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SHORT COMMUNICATION

MICROPROPAGATION AND FIELD EVALUATION OF *TINOSPORA CORDIFOLIA*: AN IMPORTANT MEDICINAL CLIMBER

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A protocol was developed for clonal propagation of *Tinospora cordifolia* through *in vitro* culture of mature nodal explants. The shoot cultures were established from nodal explants treated with mercuric chloride ($HgCl_2$) on MS (Murashige and Skoog) medium supplemented with different concentrations of 6-benzyladenine (BA). Among various doses of $HgCl_2$ and BA tested, the nodal segments treated with 0.2% $HgCl_2$ for 7 minutes and implanted on MS medium with 5 µM BA resulted in 100% sprouting and 66% aseptic cultures. Healthy shoots with 4 fold multiplication rate were obtained on MS medium supplemented with 5 µM BA + 150 µM glutamine. *In vitro* regenerated shoots inoculated on ½ MS medium enriched with 0.5 µM indole-3-butyric acid (IBA) resulted in 100% rooting. Rooted plants were successfully hardened and transferred to the field with 100% survival which showed vigorous growth after 6 months. The developed protocol can be used for *en masse* propagation and conservation of *T. cordifolia*.

Key words*: Callus, field performance, shoot multiplication, *Tinospora cordifolia

Tinospora cordifolia (Willd) Miers ex. Hook F. & Thoms. popularly known as Guduchi or Giloe, is a large deciduous climbing shrub found throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m (Anonymous 1976). Various compounds viz. glycoside, giloin, gilonin, gilosterol, berberine have been reported in this species (Anonymous 1976). It is widely used in Indian ayurvedic medicine for treating diabetes mellitus (Sai and Srividya 2002, Prince *et al*. 2004). The stem is used in the treatment of debility, dyspepsia, fever and urinary disease (Sinha *et al*. 2004) and root is known for its anti-stress, anti-leprotic and anti-malarial activities (Nayampalli *et al*. 1982). Multiple uses of the plant has attracted local people for its collection and many natural strands are under severe threat. In such circumstances, it is imperative to develop some propagation methods so that it can be conserved and cultivated for meeting commercial demand. A limited success is reported to propagate this species via cuttings (Verma and Sharma 2003, Khan *et al*. 2004). *In vitro* propagation of the species has recently been reported (Raghu *et al*. 2006, Gururaj *et al.* 2007). However, micropropagation of the species from mature cuttings has remained problematic owing to poor explant response and browning of the shoots with profuse callus formation at the cut ends (Gururaj *et al.* 2007). Here, we report a simple and improvised method to propagate *T. cordifolia* vegetatively *in vitro* for its conservation and cultivation.

Healthy vines from wild sources were collected from newly emerging lateral branches of 2 year old plant of *Tinospora cordifolia* form Dondrepal range of

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Jagdalpur Forest Division, Chhattisgarh, in the last week of February 2006 and brought to the institute. The area has wide natural distribution of the species and large collection is made by the local people. Nodal segment explants of size 1.5 cm containing single axillary bud were prepared and thoroughly washed with tap water to remove superficial contamination, and kept for 3–4 h in distilled water to facilitate leaching of phenolics. Later, the explants were successively rinsed 3-4 times with normal tap water, each of 3 min duration and once with 10% (v/v) solution of cetrimide (ICI India Ltd. Patiala, India) for 10 min. The nodal segments were made free from cetrimide foam by washing 3-4 times with distilled water followed by surface sterilization with (i) 70% ethanol for 30 seconds and (ii) 0.1% and 0.2% (w/v) solution of $HgCl_2$ for 7 minutes under aseptic condition. Later the edges of the explants were trimmed and a total of 120 nodal explants (1 cm each) containing axillary bud were implanted in MS semi solid medium containing 0, 5.0, 10.0, 15.0, 20.0 and 25.0 mM BA individually in culture tubes (2.5 cm x 15 cm, Borosil, India). Bud break started from 3rd week and the axillary shoots for *in vitro* multiplication became available after 6th week.

The shoots so obtained were subcultured after 45 days on to 5.0 µm BA supplemented medium for stock build up for further experimentation. The shoots were subcultured on to fresh medium after 15 days. After 3rd subculture cycle, the shoot cultures were noticed to be turned yellow with profuse callus formation and ultimately died. To avoid yellowing, graded doses of glutamine, i.e., 50.0, 100.0 and 150.0 µM were added in combinations with 5.0, 10.0 and 15.0 µM BA. The data on shoot multiplication was recorded after 4-5 weeks.

