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PLANT REGENERATION FROM *SOLANUM NIGRUM* L. LEAF CALLUS

M. JAWAHAR, S. VIJAYANAND, T. S. MARIA SHIBU, M. JEYASSEELAN and A.V.P. KARTHIKEYAN

Plant Tissue Culture Unit, PG & Research Department of Biotechnology, School of Life Sciences, Ponnaiyah Ramajayam College, Thanjavur-4, Tamilnadu, India.

Callus and plantlet regeneration was induced from leaf explant of *Solanum nigrum* on MS medium supplemented with different concentrations of IAA and NAA and combination with 1.0mg/l BAP in addition with 0.01 mg/l GA_3. Higher frequency of Green compact callus and multiple shoots were obtained on MS medium containing 2.0mg/l IAA + 1.0mg/l (mg/l) BAP +0.01mg/l GA_3 Rooting was obtained on the same regenerating medium. The rooted plants were successfully hardened and transferred to the field.

Keywords : Callus; Leaf; Regeneration; *Solanum nigrum* L.

Introduction

Solanum nigrum L. is an important medicinal plant. It contains steroidal glycoalkaloid Solanine and chaconine. This plant is valued for anticancerous and antibiotic properties. Tissue culture techniques are also being developed for the mass propagation of medicinal plants. There has been active research employing biotechnological methods to improve the yield of metabolites for their medicinal purposes. Most of the medicinal plants are, even today, collected only from the wild sources. The pressure on the natural population of medicinal herbs is so serious that several precious medicinal plants are likely to be listed as threatened, endangered and/or extinct. The success of in vitro regeneration from *Solanum nigrum* has been limited[1-3]. So an attempt has been made to initiate regeneration from leaf derived callus.

Materials and Methods

Leaf explants of *Solanum nigrum* L. were collected from in vivo healthy plants from the medicinal garden, PG & Research Department of Biotechnology, Ponnaiyah Ramajayam College, Thanjavur. The explants were washed with running tap water for 3-4 times and surface sterilized, initially in 70% ethanol for 2 min, followed by 0.1% (w/v) $HgCl_2$ for 1-2 min and finally in sterile distilled water for 2-3 times. Leaf explants were cultured on MS medium (Murashige and Skoog[4]) containing 3.0% (w/v) sucrose, 0.8% (w/v) agar and different concentrations of IAA & NAA (0.5-3.0mg/l) and combination with 1.0 mg/l BAP in addition with 0.01 mg/l GA_3. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes.

Results and Discussion

Callus and shoot regeneration from leaf explant was obtained through *in vitro* culture. After a week period of inoculation, callus tissue originate at the cut ends and margin of the explants. The morphogenetic response of calli varied on the different concentrations of IAA & NAA (0.5-3.0 mg/l) and combined with 0.01 mg/l GA_3. Auxin alone medium induced white friable & green friable calli (Table 1) (Fig 1&2), but in combination with cytokinin induced green compact callus (Fig 3). High frequency of callus proliferation was observed at 20-30days. The optimum level of callus occurred on the medium containing 2.0mg/l NAA + 1.0 mg/l BAP in addition with 0.01 mg/l GA_3 and followed by 2.0 mg/l IAA + 1.0 mg/l BAP in addition with 0.01 mg/l GA_3.

Green compact calli product higher frequency of shoot regeneration in 6 weeks on same medium (Fig 4). The higher frequency of shoot proliferation obtained on MS medium supplemented with 2.0 mg/l IAA and 1.0mg/l BAP in addition with 0.01 mg/l GA_3 (68.0%) followed by 2.0 mg/l NAA + 1.0 mg/l BAP in addition with 0.01 mg/l GA_3 (62.0%) (Table 2). Roots were developed with in a week on the same

Table 1. Effect of different concentration of auxins on callus induction of leaf explants of *Solanum nigrum* L.

Growth hormnes (mg/1)	% of callusing	Callus Nature
IAA		
0.5	65	W.F.
1.0	72	W.F.
1.5	85	G.F.
2.0	92	G.F.
2.5	88	G.F.
3.0	80	G.F.
NAA		
0.5	60	W.F.
1.0	69	W.F.
1.5	78	G.F.
2.0	89	G.F.
2.5	80	G.F.
3.0	75	G.F.

WF - Whitish friable, GF - Greenish friable.
Each value represents 20 replicates and each experiment was repeated atleast thrice

Table 2. Effect of different concentration of IAA and NAA in combination with 1.0mg/1 BAP in addition with 0.01mg/1 GA3 on plantlet regeneration from leaf explants of *Solanum nigrum* L.

Growth hormones (mg/1)	% of shoot proliferation	No of multiple shoots/callus (Mean ± SD)	Shoot length (Mean ± SD)
IAA			
0.5	28	1.6 ± 0.57	1.0 ± 0.00
1.0	37	2.6 ± 0.57	2.0 ± 1.00
1.5	48	4.0 ± 1.00	3.6 ± 0.57
2.0	68	10.0 ± 0.57	7.6 ± 0.57
2.5	67	6.0 ± 0.57	5.3 ± 0.57
3.0	60	5.0 ± 1.00	4.6 ± 0.57
NAA			
0.5	25	1.6 ± 0.47	0.6 ± 0.57
1.0	36	4.3 ± 0.47	2.6 ± 0.57
1.5	45	7.0 ± 0.81	3.6 ± 0.57
2.0	62	9.0 ± 0.81	6.6 ± 0.57
2.5	55	8.3 ± 0.47	5.6 ± 0.57
3.0	50	7.0 ± 0.80	4.6 ± 0.57

Each value represents 20 replicates and each experiment was repeated atleast thrice

Fig.1-4. *In vitro* cultures from *S. nigrum* leaf explants grown on MS medium supplemented with different concentrations of IAA/NAA in combination of BAP (1.0 mg/l) and GA_3 (0.01 mg/l).

