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Review Article

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# Control of browning in plant tissue culture: A review

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## ABSTRACT

Browning is one of the severe problems in plant tissue culture that hampers successful in vitro propagation of plants especially woody and perennial plants. In order to control the browning problem, different efforts has been made in vitro such as presoaking of explants in antioxidant solution, incorporation of antioxidants in to medium, culturing in the dark period and frequent subculturng of explants. Presoaking of explants in antioxidant solution like polyvinylpyrolidone (PvP) and ascorbic acid (AC) is one of the most frequently used. Incorporation of antioxidants such as 0.2-0.5g/l PvP and 15-250mg/l ascorbic acid in to MS medium are commonly used to control browning in different plants and explants followed by activated charcoal,citrc acid,MES,andAIP. Moreover, frequent sub culturing and incubation of explants in the dark period is the other alternative. This review article includes study of previous and current research achievements in a comprehensive way on the different methods to control browning problem in plant tissue culture and suggests further optimization for successful control of browning when using the same or different crops as well as explants.

**KEYWORDS:** Activated charcoal, antioxidants, browning, phenolic compounds, polyvinylpyrolidone

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## INTRODUCTION

Browning in plant tissue culture refers to a phenomenon in which the explants release brown substances or phenolics to the medium from its own tissues in the course of dedifferentiation and/or re-differentiation (George and Davies, 2008; Shen, 2005). 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 (Onuoha *et al.*, 2011).

Phenolic compounds are secreted from wounded regions of explants as a defense response (Lorenzo *et al.*, 2001), and oxidation of these compounds results in browning of culture media and plant tissues (Jones and Saxena, 2013). Phenolic compounds are oxidized by polyphenol oxidases (PPOs) to their quinine derivatives and further oxidized to form the pigment melanin, which is found in organisms and is responsible for browning reactions (Selvarajan *et al.*, 2008). Besides PPO, phenylalanine ammonia lyase (PAL) and peroxidase (POD) are also responsible for browning arising from wound as a catalyser of polyphenol biosynthesis (Krishna *et al.*, 2008).

While phenolic compounds are generally present in healthy plant tissues and can accumulate in specialized cell types (Beckman, 2000), they are produced in greater abundance and/or released as a defense response, especially following tissue wounding or stress (Beckman, 2000; Dixon and Paiva,1995) The majority of tissue culture protocols involve wounding the material in order to remove explants and culturing them in potentially stressful environments; often eliciting the production and release of phenolic compounds. As a result, this natural defense response can lead to the accumulation of toxic compounds that ultimately damage or kill plant cells and tissues.

In addition, accumulation of ethylene in the culture medium as a result of low gas exchange is another cause of browning of explants during in vitro culture (Gerszberg *et al.*, 2015). Other types of phenolic exudates appear at the end of incubation period and are apparently products of dying cells (Seneviratne and Wijesekara, 1996). The phenolic exudation is aided by light and is autocatalytic. For example, tissues cultured in the dark often display lower levels of browning than those grown in the light (Krishna *et al.*, 2008; Lainé and David, 1994; Ochoa-Alejo and Ramirez-Malagon, 2001).

The prevalence of browning varies among species, cultivars, the physiological state of the plant/tissue, and size of explants and age of explants (Dineshbabu *et al.*, 2002; Tian, 2008; Ahmad *et al.*, 2013; Ozyigit, 2008). Oxidative browning is a common problem in plant tissue culture; resulting in reduced growth (Krishna *et al.*, 2008; Uchendu *et al.*, 2011), lower rates of regeneration or recalcitrance (Laukkanen *et al.*, 2000; Aliyu, 2005; Parthasarathy *et al.*, 2007), and can ultimately lead to cell/tissue/plant death (Krishna *et al.*, 2008; Aliyu, 2005; Toth *et al.*, 1994; Panaia *et al.*, 2000; Tabiyeh *et al.*, 2006). Different

attempts has been made to eliminate browning problem in woody plant species like pre-socking of explants in antioxidants solution, incorporation of oxidants into medium, incubation of culture in to dark period and frequent sub culturing of explants (Ahmad *et al.*, 2016). However, the effectiveness of these methods varies from species to species and physiological conditions of plant.

