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Full Length Research Paper

Effect of ascorbic acid on blackening and sprouting of *Musa* spp shoot tips

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The effect of ascorbic acid (0, 5, 10, 20, 30, 40 and 150 mgl^{-1}) on blackening and sprouting of *Musa* spp genotypes was investigated using shoot tip culture. Ascorbic acid did not minimize blackening, but rather markedly reduced the sprouting of cultured shoot tip explants. Ascorbic acid, genotype and genotype x ascorbic acid interactions were significant for shoot tip blackening and spouting, though genotype was a substantial source of variation to blackening response. 'TMBX 5295 -1' (cooking banana hybrid) shoot tips exhibited maximum blackening response, while 'Agbagba' (false-horn plantain) shoot tips showed highest sprouting. An inverse / negative relationship (r = - 0.27., P < 0.001) was obtained between shoot tip blackening and sprouting. Data obtained suggest that supplementation of medium supplemented (MS) medium with ascorbic acid was not beneficial for shoot tip culture of *Musa* spp. Other techniques for effective minimization of explant blackening are proposed.

Key words: Ascorbic acid, blackening, sprouting, *Musa* spp (banana and plantain) shoot tip culture.

INTRODUCTION

Bananas and plantains (*Musa* spp) are major staple food crop for millions of people throughout the humid and subhumid tropics. Shoot tip culture of *Musa* spp is routinely used for the rapid, clonal multiplication of selected *Musa* genotypes; the production of clean, planting materials; and the exchange and conservation of *Musa* genetic resources (Simmonds, 1987). One of the problems encountered during *in-vitro* propagation of some *Musa* varieties through shoot-tip culture is blackening, caused by oxidation of polyphenolic compounds, which form a layer around wounded tissues, preventing nutrient uptake and hindering growth (Vuylsteke, 1989; Strosse et al., 2004). In nature, the layer formed around damaged or wounded tissues, due to polyphenolic oxidation, prevents the invasion of pathogens. The negative effect for the in-vitro culturist is that also it reduces the uptake of nutrients (Panis, 2000). Other scientists (Crompton and Preece, 1988; Strosse et al., 2004; Tagelsir et al., 2006; Anirudh and Kanwar, 2008) have also reported explant blackening with associated poor growth.

Strosse et al. (2004) reported that addition of ascorbic acid to the media inhibited the exudation of phenols in banana tissue cultures. *In-vitro*, ascorbic acid has been shown to act as an antioxidant/ antibrowning agent (Davis et al., 1974; Gupta, 1986), by inhibiting the formation of strongly–oxidizing quinones thought to cause poor growth and browning (Wickers et al., 1984), and by inhibiting the activity of phenolases (Khan, 1977). It has been added as a medium supplement to decrease the deleterious effects of browning associated with poor growth (Davis et al., 1974; Katterman et al., 1977; Gupta, 1986; Josekutty et al., 2002).

The objectives of this study were:

(i) To examine the influence of ascorbic acid on in-vitro blackening and sprouting of shoot tips of *Musa spp* genotypes.
(ii) To ascertain the correlation between blackening and sprouting responses of shoot tips of *Musa* spp genotypes used in this study.

MATERIALS AND METHODS

This study was carried out in the Plantain and Banana tissue

culture laboratory of the International Institute of Tropical Agriculture (IITA) Onne, Rivers State, Nigeria. Four *Musa* spp genotypes 'Agbagba' (AAB medium falsehorn plantain), 'Obino l'ewai' (AAB medium french plantain), 'Cardaba' (ABB cooking banana) and 'TMBX 5295-1' (ABB cooking banana hybrid), were used in the study.

Shoot tip explants were aseptically excised from suckers, collected from field-grown plants, and cultured following the shoot tip culture technique (Vuylsteke, 1989). All protocols were carried out under aseptic conditions.

Culture initiation

Explant disinfestation

Cubical shoot pieces (3 to 4 cm) containing the shoot tips were excised from suckers and washed under running tap water. They were surface–sterilized by rinsing in ethanol (95%) for 15 to 30 s, followed by soaking for 20 min in 0.75% ($^{w}/_{v}$) sodium hypochlorite (40% $^{v}/_{v}$ commercial bleach solution) and few drops of Tween 80 (wetting agent), to enhance penetration. Working under aseptic conditions, the bleach solution was decanted and the cubes rinsed three times with sterile, distilled water. Shoot tips (explants) of similar size (3 × 3 × 3 mm) were excised aseptically from the sterilized tissues. They were cultured in glass test- tubes containing 20 ml of modified Murashige and Skoog (1962) medium, adopted for *Musa* shoot tip culture at IITA (Vuylsteke, 1989), and supplemented with varying concentrations (0, 5, 10, 20, 30, 40, and 150 mg L^{-1}) of ascorbic acid.

