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Research Article

Induction of callus from leaf and petiole of the plant *Coleus vettiveroids* K .C. Jacob

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Abstract: *Coleus vettivereoids* K .C. Jacob belonging to the family Labiatae is a widely accepted plant known for its traditional uses such as antipyretic, antibacterial, anti-inflammatory etc. The present study aimed to evolve a protocol for rapid multiplication using culture technique. Tissue explants like leaf lamina and petiole were cultured on MS media supplemented with different concentrations of 2.4-D (0.5-8 mg/l), IAA (0.5-8mg/l) and their callusing were studied. A proper method developed for surface sterilization of explants because the major problem in the development of a callus in the case of *Coleus* species is the contamination resulting from its hairy nature. 0.1% *w/v* $HgCl_2$ for 1 min application followed by ethyl alcohol (70%) for 30 seconds was found to be most effective. The MS basal + 2, 4 D (1.5 mg/l) and MS Basal +IAA (2.00 mg/l) showed induction of callus from leaf explants and MS basal + 2,4D (1.5mg/l) showed induction of callus form petiole.
Keywords: Callus culture ,*Coleus vettiveroids*, Hrivera, Labiatae, ,leaf, petiole

INTRODUCTION

In spite of the recent developments on the synthetic chemistry, higher plants are still an important source of medicinal compounds. In recent years, it has become difficult to maintain an ample supply of medicinal plants due to several factors such as their ruthless exploitation, lack of conservation of the environment, increasing labour costs and economical or technical problems associated with the cultivation of medicinal plants. The technique of plant tissue cultures could afford possible solution to some of these problems [1].

Plant tissue culture is a modern tool for the rapid propagation of plants. It can be used to conserve rare and endangered medicinal plants and multiply them in short duration. The recent high demands for the herbal raw drugs can be easily solved with tissue culture technique and it is potentially valuable for studying the biosynthesis of secondary metabolites and also provides an efficient means of producing commercially important plant products [2, 3].

Coleus vettiveroids K. C. Jacob (*Plectranthus vettiveroids*) belonging to the family Lamiaceae is an important plant in the Indian system of medicine. The plant is cultivated in south India through vegetative cuttings and the whole plant is used in medicine. The plant is a bitter cooling, diuretic, tricogenous and antipyretic. It is useful in hyperdipsia, vitiated conditions of pitta, burning sensation, leprosy, skin diseases, leucoderma, fever, vomiting, diarrhoea and ulcers. It has antibacterial and anti inflammatory action also, and used in a number of ayurvedic formulations like Iruveli kashaya, Amritati kashaya, Vilambudadi kashaya, Dasamoolarishtam etc [4, 5, 6].

The present study aimed to develop a protocol for rapid multiplication of *Coleus vettiveroids* using callus culture technique. The explants selected were leaf lamina and petiole .The explants were cultured on MS media supplemented with different concentrations of 2.4-D (0.5-8 mg/l), IAA (0.5-8mg/l) and their callusing were evaluated

MATERIALS AND METHODS

Collection and authentication of plant material:

The plant material for the proposed study was collected from the Botanical Garden, Poojappura, Thiruvananthapuram, Kerala, India. The species of the proposed study was identified as *Coleus vettiveroids* by, Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India

Callus culture:

The Callus culture studies were performed in the Biotechnology Department of Mar -lvanios College, Thiruvananthapuram, Kerala, India.

Explant types :

The explants used for the study included leaf and petiole.

Surface sterilization:

Method (A): The explants collected from the field grown plants were washed thoroughly in running

tap water for 5 min. It was then washed in detergent (2 drops of Teepol) for 3 minutes, followed by 3-5 washes in tap water. It was then washed in distilled water (3 washes) followed by washing in sterile distilled water (2-3 washes) under sterile aseptic conditions in laminar air flow cabinet. This was followed by treatment with 70% ethanol (v/v) for 1 minute then the explants were washed in 0.1% *w/v* HgCl2 solution for 1 minute. Finally they were rinsed with sterile water (3 washes).

Method(B): In this method Laboline (2 drops) was used as detergent. Then the explants were washed thoroughly with distilled water (3-4 washes) then with 100 ml distilled water containing 4 drops of candid-B solution. It was then washed in sterile distilled water (1 wash) under sterile aseptic conditions in laminar air flow cabinet. This was followed by treatment with $HgCl_2$ solution (for 1 minute). The explants were then washed with Hg Cl2 (0.1% *W/v*) for 1 minute, followed by 70% ethanol for 30 second this with sterile water (1 wash).

Culture initiation:

Nutrient media:

The Media used was MS (Murashige and Skoog, 1962). The MS Medium was prepared using the usual stock preparation methods and also by using the commercially available readymade media (Hi Media, Mumbai). The PH of the medium was adjusted before autoclaving. The autoclaving was done at 101^0 C and 104 KPa for 20 minutes. The agar used was that of tissue culture grade (Hi Media, Mumbai). The medium was dispensed in 25mm x150mm tubes.

Plant growth Regulators Used: MS Media supplemented with different concentrations of 2.4-D (0.5-8 mg/l), IAA (0.5-8mg/l) were tried individually.

