Preliminary study of the response of some cultivars of Sugarcane (*Saccharum* spp.) to In vitro culture

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Abstract

Shoot tips of sugar cane (*Saccharum* spp.) CVS. “Measan” , “CP-70 -1133” , “ MY 5465” , “CO 845” and “E150” were cultured on MS (Murashige & Skoog) medium. The results showed that the response to growth was directed toward vegetative enhancement.

Contamination appeared on the explants of cv “E150” after the first, second and third weeks was at percentages 16.6%, 33.3% , 50% respectively and contamination percentages of explants belong to cultivars “CP-70-1133” , “MY5465” and “C0845” were 28.6% , 57.2% , 100% respectively.

دراسة اولية لاستجابة بعض اصناف قصب السكر للزراعة خارج الجسم الحي

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الخلاصة

زرعـت اطـراف السـيقان لنبـات قصـب السـكر (*Saccharum* spp.) مـن اصـناف "ميسـان" "CP-70-1133" , "MY5465" , "CO845" , "E150" علـى وسـط مـوراشـيكي وسـكوك ، اظهـرت النتـائج ان الاسـتجابة للنمـو كانـت باتجـاه تعزيـز النمـو الخضري لصنفي CP-70-1133، ميسان حيث تميزت الفروع الخضرية ، ظهور التشـوب الجرثـومي على النسيج الاصلي للصنف "E150" في الاسبوع الاول والثاني والثالث بالنسـب المئويـة 16.6% ، 33.3% ، 50% على التوالي ، بينمـا كانـت نسـب التشـوب الجرثـومي للاصـناف , :CO845" "CP70-1133" , "MY5465" هي: 28.6% و 57.22% و 100% على التوالي.

Introduction

Sugarcane *Saccharum officinarum* is an important yield crop. It is a source of table sugar which has an expensive price among food materials.

Iraq is one of the countries interested in the cultivation of sugarcane for industrial purposes. Somatic embryogenesis and organogenesis are commonly used in the propagation of sugarcane *In vitro* [Heinz & Mee, 1969]. Falco *et al* (1996) reported that sugarcane regeneration occurred from nodular callus tissues through the formation of somatic embryos or adventitious shoots.

Somatic embryogenesis was reported to occur from young leaves , apical meristem [Ahloowlia & Maretzki, 1983] and immature inflorescences [Liu, 1993]. The procedures of disinfestations and contamination rates of sugarcane explants in tissue culture were reported. [Heinz & Mee, 1969] and Salazar & Surga, 1988].

Al-Jibouri *et al*. (2002) reported that (0.5) mg/L is the best level for the vegetative propagation of sugarcane (*Saccharum officinarum* L.) cvs. CO-J-86, Co-J-64 and Measan. The aim of this study is to recognize the response of *Saccharum* spp. To *In vitro* culture.

Materials & Methods

1- Collection of Specimens:

The plants of sugarcane cvs. “Mesan” “CP-70-1133” , “MY 5465” , “CO 845” , and “E150” , were collected from agricultural field of General company for canesugar industry.

2- Media preparation :-

MS (Murashige & Skoog, 1962) mineral salts are supplemented with the following materials combinations :-

[A] (NAA) Naphthalene acetic acid 2mg/L ; Gibbrellic acid (GA) 2mg/L ; (BA) Benzyl adenine 2mg/L ; Thiamine - HC1 0.5 mg/L , Meso – Inositol 100 mg/L , NaH_2 PO_4-$2H_2O$ 170mg/L; Sucrose 30 gm/L Agar 8gm/L ; Activated charcoal 3gm/L.

[B] NAA Img/L GA3 2mg/L , Kinetin l0mg/L , Thiamine-HC1 2mg/L, Meso-lnositol 100 mg/L, NaH_2 PO_4-$2H_2O$ 170 mg/L , Sucrose 30 gm/L , Agar 4gm/L .

[C] NAA 120 mg/L, Kinetin 3mg/L , BA 3mg/L, Thiamine-HCl 0.5mg/L , Meso-lnositol 100 mg/L, NaH_2 PO_4-$2H_2O$ 170 mg/L , Sucrose 30 gm/L , Agar 8gm/L , Activated charcoal 3 gm/L .

[D] 2,4-D (2,4 Dichlorophenoxy acetic acid 50mg/L, Kinetin , 2mg/L , Thiamine-HCl Img/L , Meso-lnositol 100 mg/L , NaH_2 PO_4-$2H_2O$ 170 mg/L, Sucrose, 20 gm/L , Agar .8gm/L and Activated charcoal 3gm/L .

pH of the medium was adjusted at 5.7 before addition of Agar and activated charcoal except in conbination [B] where pH was adjusted at 5.4 . After dispensing of media in flasks and tubes , they were autoclaved at 121C for 20 minutes .

3- Excision and sterilization :-

Shoots of sugarcane cv "Measan" were dissected and shoot tips were excised and kept in antioxidant (Citric acid 150mg/L + Ascorbic acid 100 mg/) for 1.5 hr. , Then they were sterilized in 20% (v:v) commercial disinfectant (NaOCl 6%) for 20min After washing the tips with sterilized distilled water three times , they were cultured , on medium of combination [C] . Tow months-period after culturing shoot tip of cv. "Measan" was subcultured on the same medium [C].

Sugarcane shoot tips cvs. "CP 70-1133 " , "C0845" and "MY 5465" shoot tips were excised at 5^{th} and 6^{th} intenodes of stalk, they were sterilized with 95% ethyl alcohol for 0.5 min. followed by disinfectant with 20% (v:v) (NaOCl 6%) for 4-10 minutes, washing by distilled water three times and cultured on medium of combination [D] . Soot tip of "CP-1133" was subcultured firstly after 8 weeks on medium [A] then was subcultured on medium [B] for nearly more than 3 months period.

