A comparative Study On Different Carbon Source Concentrations And Gelling A Gent On In Vitro Proliferation Of Pineapple (Ananas colossus)

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Abstract: The shoots regenerated from shoot tip of Pineapple Ananas Comosus Cv. Smooth cayenne) plantlets from the establishment stage were cultured individually on Ms medium supplemented with 200 mg/L 6- benzylamin opurine (BAP). Sucrose, fructose and mannitole with concentrations (20, 30 and 40 gl/L) were tested. Various kinds of gelling agent i.e. Agar and Gerlited were tested. Data indicated that all sucrose treatment (20, 30 and 40 g/L) enhanced the proliferation percentage and shoot number compared with other treatment and control except treatment of mannitol at 40g/L improved the shoot length only. Moreover, agarasa gelling agent was better than Gelrite at proliferation stage. The best shoot length, shoot number and growth percentage were obtained when 2.0 mgL Gelrite was added to the medium. [Nature and Science. 2010;8(5):127-130]. (ISSN: 1545-0740).

Key words: Carbon Source-gelling-pineapple

1. Introduction

Pineapple (Ananas Comosus) isatroical fruit crop which originated in south America, then was transferred to many countries. In Egypt, Pineapple is a promising new crop to be grown in plastic green house, particularly, in the newly reclaimed land, pineapple can not be propageated by seeds because most varieties show strong selfin comp atibility white the others is complete parthenocarpy. Also. Buds (which are produced limited number per plant) may beused in propagation (Wakasa 1989). Sorbitol or mannitol in combination with sucrose was found beneficial for inducing differentiation in long term culture. 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 30g/L fructose (Duong et al 2006). Gelrite as an alternative gelling a gent is clearly non toxic but results in hyperhydric (vitrified) tissues.

Inan effort to overcome these problems, the controlling mechanism found in agar was examines. Hydric soluble was shown to be affected by a non – gelling, cold water soluble constituent of a commercial agar, rather than by physical properties of the gel (**Nairn et al 1995).** This study was determine the effect of different carbon sucrose concentrations and gelling agent on in vitro proliferation of pineapple plant lets.

2 - Materials and Methods

This study was carried out at the tissue culture laboratory of pamology Dept., National research Center during the period from 2008 to 2009.

Shoot tip of pineapple (Ananas Comosus Cv. Smooth Cayenne) plantlets from the establishment stage were cultured individually on Murashige and skoog medium (1962) as a basal medium supplemented with 2.00 mg/L 6- Benzylamin opurine (BAP), during the proliferation stage. The pH of the media was adjusted to 5.7 and outclaved at 121 ^{0}C and 151 b/ In^2 for 15 minutes. The culture explants were incubated under 16 hours of artificial light (Fluorescent light at 30 uM/ sec) and 8 hours of darkness at average temperature of 28 ± 2^{0}C. Thus, the following experiments were carried out.

1- Effect of carbon source and its concentration

Sucrose, fructose and mannitol were added at 20, 30 and 40 g/L Ms medium supplemented with BAP and 2.0 mg/L. shoot number , shoot length, proliferation percentage and leaf number parameters were determined.

2- Effect of type of gelling agents

Agar at 7.0 g/L or Gelrite at 2.0g/L were used in Ms medium supplemented with BAP at 2.0 mg/L. Necrosis, shootnumber, shoot length , proliferation percentage, growth percentage, leaf number, and greening were determined.

Data and Calculations

Scores were given for necrosis and greening as follows: Negative results =1 , below average = 2, average = 3, above average = 4 and excellent =5 according to pottino (1981) .

Also, shoot number (numbers), shoot length (cm), proliferation as percentage, growth (as

percentage) and number of leaves /plantlet.
Treatment were arranged in a completely randomized design , each treatment was replicated three times according to snedecrodand and cochran (1980), each replicate involved 5 Jars, Pach contained a single explant. The obtained data were statistically analyzed and the means were differentiated according to Duncan multiple range test 1% level (**Duncan, 1955).**

RESULT AND DISCUSSION

1- Effect of carbon source and its concentration

Table (1) and Photo (1) show the effect of carbon source concentrations and growth and proliferation percentage of pineapple plantlets after 6 weeks. It is clear that all sucrose treatment (20, 30 and 40 g/L) gavev the maximum proliferation percentage and shoot number as compared with the other treatment and the control. However, mannitol gave significantly the highest leaf number followed by 40g/L fructose as compared with the other carbon sucrose concentrations and the control. Meanwhile, supplementation of the culture medium with mannitol at 40g/L level maximized shoot length in comparison to the other carbon source concentrations and the control under study. In general, summarizing the above results indicated that all sucrose treatments (20,30 and 40g/L) enhanced the proliferation percentage ad shoot number compared with other treatment and control except treatment of mannitol at 40g/L improved the shoot length only. In addition, both (30, and 40 g/L) sucrose surpassed others improving leaf number. This may be due to sucrose is geneally 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 sugars are poor carbohydrate sources for the plant. These results are in coordination with the finding of Duong et al (2006) and Khafagy (2007 on Grand Naine Banana, they found that as a signle carbohydrate source in medium fructose exhibited a better growth 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 30g/L fructose.