Therefore, this review study provide comprehensive ideas on the type, effect of different browning agents on avoiding of explants with particular regards to effectiveness of the agent, concentrations as well as the type of plant/variety used and how it is applied to the explant.

## Pre-Soaking of Explants in Antioxidant Solution

The successful use of antioxidant applied during explants preparation to prevent lethal browning is reported by several authors (Table 1). Titov *et al.* (2006) reported that 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. Similarly, Ngomuo *et al.* (2014) reported that treating the explants with 1.2 g/l of ascorbic acid during explants preparation controlled the extent of lethal browning of local *Musa* spp.cv. Mzuzu. Chavan *et al.* (2000) reported that pre-socking of apical and axillary buds in 0.5% polyvinylpyrrolidone (PVP) in combination with 3% sucrose for 30 min was found effective for browning control in mango. According to Ahmed *et al.* (2016),pre-soaking of nodal explant in 1 g/l activated charcoal for 5 hours significantly reduced media browning in micro propagation of Guava (*Psidium guajava* L.). Abdelwahd *et al.* (2008) reported that treating seeds of faba bean with 1000 mg/l PVP solution for 1 h, followed by culturing in Murashige and Skoog medium (MS medium) supplemented with ascorbic acid (1 mg/l) or activated charcoal (10 g/l), greatly reduced lethal browning in explants and improved shoot regeneration. Babaei *et al.* (2013) reported that Pre-treating shoot tip of Curculigo *latifolia* with PVP, ascorbic acid and citric acid (0.1%) for 9 hr was the best technique for reducing browning. Cai *et al.* (2020) reported that dipping the explants in 0.5g/L NaCl solution was effective in suppressing browning of the petal explants of herbaceous *Paeonia Lactiflora* Pall. 'Festival Maxima. Onuoha *et al.* (2011) reported that presoaking/ pretreatment of aux bud of plantain (*Musa paradisiaca*) in 0.1-0.5 mg/mL of potassium citrate and citrate (K-C: C) for 2 hr prevented browning.

## Incorporation of Antioxidants into Medium

The addition of 0.3g/L polyvinylpyrrolidone (PVP) to the medium can effectively inhibit browning followed by 0.2 mg/L ascorbic acid in stem segments of 'Hongyang' kiwifruit (Chai *et al.*, 2018) (Table 2). Jones and Saxena (2013) reported that addition of aminoindane-2-phosphonic acid (AIP) up to 10 μM into culture media resulted in significant reductions in visual tissue browning of *Artemisia annua*. Similarly, $AgNO_3$ is a potent ethylene inhibitor and its presence in culture medium has been reported to inhibit browning in vitro shoot production in many plant species (Haque *et al.*, 2015; Kabir *et al.*, 2013; Mookkan and Andy, 2014). In addition to its role as an antibrowning agent, several reports indicate that it is effective in regulating morphogenesis and induces multiple

**Table 1: Antioxidants and their Concentrations used in presoaking of explants**

| Antioxidant | Concentration | Variety | Explants used | Reference |
|---|---|---|---|---|
| PVP+Sucrose | 0.5%+3% | Mango | Apical and axillary buds | Chavan *et al.* (2000) |
| PVP+AA[b]+CA | 0.1% | Curculigo latifolia | Shoot tip | Babaei *et al.* (2013 |
| AA[b] | 0.125 | Musa spp. | Shoot tip | Titov *et al.* (2006) |
| AA[b] | 1.2g/l | Musa spp. | Shoot tip | Ngomuo *et al.* (2014) |
| PVP | 1000 mg/l | faba bean | cotyledon | Abdelwahd *et al.* (2008) |
| NaCl | 0.5 g/L | *Paeonia Lactiflora* Pall | Petal | Cai *et al.* (2020) |