Culture media and conditions

Medium [modified Murashige and Skoog (1962) medium as used at IITA] was solidified with 2 gl^{-1} Gelrite [Vuylsteke, 1989]. (Sigma-Aldrich, St. Louis, USA). pH was adjusted to 5.8 ± 0.1 before sterilizing in autoclave at 121°C and 1.05 kg cm^{-2} (103.4 KPa), for 20 mins. Cultures were incubated at 27 ± 2°C, under a 14 h photoperiod provided by cool-white fluorescent tubes at 60 $\mu mol.s^{-1}.m^{-2}$.

Each treatment consisted of a shoot tip explant per genotype, for each ascorbic acid concentration, and was replicated six times, for the blackening and sprouting experiments. Each shoot tip explant was cultured in 20 mls of sterilized culture media contained in a test tube and the experiment was repeated twice. Data on blackening was recorded three to five days after culture initiation. Observations were quantitatively assessed, scored on a subjective rating scale of 0 to 3, and graded as follows: creamy – white (0), light-brown (1), brown (2), and black (3). Data on sprouting was recorded seven to ten days after culture initiation. Sprouting is defined here, as the observation of bud initiation and growth on cultured explants. Observations were quantitatively assessed, scored on a subjective rating scale of 1 to 3, and graded as follows: unsprouted (1); low/moderate sprouting (2) and high sprouting (3). The percent "high sprouting shoot tips'' were computed and recorded. In this study, "high sprouting shoot tips" are shoot tips with bud development and /or elongation of the shoot axis.

Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of the statistical analysis system (SAS) Institute to determine the source of variation in data obtained. Duncan's multiple range test (DMRT) was used for mean separation. Correlation analysis was performed with Pearson correlation coefficients.

RESULTS

Blackening response

Data presented on Table 1, revealed that ascorbic acid, at the concentrations tested did not minimize the blackening of *Musa* spp shoot tips. The blackening value of control shoot tips [1.67] was comparable and statistically similar to that of treated shoot tips, indicating the ineffectiveness of ascorbic acid as an antiblackening agent (Table 1). There was no significant difference among the mean values obtained from the treated shoot tips.

Anova estimates revealed that genotype effect was highly significant ($p < 0.001$) and accounted for the greatest amount of variation in blackening responses. Similarly ascorbic acid effect was significant ($p < 0.05$). Genotype x ascorbic acid interaction was also significant ($p < 0.05$). A significant ($p < 0.05$) lack of fit was obtained (Table 1).

Sprouting response

A significantly higher sprouting response [2.42] was produced from the control shoot-tip (Table 2). Incorporation of ascorbic acid, at the tested concentrations, to modified MS (Murashige and Skoog 1962) medium resulted to marked reduction of sprouting response from treated *Musa* spp shoot tips. Mean values obtained (excluding control) ranged from 1.63 to 1.98, and were statistically similar (Table 2).

The analysis of variance for sprouting response of *Musa* spp shoot tips showed that ascorbic acid ($p < 0.01$) and genotype ($p < 0.05$) effects were significant. The lack-of-fit (deviations) was also significant ($p < 0.01$). As with blackening response, a significant effect ($p < 0.01$) of genotype x ascorbic acid, was obtained, indicating differences in sprouting response of each of the four *Musa* spp genotypes, at each ascorbic acid concentration tested. Certain genotypes responded differently (in terms of yield of vigorous shoot tips) at certain ascorbic acid concentrations (Table 2). Generally, the supplementation of MS (Murashige and Skoog, 1962) medium with ascorbic acid did not improve the sprouting of *Musa* spp shoot tips.

Percent high-sprouting shoot tips

Sprouting was first observed on control explants. This treatment also produced maximum percentage (40%) of high sprouting shoot tips. Ascorbic acid significantly reduced the frequency of high sprouting vigorous shoot tips [data not shown].

