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Original Research Article

In vitro Flower Induction and Multiple Shoot Regeneration Studies in *Solanum americanum* L. (Solanaceae)

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Abstract: *In vitro* regeneration of *Solanum americanum* was achieved from explants like leaf and nodal Segments on MS medium with B5 vitamins supplemented with different concentrations and combinations of plant growth regulators. Multiple shoot induction was achieved from nodal explants cultured on MS medium supplemented with BAP 3.0 mg/L+2, 4-D 0.5 mg/L+GA3 2.0 mg/L. In vitro flower induction and multiple shoot induction was observed from leaf explants on MS medium combination with BAP 2.0 mg/L+2, 4-D 1.0mg/L+IAA 1.0 mg/L. The regenerated shoots were transferred in to MS medium fortified with NAA, IBA and IAA for root induction. Soil, compost and sand (1:1:1) mixture was the most suitable planting substrate for hardening. Regenerated plants were successfully acclimatized and survived under ex vitro conditions.

Keywords: *In vitro* flowering, Benzyl amino purine (BAP), 2, 4-Dichlorophenoxyacetic acid (2, 4-D), Gibberellin (GA3), Indole acetic acid (IAA), Indole butyric acid (IBA), Naphthalene acetic acid.

Introduction

Solanum is one of the most important and largest genera of the family Solanaceae comprising of about 84 genera and 3000 species (Yasin, 1985). *Solanum americanum* is a small, annual perennial herb plant, commonly known as American nightshade or Glossy nightshade and is native to the tropics and subtropics of the Americas, Melanesia, New Guinea, and Australia (Plant NET –The Plant Information Network System, 2013) (Zich FA *et al.,* 2001). It grows up to 1–1.5 metres tall and is an annual or short-lived perennial. The leaves are alternate on the branch, and vary greatly in size, up to 10 centimetres long and 7 centimetres broad, with a 4-centimetre petiole and a coarsely wavy or toothed margin. The flowers are about 1 cm diameter, white or occasionally light purple, with yellow stamens. The fruit is a shiny black berry 5–10 millimetres diameter, containing numerous small seeds.

Many modern researches indicate the presence of toxic glycoalkaloids and there are warnings to be careful on the use of *S.americanum* as herbal medicine and food. The green fruits of *Solanum americanum* are particularly poisonous and eating unripe berries has caused the death of children (Ayesha Mohy-ud-din *et al.,* 2010). Ripe berries and foliage may also cause poisoning. Because of its high levels of glycoalkaloids, solanine and solamargine (Lacruz, 2003). Other toxins are present in the plant, such as chaconine, solasonine, solanigrine, gitogenin and traces of saponins (Nellis, 1997), as well as the tropane alkaloids scopolamine (hyoscine), atropine and hyoscyamine (Syeda Maria Ali, 2004). Significant amounts of solasodine (0.65%) have been found in the green berries of *Solanum americanum*. The ripe fruit also contains 0.3-0.45% solasonine and acetylcholine and has a cholinesterase-inhibiting effect on human plasma (Edmonds and Chweya, 1997).

In Transkei, rural people have a high incidence of esophageal cancer thought to be a result of using *S.americanum* as a food. Livestock can also be poisoned by high nitrate levels in the leaves (Nellis, 1997). The *Solanum americanum* extracts were having selective antiviral activity against the herpes simplex type-1 virus (HSV-1) (Abdul Manaf Ali, 1996). Methanol extracts from *S. americanum* were to have high antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger* (Gugulothu valya, 2011). In China a tea from the whole plant is used to treat cancer of the cervix. It is used as folk medicine for a wide range of conditions, being applied topically and internally (Edmonds and Chweya, 1997). It is always used as a medicine in Cameroon, Kenya, Hawaii, Panama, Sierra Leone, Tanzania and Pakistan

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(Nellis, 1997). The ripe fruit is cooked into jams and preserves, or eaten raw. The leaves are used in a West Indian stew, and it is known as branched Kalaloo (Nellis, 1997). In Africa, South America, New Guinea and Oceania the young green shoots of *Solanum americanum* are cooked and eaten as greens, after boiling in water (Electronic Flora of South Australia, 2013). In Kenya, Cameroon and Papua New Guinea the leaves are sold as a leaf vegetable in the markets. In Mauritius it is cultivated and eaten as a pot-herb and used in bouillon (Edmonds and Chweya, 1997).

