Tissue Culture Studies of *Centella asiatica*

K. PURUSHOTHAM RAO.*, S. SRINATH RAO[1] AND M. SADANANDAM[2]
H.K.E.S. College of Pharmacy, Gulbarga-585 105
[1]Department of Botany, Gulbarga University, Gulbarga-585 106
[2]Jawahar Lal Nehru Technological University, Hyderabad-500 057

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To meet the growing need of drugs, research and development scientists now concentrate on the development of herbal formulations. In the present investigation, the influence of auxins and cytokinins during the production of callus in *Centella asiatica* (L) was studied. The work plan has been carried out using known procedures of tissue culture under specified conditions of inoculation and incubation in Murashige and Skoogs medium. It was noticed that stem explant, stolon proved to be the best for callus induction followed by leaf, base, kinetin supplementation at 0.25 and 0.5 mg/l along with auxins 2 mg/l 2,4-D proved to be beneficial for the growth of callus. The best combinations of growth regulators for maximum callus induction was 2 mg/l NAA+0.5 mg/l kinetin.

In recent years most of the drug research in being concentrated on herbal medications[1]. There are many medicinal plants which are to be explored for possible utiliation by the drug industries to concentration on formulative aspects to introduce in to the market. Among them, the medicinal plant *Centella asiatica* (L) from Umbelliferae family has much medicinal value in the treatment of anxiety, bacterial infections, leprosy, eczema and also as brain tonic[2]. The present investigation has been undertaken to standardize the protocols for tissue culture studies to increase callus induction and study the influence of auxins and cytokinins[3,4].

The materials used are: 2, 4-D/(2,4-dichlorophenoxy acetic acid), Kinetin (6-furfurylaminopurine), IAA(indole-3-acetic acid), IBA (indole-3-butryic acid), NAA (α-napthalene acetic acid), M.S. (Murashige and Skoog's medium). BAP (N-benzylaminopurine), 0.1N NAOH, 0.1N HCl ethanol, agar, mercuric chloride and culture tubes.

The plant stolon (1 cm), leaf base (1 cm) and the leaf were used for induction of callus. The explants were surface sterilised with 0.01% mercuric chloride solution for 5 min and then washed with sterile distilled water till traces of mercuric chloride were removed. Stock solutions were prepared (3 mg/1) with the growth regulators (2, 4-D, IAA, NAA, Kinetin, BAP) by dissolving in a small quantity of their respective solvents (0.1 N NaOH, 0.1N HCl and ethanol) and required amounts were pipetted out to supplement the basal media. The pH of the medium was adjusted to 5.7 prior to solidifying with agar (0.8%). The medium was then dispensed into culture tubes (5 x 50 cm) and closed with aluminum foils and later autoclaved at 15 lbs/sq in pressure for 20 min. The medium was cooled and checked for contamination before inoculation[6]. The cultures were incubated under continuous fluorescent lights (300 lux) at 26° and percent induction was calculated using the equation

$$\text{Frequency} = \frac{\text{No. of explants showing response}}{\text{Total no. of explants inoculated}} \times 100$$

The auxins at 0.5 to 3 mg/l concentrations were tested for callus induction using selected explants that include stolon, leaf base and leaf segments. The results are shown in table-1. The auxins 2,4-D, NAA, IAA at 0.5 mg/l failed to induce callus. NAA at 1 mg induced callus however the response was better with leaf base followed by stolon and leaf explants. The frequency of callus induction increased at 2 mg/l concentration of NAA. Similar reports were observed when studied in other medicinal plants[7] the concentration beyond 3 mg/l totally inhibited callus induction. The explants turned brown within 4 to 5 days after inoculation and further growth was arrested. Similar trends were observed with 2,4-D in all the three explants.

*For correspondence

TABLE 1 - EFFECT OF AUXINS ON THE FREQUENCY OF CALLUS INDUCTION

Auxins	Concentration mg/l	Frequency of Callus Induction (%) Source F explant		
		Leaf	Leaf Base	Stolon
NAA	0.5	0.0	0.0	0.0
NAA	1.0	30.0	38.0	34.0
NAA	2.0	68.0	72.0	68.0
NAA	3.0	26.0	28.0	28.0
2, 4-D	0.5	0.0	0.0	0.0
2, 4-D	1.0	26.0	32.0	28.0
2, 4-D	2.0	64.0	68.0	60.0
2, 4-D	3.0	18.0	24.0	26.0

NAA : (α-Napthalene Acetic Acid), 2, 4-D (1,3-Dichlorophenoxy acetic acid).