Genotypically, 'Agbagba' and 'Obino l'ewai' (the plantain genotypes) produced maximum proportion (33 and 32% respectively) of high-sprouting shoot tips and

Table 1. Effect of ascorbic acid on blackening (IB) response of shoot-tips of four *Musa* spp genotypes.

Ascorbic acid (mg L^{-1})[a]	Level of blackening				Main effect of ascorbic acid (mean)
	Agbagba	O/L'ewai	Cardaba	5295-1	
0.0	1.40	1.60	1.50	2.17	1.67a[b]
5.0	2.00	1.50	2.00	2.33	1.96[a]
10.0	1.40	1.20	1.02	2.50	1.53[a]
20.0	2.33	1.45	1.75	2.33	1.97[a]
30.0	1.83	1.20	1.60	1.50	1.53[a]
40.0	1.25	1.67	1.67	2.40	1.75a
150.0	1.83	2.40	1.75	1.80	1.95a
Main effect of genotype [Mean] [c]	1.72[b]	1.57[b]	1.61[b]	2.15[a]	
Source	Level of Significance				
Geno	*	*	*		
Ascorbic acid		*			
Geno x Ascorbic acid		*			
Lack-of-fit		*			

a = values for ascorbic treatment over the four genotypes. b = followed by the same letter are not significantly at p = 0.05, as determined by Duncan's multiple range test (DMRT). c = values for genotypes over the ascorbic acid concentrations. *Significantly different (P < 0.05). **Significantly different (P > 0.05 and 0.01). ***Significantly different (P < 0.05, 0.01 and 0.001).

Table 2. Effect of ascorbic acid on sprouting (SP) response of shoot-tips of four *Musa* spp genotypes

Ascorbic acid (mg L^{-1})[a]	Sprouting response				Main effect of ascorbic acid (mean)
	Agbagba	O/L'ewai	Cardaba	5295-1	
0.0	2.60	2.40	2.50	2.17	2.42a[b]
5.0	1.67	1.67	1.33	1.83	1.63b
10.0	2.00	2.60	1.9	1.33	1.98ab
20.0	1.83	1.80	1.75	2.00	1.85b
30.0	2.00	2.00	1.00	2.50	1.88b
40.0	2.67	1.60	2.00	1.67	1.67b
150.0	2.67	1.60	2.00	1.67	1.99ab
Main effect of genotype [Mean] [c]	2.15[a]	1.94[ab]	1.70[b]	1.87[b]	
Source	Level of Significance				
Geno		*			
Ascorbic acid			*	*	
Geno x Ascorbic acid			*	*	
Lack-of-fit			*	*	

a = values for ascorbic treatment over the four genotypes. b = followed by the same letter are not significantly at p = 0.05, as determined by Duncan's multiple range test (DMRT). c = values for genotypes over the ascorbic acid concentrations. *Significantly different (P < 0.05). **Significantly different (P > 0.05 and 0.01). ***Significantly different (P < 0.05, 0.01 and 0.001).

equally high mean sprouting values (2.15 and 1.94 respectively). In contrast, across the ascorbic acid concentrations tested, the lowest percent yield of vigorous shoot tips were recorded from the banana genotypes – 'Cardaba' (12%) and '5295 -1' (20%), similarly, the mean sprouting values for these two genotypes were 1.70 and 1.87, respectively (Figure 1, Table 2). This indicates a genotype effect in response.

Correlation coefficient between blackening and sprouting responses

Regression analysis of the data for level of blackening (lb) and sprouting (sp) values of shoot tips of the four *Musa* spp cultivars reveal a highly significant negative /inverse relationship ($r = -0.27$; $p < 0.001$) according to Pearson Correlation Coefficient (Table 3).

This inverse relationship could indicate a negative effect of polyphenol oxidation (blackening) on the sprouting of the cultured *Musa* spp shoot tips.

DISCUSSION

Blackening

Data from this investigation show that incorporation of ascorbic acid, at the concentrations tested, into the modified MS (Murashige and Skoog, 1962) medium for shoot tip culture of *Musa* spp did not minimize blackening of shoot tips of the four *Musa* spp genotypes used in the study (Table 1). This report indicates the ineffectiveness of ascorbic acid as an antioxidant /antiblackening agent in shoot tip culture of *Musa* spp, which could be attributed to its rapid decay in tissue culture.

