



Effect of plant growth regulators on immobilized cell culture of *Adhatoda Vasica* Nees

Chauhan Pradeep^{*1}, Panigrahi Mahendra Kumar², Behera Jayanti Prava³

¹*Institute of Professional Studies-College of Pharmacy, Gwalior, 474001, Madhya Pradesh, India*

²*Hi-Tech College of Pharmacy, Hi-Tech Medical College and Hospital campus, Bhubneswar, 751025, Odisha, India*

³*PG Department of Pharmacology, M.K.C.G. Medical College, Brahmapur, GM, Odisha, India*

ABSTRACT

Immobilization has been suggested as a stratagem to improve the overall productivity in plant tissue culture. Two Auxin and one cytokine used to manipulate the media and the effect was studied in terms of cell growth. Secondary metabolite production was analyzed by HPLC. The calcium alginate concentration was optimized on the basis of cell viability and cell number increase was observed in immobilized condition.

Keywords: *A. vasica*, Immobilized cell, calcium alginate, cell growth, vasicine

INTRODUCTION

Immobilized system facilitates cell viability over an extended time for secondary metabolite production. Immobilization enables reutilization of entrapped biomass, and this technique is especially suitable for slow growing plant cells. Immobilized plant cells have been employed to perform biotransformation and reported to have higher production from suspension culture cells. Enclosure in a support material exerts certain stress on plant cells leading to restricted growth, the condition normally considered as prerequisite for higher secondary metabolite production.

Immobilization separates cells from medium and the desired product is extracellular, simplifying down-stream processing. Manipulation of the auxin to cytokinin ratio in the medium can lead to the development of shoots, roots, or somatic embryos from which whole plants can subsequently be produced [1,2]. Immobilization techniques were initially developed to immobilize enzymes for applications in the industrial production of sugars, amino acids and pharmaceutical products [3].

Adhatoda vasica Nees (Vasaka) is used in various chest infections and has reputation as an expectorant in the indigenous system of medicine. The leaves, roots and flowers are extensively used for various respiratory disorders like cough, asthma, bronchitis etc. The juice of leaves used in diarrhoea and dysentery and powdered leaves in malaria in southern India [4,5,6]. It is also used for bleeding gums, due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles, and menorrhagia. It provides relief in muscular spasms, cramps or convulsions. Vasicine and vasicinone is major alkaloid responsible for pharmacological activities.

RESEARCH METHODS

Immobilization in calcium alginate

1 ml. of cell suspension, corresponding to 1 gm of fresh weight was added to 10 ml. of 2% sodium alginate (HI-media) and mixed well to achieve the uniform distribution of cells throughout the sodium alginate solution and left for some time to remove bubbles. This mixture was then added drop wise into 3mM calcium chloride solution through sterilized syringe under aseptic condition in Laminar airflow bench. This leads to form circular beads having diameter of 2.5-3.0 mm. Formed beads are then left in CaCl₂ solution for 30 minutes for stabilization. The beads were then washed with sterilized water to remove the traces of CaCl₂. Washed beads were then dispersed in 50 ml. production media in Erlenmeyer flasks in shaker cum incubator.

Optimization of calcium alginate concentration by cell viability study

Immobilized cells are subjected to check the viability in calcium alginate entrapped condition. For cell viability study 5 beads are placed in 5 ml. of potassium phosphate buffer (pH 6.5) to release the cells from calcium alginate. After complete dissolution of beads the obtained suspension was centrifuge at 2000 rpm for 2 min and 1 ml. of 23.9 mM TTC (Tri phenyl terta zolium chloride) was added in separated cells in micro centrifuge tubes.

Table 1: Percentage cell viability in different alginate gel concentration.

Time in Days	% viability in different alginate gel concentration		
	1%	2%	3%
0	73	76	71
7	69	71	64
14	Beads Dissolved	68	61
21	Beads Dissolved	65	55

Cell Number increase study in calcium alginate immobilized cells

Cell number was estimated by dissolving the randomly selected 10 beads in 10 ml. of potassium phosphate buffer (pH 6.5). After ensuring the complete dissolution of beads, the cells were counted under the microscope with cytometer.

Table 2: Cell number study in MS and B5 media immobilized in 2% calcium alginate.

