Plant Tissue Cult. & Biotech. **18**(1): 43-48, 2008 (June)

PTC&B

In vitro Regeneration and Flower Induction on *Solanum nigrum* L. from Pachamalai hills of Eastern Ghats

Amzad Basha Kolar, L .Vivekanandan and Ghouse Basha M*

P.G. and Research Department of Botany, Jamal Mohamed College, Tiruchirappalli-20. Tamil Nadu, India

Key words: *In vitro* studies, Medicinal plant, *Solanum nigrum*, Node, Callus

Abstract

Explants of *Solanum nigrum* L., collected from Pachamalai hills callused successfully on MS basal medium supplemented with IAA and BAP. The highest frequency of green compact callus and multiple shoots were obtained on MS containing 2.0 mg/l IAA and 0.5 mg/l BAP. The callus when cultured on MS basal medium fortified with different concentrations of BAP (3.0 - 8.0 mg/l) and IAA (0.5 mg/l) showed multiple shoot formation. The highest frequency of multiple shoots was obtained on MS containing 6.0 mg/l BAP and 0.5 mg/l IAA. For *in vitro* flowering, the node explants were cultured on MS fortified with different concentrations of BAP (2.0 - 7.0 mg/l) and NAA (0.5 mg/l). The highest number of multiple shoots were obtained in MS supplemented with 6.0 mg/l BAP and 0.5 mg/l NAA. The *in vitro* flowering was observed on MS containing 2,4-D and BAP 1.5 mg/l, respectively. The best rooting was obtained on MS containing 0.5 mg/l IBA. The well-rooted plants were hardened and finally planted in the garden.

Introduction

Solanum nigrum L is an important medicinal plant belonging to the family Solanaceae. The whole plant is anti-periodic, anti-phlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative. The leaves, stems and roots are used externally as a poultice, wash etc. in the treatment of cancerous soles, boils, leucoderma and wounds (Moerman 1998). Extracts of the plant are analgesic, antispasmodic, anti-inflammatory and vasodilator. The plant has been used in the manufacture of locally analgesic ointments and the juice of the fruit has been used as an analgesic for toothaches (Chiej 1984). In the present study, an attempt was made to standardize a protocol for regeneration of the plant in *in vitro* conditions.

*Corresponding author. E.mail: ghobashjmc@yahoo.co.in.

Materials and Methods

The leaf explants of *Solanum nigrum* L. were collected from Pachamalai hills of the Eastern Ghats. The leaves were washed in tap-water containing 0.1% (v/v) Tween 20. The explants were then washed in double distilled water. The plants were taken to the laminar airflow and surface sterilized by dipping in 70% alcohol for 2 min. The explants were then washed thoroughly with sterilized five times in distilled water. The explants were disinfected with 0.1% mercuric chloride for 1 min. Finally, the explants were rinsed with sterilized distilled water several times. The surface sterilized explants were cut into appropriate sizes and inoculated in MS containing 2,4 - D for callus induction. All the cultures were incubated in a growth room under a 16 h photoperiod (cool, white fluorescent light - 30 μmol m^{-2} s^{-1}) and the temperature was maintained at 25 ± 2°C with 50 - 80% relative humidity.

Well-developed callus was obtained after 15 days. The callus was excised and transferred to the shooting medium containing MS basal salts with BAP (3.0 - 7.0 mg/l) and IAA (0.5 mg/l). The well-developed shootlets were subcultured on a rooting medium containing IBA.

For *in vitro* flowering, the nodal explants of *S. nigrum* were cultured on MS containing basal salts with different concentrations of BAP (2.0 - 7.0 mg/l) and NAA (0.5 mg/l). The *in vitro* derived shoots were subcultured on MS containing different concentrations of 2,4-D and BAP for *in vitro* flower induction.