Vuylsteke and DeLanghe (1985) had reported that 10 mg^{-1} ascorbic acid did little to minimize the usual blackening of injured banana tissues caused by oxidation of polyphenols. In contrast, Ko et al. (2009) reported that addition of ascorbic acid to the surface of culture medium, not only prevented the development of lethal browning, but also greatly increased the number of Cavendish banana cv. *formosana* plantlets produced. Even at 0.005%, ascorbic acid was able to reduce the disease incidence by more than 60% and caused over 8-fold increase in number of plantlets produced.

Few papers report the use of explant pretreatment with an antioxidant (ascorbic acid and citric acid) to reduce blackening of the *Musa* shoot tips and of the culture media (Novak et al., 1986; Gupta 1986; He et al., 1995). He et al. (1995) observed that ascorbic acid pre-treatment prevented banana sucker explants from turning brown and improved plantlet production. In this study, preliminary explant pretreatment by immersing the *Musa* shoot tips, for some hours, in 100 mls solution each of the ascorbic acid treatments did not reduce explant blackening. Wu et al. (2007) reported that soaking the *P cynaroides* micrografts in pre-treatment solutions of antioxidants ascorbic acid (100 mgl^{-1}) and citric and (150 mgl^{-1}) in half-strength MS medium, *aggravated* the tissue blackening. They suggested that, a possible reason for the lack of inhibition of phenolic oxidation by the antioxidant solution may be the use of insufficient concentration of ascorbic acid and citric acid. They further proposed the use of higher antioxidant concentration for the prevention of phenolic oxidation.

Data obtained show that genotype was a highly significant ($p < 0.001$) source of variation to blackening response. The banana genotype, TMBX 5295-1 produced significantly higher mean blackening response, while Obino l'ewai (plantain genotype), displayed the lowest mean blackening response (Table 1). From his studies using banana peel extracts, Griffiths (1961) reported that dopamine was a characteristic product of the genome of *Musa acuminata* (A), but not of *Musa balabisiana* (B). He therefore suggested that the AA and AAA clones blackened strongly, while hybrids AB, AAB, ABB and ABBB blackened less in proportion, and *M. balbisiana* itself (BB) blackened very little. Since one of the parents of TMBX 5295-1 is the wild banana cv. AA ''Tjau Lagada'', it could have contributed to the significantly higher blackening response recorded from the TMBX 5295-1 shoot-tips in this study. Genotype x ascorbic acid interaction was significant ($p < 0.05$), indicating that the genotypes responded differently at each ascorbic acid concentration (Table 1).

Sprouting

The sprouting response of explants tested with ascorbic acid was markedly reduced or suppressed, compared to control explants (Table 2). Consequently, maximum mean sprouting response from *Musa* shoot tips was recorded at control (0 mgl^{-1} ascorbic acid). The rapid oxidation of ascorbic acid to dehydroascorbic acid in the culture media may be responsible for the observed significant reduction in sprouting response of the cultured *Musa* spp shoot-tips.It has been shown that DHA, at low concentration inhibits the activity of several enzymes in-vitro, including dehydrogenase,fructose1,6-biphosphate (Morell et al., 1990) and hexokinase (Fioriani et al., 2010). Moreover, root growth inhibition has been observed in response to DHA administration *in vivo*, whereas an increase in ascorbic acid content stimulates growth (Cordoba-Pedregosa et al., 1996). Liso et al. (2004) reported that both root length and number of lateral roots of *Cucurbita maxima.* L were strongly inhibited in DHA – treated roots The DHA in the culture media could have caused the suppression of the vigor of sprouted shoot tips. Tagelsir et al. (2006) showed that $150mg1^{-1}$ citric acid and 100 mgl^{-1} ascorbic depressed the vigor of guava shoot tips below the control. In their study, Bhatia and Ashwath (2008) observed that ascorbic acid did not have any significant ($p < 0.05$) effect on shoot regeneration response of tomato (*Lycopersicon esculentum* Mill cv. Red coat) cotyledonary explants. Ascorbic acid was filter-sterilized in the culture media. Chrysovalantou et al. (2007) reported that regarding ascorbic acid, no clear stimulating effect on *in-vitro* rooting of the Peach rootstock GF-677 explants was found.

