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Original Article

Micropropagation and *in vitro* flowering in *Solanum nigrum* linn. A medicinal plant

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Abstract

A protocol was developed for rapid multiplication of *Solanum nigrum* a medicinal plant, through *in vitro* culture of mature nodal explants. Multiple shoots were induced on both Murashige & Skoog's medium supplemented with varying concentrations and combinations of auxins and cytokinins (α-naphthaleneacetic acid, indolyl-3 acetic acid, 6-benzyladenine and kinetin). Maximum number of shoots was developed with medium fortified with 13.5 μM BAP. For rooting of the excised shoots were rooted on half strength woody plant medium supplemented with IAA 5.58 μM and IBA 4.92 μM were used. Thus, a reproducible protocol has been established for micropropagation of this species.

Keywords: *Solanum nigrum*; Multiple shoots; BAP; IAA; Murashige & Skoog's medium; axillary shoot

Introduction

Solanum nigrum is an erect annual herb. The juice of the plant is diuretic and used to cure chronic enlargement of liver, piles, dysentery and fever (Kumar *et al*., 1997). The drug made from this plant acts as laxative, improve appetite and this is administered against asthma, leprosy skin diseases (Bhattacharjee 2001). Due to large scale and unprohibited exploitation of the natural resource by the pharmaceutical industry, the wild stock of this medicinally important plant has been markedly depleted. *S. nigrum* can be propagated by seeds and vegetative cuttings. Root behaviour of stem cutting and non availability of seeds due to over exploitation are major get back for plant propagation processes are season dependent and can be achieved only during monsoon period. The application of *in vitro* techniques might be of great value as an alternative method to achieve rapid multiplication independent of Season (Fay,1994).

In the present study, a reliable protocol has been developed for large scale propagation of this important medicinal plant using multiple axillary shoot proliferation from single node cultures is described. There is no earlier report on *in-vitro* micropropagation of this useful plant.

Materials and Methods

Plants of *S. nigrum* were collected from Tirunelveli hills of Southern Western Ghats and established in the herbal garden and green house attached to the Centre for Biodiversity and Biotechnology, St. Xavier's College, Palayamkottai. Young shoots (5cm length) were collected from the green house. Plants were defoliated and washed in running tap water for 10 min. Surface decontamination of the shoots consisted of passage through 0.1% $HgCl_2$ (w/v) for 1½ min and three washes in sterilized distilled water. Then single nodes of 1-1.5 length were dissected and inoculated aseptically on to Murashige & Skoog's (1962)solidified with agar 0.5%,Himedia,Mumbai) and supplemented with 3% of sucrose and various concentration and combinations of plant growth regulators, viz 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA).

The pH of the medium was adjusted to 5.8 before adding 0.5% W/A agar and autoclaved at 121°C for 15 minutes in Astell (Scientific - U.K.) Autoclave. All the cultures were incubated at 25° ± 2°C under cool white fluorescent tubes (1500 - 2000 lux) for 16 hours/days. After few weeks , the multiple shoots formed, were cultured onto root inducing medium ½ M.S supplemented with 2% of Sucrose and different concentration of Auxin Indole-3 Butric Acid (IBA), Indole – 3 Acetic Acid (IAA) and NAA. After the shoot formation the *in vitro* raised plantlets were removed from culture tube, washed thoroughly in running tap water before transplanting into small polycups containing mixture of sterilized sand and garden soil and irrigated with 1/10 diluted liquid MS medium and covered with poly bags for *in vitro*

hardening. After hardening in polycups they were subsequently transferred to 15 cm diameter pots containing, sand and compost (2:1:1) and maintained under mist irrigation. Then they were shifted for field planting. Experiments were performed with a minimum of 15 replicates for initiation and were repeated at least twice.

Results and Discussion

When MS medium supplemented with different concentration of BAP was used, multiple shoots emerged from the nodal explants after 12 days of inoculation. The effect of BAP on shoot multiplication from nodal explant is shown in Table 1. The medium containing 13.5 μM of BAP induced multiple shoots (3-5) with maximum percentage of responding cultures (75%). The concentration of BA at 0.88 μM was too low to induce only 1 shoot / node.

Shoot elongation did not correlate well with shoot production with short length and number of nodes / shoot got consistently declined with increasing concentration (22.2 μM) of BAP. Perusal of literature suggests that BAP as a cytokinin is most active at concentration in many plant systems (Scott *et al*., 1995, Kathiravan & Ignacimuthu 1999). Therefore the maximum caulogenic response observed in this study at low concentration (0.44 μM) together with a decreasing response at concentration exceeding 8.88 μM is somewhat different. Similarly though the negative influence of BA at higher concentration on shoot and inter nodal length is known (Emmanuel *et al*. 2000). Hence the optimal shoot production at 0.44 μM BAP was achieved with less than satisfactory shoot elongation and number of node formation. Maximum number of flowers was also obtained on the MS medium supplemented with 2.22 μM of BAP. Shoot elongation can be achieved by transferring shoot clusters to a fresh medium with decreased concentration of cytokinin or by substituting less active cytokinin at dark conditions, the one which was employed for shoot induction (George,1996).

Flowering was considered to be a complex processes regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare (Stephan and Jayabalan, 1998).

