BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **44(4)**, 453-456, 2009

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

In vitro Shoot Proliferation and Plant Regeneration of *Physalis minima* L. - a Perennial Medicinal Herb

Farhana Afroz, A. K. M. Sayeed Hassan, Laila Shamroze Bari, Rebeka sultana, Nadira Begum , Miskat Ara Akter Jahan and Rahima Khatun

Biological Research Division, BCSIR Laboratories, Dhaka-1205, Bangladesh.

Abstract

The present study describes a protocol for high frequency plant regeneration of *Physalis minima*. Shoots were induced by culturing nodal segments and shoot tips from 15 day old seedlings. About 29 and 32 shoots were found to be induced from nodal segment and shoot tip explants, respectively, cultured on MS medium supplemented with 1.0 mg/l BAP. When shoots were subcultured on the fresh medium with same component as mentioned above, the shoots were elongated. Shoots rooted well when they were excised individually and implanted on half-strength MS medium with 0.3 mg/l NAA, where 98% shoots rooted within 12-15 days. *In vitro* grown plantlets with strong root system were successfully established in normal room temperature for seven days before transplanting in pots where they were reared for three weeks through successive acclimatization. The regenerated plants were successfully transferred to the soil with 90% survival rate.

Key words: *Physalis minima*, Medicinal plant, Shoot proliferation, Micropropagation, Regeneration

Introduction

Physalis minima L., commonly known as 'ban tipariya/ Kopal fatki' and belongs to the family Solanaceae, is a small, delicate, erect, annual, pubescent herb. Its fruit is edible (Crib 1976). The fruit is said to be appetizer, bitter, diuretic, laxative and tonic (Parmar and Kaushal 1982, Chopra *et. al.* 1986). Extracts of the plant have anticancer activity (Duke and Ayensu 1985) and extracts from leaves have antimicrobial activity (Nayeemulla *et al.* 2006). The juice of the leaves, mixed with mustard oil and water, has been used as a remedy for earache (Chopra *et. al.* 1986).

Though the plant has immense medicinal value it is gradually declining from the nature due to over exploitation and environmental pollution. Fast forest cleaning activities of Bangladesh is leading to a depletion of valuable plant resources; the conservation of these valuable genotype is imperative (Karuppusamy *et. al.* 2006). That is why there is an urgent need of replenishment of the short supply and conservation of this plant resource. Micropropagation is an effective approach to conserve such germplasm. Further, genetic improvement is another approach to augment drug-yielding capacity of the plant. Therefore it is important to develop an efficient micropropagation technique for *Physalis minima* to rapidly disseminate superior clones once they are identified. Tissue culture technique can play an important role in the clonal propagation of elite clones and germplasm conservation of this medicinal hurb. In recent years, numerous studies on *in vitro* propagation of different plant species have shown that this technique may be a solution for rapid propagation of selected plant species (Bonga and Durjan 1987, Chalupa 1987, McCown 1987, Boulay 1987). The present communication deals with the development of a protocol for *in vitro* asexual multiplication in *Physalis minima* L.

Materials And Methods

The shoot tips and nodal explants of *Physalis minima* L. were collected from the medicinal plant garden of BCSIR Laboratories, Dhaka and used as explants for this experiment. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent (0.1% Tween-20) for about 30 min, washed three times again by running tap water and dipped in 70% (v/v) ethanol for 1 min. They were then surface sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five rinses with sterile distilled water in a laminar air flow cabinet. The surface sterilized explants were sized to 1.0-1.5 cm in length containing a single node with an axillary bud or a shoot tip with an apical bud. The

* Corresponding author: E-mail: farhana04@yahoo.com

explants were placed vertically on the culture medium.

For shoot induction and proliferation agar gelled MS (Murashige and Skoog 1962) basal media supplemented with BAP and coconut water (CW) at varying concentrations were prepared. Shoots induced from the *in vitro* cultures were further used as explants for adventitious shoot proliferation. For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted on half strength MS media with different concentrations and combinations of IBA, NAA and BAP. All media were supplemented with 30 g/l sucrose and 7 g/l agar (Difco). The pH of each medium was adjusted to 5.8 before autoclaving at 121^{O}C for 20 min and the medium was dispensed into 25x150 mm culture tubes or 250 ml conical flasks. The cultures were incubated for 16 h photoperiod at 24 ± 2^{O}C under a fluorescent light (3000 lux).

