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Reduction of Exudates (Browning) in Sugarcane Micro Propagation

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Abstract:
Ex plant preparation in the course of sugarcane micro propagation is known to release exudates which lead to the death of sugarcane *in vitro*. An industrial sugarcane variety (NCS008) was used for the study and was observed to die in less than 14 days *in vitro.* In a study to reduce the death of this variety, ascorbic acid and citric acid was added as constituent of the media using MS and stock at different concentrations. The browning was reduced drastically at the addition of 0.1g/litre ascorbic acid and 0.15g/litre citric acid.

Key words: reduction, exudates browning, micro propagation, sugarcane.

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Introduction

The improvement, development and sustainability of our sugarcane varieties have been widely recognized as the basic raw material of the sugar industries. This is as a result of the necessity to meet the need for the diverse use of sugar which has presently been in high demand and currently not meeting its economic and social demand. Sugarcane (Saccharum officinarum) is one of the most preferred crops for sugar production, but the sustainable supply of adequate quantity of good quality sugarcane seedlings that can cover tens of thousands of hectares of land required for industrial sugar production remains a major constraint to sugarcane-based production programs in Nigeria. The biotechnology method of plant tissue culture has been demonstrated as a powerful tool that can guarantee the propagation of millions of disease-free seedlings of the required varieties of sugarcane within a short period.

In trying to meet the demand of the products of this economically important grass, scientists have therefore resulted to the use of biotechnology to mass produce this plant. One of the problems encountered is browning as a result of exudates from wounds in the course of ex-plant preparation. This paper discussed how the browning can be controlled and rapidly produce shoot from explants *in vitro*.

Materials and Method

The newest industrial sugarcane variety developed by NCRI (NCS008) was used for this study. MS media was first prepared in the biotechnology laboratory of the institute using the following concentration (M.S:4.3g/litre, Sucrose: 30g/litre, Myoinositol: 0.1g/litre, Vitamin: 5mls/litre, Agar: 7g/litre, pH: 5.8 and Autoclaved at 121°C for 15min at 15psi). Ascorbic acid of 0.1g/litre was added into the media being prepared. This was repeated with the addition of Ascorbic and Citric acid.

Another media was prepared with the use of stock solutions having the same constituents with the addition of ascorbic acid, citric acid and ascorbic acid differently at different concentrations. The explants were prepared accordingly with the use of 70%ethanol, tween 20, Sodium hypochlorite and distilled water after which it was initiated into different media. Initiation was done under the laminar air flow hood where the explants were excised and prepared using sterile forceps, surgical blade and scalpels to get incisions close to the meristem tip. After

initiation, culture vessels (vials) were parafilmed and labeled accordingly. The cultures were grown in the culture room (growth room) at 25±2^{0}c at 1500-2000 flux.

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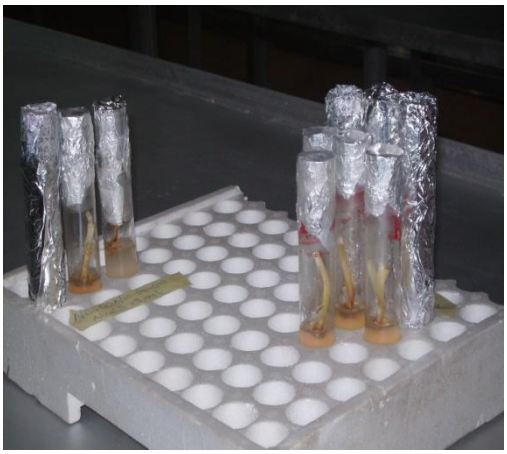
5 

Plates 1: media preparation 2: Explants harvest 3: Explants preparation (surface sterilization) 4: Aseptic manipulation (inside the flow hood) 5: culture room

Results

The cultures started releasing exudates immediately they were initiated for media that contained only ascorbic acid or citric acid. The control experiment having only the MS and Stock media also had the same problem. Those that had additions of both ascorbic and citric acid contained the exudates. The tables below show how the cultures performed.

Table 1. Showing Performance of Citric Acid Addition Into Media Within The First 2weeks of Initiation (10 Explants Per Concentration Column).