TABLE 2 - INFLUENCE OF KINETIN ON CALLUS INDUCTION

Auxins	Concentration Mg/l	Frequency of Callus Induction (%) Source F Explant		
		Leaf	Leaf Base	Stolon
	(NAA+Kinetin)			
NAA+Kinetin	2+0.25	72.0	76.0	70.0
NAA+Kinetin	2+0.5	82.0	88.0	82.0
NAA+Kinetin	2+1.0	70.0	72.0	68.0
	(2,4-D+KINETIN)			
2,4-D+Kinetin	2+0.25	66.0	74.0	66.0
2,4-D+Kinetin	2+0.5	80.0	82.0	74.0
2,4-D+Kinetin	2+1.0	64.0	64.0	58.0

NAA : (α-Napthalene Acetic Acid), 2,4-D(1,3-Dichlorophenoxy acetic acid).

The auxins NAA and 2,4-D gave better response to callus induction than IAA. Therefore, to see the effect of kinetin (0.25 to 1 mg/l) on the frequency of callus induction, 2,4-D and NAA only at 2 mg/l were used. From the results tabulated in Table-2, it is clear that kinetin at 0.25 to 0.5 mg/l enhanced the frequency of callus induction when used either with 2,4-D or with NAA and at a concentration of 1 mg/l, the frequency of callus induction decreased. The best combination of growth regulator was 2 mg/l NAA + 05 mg/l kinetin and for leaf base explant where the maximum frequency of 88% callus induction was noticed. The same trends were observed previously with other medicinal plants also[9].

It was noticed in all the species investigated, that stem explant and stolon proved to be the best for callus induction followed by leaf base. Supplementing with kinetin at 0.25 and 0.5 mg/l along with auxins proved beneficial for the growth of callus. The best combinations of growth regulators for maximum callus induction was, found to be 2 mg/l NAA+0.5 mg/l kinetin.

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A New Spectrophotometric Method for the Determination of Nitrendipine

K. GIRISH KUMAR, K.P.R. CHOWDARY[1] AND G. DEVALA RAO[1]*
Department of Chemistry, Gandhigram Rural Institute, Gandhigram-624 302, Tamil Nadu
[1]KVSR Siddartha College of Pharmaceutical Sciences, Vijayawada-520 010, A.P.

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A simple and sensitive spectrophotometric method for the determination of nitrendipine is described. The method is based on the reaction of reduced nitrendipine with 3-methyl-2-benzo-thiazolinone hydrazone hydrochloride (MBTH) in the presence of ferric chloride to form a green colored chromogen with an absorption maximum of 670 nm. The color obeyed Beer's law in the concentration range of 2-10 µg/ml.

Nitrendipine[1,2] chemically ethyl 1,4-dihydro-5-(acetoxy carbonyl)-2,6-dimethyl-4-(3-nitrophenyl)-3-pyridine carboxylate, is a relatively new antianginal drug. It is not yet official in any pharmacopoeia. A survey of the literature revealed that HPLC[3-6] and few spectrophotometric methods[7-8] are reported earlier for the determination of nitrendipine in biological fluids and in dosage forms. Huning and Fritsch[9] had described the oxidative coupling of MBTH with aromatic amines in the presence of an oxidant under acidic conditions. Sawicki *et al.*[10] investigated the reaction of MBTH with a number of amines and found that the reagent MBTH reacts readily with most aromatic amines resulting in the formation of an intensely colored azodye cation. The presence of primary aromatic amino group in the reduced nitrendipine enable the use of MBTH-Fe (III) reagent to form a green colored chromogen.

An ELICO model SL-150 UV-VIS spectrophotometer with 1 cm matched quartz cells was used for all absorbance measurements. All the chemicals used were of AnalaR grade. An aqueous solution of MBTH (0.2% w/v) and solution of ferric chloride (0.7% w/v) in 0.5 N hydrochloric acid were prepared. Nitrendipine was obtained as gift sample from a local industry.

Nitrendipine (20 mg), was accurately weighed and dissolved in 20 ml of methanol and treated with 5 g of zinc dust and 4 ml of concentrated hydrochloric acid. After keeping for 1 h at room temperature, the solution was filtered through cotton wool, and the residue was washed with 3x10 ml portions of methanol and the total volume was brought to 100 ml with distilled water. Working standard solutions were obtained by appropriate dilution of the standard solution.

In a series of 10 ml volumetric flasks, aliquots of reduced nitrendipine solutions (1.0-5.0 ml, 20 µg/ml) were placed. A 1.5 ml portion of MBTH solution was added to each flask and kept aside for 2 min at room temperature. Then 2.0 ml of ferric chloride solution was added, kept for 10 min and diluted to the mark with distilled water. The absorbances were measured at 670 nm against a reagent blank.

* For correspondence