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Control of lethal browning by using ascorbic acid on shoot tip cultures of a local *Musa* spp. (Banana) cv. Mzuzu in Tanzania

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The use of ascorbic acid during explants preparation and the effect of different concentrations of ascorbic acid in controlling lethal browning and survival of the explants in local banana cv. Mzuzu banana were investigated. The explants were taken from young suckers. The shoot tips were cultured on Murashige and Skoog's media supplemented with 5 mg/l of benzylaminopurine (BAP) and different concentrations of ascorbic acid (0, 50, 100 and 200 mg/l). Completely randomized design was used in this study. The results indicate that the use of ascorbic acid as an antioxidant during explants preparation significantly reduced the extent of lethal browning and survival of the explants followed by 100 mg/l of ascorbic acid applied directly into the media.

Key words: Micro propagation, surface sterilization, survival of explants, tissue culture.

INTRODUCTION

Banana contains constituents of phenolic enzymes principally polyphenoloxidase enzyme. Polyphenoloxidase enzymes serve as a very important phyto auxine in banana and help to defend the plant against infection from fungi, viruses and bacteria when injured (Chiremereze et al., 2011). The constituent of phenols in *Musa* spp. are principally dopamine, catechin, chlorogenic acid, cinnamic acid, hydroxyl benzoic, Resorcinol, progallic acid, salicylic acid, ferulic acid, vanillin coumurin, P-coumaric acid and phenol (Khalil et al., 2007). Browning reactions and astringency of the fruit caused by phenolic compounds are responsible for high mortality rate (lethal browning) in third generation of tissue culture. This process is initiated by browning of the surface of plant tissues due to the oxidation of phenolic compounds resulting in the formation of quinines which are highly reactive and toxic to plant tissue (Titov et al., 2006).

Apart from being an important group of secondary metabolites, phenolics may act as modulators of plant development by regulating indole acetic acid (IAA) catabolism (Ozyigit et al., 2007). They also play effective

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Abbreviations: MS, Murashige and Skoog (1962); **AA**, ascorbic acid; **BAP**, benzylaminopurine; **AC**, activated charcoal.

role in plant growth regulation, cell differentiation and organogenesis (Mato et al., 2006). Their concentration is often affected by several internal and external factors (North et al., 2011). Other factors include stress factors such as drought, water, radiation and pathogen infection from injured surfaces, which directly affects the concentrations of phenolics in plants (Kefeli et al., 2003).

Control of lethal browning in tissue culture of banana has been reported by different studies. Chimereze et al. (2011) reported the use of antioxidant potassium citrate and citrate (K-C:C) in prevention of browning in plantain culture. As an antioxidant, potassium citrate-citrate reduced browning within 2 h before culturing the tissues (Chimereze et al., 2011). Ascorbic acid is also an antioxidant used to control oxidation of phenols (Bharadwaj and Ramawat, 1993; Chawla, 2002; Abeyaratne and Lathiff, 2002). In controlling lethal browning in faba beans, Abdelwahd et al. (2008) reported that the use of activated charcoal, ascorbic acid, cystine and silver nitrate had a significant effect on the number of shoots and the length of shoots regenerated per explants. Strosse et al. (2004) reported that addition of cysteine to the growth media reduced explant blackening in banana tissue culture (Strosse et al., 2004). Understanding the processes contributing to the oxidation of phenols and how these can be minimized when initiating banana tissue culture is critical for successful *in vitro* culture, not only of banana but also of other crops.

Mzuzu variety is among the banana varieties susceptible to tissue browning and elimination or reduction of this process is necessary requirement for successful culture establishment. Development of suitable and efficient treatment to minimize tissue browning of *Mzuzu* variety was the objective of this study. The emphasis is particularly focused on the use suitable antioxidant concentration in the media and method of application during explant preparation.

MATERIALS AND METHODS

Plant materials

Young banana suckers of variety *Mzuzu* were collected from banana plantation grown at Chambezi outside Dar es salaam City near Bagamoyo.

Sterilization

The banana suckers were trimmed to remove extraneous matters and roots. They were then washed with tap water and a liquid detergent. The suckers were further trimmed and cut to a size of 10 cm by 5 cm to form explants which were soaked in a solution of distilled water and in a solution with concentration of 1.2 g/l of ascorbic acid. The explants were sterilized with 70% alcohol for 1 min and then rinsed three times with sterile distilled water. The explants soaked in 3.85% solution of sodium hypochlorite (NaOCl) for 1 h and two drops of tween 20. The explants were further sterilized with 1.925% solution of sodium hypochlorite. The explants were trimmed to remove the excess hypochlorite and aseptically placed on the culture media.

