(B)** 10 g/L glucose, **(C)** 20 g/L glucose, **(D)** 30 g/L glucose, **(E)** 10 g/L fructose, **(F)** 20 g/L fructose, **(G)** 30 g/L fructose, **(H)** 10 g/L sucrose, **(I)** 20 g/L sucrose, and **(J)** 30 g/L sucrose.

boric acid (12.4 mg/L) as compared with low Boric acid concentration (9.3 mg/L) and the control. However, increasing the concentrations of copper sulfate to 2 folds (0.05 mg/L) significantly increased vitality as compared with the control and treatments. Increasing Boric acid to 1.5 and 2.0 folds (9.3 mg/L and 12.4 mg/L) as well as copper sulfate to 2.0 folds (0.05 mg/L) significantly reduced necrosis whilst significantly increased rooting percentage as compared with other treatments and the control. In contrast; higher concentrations of copper sulfate (0.05 mg/L) had the best significant effect on growth and greening.

DISCUSSION

Sucrose at different concentration enhanced the proliferation and rooting, and decreased necrosis of pineapple plant. Glucose and fructose surpassed others in improving shoot length. This may be due to that sucrose is generally regarded as the best carbon source in plant tissue culture and is universally used as the principal energy source. In certain cases glucose and fructose may be substituted, but most other sugars are poor energy sources for the plant. These results are in coordination with the findings of Baaya (2002) and Abd El-Gawad (2006). They found that the higher proliferation and rooting rates for Pineapple were obtained when MS medium was supplemented with sucrose at 30 g/L. In addition, Duong *et al.* (2006) stated that as a single carbohydrate source in medium, fructose exhibited a better growth of cell of *Taxus wallichiana* when compared with sucrose or glucose. In case of the combination of two hexoses (glucose and fructose) at different concentrations, the best proliferation of cell was obtained at the combination of 30 g/L glucose and 30 g/L fructose.

However increasing copper sulfate to 2 folds (0.05 mg/L) is important for growth and proliferation. On the other hand, Boric acid at the rate of 12.4 mg/L is the most suitable concentration for improving rooting for

Table (3): Effect of Boric acid and copper sulphate levels on growth and proliferation of Pineapple.

Treatments	Necrosis	Shoot length (cm)	Proliferation %	Greening
Control (0.0258 Cu So_4 + 6.2 H3 Bo4 mg/L)	2.00^{A}	1.67^{E}	60.00^{E}	2.61^{C}
Boric acid 9.3 mg/L	2.01^{A}	2.02^{D}	66.67^{D}	2.68^{C}
Boric acid 12.4 mg/L	1.00^{B}	2.63^{C}	73.33^{C}	3.63^{B}
Cu So_4 0.0375 mg/L	2.00^{A}	3.02^{B}	86.67^{B}	3.67^{B}
Cu So_4 0.0500 mg/L	1.00^{B}	3.61^{A}	95.33^{A}	5.00^{A}

Means followed by the same letter are not significantly different from each other at 1% level.

Table (4): Effect of Boric acid and copper sulphate levels on growth and root formation of Pineapple.

Treatments	Necrosis	Growth %	Rooting %	Vitality
Control (0.0258 Cu So_4 + 6.2 H3 Bo4 mg/L)	4.17^{A}	73.37^{D}	60.33^{BC}	1.60^{D}
Boric acid 9.3 mg/L	1.00^{C}	73.37^{D}	86.67^{AB}	2.00^{C}
Boric acid 12.4 mg/L	1.00^{C}	82.00^{C}	93.33^{A}	2.17^{C}
Cu So_4 0.0375 mg/L	3.27^{B}	91.00^{B}	60.00^{BC}	3.67^{B}
Cu So_4 0.0500 mg/L	1.33^{C}	100.00^{A}	73.33^{B}	4.83^{A}

Means followed by the same letter are not significantly different from each other at 1% level.

