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Standardization of efficient *in vitro* callus Induction protocol for *Solanum pubescens* Willd

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Abstract
An efficient protocol has been developed for a rapid callus induction from *Solanum pubescens* willd. Shrub found in the foot hill areas of southern India. In Important medicinal plant which belongs to the family Solanaceae. It is widely used as folk medicine against many diseases. The *in vitro* callus induction was achieved from the node, internode and leaf explants of *Solanum pubescens*. Sterilized explants were prepared by using 0.1% $HgCl_2$ and 1.0 % Bavistin and callus was obtained when cultured onto Murashige Skoog's (MS) medium by using different concentrations and combination of BAP, NAA and 2, 4-D. The high amount of Callus induction was achieved from nodal and internodal explant in BAP 1.5 mg/l + NAA 1.0 mg/l + 2, 4-D 0.5 mg/l(87.5 %).The Callus induction was achieved from leaf explant on MS medium supplemented with BAP 3.0 mg/l + NAA 1.0 mg/l +GA3 0.5 mg/l (86.3 %). Hence, in the present article, an attempt has been made to overview *in vitro* callus induction studies in *Solanum pubescens* which serves as a potential source for contribution in the modern system of herbal medicine.

Keywords: Medicinal plant, Callus induction, MS medium, NAA.

1. Introduction

Solanum pubescens is a wild shrub grows up to the height of 4-6 feet found at mid elvations (500-700m) especially in the deciduous forests of peninsular India. It is very closely related to the Turkey berry (*Solanum torvum*). Leaves are smaller in size covered with dense sticky hairs. Flowers are larger, purple to violet, the flowering and fruiting is seasonal in *S. pubescens*. Flowering was hotzed from August to December. The fruit are around 1.2 cm diameter and bitterer. Matured fruits change in color to orange. In the absence of a reliable liver drug in modern medicine. There are numbers of medicinal preparations available in Ayurveda medicinal system for the treatment of liver disorders called as pajarito [4]. The extract has been already proved with some phytoconstituents like flavonol-B-o-methyl ethers, Solanopubamine a steroidal alkaloid and with different pharmacological property [8, 21].*in vitro* culture methods have been reported for so much species of medicinal plants [19,12]. However to our knowledge there is no information available concerning the micropropagation of *Solanum pubescens* earliest and the performance of tissue culture-derived plants under field conditions. Many scientists have reported *in vitro* tissue culture was monitored by different combinations of plant growth hormones [14, 5, 13]. Plant tissue culture has been identified as an excellent surrogate method to overcome the problems connected with utilization and conservation of medicinal plants [2]. Callus culture has a model system for several biological investigations. Even callus has proved better for the synthesis of alkaloids in several cases [3, 9]. India has a great environmental and biological diversity with a range of medicinal plants. An anti-diarrheal property has been widely used by the traditional healers; however, the efficacy of these anti-diarrheal traditional medicines has not been scientifically evaluated [10]. Callus induction and regeneration was previously reported by several workers [22, 25].The present investigation was aimed to develop a simple and efficient tissue culture protocol for *Solanum pubescens* with connection the study can impose the production of the medicinal plant by an alternate and cost effect approach.

2. Materials and Methods

Solanum pubescens Willd. Were collected from the Western Ghats, Sirumalai, Dindugal district, Tamilnadu. (Plate – 1, Fig: - A) The explants were taken from 4-5 months old plants. All the explants were washed thoroughly with running tap water for 20 min, then they were soaked at liquid detergent Tween 20 (1%v/v) for 5-10 min and rinsed with sterile double distilled water, followed by explants sterilized with 0.01% $HgCl_2$ (w/v) solution for 2 min and finally rinsed with double sterile for distilled water three – four times to remove traces of $Hgcl_2$. Surface sterilized explants were aseptically inoculated in MS medium (11) supplemented with B5 vitamins (6) 30 % sucrose and agar 0.8% the MS with different combination and concentration of BAP (0.5-3.0 mg/l), NAA (1.0 mg/l), 2, 4-D (0.5mg/l) and GA_3 (0.5 mg/l) were used P^H was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl before autoclaving at 121° C and 15 lb for 20 min. All cultures were maintained at 25 ± 1°C less than 16 hrs photoperiod at a photosynthetic flux of 12.6 µmol2/s, which was provided by cool daylight fluorescent lamps. Callus induction rate on each media formulations were calculated using the following equation.