Influence of Activated Charcoal (AC):

To find out whether the activated charcoal had any effect on the germination and growth of explants, activated charcoal was added to the medium in the range of 0.1 -0.2°6 *W/V*.

Culture Environment:

After inoculation of explants, all the cultures were incubated at 25 ± 2° C under cool white fluorescent light at the irradiance of 40 μmol^{-2} S^{-1} and a 16 h light /8h dark photoperiod. The cultures were monitored periodically and the data were recorded after 4 weeks [7, 8, 9, 10].

RESULTS AND DISCUSSION

Callus Culture Study

Surface sterilization:

The rate of contamination was high in the explants taken directly from the field grown plants. The contamination percentage of 90-100% was observed in these explants. Of the different sterilants used 0.1°6 *w/v* HgCl2 for 1 min application followed by ethyl alcohol (70%) for 30 seconds was found to be most effective.

The major problem in the development of a callus in the case of *Coleus* sp is the contamination resulting from its hairy nature. So through surface sterilization with least tissue damage is necessary. The above method of surface sterilization also produced least tissue damage.

Culture initiation:

Among the different plant growth regulators used, i.e., MS basal + 2,4-D (0.5- 8 mg/l) and MS basal + IAA (0.5-8mg/l)The MS basal + 2,4 D (1.5 mg/l) and MS Basal +IAA (2.00 mgl) showed induction of callus from leaf explants and MS basal + 2,4D (1.5mg/l) showed induction of callus form petiole.

In the present study, it was observed that that activated charcoal has no influence in the callus formation.

The callus obtained from leaf was friable type and MS basal media + IA,A (2.00mg/l) shows more browning. Translucent callus obtained from petiole using MS basal media + 2,4D (1.5 mg/l).

CONCLUSION:

The plant *Coleus vettiveroids* is an important plant in the indigenous system of medicine. The plant is used in so many ayurvedic formulations. The major problem in the development of a callus in the case of *Coleus* sp is the contamination resulting from its hairy nature. By this study we can develop a suitable method for surface sterilization with least tissue damage. 0.1% *w/v* $HgCl_2$ for 1 min application followed by ethyl alcohol (70%) for 30 seconds was found to be most effective. The MS basal + 2, 4 D (1.5 mg/l) and MS Basal +IAA (2.00 mg/l) showed induction of callus from leaf explants and MS basal + 2,4D (1.5mg/l) showed induction of callus form petiole.

This method can be utilized as a fundamental reference method for the tissue culture studies of *Coleus vettiveroids*

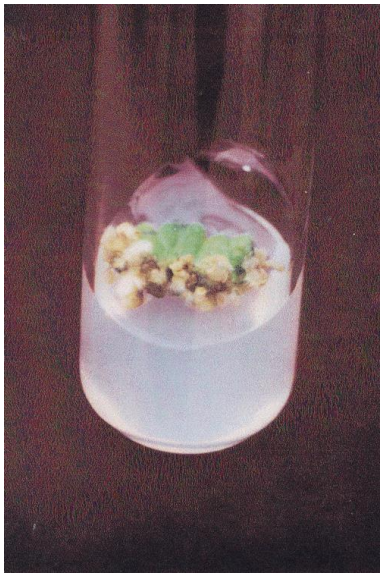

Figure 1: Leaf cultured on MS Basal+ IAA (2.00mg/l)

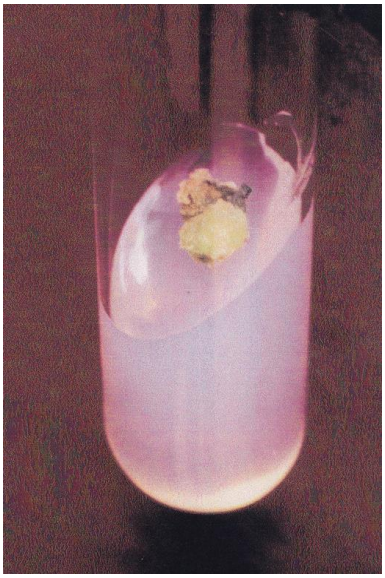

Figure 2: Petiole cultured on MS basal + 2 ,4 D (1.5 mg/l)

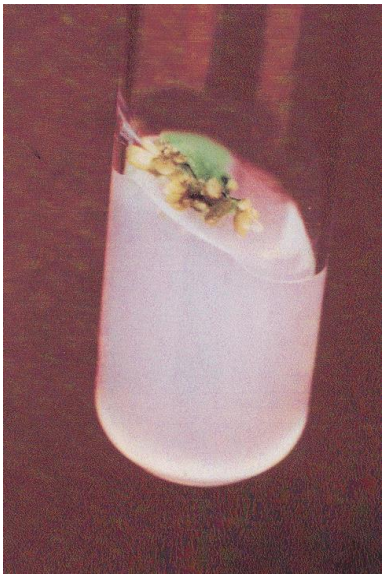

Figure 3: Leaf cultured on MS basal + 2, 4 D (1.5 mg/l)

Fig: In vitro micro propagation of *Coleus vettiveroids*

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