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In vitro Shoot Multiplication Studies on *Solanum pubescens* Willd an Important Antiepileptic Activity Plant

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ABSTRACT

Solanum pubescens Willd is an important medicinal plant which belongs to the family Solanaceae. It is widely used as folk medicine and treating for many diseases. Efficient in vitro regeneration of Solanum pubescens was achieved from node and Internode explants on MS medium with B5 vitamins and different concentrations and combinations of PGRs like BAP, GA_3 and KIN. The maximum numbers of multiple shoots were achieved from nodal and internodal explants on 3.0 mg/l BAP+1.0mg/l GA3+0.5mg/l KIN (80.5%). The regenerated shoots were transferred in to half strength MS medium fortified with IBA for root induction. Rooted plantlets were successfully acclimatized. The current study showed efficient in vitro shoot regeneration capabilities of Solanum pubescens.

Keywords: Multiple shoots, *Solanum pubescens*, MS medium, BAP, GA_3, KIN

INTRODUCTION

Solanum pubescens is a Willd. plant it is an annual erect, unarmed shrub growing up to 1.5 m tall abundantly growing as weed of forest and the hills of South-Eastern Ghats in Andhra Pradesh peninsular India and Sri Lanka. Commonly known as Ushtichettu, Kasivuste and pajarito in Telugu and Kaattu sundai kaai in Tamil, flowering and fruiting is in the month of July to February. *Solanum pubescens* is a traditional medicine plant for the treatment of headache, menstrual pain, rheumatoid arthritis, tuberculosis, ulcers, etc [1]. It exhibits gastro protective activity [2]. The use of herbal preparations in the management of various forms of epilepsies is very common in many parts of the world. Epilepsy affects more than 50 million persons worldwide [3]. Seizure is a characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain and status epilepticus is characterized by repeated episodes of epilepsy without the patient having recovered from the previous attack [4]. There are many classes of anticonvulsants that are of clinical usefulness with good prognosis for controlling seizures in most patients [5]. These and the treatment cost have made traditional herbs and herbalists very useful and indispensable in the struggle for seizure management and future antiepileptic drug development. Despite this many patients have seizures that are not adequately managed by the established antiepileptic drugs [6] for this above medicinal purposes; this plant is highly focused in many countries and pharmaceutical industries. Tissue culture plays an important key role for medicinal plants in rapid propagation, conservation and enhanced the production of secondary metabolites. The secondary metabolites production can be possible through *in vitro* plant cell culture [7,8]. In this present investigation was undertaken with an objective to develop an efficient *in vitro* Shoot regeneration protocol for important medicinal plant *Solanum pubescens* through nodal and internodal explants.

MATERIALS AND METHODS

Plant material and surface sterilization

Healthy plant of *Solanum pubescens* were collected from Western Ghats, Sirumalai, Dindugal district, Tamilnadu. The node and Internode explants were taken from 4-5 months old plants. All the explants were washed thoroughly with running tap water for 20 mins. The sterilization of explants was done by dipping them in 70% ethanol for 10 seconds followed by continuous shaking. Then the explants were washed with detergent Tween-20 for 5 mins and after that explants were surface sterilized by 0.1% mercuric chloride ($HgCl_2$) for 1 min then finally rinsed for 3 times with sterilized distilled water the explants were aseptically transferred onto nutrient medium for shoot induction.

Shoot regeneration

In a laminar air flow cabinet, sterilized node and Internode explants (about 1.0 cm in length) were inoculated in culture tubes (22 × 150 mm) containing 25 ml of sterile Murashige and Skoog (MS) [9] with B5 vitamins [10] supplemented with 3% (w/v) sucrose, 8 g/l agar, and pH adjusted to 5.8. Explants were maintained in a growth room in the dark at a temperature of 25ºC. After 20 days, shoot induction. The basal medium was supplemented with different growth regulators in different concentrations and combinations. In the present study, two types of media were employed on the basis of growth regulator used. The shoot differentiating medium. The former medium was fortified with various concentrations of N6-benzylaminopurine BAP; 1.0–5.0 mg/l alone or in combination with α-naphthalene acetic acid KIN; 0.5 mg/l and GA_3 1.0 mg/l the shoot was periodically sub cultured on MS medium supplemented with 4.0 mg/l BAP for shoot regeneration from node and Internode were transferred to each culture tube. Data on percentage of shoot forming shoots and mean shoot number and length of differentiated shoots were recorded after 45 days of culture. MS medium lacking growth regulators served as the control.

