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# Plant Regeneration from Node and Internode Explants of *Solanum trilobatum* L.

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## Abstract

Shootlets were regenerated from node and internode explants of *Solanum trilobatum* L. through direct and indirect organogenesis. The induction of multiple shoots from nodal explants was high in LS medium supplemented with a combination of 5 mg/l BAP and 0.05 mg/l IAA. Shootlet regeneration from internodal explants was also high in LS medium with a supplement of 5 mg/l BAP and 0.05 mg/l NAA. The regenerated shootlets were rooted on LS medium fortified with different concentrations of IBA. The maximum percentage of rooting was obtained with 1 mg/l IBA. The rooted plantlets were hardened and successfully established in soil.

## Introduction

*Solanum trilobatum* L. is one of the important medicinal plants, considered to be a home remedy for the treatments of all kinds of cough. The decoction is used in chronic bronchitis (Kirtikar and Basu 1975). In the present study a simple protocol has been developed to propagate *Solanum trilobatum* L. through tissue culture methods in order to ensure abundant supply of this plant material for the preparation of herbal medicine.

## Materials and Methods

Seeds of *Solanum trilobatum* L. were collected from wild plants, treated with 5% Bavistin, sun-dried and surface sterilized with 0.1% $HgCl_2$ for 3 min and washed thrice with double distilled water inside the Laminar Air flow

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chamber. Surface sterilized seeds were inoculated in plain agar medium containing 3 % sucrose for germination. The aseptically germinated seedlings were used for further studies. The various explants such as nodes, internodes and leaves were inoculated on Linsmaier and Skoog (1965) medium fortified with different concentrations of auxins (2,4-D, NAA and IAA) and cytokinins (BAP). pH of the medium was adjusted to 5.8, solidified with 0.8 % agar and autoclaved at 121_C for 15 minutes. The cultures were incubated at 25 ± 2_C with 16 h photoperiod.

## Results and Discussion

Nodal explants were incubated on LS medium fortified with different concentrations of BAP (3 - 6 mg/l) and IAA (0.5 mg/l). Within three weeks multiple shoots emerged directly from the explants. The response was good at 5 mg/l BAP + 0.5 mg/l IAA combination (Table 1) where 10 - 15 shoots developed (Fig. A). At 4 mg/l BAP + 0.5 mg/l IAA only one or two shoots developed and at 3 mg/l BAP + 0.5 mg/l IAA, the response was very poor and no shoot formation took place. Only green, hard callus induction was observed.

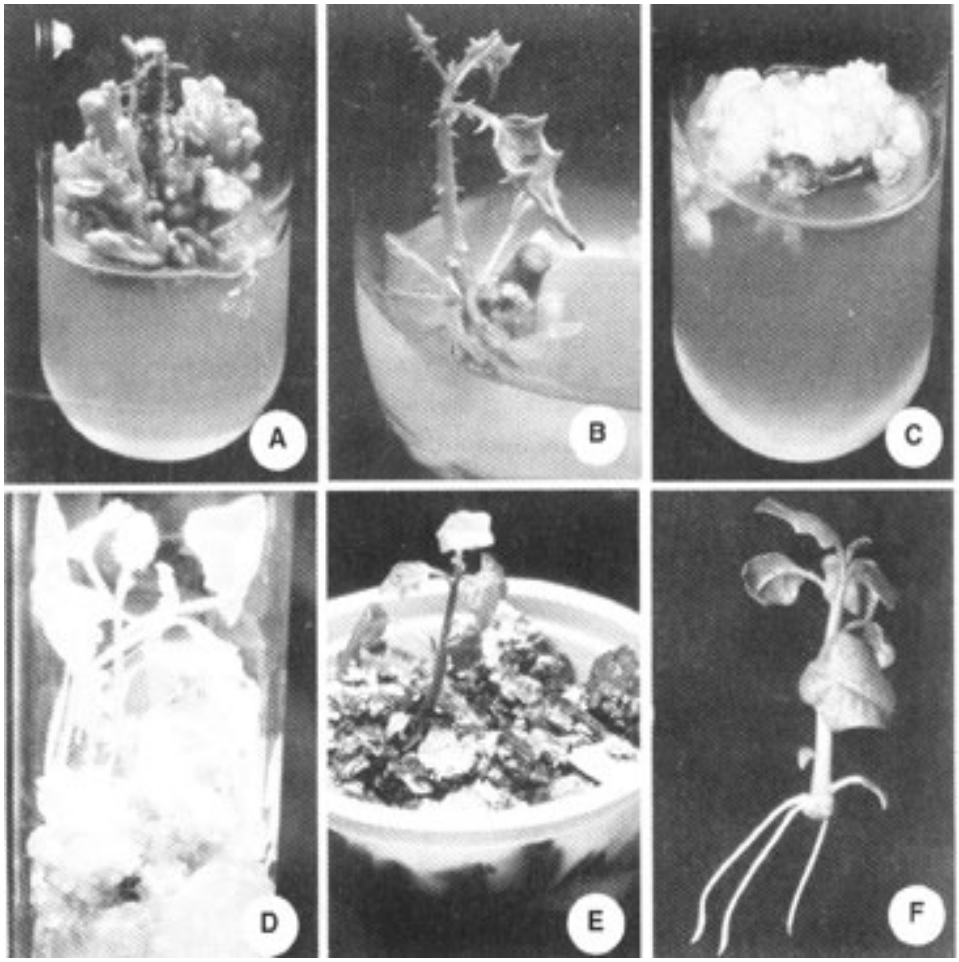
**Table 1. Effects of different concentrations of BAP and IAA on multiple shoot induction from nodal explants of *S. trilobatum* L.**

| Hormonal conc. (mg/l) | | % of multiple shoot proliferation | No. of shoots/ explant (mean ± SD) |
|---|---|---|---|
| BAP | IAA | | |
| 3 | 0.5 | – | – |
| 4 | 0.5 | 45 | 4.0 ± 0.8 |
| 5 | 0.5 | 87.5 | 13.3 ± 1.2 |
| 6 | 0.5 | 66 | 8.3 ± 1.6 |

