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Short communication

In vitro study of *Tinospora cordifolia* (Willd.) Miers (Menispermaceae)

Aditi Singh[1], Saroj K. Sah[2*], Aunji Pradhan[1], Sabari Rajbahak[2] and Niran Maharajan[2]

*[1]College for Professional Studies, Maitidevi, Kathmandu, Nepal; [2]Department of Plant Resources, Thapathali, Kathmandu, Nepal; *Corresponding author, e-mail: saroj1021@yahoo.com*

Abstract

In vitro study was carried out in an important medicinal plant *Tinospora cordifolia* (Willd.) Miers belonging to the family Menispermaceae. Vegetative parts such as stem, leaf and nodal explants were excised from an elite *in vivo* grown mature plant and thereafter cultured on Murashige-Skoog (MS) medium supplemented with different hormonal concentrations for callus induction and organogenesis. Callus formation occurred from nodal segments, leaf and inter-node explants when planted on different combinations of hormones. *Tinospora cordifolia* showed response for *in vitro* shoot growth from the nodal segment. The best shoot growth was observed on MS medium supplemented with kinetin (1.5 mg/l). Similarly, the best result for root induction was obtained on MS medium supplemented with 6-benzylaminopurine (1.0 mg/l) and *naphthaleneacetic acid* (2.5 mg/l).

Key-words: callus induction, explants, medicinal plant, MS medium, tissue culture.

Introduction

Tinospora cordifolia (Willd.) Miers is an extensively spreading, glabrous, succulent, climbing shrub belonging to the family Menispermaceace. It is distributed throughout the tropical region of Nepal, India, Sri Lanka and China, ascending to an altitude of 1,200 m asl. It thrives in the tropical region in forests and other habitats (DPR 2007). Flowering takes place in summer and fruiting in winter.

Stems, roots, leaves and starch obtained from the roots and stems are used for medicinal purpose, especially in Ayurveda (Sinha *et al*. 2003). The root is a powerful emetic and is used for visceral obstructions; its water extract is used in leprosy (Nayampalli *et al*. 1982; Sharma *et al*. 1998). The root also exhibit antidiabetic effect (Gupta *et al*. 1967). The extracts of stem, leaves, barks and roots show strong antioxidant activities (Stanley *et al*. 1999). The bitter principle present in the stem is used in the treatment of debility, dyspepsia, fever and urinary disease and the decoction of the leaves is used for the treatment of gout (Sinha *et al*. 2003). The pharmaceutical significance of this plant is mainly due to the presence of various bioactive compounds, such as glucosides and alkaloides including berberine (Singh *et al*. 2003).

Various reports on its multiple medicinal use attracted attention for commercial exploitation of the plant to meet the requirements of the growing pharmaceutical industry. *T. cordifolia* natural stands are now fast disappearing and are threatened due to indiscriminate collection and over-exploitation. Conventional vegetative propagation of this plant has limited potential for large scale cultivation. Micropropagation technique can be most useful for its mass propagation as well as for its conservation. This paper highlights the results of an *in vitro* study of this plant.

Materials and Methods

Shoot tips, leaves and nodal segments used as explants in this experiment were collected from 12-14 months old *in vivo* grown plants. The explants were cut into small pieces (about 1.5 cm long) and then washed for 1 h under running water with liquid detergent, Polyoxyethylene Sorbitan Monolaurate (teepol), and washed thoroughly with distilled water (7-8 times). The explants were surface sterilized with 0.1% mercuric chloride solution for 5 minutes under aseptic condition followed by washing with sterile water (7-8 times). Then explants were inoculated aseptically on culture medium.

MS (Murashige-Skoog 1962) media supplemented with auxin [naphthalene acetic acid (NAA)] or cytokinins [6-benzylaminopurine (BAP) or kinetin (Kn)] at varying concentrations singly or in combinations were prepared.

After mixing all stock solutions (for preparation of MS media) 3% sugar was added then the pH of the media was adjusted to 5.7–5.8. The agar (0.8%) was dissolved and the medium was dispensed in the test tubes and capped with aluminium foil. Media were then autoclaved at 121^0C at 15 psi for 20 minutes. After surface sterilization, the explants were cut into small pieces (*ca*. 1 cm for shoot and node and 1 cm^2 for leaves) and inoculated into the media. All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. All cultures were grown in an air-conditioned culture room illuminated by 40 W white fluorescent tubes with an intensity varying from 2000 to 3000 lux. The photoperiod was maintained as 16 h light and 8 h dark. The temperature of the culture room was maintained at 25 ± 2^0C. Visual observation of culture was made every week. Data were recorded for days of initiation and growth of shoots and roots.