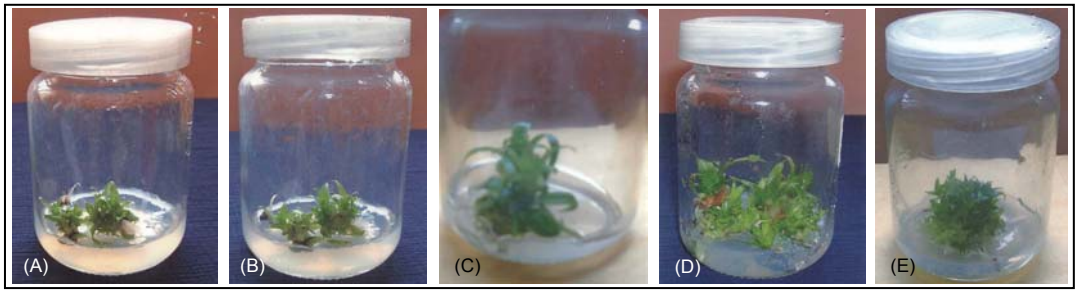

Photo (3): Effect of Boric acid and copper sulphate on growth and proliferation of Pineapple**; (A)** Control (0.0258 Cu So_4 + 6.2 H_3Bo_4 mg/L), **(B)** 0.0258 $CuSo_4$ + 9.3 H_3Bo_4 mg/L, **(C)** 0.0258 Cu So_4 + 12.4 H_3Bo_4 mg/L, **(D)** 0.0375 Cu So_4 + 6.2 H_3Bo_4 mg/L, and **(E)** 0.0500 Cu So_4 + 6.2 H_3Bo_4 mg/L.

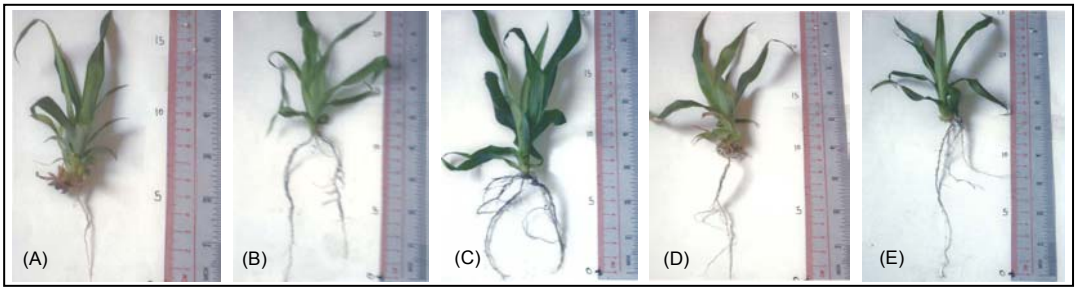

Photo (4): Effect of Boric acid and copper sulphate on growth and root-formation of Pineapple; **(A)** Control (0.0258 $CuSO_4$ + 6.2 H_3Bo_4 mg/L), **(B)** 0.0258 $CuSo_4$ + 9.3 H_3Bo_4 mg/L, **(C)** 0.0258 $CuSo_4$ + 12.4 H_3Bo_4 mg/L, **(D)** 0.0375 $CuSo_4$ + 6.2 H_3Bo_4 mg/L, and **(E)** 0.0500 $CuSo_4$ + 6.2 H_3Bo_4 mg/L.

in vitro grown pineapple percentage. These results coordinate with the findings of Marschner (1995) who said that the polyphenoloxidase activey was much lower in copper deficient plants, but the activities of IAA oxidase and peroxidase were also lower. Meanwhile, Kintzios *et al*. (2001) found that, adding Copperat a ten fold as the standard MS concentration is favored for somatic embryogenesis of chili pepper leaves.

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تأثير كل من مصادر الكربون وبعض العناصر الصغرى على الزيادة العددية والتجذير فى الأناناس معملياً

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الملخص العربـــى

أجرى هذا البحث بمعمل زراعة الأنسجة، قسم البساتين، بكلية الزراعة، جامعة الزقازيق بمشتهر خلال الفترة من ٢٠٠٥ إلى ٢٠٠٦ بهدف دراسة تأثير أنواع مختلفة من السكريات كمصدر للكربون وبعض العناصر الصغرى على الزيادة الخضرية والتجذير في الأناناس فى مزارع الأنسجة، وقد تم فصل القمة النامية من النموات الصغيرة للأناناس (صنف سيموس كابين) حيث زرعت على بيئة موراشيج وسكوج الصلبة المضاف إليها ٢ ملليجرام/لتر و٢ أيزو بينزيل أمينوبيورين في مرحلة الزيادة العددية و٢ ملليجرام/لتر من هرمون إندول ٣ حمض البيوتريك في مرحلة التجذير، وقد اختيرت ثلاثة أنواع من السكريات (السكروز، الجلوكوز، الفركتوز) بتركيزات مختلفة (١٠، ٢٠، ٣٠ ملليجرام/لتر). كما اختيرت مركبات العناصر الصغرى (سلفات النحاس وحمض البوريك) بتركيزات مختلفة (١، ١,٥، ٢) من تركيزهم داخل بيئة زراعة الأنسجة.

وقد أثبتت النتائج تفوق السكروز عن نظيره من الجلوكوز والفركتوز، فقد أعطت جميع تركيزات السكروز أعلى نسبة زيادة خضرية وتجذير. كما وجد أن زيادة تركيز سلفات النحاس إلى الضعف تؤدى إلى أعلى نسبة زيادة خضرية فى حين أن زيادة حمض البوريك إلى الضعف أعطت أعلى نسبة تجذير.