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# Performance of Nodal Explants of *Pycnanthus angolensis* on different Ascorbic Acid Concentrations

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**Abstract:** Performance of nodal explants of *Pycnanthus angolensis* on different ascorbic acid concentrations was carried to determine Number of nodes, Mean number of callused explants and size of shoot, Nature of leaf, Days of sprouting (days) and Colour of callus formed. **Number of nodes:** Explants at 100mg/l and 500 mg/l produced the highest number of nodes (3), while control produced lowest number of nodes (1) **Mean number of callused explants and size of shoot:** 100mg/l produced highest number of callused explants at 4, 80% and shoots height at 1.00cm, B and C at 60%., 250mg/l, zero. Control performed poorly on number of nodes (1), shoot height (0.40cm) and callus formation at 20%. Abscission was observed at lowest concentration of ascorbic acid (100mg/l).**Nature of leaf:** A- containing 100mg/l at 17th day, C containing 500mg/l at 10th days while the AA-free medium produced leaves at 14th day. Early production of leaves 10th day was observed in 500mg/l while low concentration of AA containing 100mg/l produced leaves late at 17th day. **Days of sprouting (days):** Early days of sprouting occurred in ascorbic acid (AA) free medium in 13.6 days, followed by 14.2 days in C containing 500mg/l, A containing 100mg/l (14.4days) while B-containing 250mg/l of ascorbic acid had late days to sprouting in 20.0 days. Early days of sprouting occurred in ascorbic acid (AA) free medium this could be as a result of hormonal combinations and concentrations (2.0KIN+ 10 IBA). **Colour of callus formed** was mostly brown to chocolate and white. Generally it was revealed that there was no browning with an increase in concentration of ascorbic acid whereas, the study showed that the highest rate of colouration due to phenolic compounds was obtained in the culture medium without ascorbic acid (Control). The study established that the best results for controlling browning were obtained when *P. angolensis* nodal segments were cultured on MS medium supplemented with 2.0 mg/l KIN + 10mg/l IBA while incorporated with 500 mg/l of ascorbic acid.

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**Keywords:** Performance, Nodal Explants, *Pycnanthus angolensis* Ascorbic Acid, Concentrations

## 1. Introduction

Problem often encountered in mass micropropagation of *Pycnanthus angolensis* is the explant browning. Explants' browning is caused by oxidation of phenolic compounds resulting from injuries the isolation of explant (North *et al.,* 2010). It leads to the death of explants and failure of shoot regeneration (Pierik. 1987). This is due to quinones produced by oxidation of phenolic compounds (North *et al.,* 2010) are toxic (Ziv and Halevy, 1983) and it diffuses into culture media causing tissue necrosis and death of explants (Laukkanen *et al*., 1994). Phenolic browning of explant, media composition and culture conditions greatly affect shoot regeneration (Nayanakantha *et al.,* 2010). The concentration and combination of auxin and cytokininin in culture media is a key factor which determines successful shoot regeneration (North *et al.*, 2010).Besides growth hormone, other compounds such as ascorbic acid and activated carbon can be added to culture media. Both compounds are able to reduce the oxidation of phenolic compounds that can prevent death due to explant browning and increase shoot regeneration (Abdelwahd *et al*., 2008). Ascorbic acid is an antioxidant that is able to prevent or inhibit oxidation process (Babbar *et al.,* 2010). Besides its role as an antioxidant, ascorbic acid is involved in cell division and elongation Smirnoff. 1996). Research by Ko *et al*. (2009) on culture of banana cultivar Cavendish showed that ascorbic acid not only prevent death due to explants browning, but also can increase the number of shoots growing on explants. Activated charcoal is an essential component of plant tissue culture media. It is a strong adsorbent that can absorb toxic substances (Zhou *et al.,* 2010). Effect of light duration on regeneration shoots is also evaluated. The main objective of this research was to investigate

the effect of ascorbic acid in culture media on shoot regeneration on *Pycnanthus angolensis in vitro* culture.