Days	Cell number/ mm3 in MS Media Supplemented with (cell number X 105)			Cell number/ mm3 in B5 Media supplemented with (cell number X 105)		
	2 ppm 2,4-D 1 ppm Kin. 0.5 ppm IAA	2 ppm 2,4-D 0.5 ppm Kin. 0.5 ppm IAA	1.5 ppm 2,4-D 1.0 ppm Kin. 1.0 ppm IAA	2 ppm 2,4-D 1 ppm Kin. 0.5 ppm IAA	2 ppm 2,4-D 0.5 ppm Kin. 0.5 ppm IAA	1.5 ppm 2,4-D 1.0 ppm Kin. 1.0 ppm IAA
0	3.6	3.62	3.54	3.3	3.61	3.42
7	3.67	3.75	3.6	3.41	3.75	3.42
14	3.84	4.14	3.74	3.48	3.76	3.46
21	3.86	4.16	3.77	3.52	3.80	3.50

Value represent the mean \pm SE (n=3)

HPLC Quantitative estimation of Vasicine

The Quantitative estimation of Vasicine was analyzed qualitatively was done by High Performance Liquid Chromatography (HPLC) method given in Indian Herbal Pharmacopoeia, 1998 [7]. The different dilutions dilutions of known concentration of vasicine (Provided by Laila Impex, Vijaywada) were prepared by dissolving them in methanol to get the concentration range between 10-60 μ g/ml.

Sample preparation

For quantitative estimation of vasicine in immobilized cell culture, 25 ml production media was taken out from four flasks and pooled together to make it 100 ml. This is then subjected for acid-alkali method 7 to extract alkaloid fraction. At the end of process a semi solid mass was remains that further dissolved in methanol and use for HPLC estimation.

HPLC Standard curve for Vasicine

Instrument: Shimadzu, Mobile phase: Methanol: water (2:3), Flow rate: 0.7 ml/min.
Column: Resolve C18 Spherical 5 μ (3.9 mm X 15 cm), Detector: SPD-M10 Avp.
Wavelength: 298 nm, Retention time: 3.8 min.

Table 3: Vasicine content in immobilized cell culture.

Time in days	Vasicine content ($\mu\text{g/ml}$ / gm fresh weight) of <i>A. vasica</i> cells immobilized in calcium alginate					
	MS medium supplemented with			B5 medium supplemented with		
	2,4-D (2.0 ppm) Kinetin (1.0 ppm) IAA (0.5 ppm)	2,4-D (2 ppm) + Kinetin (0.5 ppm) + IAA(0.5 ppm)	2,4-D (1.5 ppm) + Kinetin (1.0 ppm) + IAA (1.0 ppm)	2,4-D (2 ppm) + Kinetin (1.0 ppm) IAA (0.5 ppm)	2,4-D (2 ppm) Kinetin (0.5 ppm) + IAA (0.5 ppm)	2,4-D (1.5 ppm) + Kinetin (1.0 ppm) + IAA (1.0 ppm)
01	0.17 \pm 0.03	0.30 \pm 0.05	0.20 \pm 0.05	0.29 \pm 0.05	0.31 \pm 0.09	0.19 \pm 0.04
07	0.48 \pm 0.03	0.92 \pm 0.07	0.61 \pm 0.07	0.49 \pm 0.07	0.90 \pm 0.07	0.58 \pm 0.07
14	1.59 \pm 0.14	2.51 \pm 0.21	2.1 \pm 0.07	1.98 \pm 0.04	1.87 \pm 0.17	1.75 \pm 0.04
21	1.73 \pm 0.09	2.55 \pm 0.14	2.20 \pm 0.09	2.26 \pm 0.12	2.50 \pm 0.12	2.49 \pm 0.17

Value represent the mean \pm SE (n=3)

RESULTS AND DISCUSSION

From the above results it was observed that the immobilization of *A. vasica* cells in calcium alginate gel is showing promising results. 2% calcium alginate was found optimum for immobilization as it maintains higher degree of cell viability. Immobilization restricted cell number increment, which stimulates secondary metabolite productions. MS media was found suitable for more vasicine production, maybe due to higher concentration of ions and nutrient supplements, which provide necessary material for de-novo synthesis of vasicine. The above study showed that MS media supplemented with 2,4-D (2 ppm), Kinetin (0.5 ppm) and + IAA(0.5 ppm) is suitable for more vasicine production.

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