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In Vitro Prevention of Browning in Plantain Culture

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Abstract: Problem statement: The *in vitro* propagation of plantain (*Musa paradisiaca*) is still faced with lots of challenges such as blackening or browning of tissues prior to culture due to the oxidation of phenolic compound by polyphenolic oxidase enzyme present in the tissue when excised. **Approach:** Understanding browning processes in plantain and possible ways of minimizing it during excision of explants with particular emphasis on the use of antioxidants was purposed. Tissues were surfaced sterilized with 0.1% (w/v) $HgCL_2$ for 1-6 min to get a pure culture and then treated for 2 h with different concentrations of 0.1-0.5 mg mL^{-1} of potassium citrate and citrate (K-C: C) as an antioxidant to check browning while sterile distilled water was used as control. **Results and Conclusion:** The result showed that contamination free culture (100%) was achieved in the explants treated with $HgCL_2$ for 6 min. Also the various concentration of K-C: C prevented browning within 2 h before culturing the tissues inferring that browning in young plantain excised tissue can be greatly reduced by presoaking or pretreatment with antioxidant solution of potassium citrate-citrate before culturing them.

Key words: *In vitro*, tissue browning, young plantain, *Musa paradisiaca*, culture vessel, potassium citrate, phenolic compound, antioxidant solution, lethal browning, phenolic compounds, Sterile Distilled Water (SDW)

INTRODUCTION

Plantain (*Musa Pardisiaca*) belongs to the Emusa section of the genus *Musa* in the family Musaceae and is natural hybrid polyploids, diploids, triploids, or tetraploids). They are cultivated over a wide range of agro ecological zones and produce fruit all year round and contributing to their importance as staple food and valuable export commodity. It is a nutritious fruit rich in carbohydrates and a good source of iron and vitamins the plant is found in several countries and continents of the tropical regions such as southeast Asia and Oceania including the modern Indonesia, Malaysia and the Philippines and northern Australia. Establishment of plantain plantation has some economical implications, which directly or indirectly stimulates agricultural and commercial ventures. This leads to accelerated developments crucial to national economic growth.

The development of micro propagation techniques has been a major focus of Musa research during the past two decades and such technologies have now been well established (Vuylsteke, 1998; Gowen, 1995). Plantain contain constituents of phenolic enzymes principally polyphenoloxidase enzymes. They serve as a very important phyto auxine in plantain and help to defend the plant against infection from fungi viruses and bacteria when injured.

Phenols are chemical compounds that embraces a wide range of plant substances which posses in common, an aromatic ring bearing one or more hydroxyl constituents. Phenolic substances tend to be water soluble since they most frequently occur combined with sugar as glycosides and are usually located in the cell vacuoles. Phenols are collectively called polyphenols. They are a group of chemical substances found in plant, characterized by the presence of more than one phenol unit or building block per molecule. The constituent phenols in *Musa spp* are principally doparnine, catechin, chlorogenic acid, cinnamic acid, hydroxylbenzoic, Resorcinol, progallic acid, salicylic acid, ferulic acid, vanillin, coumurin, P-coumaric acid, phenols (Khalil *et al*., 2007). These are localized mainly in the later vessel of the pulp peep, cells and tissues disposed in the latter.

However, these phenolic compounds are actively responsible for certain browning reactions and astringency of the fruit and its responsible for high mortality rate (lethal browning) in third generation of tissue culture (Ko *et al*., 2008).

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These form a problem of in in-vitro culture of explants, accompanied by the darkening of medium an attribute of the phenolic compound exuded from the plant tissue and accumulating in the culture medium. This browning of the surface of the explants is due to the oxidation of phenolic compounds resulting in the formation of quinines which is highly reactive to the plant tissue.

Plantain and suckers (stem) are susceptible to tissue browning and elimination or minimization of this process is an essential prerequisite to successful culture establishment. Therefore identification of a suitable treatment to minimize tissue browning in the explants with particular emphasis on the use of antioxidants is the main objective of this study.

MATERIALS AND METHODS

Collection of buds or mini suckers (meristem buds): Mini suckers of plantain was collected from the field with a machete and carried to the lab for processing. The mini suckers contain/have the buds or meristem in them.

The peduncle was removed in a stepwise manner until they became too small to remove by hand peeling. Working with a dissecting scalpel and forceps, the remaining leaf/peduncle was removed and reduced to 1cm long showing the rounded growing points.

Disinfection/sterilization procedure: The surface sterilization procedure began with dissection of explanted material into manageable units. The auxiliary buds or meristem was treated by initially removing the small leaflets and cleaning away surface detritus under running tap water for 1-2 min. A beaker (250 mL) was used for treatment with sterilant solution. Sterilization was undertaken for 6mins using 0.1% (w/v) $HgCL_2$. Explants were transferred to a separate beaker for the washing phase, in three changes of Sterile Distilled Water (SDW) and they were cultured in culture vessels to check contamination and sterilization percentage.