PVP=polyvinylpyrolidone, AA[b]=ascorbic acid, CA=citric acid

**Table 2: Incorporation of antioxidants into medium**

| Antioxidants | Concentration | Variety/plant name | Explants used | Reference |
|---|---|---|---|---|
| Pvp | 0.3g/l | Hongyang kiwifriut | Stem segments | Jiufeng *et al.*, 2018 |
| AA[b] | 0.2g/l | Hongyang kiwifriut | Stem segments | Jiufeng *et al.*, 2018 |
| AIP | 10M | Artmesia annua | - | Jones and Saxena (2013) |
| AA[b] | 15mg/l | Okra | Node | Mohammed *et al.* (2018) |
| MES | 1g/l | *Sideritis trojana* | - | Corduk and Aki (2011) |
| PVP | 0.2g/l | *C86-56* | | Shimelis *et al.* (2015) |
| PVP | 0.3g/l | *C86-12* | | Shimelis *et al.* (2015) |
| AA[b] | 15mg/l | Okra (Abelmoschus esculentus L.) | cotyldonary node | Muhammad *et al.* (2028) |
| PVP | 0.5 g·L-1 | *Paeonia Lactiflora* Pall | Petal explants | Xuan *et al.* (2019) |
| AA[b] | 200-250 mg/litre | *Brahylaena huillensis.* | Node | Ndakidemi *et al.* (2014) |
| AA[c] | 200 mg/l | *Punica granatum* L | Node | Singh and Patel (2016) |
| AA[b]+CA | 100 mg/l+50 mg/l | Sideritis trojana bornm | Leaf | Corduk and Aki (2011) |

A A[b]=ascorbic acid, AA[c]=activated charcoal, CA=citric acid, MES=morpholine ethane sulfonic acid, AIP=aminoindane-2-phosphonic acid, PVP=polyvinylpyrolidone

shoot production (Fernandez *et al.*, 1999; Kumar *et al.*, 2016). Activated charcoal adsorbs the free phenolic compounds secreted by explants into the culture medium (Thomas, 2008) and prevents tissue browning.

According to Irshad *et al.* (2018), supplementation of 15 mg/L ascorbic acid to basal media minimized the phenolic secretion, improved culture quality, and survival from cotyldonary node explant of Okra (Abelmoschus esculentus L.). Similar effects of ascorbic acid in tissue culture medium were reported by (Ko *et al.*, 2009) during the micro propagation of Cavendish banana and in V. faba (Abdelwahd *et al.*, 2008). AA has been shown to inhibit the browning of cultured tissues and improve morphogenesis in Cavendish banana (Ko *et al.*, 2009) and Brachylaena huillensis (Ndakidemi *et al.*, 2014). In addition, this compound has an essential role during plant morphogenesis (Horemans *et al.*, 2000) and is involved in cell division, cell differentiation, and cell elongation of apical meristems of Aloe barbadensis Mill (Kaviani, 2014) and cotyledonary nodes of Vicia faba (Abdelwahd *et al.*, 2008). Ascorbic acid contains ascorbate that has a direct inactivating effect on PPO (Ndakidemi *et al.*, 2014). In addition, AA converts colorless o-quinones resulting from PPO action back to diphenols and prevents browning (Martinez and Whitaker, 1995). According to Titvo *et al.* (2006), AA scavenges oxygen radicals to prevent the oxidation of phenolic compounds in wounded tissues, thereby reducing tissue browning

Corduk and Aki (2011) reported that the addition of 1.0 g/L morpholine ethane sulfonic acid (MES) into MS medium significantly reduced browning in *Sideritis trojana*. Shimeles *et al.* (2015) reported that Murashige and Skoog medium supplemented with 0.2 gL-1and 0.3 gL-1 of Polyvinylpyrrolidone has gave 100% and 80% survived explants of C86-56 and C86-12 sugarcane genotypes respectively after 30 days of culturing. The results of study conducted by Cai *et al.* (2020) demonstrated that dipping excised explants in a 0.5 g·L-1 NaCl solution, adding 0.5 g·L-1 PVP to the medium, storing planted explants at 4 °C for 24 h, and transferring planted explants to the same fresh medium after 24 h could effectively inhibit browning in Petal explants of *Paeonia Lactiflora* Pall. Peach can be successfully propagate in media supplemented with 50m/l ascorbic acid, 20 mg/l stabs vitamin mixture (Miller *et al.*, 1982).

