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In vitro Callus Culture of Adhatoda Vasica: A Medicinal Plant

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ABSTRACT

Callus culture of Adhatoda vasica Nees were established from leaf explant. Different growth regulators greatly influenced the growth of callus cultures. Leaf derived callus grown on Murashige and Skooge (MS) medium fortified with different concentration (1, 1.5, 2,2.5 ppm) of Indole Acetic Acid (IAA), 6-Benzyladenine (BA), Kinetin (Kn), 2, 4- Dichlorophenoxyacetic Acid (2, 4-D). Accumulation of a bioactive pyrroloquinazoline alkaloid, vasicine and vasicinone in callus culture was detected and confirmed by thin layer chromatography. The R_f values of vasicine and vasicinone were found as 0.52 and 0.60

Keywords: Adhatoda vasica, Callus culture, Pyrroloquinazoline, Vasicine, Vasicinone

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INTRODUCTION

Adhatoda vasica a member of the *family Acanthaceae*, commonly called as Vasaka or Arusha. It is an important medicinal plant found in India and utilized in rural areas for several ailments [14]. The Vasaka plant perennial, evergreen and highly branched with unpleasant smell and bitter taste, the plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub, having 1.0–2.5 m height, with opposite ascending branches. The drug contains stem, leaf, flower, fruit and seeds [2, 4]. Vasaka is a bitter quinazoline alkaloid, the major alkaloids are vasicine and vasicinone which is present in all parts of the plant. the leaves contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, steroids, carbohydrate and alkanes . In the flowers triterpines (a-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin, vitexin) have been found [1, 3, 5]. This plant has medicinal uses, mainly antispasmodic, fever reducer, anti-inflammatory, anti bleeding, branchodilator, antidibetic, antihelminthic, disinfectant, anti-jaundice, antiseptic, oxitocic and expectorant and has many other medicinal applications [10,13,15]. There is a considerable demand of this plant in India and this demand is met from the natural habitat. This plant show low seed germination and conventional propagation through cutting is slow [16,17]. This leads to rapid depletion of plant material due to over exploitation of this important plant. Pant tissue and cell culture system are being exploited for the accumulation of the variety of natural products [6,

7]. The tissue culture systems for a number of medicinal plants have been established, and this enables the analysis of callus and suspension for the presence of the various secondary metabolites [11].

MATERIAL AND METHODS

The leaves of ***Adhatoda vasica*** were collected from the medicinal garden of pharmacy, Integral University, Lucknow. Sample of plant material was given to NBRI Lucknow, India for identification and taxonomic authentification. The test report from CIF, NBRI, Lucknow, conformed the taxonomic authentification of plant material sample. Specification No.: NBRI-SOP-202, Receipt No. & Date : 19/76, 27-2-2009

For callus initiation from leaf of *adhatoda vasica*, explants were cultured on Murashige and Skoog (MS) medium [8]. Establishment of callus cultures, young leaves from plants of *Adhatoda vasica* were collected from the botanical garden and washed under running tap water. Explants were immersed in a detergent for 5 min, washed in water once, and then surface sterilized with mercuric chloride (0.1%) for 4-5 minutes, and then explants were washed thrice with double-distilled water. The surface sterilized leaves cut in to small pieces. The cut segments were then cultured individually on MS medium containing different concentration (1, 1.5, 2, 2.5 ppm) of Indole Acetic Acid (IAA), 6-Benzyladenine (BA), Kinetin (Kn), 2, 4-Dichlorophenoxyacetic Acid (2, 4-D). Phyto-hormones are added in the MS basal medium and then pH of the medium was adjusted to 5.7 to 5.8. The agar is added in the medium before autoclaving at 121ºC for 20 minutes at 15 psi.