Media preparation in 1 liter: Ms Medium supplemented with 20 mg mL^{-1} Ascorbic acid, 4 mg mL^{-1} BAP, O.1mg mL^{-1} 1AA, 100 mg mL^{-1} myo-Inositol, sugar 30g & 2.5g phytagel Table 1.

The medium was stirred with a stirrer and made up to 1000mLs (IL) with distilled water using a measuring cylinder. The medium was pour into a 1 L beaker and 2.5g of phytagel was added. The medium was micro waved (or heated) to melt/dissolve the phytagel for 8mins. It was stirred for even distribution using a magnetic stirrer.

Table 1: Medium composition

Composition	M/L/g
Micro stock×200	5mLs
Iron stock×200	5mLs
Vitamins stock×200	5mLs
Ascorbic acid (20mg mL^{-1})	5mLs
BAP (1mg mL^{-1})	4.5mLs
IAA (o. 1mg mL^{-1})	1.8mL
Sugar	30g
Myo-Inositol (100g)	0.1g
PH	5.8 (Adjust with 1M NAOH)

Table 2: Antioxidant treatments

Treatments	Volume of antioxidant treatment (ML)
Expose to air, cut on wet filter for pear	0
Cut in Petri dish plus H_2o (SDW)	100
Cut in k-c: c (0.1mg mL^{-1})	100
Cut in k-c: c (0.2mg mL^{-1})	100
Cut in k-c: c (0.2mg mL^{-1})	100
Cut in k-c: c (0.3mg mL^{-1})	100
Cut in k-c: c (0.4mg mL^{-1})	100
Cut in k-c: c (0.4mg mL^{-1})	100
Cut in k-c: c (0.125/v) + L-cysteine	100
Cut in k-c: c (0.125) + L-cysteine + Ascorbic acid	100

Dispense 20 mL in culture vessel and autoclave at 121°C for 15mins under 15psi. Allow culture to cool and store in a cabinet to check for any growth or contamination before using.

Antioxidant treatment: A stock solution of potassium citrate and citrate (K-C:C) was made up using 1g k-c and 0.25g citrate and dissolved in 10 mL of SDW. The concentration was then diluted and used at a final concentration of 0.125%. The excised buds (meristem) were placed in Petri dishes containing the treatment (the antioxidant, k-C:C), 0.125% of K-C:C (as stock solution) in the concentration of 0.1-0.5 mg $m1^{-1}$ of K-C:C to check the strength of K-C:C i.e. how long and at what concentration will the start or stop browning. Also the disc section was placed on filter paper and exposed to Air. It was place in water and sterile distilled water to check browning, these served as a control Table 2.

These treatments were placed in a Petri dish and results were recorded at time interval of 0, 6, 30, 60 and 120mins. Observations of the extent of browning were recorded. The treated explants were placed in a test tube containing 20mLs of the media under a laminar flow hood and kept in the growth room to grow.

RESULTS

Results of the surface sterilization procedure for explant is summarized in Table 3 and Fig. 1 and 2. It showed that after few days of culture, contamination free culture (100%) was achieved in the explants treated with HgCL2 for 6 min (Fig. 3).

Table 3: Surface sterilization of explant using $Hgcl_2$ treatment

Treatment duration (min) with 0.1% $HgCl_2$	Number of explant	Percentage contamination	Percentage contamination free
1	1	100%	0
2	1	100%	0
3	1	100%	0
4	1	100%	0
5	1	100%	0
6	1	0	100%

Table 4: Antioxidant treatment relative browning of disc section of plant tissue treated with antioxidant for a period of 2 hrs

Time (min)	Treatment number									
	1	2	3	4	5	6	7	8	9	10
0	+++	++	+	-	-	-	-	-	-	-
16	+++	+++	++	-	-	-	-	-	+	-
30	+++	+++	+++	-	-	-	-	-	+	+
60	+++	+++	+++	-	-	-	-	-	++	++
120	+++	+++	+++	-	-	-	-	-	+++	+++

Keys: - = No oxidation of phenol (Thus no browning),, + = Low oxidation,++ = Medium oxidation, +++ = High oxidation, Treatment number: 1→Expose to air, cut on filter paper,2→Cut in Petri dish plus H_20 (SDW), 3→Cut in Petri dish plus H_2O , 4→Cut in K-c: c (0.1mg mL^{-1}), 5→Cut in K-c: c (0.2mg mL^{-1}), 6→Cut in K-c: c (0.3mg mL^{-1}), 7→Cut in K-c: c (0.4mg mL^{-1}), 8→Cut in K-c: c (0.5mg mL^{-1}), 9→Cut in K-c: c (0.125 w/v) + L-cysteine, 10→ Cut in K-c: c (0.125 w/v) + L-cysteine + Ascorbic acid

Table 4 showed the result of the antioxidant treatment. The buds or meristem disc section from young plantain mini suckers were treated with various concentration of antioxidant solution and a control with water, Sterile Distilled Water (SDW) and air for duration of 2 h.