Contrary to these reports, Sharma and Chandel (1992)

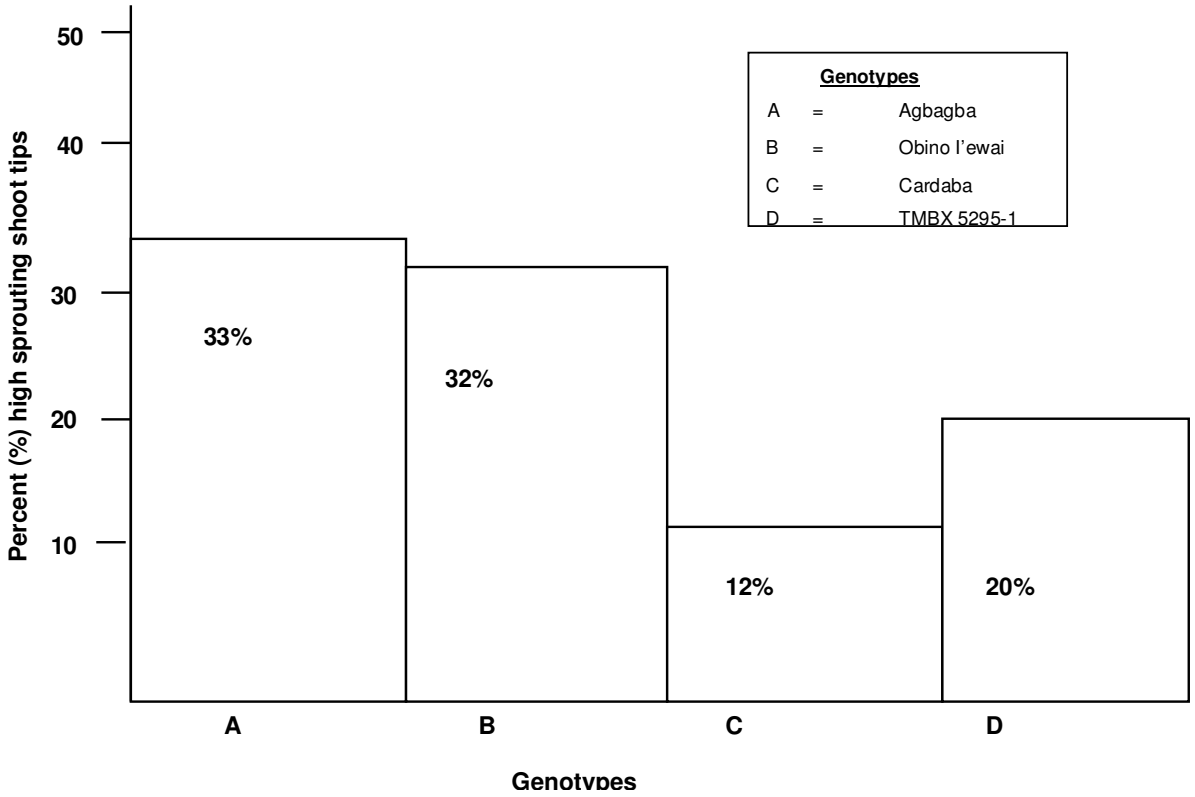

Figure 1. Percent distribution of high sprouting shoot-tips of *Musa* spp genotypes across seven ascorbic acid concentrations. Values were computed as number of high sprouting shoot-tips over total number of surviving shoot-tips per genotypes.

Table 3. Correlation matrix of level of blackening (LB) and sprouting (SP) responses of shoot-tips of four *Musa* spp genotypes, treated with ascorbic acid.

shoot-tips response	LB	SP
LB	1.00000	- 0.27209 * * *
		0.0011
SP	- 0.27209	1.00000
	0.0011	

Pearson Correlation Coefficients, N = 168. Prob. > /r/ under HO: Rho = O. * * *Significant at p < 0.001.

reported that addition of ascorbic acid was essential to induce sprouting of axillary buds of *Tylophora indica* (Burm. f). Merrill. Optimum multiplication was observed on MS medium containing 6-benzylammopurine (5.0 mgl^{-1}), ∝ -naphthalene acetic acid (0.5 mgl^{-1}) and ascorbic acid (100 mgl^{-1}). Flores *et al* (1998) demonstrated that solution containing 0.05% ascorbic and showed the best regeneration rate and the slowest oxidation rates during *in-vitro* regeneration of *Maytenus ilicifolia*. Prakash et al. (2006) observed that combinations of serial transfer technique and incorporation of antioxidants (250 mg/L <emphasis> ascorbic acid and 500 mg/L citric acid) into the culture medium helped to minimize medium browning and improved explant survival during shoot sprouting.