Results

After 2-3 weeks of primary culture, explants showed growth response on different culture medium. Callus formation occurred from nodal, inter-nodal and leaf explants when planted on the medium containing the combination of BAP and NAA (Table 1). In the hormone-free basal medium and the medium containing Kn, no response was shown by nodal and inter-nodal explants (Table 1). In such conditions, callusing was seen only on leaf explants. Shoot growth was observed from callus after 40 days of culturing. However, shoot was induced only from nodal explants and root from nodal and inter-nodal explants (Table 2 and 3). The shoot and root induction from cultured explants were remarkably influenced by the type and concentration of hormone.

The shoot was induced from nodal explants on MS medium supplemented with different concentrations of Kn (0.5-3.0 mg/l) (Table 2). However, the ideal condition for shoot induction was observed on the medium containing 1.5 mg/l Kn within 15 days of culture with a shoot length of 2.4 cm (Table 2). Nodal explants cultured on MS medium supplement with higher concentrations of BAP (2.0 and 2.5 mg/l) in combination with 1.0 mg/l NAA also showed good response on shoot induction (Table 2; Fig. 1a). The influence of combination of NAA and BAP in lower concentration was not effective for shoot induction.

Rooting was initiated in the medium containing different combination of BAP and NAA (Table 3; Fig. 1b), but root induction was not observed in the medium containing Kn alone. The best condition for root development was observed in the medium containing 1 mg/l BAP and 2.5 mg/l NAA, with the production of multiple numbers of roots (length 3.5 cm) (Table 3). In the medium containing 1 mg/l BAP along with 2 mg/l and 3 mg/l NAA, the root length was high but root number was low, whereas in the other combinations of BAP and NAA small and single root was developed.

Discussion

Callusing was seen from the nodal, inter-nodal and leaf explants on the medium supplemented with different concentrations of BAP and NAA. However, late shoot growth was seen from the callus on the medium supplemented with high concentration of NAA. Nakano *et al*. (1994) found that NAA and 2,4-D alone could initiate callusing from stem, leaf and nodal segments but callus may grow slowly. Kn along with auxins considerably enhanced callus growth.

Shoot was induced from nodal explants on MS medium supplemented with higher concentrations of Kn, whereas nodal and inter-nodal explants cultured on MS medium supplemented with NAA and BAP produced roots. Gururaj *et al*. (2007) also reported that higher concentrations of Kn induce shoots in nodal explants of *Tinospora*. Similar to our findings, they also observed induction of single shoot by higher concentrations of BAP. However, Raghu *et al*. (2006) reported benzyl adenine to be effective than Kn for auxiliary shoot proliferation while Kn is better for shoot elongation.

Table 1. Effect of different hormone concentrations on the callus induction in *Tinospora cordifolia*.

S.N	MS medium + growth hormones			Callusing		
	BAP (mg/l)	NAA (mg/l)	Kinetin (mg/l)	Node	Inter-node	Leaf
1	1	0.5	-	+	+	+
2	1	1	-	+	+	+
3	1	1.5	-	+	+	+
4	1	2	-	+	+	+
5	1	2.5	-	+	+	+
6	1	3	-	+	+	+
7	0.5	1	-	+	+	+
8	1.5	1	-	+	+	+
9	2	1	-	+	+	+
10	2.5	1	-	+	+	+
11	3	1	-	+	+	+
12	-	-	1	-	-	+
13	-	-	0.5	-	-	+
14	-	-	1.5	-	-	+
15	-	-	2	-	-	+
16	-	-	2.5	-	-	+
17	-	-	3	-	-	+
18	-	-	-	-	-	+

- sign indicates no response, and + indicates basal callusing. For culture conditions see Table 1.

Table 2. Effect of different hormone concentrations on the shoot growth of *Tinospora cordifolia*.

S.N.	MS medium + growth hormones			Shoot length (cm) from nodal explants	Shoot condition
	BAP (mg/l)	NAA (mg/l)	Kinetin (mg/l)		
1.	2.0	1.0	-	1.6	Not so good
2.	2.5	1.0	-	2.0	Better
3.	-	-	1.0	0.5	Not so good
4.	-	-	0.5	0.8	Not so good
5.	-	-	1.5	2.4	Best
6.	-	-	2.0	0.9	Not so good
7.	-	-	2.5	1.6	Better
8.	-	-	3.0	1.2	Good
9.	-	-	-	1.0	Good

- sign indicates no shoot growth.

Culture conditions: MS media with different growth hormones; 4 replicates for each concentration were used (incubation temperature: 25 ± 2^0C; photoperiod: 16 h).