Plant tissue culture is an alternative method of commercial propagation (George and Sherrington, 1984) and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants. Application of traditional medicinal plants for human use has also been reported (Shimomura *et al*., 1997). The present investigation was carried out to develop a simple and efficient protocol for rapid micropropagation and conservation of *Solanum americanum*. This is an alternative and cost effective method to improve the crop production of medicinal plant.

Materials and Methods

Plant Material

Solanum americanum plants were collected from Samuthiram, Tiruchirappalli district, Tamilnadu, India 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 nodal and leaf explants were collected from two months old plant.

Selection and Surface sterilization of explants

After selection of nodal segment and 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), Indole acetic acid (IAA), 2,4-Dichlorophenoxyacetic acid (2, 4-D) and Gibberellin (GA_3). All explants were first washed under running tap water for 30 min and then washed with 2-3 drops of Tween 20 detergent 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 (Murashige and Skoog, 1962) with B5vitamins (Gamborg *et al*., 1968) 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 enriched with varying concentrations of BAP and BAP in combination with IAA, 2, 4-D, GA_3 were used further to determine the optimum growth regulator levels. The concentrations tested for BAP (0.5-4.0 mg/l), IAA (1.0-3.0 mg/l), 2, 4-D (1.0-3.0 mg/l) and GA_3 (1.0-3.0mg/l). Molten media were dispensed into test tubes (Borosil, India) (25×150mm; 10ml) and closed with non-absorbent cotton plugs and media were autoclaved at 104 kpa and 121^0C for 20 min. The cultures were maintained at 25± 2^0C under a 16 hour photoperiod of 35µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent light with 55-65% relative humidity. For hardening-off, 7 to 8 weeks old rooted shoot lets were removed from the culture flacks. After freeing the agar with the running water they were transferred into small polythene bags containing sterilized cow-dung, sand and red soil (1: 1: 1) and kept in a mist house. After acclimation in the mist house for 2 months, they were transferred to green house.

Results

Shoot multiplication and Flower induction

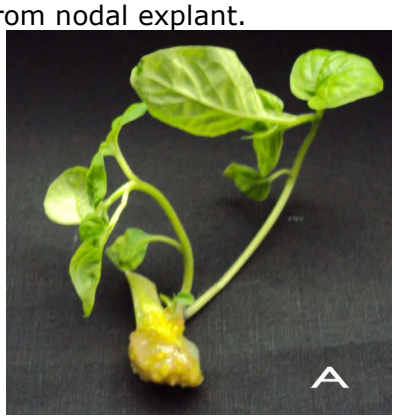
MS basal medium with B5 vitamins supplemented with various concentrations and combination of BAP (0.5-4.0mg/l), IAA (1.0-3.0 mg/l), 2, 4-D (1.0-3.0 mg/l) and GA_3 (1.0-3.0 mg/l) were used for culture initiation and multiplication of shoots. After 2 weeks of inoculation multiple shoots was observed from nodal and leaf explants. In leaf explant flower induction was observed along with shoot multiplication. 3 weeks after inoculation all the cultures were transformed to the fresh

medium. The mean numbers of multiple shoots were evaluated after 2 weeks of inoculation.

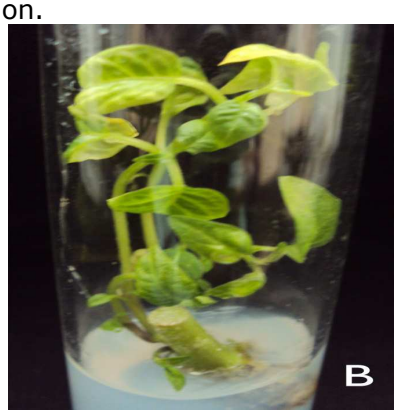
Discussion

Solanum americanum plants were efficiently regenerated from field-grown young plants on MS medium with B5 vitamins supplemented with different types of growth hormonal concentrations were used to study their effect on shoot multiplication and flower induction from nodal and leaf explants. In the present study, the various concentration and combination of plant growth regulators like BAP (0.5-4.0mg/l), 2, 4-D (0-0.5 mg/l) and GA_3 (0-3.0 mg/l) were used for multiple shoot induction from nodal explants. The maximum number of multiple shoots were obtained from nodal explant of *Solanum americanum* at BAP (3.0 mg/l) + 2, 4-D (0.5mg/l) + GA3 (2.0mg/l) showed the mean value 13.5 is the best response (Table 1) (Fig.1. A & B).