DAY	(CITRIC ACID + M.S MEDIA) NO OF CULTURES THAT BROWNED (g/litre)					(CITRIC ACID + STOCK SOLUTION) NO OF CULTURES THAT BROWNED (g/litre)				
	0.13	0.14	0.15	0.16	0.17	0.13	0.14	0.15	0.16	0.17
1	2	-	-	-	6	3	10	-	-	-
2	-	4	-	3	2	-	-	-	6	-
3	-	-	-	-	-	7	-	-	-	8
4	2	3	2	7	2	-	-	-	-	-
5	-	-	-	-	-	-	-	5	3	-
6	5	-	-	-	-	-	-	-	-	1
7	1	-	-	-	-	-	-	1	-	-
8	-	3	3	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	3	-	-	-	-	-	1	1
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-

Table 2. Showing Performance of Ascorbic Acid Addition Into Media Within The First 2weeks of Initiation (10 Explants Per Concentration Column).

DAY	(ASCORBIC ACID + M.S MEDIA) NO OF CULTURES THAT BROWNED (g/litre)					(ASCORBIC ACID + STOCK SOLUTION) NO OF CULTURES THAT BROWNED (g/litre)				
	0.08	0.09	0.10	0.11	0.12	0.08	0.09	0.10	0.11	0.12
1	4	-	-	3	-	6	10	-	-	-
2	-	3	-	3	4	-	-	-	8	4
3	4	7	7	-	-	4	-	6	-	-
4	-	-	-	4	6	-	-	-	2	4
5	2	1	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	2
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	-	2	-	-	-	-	4	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-

Table 3. Showing Performance of Citric And Ascorbic Acid Addition Into Media Within The First 2weeks of Initiation (10 Explants Per Concentration Column).

DAY	(CITRIC AND ASCORBIC ACID + M.S MEDIA) NO OF CULTURES THAT BROWNED (g/litre)					(CITRIC AND ASCORBIC ACID+STOCK SOLUTION) NO OF CULTURES THAT BROWNED (g/litre)				
	0.13 0.08	0.14 0.09	0.15 0.10	0.16 0.11	0.17 0.12	0.13 0.08	0.14 0.09	0.15 0.10	0.16 0.11	0.17 0.12
1	-	-	-	-	-	-	-	-	-	1
2	-	-	-	-	-	-	-	-	-	-
3	4	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	5	-	-	-	-	2	-	-	-
6	2	-	-	3	4	-	-	-	-	
7	-	-	-	4	-	6	-	-	-	7
8	-	-	-	-	1	-	-	-	-	2
9	-	3	-	-	-	1	-	-	-	-
10	1	-	-	3	4	-	7	-	6	-
11	-	-	-	-	-	3	-	-	-	-
12	-	12	-	-	-	-	-	3	-	-
13	-	-	2	-	1	-	-	-	3	-
14	3	-	-		-		1	-	-	-

Table 4. Performance of the control experiment within the first 2 weeks of initiation. (50 explants were initiated as control)

DAY	(M.S MEDIA ALONE) NO OF CULTURES THAT BROWNED (g/litre)					(STOCK SOLUTION ALONE) NO OF CULTURES THAT BROWNED (g/litre)				
1	25	-	-	-	-	31	-	-	-	-
2	8	-	-	-	-	5	-	-	-	-
3	12	-	-	-	-	7	-	-	-	-
4	-	-	-	-	-	4	-	-	-	-
5	5	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	3	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-

Discussion

In the evaluation of the effect of the browning on the sugarcane variety NCSS-008, the differences found from the control experiment was glaring and has been proven beyond reasonable doubts that the addition of ascorbic and citric acid in the course of media preparation reduces browning. This browning is a problem encountered in the invitro culture of some plant

materials. Browning (or sometimes blackening) of cultured plant materials occur when phenolic substances such as tannins or other hydroxyphenols are oxidized to highly active quinone compounds which then cyclise, polymerise (oxidize) to form increasingly melanic compounds. The result of these is that the plant tissues become brown or black and growth of the plant cell is inhibited, Abdelrahaman (2007). On the other hand, one aspect of the present invention has provided a method of reducing browning of plant cell culture, comprises introducing into the plant cell culture mucilaginous materials obtained from sugarcane materials by at least partially submerging the sugarcane material in an aqueous medium. The present invention provides a method of plant cell culture in which the browning is controlled, improving the growth of the cultured plant cells. The method of the present invention is applicable to a wide range of plant and cell types, since browning is a wide spread problem in plant cell culture, Adelrahaman (2007).

The way the explants are being prepared is also instrumental to the reduction of the browning because explants that were carefully excised in such a way that the incision made left whole part of the leaf primodium without scares did well. But those that were badly handled continued to release exudates. Age of the plants is also a very vital aspect of the experiment in sugarcane micro propagation. It was observed that those sugarcane that were more than four months old did not survive the experiment. Thus the present study suggests that the use of ascorbic and citric acid as a media component in sugarcane micro propagation reduced browning.

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