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

3. Result and Discussion

Initially all the three explants such as node, internode and leaf were cultured on MS medium supplemented with different concentration of BAP, NAA, GA_3 and 2, 4-D alone or in combination for callus induction. The node and internode explant produce more amount of callus induction in BAP 1.5 mg/l +NAA 0.5 mg/l + 2, 4-D 0.5 mg/l (21 / 24 (87.5 %),(Plate:1, Fig. B and C; Table: 1)both node and internode explants produce greenish yellow and softy nature of the callus induction. The maximum amount of callus induction was observed from the leaf explant on MS medium supplement with BAP (3.0 mg/ l) + NAA (1.0mg/ l) + GA_3 (0.5mg/l) (Plate:1, Fig. D; Table-2**)** This showed best results when compared with other combination of BAP. The similar results has also suggested by [16] on *Solanum melongena* in MS media supplemented with NAA (2 mg/1) was suitable for the study of callus induction and IAA + BAP (0.5+3.0 mg/1) through Eggplant. *Withania somnifera* [7] *Eclipta alba* [1] *Abutilon indicum* [15] *Glinus lotoides* [18] *Solanum hainanense* [20] From this present investigation it is new opening to researchers for doing genetic manipulation of *Solanum pubescens* for disease, pest resistance or enhancing secondary metabolites using a rapid callus induction protocol that a suitable concentration of growth regulating substance is fruitful in tissue culture for further propagation.

Table 1: Effect of grouth regulators for Callus induction from node and internode Explants of *Solanum pubescens*

Plants Grouth regulators (mg/l)			Node and Internode explants [Number of explants responded/ inoculated (%)]	Nature of the callus (GFC)
BAP	2.4-D	NAA		
0.5	0.5	1.0	16 / 24 (66.6)	Greenish and Yellow Callus
1.0	0.5	1.0	18 / 23 (78.2)	Greenish and Yellow Callus
1.5	0.5	1.0	21 / 24 (87.5)	Greenish and Yellow Callus
2.0	0.5	1.0	17 / 22 (77.2)	Greenish and Yellow Callus
2.5	0.5	1.0	16 / 25 (64.0)	Greenish and Yellow Callus
3.0	0.5	1.0	15 / 23 (65.2)	Greenish and Yellow Callus

Table 2: Effect of grouth regulators for Callus inductions from leaf explants of *Solanum pubescens*

Plants Grouth regulators (mg/l)			leaf explants [Number of explants responded/ inoculated (%)]	Nature of the callus (GFC)
BAP	NAA	GA_3		
1	1.0	0.5	10 / 21 (47.6)	Greenish and Yellow Callus
2	1.0	0.5	17 / 24 (70.8)	Greenish and Yellow Callus
3	1.0	0.5	19 / 22 (86.3)	Greenish and Yellow Callus
4	1.0	0.5	10 / 23 (43.3)	Greenish and Yellow Callus
5	1.0	0.5	12 / 21 (57.1)	Greenish and Yellow Callus

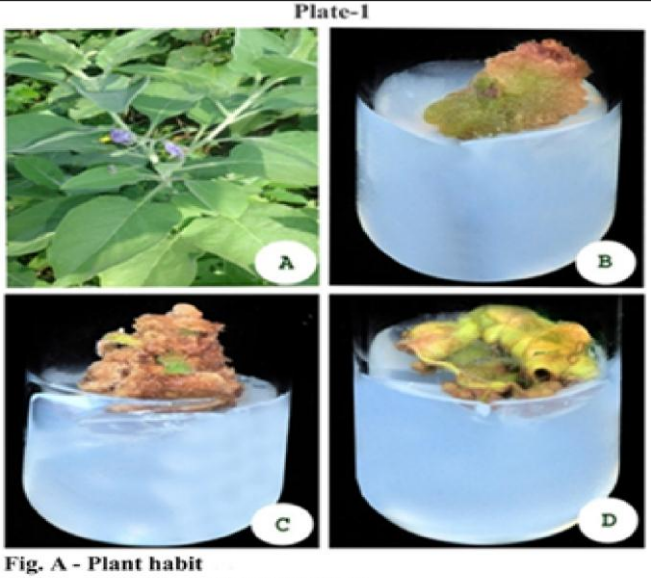

Fig. A - Plant habit
Fig. B - Callus induction from node
Fig. C - Callus induction from internode
Fig. D - Callus induction from leaf

4. Conclusion

In this present investigation, we have reported a very simple and efficient protocol for *in vitro* callus induction on *Solanum pubescens*. This is an essential tool for the sustainable supply of plant materials to the pharmaceutical industries and also to conserve the elite germplasm.

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