Visual observation of culture was made every week. Data on shoot proliferation and root induction were recorded after three weeks of inoculation and used for calculation. For each treatment 15 explants were used for shoot proliferation and 15 shoots were used for rooting. All the treatments were repeated thrice. Data were analyzed statistically according to Mian and Mian (1984). The healthy plantlets were taken out from the culture tubes, washed to make free from agar gel with running tap water and transplanted to plastic pots containing soil, sand and compost (1:1:1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity

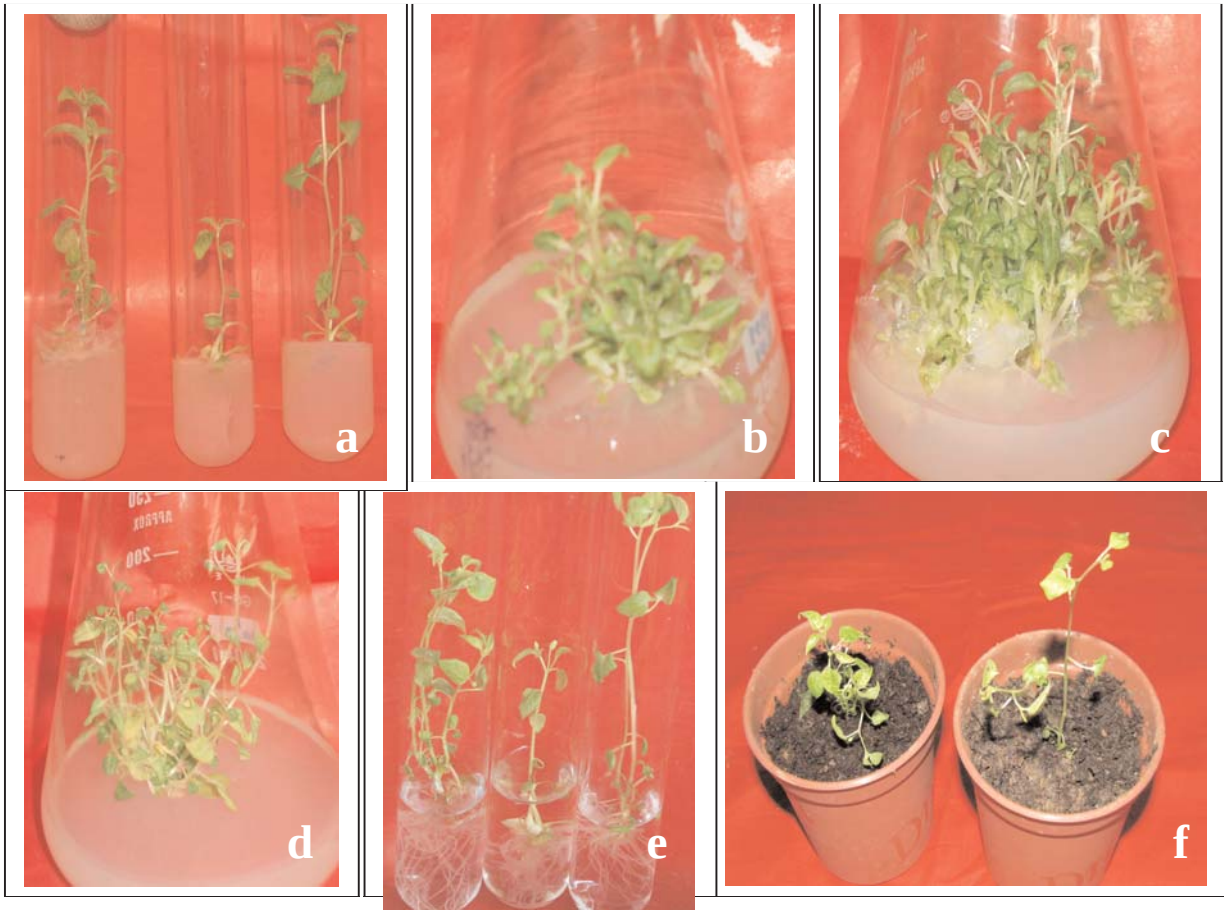

Fig 1. Regeneration of plantlets from nodal explants and shoot tips obtained from fifteen day old seedling *in vitro*.