Huang CM *et al.* (2003) found 60% and 40% browning free explants for two different sugarcane genotypes at a PVP concentration of (0.5-1) gL-1. This could be due to genotypic differences among the materials used. MS medium supplemented with 0.5 gL-1 PVP resulted in successful initiation of large embryogenic callus ranging from 80 to 90% which were free of browning (Michael, 2007). This difference may be happened due to the difference in genotypes and the type of *in vitro* regeneration path used.

Ndakidemi *et al.* (2014) reported that incorporation of 200-250 mg/litre of ascorbic acid into the medium significantly controlled lethal browning in nodal culture of *Brahylaena huillensis*. Supplementation of 0.5% PVP into culture medium prevented explants browning in callus culture of nodal explant of *Spartium junceum L.* (Taghizadeh and Dastjerd, 2021). According to Corduk and Aki (2011) reported that adding a combination of 100 mg/l ascorbic acid and 50 mg/l citric acid to the murashige and skoog (MS) medium was found as the most effective treatment during micro propagation of Sideritis trojana bornm, an endemic medicinal herb of Turkey. According to Singh and Patel (2016), addition of 200 mg/L activated charcoal into the medium was found quite effective to minimize browning problem in nodal segment of mature explant *Punica granatum* L s.

Assis *et al.* (2018) reported that 300 mg L PVP and in conjunction with 2 g L-1 activated charcoal ascorbic acid, is recommended for minimizing the effects of phenol oxidation in nodal segments of *E. pyriformis*. Pre-socking of apical and axillary buds in 0.5% polyvinylpyrrolidone (PVP) + 3% sucrose for 30 min was found effective for browning control in mango (Chavan *et al.*, 2000). Patil *et al.* (2011) found best results in browning control with 150 mg/L ascorbic acid and 100 mg/L citric acid in pomegranate.

The addition of 15 mL L-1 ascorbic acid to the MS culture medium was efficient in preventing oxidation in banana tree explants *Musa* spp. (Anicezio, 2012). Sanyal *et al.* (2005) reported that adding the antioxidants cysteine and silver nitrate improved the maximum recovery of chickpea plantlets *in vitro* after agro-inoculation. Similarly, Strosse *et al.* (2004) reported that addition of cysteine to the growth media reduced explant blackening in banana tissue culture. Sharada *et al.* (2003) and Prajapati *et al.* (2003) found that adding activated charcoal to the culture medium prevented the effect of leached phenolics that hindered regeneration of *Celastrus paniculatus* and *C. orchioides* respectively,

## Frequent Sub Culturing

Quick transfer of explants within the same spell or to fresh medium 2 or 3 times, at short intervals, is the simplest and fairy successful method to protect the explants from the detrimental effect of oxidative browning (Kotomari and Murashige, 1965). Frequent transfer of explants within the same medium or into fresh medium fairly prevents *in vitro* browning of explants (Kotomory and Murashige, 1965; Block and Lankes, 1996). During this period the cut ends of explant may become sealed up and the leaching of phenolics stops. Murkute *et al.* (2003) reported that sub culturing of explant consecutively thrice at an interval of 24 hours controlled browning completely in pomegranate. Muralikrishna (1988), Singh and Khawale (2006) and Singh *et al.* (2011) claimed that the subsequent transfer of explants on fresh medium resulted in complete disappearance of browning in nodal segment explants of mature plants in pomegranate. Singh and Patel (2016) reported sub culturing of nodal explants of *Punica granatum* L.twice, at the first day and third day of inoculation was effective in browning control. Pushpraj and Patel (2016) result revealed, that the most effective browning control was observed in sub culturing of nodal explants twice, at the first day and third day of inoculation, which also found better in establishment of explants of pomegranate.

### Incubation of Culture into Dark Period

Presence of light and high temperature raise browning rate by increasing the enzyme activity

(Dobránszki and Teixeira, 2010). For example, tissues cultured in the dark often display lower levels of browning than those grown in the light (Krishna *et al.*, 2008; Laine and David, 1994; Ochoa-Alejo and Ramirez-Malagon, 2001). MS media supplemented with 1.6 mgl-1 IAA and 4.0 mgl-1 BAP without ascorbic acid and activated charcoal in darkness for 4 weeks was the most suitable media for shoot regeneration (Nisyawati & Kusuma, 2013). Keeping the cultures initially in the dark may also help to reduce browning problem (George and Sherington, 1984) by preventing or reducing the activity of the enzymes concerned with both biosynthesis and oxidation of phenols Titov *et al.* (2006).

