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IN VITRO DIRECT MULTIPLE SHOOT INDUCTION FROM LEAF EXPLANTS OF *SOLANUM PUBESCENS* WILLD

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Abstract

Efficient *in Vitro* direct multiple shoot regeneration from *Solanum pubescens* was achieved from leaf explants on MS medium Sublimated with B5 vitamins and different concentrations and different combinations of PGRs like BAP, NAA and GA3. The maximum numbers of multiple shoots were achieved from leaf explants on 3.0 mg/l BAP + 1.0mg/l GA3. The regenerated shoots were transferred in to half strength MS medium fortified with IBA for root induction. Rooted plantlets were successfully acclimatized. This new and transfer into the field Conditions. Standardized and reproducible protocol useful the mass propagation of *Solanum pubescens*.

Keywords: Multiple Shoots; Solanum pubescens; MS Medium: BAP and GA_3.

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1. Introduction

Solanum pubescens is a wild plant. It is an annual erect, unarmed shrub growing up to 1.5m tall abundantly growing as weed of forest and the hills of Western Ghats, Sirumalai, Dindugal district, Tamilnadu. It is commonly known as Ushtichettu, Kasivuste and pajarito in Telugu and Kaattusundaikaai in Tamil, flowering and fruiting is in the month of July to February. Solanum pubescens is a traditional medicinal plant for the treatment of headache, menstrual pain, rheumatoid arthritis, tuberculosis, ulcers, etc (1). Medicinal plants are of great importance to the health of individuals and communities in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants (2). Tissue culture technique can play an important role in clonal propagation and quantitative improvement of this medicinally important plant. Regarding this shoot multiplication of

Ashwagandha from different explants such as hypocotyl and cotyledonary leaf (3) leaf (4) and shoot tip (5) has been reported. To date there have been few reports on *in vitro*shoot multiplication of Ashwagandha via direct organogenesis (6), (7).The present study was undertaken to examine the potential, of different explants, to respond under in vitro conditions with the possibility of developing a protocol for the *in vitro* multiplication of *Solanum pubescens.*

2. Materials and Methods

Plant Material *Solanum pubescens* plants were collected from Western Ghats, Sirumalai, Dindugal district, Tamilnadu and were successfully planted in National College herbal garden for further use. The plant Specimens are maintained in the department of Botany, National College. For the initial experiments, healthy leaf explants were collected from two months old plant. Selection and Surface sterilization of explants after selection of leaves as ideal explants for our experimentation, we have chosen them for further studies on the effect of growth hormones like Benzyl amino purine (BAP), and Gibberellin (GA3). All explants were first washed under running tap water for 30 min and then washed with 2-3 drops of tween 20detergent solution for 15-20 min. Traces of Tween 20 solution were removed by washing 6-7 times with distilled water and transferred to Laminar air flow chamber. The explants were surface sterilized with 0.1 % (w/v) HgCl2 solution for 4 min, and then washed with sterilized distilled water. The 70% ethanol was added and waits for 5 min and then explants were washed with sterilized distilled water for 5-7 times. Now the explants were cut to the required size and inoculated onto culture medium. All the explants were placed horizontally on the medium, and the leaves were placed with their dorsal side in contact with the medium. Culture Medium and Conditions the culture medium used for the explants selection was MS medium (8) with B5vitamins (9) supplemented with 3% (w/v) sucrose and pH was adjusted to 5.8 with 1N NaOH or HCl before addition of 0.8%(w/v) agar (Hi media, India) and enrichedwith varying concentrations of BAP incombination with IAA, 2, 4-D, GA3 were usedfurther to determine the optimum growthregulator levels. The concentrations tested forBAP (0.5-4.0 mg/l), IAA (1.0-3.0 mg/l), 2, 4- D (1.0-3.0 mg/l) and GA3 (1.0-3.0mg/l).Molten media were dispensed into test tubes(Borosil, India) (25×150mm; 10ml) andclosed with non-absorbent cotton plugs andmedia were autoclaved at 104 kpa and 121^0Cfor 20 min. The cultures were maintained at25± 20C under a 16 hour photoperiod of35µmol m-2 s-1 irradiance provided by coolwhite fluorescent light with 55-65% relativehumidity. For hardening-off, 7 to 8 weeks oldrooted shoot lets were removed from theculture flacks. After freeing the agar with therunning water they were transferred intosmall polythene bags containing sterilizedcow-dung, sand and red soil (1: 1: 1) andkept in a mist house. After acclimation in themist house for 2 months, they weretransferred to green house.

