

Role of Silver Nitrate on *in Vitro* Flowering and Shoot Regeneration of *Solanum nigrum* (L.)—An Important Multipurpose Medicinal Plant

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Abstract

Silver nitrate induced MS media enhanced multiple shoot regeneration and *in vitro* flowering from axillary bud and leaf explants of *Solanum nigrum* (L.) an important antiulcer medicinal plant. Healthy axillary bud and leaf explants were inoculated on MS medium supplemented with BAP/Kn (1.0 - 2.0 mg/l) in combination with IAA/NAA (0.5 mg/l) and AgNO₃ (0.1 - 1.0 mg/l). The explants were responded effectively and good regeneration frequencies were observed in all the combinations of silver nitrate tested when compared to control. Maximum number of multiple shoots (34.3) was found in leaf explants cultured on MS media supplemented with BAP (2.0 mg/l), NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l). These regenerated shoots were sub-cultured on to the flowering media. Maximum number of *in vitro* flowers (12) was obtained from axillary bud explants in BAP (2.0 mg/l), Kn (1.0 mg/l), IAA (0.5 mg/l) and AgNO₃ (6.0 mg/l) supplemented media when compared to leaf (4) and control. All the *in vitro* raised shoots were transferred to rooting medium supplemented with NAA, IBA (1.0 - 2.0 mg/l) and AgNO₃ (0.1 - 0.6 mg/l). The best rooting response (24.6) was observed in 2.0 mg/l IBA + 0.4 mg/l AgNO₃. The well rooted plantlets were transferred to polybags containing soil + vermiculate in 1:1 ratio for hardening and finally the hardened plantlets were transferred to field conditions.

Keywords

In Vitro Flowering, Micropropagation, Silver Nitrate, Ethylene Inhibitor, *Solanum nigrum*

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1. Introduction

Solanum nigrum (L.) commonly known as black night shade, an important medicinal plant of family Solanaceae. It is grown in dry parts of India up to an elevation of 2100 mts. It is an important herbaceous medicinal plant, generally used in traditional and folklore medicines. Commonly it is used as leafy vegetable in preparing traditional dishes. The herb is antiseptic, antidiarrhetic and diuretic used in the treatment of cardiac, skin diseases, psoriasis, herpesvirus and inflammation of kidney. The leaves, stems and roots are used externally as poultice; wash etc., in the treatment of cancerous sores, boils, leucoderma and wounds [1]. The fruits and leaves have been traditionally used against various nerve disorders [2]. Root bark is laxative, useful in the treatment of neck, burning of throat, inflammation of liver and chronic fever. Berries are bitter, pungent and are useful in the disease of heart, piles and dysentery [3].

Most important aspect of this medicinal plant is that it contains two important alkaloids solamargin and solasonine which yield solasodine glycone [4]. Solasodine has embryogenic, teratonic as well as antifungal, antiviral and molluscicidal effects [5]. Solasodine has great demand in pharmaceutical industry; owing to its demand in pharma industry, the plant is extensively harvested. So it is necessary to establish an efficient protocol for *in vitro* propagation of this important herbaceous medicinal plant. *In vitro* flowering bears immense importance in selective hybridization especially in plants that use pollens from rare stocks. *In vitro* flowering may help in obviating the intricate interactions present in whole plants. It also facilitates in understanding the nature of factors which influences the flowering.

Ethylene is a ubiquitous gaseous hormone which influences the growth as promoter or inhibitor based on the species used [6]. It is a simple hydrocarbon (C₂H₄), but has maximum impact on growth, cellular differentiation, fruit ripening and senescence in plants even at concentrations as low as 0.01 µl·L⁻¹ or 10⁻⁶ % v/v [7]. Wounding during explants preparation, presence of high auxins and cytokinins concentration in media further increase ethylene production in *in vitro* conditions. So excess accumulation of gases directly influences the success of *in vitro* regeneration. Influence of ethylene in tissue culture propagation had been well documented previously. Ethylene suppresses the growth and morphogenesis of explants depending on the species and stage of the culture [8]. To overcome this problem, for the first time we had made an attempt to study the effect of AgNO₃, ethylene inhibitor to enhance the shoot and root proliferation and *in vitro* flowering in *Solanum nigrum*.

2. Materials and Methods

2.1. Source of Plant Material

Healthy axillary bud and leaf explants of *Solanum nigrum* (L.) were collected from two-month-old seed germinated field grown plants growing in the Herbal garden of Dravidian University, Kuppam, Andhra Pradesh, India.