## 2. Material and Methods

### Explants preparation and sterilization

Nodal stem segments were prepared from 4months old nursery-growing seedlings with leaves by discarding internodes and were then used as the experimental materials. The nodal stems and leaves were washed severally by immersing the explants into mixture containing 100% Chlorox with 2000litres of distilled water, rinsed under running water and were surface cleaned in 70% ethanol for 2 minutes, decanted and transferred to 50% Clorox (Sodium hypochlorite solution) for additional 5 minutes, and then rinsed thoroughly with sterile distilled water (Bello and Akinyele, 2016) in the laminar flowhood. Nodal stem segments were then excised into desired size aseptically and Leaf explants were then cut with mid rib and were individually inoculated in medium enhanced with levels of auxin NAA alone and combined with cytokinin modified with 10mg/l Indole butyric acid (IBA) (Table 1).

Table 1. Plant growth regulators of *P. angolensis*

| **CYTOKININ (Mg/l)** | | | | |
|---|---|---|---|---|
| | | **AUXINS** | | |
| **NAA (mg/l)** | | | | |
| **K** | **0** | **1** | **2** | **3** |
| A 0 | $K_0N_0$ | $K_0N_1$ | $K_0N_2$ | $K_0N_3$ |
| B 1 | $K_1N_0$ | $K_1N_1$ | $K_1N_2$ | $K_1N_3$ |
| C 2 | $K_2N_0$ | $K_2N_1$ | $K_2N_2$ | $K_2N_3$ |
| D 3 | $K_3N_0$ | $K_3N_1$ | $K_3N_2$ | $K_3N_3$ |
| **BAP (B)** | | | | |
| E 0 | $B_0N_0$ | $B_0N_1$ | $B_0N_2$ | $B_0N_3$ |
| F 1 | $B_1N_0$ | $B_1N_1$ | $B_1N_2$ | $B_1N_3$ |
| G 2 | $B_2N_0$ | $B_2N_1$ | $B_2N_2$ | $B_2N_3$ |
| H 3 | $B_3N_0$ | $B_3N_1$ | $B_3N_2$ | $B_3N_3$ |

Where:

K=Kinetin, BAP (B) = Benzyl Amino Purine, NAA= Naphthalene Acetic Acid

### Data collection and analysis

The NL, NR and number of nodes (NN) were counted visually, callus formed was also counted and days of sprouting were recorded to determine the influence of ascorbic acid concentrations.