Shoot elongation, rooting and field transfer

The shoots below 2.5 cm in length were excised and sub cultured on MS medium supplemented with 4.0 mg/l BAP for shoot elongation. The shoots having approximately 3.0 cm in length were harvested from the shoot elongation medium and cultured on half strength MS medium supplemented with various concentrations of indole-3-butyric acid (IBA; 0.5-2.5 mg/l) or NAA (0.2–1.0 mg/l) for root induction. Data were recorded for percent rooting, root number and length after 45 days of transfer on rooting medium. Plantlets with well developed roots were removed from culture tubes, washed well to remove the remnants of agar from roots and transplanted to plastic cups (6 cm diameter) containing garden soil and sand (1:1). The plantlets were placed in a glasshouse set at 24 ± 2ºC, 82–88% relative humidity and irradiance (60 mg/l m−2s−1) provided by cool white fluorescent tubes. Plants were irrigated with half-strength MS salt solution for 3 weeks and thereafter with water. After two months the plants were transferred to larger pots and kept under shade in a net house for another two weeks before transferring outside under full sun to develop into mature plants.

Culture conditions

The pH was adjusted to 5.8 with 1.0 N HCl or 1.0 N NaOH before autoclaving the medium at 1.06 kg cm^{-2} and 121ºC for 20 mins. The cultures were maintained in a culture room with a 16 hrs /8 hrs light/dark photoperiod at 23 ± 2ºC unless otherwise mentioned. Light was supplied at intensity of 80 _mol m−2s−1 supplied by two Philips TL 40W cool-white fluorescent lamps. Each treatment consisted of 20 tubes and all experiments repeated 3 times. The data were presented as mean and its standard deviation (mean ± SD) and comparisons of means were carried out with Duncan's multiple range tests [11] at 0.05% significance.

RESULT

Plant regeneration

The Node and Internode explants were inoculated on MS medium with B5 vitamins supplemented with various concentrations of BAP (0.5–5.0 mg/l) alone or in combination with KIN (0.5 mg/l) and GA_3 (1.0 mg/l) were used for culture initiation and multiplication of shoots. After 12 days of inoculation multiple shoot induction was observed from the explants. The mean number of multiple shoots was recorded on after 4 weeks of inoculation. In nodal and internodal explants were showed the maximum number of multiple shoots on BAP (3.0 mg/l)+GA_3 (1.0 mg/l) KIN (0.5 mg/l) and obtained the best response (Figures 1a-1f).

Shoot elongation, rooting and field transfer the length of some shoots (about 22%) were less than 1.0 cm and not suitable for root induction. Therefore, such shoots were excised from the shoot clumps and transferred to a fresh MS medium containing BAP (3.0 mg/l). The shoots elongated to a mean Number of shoots of 8.6 ± 0.32, Response 99% with minimum 5 nodes in 4 weeks. Although rooting was observed on half strength MS basal medium, the percent response and number of roots were low. Hence, further experiments were carried out with the half strength MS medium supplemented with NAA or IBA. The elongated shoots measuring a size of 8.6 ± 0.32 were transferred to half strength MS medium supplemented with NAA (0.2–1.0 mg/l) or IBA (0.5–2.5 mg/l). Comparatively, IBA was more effective for root induction than NAA, as the former resulted in optimum rooting frequency (98%) than the latter (68%). Half strength MS medium supplemented with 2.0 mg/l IBA was the best for percentage induction (98%) and average number of roots per culture the rooted shoots were successfully transplanted to thermocol cups containing sand: soil (1:1) and acclimatized two months after transplantation of the 98 plants transplanted to soil 83 survived they grew well with irrigation and showed new growth after 4 weeks (Graph 1).

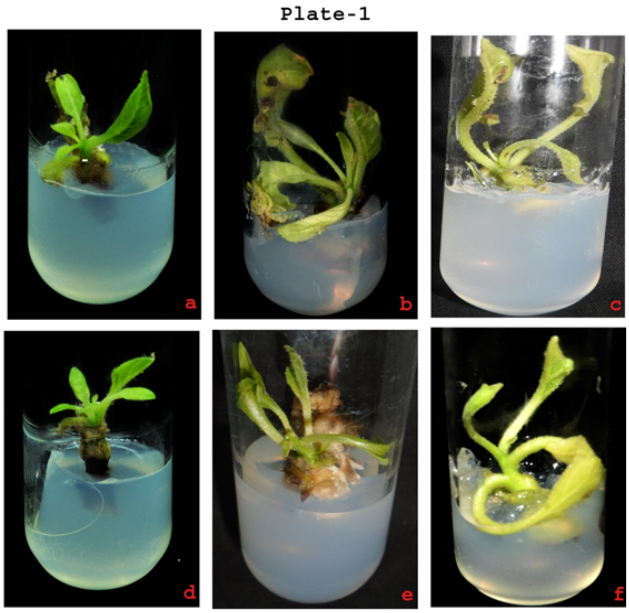
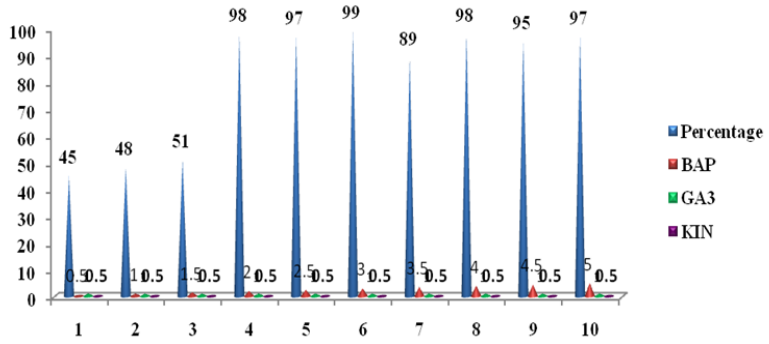


Figure 1: a-Shoot induction from intermodal explants, b-Multiple shoot induction, c- Shoot elongation, d-Shoot induction from nodal explants, e- Multiple shoot induction, f- Shoot elongation.