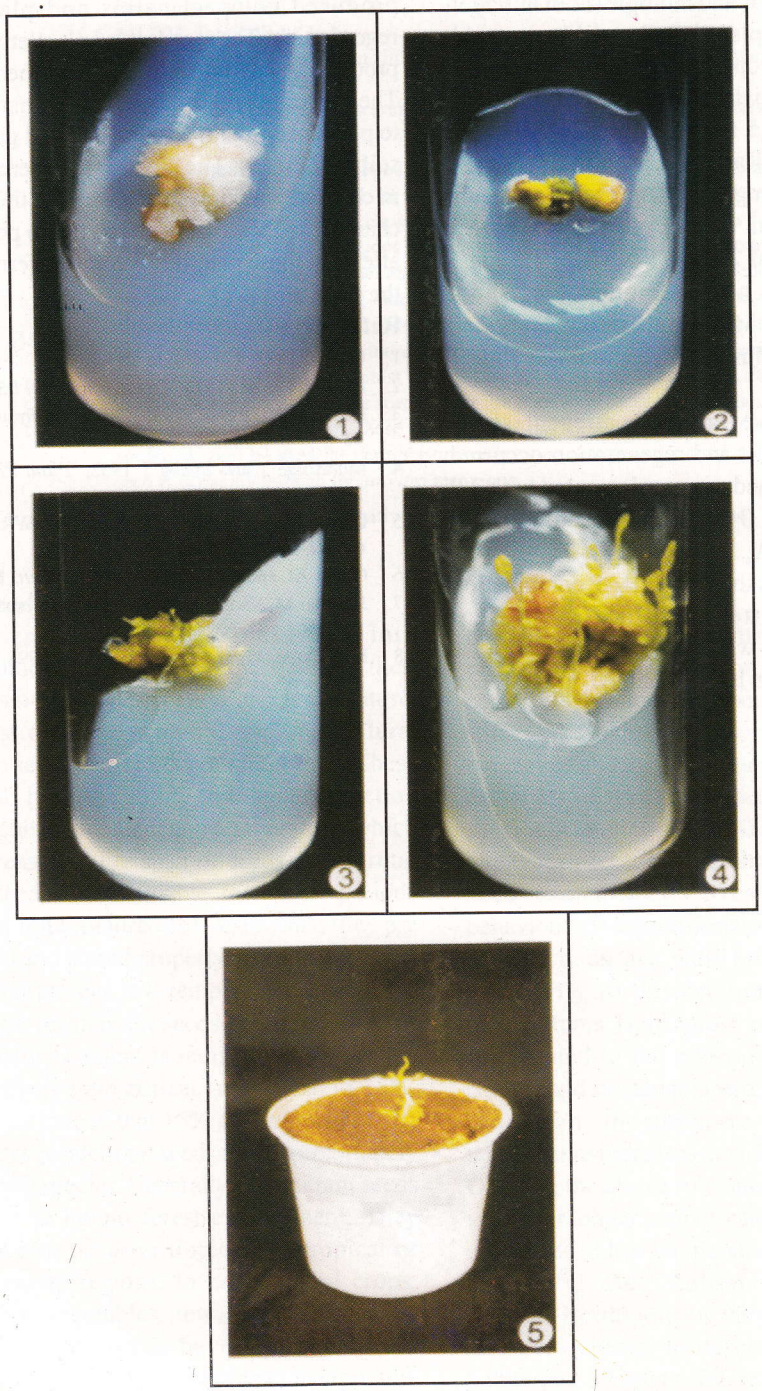

Fig. 1. White friable callus proliferation; Fig. 2 Green friable callus proliferation;
Fig. 3 Green compact callus proliferation; Fig. 4 Multiple shoot induction from leaf callus;
Fig. 5 Hardening of well developed plantlet.

medium. In the present investigation IAA and BAP in addition with GA_3 provide optimum callus and multiple shoot initiation from leaf explants. Padmanabhan *et al.*[5], reported that optimum callus induction and regeneration was obtained on MS medium containing 0.5 mg/l IAA + KN 3.0 mg/l. Gunnay and Rao[6] suggested that 0.5 mg/l IAA & 2.0 mg/l BAP provide optimum regeneration (60%). Jawahar *et al.*[7], suggested that 2.5 mg/l NAA & 1.5 mg/l KN induced higher frequency of shoot proliferation. Muthukumar *et al.*,[8] reported maximum callus and regeneration at 2 mg/l BAP alone.

However in the present study optimum callus and regeneration occurred only on MS medium supplemented with 2.0 mg/l IAA + 1.0 mg/l BAP in addition with 0.01 mg/l GA_3.

Thus the IAA & BAP combination provide better results in present investigation, with 75% of callus and 68% of regeneration being obtained (Table 1&2). IAA and NAA alone only induced friable callus, where as combined with BAP produced compact callus and plantlet regeneration. The regenerated plantlets were produced roots on same parental medium. The rooted plantlets were first transferred to plastic cups with vermiculate and garden soil 3:1 (Fig. 5). The plastic cups were kept in covered glass trays for a week in the first chamber at 25 ± 2^0c under 16h photo period, after that these plantlets were transferred to the field.

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