## CONCLUSION

The purpose of this review was to systematically analysis the previous research done to control browning problem in plant tissue culture. It is clear from the research reviewed that browning of culture media is a critical problem that hampers successful in vitro propagation of plants especially woody and perennial plants. A number of authors have studied different attempts for browning control; such as presoaking of explants in antioxidant solution, incorpoation of antioxidants in to MS medium, frequent subcultuirng of explant and incubation into dark period. Moreover, it is essential that the mother plant should be grown in the greenhouse or lath house than field grown so that the browning intensity can be minimized.

## REFERENCES


Abdelwahd, R., Hakamu, N., Labhilili, M., & Udupa, S. M. (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.

Ahmad, I., Hussian, T., Ashraf, I., Nefees, M., Marayam, M. R., & Iqbal, M. (2013). Lethal effects of secondary metabolites on plant tissue culture. *American-Eurasian Journal of Agricultural & Environmental Sciences, 13*(4), 539-547.

Ahmad, I., Jaskani, M. J., Nafees, M., Ashraf, I., & Qures, R. (2016). Control of media browning in micro propagation of guava (*Psidium guajava* l.). *Pakistan Journal of Botany, 48*(2), 713-716.

Aliyu, O. M. (2005). Application of tissue culture to cashew (Anacardiumoccidentale. p. L.) breeding: An appraisal. *African Journal of Biotechnology, 4*(13), 1485– 1489.

Anicezio, L. C. (2012). Efeito de antioxidantes e descontaminantes no estabelecimento de explantes de bananeira (*Musa* spp) *in vitro*. *Uniciências, 16*(1), 9-16.

Assis, F. A., Rodrigues, F. A., Pasqual, M., Assis, G. A., Luz, J. M. Q., Anoni, F., Costa, I. D., Costa, B. N. S., & Soares, J. D. R. (2018). Antioxidants in the control of microorganism contamination and phenol oxidation in *eugenia pyriformis*. *Bioscience Journal, 34*(1), 49-58. https://doi.org/10.14393/BJ-v34n1a2018-36311

Beckman, C. H. (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiological and Molecular Plant Pathology, 57*(3), 101–110. https://doi.org/10.1006/pmpp.2000.0287

Cai, X., Wei, H., Liu, C., Ren, X., Thi, L. T., & Jeong, B. R. (2020). Synergistic Effect of NaCl Pretreatment and PVP on browning suppression and callus induction from petal explants of *Paeonia Lactiflora* Pall. 'Festival Maxima'. *Plants (Basel, Switzerland), 9*(3), 346. https://doi.org/10.3390/plants9030346

Chai, J., Gao, Y., Dong, Y., Kong, L., & Zhang, Y. (2018). Browning Treatment in Tissue Culture of 'Hongyang' Kiwifruit. *IOP Conference Series Materials Science and Engineering, 452*(2), 02207. https://doi.org/10.1088/1757-899X/452/2/022075

Chavan, S. S., Ranade, S. S., Deore, A. C., Deshpande, R. S., & Dhonukshe, B. L. (2000). Cloning of Alphonso mango through vegetative explants. *Annals of Plant Physiology, 14*(2), 178-181.

Corduk, N., & Aki, C. (2011). Inhibition of browning problem during micro propagation of *Sideritis trojana* bornm, an endemic medicinal herb of Turkey. *Romanian Biotechnological Letters, 16*(6), 6760- 6765.