3. Results and discussion

Direct multiple shoot induction was achieved from leaf explants on MS basal medium with B5 vitamins and various concentrations of plant growth regulators were used in leaf explants, direct multiple shoots was observed at BAP 3.0 mg/l + GA3 1.0 mg/l. The highest number of multiple shoot percentage in leaf explants showed at 99.2% the best response. (Table 1) (Fig. 1 -a, b, c, d).The similar result has also suggested by (10). In shoot tip and nodal explants, direct multiple shoots was observed at BAP 2.0 mg/l + NAA 1.0 mg/l. The highest number of multiple shoot

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percentage in shoot tip explants showed at 87.5% and nodal explants showed at 89.2%. The multiple shoots were sub cultured on shoot elongation medium containing GA3 and NAA. In shoot elongation was observed at the concentration of GA3 1.0 mg/l + NAA 1.5 mg/l and showed the best response. The meristem tic cells of the nodal region were induced to produce fresh shoots in many plant species (11, 12), (13)and (14). Cytokinins were proved responsible for cell division, cell elongation and to induce shoots from nodal meristem of explantThe superiority of BAP over Kin in bud initiation from the explants has been proved in many plant species such as *Holarrhenaantidysenterica*, *Arnebiahispidiss*ima, *Randiadumetorum*, *Tectonagrandis*, *M. citrifolia* and *Turneraulmifolia* (15, 16,17, 18, 19,20 and 21). MS medium with BAP/Kin + IAA induced callus fromthe base of the explants and less number of shoots. The in vitro regeneration of medicinal plant Physalis minima Revealed that the tissue culture showed good response in proliferation of multiple shoots in MS medium by supplement in with BAP, NAA and GA3. This present study was for *Solanum pubescens* which can be used for easier cultivation, propagation and plant genetic studies.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 (0.1-0.5 mg/l) or IBA (0.2–1.0 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 0.8 mg/l IBA was the best for percentage induction (98%) (Graph: 2 : Table:- 2, Fig:- E) 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 50d. In thispresent invsearctigation has also opened new researchers for genetic manipulation of *Solanum pubescens* for disease, pest resistance or enhancing secondary metabolites using a rapid regeneration protocol. The rooted plants were successfully acclimatized and survived under *Ex Vitro* conditions.

Table 1: Effect of different concentrations of bap in combination with GA_3 on multiple shoot induction from leaf explants of *Solanum pubescens.*

Plant growth regulators (mg L−1)		**No. of shoots per explant**	**Shoot height (cm)**	**% response**
BAP	GA_3			
1	0.0	1.5 ± 0.1 c	2.1 ± 0.1 b	55.3
2	0.5	2.3 ± 0.1 b	4.1 ± 0.2 a	95.5
3	**1.0**	**3.5 ± 0.2 b**	**4.5 ± 0.3 a**	**99.2**
4	1.5	3.3 ± 0.1 a	3.8 ± 0.1 a	89.1
5	2.0	2.8 ± 0.2 b	2.5 ± 0.2 b	60.8

