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Tissue Culture and Phytochemical Studies of *Nicotiana tabacum* L.

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ABSTRACT

Nicotiana tabacum L. is an important medicinal plant found mostly in tropical and subtropical America. The soil required for growth should be without nitrogen. It is commonly known as Tobacco and Tambakhu. The Present work is divided in two parts: *in vitro* propagation and phytochemical analysis. The explants used for *in vitro* propagation are seed and leaf .Maximum callus was obtained in M.S medium supplemented with 2, 4-D (1) and Kin (0.5). Organogenesis responses better in M.S. medium supplemented with N.A.A. (0.5) and Kin (1). Obtained callus, roots and leaves are further used for phytochemical analysis which includes the test of phenols, proteins, nucleic acid (DNA and RNA) and alkaloids.

KEYWORDS: *Nicotiana tabacum*, Phytochemical, *In Vitro* propagation.

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INTRODUCTION

Nicotiana tabacum L. belongs to family Solanaceae. It is commonly known as Tambakhu [1] All parts of the plant contain nicotine, which can be extracted and used as an insecticide. The juice of the leaves can be rubbed on the body as an insect repellent. The leaves can be dried and chewed as an intoxicant. This is the main species that is used to make cigarettes, cigars, and other smokable tobacco preparations. It is an Annual herb, shrub or small tree; from 0.90 to 1.50 tall according to the variety. The leaves are elliptic or oblanceolate; flowers clustered at the end of branches; have a cylindrical calyx and are greenish or reddish in the upper part. Fruit has different form with globular seeds.[2] Whole plant or mainly the leaves are used in medicines. At least 12 different alkaloids have been identified in the genus *Nicotiana*[3], but nicotine, nornicotine, analysine and anatabine are considered the main alkaloids. Nicotine has a number of important pharmacological effects like a muscle relaxant

MATERIAL AND METHODS

Collection and identification of plant material

The plant material of *Nicotiana tabacum* L. was collected from Botanical garden, Department of Botany, Nowrosjee Wadia College, Pune. Efforts were made to collect the plant in flowering and fruiting condition for the correct botanical identification and authentication. It was identified with help of Flora of Presidency of Bombay [4]

InVitro propagation

Preparation of explant

The fresh mature leaves and seeds of *N. tabacum* were collected for callus induction and organ development.

Sterilization of explant

All the explants were washed thoroughly with running tap water for 20 min, after that it will be immersed in Teepol for 2-3 min and washed thoroughly with D.W. than it was dipped in the solution of Savlon for 1-2 min and again washed with D.W. The explants were surface sterilized with 0.1% $Hgcl_2$ for 1-2min and again washed well in D.w for 3-4 times to remove the traces of $Hgcl_2$. The explants were inoculated on different concentration of plain Murashige and Skoog medium. The pH of all the concentrations was adjusted to 5.7 with 1N NaOH/1N HCl, before addition of 0.8% agar and autoclaved at 15 lb/Inch 2 pressures and 121oC temperature for 20 minutes. In the initial stage of callusing and organ development, cultures were kept in dark at 25oC and 90% humidity, in Environmental test

chamber, for 4-5 days. Then the cultures were transferred to culture room, where they were maintained at 25o±2oC and 16/8 hours (light/dark) photoperiod provided through white fluorescent tubes with light intensity of 3000 lux. The effect of treatments toward callusing and organogenesis was weekly recorded and also kept in daily observation.

Callus and Organ development (Root and Shoot)

The mediums used for callus/organ development were Full MS. The culture vessels were maintained in the same culture room. The growth responses of shoot and root were also weakly observed. The experiments were terminated when the shoots were fully developed (belonging roots, 3- 4 leaves, and 4-5 cm high) and ready for acclimatization.

Phytochemical analysis-

Phytochemical Study of *In Vitro* and *In vivo* plants–

The leaf of *N. tabacum* (*In vitro* & *In vivo*) were analyzed by Harborne, J.B method[5] .,

Protein estimation-

It was estimated by the Lowry's *et al*. Method [6]

Phenol estimation-

Phenol content is estimated by Swain and Hills *et al*. method [7]

Nucleic acid-

It was estimated by Witham *et al*. method [8].

Alkaloids estimation-

Singh *et al*. method used for alkaloid estimation.[9]

RESULTS AND DISCUSSION

Characteristic of explant

The leaves of *N. tabacum* were used as explants that have greenish color. The leaves are elliptic or oblanceolate.

Callus and Organ development

Tissue culture studies were made on Murashige and Skoog's (M.S.) medium supplemented with different growth regulators. The explants used as leaf of *N. tabacum.* Maximum callusing was obtained

from leaf explants by using MS medium supplemented 2, 4-D (1) and Kin (0.5). Organogenesis took place better in M.S. medium supplemented with N.A.A. (0.5) and Kin (1). (Table 1).