Dineshbabu, K., Sathiamoorthy, N., Chezhiyan, N., Kapil, D., & Singh, N. (2002). *In vitro* establishment of gynodioecious papaya variety CO-7 influenced by age of mother plants. *Orissa Journal of Horticulture, 30*(1), 5-7.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell, 7*(7), 1085–1097. https://doi.org/10.1105/tpc.7.7.1085

Dobránszki, J., & Teixeira da Silva, J. A. (2010). Micropropagation of apple—A review. *Biotechnology Advances, 28*(4), 462–488. https://doi.org/10.1016/j.biotechadv.2010.02.008

Fernandez, S., Michaux-Ferriere, N., & Coumans, M. (1999). The embryogenic response of immature embryo cultures of durum wheat (*Triticum durum* Desf.): Histology and improvement by $AgNO_3$. *Plant Growth Regulation, 28*, 147–155.

George, E. F., & Davies, W. (2008). Effects of the Physical Environment. E. F. George, M. A. Hall, & G. J. D. Klerk (3rd Ed.). *Plant Propagation by Tissue Culture* (Vol. 1, pp. 423–464) Dordrecht, The Netherlands: Wiley-Blackwell.

George, E. F., & Sherington, P. D. (1984). Plant propagation by tissue culture (pp. 690). England: Exegentics Limited.

Gerszberg, A., Hnatuszko-Konka, K., & Kowalczyk, T. (2015). In vitro regeneration of eight cultivars of *Brassica oleracea* var. capitata. *In vitro Cellular & Developmental Biology. Plant*, *51*(1), 80–87. https://doi.org/10.1007/s11627-014-9648-7

Haque, M., Siddique, A. B., & Islam, S. S. (2015). Effect of silver nitrate and amino acids on high frequency plants regeneration in barley (*Hordeum vulgare* L.). *Plant Tissue Culture and Biotechnology, 25*(1), 37–50. https://doi.org/10.3329/ptcb.v25i1.24124

Irshad, M., Rizwan, H. M., Debnath, B., Anwar, M., Li, M., Liu, S., He, B., & Qiu, D. (2018). Ascorbic acid controls lethal browning and pluronic f-68 promotes high-frequency multiple shoot regeneration from cotyldonary node explant of okra (Abelmoschus esculentus L.). *Hortscience*, *53*(1), 183–190. https://doi.org/10.21273/HORTSCI12315-18

Jones, A. M., & Saxena, P. K. (2013). Inhibition of phenylpropanoid biosynthesis in *Artemisia annua* L.: a novel approach to reduce oxidative browning in plant tissue culture. *PloS one, 8*(10), e76802. https://doi.org/10.1371/journal.pone.0076802

Kabir, K. M. R., Kwon, S.W., & Park, Y. J. (2013). Application of cobalt chloride and silver nitrate for efficient microspore culture of *Brassica rapa* ssp. *Plant Tissue Culture and Biotechnology*, *23*(1), 1–10. https://doi.org/10.3329/ptcb.v23i1.15554

Kaviani, B. (2014). Effect of ascorbic acid concentration on structural characteristics of apical meristems on *in vitro Aloe barbadensis* Mill. *Acta Scientiarum Polonorum, Technologia Alimentaria, 13*, 49–56.

Ko, W. H., Su, C. C., Chen, C. L., & Chao, C. P. (2009). Control of lethal browning of tissue culture plantlets of *Cavendish banana* cv. Formosana with ascorbic acid. *Plant Cell Tissue and Organ Culture, 96*(2), 137-141. https://doi.org/10.1007/s11240-008-9469-7

Kotomory, S., & Murashige, T. (1965). Some aspects of aseptic propagation of orchids. *American Orchid Society Bulletin, 34*, 484-489.

Krishna, H., Sairam, R. K., Singh, S. K., Patel, V. B., Sharma, R. R., Grover, M., Nain, L., & Sachdev, A. (2008). Mango explants browning: Effect of ontogenic age, mycorrhization and pre-treatments. *Scientia Horticulturae, 118*(2), 132-138. https://doi.org/10.1016/j.scienta.2008.05.040

Kumar, G. P., Sivakumar, S., Siva, G., Vigneswaran, M., Kumar, T.S., & Jayabalan, N. (2016). Silver nitrate promotes high-frequency multiple shoot regeneration in cotton (Gossypium hirsutum L.) by inhibiting ethylene production and phenolic secretion. *In Vitro Cellular & Developmental Biology - Plant, 52*, 408–418.