The well-rooted plantlets were removed from the agar medium, washed thoroughly in tap-water, dipped in liquid MS and transplanted to sterile vermiculite and irrigated with half strength of MS basal liquid medium. After three weeks, the plantlets were transferred to plastic cups containing equal ratios (1 : 1 : 1) of sterilized vermiculite, garden soil and farmyard soil. They were kept under shade for three weeks. The hardened plants were finally planted in the garden.

Results and Discussion

The leaf explants of *S. nigrum* showed callus initiation after 15 days of inoculation and the well-developed callus was obtained after 20 days (Fig. 1a,b). The highest frequency of green, compact callus was obtained on MS supplemented with 2.0 mg/l IAA and 0.5 mg/l BAP. The results obtained by Shahzad et al. (1999) was similar to our findings.

The well-developed callus was excised and inoculated on MS shooting medium. A combination of 6.0 mg/l BAP and 0.5 mg/l IAA was found to be the suitable concentration for high frequency of multiple shoots (Table 1, Fig. 1c), whereas in *S. trilobatum*, a combination of 5.0 mg/l BAP and 0.5 mg/l IAA was

reported to be the most suitable concentration for multiple shoot formation (Arokiasamy et al. 2002).

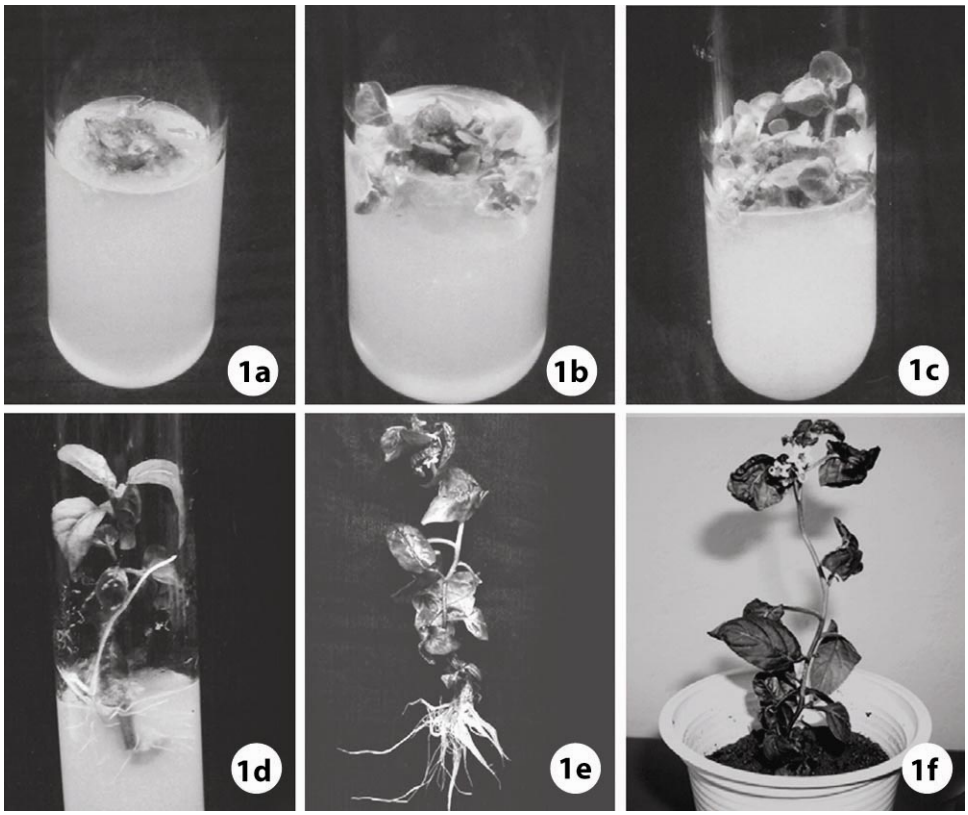

Fig. 1. *In vitro* regeneration of *S. nigrum* from leaf explant. (a) Leaf explant, (b) Initiation of callus, (c) Multiple shoot proliferation, (d) Rooting of the shootlet, (e) Regenerated plantlet, (f) Hardening.