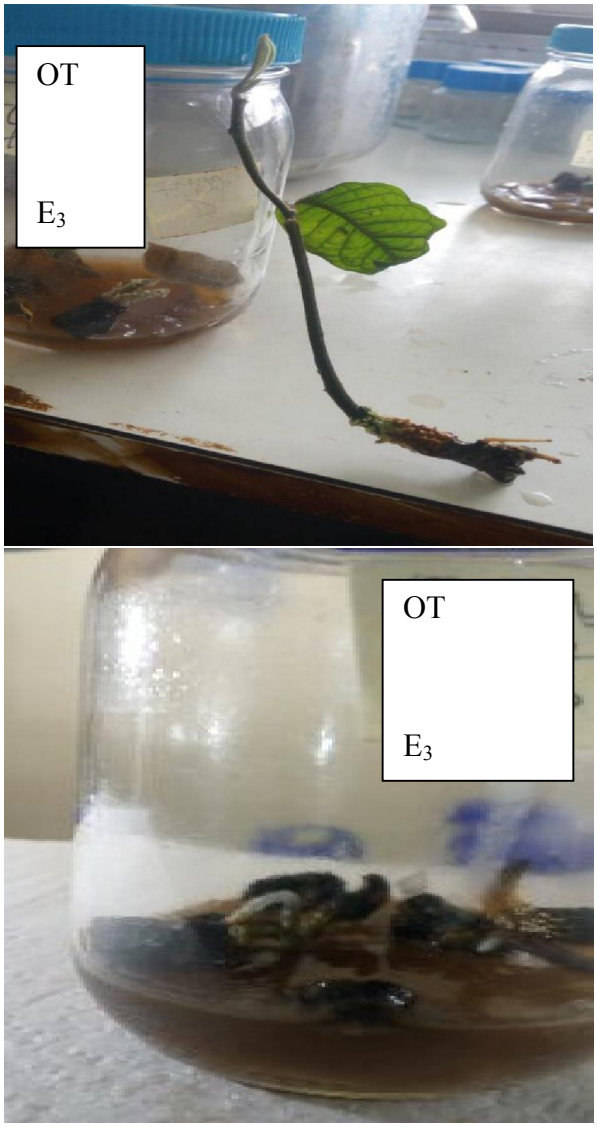


**Plate 1:** Media browning

## 3. Results

Explants on ascorbic acid free media represented as D produced a higher number of shoots than those on media with addition of ascorbic acid in concentrations of 100mgl-1 (A), 250mgl-1 (B) and500 mgl-1 (C). Ascorbic acid is known to decay rapidly in plant tissue culture media. Ascorbic acid is oxidized by reactions catalysed by Cu (II) and Fe (III), both of which are component of Murashige and Skoog media (Elmore *et al*., 1990). Also, light and pH accelerated the decay (Elmore *et al*., 1990). Ascorbic acid was most stable at pH 4.5 (Elmore *et al*., 1990) but pH 5.7 was used in this media culture for the study. Since ascorbic acid is an ephemeral component of culture media, it is quite possible that none exist when explant is inoculated.

**Number of nodes (NN)**

Explants inoculated at 100mg/l and 500 mg/l concentration of ascorbic acid produced the highest number of nodes (3) (NN), while ascorbic free medium produced lowest number of nodes (1) (Tables 2 and 3).

**Mean number of callused explants and size of shoot**

The sprouting response of explants tested with ascorbic acid was markedly increased, compared to control explants. Low ascorbic acid concentration at 100mg/l produced highest mean number of callused explants at 4, 80% and shoots height at 1.00cm Followed by B and C at 60%. At 250mg/l ascorbic acid medium, nodal explants did not shoot at all. Ascorbic acid free medium performed poorly on number of nodes (1), shoot height (0.40cm) and callus formation at 20% (Table 2). This could be as a result of absence of ascorbic acid to enhance growth. Callus formation occurred mostly at the base, axillary and over all the explants (Plate 2). It occurred at base only in 100mg/l, axillary in ascorbic acid free medium while it occurred both at the base and axillary in 250mg/l. Abscission was observed at lowest concentration of ascorbic acid (100mg/l).

**Nature of leaf**

Leaf production occurred from explants cultured on A- containing 100mg/l at 17$^{th}$ day, C containing 500mg/l at 10th days while the AA-free medium produced leaves at 14$^{th}$ day. Early production of leaves 10$^{th}$ day was observed in maximum concentration of ascorbic acid containing 500mg/l while low concentration of AA containing 100mg/l produced leaves late at 17$^{th}$ day (Table 2). The result indicated that higher concentration of ascorbic acid produced leaf earlier than low concentration of ascorbic acid. Two leaves were produced in A, C and D produced (1) (Tables 2 and Plate 3):

**Days of sprouting (days)**

Early days of sprouting occurred in ascorbic acid (AA) free medium in 13.6 days, followed by 14.2 days in C containing 500mg/l, A containing 100mg/l (14.4days) while B-containing 250mg/l of ascorbic acid had late days to sprouting in 20.0 days (Table 3). Early days of sprouting occurred in ascorbic acid (AA) free medium this could be as a result of hormonal combinations and concentrations (2.0KIN+ 10 IBA). Late sprouting could be due to quantity of ascorbic acid used. No root formation in all the explants used for the study but callus was mostly formed (Tables 3).