Phytochemical studies (Phenols, total proteins, Nucleic acids and Alkaloids) of fresh leaf and root (*in vivo*) and callus obtained from root and leaf explants (*in vitro*) were carried out. It was observed that, there was increased level of phenols in leaf (3.244 µg/g) as compare to root and callus. Maximum protein contains reported in callus where as minimum proteins are observed in roots. More D.N.A. and R.N.A. are present in leaf (1.324µg/g) as compare to callus and fresh roots. Alkaloids are reported in fresh leaf and roots as well as in callus[10] This finding will help to get diseased free *Nicotiana tabacum* L by using callus to extract active principal of this plant. (Table-2).

Table 1 : I*n Vitro* propagation of *Nicotiana tabacum* L Growth Response on M.S. Medium

Sr. No.	Growth Regulators (mg/l)	Explant	Callus	Root	Shoot
1.	2, 4-D (1) + Kin (0.5)	ROOT& LEAF	+ + +	_ _ _	+ + +
2.	I.A.A. (1) + Kin (1)	ROOT& LEAF	+ +	_ _ _	_ _ _
3.	N.A.A. (0.5) + Kin (1)	ROOT& LEAF	_ _ _	+ + +	+ + +
4.	N.A.A. (0.5) + B.A.P. (0.5)	ROOT& LEAF	_ _	_ _ _	_ _ _
5.	N.A.A. (0.5) + B.A.P. (1)	ROOT& LEAF	+ + +	+ + +	_ _ _
6.	N.A.A. (0.5) + I.A.A. (1)	ROOT& LEAF	_ _	_ _ _	_ _ _

- indicates no growth.

+ to +++ indicates weak to vigorous growth

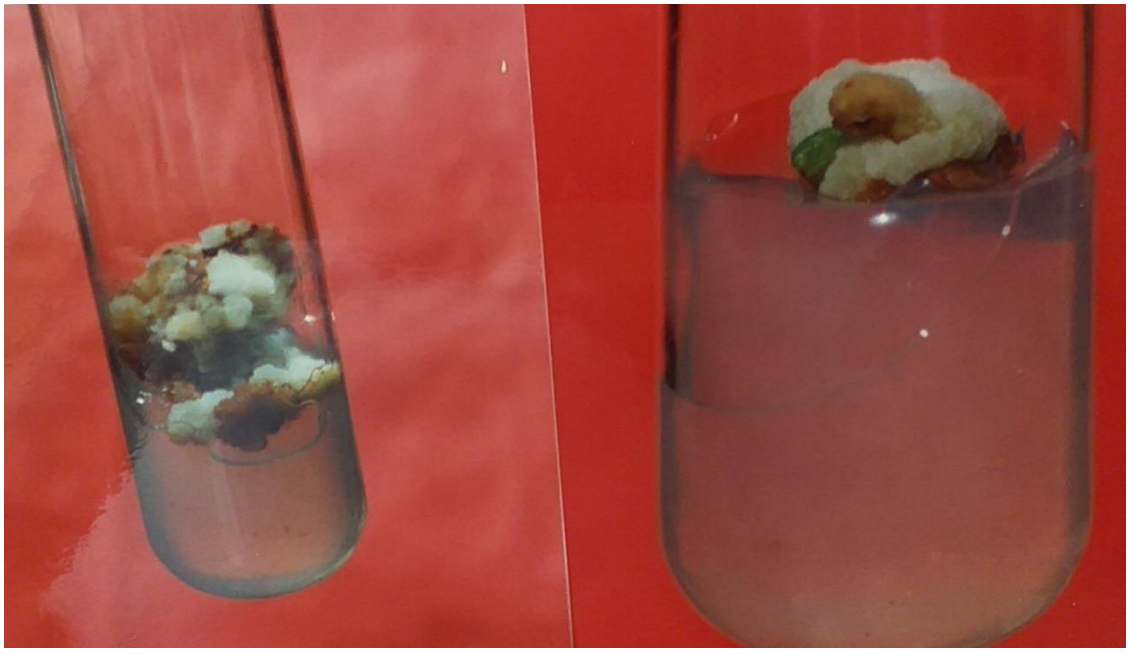

Fig 1- Callogenesis and Organogenesis in *N. tabacum*

Table 2: Estimation of phytochemical present in the *Nicotiana tabacum* L.

Sr. no	Chemical constituent	Callus (µg/g)	Roots (µg/g)	Leaf (µg/g)
1	Proteins	1.212	0.356	1.156
2	Phenols	1.412	1.477	2.224
3	Alkaloid	0.751	0.668	1.140
4	DNA	0.388	0.106	1.224
5	RNA	0.106	0.213	1.060

CONCLUSIONS

As the selected plant *Nicotiana tabacum* L is an ayurvedic plant so its demand is increasing day by day. As the explant used for the *in vitro* propagation is responding a very good result it is concluded that the experiment was successfully achieved. The best medium for rooting and shooting of *Nicotiana tabacum* L was MS medium with N.A.A and Kinetin. The plantlets of *Nicotiana tabacum* L were resulted from these research more than 200 individuals. This study will be helpful for the conservation of diseased free plants and to meet the more demand of *Nicotiana tabacum* L for the preparation of traditional and other pharmaceuticals remedies.

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