Graph: 1 Effect of various media compositions of plant growth regulators on multiple shoot induction from *Solanum pubescens* Willd.

DISCUSSION

The aim of the present investigation was to obtain high frequency shoot regeneration from nodal and internodal explants. There are some reports available on another species *Withania somnifera* [12] and [13]. We have used several explants for callus induction as a preliminary study. However, the satisfactory result was obtained with nodal and internodal explants only. Therefore, only the nodal and internodal explants were used for the present study. Similar results on superiority of intermodal explants on callus induction and shoot organogenesis have been reported in other systems like *Achillea millefolium* L [14]. The optimum plant growth regulator combination for callus induction was 4.0 mg/l 2, 4-D and 0.5 mg/l Kin. This study is in agreement with other systems where 2, 4-D and Kin induced callus has been reported in other systems like *Diplocyclous palmatus* L [15]. In this study, the light yellow, friable calli formed on all plant growth regulator combinations exhibited organic potential in the present study, MS medium supplemented with 4.0 mg/l BAP, GA3 1.0 mg/l and 0.5 mg/l NAA was optimum for shoot organogenesis from callus. The present report is in agreement with several previous reports where an auxin-cytokine combination provided maximum shoot induction response from callus. In *Physalis peruviana* highest shoot regeneration from callus was noted on MS medium supplemented with BAP 2.0 mg/l +NAA1.0 mg/l+GA_3 1.0 mg/l [16]. The two cytokinins used for shoot induction from callus, BAP was superior to Kin. It has been reported that BA being considered as one of the most potential cytokinin for shoot induction and used for micro propagation of several plants such as *Valeriana jatamansi Jones* [17], *Gentiana dinarica* [18], *Mentha piperita* [19], *Ajugabracteosa* [20] and *Stevia rebaudiana* [21]. Because of high frequency proliferation and regeneration potential, the present callus lines could be an ideal target material for Agro bacterium mediated or direct gene transfer (Table 1).

Table 1: Effect of various media compositions of plant growth regulators on multiple shoot induction from node and Internode Medium: MS; culture period: 20 days

Plant growth regulators (mg/l)			Number of shoots/explants (Mean ± SD)	% Response
BAP	**KIN**	**GA3**		
0.5	0.5	1.0	3.4 ± 0.89	45
1.0	0.5	1.0	5.3 ± 0.13	48
1.5	0.5	1.0	6.5 ± 0.56	51
2.0	0.5	1.0	7.5 ± 0.21	98
2.5	0.5	1.0	5.7 ± 0.26	97
3.0	0.5	1.0	8.6 ± 0.32	99
3.5	0.5	1.0	7.4 ± 0.47	89
4.0	0.5	1.0	7.5 ± 0.21	98
4.5	0.5	1.0	5.2 ± 0.57	95
5.0	0.5	1.0	4.8 ± 0.36	97

Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range tests ($P \geq 0.05$). The values represent the means (± SE) of three independent experiments. At least 20 cultures were raised for each experiment.

Shoot elongation 3.0 mg/l BA was optimum for *Solanum pubescens*. According to [22] *Withania coagulans,* BAP is the key plant growth regulator for shoot elongation and low concentration of BAP is ideal for optimum result. In this study full strength MS medium produced callus at the basal cut end and was not suitable for root induction as reported in other systems *Withania somnifera* [23]. Therefore, half strength MS medium was employed for the present study. Our study is in line with other reports where half strength MS medium produced optimum rooting response *Silybum marianum* and *Silymarin accumulation* [24] and [25] during the present investigation, it has been clearly indicated that the highest root induction from shoot was obtained on IBA. These results are comparable with those described by other researchers for IBA induced rooting in other species *Pelargonium graveolens* [26]. *Azadirachta indica* [27]. *Jatropha curcas* L [28], *Withania somnifera* [29].

CONCLUSION

In the present investigation, we have reported very simple and efficient protocol for *in vitro* shoot regeneration of *Solanum pubescens* as compared to the methods described for other members of Solanaceae. This will be useful for conservation and sustainable utilization of this medicinal Shrub. These are the first protocol for *in vitro* shoot regeneration of *Solanum pubescens*. Further, this approach can be used for mass multiplication of targeted medicinal plants in short span of time to cater to the need of pharmaceutical industries. Our success with in-vitro establishment clearly indicates that micro propagation is an effective and useful technique for the reproduction of this species.

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