2.2. Surface Sterilization

Explants were washed thoroughly under running tap water to remove traces of dust etc. followed by treatment with 10% teepol or tween -20 for 5 minutes and 0.4% bavistine (fungicide) for 10 - 15 minutes. Then the explants were sterilized in 70% alcohol for a minute, and finally with 0.01% mercuric chloride for 1 - 2 minutes and washed 3 - 4 times with sterile double distilled water.

2.3. Culture Medium

The explants were inoculated on MS medium [9] containing 3% sucrose and gelled with 0.8% agar, supplemented with various concentrations of BAP, Kn in combination with IAA or NAA and AgNO₃. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 20 minutes at 121°C and 15 lbs pressure.

2.4. Sub Culturing

The cultures were maintained by regular subculture at 4 week intervals on fresh MS medium.

2.5. Culture Conditions

All cultures of *Solanum nigrum* were maintained in a culture room at temperature of 24°C ± 2°C and 55% - 65%

RH with 16 h/8h photoperiod at a photon flux density of 3000 lux or $50 - 70 \text{ Em}^{-2}\cdot\text{s}^{-1}$ provided by cool white fluorescent tubes.

2.6. Data Collection and Statistical Analysis

Visual observations were recorded on the frequency in terms of number of cultures responding for axillary shoot proliferation, shoot development, number of shoots per explant, average length of the regenerated shoots, number of flowers and number of roots per shoot and average root length.

Despite scarcity and limitations encountered with the plant material, for most of the treatment a minimum of 10 replicates were used. All the experiments were repeated at least twice/thrice and the cultures were observed at regular intervals. The qualitative data were subjected to statistical analysis by using standard error (SE \pm) for shoot length, rate of shoot multiplication and then number of roots per shoot.

3. Results and Discussion

3.1. Effect of Silver Nitrate on Axillary Bud Induction and Multiplication

Axillary buds excised from field grown plants of two month old have shown shoot proliferation response. Efforts have been made to study the synergetic effect of AgNO_3 along with various concentrations of cytokinins such as BAP, Kn in combination with auxins such as NAA and IAA. In axillary bud explants shoot buds initiated after 7 - 8 days of inoculation on MS medium supplemented with various plant growth regulators along with silver nitrate with different concentration of BAP (1.0 - 2.0 mg/l) and Kn (1.0 - 2.0 mg/l). Observations recorded in **Table 1** and **Table 2** signifies, 0.4 mg/l AgNO_3 at all cytokinin and auxin concentrations initiated better response in relation to shoot regeneration and also induced higher number of shoots per explants. Significantly very high shoot regeneration frequency (95%) was observed in 0.4 mg/l AgNO_3 supplemented media along with 2.0 mg/l BAP and 0.5 mg/l NAA and the highest number of multiple shoots (29.5 ± 0.40), whereas further increase in the concentration of AgNO_3 supplementation in the media had resulted in poor regeneration frequency (60% at 1 mg/l AgNO_3 , BAP 1.0 mg/l and NAA 0.5 mg/l). The frequency, number of multiple shoots induced in this combination were less when compared to BAP (2.0 mg/l), IAA (0.5 mg/l) and AgNO_3 (0.4 mg/l) (20.7 ± 0.70) respectively. Results considerably enlighten the fact that presence of silver nitrate in the media has increased number of shoots per explants over three folds as compared to those formed on control. Similar type of enhancement was observed in *Coffea arabica* [10] [11] and *Vanilla planifolia* [12].

Supplementation of 0.4 mg/l AgNO_3 in the media had effectively enhanced the shoot length similar to multiple shoot regeneration. The maximum shoot length was observed in AgNO_3 (0.4 mg/l), BAP (2.0 mg/l) and NAA (0.5 mg/l) (12.7 ± 0.85 cm) and the same concentration in case of IAA (0.5 mg/l) the shoot length was (8.46 ± 0.05 cm) respectively. Multiple shoots can be sub cultured repeatedly by excising the shoots from the cluster and transferring them on to fresh medium with four weeks passage time. Large clumps of multiple shoots were separated and sub cultured as small clusters for further proliferation in nutrient media supplemented with different concentrations and combinations of AgNO_3 , cytokinins (BAP, Kn) and auxins (IAA, NAA). Between the two cytokinins tested, BAP was found to be more effective than Kn in the induction of multiple shoots from the axillary bud explants. AgNO_3 at the concentration of 0.4 mg/l in the media had efficiently improved the shoot regeneration in axillary bud cultures, hence further experiments were conducted with the same concentration (0.4 mg/l) of AgNO_3 (**Figure 1**).