Table 3. Effect of different hormone concentrations on the root growth of *Tinospora cordifolia*.

S.N.	MS medium + hormones		Root length (cm)		Root condition
	BAP (mg/l)	NAA (mg/l)	Nodal explants	Inter-nodal explants	
1	1	0.5	2.5	6	Single root
2	1	1	3.8	-	Single root
3	1	1.5	1	2.8	Not so good
4	1	2	5	1.5	Good
5	1	2.5	3.5	-	Best growth
6	1	3	4.6	4.5	Fat and best
7	0.5	1	2	-	

- sign indicates no root growth. For culture conditions see Table 1.

Cytokinins are one of the most important hormones for shoot proliferation (Lane 1979; Bhojwani 1980). Similar to our present findings, Jian-Ping Luo *et al*. (2009) demonstrated that Kn alone is enough for shoot elongation of *Dendrobium huoshanense*. However, a wider survey of literature suggests that BAP is the most reliable and effective cytokinin (Sudhersan *et al*. 2001). Mao *et al*. (1995) reported requirement of higher concentrations of BAP to induce highest number of shoots through nodal segments of *Clerodendrum*. Different studies reported the caulogenic effect of BAP, as also observed in the present study (Khan *et al*. 1998).

Based on the present study it is concluded that Kn is best hormone for the *in vivo* shoot growth from nodal explants. Kn can be used for the micropropagation and large scale production of the plant which can be economically beneficial for country as whole.

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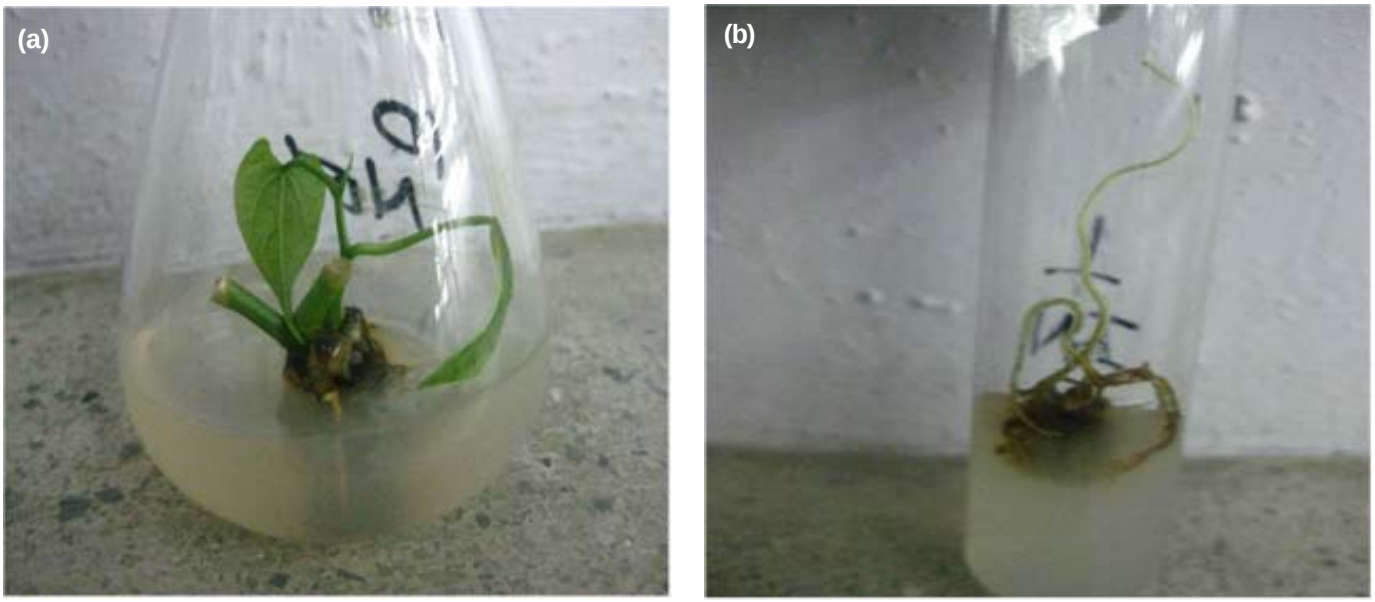

Fig. 1. *In vitro* study of *Tinospora cordifolia*: (a) shoot growth from nodal explants after basal callusing on MS medium supplemented with 2.5 mg/l BAP + 1 mg/l NAA after 40 days; (b) root growth with maximum length after basal callusing from inter-node on medium supplemented with 1 mg/l BAP + 0.5 mg/l NAA.