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ATTEMPTS TO CONTROL PHENOLIC EXUDATION FROM NODAL EXPLANTS OF *MANGIFERA INDICA* DURING *IN VITRO* CULTURE

[1,*]Ranjana Roy Mishra, [2]Veena Agrawal, [2]Shrish C. Gupta

[1]Department of Botany, Kalindi College, University of Delhi, India.
[2]Department of Botany, University of Delhi, India.

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ABSTRACT

The release of phenolic compounds from many plants causes death of explants within a few days of *in vitro* culture. This happens due to the toxic effect of phenolics released in media. These phenolics thus need to be controlled for establishing explants inasepticculture. In the present study, phenolic release in the culture media was encountered during nodal culture of *Mangifera indica*. The exudation of phenolic compounds from nodal explants caused browning within 7-8 days and ultimately they died in 25-30 days of culture. Various strategies were applied to control phenolics released in the media so that cultures could be established. Different experiments using antioxidants, adsorbing agents and liquid media were conducted and the results are presented here.

Keywords: Mango, Phenolics, in vitro culture, Micropropagation. Tissue culture, Nodes

Abbreviations: AA- Ascorbic Acid, CA- Citric acid, HgCl2 – Mercuric Chloride, MS- Murashige & Skoog, PVP- Polyvinylpyrollidone, DOI- Days of inoculation.

INTRODUCTION

There are many plants which are considered recalcitrant and unresponsive in tissue culture. Mango is one of them due to release of phenolics after cutting and wounding. Phenolic exudation in high quantity during excision, medium browning, very slow *in vitro* growth and endogenous bacterial contamination has made mango a very difficult crop to work with (Thomas & Ravindra 1997; Raghuvanshi & Srivastava, 1995). Wounding during plant collection results in phenolics exudation from the cut ends. These phenolics oxidize to highly toxic black quinones and cause browning of the medium and ultimately the explant die on culture media. Similar problems have been reported by earlier workers using leaf (Raghuvanshi & Srivastava, 1995) and shoot explants (Chandra *et al.,* 2004; Krishna *et al.,* 2008, Petri *et al.,* 2021; Conde *et al.,* 2023).

Successful reports on mango micropropagation has been reported with nucellus explants (Litz *et al.,* 1982; Jana *et al.*, 1994; Ara *et al.,* 1999) but little success has been achieved with other explants. There are many advantages of explants other than nucellus. Regeneration protocols using other explants like leaf and nodal segments ensure their availability all round the year while nucellar explants are available only during fruiting season. During the present study nodal explants were taken for *in vitro* culture of mango cv. 'Amrapali'. Attempts were made to control phenolic exudation during tissue culture. A number of combinations of adsorbing agents and antioxidants were tried with little success.

MATERIALS & METHODS

The nodal explants used during the study were excised from trees of *Mangifera indica* L. cultivar "Amrapali", growing in the mango gardens of Indian Agriculture Research Institute, New Delhi. The explants were directly collected either in ascorbic acid solution (100 mg l^{-1}), citric acid solution (150 mg l^{-1}) or PVP solution (500 mg l^{-1}).

***Corresponding Author: Ranjana Roy Mishra,**
1Department of Botany, Kalindi College, University of Delhi, India.

Sterilization and inoculation of explant

The experimental plant materials collected from trees were washed in tap water for 30 min to remove dust and contaminants. Then they were washed in 1% Polysan (a germicide detergent) for 30 minutes. The nodal explants were then soaked in 0.3% Bavistin (a fungicide) solution for 25-30 minutes. Then they were washed with tap water and the explants were soaked in 0.1% $HgCl_2$(Qualigens) for 10 minutes in laminar flow cabinet. After washing 3-5 times with autoclaved water, explants were kept in 95% ethanol for 2 min. The explants were finally washed with autoclaved sterile water two to three times.

After sterilization process, the nodal explants were inoculated on semi solid medium. Murashige and Skoog (1962) basal medium was used for majority of the studies. Explants were also cultured on medium with adsorbing agents like PVP (Polyvinylpyrrolidone) or antioxidants (citric acid and ascorbic acid) to control phenolics. Sucrose (3%) was used as a source of carbohydrate. The medium was gelled with 0.8% agar (Qualigens) and at pH adjusted to 5.8 before autoclaving. The cultures were maintained at a temperature of 25± 2°C. Photoperiod for 16-h was maintained inside culture room where cultures were kept. Each experiment was repeated thrice with 24 explants.