3.2. Effect of Silver Nitrate on Direct Organogenesis from Leaf Explants

Effect of two different cytokinins (BAP, Kn) in combination with auxins (NAA, IAA) along with ethylene inhibitor AgNO_3 on direct shoot induction was examined (**Table 3**, **Figure 2**). In leaf explants after three weeks of inoculation leaf folding, leaf enlargement were noticed. Shoot bud primordia were emerged at the cut portions and midrib regions of young leaf explants, later they were developed as shoots. Silver nitrate is added to the culture medium with growth regulators significantly improved the initial shoot regeneration. In BAP (1.0 - 3.0 mg/l), NAA (0.5 mg/l) and AgNO_3 (0.4 mg/l) combinations multiple shoots were induced with mean shoot number in the range of (17.3 - 34.3) and the mean shoot length of (4.3 - 10.3 cms) respectively. The highest number of shoots (34.3 ± 0.25) were obtained in BAP (2.0 mg/l), AgNO_3 (0.4 mg/l) and NAA (0.5 mg/l) supplemented medium. Similar results were observed in *Brassica napus* [13], *Gloxinia (Sinningia speciosa)* [14].

Table 1. Effect of different concentrations of BAP, NAA and IAA in combination with AgNO₃ on multiple shoot regeneration from axillary bud explants of field grown *Solanum nigrum* plants. Observation: After 8 weeks, values are mean ± S.E. of 20 independent determinants; Intensity of callus: C⁺ = very low, C⁺⁺ = low.

Plant growth regulators (mg/l)			Concentration of AgNO ₃ (mg/l)	Regeneration frequency (%)	Mean no. of shoots/explant	Mean shoot length (cm)	Callus formation
BAP	NAA	IAA					
1.0	0.5	-	-	80	9.4 ± 0.35	4.5 ± 0.2	-
1.0	0.5	-	0.1	82	16.9 ± 0.25	5.6 ± 0.36	C ⁺
1.0	0.5	-	0.2	85	19.6 ± 0.20	7.63 ± 0.75	-
1.0	0.5	-	0.4	90	25.8 ± 0.55	9.5 ± 0.3	-
1.0	0.5	-	0.6	75	17.4 ± 0.40	6.76 ± 0.61	-
1.0	0.5	-	0.8	70	10.4 ± 0.26	6.3 ± 0.20	-
1.0	0.5	-	1.0	60	7.1 ± 1.11	3.93 ± 0.45	C ⁺⁺
1.0	-	0.5	-	70	7.7 ± 0.2	3.43 ± 0.15	-
1.0	-	0.5	0.1	75	14.7 ± 0.15	5.93 ± 0.85	-
1.0	-	0.5	0.2	80	17.9 ± 0.87	7.4 ± 0.36	C ⁺
1.0	-	0.5	0.4	85	22.5 ± 0.35	9.1 ± 0.52	-
1.0	-	0.5	0.6	75	15.9 ± 0.50	6.58 ± 0.08	C ⁺
1.0	-	0.5	0.8	72	7.9 ± 0.62	3.63 ± 0.30	-
1.0	-	0.5	1.0	65	5.6 ± 0.37	3.43 ± 0.20	-
2.0	0.5	-	-	80	12.7 ± 0.2	4.43 ± 0.25	C ⁺
2.0	0.5	-	0.1	85	18.6 ± 0.26	5.83 ± 0.51	-
2.0	0.5	-	0.2	90	22.6 ± 0.25	9.6 ± 0.26	-
2.0	0.5	-	0.4	95	29.5 ± 0.40	12.7 ± 0.85	-
2.0	0.5	-	0.6	85	19.5 ± 0.15	7.3 ± 0.30	-
2.0	0.5	-	0.8	80	10.7 ± 0.65	4.93 ± 0.15	-
2.0	0.5	-	1.0	75	6.7 ± 0.30	3.9 ± 0.7	C ⁺
2.0	-	0.5	-	70	8.5 ± 0.15	3.6 ± 0.25	-
2.0	-	0.5	0.1	80	15.5 ± 0.30	4.46 ± 0.40	-
2.0	-	0.5	0.2	85	17.2 ± 0.15	5.73 ± 0.65	-
2.0	-	0.5	0.4	90	20.7 ± 0.70	7.93 ± 0.25	-
2.0	-	0.5	0.6	80	16.6 ± 0.36	6.36 ± 0.30	-
2.0	-	0.5	0.8	75	8.5 ± 0.20	4.6 ± 0.32	C ⁺
2.0	-	0.5	1.0	70	5.76 ± 0.47	4.83 ± 0.51	-