2- Effect of gelling agent:

Data in table (1) and Photo (1) shoe the effect of using (celrite of agar on parameters of pineapple shoots at multiplication stage. It is obvious that using Gelrite was significantly more superior than using agar in increasing shoot number, shoot length, greening and growth percentage and decreasing necrosis. Meanwhile, suing agar was more effective in increasing proliferation percentage as compared with Gelrite. On the other han, statistical differences were nil between Gelrite and agar when leaf number parameter was considered.

Generally, the above results can recommended that Gelrite gave the highest shoot length, shoot number and growth percentage in pineapple plantlets. Meanwhile, agars as a gelling agent was better than Gelrite at proliferation stage. These results agree with the findings of Arrequi et al (2003) found that tuberizalion was higher when phytogel(TM) was used rather than Difco Bacto agar for all cultivars. Also Taha (2009) who found that Gelrite gave the highest average shoot number and shoot length compared with agar.

Table (1) : Effect of carbon sucrose concentration on growth and proliferation of pineapple plantlets after 6 weeks.

Measurement	Shoot number	Shoot length	Proliferation (%)	Leaf number
Control	$26.00^{\pm}$	10.33^{b}	40.33^{f}	4.92C
20 g/L sucrose	29.76^{C}	11.36^{ab}	97.63^{a}	3.67E
30 g/L sucrose	31.00^{b}	11.67^{ab}	99.00^{a}	4.30C
40 g/L sucrose	32.00^{a}	11.33^{ab}	100.00^{a}	4.30C
20 g/L fructose	25356^{f}	11.00^{ab}	73.30^{cb}	4.00D
30 g/L fructose	25.00^{g}	10.36^{b}	87.33^{b}	4.67C
40 g/L fructose	24.00^{h}	10.67^{b}	89.63^{b}	5.20B
20 g/L Mannitol	26.67^{f}	11.67^{ab}	54.67^{e}	5.67B
30 g/L Mannitol	26.99^{e}	11.67^{ab}	66.33^{d}	6.30A
40 g/L Mannitol	29.0^{d}	12.63^{a}	81.36^{b}	6.32A

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

Photo (1): Effect of carbon source concentrations on grow th and proliferation of pineapple plantlets after 6 weeks.
A = 30 g/L sucrose
B = 30 g/L fructose
C = 30 g/L mannitol

Table (2): Effect of gelling agent on growth and proliferation of pineapple plantlets after 6 weeks

Measurement	Necrosis (secores)	Shoot number (N)	Shoot length (cm)	Proliferation (%)	Growth (%)	Leaf number (N)	Greening (secores)
Gelrite	1.00A	6.50A	1.37A	86.73B	88.33A	4.33A	5.00A
Agar	1.33B	4.36B	0.89B	99.80A	73.00B	4.00A	3.67B

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

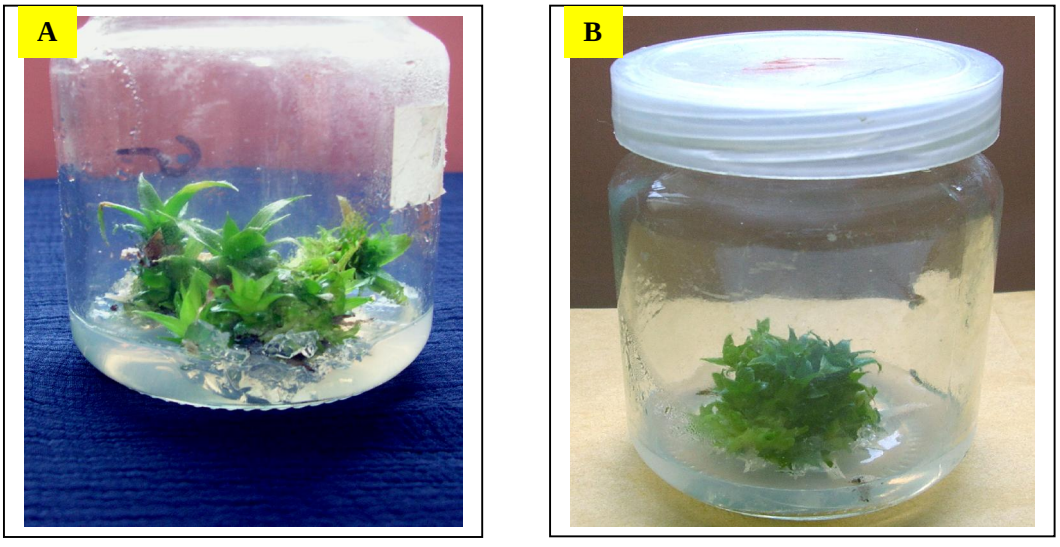

Photo (2): Effect of gelling agent on growth and proliferation of pineapple plantlets after 6 weeks.
A = 2g/L Gelrite.
B = 7 g /L Agar.

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