Fig.1: [A & B] *In vitro* shoot development stages from nodal explant.



A. Multiple shoot induction from nodal explant on MS medium B5 vitamins supplemented with 3.0 mg/l of BAP, 0.5 mg/l of 2, 4-D and 2.0 mg/l of GA_3 two weeks after inoculation.

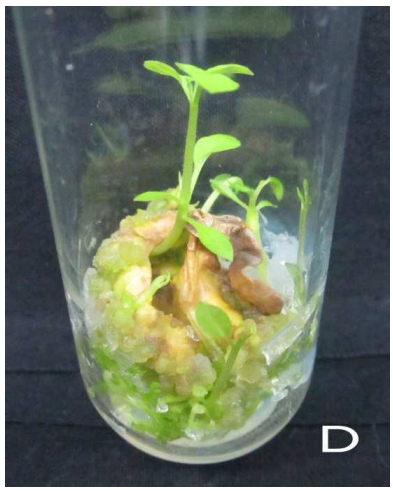


B. Multiple shoot elongation from nodal explant on MS medium B5 vitamins supplemented with 3.0 mg/l of BAP, 0.5 mg/l of 2, 4-D and 2.0 mg/l of GA_3 four weeks after inoculation.

Similar results were reported on *Citrus aurantifolia* where BAP (2.0 mg/l), KIN (1.0 mg/l) and NAA 1.0 (mg/l) were used (Abdulaziz and Al-Bahrany 2002). The maximum number of shoots was obtained from nodal explant of *Aegle marmelos* (L.) at BAP (2.5mg/l) and IAA (1.0 mg/l) (Ajithkumar and Seeni 1998). In different concentration and combination with plant growth regulators like BAP (0.5-4.0mg/l), IAA (1.0-3.0 mg/l) and 2, 4-D (1.0-3.0 mg/l) were used for shoot multiplication and flower induction from leaf explants. The large numbers of multiple shoots were obtained from leaf explant of *Solanum americanum* at BAP (2.0 mg/l) + IAA (1.0mg/l) + 2, 4-D (1.0mg/l) showed the mean value 14.0 is the best response (Table 2) (Fig.2. C & D).

Figure.2: [C & D] *In vitro* multiple shoot development stages from leaf explant.

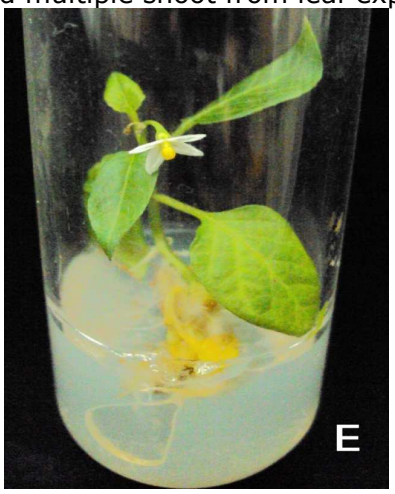
C. Multiple shoot induction from leaf explant on MS medium B5 vitamins supplemented with 2.0 mg/l of BAP, 1.0 mg/l of IAA and 1.0 mg/l of 2, 4-D two weeks after inoculation.



D. Multiple shoot elongation from leaf explant on MS medium B5 vitamins supplemented with 2.0 mg/l of BAP, 1.0 mg/l of IAA and 1.0 mg/l of 2, 4-D four weeks after inoculation.