In Kn-AgNO₃-NAA/IAA combination less number of multiple shoots formed when compared to BAP combination. Silver nitrate at 0.4 mg/l significantly enhanced shoot regeneration compared with higher concentrations. Chraibi *et al.* [15] have shown similar results from cotyledons of Sunflower. Most of the plant tissues have to produce ethylene. The effect of ethylene on *in vitro* culture, as with other hormones, depends on its concentration in and around the cultured tissues, as well as their sensitivity to it [16]. Silver nitrate is usually employed in *in vitro* studies for inhibiting ethylene action. In silver nitrate, Ag⁺ ions can prevent a wide diversity of ethylene induced plant responses, the growth inhibition the effect was assumed to mediate the inhibition of the physiological action of ethylene [17], a potential inhibitor of many plant regeneration systems.

Table 2. Effect of different concentrations of Kn, NAA and IAA in combination with ethylene inhibitor AgNO₃ on multiple shoot regeneration from axillary bud explants of field grown *Solanum nigrum* plants. Observation: After 8 weeks, values are mean ± S.E. of 20 independent determinants; Intensity of callus: C⁺ = very low, C⁺⁺ = low.

Plant growth regulators (mg/l)			Concentration of AgNO ₃ (mg/l)	Regeneration frequency (%)	Mean no. of shoots/explant	Mean shoot length (cm)	Callus formation
Kn	NAA	IAA					
1.0	0.5	-	-	65	3.4 ± 0.40	2.76 ± 0.30	-
1.0	0.5	-	0.1	70	11.8 ± 0.15	4.8 ± 0.55	-
1.0	0.5	-	0.2	75	14.8 ± 0.70	5.3 ± 0.25	C ⁺
1.0	0.5	-	0.4	80	18.5 ± 0.26	7.96 ± 0.47	-
1.0	0.5	-	0.6	72	10.9 ± 0.3	4.83 ± 0.15	-
1.0	0.5	-	0.8	60	8.0 ± 0.49	3.4 ± 0.26	C ⁺⁺
1.0	0.5	-	1.0	55	4.4 ± 0.32	2.93 ± 0.35	-
1.0	-	0.5	-	60	3.2 ± 0.41	3.96 ± 0.61	-
1.0	-	0.5	0.1	65	7.9 ± 0.83	5.46 ± 0.32	-
1.0	-	0.5	0.2	68	12.2 ± 0.20	7.5 ± 0.17	C ⁺
1.0	-	0.5	0.4	75	15.9 ± 0.36	9.9 ± 0.52	-
1.0	-	0.5	0.6	65	9.9 ± 0.72	6.93 ± 0.25	-
1.0	-	0.5	0.8	60	5.4 ± 0.3	4.53 ± 0.49	-
1.0	-	0.5	1.0	50	3.9 ± 0.60	2.32 ± 0.37	-
2.0	0.5	-	-	70	5.8 ± 0.32	4.6 ± 0.15	C ⁺
2.0	0.5	-	0.1	80	13.8 ± 0.49	6.5 ± 0.56	-
2.0	0.5	-	0.2	85	21.5 ± 0.36	6.63 ± 0.45	-
2.0	0.5	-	0.4	90	18.9 ± 0.81	10.5 ± 0.20	-
2.0	0.5	-	0.6	80	15.4 ± 0.32	8.63 ± 0.15	-
2.0	0.5	-	0.8	70	8.1 ± 1.02	6.2 ± 1.15	C ⁺
2.0	0.5	-	1.0	65	5.9 ± 0.3	3.6 ± 0.08	C ⁺⁺
2.0	-	0.5	-	65	4.7 ± 0.56	2.8 ± 0.65	-
2.0	-	0.5	0.1	70	6.5 ± 0.36	4.6 ± 0.32	-
2.0	-	0.5	0.2	80	14.3 ± 0.25	5.7 ± 0.26	-
2.0	-	0.5	0.4	85	19.4 ± 0.3	8.46 ± 0.05	-
2.0	-	0.5	0.6	65	10.2 ± 0.17	6.63 ± 0.37	C ⁺
2.0	-	0.5	0.8	55	5.4 ± 0.25	3.4 ± 0.26	-
2.0	-	0.5	1.0	50	3.9 ± 0.3	2.6 ± 0.1	-

