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Micro propagation mediated indirect organogenesis in *Naravelia zeylanica* (L.) DC.

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Abstract
Naravelia zeylanica is a rare medicinal plant belonging to the family Rannunculaceae. In the present investigation for tissue culture studies to test the callus regeneration from the internodal explants were inoculated on MS medium supplemented with varying concentrations of auxins and cytokinins. Organogenic callus derived from the internodal explants inoculated on MS medium supplemented with 1 mg/l BA and 0.5 mg/l IAA.

Keywords: *Naravelia zeylanica*, Callus, Indirect Organogenesis

Introduction

Naravelia zeylanica Linn. Dc or *Atragene zeylanica* Linn. (Rannunculaceae) includes about fifty genera and about 2000 species (Cronquist, 1981) [5]. The plant is useful on vitiated conditions of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers. The roots and stems have a strong penetrating smell (Warrier *et al.*, 1995) [19]. In Kerala *Naravelia zeylanica* is used as a source of the drug for intestinal worms, skin disease, leprosy, toothache and headache (Sivarajan and Balachandran, 1958) [15]. It is a scan dent climbing shrub found in tropical forest of eastern Himalayas, Assam, Bengal and Deccan peninsula (Sahib and Rao, 1914). The plant has tuberous roots, wiry stem and strong tendril. Leaves are trifoliate, opposite with terminal leaflet modified in to a trifoliate tendril. Flowers are yellow, fragrant, in axillary and terminal panicles, sepals downy, petals linear-clavate and elongate. Petals are tubular or club shaped and honey secreting. Fruits are aggregate of achene ending in twisted feathery tails (Warrier, *et al*, 1995) [19].

Plant tissue culture involves a collection of experimental method of growing large number of isolated cells or tissues under sterile and controlled conditions. A method of culturing plant cells and tissues provides a new means for the commercial processing of rare and exotic plants. Micro-propagation is one of the basic techniques used in plant biotechnology. It is a process by which tissues as well as single cells are grown *in vitro* in sterile culture medium containing a carbon source, minerals, growth factors and growth regulators such as hormones. The totipotancy of plant cells has greatly contributed to the progress in this area. The organogenesis is a biotechnological tool used for obtaining mass production of mother plant with high quality of health. The produced callus can be utilized to regenerate plantlets or to extract or manipulate some primary and secondary metabolites (Pande and Gupta, 2013) [12]. Plant mass production can be affected by several factors such as light, temperature, plant varieties, and type of explants, components of media, sources and orientation of explants. The most common culture temperature range has been between 20 and 27°C, but optimal temperatures vary widely, depending on genotype (Kumar and Raddy, 2011) [8].

Genetic variability develops spontaneously during tissue culture. Larkin and Scowcroft studied these genetic variability in detail and proposed the term somaclonal variation (Larkin and Scowcroft, 1981) [9]. The understanding of plant organogenesis and the initial developmental stages of meristemoids require observation of subcellular level changes and their correlation with biochemical alterations (Pihakashi-Maunsbach *et al*. 1993) [13]. Studies on ultrastructural alterations during organogenesis *in vitro* to characterize the meristemoidal cells responsible for bud formation are scarce (Villalobos *et al*. 1985, Pihakashi-Maunsbach *et al*. 1993, Arai *et al*. 1997) [17, 13, 2]. These studies do not make comparative analyses between the direct and indirect regeneration systems or relate the ultrastructural aspects to the organogenic potential. Some authors, however, have attempted to relate ultrastructural characteristics to embryogenic potential (Konar *et al*. 1972, Radojevic *et al*. 1975, Vujicic *et al*. 1979) [7, 14, 18].

Materials and methods

Naravelia zeylanica belonging to the family Rannunculaceae was selected for the present study. The plant was collected from T.B.G.R.I., Palode and maintained in the green house of Department of Botany. The explants from stem such as internodes were used for the present study. The intermodal explants were washed thoroughly under running tap water for 30 minutes, followed by washing in 10% labolene. Then treated with 0.1% Mercuric Chloride ($HgCl_2$) (w/v). The sterilized explants were washed using autoclaved double distilled water. The cultures were maintained at a temperature of 25 ± 1°C and incubated at 16 hour photoperiod at a light intensity of 2500 lux from cool fluorescent tubes. Internodal explants were used for callus induction. Callus obtained from internodal explants were sub cultured on MS medium supplemented with BA or Kin in combination with IAA or NAA to assess the regeneration potential of callus. The callus obtained after 30 days were subculture with intervals for analyzing the organogesis regeneration potential.