The Similar results have also suggested the same type of explants for propagation of other medicinal plants, such as, the highest number of multiple shoots on *Pistacia vera* L. were regenerated from leaf explants at BAP (1.0 mg/l) (E. Tilkat *et al*., 2009). The highest rate of shoot multiplication was obtained from leaf explants of *Phellodendron amurense* at 1.5μM BAP and 1.0 μM NAA (Yoshizawa, 2005). High amount of shoot regeneration achieved from *Echinacea purpurea* at BAP (4.44μM) and NAA (0.054μM) (Simon, 2002). As the concentration of BAP increased or decreased in the basal medium, the number of shoots was decreased. In this present study, we showed that the BAP play a key role of shoot multiplication from nodal and leaf explants in *Solanum americanum.*

Figure.3: *In vitro* flower inductions on elongated multiple shoot from leaf explant.



In vitro flower inductions on elongated multiple shoot from leaf explant on MS medium B5 vitamins supplemented with 2.0 mg/l of BAP, 1.0 mg/l of IAA and 1.0 mg/l of 2, 4-D.

Table 1: Multiple shoot induction from nodal explants with various concentrations of plant growth regulators

S. No.	Different Concentration of Growth regulators			No. of shoots / explants Mean ± SD.
	BAP	NAA	GA_3	
1.	0.5	0	0	10.5 ± 0.30
2.	1.0	0	0	10.6 ± 0.29
3.	1.5	0	0	10.8 ± 0.26
4.	2.0	0	1.0	9.9 ± 0.26
5.	2.5	0.5	1.5	11.0 ± 0.21
6.	3.0	0.5	2.0	13.5 ± 0.28
7.	3.5	0.5	2.5	11.4 ± 0.22
8.	4.0	0.5	3.0	12.1 ± 0.23

Values are mean ± SE.

Table 2: Multiple shoot induction from leaf explants with various concentrations of plant growth regulators.

S. No.	Different Concentration of Growth regulators			No. of shoots / explants Mean ± SD.
	BAP	IAA	2, 4-D	
1.	0.5	0	0	7.8 ± 02.90
2.	1.0	0	0	9.5 ± 0.302
3.	1.5	1.0	0	13.0 ± 0.350
4.	2.0	1.0	1.0	14.0 ± 0.260
5.	2.5	1.5	1.5	9.0 ± 0.258
6.	3.0	2.0	2.0	9.5 ± 0.302
7.	3.5	2.5	2.5	8.5 ± 0.302
8.	4.0	3.0	3.0	9.1 ± 0.260

Values are mean ± SE.

The elongated shoots from leaf explant were showed best response of flower induction at BAP (2.0 mg/l) + IAA (1.0mg/l) + 2, 4-D (1.0mg/l) (Table 3) (Fig.3.E). Some similar results were reported on *in vitro* flowering from *Vitex negundo* at 4.4 μM BAP and 0.53 μM NAA were used (Vadawale *et al*., 2006). In vitro flowering achieved from *Ocimum basilicum* at BAP 5.0 mg/l and IAA 1.0 mg/l (Sudhakaran and Sivasankari, 2002). The flowers produced *in vitro* appeared morphologically normal, greenish white in colour. However, some exhibited growth and developed into white flowers, which matured. From the result it is showed that BAP play an important role for flower induction from *Solanum americanum*. The Empherical guidences show that exogenous auxin could act as a principal floral inhibitor, but in the present investigation IAA in the medium did not inhibit in vitro flower formation from leaf explant. *In vitro* flowering was also influenced by the levels and ratios of the two major components, carbohydrates and minerals. The presence of a carbohydrate source is a ubiquitous requirement for reliable induction and development of flowers *in vitro* (Scorza, 1982).

Table 3: *In vitro* flower induction from multiple elongated shoots for leaf explants

S. No.	Different Concentration of Growth regulators			Flower Response
	BAP	IAA	2, 4-D	
1.	0.5	0	0	No response
2.	1.0	0	0	No response
3.	1.5	1.0	0	Flower response
4.	2.0	1.0	1.0	Flower response
5.	2.5	1.5	1.5	No response
6.	3.0	2.0	2.0	No response
7.	3.5	2.5	2.5	No response
8.	4.0	3.0	3.0	No response

Conclusion

In the present investigation, we have reported very simple and efficient protocol for *in vitro* flower induction and regeneration of *Solanum americanum* as compared to the methods described for other members of Solanaceae. This will be useful for conservation and sustainable utilization of this medicinal herb. This is the first protocol for *in vitro* flower induction and regeneration of *Solanum americanum.* 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 micropropagation is an effective and useful technique for the reproduction of this species.

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