**Colour of callus formed**

Under treatment of nodal explants with different concentrations of ascorbic acid, colour of callus produced from nodal explants of *P.angolensis* was mostly brown to chocolate and white (Table 2 and Plate 2). Generally it was revealed that there was no browning with an increase in concentration of ascorbic acid whereas, the study showed that the highest rate of colouration due to phenolic compounds was obtained in the culture medium without ascorbic acid (Control) (Plates 3 and 4). The study established that the best results for controlling browning were obtained when *P. angolensis* nodal segments were cultured on MS medium supplemented with 2.0 mg/l KIN + 10mg/l IBA while incorporated with 500 mg/litre of ascorbic acid.

**Table 2: The growth related parameters of Ascorbic acid effects on Nodal explants of *P. angolensis***

| Treatment (mg/ L) | Size of shoot (cm) | Size of root (cm) | Nature of leaf | Number of Node | Number of callus formed/ colour | Days of sprouting | % of callus formed |
|---|---|---|---|---|---|---|---|
| A (100) | | | | | | | |
| 1 | 1.00 | 0No root | 2 | 3 | 0 | 17 | Abscission at 23rd day |
| 2 | 0.5 | 0 | 0 No leaf | 0 | 1 (brown) | 10 | Base |
| 3 | 0 | 0 | 0 | 0 | 1 (white) | 10 | Base |
| 4 | 0 | 0 | 0 | 0 | 1 (brown) | 15 | Base |
| 5 | 0 | 0 | 0 | 0 | 1 (cream) | 10 | Base |
| B (250) | | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 1 (white) | 59 | Base |
| 2 | 0 | 0 | 0 | 0 | 0 | 13 | Axillary |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | Bud |
| 4 | 0 | 0 | 0 | 0 | 1 (brown) | 13 | Base |
| 5 | 0 | 0 | 0 | 0 | 1 (brown) | 15 | Base |
| C (500) | | | | | | | |
| 1 | 1.00 | 0 | 2 | 3 | 0 | 10 | Base |
| 2 | 0 | 0 | 0 | 0 | 1 (chocolate) | 14 | Base |
| 3 | 0.2 | 0 | 0 | 0 | 0 | 16 | Base |
| 4 | 0 | 0 | 0 | 0 | 1 (wet brown) | 16 | All over |
| 5 | 0 | 0 | 0 | 0 | 1 (cream) | 15 | Base |
| D (Control) | | | | | | | |
| 1 | 0.6 | 0 | 2 | 1 | 0 | 14 | Axillary bud |
| 2 | 0.5 | 0 | 0 | 0 | 0 | 16 | Axillary bud |
| 3 | 0.1 | 0 | 0 | 0 | 0 | 13 | Axillary bud |
| 4 | 0 | 0 | 0 | 0 | 0 | 10 | Axillary bud |
| 5 | 0 | 0 | 0 | 0 | 1 (brown) | 15 | Axillary bud |

**Table 3: The Ascorbic acid effects on Nodal explants of *P. angolensis***

| Treatment (mg/ L) | Nature of leaf | Number of Node | Number of callus formed/ (%) | Mean size of shoot(cm) | Mean Days of sprouting (Days) |
|---|---|---|---|---|---|
| A (100) | 2 | 3 | 4 (80) | 1.00 | 14.40 |
| B (250) | 0 | 0 | 3(60) | 0.00 | 20.00 |
| C (500) | 2 | 3 | 3 (60) | 0.60 | 14.20 |
| D (Control) | 2 | 1 | 1(20) | 0.40 | 13.60 |