3.3. Effect of Silver Nitrate on *in Vitro* Flowering from Axillary Bud and Leaf Explants

The multiple shoots formed from different explants like axillary buds and leaves, showed *in vitro* flowering on MS medium supplemented with different combinations and concentration of cytokinins such as BAP/Kn (1.0 - 2.0 mg/l), AgNO₃ (2.0 - 8.0 mg/l) and auxins IAA/NAA (0.5 mg/l) (Table 4, Figure 3). This exogenous hormone supply might have been added up to the endogenous contents, raising the hormonal level required for triggering the flowering. In the present investigation flowering has been induced by the presence of lower concentrations of auxin (0.5 mg/l IAA) and higher concentrations of cytokinin (1 mg/l BAP) along with the presence of additional nitrate in the form of silver nitrate. Cytokinin promotes the transition in some higher plants to

Table 3. Effect of different concentrations of BAP, Kn, NAA and IAA in combination with AgNO₃ on direct multiple shoot organogenesis from leaf explants of field grown *Solanum nigrum* plants. Observation: After 8 weeks, values are mean ± S.E. of 20 independent determinants; Intensity of the callus: C⁺ = very low, C⁺⁺ = low.

Plant growth regulators (mg/l)				Concentration of AgNO ₃ (mg/l)	Regeneration frequency (%)	Mean no. of shoots/explant	Mean shoot length (cm)	Callus formation
BAP	Kn	NAA	IAA					
1.0	-	0.5	-	-	70	9.5 ± 0.36	3.4 ± 0.29	C ⁺
1.0	-	0.5	-	0.4	75	18.4 ± 0.21	6.5 ± 0.1	-
2.0	-	0.5	-	-	85	10.4 ± 0.10	7.4 ± 0.26	-
2.0	-	0.5	-	0.4	90	34.3 ± 0.25	10.3 ± 0.31	-
3.0	-	0.5	-	-	70	6.5 ± 0.36	4.7 ± 0.05	C ⁺⁺
3.0	-	0.5	-	0.4	75	17.3 ± 0.10	4.3 ± 0.15	-
1.0	-	-	0.5	-	65	7.9 ± 0.61	3.4 ± 0.2	-
1.0	-	-	0.5	0.4	70	15.5 ± 0.32	5.8 ± 0.25	-
2.0	-	-	0.5	-	75	12.2 ± 0.15	6.5 ± 0.18	-
2.0	-	-	0.5	0.4	80	22.4 ± 0.23	11.3 ± 0.37	-
3.0	-	-	0.5	-	70	9.8 ± 0.55	2.4 ± 0.18	-
3.0	-	-	0.5	0.4	65	13.5 ± 0.15	8.7 ± 0.26	-
-	1.0	0.5	-	-	60	6.9 ± 0.73	4.3 ± 0.37	C ⁺
-	1.0	0.5	-	0.4	70	14.7 ± 0.45	6.5 ± 0.20	-
-	2.0	0.5	-	-	65	11.4 ± 0.22	5.5 ± 0.35	C ⁺
-	2.0	0.5	-	0.4	82	16.4 ± 0.32	8.5 ± 0.2	-
-	3.0	0.5	-	-	60	5.5 ± 0.20	3.8 ± 0.36	-
-	3.0	0.5	-	0.4	70	12.7 ± 0.4	5.2 ± 0.20	-
-	1.0	-	0.5	-	55	5.7 ± 0.14	3.8 ± 0.25	-
-	1.0	-	0.5	0.4	65	10.5 ± 0.34	5.4 ± 0.30	-
-	2.0	-	0.5	-	65	8.98 ± 0.42	6.4 ± 0.18	-
-	2.0	-	0.5	0.4	70	14.2 ± 0.15	7.5 ± 0.34	-
-	3.0	-	0.5	-	60	4.5 ± 0.16	4.2 ± 0.14	-
-	3.0	-	0.5	0.4	65	12.4 ± 0.2	4.9 ± 0.26	-

their reproductive stage *in vitro*.

The first flower bud was observed in axillary bud culture after three weeks of sub culturing on to a fresh medium MS medium supplemented with BAP (1.0 mg/l), AgNO₃ (2.0 mg/l) and NAA (0.5 mg/l). Maximum number of *in vitro* flowers (12) per culture were obtained in (2.0 mg/l) BAP, Kn (1.0 mg/l), AgNO₃ (6.0 mg/l) and IAA (0.5 mg/l) supplemented MS medium. In the present study IAA in combination with Kn also induced *in vitro* flowers. The flower buds were appeared after four weeks of subculture on to a fresh MS medium along with silver nitrate. Similar results were obtained in *Rosa indica* [18]. The addition of AgNO₃ in the medium along with low concentration of auxin induced *in vitro* flowering. The number of flowers formed in silver nitrate treated plants was more when compared to control ones (without silver nitrate).