Table 1. Effect of BAP and IAA for multiple shoot initiation from leaf explants of *S.nigrum.*

Concentrations (mg/l)		No. of tubes responded/total No. of tubes inoculated	Percentage of multiple shoot proliferation	No. of shoots/ explant (Mean ± Sd)
BAP	**IAA**			
3.0	0.5	1/20	5	2.00 ± 0.18
4.0	0.5	9/20	45	4.40 ± 1.81
5.0	0.5	16/20	80	12.75 ± 2.79
6.0	0.5	18/20	90	19.40 ± 4.25
7.0	0.5	12/20	60	11.83 ± 2.62
8.0	0.5	8/20	40	8.37 ± 2.06

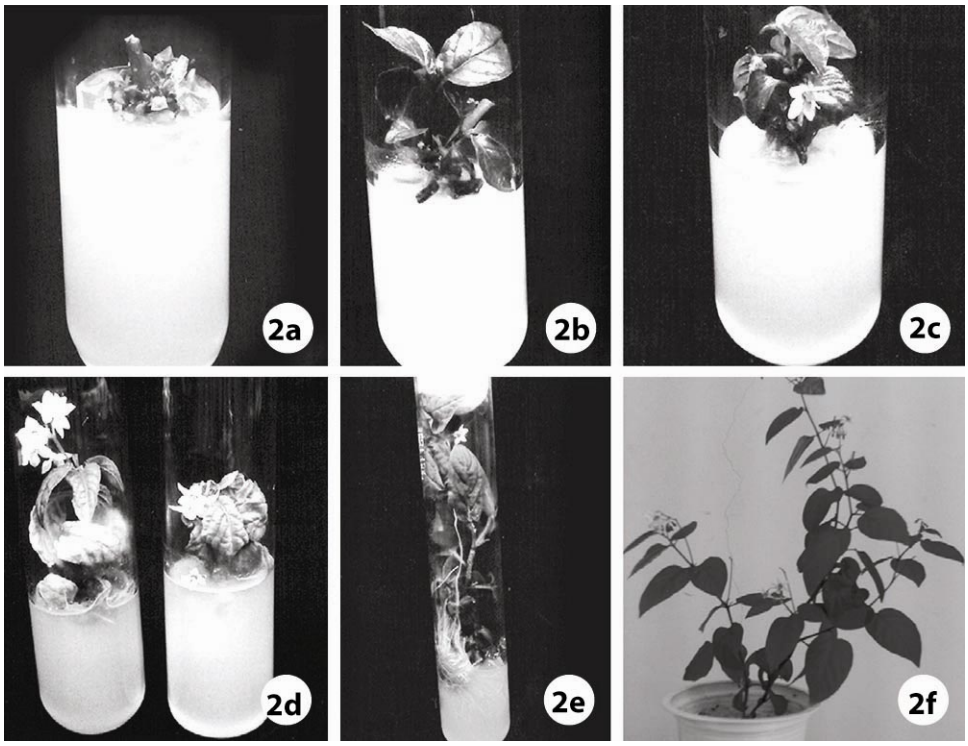

Fig. 2. *In vitro* flowering of *S. nigrum* through nodal explant. (a) Multiple shooting from nodal explant. (b) Proliferated multiple shoots. (c,d) *In vitro* flowering. (e) Root induction. (f) Hardening.

Table 2. Effect of different concentrations of BAP and NAA on shoot regeneration from nodal explants of *S. nigrum*.