The results reveal a significant effect ($p < 0.05$) of genotype for shoot tip sprouting response. Ascorbic acid effect was also significant. The genotype × ascorbic acid interaction was also significant ($p < 0.01$) indicating differences in sprouting response of each of the four *Musa spp* genotypes, at each ascorbic acid concentration tested. Certain genotypes responded differently (in terms of yield of vigorous shoot tips) at certain ascorbic acid concentrations (Table 2). Agbagba produced significantly higher sprouting response, than Cardaba and TMBX

5295-1. Clearly, the plantain genotypes yielded more vigorous-growing healthy shoot tips than the bananas in this study.

Correlation coefficients between the blackening and sprouting responses of *Musa* spp shoot tip

The results from this study show an inverse/negative (r -0.27; $p < 0.001$) relationship between *in-vitro* blackening, due to oxidation of polyphenol exudates, and sprouting responses of *Musa spp* shoot tips, as revealed by Pearson Correlation Coefficients (Table 3). This suggests a negative effect of polyphenol blackening of the cultured *Musa* spp shoot tips used in this study, on their sprouting vigor.

A possible explanation for the observed inverse/ negative relationship between *in vitro* blackening and sprouting responses of the shoot-tips could be the formation of the protective layer around the damaged/excised explants tissues, resulting from blackening due to polyphenol exudation (Panis, 2000).This layer reduces the uptake of nutrients by the shoot- tips, thus contributing to the observed reduction in shoot-tip sprouting. Strossel et al. (2004) also reported that oxidation of phenolic compounds formed a barrier, around the banana tissues (shoot-tips) preventing nutrient uptake and hindering growth in the cultured shoot- tips. According to Cromptom and Preece (1988) exudation is likely a complex mixture of substances that can indeed be toxic. In-vivo, these compounds may function to protect injured tissue from invasion by various pests, in-vitro, these oxidation products are auto-inhibitory to the explants and may be viewed similar to waste products. Anirudh and Kanwar (2008) observed that explants of Wild Pear (*Pyrus pyrifolia* Burm .F) that turned dark-brown, or black, released compounds from the cut ends that led to browning of medium and did not sprout. However, Ettinger and Preece (1985) reported that visible exudation from shoot tips of Rhododendan "P.J.M" hybrids did not affect explant survival or growth; therefore rapid transfers were not necessary.

Phenolic compounds have also been shown to have both stimulatory and inhibitory effects on plant development. They can influence explants growth by promoting leaf expansion, stimulating callus growth and increasing rooting of cuttings. Saunders (1982) found that callus was three times more likely to arise from sugar beet petiole segments, which blackened, than on those which remained green.

Conclusion

Data from this investigation show that the incorporation of ascorbic acid as an antiblackening agent in modified MS (Murashige and Skoog, 1962) medium did not minimize explant blackening, nor enhance/ improve the sprouting of the cultured shoot- tips, and thus was not beneficial to the *Musa* spp shoot tips. The results obtained reveal that ascorbic acid, genotype and genotype x ascorbic acid interactions were significant sources of variation to explants' response (Tables 1 and 2). The data also suggest that explant blackening may have contributed to decreased sprouting of the cultured *Musa* spp shoot tips (Table 3).

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REFERENCES

Anirudh T, Kanwar JS (2008). Micropropagation of "Wild Pear" Pyrus pyrifolia, (Burm. F.) Nakai T. Explant establishment and shoot multiplication. Notulae Botanicae Horti. Agrobotania. Cluj 36 (1) 103-108.

Bhatia P, Ashwath N (2008). Improving the quality of *in-vitro* cultured shoots of Tomato (*Lycopersicum esculentum* Mill. Cv. Red Coat). Biotechnol., 7(2): 188-193.

Crompton ME, Preece JE (1988). Response of tobacco callus to shoot tip exudation from five species. Hortic. Sci., 23(1): 208-210.

Cordoba – Pedregosa, MC, Gonzalez-Reyes JA, Canadillas MS, Navas P, Cordoba,F (1996). Role of apoplastic and cell – wall peroxidases on the stimulation of root elongation by ascorbate. Plant Physiol. 112: 1119-1125.