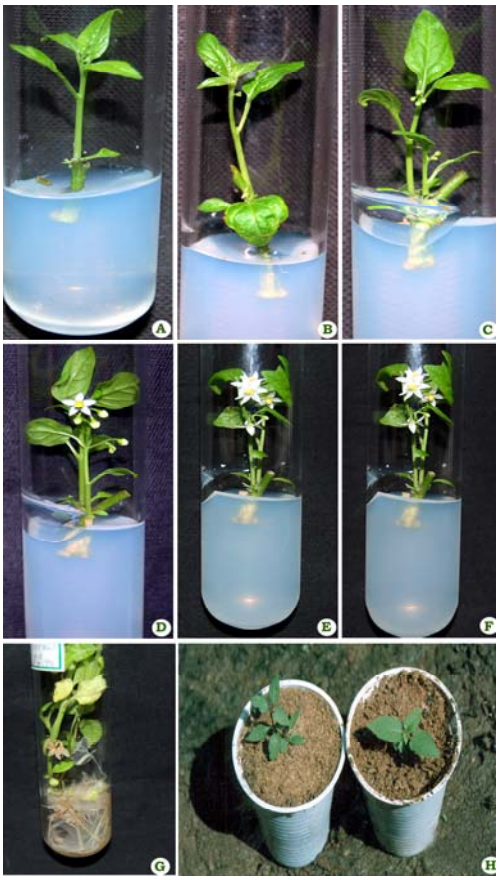

Fig.1: Micropropagation stages of *Solanum nigrum* Linn.
A – C: Shoot formation from nodal segment of *Solanum nigrum* on M.S Medium containing BAP & NAA; D - F. Flower bud initiation; G. Rooting of shoots on MS Medium Containing IBA;Transplanted plant in pot of *Solanum nigrum;* Hardened plantlets of *Solanum nigrum*

In vitro flowering was observed within 15 days of Culture (Fig. 1 D –F). When in vitro shoots, derived from nodal explants were transferred to rooting medium. For in vitro flowering, the response the response of IBA and NAA is better than IAA. High frequency and maximum number of flowers per explants was more on MS-medium supplemented with 0.8mg/lof IBA and 0.3mg/l of NAA compare to other concentration. Auxin support in vitro flowering, similar results were observed by patil *et al*., (1993) in Sun Flower, Vandana *et al*.,(1995)in Cauli flower, Naik and Lata (1998) in Coriander . Cytokinin did not support in vitro flowering either singly or in combination with auxins.

Table- 1: Effect of BAP on shoot production from the nodal segments of S*olanum nigrum* L.on ms medium

Sl. No.	BAP concentration (μM/*l*)	Percentage of shooting	No. of shootlets/ node ± SD (after 5 weeks of culture)	Mean length of shootlets (cm)
1	0.44	60	1.58 ± 0.06	2.36±0.185
2	0.88	65	1.46± 0.13	1.62±0.189
3	2.22	72	1.67 ± 0.32	1.95±0.404
4	4.44	75	1.56 ± 0.33	2.20±0.496
5	8.88	74	1.70 ± 0.41	2.03±0.536
6	13.50	75	1.57 ± 0.21	2.07 ±0.500
7	17.75	74	1.59 ±0.42	2.00±0.489
8	22.20	72	1.57 ± 0.48	2.02±0.459

Each experiment was performed with 10 replicates and was repeated thrice.

Table- 2: Effects of auxins on rooting of *in vitro* shoots of S*olanum nigrum* L..in half strength ms medium

Concentration μM/*l*			% of rooting response	Mean no. of roots / shootlets ± S.D.	Mean length of rootlets (cm)
IAA	IBA	NAA			
5.58	4.92	-	79	3.35 ± 0.30	1.42± 0.19
1.12	9.84	-	57	1.35 ± 0.22	1.39± 0.20
-	19.68	-	60	2.02 ± 0.51	2.03± 0.17
-	0.98	21.48	62	2.46 ± 0.21	1.78± 0.93
1.12	4.92	-	61	1.37 ± 0.23	1.51± 0.03
-	9.84	11.16	61	1.70 ± 0.20	1.44± 0.20
-	14.76	1.12	62	2.19 ± 0.24	1.97± 0.57
-	19.68	1.12	62	1.45 ± 0.28	1.14± 0.17

Each experiment was performed with 10 replicates and was repeated thrice.

The *in vitro* raised multiple shoots were excised and transferred individually to half strength MS medium supplemented with varied concentration of the root inducing auxins IBA, NAA and IAA. IBA, IAA was more effective than NAA in inducing robust roots in shoot cultures. Of the concentration tested, IAA (5.58μM) IBA (4.92 μM) gives maximum number of rootlets and root length were also observed in the same concentration of IAA, IBA. The percentage of rooting response was also the highest (79%). These results are in accordance with those of Mustafa Anand *et al*. (1997) on *Kaempteria rotunda*, Manickam *et al*. (2000) on *Withania somnifera* (Indian ginsens) and Segio *et al*. (2000) on *Anthemis robilis*. When IAA was tested for rooting, there was not only a decrease in the rooting response but also enhance the callus formation. Plantlets(30 day old) were transferred to polycups contain the mixture of soil and vermiculite(1:1) for hardening. After 4 weeks 25 *in vitro* rooted plantlets were transferred to pots and to the field after few weeks. The survival percentage was 65%. The present investigation has resulted in a protocol which could be used for mass propagation of *Solanum nigrum* to meet the increasing demand of the pharmaceutical industry as well as for conservation of this important medicinal plant.

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