In leaf culture *in vitro* flower initiation was observed after 5 - 6 weeks of culture on MS medium along with silver nitrate supplemented with BAP (1.0 mg/l), AgNO₃ (2.0 - 8.0 mg/l) and IAA (0.5 mg/l). Maximum number of *in vitro* flowers (4) per culture were obtained in BAP (1.0 mg/l), AgNO₃ (6.0 mg/l) and IAA (0.5 mg/l). But the number of flowers (12) produced from axillary bud explants was more when compared to leaf (4) explants and control.

Table 4. Effect of different concentrations of BAP, Kn, NAA and IAA in combination with AgNO₃ on *in vitro* flowering from axillary bud and leaf explants of *Solanum nigrum*. Observation: After 8 weeks, values are mean ± S.E. of 20 independent determinants. =No flowering.

Plant growth regulators (mg/l)				Concentration of AgNO ₃ (mg/l)	Explant	Number of flowers
BAP	Kn	NAA	IAA			
1.0	-	0.5	-	-	Axillary bud	1
1.0	-	0.5	-	2.0	Axillary bud	5
1.0	-	0.5	-	4.0	Axillary bud	8
1.0	-	0.5	-	6.0	Axillary bud	4
1.0	-	0.5	-	8.0	Axillary bud	2
2.0	-	0.5	-	-	Axillary bud	-
2.0	-	0.5	-	2.0	Axillary bud	4
2.0	-	0.5	-	4.0	Axillary bud	7
2.0	-	0.5	-	6.0	Axillary bud	9
2.0	-	0.5	-	8.0	Axillary bud	6
1.0	-	-	0.5	-	Leaf	-
1.0	-	-	0.5	2.0	Leaf	2
1.0	-	-	0.5	4.0	Leaf	4
1.0	-	-	0.5	6.0	Leaf	3
1.0	-	-	0.5	8.0	Leaf	1
2.0	1.0	-	0.5	-	Axillary bud	2
2.0	1.0	-	0.5	2.0	Axillary bud	5
2.0	1.0	-	0.5	4.0	Axillary bud	9
2.0	1.0	-	0.5	6.0	Axillary bud	12
2.0	1.0	-	0.5	8.0	Axillary bud	4

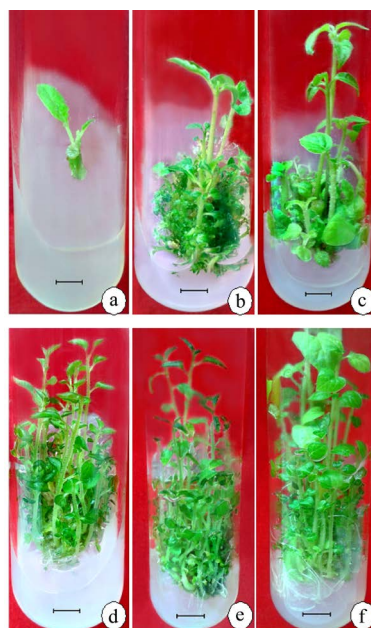


Figure 1. Direct shoot organogenesis from axillary bud explants: (a) Shoot initiation from axillary bud explants cultured on MS media containing BAP (1.0 mg/l), NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) (b) Shoot initiation from explants on MS media with BAP (2.0 mg/l), IAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) (c) Formation of multiple shoots in MS + Kn (1.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.1 mg/l) supplemented media (d) Formation of multiple shoots in MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) media (e), (f) Elongation of multiple shoots on MS + Kn (2.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) media.



Figure 2. Direct shoot regeneration from leaf explants: (a) Shoot initiation from leaf explants cultured on MS media containing BAP (1.0 mg/l), NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l). (b) Shoot initiation from explants on MS media with BAP (2.0 mg/l), IAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) (c) Formation of multiple shoots in MS + Kn (1.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.1 mg/l) supplemented media. (d) Formation of multiple shoots in MS + BAP (2.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) media (e), (f) Elongation of multiple shoots on MS + Kn (2.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (0.4 mg/l) media.