Davis DG, Dusabek KE, Hoeraug RA (1974). *In-vitro* culture of callus tissues and cell suspensions from Okra *(Hibiscus esculentus* .L) and cotton (*Gossypium hirsutum); In-Vitro* (Rockville) 9: 395-398.

Ettinger TL, Preece JE (1985). Aseptic micropropagation of Rhododendron P.J.Hybrids. J. Hortic. Sci., 60: 269-274.

Flores R; Stefanello S; Franco E.T.H and Mantovani N (1998). Regeneracao *in-vitro* de EspinHeira-Santa (*Maytenus ilicifolia*. Mart). Rev. Bras de Agrociencia. 4(3): 201-205.

Gupta PP (1986*)* Eradication of mosaic disease and rapid clonal multiplication of bananas and plantains through meristem-tip culture. Plant Cell Tissue and Organ Cult., 6: 33-59.

He-O Y, Zhag-D. F, Wang RH (1995). A preliminary study on preventing browning of sucker explants from banana by ascorbic acid pre-treatment. J. South China Agricultural University. 16(3): 72-82.

Hegedus P, Phan CT (1982) Malformation chez le pommier M-26 cultive *in vitro*:action de la phloroidzine.*Ann. AC-FAS* 49:35.

Josekutty PC, Kilafwasru NT, George RA, Salik SC (2002). Micropropagation of endangered, vitamin A-rich bananas (*Musa troglodytarum)*. In: Proceedings of the 7th Annual meeting, IAPTC *& B,* University of New England, Armidale, Australia. Pp 173-176.

Khan V (1977). Some biochemical properties of polyphenol oxidase from two avocado varieties differing in their browning rate. J. Food Sci., 42:38-43.

Ko WH, Su CC, Chen CL, Chao CP (2009) Control of lethal browning of tissue culture plantlets of Cavendish banana cv. Formosana with ascorbic acid. Plant Cell Tissue Organ Cult., 96(2): 137-141.

Liso R, De Tuillo MC, Ciraci. S, Balestrini R, La Rocca N, Bruno L, Chiapetta A, Bitonti MB, Bonafante P, Arrigoni O (2004) Localization of ascorbic acid, ascorbic acid oxidase of Curcubita maxima.L., J. Exp. Bot., 55(408): 2589-2597.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Novak FJ, Afza R, Phadvibulya V, Hermelin T, Brunner H, Donini B (1986). Micropropagation and radiation sensitivity in shoot-tip cultures of banana and plantain. In: Nuclear Techniques and In-Vitro Culture for Plant Improvement: 167-174. International Atomic Energy Agency, Vienna.

Prakash E, Sha VK, Patan S, S Sreenivasa R, Thoguru JW, Meru ES (2006). Micropropagation of red sanders (*Pterocarpus sanalinus* L.) using mature nodal explants. J. For. Res., 11(5): 329-335 (Abstract).

Sharma N, Chandel KPS (1992) Effects of ascorbic acid on axillary shoot induction in *Tylophora indica* (Burm. F.) Merrill. Plant Cell, Tissue Organ Cult., 29: 109-113.

Simmonds NW (1966*). Bananas* Longman, London .UK.

Strosse HI, Van den Houwe, Banis P (2004) Banana Cell Tissue Culture - review In: Jain SM and Swennem R (eds) Banana Improvement: Cellular, Molecular Biology, and Induced Mutations. Science Publishers, Inc, Enfield .NH. USA. Pp1-12.

Tagelsir MI, Ei-Faith MM, Abdelghaffar ES (2006). Enhancement of growth and control of browning of tissue cultures of Guava (*Psidium guajava* .L.). J. Sci. Technol., 7(1): 74 -83.

Vuylsteke DR (1989). Shoot-tip culture for the propagation, conservation and exchange of Musa germplasm. International Board for Plant Genetic Resources, Rome. Pp 9,

Vuylsteke D, De Langhe EA (1985). Feasibility of *in-vitro* propagation of Bananas and Plantains. Trop. Agric. (Trinidad), 62(4):323-328.

Wu HC, Du Toit ES, Reinhardt CF (2007). Micrografting of Protea cynaroides Physiol. Plant, 15: 473-497.