Culture conditions and media

The explants were placed in culture vessels containing 20 ml of culture media with MS basal salts supplemented with 20 g/l sucrose, vitamins glycine 2 g/l, pyridoxine 0.5 g/l, Nicotinic acid 0.5 g/l, Thiamine 0.1 g/l and Myo inositol at 0.1 g/l. The media will also be supplemented with 5 mg/l of BAP and solidified with 4.0 g/l of agar. The pH of the media was adjusted to 5.8 prior to autoclaving at 121°C for 15 min.

The experiment consisting of five treatments of ascorbic acid (0 mg/l, 0 mg/l but soaked in 1.2 g/l of ascorbic for 1 h during explants preparation, 50, 100 and 200 mg/l) investigated the use of ascorbic acid in explants preparation and the effects of ascorbic acid concentration in controlling lethal browning during culture initiation of banana. Five culture vessels were used for each treatment; each culture vessel contained one explant and each treatment was replicated three times.

Data collection and analysis

Data on number of healthy and growing plantlets and numbers of diseased plantlets were collected on weekly intervals for a period of four weeks after initiation. Based on visual observation, the extent of media discoloration was assessed. Media discoloration was rated on scale of 1 to 4 (1 implies no discoloration, 4 implies extreme media discoloration). Data collected were analyzed for statistical significance using analysis of variance (ANOVA). These computations were done by using a statistical software program STATISTICA software Programme version 2013 (StatSoft Inc., Tulsa, OK, USA). Fisher least significance was used to compare means at P = 0.05 level of significance.

RESULTS AND DISCUSSION

Effect of various concentrations of ascorbic acid in controlling the extent of lethal browning

In this study, the extent of browning and death of explants was observed on weekly basis for four weeks. Generally, lethal browning increased with time, but decreased with increased concentration of ascorbic acid and then declined at highest concentration (Table 1). During this experiment, highest degree of lethal browning was observed in control treatment (Table 1). The mortality of explants was also high in the control treatment (Table 2) and (Figure 1). It is well established that injured tissues normally stimulate the production of phenols (Dodds and Roberts, 1995). This is a defensive mechanism common in plants in response to tissue damage (Pan and van Staden, 1998; Ndakidemi and Dakora, 2003). The production of these compounds in excess results in browning and eventually death of explant (Figure 1). The darkening or browning of the media in tissue culture is caused by exudation and oxidation of phenollic compounds which results in the formation of quinones which are highly reactive and toxic to plant tissues (Ko et al., 2009).

In this experiment, the use of ascorbic acid during

Table 1. Extent of media discoloration after four weeks.

Treatment	Time (week)			
	1	2	3	4
Concentration				
0 mg.l^{-1} A.A	3.00 ± 0.33^{a}	3.40±0.2^{a}	3.60±0.16^{a}	3.70±0.15^{a}
0 mg.l^{-1} washed with A.A	1.50 ± 0.20^{b}	1.93±0.3^{b}	2.00±0.33^{b}	2.00±0.33^{c}
50 mg.l^{-1} A.A	1.91 ± 0.31^{b}	2.63±0.36^{ab}	2.63±0.36^{ab}	3.09±0.34^{ab}
100 mg.l^{-1} A.A	1.53 ± 0.21^{b}	2.07±0.34^{b}	2.07±0.34^{b}	2.13±0.36^{c}
200 mg.l^{-1} A.A	1.23 ± 0.21^{b}	2.31±0.38^{b}	2.23±0.38^{b}	2.31±0.36^{bc}
One way ANOVA (F- Statistic)				
Main effect				
Concentration	7.52**	2.63*	3.31*	4.17*

*, P≤0.05; **, P≤0.001; ns, not significantly different. Values (Mean ± SE) followed by dissimilar letters in a column are significantly different by Least significant difference (LSD) test at P=0.05. The rating scale is 1 to 4 (1, No media discoloration; 4, extreme discoloration).