Concentrations (mg/l)		No. of tubes responded/total No. of tubes inoculated	Percentage of multiple shoot proliferation	No. of shoots/ explant (mean ± SD)
BAP	NAA			
2.0	0.5	7/20	35	2.70 ± 1.49
3.0	0.5	10/20	50	5.20 ± 1.93
4.0	0.5	12/20	60	6.81 ± 1.83
5.0	0.5	15/20	75	8.57 ± 3.03
6.0	0.5	18/20	90	12.94 ± 2.81
7.0	0.5	11/20	55	6.90 ± 2.73

When nodal explants were cultured on MS fortified with 6.0 mg/l BAP and 0.5 mg/l NAA, the highest percentage of shoots were observed (Table 2, Fig. 2a,b). Our results are similar to Reddy et al. (1998) who obtained maximum number of shoots in the medium fortified with 5.0 mg/l BAP and 2.0 mg/l NAA.

The *in vitro* shoots were then transferred to MS containing 1.5 mg/l 2,4-D and BAP each of which was found to be the suitable concentration of growth regulators for *in vitro* flower induction (Table 3, Fig. 2c,d). Our results are similar to those of Saravanan et al. (2007) who induced *in vitro* flowering in *Pedalium murex* L. using same hormonal concentrations with nodal and internodal explants.

Table 3. Effect of 2,4-D and BAP on *in vitro* flowering in *S.nigrum.*

Concentrations (mg/l)		Culture showing shooting response (%)	Av. No. of shoots formed	Av. shoot length (cm)	Result
2,4 - D	BAP				
0.5	1.0	15	1.0	2.0	No flowering
1.0	1.0	35	2.0	2.8	No flowering
1.5	1.5	70	3.5	3.0	*In vitro* flowering

Table 4. Effect of IBA on root formation from the regenerated shoots of *S. nigrum.*

Concentrations (mg/l)	Percentage of root induction	No. of roots/shoot (mean ± Sd)
IBA		
0.5	71	7.2 ± 2.14
1.0	58	4.7 ± 1.88
1.5	48	2.7 ± 1.33
2.0	32	2.0 ± 0.81
2.5	-	Callus

The elongated shoots were subsequently rooted on MS containing IBA. The highest rooting percentage was obtained when MS supplemented with 0.5 mg/l IBA (Table 4, Figs. 1d, 2e). Similar observation was reported in earlier studies on *Anisochilus carnosus*, *Annona squamosa, Quisqualis indica* by Jeyachandran (2004), Roxana Ahmed (2005) and Poornima and Shivamurthy (2005), respectively. The regenerated plantlets were finally subjected to hardening (Figs. 1e,f, 2f).

Acknowledgements

Authors are thankful to the authorities of the Department of Science and Technology, New Delhi, for providing the DST FIST – Sponsored program to our Department. Authors are grateful to the University Grants Commission, New Delhi, for providing financial support.

References

Arokiasamy DI, Muthukumar B, Natarajan E and **John Britto S** (2002). Plant regeneration from node and internodal explants of *Solanum trilobatum* L. J. Plant Tissue Cult. **12**(2): 93-97.

Chiej R (1984). Encyclopedia of Medicinal plants. Mac Donald. ISBN 0-356-10541-5.

Jeyachandran R (2004). *In vitro* culture root formation in *Anisochilus carnosus*. J. Swamy Bot. Club. **21**: 27-30.

Moerman D (1998). Native American Ethnobotany. Timber Press *Oregon*. ISBN 0-88192-453-9.

Poornima D and **Shivamurthy GR** (2005). Root formation in *Quisqualis indica* L. J. Swamy Bot. Club. **22**: 37-38.

Reddy PS, Gopal GR and **Sita GL** (1998). *In vitro* multiplication of *Gymnema sylvestre* R.Br - An important medicinal plant. Cur. Sci. **75**: 843-845.

Roxana Ahmed (2005). Best root formation in *Annona squamosa.* Asian J. Microbial Biotech. Env. Sc. **7**(2): 191-194.

Saravanan R, Ghouse Basha M, Amzad Basha K and **Vivekanandan L** (2007). *Ex situ* culture studies on a medicinal plant *Pedalium murex* L . Indian J. Plant Physiol . **12**(2): 178-180.

Shahzad A (1977). Somatic cell genetics and plant improvement. Ann. Agron. **20**: 39-81.