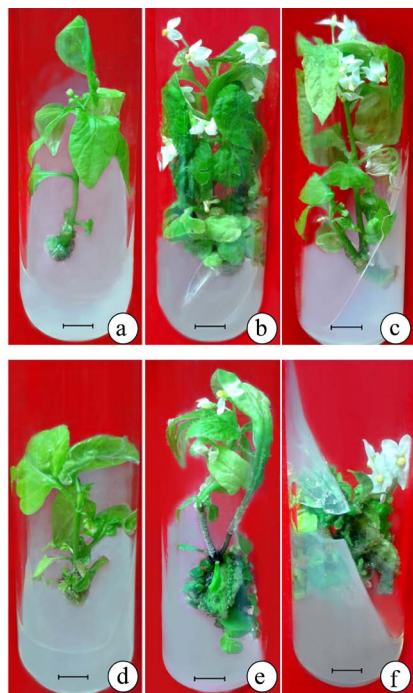


Figure 3. *In vitro* flowering from axillary bud and leaf explants: *In vitro* flower initiation from axillary bud explants cultured on. (a) MS + BAP (1.0 mg/l) + NAA (0.5 mg/l) and AgNO₃ (2.0 mg/l) (b) MS + BAP (2.0 mg/l) + IAA (0.5 mg/l) and AgNO₃ (4.0 mg/l) (c) MS + BAP (2.0 mg/l) + Kn (1.0 mg/l) + IAA (0.5 mg/l) and AgNO₃ (6.0 mg/l). *In vitro* flower initiation from leaf explants cultured on. (d), (e) MS + BAP (1.0 mg/l) + IAA (0.5 mg/l) and AgNO₃ (2.0 mg/l) (f) MS + BAP (1.0 mg/l) + IAA (0.5 mg/l) and AgNO₃ (6.0 mg/l).

3.4. Effect of Silver Nitrate on *in Vitro* Rooting

The shoots developed *in vitro* (3.0 - 5.0 cm) were excised from shoot clumps and transferred to MS medium supplemented with silver nitrate and different concentrations of auxins such as IBA, NAA and IAA. A marked difference with declined rate of rooting was observed (Table 5, Figure 4), when culture was supplemented with either lower or higher concentrations of auxins. Similar to the other parameters under study, rooting of *in vitro* grown shoots also responded well in the presence of 0.4 mg/l AgNO₃ which was almost enhanced by 2 to 2.5 folds compared to control. In all the concentrations tested, root primordia appeared between 6 - 10 days after transferred *in vitro* raised shoots to rooting medium. When *in vitro* shoots transferred to MS media fortified with IBA, NAA, IAA and AgNO₃ 95% of rooting frequency with highest number of roots (24.6 ± 0.26) were obtained in the IBA (2.0 mg/l) and AgNO₃ (0.4 mg/l) and maximum mean root length (6.5 ± 0.36 cm) was observed. But plenty of hairy root emergence was observed when auxin concentration in the media increases.

Table 5. Effect of AgNO₃ on different concentrations of IBA, NAA and IAA on *in vitro* rooting using MS medium. Observation: After 8 weeks, values are mean ± S.E. of 20 independent determinants.

Plant growth regulators (mg/l)			Concentration of AgNO ₃ (mg/l)	Regeneration frequency (%)	Mean no. of roots/shoot	Mean root length (cm)
IBA	NAA	IAA				
1.0	-	-	-	65	7.6 ± 0.20	2.76 ± 0.31
1.0	-	-	0.1	75	11.9 ± 0.32	3.5 ± 0.19
1.0	-	-	0.2	80	15.5 ± 0.35	4.3 ± 0.20
1.0	-	-	0.4	85	19.5 ± 0.23	5.9 ± 0.73
1.0	-	-	0.6	75	8.5 ± 0.18	3.0 ± 0.34
2.0	-	-	-	70	9.6 ± 0.3	3.54 ± 0.28
2.0	-	-	0.1	80	13.9 ± 0.14	4.3 ± 0.37
2.0	-	-	0.2	85	17.4 ± 0.40	4.6 ± 0.29
2.0	-	-	0.4	95	24.6 ± 0.26	6.5 ± 0.36
2.0	-	-	0.6	80	10.3 ± 0.21	3.2 ± 0.35
-	1.0	-	-	60	6.7 ± 0.19	2.2 ± 0.14
-	1.0	-	0.1	70	11.5 ± 0.36	2.7 ± 0.15
-	1.0	-	0.2	75	14.5 ± 0.14	3.3 ± 0.32
-	1.0	-	0.4	80	17.9 ± 0.36	4.0 ± 0.41
-	1.0	-	0.6	72	9.0 ± 1.3	1.8 ± 0.1
-	2.0	-	-	65	9.5 ± 0.32	2.4 ± 0.28
-	2.0	-	0.1	70	12.4 ± 0.15	3.6 ± 0.30
-	2.0	-	0.2	78	15.4 ± 0.29	5.3 ± 0.61
-	2.0	-	0.4	85	19.8 ± 0.56	4.56 ± 0.32
-	2.0	-	0.6	70	10.6 ± 0.30	3.1 ± 0.43
-	-	1.0	-	60	7.7 ± 0.26	1.4 ± 0.26
-	-	1.0	0.1	65	9.5 ± 0.15	2.47 ± 0.16
-	-	1.0	0.2	70	11.8 ± 0.40	3.62 ± 0.30
-	-	1.0	0.4	78	14.4 ± 0.36	4.4 ± 0.41
-	-	1.0	0.6	62	10.3 ± 1.22	2.3 ± 0.2
-	-	2.0	-	65	9.4 ± 0.36	2.0 ± 0.45
-	-	2.0	0.1	70	11.4 ± 0.21	2.8 ± 0.11
-	-	2.0	0.2	75	13.3 ± 0.15	3.5 ± 0.26
-	-	2.0	0.4	80	17.2 ± 0.92	4.42 ± 0.31
-	-	2.0	0.6	65	11.6 ± 0.34	1.39 ± 0.14