Figure 1. Dead explant due to browning (the picture was taken at the fourth week of the experiment).

explants preparation showed the best results in controlling lethal browning throughout the experimental period. The analysis indicated that there were significant ($p \leq 0.05$) differences in the extent of lethal browning in control treatment (3.70) relative to the use of ascorbic acid (2.0) during explants preparation (Table 1). The successful use of antioxidant applied during explants preparation to prevent lethal browning is also reported by Titov et al. (2006), in which an antioxidant wash of 0.125% potassium citrate:citrate (K-C:C in a ratio of 4:1 w/w) solution was useful for explants preparation of *Musa* spp.cv. Kanthali (Titov et al., 2006).

Good control of lethal browning in terms of concentration of ascorbic acid applied on the media was observed at the concentration of 100 mg/l followed by the concentration of 200 mg/l (Table 1). At the concentration of 100 mg/l, ascorbic acid significantly reduced the extent of lethal browning on the explants at fourth week compared with the rest of other treatments. Similar to our study, Strosse et al. (2004) indicated that antioxidants such as ascorbic acid or citric acid in concentrations ranging from 10 to 150 mg/l added to the media reduced browning in banana varieties.

The wide range in concentration of antioxidant added to the media to control browning is due to the fact that the extent of lethal browning is genotype specific as it depends on the cultivar or variety. In our study, ascorbic acid at concentrations of 100 and 200 mg/l showed good control

Table 2. The effect of ascorbic acid concentration on survival of explants.

Concentration	Number of explants	Total number of dead explants on weekly basis [Time (weeks)]				Percentage of surviving explants after 4 weeks
		1	2	3	4	
0 mg.l^{-1} A.A	10	4	5	7	10	0
0 mg.l^{-1} A.A, soaked in A.A	14	0	0	4	5	64.3
50 mg.l^{-1} A.A	11	1	2	7	9	18.2
100 mg.l^{-1} A.A	15	0	4	7	7	53.3
200 mg.l^{-1} A.A	13	0	3	6	6	53.8

Figure 2. Surviving explant due to application of ascorbic acid.

of lethal browning.

During the first week of this experiment all treatments were significantly ($p \leq 0.001$) not different except for a control which was significantly different to the rest of the treatments. This indicates that even at low concentration of 50 mg/l, ascorbic acid was able to control lethal browning at least in the first week. Ko et al. (2008) reported similar results where a low concentration of ascorbic acid (0.0005%) applied directly on the surface of the media after autoclaving was able to reduce the number of diseased plantlets per flask from 10.7 without ascorbic acid to 4 and increased the number of healthy plantlets from 1.7 in control to 15.0. However, from second week, the low concentration of ascorbic acid was not significantly different to the control (Table 1).

Effect of various concentrations of ascorbic acid on survival of explants

The death of explants due to lethal browning was mostly observed in the control treatment. Generally, the highest death of explants was observed on third and fourth week of the experiment (Table 2). The observed death of the explants with time was attributed to the oxidation of polyphenolic compounds released from the wounded tissues which formed the barrier round the tissues preventing nutrient uptake and hindering growth (Strosse et al., 2004). The death pattern of these explants was similar to the extent of browning in the respective treatments. The lowest survival of explants was observed in the control treatment where there was no explant which survived (Table 2). The highest survival of explants was observed in treatment of soaking the explants for 1 h in 1.2 g/l of ascorbic acid during explants preparation. About 64.3% of the explants survived the incidence of lethal browning (Figure 2). This was followed by the use of ascorbic acid at concentration of 200 mg/l applied directly to the media where 53.8% of the explants survived (Table 2).

Conclusion

This study indicated that lethal browning in cv. Mzuzu can be controlled by the use of ascorbic acid during explants

preparation. Treating the explants with 1.2 g/l of ascorbic during explants preparation and addition of 100 mg/l in the growth medium controlled the extent of lethal browning of the explants significantly compared with the rest of different concentrations of ascorbic acid added to the media after four weeks of experimentation. In order to minimize the cost of tissue culture and the losses associated with death of explants, it is recommended that the use of ascorbic acid as an antioxidant can be applied during explants preparation to avoid addition directly to the media which might cause unforeseen problems to nutrient absorption and general availability of nutrients. It can be concluded that application of ascorbic acid directly to the media at appropriate concentration can control lethal browning in this variety but high concentration can be deleterious to the explants, while low concentration might be ineffective. To produce an optimized culture with low mortality of explants, ascorbic acid should be applied before surface sterilization.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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