Lainé, E., & David, A. (1994). Regeneration of plants from leaf explants of micropropagated clonal *Eucalyptus grandis*. *Plant Cell Reports*, *13*(8), 473–476. https://doi.org/10.1007/bf00231970

Laukkanen, H., Rautiainen, L., Taulavuori, E., & Hohtola, A. (2000). Changes in cellular structures and enzymatic activities during browning of Scots pine callus derived from mature buds. *Tree physiology*, *20*(7), 467–475. https://doi.org/10.1093/treephys/20.7.467

Lorenzo, J. C., de los Angeles Blanco, M., Pelaez, O., Gonzalez, A., Cid, M., Iglesias, A., Gonzalez, B., Escalona, M., Espinosa, P., & Borroto, C. (2001). Sugarcane micropropagation and phenolic excretion. *Plant Cell, Tissue and Organ Culture, 65*, 1–8.

Martinez, M. V., & Whitaker, J. R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science & Technology, 6*,195–200.

Miller, G., Coston, D., Denny, E., & Romeo, M. (1982). *In vitro* propagation of 'Nema-guard' peach rootstock. *HortScience, 17*, 194.

Mookkan, M., & Andy, G. (2014). AgNO3 boosted high-frequency shoot regeneration in Vigna mungo (L.) Hepper. *Plant Signaling & Behavior*, *9*(10), e972284. https://doi.org/10.4161/psb.32165

Muralikrishna, A. (1988). Development of micropropagation strategies in pomegranate, grape and guava cultivars. *Physiologia Plantarum, 15*, 473-497.

Murkute, M., Patil, S., & Singh, S. K. (2004). *In vitro* regeneration in pomegranate cv. Ganesh from mature plant. *Indian Journal of Horticulture, 61*(3), 206-208.

Ndakidemi, C. F., Mneney, E., & Ndakidemi, P. A. (2014). Effects of ascorbic acid in controlling lethal browning in in vitro culture of *Brahylaena huillensis* using nodal segments. *American Journal of Plant Sciences 5*(1), 187–191. https://doi.org/10.4236/ajps.2014.51024

Ngomuo, M., Mneney, E., & Ndakidemi, P. (2014). Control of lethal browning by using ascorbic acid on shoot tip cultures of a local *Musa* spp. (Banana) cv. Mzuzu in Tanzania. *African Journal of Biotechnology, 13*(16), 1721-1725. https://doi.org/10.5897/AJB2013.13251

Ochoa-Alejo, N., & Ramirez-Malagon, R. (2001). *In vitro* chili pepper biotechnology. *In Vitro* Cell Dev Biol Plant. *In Vitro Cellular & Developmental Biology – Plant, 37*(6), 701–729. https://doi.org/10.1007/s11627-001-0121-z

Onuoha, I. C., Eze, C. J., & Unamba, C. I. (2011). *In vitro* prevention of browning in plantain culture. *OnLine Journal of Biological Sciences, 11*(1), 13-17.

Ozyigit, I. I. (2008). Phenolic changes during *in vitro* organogenesis of cotton (*Gossypium hirsutum* L.) shoot tips. *African Journal of Biotechnology, 7*(8), 1145-1150. https://doi.org/10.5897/AJB07.396

Panaia, M., Senaratna, T., Bunn, E., Dixon, K. W., & Sivasithamparam, K. (2000)

Micropropagation of the critically endangered Western Australian species, *Symonanthus bancroftii* (F Muell.)L. Haegi (Solanaceae). *Plant Cell, Tissue and Organ Culture, 63*, 23–29. https://doi.org/10.1023/A:1006457624282

Parthasarathy, V. A., Keshavachandran, R., Nazeem, P., Girija, D., John, P. S Peter, K. V. (2007). High tech propagation of horticultural crops-accent on recalcitrance. Recent trends in horticultural biotechnology, Vol. I and II (pp. 85–91). ICAE national Symposium on Biotechnological Interventions for Improvement of Horticultural Crops: Issues and Strategies. Vellanikkara, Kerala, India: New India Publishing Agency.