Observation

Internodal explants inoculated on NAA and IAA alone produced pale green callus and poor callus proliferation. Internodal explants inoculated on MS medium augmented with NAA: BA, IAA and BA, NAA: Kin in combination induced callus proliferation. In this combination callus morphology varied from yellowish green compact to green compact. Lower and higher concentration of IAA along with different concentrations of Kin produced more callusing. The callus morphology varied from friable pale brown yellow to dark green compact. The indirect organogenesis was recorded in the combinations of NAA: BA, BA:IAA, NAA: Kin after 60 days of sub culturing. The sub cultured callus in the combinations of NAA: BA (1.0:0.5) and BA: IAA (1.0:0.5,1.0:2.0) induced shoot having different lengths. The hormonal combinations of NAA: Kin (0.5:2.0) induced roots via callusing after 50 days of sub culturing (Table: 1 & Figure:1).

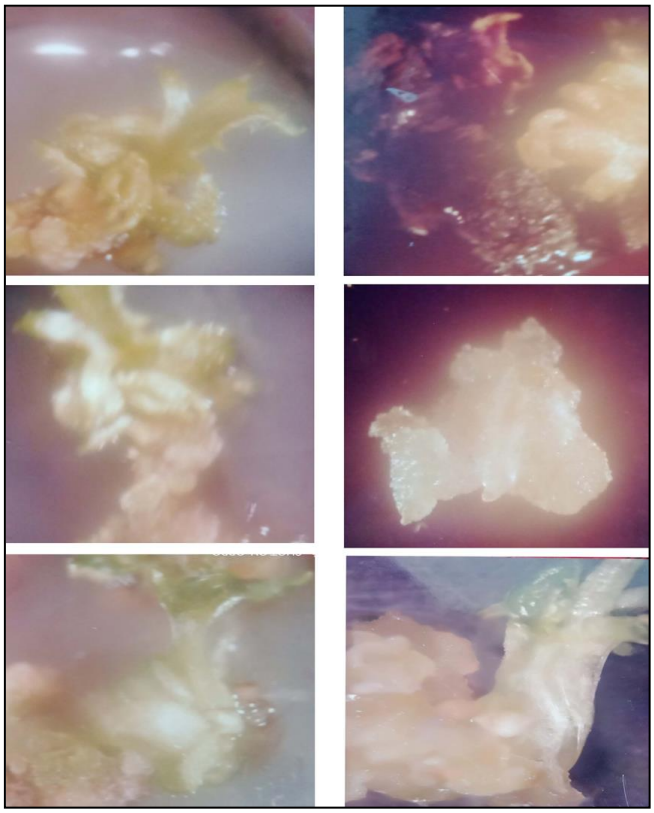

Plate 1: Showing Indirect organogenesis in different hormonal cocentrations

Discussion

Cytokinin type and different hormonal concentration also affected the frequency of shoot induction (Maheshwari and Kovalchuk, 2011). Vieitez and Vieitez (1980) reported that 6-benzylaminopurine (BAP) showed the most satisfactory effect on promoting the proliferation of axillary shoots, whereas zeatin slightly inhibited the development of axillary shoots but increased the induction rate and caused more vigorous shoots. Successful adventitious bud regeneration in *Adhatoda vasica* was limited to reports of shoot formation from callus through internode segments of mature plants (Azad and Amin, 1998) and young leaves and cotyledon explants (Amin *et al.* 1997; Azad *et al*. 1999). Besides, a high local auxin concentration as a major signal redirects the cytokinin stimulated growth to new organ initiation and positioning (Murray *et al.*, 2012) [11]. Erisen *et al*. (2010) [6] who achieved optimum number roots of *in vitro* shoot cuttings of *Austragalus cariensis* on MS medium supplemented with IBA.

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