(a) Inoculation of explants into regeneration medium at fifteen days (b) Shoot regeneration from nodal explants after three weeks (c) Shoot regeneration from nodal explants on MS containing 1.0 mg/l BAP after three weeks (d) Shoot regeneration from shoot tips on MS containing 1.0 mg/l BAP after three weeks (e) Adventitious root formation on regenerated shoots on half MS containing 0.3 mg/l NAA after two weeks (f) Establishment of in vitro grown *Physalis minima* plantlets in outside pot after two months.

Table I. Effect of growth regulators in MS medium on morphogenic response of *Physalis minima* L. nodal segments and shoot tips

Growth regulators (mg/l)		Nodal segments		Shoot tips	
BAP	CW	% of explants forming shoots	Mean No. of Shoot/explant	% of explants forming shoots	Mean No. of Shoot/explant
0.0	0.0	-	-	-	-
0.1	0.0	92±0.8	30±0.12	89±2.3	28±0.1
0.5	0.0	93±0.8	25±0.25	88±1.8	23±1.4
1.0	0.0	95± 1.2	32±0.46	90±0.8	29±0.5
1.5	0.0	88±1.4	26±0.50	89±0.1	22±0.8
2.0	0.0	89±0.8	23±0.96	87±0.5	18±0.3
1.0	5%	94±0.5	29±0.30	90±0.7	26±1.4
1.0	10%	92±1.0	26±1.44	91±0.1	22±0.1

Results are mean ± SE of three experiments with 15 replications.

ity, 32 ± 2^{O}C under 12 h photoperiod for acclimation. Established plantlets were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tips and nodal segments of *Physalis minima* were cultured on MS media supplemented with various concentrations of BAP alone, BAP + 5% or 10% CW for shoot regeneration and IBA, NAA, as well as BAP + NAA for root induction. The explants were found to be swollen and they produced two to five shoots within two weeks after inoculation (Fig. 1b). The highest percentage of response of the inoculated explants for shoot induction was found to be 95%. Maximum number of shoots induced when the nodal segments were cultured on MS medium supplemented with 1.0 mg/l BAP. In case of shoot tips, the highest number of micro shoots was found on MS medium with 1.0 mg/l BAP. Both the explants responded in the same medium but highest number of micro shoots were observed in nodal explants. Ramirez-Malagon and Ochoa-Alejo (1991) reported that the highest number of shoot formation was observed on media containing 2.5 mg/l BAP and 1.0 mg/l NAA. In the present investigation the highest number of shoots was found to be 32.0 when cultured on MS medium supplemented with 1.0 mg/l BAP alone. Rapid increase of micro shoots were observed when the above shoot containing explant was repeatedly subcultured on the same medium. Effect of CW on shoots proliferation was reported by Roy *et al.* (1998) and Hassan and Roy (2004 and 2005) for *Elaeocarpus robustus, Smilax zeylania* and *Gloriosa superba* culture, respectively. But in this investigation for further shoot multiplication and growth, addition of 5% and 10% CW in the nutrient medium was not found to be effective. The maximum number of roots were observed in regenerated shoots of Physalis minima when the excised shoots were cultured individually on half-strength MS medium supplemented with 0.3 mg/l NAA within 15 days (Table II), (Fig. 1e). Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand 1998, Usha *et al*. 2007, Vadawale *et. al*. 2006, Hassan and Roy 2005, Rahman *et al.* 2006, Baksha *et al.* 2007). After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under

Table II. Effect of auxin(s) on root induction in regenerated shoots of *Physalis minima* L. cultured on half strength MS medium

Auxins (mg/l)	No. of roots per shoot (±SE)	Days required for root induction (±SE)
IBA 0.1	18.67±3.98	26±0.1
IBA 0.2	23.33±3.20	21±0.3
IBA 0.5	19.67±2.87	24±0.8
IBA 1.0	16.40±4.39	24±0.5
IBA 2.0	21.75±2.09	25±1.0
NAA 0.1	28.25±2.09	21±0.1
NAA 0.2	27.50±5.09	18±1.4
NAA 0.3	37.50±3.98	15±0.7
NAA 0.4	22.22±0.61	19±0.3
NAA 0.5	17.00±1.16	18±0.8
NAA 1.0	12.33±2.44	25±0.3
BAP 0.1 + NAA 1.0	7.67±0.13	23±0.5
BAP 0.5 + NAA 0.5	14.33±0.51	24±0.2

Results are mean ± SE of three experiments with 15 replications.

natural conditions. About 80 percent of the transplanted plants of *Physalis minima* survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30 ± 2^{O}C) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green looking good and healthier (Fig. 1f).