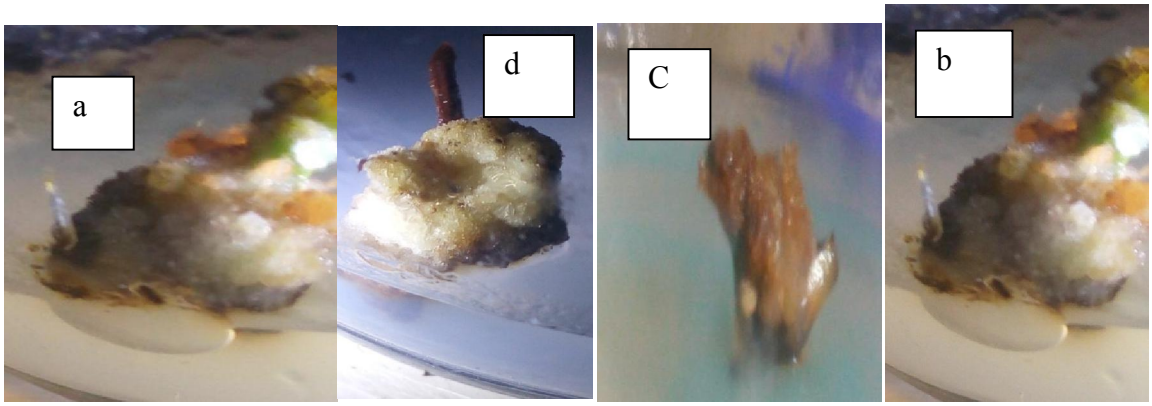


**Plate 2: Performance of nodal explants of *P. angolensis* on different ascorbic acid concentrations**

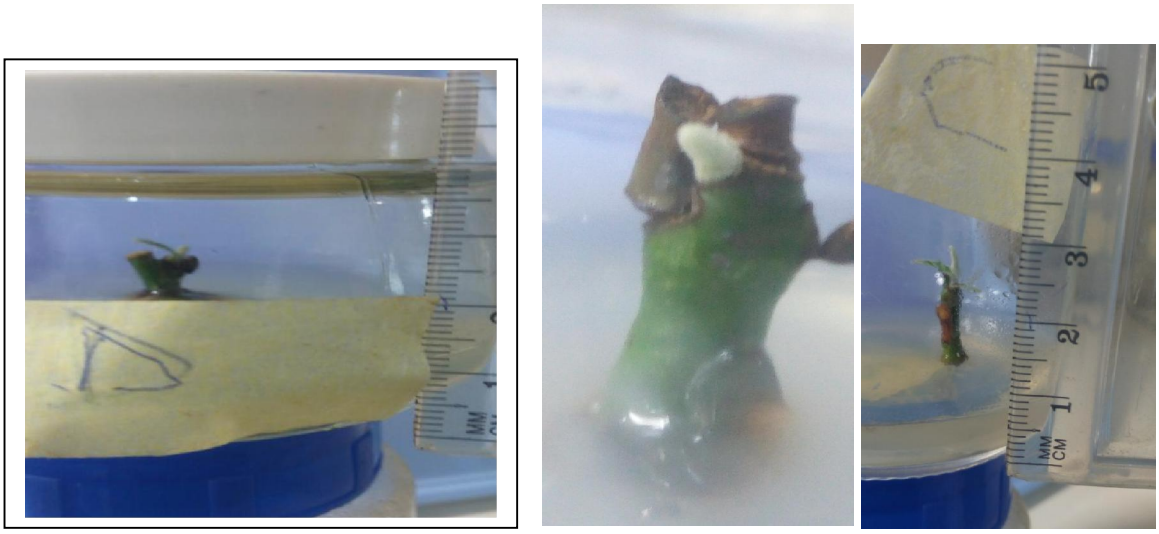

**Plate 3: Nodal segment showing growth of axillary bud on MS medium (free from browning) containing 2.0mg/l NAA+10.0 mg/l IBA + ascorbic acid Concentrations (a) 100mg/l, (b) 500mg/l (d) 0 mg/l (control) of ascorbic acid).**