In the present study 5 mg/l BAP + 0.5 mg/l IAA was found to be the ideal concentration for high frequency multiple shoot induction, while in *Solanum viarum* (another important medicinal plant belonging to the same family) a combination of 8 mg/l 2ip + 1 mg/l IAA was reported to be the most suitable concentration for multiple shoot induction (Tejavathi and Bhuvana 1998). The developing shoots elongated (Fig. B) by subculturing on the same medium using the same concentrations of growth hormones. Later on elongated shoots were excised and used for root regeneration.

Internodal explants were inoculated in LS medium supplemented with different concentrations of BAP (1 - 5 mg/l) and NAA (0.05 - 0.25 mg/l) for

regeneration of shoots. A pale greenish hard callus (Fig. C) was obtained within two weeks of inoculation at 5 mg/l BAP + 0.05 mg/l NAA (Table 2). Other combinations of BAP and NAA yielded slightly friable and greenish callus.




Figs. A - F : Plant regeneration from nodal and internodal explants. A. Induction of multiple shoots from nodal explants. B. Elongation of regenerated shoots. C. Induction of callus from internodal explants. D. Regeneration of shoots from the internodal callus. E. A rooted plantlet. F. Hardening of plantlet.

Well grown calli were subcultured on the same medium with the same concentrations of plant growth regulators. Of various concentrations of BAP and NAA, the combination of 5 mg/l BAP + 0.05 mg/l NAA was found to be ideal for shoot regeneration. In this combination shoot proliferation was as high as

86.5 %. Any deviation in concentration, high or lower adversely affected shoot proliferation. The regenerated shoots (Fig. D) from internodal explants were excised and subcultured for root regeneration.

Repeated callus subcultures maintained at 5 mg/l BAP + 0.05 mg/l NAA combination, resulted in increased shoot proliferation till 90 days (six subcultures). Thereafter the vigour decreased substantially (Table 3).

**Table 2. Effects of different concentrations of BAP and NAA on shoot regeneration from internodal explatns of *S. trilobatum* L.**

| Hormonal conc. (mg/l) | | % of multiple shoot proliferation | No. of shoots/ explant (mean ± SD) |
|---|---|---|---|
| BAP | NAA | | |
| 2 | 0.05 | 36 | 2.3 ± 0.47 |
| 3 | 0.05 | 46 | 4.6 ± 0.92 |
| 4 | 0.05 | 61.5 | 7.6 ± 0.94 |
| 5 | 0.05 | 86.5 | 12.3 ± 0.47 |
| 6 | 0.05 | 76 | 9.3 ± 0.47 |

**Table 3. High frequency of successive shoots harvested from explants after multiple shooting.**

| Days after inoculation | Serial no. of harvest | No. of shoots harvested | No. of plantlets successfully planted |
|---|---|---|---|
| 15 | 1 | 18 | 12 |
| 30 | 2 | 23 | 18 |
| 45 | 3 | 27 | 22 |
| 60 | 4 | 30 | 25 |
| 75 | 5 | 30 | 25 |
| 90 | 6 | 35 | 27 |
| 105 | 7 | 31 | 23 |

The excised shoots from the nodal and internodal explants were inoculated on LS medium containing different concentrations of IBA (0.5 - 2.5 mg/l) for rooting. Maximum root induction was observed at 0.5 mg/l IBA (Table 4). The root induction gradually decreased with increasing concentration of IBA. At 2 and 2.5 mg/l IBA callus induction was observed from the cut portion of the shoot. At 0.5 mg/l IBA there was no callus induction (Fig. E) proving ideal

concentration for root induction; compared to this in *Datura metel* (another important medicinal plant of the Solanaceae) 2 mg/l IBA was reported to be the most suitable concentration for root induction (Arockiasamy et al. 1992).

**Table 4. Effect of different concentrations of IBA on root induction.**

| Hormonal conc. (mg/l) | % of multiple shoot proliferation | No. of shoots/ explant (mean ± SD) |
|---|---|---|
| 0.5 | 71.0 | 6.8 ± 1.4 |
| 1.0 | 58.5 | 4.3 ± 0.78 |
| 1.5 | 48.0 | 36 ± 0.49 |
| 2.0 | – | Callus |
| 2.5 | – | Callus |

Rooted plantlets of *S. trilobatum* were taken out from culture tubes, and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were grown on polythene cups containing vermiculite (Fig. F) and kept inside the culture room. Plantlets were nourished with half strength LS liquid medium for two weeks. Then they were transferred to polythene bags consisting of a soil mixture of sand and red soil at the ratio of 1 : 1 and after two weeks the hardened plantlets were planted in soil.

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