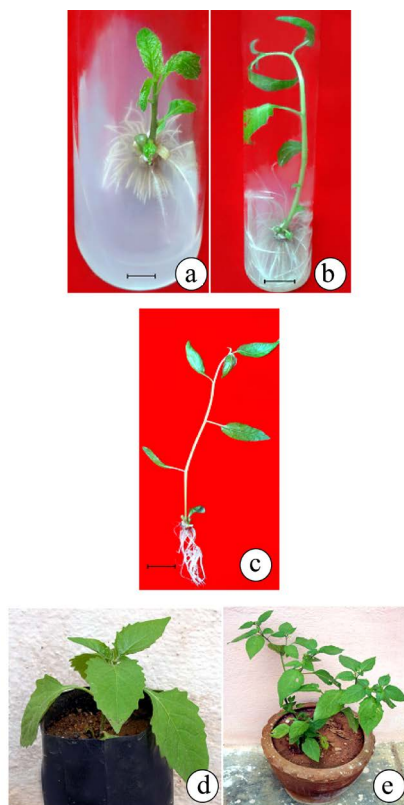


Figure 4. Rooting and acclimatization of *Solanum nigrum*: (a), (b) Root formation from shootlets inoculated on MS media with IBA (1.0 mg/l) and AgNO₃ (0.4 mg/l) (c) Plantlet showing elongated root system (d) Hardened plantlet in polybags containing soil and vermiculate in 1:1 ratio (e) Plantlet in field condition.

Presence of silver nitrate in *in vitro* propagation showed marked significance in almost all parameters under present investigation. Media without silver nitrate showed lesser response. This may be due to presence of silver nitrate, which might have suppressed the activity of excess of ethylene present in the *in vitro* culture tubes and further promoted for easy translocation and assimilation of these energy sources available in the media by the explants resulting in cell division and leading to vigorous growth. Similar type of results was documented in *Decalepis hamiltonii* [19], *Vanilla planifolia* [20].

3.5. Acclimatization and Hardening

The well rooted shoots were removed from the culture tubes and washed thoroughly to remove the traces of agar. Acclimatization of the regenerated plants to the external environment is the last and very important stage of micropropagation and its success depends upon different factors as suggested by various researchers [21] [22]. The plantlets of *in vitro* grown *Sphaeranthus indicus* with well developed roots and shoots were transplanted to plastic cups containing autoclaved vermiculite and soil (1:1). About 90% of the transplanted plantlets survived after acclimatization and showed healthy growth without any morphological variations. Finally after one month the hardened plants were transferred to pots containing garden soil and sand (2:1) and were allowed to grow under nursery shade conditions. These plants were watered at 3 days intervals and were finally planted in field condition. All the plantlets were phenotypically indistinguishable from the parent plants.

4. Conclusion

In the present investigation, an attempt has been made to study the effect of AgNO₃ on *in vitro* flowering and multiple shoot regeneration for the first time in medicinal plants like *Solanum nigrum*. Addition of AgNO₃ along with low concentrations of cytokinins and auxins in the medium induced *in vitro* flowering. The present study may offer to achieve better genetic varieties of plants which fail to produce seeds.

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Abbreviations

BAP	6-benzyl amino purine
Kn	Kinetin
AgNO ₃	Silver nitrate
NAA	α -naphthalene acetic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
MS	Murashige and Skoog