Elimination of phenols

Six sets of experiments were carried out to control phenolics.

I The explants were kept on shaker at 150 rpm before surface sterilization and inoculation
   (a) in aqueous solution of citric acid (150 mg l^{-1}) for 4 hrs.
   (b) in aqueous solution of ascorbic acid (100 mg l^{-1}) for 4 hrs.

II Shaking the explants on shaker at 150 rpm before surfacesterilization and inoculation
   (a) in citric acid solution (150 mg l^{-1}) for 24 hrs.
   (b) in ascorbic acid solution (100 mg l^{-1}).

III Polyvinyl pyrollidone (PVP) treatment (500 mg l^{-1}).

This included 8 different treatments which are as follows:

a. Explants cultured on PVP(500mg l^{-1}) adjuvanted to MS basal medium and culture kept in light for 16h photoperiod.
b. Explants cultured on PVP(500mg l^{-1}) adjuvanted to MS basal medium and culture kept in dark throughout the experiment.
c. PVP pretreatment (Agitation of explants in aqueous solution of PVP for 5 hrs. on shaker and cultures kept in light for 16 h photoperiod).
d. PVP pretreatment (Agitation of explants in aqueous solution of PVP for 5 hrs. on shaker and cultures kept in dark).
e. PVP in medium + PVP pretreatment - Culture kept in light for 16h photoperiod.
f. PVP in medium + PVP pretreatment -Cultures kept in dark
g. Control (no pretreatment, no PVP in medium)- Culture kept in light for 16h Photoperiod.
h. Control (no pretreatment, no PVP in medium)- Cultures kept in dark.

IV Citric Acid treatment (150 mg l^{-1}).

This also included 8 different treatments as described above:

a. CA in medium (light)
b. CA in medium (Dark)
c. CA pretreatment (light)
d. CA pretreatment (Dark)
e. CA in medium + pretreatment (light)
f. CA in medium + pretreatment (Dark)
g. Control (light)
h. Control (dark)

V Shaking of explants (after surface sterilization) in MS liquid media for 4 hrs before culturing on semi- solid media.

VI Frequent sub culturing explants cultured in citric acid (150 mg l^{-1}) adjuvanted medium for 7 days and subsequently transferring them on MS basal semi-solid media at intervals of 7 days for 3 weeks.

RESULTS

Phenolic release was observed on culture media soon after inoculation. Cut ends and medium started turning brown within a day of culture(Fig. 1A-C). With the passage of time the release of phenols increased and explants turned black and ultimately died after 25-30 days of inoculation (DOI). The quantity of phenolics exudation was observed more in explants excised from fresh flushes than those collected from old parts.

Negligible difference was observed between control and treated explants on shaking in antioxidant solutions like ascorbic acid or citric acid. Treatment with PVP solution also showed almost same results and not much difference was observed between explants kept in light and dark. PVP pretreatment of explants or culturing on PVP adjuvanted medium also showed same results but the rate of phenolics exudation was less than ascorbic acid or citric acid treated explants. Dark treatment did not make any difference.

In some of the cultures, explants showed swelling (Fig. 1A), falling of petioles (Fig. 1B) and opening of bracts at nodes (Fig. 1C) but finally they turned brown and ultimately died after 25-30 days of culture.

Figure 1 A-C: Response of nodal explant of mango during *in vitro* culture. (A) Nodal explant showing browning of the cut ends and swelling at lower end after 25 days of Inoculation on PVP containing medium. (B) Nodal explant showing petiole falling and browning of medium due to phenolics exudation from the lower cut end. (C) Nodal explant showing bracts opening (arrow) during in vitro culture on PVP containing medium.

Citric acid treatment also showed same results as mentioned for PVP treatment. In this set of experiments, some of the explants were shifted to light after keeping in dark for one week. But there was no difference in cultures kept in light or dark. A few explants were sub cultured at 1 week interval for 3 weeks after keeping in citric acid medium for 1 week. This treatment increased the bacterial infection at each subculture and ultimately led to browning and death of explants within 3 weeks. Liquid media pretreatment for 4 hrs (liquid media changed at 1 hour interval) showed some positive result as most early response in terms of swelling of explant, falling of petiole and opening of bract was seen with this treatment. Explants swelled and showed bracts opening after 10 or 11 days of inoculation compared to cultures inoculated on PVP containing medium where this response was observed after 25-30 DOI. The results of the various treatments are summarized in table 1.