References

Baksha R., Jahan M. A. A., Khatun R. and Munshi J. L. (2007) *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. *Bangladesh J. Ind. Res*. **42**(1): 37-44.

Bonga J. M. and Durzan D. J. (1987) Cell and Tissue culture in Forestry. (Martinus Nijhoff Publishers, Dordrecht). **3**: 272-284.

Boulay M. (1987) *In Vitro* Propagation of Tree Species (*Plant Tissue and Cell Cult*. Alan, R. Liss. Inc, New York). pp 367-381.

Chalupa V. (1987) Clonal propagation of broad-leaved forest trees. *In Vitro Commun. Inst. Forest. Cechosl*.**12** : 255-257.

Chopra R. N., Nayar S. L. and Chopra. I. C. (1986) Glossary of Indian Medicinal Plants (including the supplement). Council of Scientific and Industrial Research, New Delhi..

Cribb A. B. and J. W. (1976) *Wild food in Australia.* Fontana ISBN 0-00-634436-4.

Duke J. A. and Ayensu. E. S. Medicinal plants of China Reference Publications, Inc 1985, **2**: 705.

Hossain S. S., Rahman S., Joydhar A., Islam S. and Hossain M. (2000) *In vitro* propagation of thankuni (*Centella asiatica* L.). *Plant Tissue Cult.*; **10**(1) : 12-23.

Hassan A. K. M. S. and Roy S. K. (2004) Micropropagation of *Smilax zeylanica* L., a perennial climbing medicinal shrub, through axillary shoot proliferation. *Bangladesh J. Life Sci.* **16**(1): 33-39.

Hassan A. K. M. S. and Roy S. K. (2005) Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. *Plant Tissue Cult. and Biotech.* **15**(1): 67-74.

Karuppusamy S., Kiranmai C., Aruan V. and Pullaiah T. (2006) Micropropagation of *Vanasushava pedata*- An endangered medicinal plant of South India. *Plant Tissue Cult. & Biotech*. **16:** 85-94.

McCown D. D. and McCown B. H. (1987) Northern American Hard Woods. *In: Cell and Tissue Cult. in Forestry* Volm. 3. Martinus Nijhoff Publishers, Dordrecht.

Mian M. A. and Mian M. A. (1984) An Introduction to Statistics. 4th Ed. (Ideal library, Dhaka) pp. 125-129.

Murashige T. and Skoog, F. (1962) A received medium for rapid growth and bioassay with tobacco tissue cult. *Physiology of Plants*. **11**: 47-53.

Nayeemulla S., Sudarshana M. S., Umesha S. and Hariprasad P. (2006) Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *African Journal of Biotechnology.* **5**(10): 946-950.

Parmar C. and Kaushal M. K. (1982) Wild fruits of the Subhimalayan Region. Kalyani Publishers. New Delhi. pp 23-25.

Rahman S. M. R., Afroz F., Sultana K., Sen P. K. and Ali M. R. (2006) Effect of growth regulators and state of medium on micropropagation of *Adhatoda vasica* (Nees.) *Khulna University Studies*, Special Issue (1st Research Cell Conference): 55-59.

Roy S. K., Islam M. S. and Hadiuzzaman, S. (1998). Micropropagation of *Elaeocarpus robustus. Plant Cell Rep*. **17**: 810-813.

Sahoo Y. and Chan P. K. (1998) Micropropagation of *Vitex negundo* L., a woody aromatic medicinal plant shrub, through high frequency axillary shoot proliferation. *Plant Cell Rep.* **18:** 301-307.

Ramirez-Malagon R. and Ochoa-Alejo N. (1991) Adventitious shoot formation and plant regeneration from tissues of tomatillo (*Physalis ixocarpa* Brot.). *Plant Cell, Tissue and Organ Culture*. **25:** 185-188.

Usha P. K., Benjamin S., Mohanan K. V. and Raghu A. V. (2007) An efficient micropropagation system for *Vitex negundo* L., an important woody aromatic medicinal plant, through shoot tip culture. *Research journal of Botany.* **2:** 102-107.

Vadawale A. V., Barve D. M. and Dave A. M. (2006) In vitro flowering and rapid propagation of *Vitex negundo* L.- a medicinal plant. Indian *Journal of Biotechnology*. **5:** 112-116.

Received : Nobember 11, 2008;
Accepted : June 06, 2009