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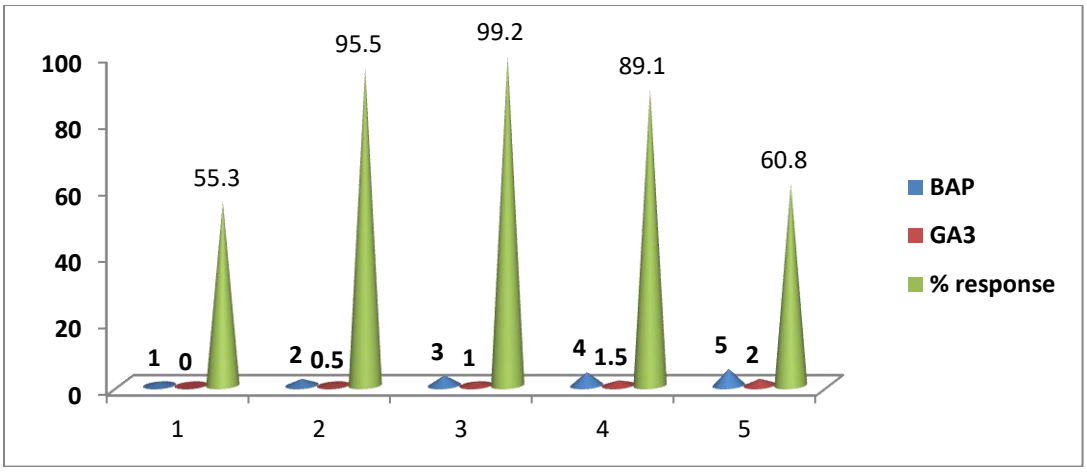

Graph 1: Directmultiple shoot induction from leaf explants

Figures 1: (PLATE-1)

Table 2: Effect of various concentrations of IBA or NAA on rooting of shoots in S. pubescens. Medium: Half strength MS; culture period: 50 d.

Plant growth regulators (mg/l)		**% Response**	**Mean root number a**	**Mean root length (cm)a**
IBA	NAA			
0.2	0.1	68	6.8±0.3b	0.2±0.07a
0.4	0.2	75	7.5±0.6a	0.4±0.06b
0.6	0.3	90	9.0 ± 0.9a	0.5±0.07a
0.8	**0.4**	**98**	**9.9± 0.2a**	**0.7±0.08a**
1.0	0.5	96	9.7 ± 0.5a	0.8±0.05c

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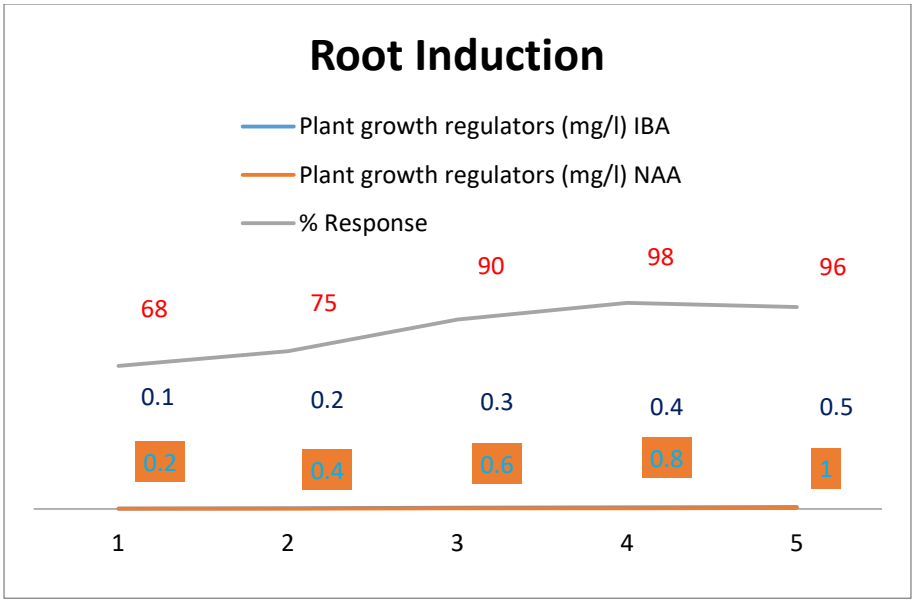

Graph 2: Root induction from leaf explant

4. Conclusion

In the present investigation, we have reported very simple and efficient protocol for*in vitro*multiple 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. This is the first protocol for in vitro multiple 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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