Effect of Anti-Browning on Initiation Phase of Musa Species Grand Naine in Vitro

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Abstract—This study was carried out at the tissue culture laboratory of Sudan University of Science and Technology during the period from March to May 2009 for manipulation problems of oxidation in the initiation phase by use chemical and nonchemical addenda by dipping in vitro. After excision and before disinfection explants (small pieces of plant) were dipped in vitro into the following solutions, citric acid 150mg/l + ascorbic acid 100mg/l, cysteine 100mg/l, ginger 10g/l, activated charcoal 3g/l for prevention of browning or blackening. Results indicated that the pretreatment of dipping in solutions used gave positive results to counteract oxidation in medium and explants.

Index Terms—Musa species, Banana, Tissue culture, Browning

I. INTRODUCTION

Banana belong to the family Musaceae which consists of large to gigantic perennial, monocotyledonous plants. Bananas are the most important food crop in sub-Saharan Africa worldwide and ranks the fourth most important staple crop in developing countries [1].

It is the fourth most important food crop after sorghum, millet and wheat in developing countries which a combined production of more than 90 million tones [2] and is a staple for nearly 400 million people throughout the developing world [3]. Banana can be divided into two main groups: dessert bananas which constitute 43% of world production they are eaten raw when ripe, sugary and easily digestible, and account for the second group 57% of world production and this group is cooking bananas, members of this group are usually starchy when ripe and need to be cooked by boiling or roasting to provide a starchy food which is nutritionally similar to the potato [4]. Most of the world production is consumed locally leaving 15% for export [5].

In Sudan banana is produced commercially in small scattered gardens along the Nile banks and in small plantations in Kassala and in large plantation in Sinnar and Damazine.

Tissue culture is an inclusive term used for the range of procedures use to maintain and grow plant (cell, tissue or organ) in aseptic culture under control condition. The technique is used for propagation, genotype modification, biomass production of biochemical secondary products, plant pathology, germplasm preservation, and scientific investigations.

Micropropagation of banana is highly efficient, allowing a large turnover of plants in very short period of time within very little space [6]. Browning of tissues and/or media is one of the most studied known hypersensitivity reactions under in vitro conditions [7]. Some plants have the unpleasant characteristic of exudating brown/black pigments upon wounding, often making growth and development impossible especially woody plants and bananas [8].

This phenomenon results from physiological changes within the cultured tissues that lead to gradual browning and eventual death of tissues. The browning appears due to the oxidation of phenols within the tissues [9].

The objectives of this study were to prevent browning or blackening by use of different organic and inorganic substances.

II. MATERIALS AND METHODS

Plant material was brought from Horticulture department of Khartoum.

All experiments were carried out at the tissue culture Laboratory of Sudan University of Sciences and Technology for manipulation of browning in initiation phase.

Suckers were brought from the field, the superfluous tissue was removed by trimming away the outer leaf sheaths, leaf bases and corm tissues until a 5 - 7 cm cube enclosing the
shoot apex was obtained. The cubes tissue were washed under running tap water for about 1 hour. The cubes were then disinfected under laminar air flow cabinet by soaking for 30 min in commercial bleach(5.25% NaOCl) diluted to 30%(v/v) with two drops of Tween 20 per 100ml. This was followed by rinsing for 3 times autoclaved distilled water [10]. Explants were then placed on sterile petri dishes. All surface brown tissue and outer leaves were removed until the size of explants become about 1.5-2 cm in length then culture explants in the initiation media [10].

The antioxidants solution included:
- a- Citric acid150mg/l + Ascorbic acid 100mg/l.
- b- Cysteine 100mg/l.
- c- Ginger 10g/l.
- d- Activated charcoal 3g/l.

The basal media used was the recommended medium used at the tissue culture laboratory, University of Sudan. It consisted of full MS basal Salts (Murashige and Skoog, 1962)[11] strength additional Phosphate(KH_{2}PO_{4} 17 g/l)full MS vitamins (Murashige and Skoog, 1962)[11] mixture with 30 g/l of sucrose, 5mg/l BAP,0.2 mg/l NAA , 7 g/l agar agar ,the pH was adjusted to 5.7 ± 0.1 with Na OH acid HCl prior to addition of agar.

Media and dishes were sterilized by autoclaving at 121C0 for 20 min under a pressure of 15 psi[10]. Forceps and dissecting blades dipped in pure ethanol and exposed to flame gas. The laminar Air-Flow cabinet was sterilized by spraying and wiping with 70% Ethanol .It was switched on 15 minutes before use .The U.V lamp in the culture room was switched on during the night.

Depending on the objective of the experiment, culture was maintained either in the dark chamber or light under 16 hours light exposure of 1000 lux. Using white cool fluorescent lamps and the culture room temperature was maintained at 25 ± 2 C0. To define an explants that is most responsive Grand Nain plants AAA groups were used as explants for culture initiation. After excision and before disinfection explants were dipped into the following prior solutions for about half an hour and then rinsed 3 times with autoclaved distilled water before culturing on basal medium.

### III. RESULTS AND DISCUSSION

Table (1) and plate (1) indicated that the pretreatment of dipping in solutions of cysteine 100mg/l, citric acid 150mg/l+ ascorbic acid 100mg/l , ginger 10g/l gave positive results to counteract oxidation of medium than activated charcoal. However comparable results were obtained in all treatments regardy reviewing of explants. This result is in agreement with many investigators [12];[13];[14] placed explants of Musa textilis in an ascorbic and citric acid solution (1.0 and 1.5% w/v) respectively before surfacedisinfection and also supported by [15] who added the reducing agents citric acid (50 mg/l) and ascorbic acid (40 mg/l) to disinfecting solution itself. [16] indicated that activated charcoal is well known for its role in adsorption of toxic brown/black pigmentation of phenolic compounds, and other unknown color compounds. No reports for Ginger as antioxidant are available but it gave satisfactory result in this study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Browning intensity</th>
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<tbody>
<tr>
<td>Media</td>
<td>Explants</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>x</td>
</tr>
<tr>
<td>Citric + ascorbic acid</td>
<td>-</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-</td>
</tr>
<tr>
<td>Ginger</td>
<td>-</td>
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Data based on 10 cultures per sample.
Key:
- x = no
- x = poor
- xx= medial
- xxx= heavy.

### VI. CONCLUSION

1- Cysteine,citric+ascorbic acid and ginger in pretreatment proved to be the effective for preventing browning of medium and reduce browning of explants.

2- Activated charcoal also reduced browning of medium and explants.

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Plate 1. Response of banana explants to dipping in antioxidants after three weeks incubation

REFERENCES