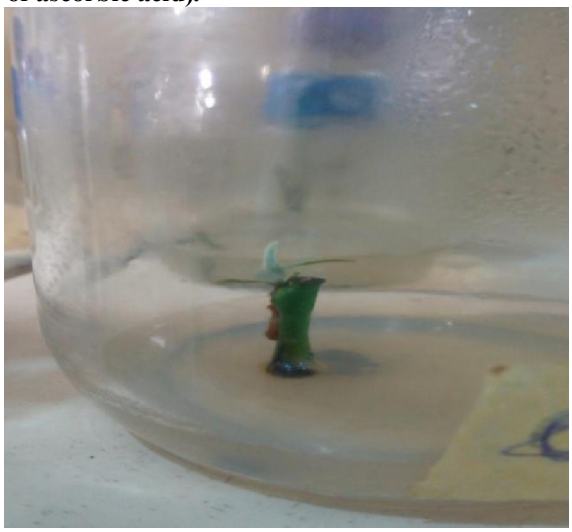

**Plate 4: Shoot regeneration from nodal explants at 500mg/l of Ascorbic acid**

## 4. Discussions

Explants on ascorbic acid free media (D) produced a higher number of shoots than those on media with addition of ascorbic acid in concentrations of 100mgl-1 (A), 250mgl-1 (B) and500 mgl-1 (C). This result is in agreement with Chikezie (2012) who reported that ascorbic acid significantly reduced the frequency of high sprouting vigorous shoot tips of *Musa spp.* Ascorbic acid is known to decay rapidly in plant tissue culture media. Ascorbic acid is oxidized by reactions catalysed by Cu (II) and Fe (III), both of which are component of Murashige and Skoog media (Elmore *et al*., 1990). The sprouting response of explants tested with ascorbic acid was markedly increased, compared to control (0 mg/l) and explants with Low ascorbic acid concentration (100mg/l) produced highest mean number of callused explants (4, 80%) and shoot height (1.00cm). followed by B and C (60%). At 250mg/l ascorbic acid medium, nodal explants did not shoot at all. Ascorbic acid free medium performed poorly on number of nodes (1), shoot shoot height (0.40cm) and callus formation (20%) (Table 3). This could be as a result of absence of ascorbic acid to enhance growth. In contrary to Chikezie (2012) who reported that the sprouting response of explants tested with ascorbic acid was markedly reduced or suppressed compared to control explants.

Incorporation of antioxidant in culture medium was shown to control tissue browning in this study. This is similar to Onuoha *et al*. (2011). Antioxidant was also tested to check their ability to control browning. Antioxidant treatment at different concentrations (ascorbic acid @ 100, 250 and 500 mg/l) given to the culture medium resulted in reduced intensity of browning. Similarly, use of antioxidants was also reported by Murkute *et al.* (2004). Therefore, it is evident from this study that addition of ascorbic acid (AA) effectively reduces explant browning and tissue death, thus improving success rate in micropropagation. The study showed that ascorbic acid not only prevent death due to explant browning, but also can increase the number of shoots, nodes growing on explants and this is in agreement with Ko *et al*. (2009) who 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 while Ascorbic acid free medium performed poorly on number of nodes (1), shoot height (0.40cm) and callus formation (20%) (Table 1). This could be as a result of absence of ascorbic acid to enhance growth. Ascorbic acid is involved in cell division and elongation and this result is in consonant with Smirnoff, (1996).

Early production of leaves on the 10$^{th}$ day was observed in maximum concentration of ascorbic acid (500mg/l) while low concentration of AA (100mg/l) produced leaves late (17$^{th}$ day) (Table 2). The result indicated that higher concentration of ascorbic acid produced leaf earlier than low concentration of ascorbic acid.

The study showed that browning occured in ascorbic acid free medium. Thus, as observed in this study, the ascorbic acid was useful and effective in managing the problem of browning caused by phenolic exudates and hence improving plant survival *in vitro* (Abdelwahd *et al*., 2008).

