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IN VITRO TISSUE BROWNING AND CALLUS INDUCTION IN *IPOMOEA BERAVIENSIS* VATKE

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ABSTRACT

Abstract: Cell proliferation, callus induction and morphogenesis are limited by explant produced toxic metabolites during *in vitro* tissue culture technique. Secondary metabolites, released or synthesized during leaf explant culture of *Ipomoea beraviensis* resulted in browning of tissue and without induction of callus. Initial Repeated fresh cultures and addition of activated charcoal reduced the exudation of Secondary metabolites and resulted in proliferative callus induction.

IndexTerms*: Tissue browning, repeated culture, activated charcoal, young and mature explants*

INTRODUCTION

Ornamental climbers with bright, colourful flowers are important components of gardens, parks and such other amenity areas (Poornima and Shivamurthy, 2005). Woody Ornamental perennials are attractive subjects in bio-aesthetic planning. Plant tissue culture has the ability to provide different techniques of propagating ornamentals on a large scale under limited space. *In vitro* culture studies were undertaken for the mass propagation of *I. beraviensis, a w*oody, flowering climbers with densely pubescent young and glabrescent yellowish older stems and attractive large leaves with mucronate apex along with long petiole. Corolla is very bright crimson with base of tube orange-yellow and funnel-shaped.

The only set back in the establishment of culture of *I. beraviensis,* was browning of medium due to leaking of metabolites and reduced culture response of explants for cell multiplication and no induction of callus formation. Tissue blackening results through the action of copper containing oxidase enzymes like polyphenol oxidases and tyrosinase (Lerch, 1981) which are synthesized or released and presented with oxidative conditions when tissues are wounded. In the present study, browning of culture media *in vitro* is due to phenolic compounds exudation. Test for phenols confirmed the presence of phenolic compounds. To achieve growth and morphogenesis of tissue, neutralization or elimination of phenolic compounds is most important step in culture system. Different trials were conducted to minimize the exudation of phenolic compounds.

MATERIAL AND METHODS

Test for phenols

Test for phenols was conducted taking 1 ml of extract and adding 0.5 ml of ferric chloride which turned olive brown color indicating presence of phenols (Gibbs, 1974, Fiegel, 1960)

Explants selection and Sterilization

Young, mature leaf and inter nodal segments of stem were collected from healthy garden growing climber. The explants (2.0 × 2.0 cm-leaf, 2.5 cm-internodes) were soaked in 1% detergent solution, washed thoroughly under tap water repeatedly without making injury. Explants were Surface sterilized using 1.0% w/v bavistin for 4 min followed by 0.1% w/v mercuric chloride for 4.0 min which was freshly prepared. The sterilants were removed by 2-3 times washings with sterile tap water. Under aseptic condition explants were again rinsed 3-4 times with sterile distilled water. The explants were trimmed to appropriate size, leaf- 1.5 cm^2acrossand , stem 1.5 -2.0 cm) before explants inoculation.

Culture medium and Physical conditions

MS medium (Murashige and Skoog, 1962) supplemented with (0.5 mg/l) of either 2, 4-D (2, 4-dichlorophenoxy acetic acid or NAA (Naphthalene acetic acid) was used for inoculation of explants. The medium contained 3.0% w/v sucrose as carbohydrate source and 0.8% w/v agar for gelling and pH5.6 was adjusted before autoclaving. The cultures were incubated at 22.0± 2.0 °C and under 14 hr photoperiod using cool day light fluorescent tubes (50 M $m^{-2}s^{-1}$). Two sets of experiments were performed having six culture tubes. In first set, leaf, stem – young and mature explants which were cultured were repeatedly transferred to fresh media with an interval of 3 days for three subsequent cultures. In the second set, the explants were inoculated on MS medium supplemented with activated charcoal (3.0 % w/v).

RESULTS AND DISCUSSION

Cultures	Young explants		Mature explants	
	Leaf	Inter node	Leaf	Inter node
Without treatment	++	++	--	--
Repeated cultures	++	++	++	++
Activated charcoal	++	++	++	++

++ Reduced phenolics, -- no change

The explants of *I. beraviensis,* showed controlled phenolic exudation when they repeatedly cultured on fresh media. Similar result

has been noticed in *Adenocalymma allicea* and *Hiptage madoblota* (Poornima and Shivamurthy, 2006) Muriithi et al. (1982) reduced the possible inhibitory effect of oxidation of phenolic compounds by frequent transfer of Fig shoot tips on fresh media at seven days of interval. Young tissues show reduced browning or less prone to browning on excision than older ones (George and Sherrington, 1984). Young leaves of Oil palm proved to be the best explants towards reduced browning (Rabechault and Martin, 1976). In the present study, only young leaf and internodal explants showed good response towards the control of phenolics and subsequent callus proliferation in the culture system compared to mature explnats.

Amin and Jaiswal, 1988 and Gupta et al. (1980) have reported that use of polyvinyl pyrrolidone (PVP) and polyvinyl poly pyrrolidone (PVPP) in case of guava and teak respectively have neutralized phenolics. Cresswell and Nitsch, 1975) have partly overcome the difficulty of browning by keeping the nodal explants of *Eucalyptus* in sterile water for 02 - 03 hrs before inoculation. Wang and Haung, 1976 have reported the beneficial effects of activated charcoal on plant tissue and organ culture. Inclusion of activated charcoal (0.1%) for multiple shoot induction and elongation was reported by Amerson et al. (1988) and Boulay, 1979. Addition of 3.0 gm/l activated charcoal (AC) to the medium resulted in minimized phenols leaking and proliferation of callus in both leaf and intermodal explants of *I. beraviensis* .

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