Patil, V. M., Dhande, G. A., Thigale, D. M., & Rajput, J. C. (2011). Micropropagation of pomegranate (*Punica granatum* L.) 'Bhagava' cultivar from nodal explant. *African Journal of Biotechnology, 10*(79), 18130-18136. https://doi.org/10.5897/AJB11.1437

Prajapati, H. A., Patel, D. H., Mehta, S. R., & Subramanian, R. B. (2003). Direct *in vitro* regeneration of *Curculigo orchioides* Gaertn., an endangered anticarcinogenic herb. *Current Science, 84*(6), 747-749.

Sanyal, I., Singh, A. K., Kaushik, M., & Amla, D. V. (2005). *Agrobacterium* mediated transformation of chickpea (*Cicer arietinum* L.) with *Bacillus thuringiensis* cry1Ac gene for resistance against pod borer insect *Helicoverpa armigera*. *Plant Science, 168*, 1135-1146. https://doi.org/10.1016/j.plantsci.2004.12.015

Selvarajan, E., Veena, R., & Kumar, N. M. (2008). Polyphenol oxidase, beyond enzyme browning, a review. In J. Singh, D. Sharma, G. Kumar & N. Sharma (Eds.),

*Microbial Bioprospecting for Sustainable Development* (pp. 203-222) Singapore: Springer.

Sharada, M., Ahuja, A., & Kaul, M. K. (2003). Regeneration of plantlets via callus cultures in *Celastrus paniculatus* Willd–A rare endangered, medicinal plant. *Journal of Plant Biochemistry and Biotechnology, 12*, 65-69.

Shen, H. L. (2005). *Plant Tissue Culture* (Vol. 3, pp. 256-261) Beijing, China: China's Forestry Press.

Shimelis, D., Bantte, K., & Feyissa, T. (2015). Effects of Polyvinyl Pyrrolidone and Activated Charcoal to Control Effect of Phenolic Oxidation on *In Vitro* Culture Establishment Stage of Micropropagation of Sugarcane (*Saccharum Officinarum L.*). *Advances in Crop Science and Technology, 3*(4), 184. https://doi.org/10.4172/2329-8863.1000184

Singh, N. V., Singh, S. K., & Patel, V. B. (2011). *In vitro* culture establishment studies on pomegranate. *Indian Journal of Horticulture, 68*(3), 307-311.

Singh, P., & Patel, R. M. (2016). Factors affecting *in vitro* degree of browning and culture establishment of pomegranate. *African Journal of Plant Science, 10*(2), 43-49. https://doi.org/10.5897/AJPS2013.1119

Singh, S. K., & Khawale, R. N. (2006). Plantlet regeneration from nodal segments of pomegranate (*Punica granatum*) cv. Jyoti. Plant Biotechnology and its applications in tissue culture (Chapter 12, pp. 107-113).

Strosse, H., Van den Houwe, I., Banis, P. (2004). Banana Cell Tissue Culture – review. In: S. M. Jain & Swennem, R. (Eds.), *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations* (pp. 1-12) Enfield, USA: Science Publishers, Inc.

Tabiyeh, D. T., Bernard, F., & Shacker, H. (2006). Investigation of Glutathione, Salicylic Acid and GA∼ 3 Effects on Browning in Pistacia vera Shoot Tips Culture. *Acta Horticulturae, 726*, 201-204.

Taghizadeh, M., & Dastjerd, M. G. (2021). Inhibition of browning problem during the callogenesis of *Spartium junceum* L. *Ornamental Horticulture, 27*(1), 68-77. https://doi.org/10.1590/2447-536X.v27i1.2230

Tian, D. (2008). *Container production and post-harvest handling of Lotus (Nelubo) and micropropagation of herbaceous peony (Paeonia)*. Ph.D. Thesis, Auburn University, USA.

Titov, S., Bhowmik, S. K., Mandal, A., Alam, M. S., & Uddin S. N. (2006). Control of phenolic compound secretion and effect of growth regulators for organ formation from Musa spp. cv. Kanthali floral bud explants. *American Journal of Biochemistry and Biotechnology, 2*(3), 97–104. https://doi.org/10.3844/ajbbsp.2006.97.104