DISCUSSION

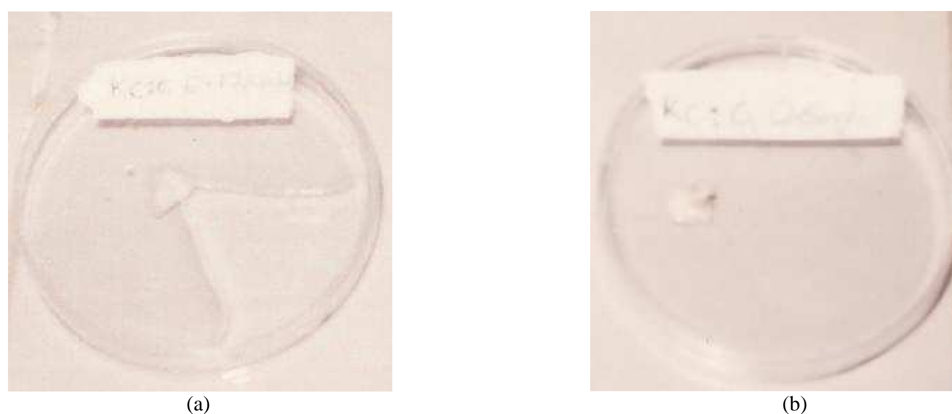
The results of the treatment showed that antioxidants are electron donors (reducing agent) which inhibits the oxidations of labile substrates as described by George and Sherringyon (1984). The antioxidant compounds utilized in the experimental work were selected because they have been used successfully in the past to delay browning in arborescent monocotyledons species (Khatri *et al*., 1997). The disc section selected were young sucker which is highly prone to browning or oxidation of phenolic compounds which is a common problem in the establishment of plantain in-vitro culture. All cut surfaces in the control experiment appeared to oxidize phenol rapidly once exposed to air evidenced by tissue browning. Subsequently, tissue exercised in water and SDW oxidized phenolic compounds and the tissues turned brown. The cut surface of any damaged area of untreated tissue turned brown in less than 15 minutes after excision. These explants continued to oxidize phenolic compounds and were completely brown after 2 h. and were subsequently discovered. According to Titov *et al*. (2006), L-cysteine and ascorbic aid in combination with K-C:C initially reduced or delayed browning but oxidation of phenolic compounds resumed after 30 min of treatments and continued the oxidation of these compound until tissues turned brown.

(a)

(b)

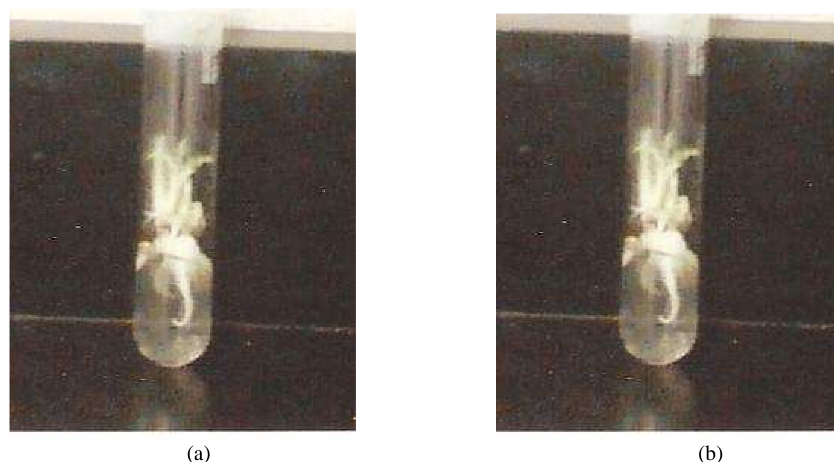
Fig. 1: Plantain tissue,(a) Exposed to air cut on filter paper, (b) Cut in Petri dish plus water (SDW)

Potassium citrate-citrate combination as an antioxidant treatment for excised plantain tissue proved to be the best treatment type from the treatment result (Table 2 from no. 4-8) and Fig. 1a-b. The various concentration of K-C: C reduced or prevented browning within 2 h before culturing the tissues.



(a) (b)

Fig. 2: Treated plantain tissues



(a) (b)

Fig. 3: Plantain tissue culture showing proliferation

The citrate in citric acid work as a chelating agent (i.e., has the ability to interfere with the action of the peroxidase enzymes) bonding to ions responsible for activating polyphenolic oxidative enzymes (PPO). Ascorbates behave as a reducing agent and are converted to dehydro-ascorbic acid. The Ascorbates are able to scavenge oxygen radicals produced when tissue is damaged and therefore cells are protected from oxidative injury. Oxygen radicals attributed to exacerbating oxidative injury. These free radicals can be detoxified by Antioxidants containing citrate and Ascorbates, thus reducing browning of tissues (Titov *et al*., 2006).

CONCLUSION

It can be seen from the result that contamination free culture (100%) during *in vitro* propagation of plantain (*Musa paradisiaca*) was achieved in the explants treated with HgCL2 for 6mins. Also the various concentration of K-C: C prevented browning within 2 hours before culturing the tissues thus inferring that browning in young plantain excised tissue can be greatly reduced by presoaking or pretreatment with antioxidant solution of potassium citrate-citrate before culturing them.

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