Table 1: Response of nodal explants of mango cultivar 'Amrapali' during in vitro culture to different treatments for control of phenolics.

Treatment	Phenolics exudation	Response of nodal explant
Control (Light)	+++++++	Browning, death after 25-30DOI
Control (Dark)	+++++++	Browning, death after 25-30DOI
Shaking in AA solution (4 h, 24 h)	+++++++	Browning, death after 25-30DOI
Shaking in CA solution (4 h, 24 h)	+++++++	Browning, death after 25-30DOI
PVP treatment		
PVP in medium (light, Dark)	+++++++	Browning, death after 25-30DOI
Shaking in PVP solution (5 h, light, dark)	+++++++	Browning, death after 25-30DOI
Shaking in PVP solution plus PVP in medium (Light, Dark)	+++++	Swelling of explants, Bracts opening after 25-30 DOI
Citric Acid treatment		
Citric acid in medium (light, Dark)	+++++++	Browning, death after 25-30DOI
Shaking in citric acid solution (5 h, light, dark)	+++++++	Browning, death after 25-30DOI
Shaking in citric acid solution plus PVP in medium(Light, Dark)	+++++	Browning, death after 25-30DOI
Frequent sub-culturing	+++++++	Browning, death after 25-30DOI
Shaking of explants in MS liquid medium for 4 h (Medium changed at every 1 h)	++++	Swelling of explants, Bracts opening after 10-11 DOI

DISCUSSION

The main problem encountered in present study was release of phenolics from cut ends of explants in culture medium. There are previous reports of using certain methods to reduce release of phenols from the cultured explants (Raghuvanshi & Srivastava, 1995) and shoot explants (Thomas & Ravindra 1997; Krishna *et al.,* 2008, Petri *et al.,* 2021; Conde *et al.,* 2023).

Several of the previously reported methods were tried to control phenolic in present study (Litz, 1984; Dewald *et al.,* 1989; Yang & Ludders, 1993; Raghuvanshi & Srivastava, 1995; Ara *et al.,*1999). Some of these include treatment of explants using liquid shaker prior to culture, augmenting the medium with antioxidants and adsorbing agent like PVP, frequent sub culturing etc. Cultures were kept in dark to prevent oxidation of phenols to quinones. The explants were pretreated with antioxidants and adsorbing agents but, none of the combination(s) gave satisfactory results.

Shaking the culture in liquid media prevents the accumulation of released phenols at cut ends. In the present study, liquid media pretreatment was somewhat stimulatory in controlling phenolics and enhanced response of explants in terms of swelling and falling of petioles and opening of bracts.

Antioxidants like citric acid and ascorbic acid prevents oxidation of phenols to quinones. Frequent sub culturing also helps in avoiding accumulation of phenols at cut ends.

In the present study, all these methods and chemicals were not effective in controlling the release of phenols from cut ends of nodal explants. The explants started turning brown within a few days of culture and ultimately died. In a few explants, nodal cut ends showed swelling after some days but no further response was observed thereafter. Swelling of cut ends was observed more on explants cultured on PVP containing medium . Some nodal explants showed falling of petioles and opening of bracts on PVP containing medium but no further response was observed in any explant.

Out of all treatments tried to control phenolics, culturing nodal explants on PVP containing medium showed little response like falling of petiole and opening of bracts , So, it was concluded that PVP containing medium will be used in further experiments to control phenolics.

It was also observed that release of phenolic compounds was less in the explants excised from older portion of the tree as compared to that in fresh flushes. So second conclusion was to do further experiments with older explants.

Mango is known to be recalcitrant plant in tissue culture studies. The present study was done to develop a micro-propagation protocol through nodal explants of mango. Attempts were done to control phenolic exudation in medium which was a major deterrent in showing response. Little or no response was observed by the combinations tried in present study. The present study can be helpful for researchers working on the control of phenolics and development of micropropagation protocolthrough nodes.

There are many reports of propagation of mango *via* somatic embryogenesis through nucellar explants but a handful through other explants. Mango is a popular fruit of India and its propagation with nodal explants will be helpful in the generation of large number of plants in short time and space. To the best of our knowledge, till date, satisfactory protocol to control phenolic exudation and culture establishment from nodal explants is not available. Experiments are underway to control phenolics release and develop a protocol for micro propagation via nodal explants of *Mangifea indica*.

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