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In vitro propagation of *Phyllanthus niruri* L. – a medicinal plant

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Abstract

An efficient protocol for *in vitro* propagation of *Phyllanthus niruri* L. has been developed. MS medium supplemented with NAA induced prolific callus from leaf, shoot tip and nodal explants. Organogenic and chlorophyllous calli produced well developed prominent shoots with thick and long roots in the medium supplemented with NAA (1 mg/l) and BAP (1 mg/l). Of the *in vitro* grown 120 plantlets transferred to the field, 92% survived after two months of transplantation to natural environment.

Keywords : *In vitro* propagation, *Phyllanthus niruri*, medicinal plant

INTRODUCTION

Phyllanthus niruri L. commonly known as 'Kizhanelli' in Tamil belongs to the family Euphorbiaceae. It is a small, erect, annual herb and grows to 30-40 cm height, and found in the tropical regions of the South India. The Spanish name of this plant is '*Chanca piedra*' which means "stone breaker" or "shatter stone". It was named for its effective use in eliminating gall stones and kidney stones. This plant is used to treat colic diseases, diabetes, malaria, dysentery, fever, flu, tumours, jaundice, vaginitis, gonorrhea and dyspepsia, to reduce pain, to stimulate and promote digestion, to expel worms and as a mild laxative. Many of the active constituents are attributed to biologically active lignins, glycosides, flavanoids, alkaloids, ellagitannins and phenyl propanoids found in the leaf, stem and root of the plant (Kirtikar and Basu, 1975).

In the recent years, the demand for *P. niruri* has increased owing to its antiviral property (Unader, 1991). This led to its indiscriminate harvesting and as a result it has become threatened. Hence it imperative to establish a suitable protocol for conservation of the species through micropropagation. There are only few reports available on the tissue culture studies in the members of the genus *Phyllanthus* (e.g., Bhattacharya and Bhattacharya, 2001).

The present paper deals with the continuous supply of *P. niruri* through micropropagation using shoot tip and nodal explants which could serve as a source of raw material for the manufacturing of drugs and also in drug research.

MATERIALS AND METHODS

Healthy plants of *Phyllanthus niruri* L. were collected from Thanjavur, Tamil Nadu, India. Shoot tip and nodal explants (1 cm long) were excised from two months old plants. They were pretreated with detergent (Teepol 5% v/v) solution for 5 minutes and disinfected with 0.1% mercuric chloride solution for 3 minutes followed by repeated washings with sterile distilled water under aseptic conditions in a sterile flask. The shoot tips and nodal explants were inoculated by inserting their cut-ends in the MS medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/l of BAP, NAA and BAP + NAA to induce multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. It was adjusted to pH 5.8 and autoclaved at 121°C, 15 lb pressure for 15 minutes. The cultures were maintained at 25 ± 2°C under a light intensity of 3000 lux provided by cool-white fluorescent lamps.

Each treatment consisted of fifteen replicates and the experiments were repeated three times. A complete randomized block design was used in all experiments. Analysis of variance and Duncan's Multiple Range Test (DMRT) were carried out and the significance was assessed at 5% level.

RESULTS AND DISCUSSION

Friable callus was induced from the shoot tip explant on MS medium fortified with 0.5 mg/l NAA after 7 days of culture (Fig. 1a). When the medium was supplemented with 2 mg/l NAA, prolific callus induction occurred in leaf and shoot tip explants (Table 1) (Fig.1a). In this medium the callus was comparatively big, friable and white-pale green in colour (Fig.1b). Further more, it doubled in size for every 15 days with routine subcultures. When nodal explants were grown in the medium supplemented with low concentrations of NAA (1.0 mg/l) and BAP (1.0 mg/l), they produced compact chlorophyllous calli with shoots (Fig.1c). This may be attributed to differential physiological status and endogenous concentration of growth regulator as revealed by the studies on *Justicia gendarussa* for Agastian *et al.* (2006).