The results of the present study showed that almost all explants on the medium without ascorbic acid, browned extremely. This may be attributed by the fact that exudation of phenolics is a natural mechanism in plants not only that but also many plants produce dark phenolic substances after wounding and the accumulation of phenolic compounds in medium adversely affects the growth and survival of *in vitro* explants as seen in Plate 4.20 where there was leaf fall as a result of browning. This is in consonant with Roussos and Pontikis (2001) and Arnaldos, (2001) who showed that accumulation of these compounds leads to browning and possibly death of the explants.

## 5. Conclusion

Data from this investigation show that the incorporation of ascorbic acid as an antiblackening agent in modified MS (Murashige and Skoog, 1962) medium minimized explant blackening/ browning and also enhanced/ improved the sprouting of the cultured nodal explants, and thus was beneficial to the *Pycnanthus angolensis* nodal explants. Thus, the study revealed that for successful control of oxidative lethal browning the concentration of antioxidant ascorbic acid is of great importance.

Ascorbic acid free medium performed poorly on number of nodes (1), shoot height (0.40cm) and callus formation (20%) and this could be as a result of absence of ascorbic acid to enhance growth. Early production of leaves 10$^{th}$ day was observed in maximum concentration of ascorbic acid (500mg/l) while low concentration of AA (100mg/l) produced leaves late (17$^{th}$ day). The result indicated that higher concentration of ascorbic acid produced leaf earlier than low concentration of ascorbic acid. Under treatment of nodal explants with different concentrations of ascorbic acid, colour of callus produced from nodal explants of *P. angolensis* was mostly brown, chocolate and white.

**References**


[1]. North, J. J.,Ndakidemi, P.A &. Laubscher, C.P. (2010). The potential of developing an *in vitro* method for propagating Strelitziaceae. *African Journal of Biotechnology* 9(45): 7583--7588.

[2]. Pierik, R. L. M. (1987). *In vitro* culture of higher plant. Martinus Nijhoff Publishers, Dordrecht: v + 344 pp.

[3]. Ziv, M. &Halevy, H. A., (1983). Control of oxidative browning and *in vitro* propagation of Strelitzia reginae. *Horticulture Science* 18(4): 434--436.

[4]. Laukkanen, H., Haggman, H., Kontunen-Scoppia, S., & Hohtola, A., (1999). Tissue browning of *in vitro* culture of Scot pine: Role of peroxidase and polyphenol oxidase. *Physiological Plantarum* 106: 337--343.

[5]. Nayanakantha, N. M. C., Singh, B.R., & Kumar, A., (2010). Improved culture medium for micropropagation of *Aloe vera* L. *Tropical Agricultural Research & Extension* 13(4): 87--93.

[6]. R. Abdelwahd, N. Hakam, M. Labhilili & S.M. Udupa. 2008. Use of an adsorbent and antioxidants to reduce the effects of leached phenolics in in vitro plantlet regeneration of faba bean. *African Journal of Biotechnology* 7(8): 997--1002.

[7]. N. Babbar, H.S. Oberoi, D.S. Uppal & R.T. Patil. 2010. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. Food Research International 44: 391--396.

[8]. N. Smirnoff. 1996. The function and metabolism of ascorbic acid in plants. *Annals of Botany* 78: 661—669

[9]. W. H. Ko, C.C. Su, C.L. Chen & C.P. Chao. 2009. Control of lethal browning of tissue culture plantlets of Cavendish banana cv. Formosana with ascorbic acid. *Plant Cell Tissue Organ Culture* 96: 137--141.

[10]. Bin Zhou, Xinfang Wei, Rongting Wang & Jingming Jia. 2010. Quantification of the enzymatic browning and secondary metabolites in the callus culture system of *Nigella glandulifera* Freynet Sint. *Asian Journal of traditional medicines* 5(3):109--116


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