Shoots of sugarcan cv. "E150" were dissected by removing the leaves up to shoot tip that was excised , trimmed and sterilized by 20% (v:v) (NaOCl 6%)

After washing the sterilized shoot tip with sterilized distilled water three times , they were cultured on medium of combination [D].

Results & Discussion

1- Contamination

Table (I) showed that contamination percentages of sugarcane cv. "El50" explants after: first, second and third weeks were 16.6% , 33.3% and 50% respectively , whereas the percentages of browning were 16.6% 50% and 50% respectively .

Contamination percentages of explants of cultivars :CP70-1133" , "MY5465" and "CO845" were 28.6% , 57.2% and 100% respectively [Table II]

Table (I) : The contamination and browning percentages of sugarcane explants of cv. "E150" after the 1st , 2nd and 3rd weeks.

Time period	1st week	2nd week	3rd week
Total number of explans	12	12	12
Number of contaminated explants	2	4	6
Number of browning explante	2	6	6
%contamination	16.6%	33.3%	50%
% Browning	16.6	50%	50%

Table (II) : The contamination percentages of sugarcane explants cvs "CP 70-1133" ; "MY 5465" ; "CO 845" .

Cultivar	CP.70-1133	MY 5465	CO 845
Total number of explans	7	7	7
Number of contaminated explants	2	4	7
% contamination	28.6%	57.2%	100%

The increase of contamination at the end of third week may be due to different kinds of microorganisms found in the explants. The difference may be due to the inherited resistance specific to variety; the field conditions also contribute to infection intensity , however , Salazar and Surga (1988) found that explants collected in rainy season showed higher contamination than those collected in dry season and this contamination may be due to the size of explant ; Salazar and Surga (1988) also , found that the larger explant showed higher contamination , however , some authors used different treatments in sterilization of sugarcane explants , Heinz and Mee (1969) used 95% ethyl alcohol then phenyl mercuric acetate and clorox or ethyl alcohol, then wescodyne (detergent) . Salazar and Surga (1988) , obtained the best results when the explants were immersed in 50°C water bath for 60 min then into 10%NaClO solution.

3-Response of tissue to growth :-

Shoot tip of sugarcane cultivar "CP70-1133" showed the development of shoot after repeated subculture on the medium combination [A] (Fig.-l). Another shoot tip gave rise shoot [Fig. 2], after separartion of this shoot from shoot tip that gave rise another shoot on the medium combination [B] [Fig.3].

The enhancement of the growth of shoots from axillary buds is usually due to high level of cytokinin and low level of auxins in addition to the presence of gibbrellin.

Hartmann & Kester (1984) stated that the lateral growth below the apical meristem are stimulated to grow if the apical meristem is inhibited or removed.

Sugarcane culture cv. "Measan" showed the growth of shoot tip on the medium combination C [Fig.-4] which resulted in the differentiation of shoots [Fig.-5] .

High level of cytokinin in 3mg/L. Benzyl adenine + 3mg/L of kinetin may be the cause of the appearance of the shoot growth .

This result is supported by Sachs & Thiamann (1964) who stated that the apical dominance is controlled by the interaction of growth regulators , the effect of cytokinin to stimulate axillary bud to grow is temporary as the effect of exogenous growth regulators diminishes.

Fig 1- Development of apical of sugarcane explant cv "CP70-1133"

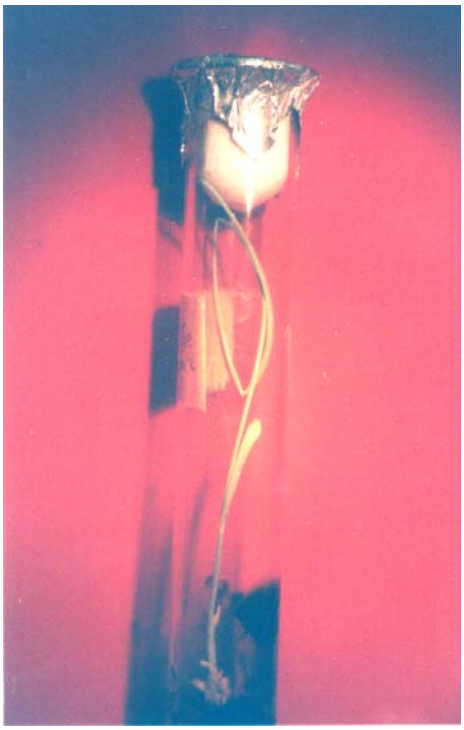

Fig 2-Shoots differentiated from subcultured shoot tip segment of sugarcane cv. "CP70-1133"

Fig 3- Shoot differentiated from sugarcane shoot tip cv. "CP70-1133".

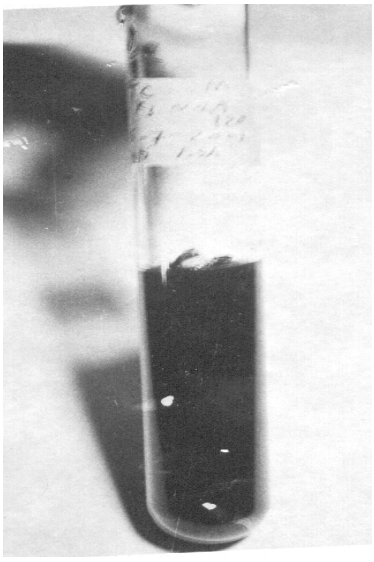

Fig 4- Grwth of sugarcan cv. "Mesan" shoot tip.

Fig 5- Differentiated shoot from sugarcane shoot tip cv. "Mesan".

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