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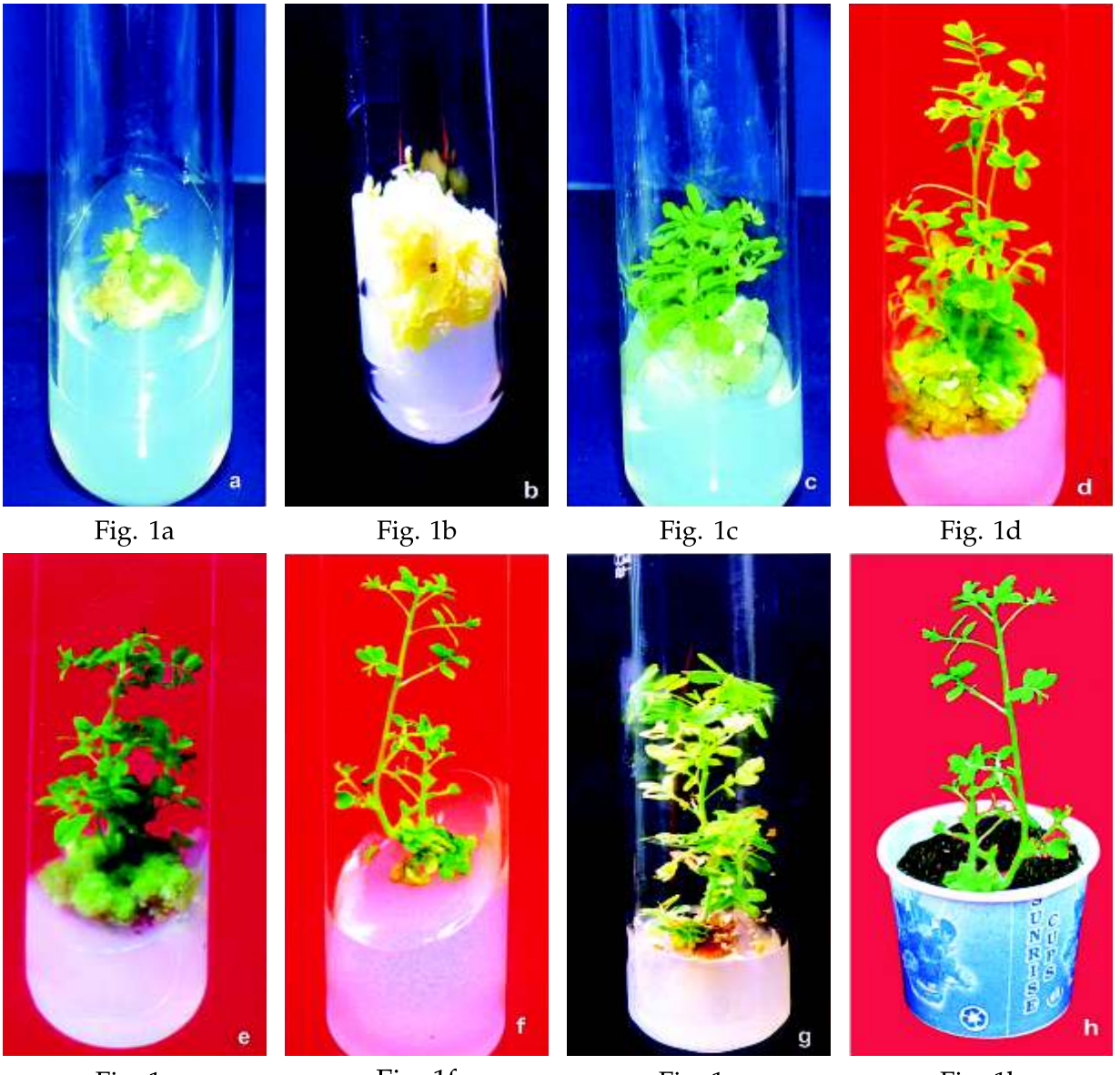

Fig. 1a Fig. 1b Fig. 1c Fig. 1d

Fig. 1e Fig. 1f Fig. 1g Fig. 1h

Figure 1. Micropropagation of *P. niruri*

a) Induction of friable callus from the shoot tip explant on MS medium fortified with 0.5 mg/l NAA after 7 days of culture.
b) Friable, bulky, organogenic and chlorophyllus callus was formed from nodal explants on MS medium supplemented with 2 mg/l NAA after 15 days of culture.
c) Multiple shoot regeneration was formed from nodal explant culture on MS medium with BAP and NAA (each 1 mg/l) after 20 days culture.
d) Multiple shoot regeneration with callus formed from shoot tip explants cultured on MS medium supplemented with 2 mg/l NAA after 25 days of culture.
e) Initiation of shoot formation from leaf explants culture MS medium containing (0.5 mg/l) NAA and BAP (1.0 mg/l) after 25 days of culture.
f) Elongated shoot was obtained on MS medium containing BAP (2.0 mg/l) after 15 days of culture.
g) Rooting was obtained on MS medium containing BAP (1 mg/l) and NAA (1.5 mg/l) after 30 days of culture.
h) Hardened plant in paper cups containing vermiculite and soil.