While working inside laminar flow, hand washed with denatured spirit. Then transfer the material in fresh culture medium. Before and after each transferred material forceps and scalpel washed with spirit and also burn with the help of spirit lamp. After transferring explants in the nutrient medium the boiling tube should plugged tightly, which then inoculated under controlled condition where they multiply and grow properly. All cultures were maintained under cool white, fluorescent light with a 16 h photoperiod, the intensity of lights is 3000 lux, at 24± 2ºC temperature. The response of these inoculated explants was observed after 3 weeks of culture. The present frequency of callus induction was determined by counting the number of explants producing callus as the percentage of the total number of explants. The callus induced was subculture at an interval of 21 days on the same medium. They were monitored, developed and maintained on the same medium. Callus was healthy over a period of time on this medium. Callus growth was measured after 35 days of culture in term of fresh weight and dry weight. The dry weight was used for alkaloid analysis.

Isolation of alkaloids

For isolation of the alkaloids, 2g of dried callus sample was suspended in 75 ml of methanol, stirred for 15 min and filtered. Callus tissues were washed in boiling methanol (100ml) and the combined methanolic extracts were concentrated in vacuum, and the residue partitioned between 75 ml each of ethyl acetate and 1N HCl. The ethyl acetate portion was neutralized with sodium bicarbonate, and ethyl acetate was then added [12]. The combined ethyl acetate fractions were evaporated and the residue was titrated with ethyl acetate and then filtered. The presence of alkaloids in the callus was confirmed by the Dragendorff reagent test. The solvent system comprised of methanol and chloroform in 1:9 ratio [9]. Observe the plate under UV 254 nm & spray the plate with Dragendroff's reagent note the R_f values of the compound in the callus.

RESULTS AND DISCUSSION

Three boiling tubes of each combination of media were used, in each boiling tube single explants were inoculated. To initiate callus from leaf explants, explants were grown on different combinations and concentrations of growth regulators (Table 1). Each boiling tubes containing 15ml of Murashige and Skoog's basal medium Supplemented with sucrose (3%) and agar (0.8%) (Table 2).

Table 1: Effect of phytohormones on callus induction from cultured leaf of A. vasica

S. No.	Combinations of phytohormones				Conc. (ppm)	Frequency of Callus induction (%)	Observation
1	IAA	BA	Kn		1	62	Callus
2	IAA	BA	Kn	2,4-D	1.5	75	Callus
3	IAA		Kn		1	Nil	No callus
4	IAA	BA		2,4-D	1.5	54	Callus
5	IAA		Kn		1.5	27	Callus
6	IAA	BA			1	52	Callus
7	IAA	BA		2,4-D	2.5	Nil	No callus
8	IAA	BA	Kn		2.5	48	Callus
9	IAA	BA	Kn		2	67	Callus
10	IAA		Kn	2,4-D	2	35	Callus

*IAA:*Indole Acetic Acid; *BA:*6-Benzyladenine; *Kn:*Kinetin; *2,4-D:* 2,4-dichlorophenoxyacetic Acid

Table 2 : Composition of MS basal medium (100ml)

S.No.	Compound	Amount
1	Major	10 ml
2	Minor	1 ml
3	Vitamins	1 ml
4	Iron	0.5 ml
5	Sucrose	3 gm
6	Inositol	100 mg
7	Agar	0.8 gm
8	Double distilled water	Quantity sufficient

The frequency of callus initiation from leaf explants is presented in Table 1. When leaf explants were cultured on MS media supplemented with various combinations of auxin and cytokinin, callus formation was observed in 15 and 21 days, respectively. The maximum frequency of callus initiation was 75% in MS medium containing *1.5 ppm of* Indole Acetic Acid (IAA), 6-Benzyladenine (BA), Kinetin (Kn), 2, 4-Dichlorophenoxyacetic Acid (2, 4-D) (Table 1). The maximum fresh weights of leaf calli were 18.164 g and the corresponding dry weight were 2.015 g on MS media. When sample spotted on thin layer chromatographic plate was run in a solvent system comprising methanol and chloroform in the ratio of 1:9, the R_f values of vasicine and vasicinone were found as 0.52 and 0.60 (Table 3) by detecting under ultraviolet light at wavelength of 254 nm.

Table 3: R_f values by TLC of vasicine and vasicinone

S.No.	Compound	R_f Value
1	Vasicine	0.52
2	Vasicinone	0.60

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