Table 1. Effect of NAA and BAP on callus induction and organogenesis from nodal explants of *P. niruri* (n = 15)

Medium	Hormonal concentrations (mg/l)		Percentage of response	Mean Number of shoots	Mean Length of shoots (cm)	Nature of callus
	Callus induction	Shoot formation				
MS	-	-	50.14 (2.61)*	1.00# (0.09)	4.6 (0.42)*	Compact. very slow growth
MS + NAA	2.0	1.0	94.66 (2.40)*	4.11 (0.70)*	5.3 (0.51)*	Friable, bulky, organogenic
MS + BAP	1.0	2.0	73.32 (2.01)*	6.33 (1.23)*	6.2 (0.48)*	Compact, slow growth with thick roots
MS+NAA+ BAP	1.0	1.0	80.21 (1.20)*	5.01 (1.24)*	7.6 (0.75)*	Friable, bulky, organogenic with lengthy shoot and roots

(values in parenthesis are SD) (* LSD at 5% level = 0.90) (# LSD at 5% level = 0.66)

The roots developed from the callus after 12-16 days of culturing in rooting medium exhibited varied morphological features. Lower concentrations of NAA (0.1 to 1.0 mg/l) without BAP gave maximum number of tender roots (8 / nodal explant). Similar results were also recorded earlier by Natarajan *et al.* (1999) in *Hybanthus enneaspermus* and *Datura metel* (Muthukumar *et al.*, 2001). Thick and long (20 cm) roots were developed from the callus in large numbers in the medium supplemented with NAA (1 mg/l) and BAP (0.1 mg/l). Very minute dense root hairs were produced from the thick roots (Fig.1g).

The inclusion of cytokinins and auxins to the culture medium stimulated *in vitro* multiplication and growth of shoots in several plant species. In the present study shoots were produced from 15th day onwards and final observations were made on 25th day. All combination of NAA and BAP produced long shoots (7.6 ± 0.75 cm) with five or six shoots per callus (Figs.1d and 1e). Dwarf shoot appeared at higher concentration of NAA and BAP.

Nodal explant produced calli with long shoots (10.8 cm) and profuse rooting occurred at 1.5 mg/l NAA and 1 mg/l BAP. Elongated shoots were also obtained on MS medium supplemented with BAP at 2 mg/l (Fig.1f). Similar findings have been reported in *Justicia gendarussa* (Agastian *et al.*, 2006). When the plantlets with well developed shoot and root systems were transferred to plastic cups containing a mixture of vermiculite and soil, they readily hardened (Fig.1h), and when the acclimatized plantlets were successfully transplanted, they exhibited 92% survival rate in pots under field conditions after 2 months.

Thus it is concluded that both BAP and NAA induced organogenesis and obviously BAP is very essential to induce organogenesis. Similar observations have been reported in legumes (Vijayakumari *et al.*, 2001) and foxtail millet (Osuna-Avila, 1995). Higher concentration of NAA (>1.5 mg/l) and BAP induced thick and compact callus with no shoot formation. These results presumably indicate a threshold level of endogenous hormone in the explants.

REFERENCES

Agastian, P., Lincy Williams and Ignacimuthu, S. 2006. *In vitro* propagation of *Justicia gendarussa* Burm. f. – A Medicinal Plant. *Indian J. Biotechnol.* 5: 246-248.

Bhattacharya, R. and Bhattacharya, S. 2001. High frequency of *in vitro* propagation of *Phyllanthus amarus* by shoot tip culture. *Indian J. Exp. Biol.* 39: 1184-1187.

Kirtikar, K.R. and Basu, B.D. 1975. *Indian Medicinal Plants,* Lalit Mohan Basu, Allahabad. 3: 2225-2227.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tissue culture. *Physiol. Plant* 15: 473-497.

Muthukumar, B., Arokiasamy, D.I. and Britto, S.J. 2001. Response of leaf explants of *Datura metel* L. to different types auxins and cytokinins in LS and MS medium. *J. Phytol. Res.* 14: 13-18.

Natarajan, E., Arokiasamy, D.I. and Britto, S.J. 1999. Regeneration of plantlets from the callus of stem explants of *Hybanthus enneaspermus* (L.) F. Muell. *Plant Tissue Cult.* 9: 167-172.

Osuna-Avila, P. 1995. Plant regeneration from shoot apex explants of foxtail millet. *Plant Cell Tissue Organ Cult.* 42: 33-45.

Unader, D.W. 1991. Callus induction in *Phyllanthus* species and inhibition of viral DNA polymerase and reverse transcriptase by callus. *Plant Cell. Rep.* 10: 461-466.

Vijayakumari, P., Kavi Kishore, P.B. and Bhalla, J.K. 2001. *In vitro* plant regeneration in pigeon pea (*Cajanus cajan* (L.) Millsp.) via organogenesis. *Plant Cell Biotechnol. Mol. Biol.* 2: 49-56.