Shoots of 2.5-3.0 cm were excised and implanted on ½ MS rooting medium, individually supplemented with 0 µM, 0.05 µM, 0.5 µM and 5.0 µM IBA. The data of rooting percentage and root length were scored after 6 weeks of inoculation. The *in vitro* raised plantlets were deflasked and washed in running tap water to remove adhered medium from the surface of plantlets followed by washing with 0.2% (w/v) Bavistin®, (BASF India Ltd, Mumbai, India) and tap water. The washed plantlets were transferred to root trainers (Neevedita Plastic Industries, Nagpur, India) comprising 25 cells each of 150cc, filled with a mixture of autoclaved soilrite: compost (1:1). The planted root trainers shifted to plastic tray filled with half strength of iron free MS salts, covered with perforated transparent polythene sheet and maintained at room temperature. After an acclimatization period of 2-3 weeks when new leaves had emerged, the plantlets were transferred to shadehouse and maintained for 2 weeks. Finally, the hardened plantlets were transplanted in polythene bags containing soil: sand: farmyard manure (1:1:1) and irrigated once in a week under shadehouse condition. After three month the hardened plants were used for planting in the field.

For all experiments, the medium contained 3% (w/v) sucrose as carbon source, 0.8% (w/v) agar (Loba Chemie Ltd. India) as gelling agent. The pH of the medium was adjusted to 5.8 before autoclaving for 15 min at 1.06 kg cm^{-2} (121^0C). For bud induction the nodal segments were inoculated in test tube containing 10 ml of semi solid medium. The shoots were cultured in 200 ml glass jar containing 25ml of semi-solid medium for *in vitro* shoot multiplication and rooting experiments. The cultures were incubated at a temperature of 25 ± 2^0C and 60% RH under 16 h illumination with fluorescent light (approx. 45 µmol $m^{-2}s^{-1}$). Each experiment was conducted twice following completely randomized design with ten replicates. The data representing means of two experiments were analyzed with SX statistical package. Least significant differences (LSD) values were calculated at $p= 0.05$ for comparing means of the treatments. Arc sin transformation was used for the data expressed as percentage.

Twelve plants each with one shoot of approximately 10 cm length were field planted with three replications in randomized block design (RBD). Plants were given support for proper growth. Data on plant height (cm), branch number, leaf length (cm), and leaf breadth (cm) of twelve randomly selected plants from each replication were recorded after 3 and 6 months. HeightGrowth was measured in terms of total length of the shoot from the ground level to the tip. Leaf length and leaf breadth were measured on ten randomly selected leaves above 0.5 m from ground level.

Initiation of sprouting of axillary shoots was observed after 5 days of inoculation and clearly visible after 15 days in all treatments of BA. After 45 days most of them grew into shoots of 1.5-1.8 cm (Fig. 1a). Response of axillary bud sprouting was affected by the concentrations of BA and $HgCl_2$ used. The maximum sprouting, aseptic culture establishment and shoot number were obtained in the explants treated with 0.2% $HgCl_2$ (92.2%, 37.77% and 1.11, respectively) (data not shown). The results exhibited a non significant difference among the different concentrations of BA used for sprouting response, which were comparable with control (68.33%). However, the maximum aseptic culture establishment (66.66%) and shoot number (3.33) were obtained in the explants inoculated on 5.0 µM BA supplemented medium which was significantly higher than all other BA concentrations.

The growth regulator and $HgCl_2$ interaction studies exhibited that explants treated with 0.2% $HgCl_2$ and inoculated on 5.0 µM BA supplemented medium recorded maximum value for sprouting (100%), aseptic culture establishment (66.66%) and shoot number (4.33) which were significantly higher than all BA treatments for above characters in the explants treated with 0.1% $HgCl_2$ (data not shown). Working with the same species Gururaj *et al*. (2006) also found 100% sprouting response in the nodal explants treated with more than 0.1% $HgCl_2$. Shukla *et al*. (2007) and Kantharaju *et al*. (2008) used 0.2% $HgCl_2$ for better response of nodal explants of *Curcuma angustifolia* and *Strelitzia reginae*, respectively. Callus formation was common in all BA treated explants but with increased concentrations of BA (>5.0 µM) intensity of callus formation was higher. Only 1-2 shoots per explant was frequent at 10 µM BA, whereas higher concentrations (>15 µM) were noticed to be inhibitory and not able to convert sprouting in shoots due to profuse callus formation (Fig. 1b). On the contrary, callus inhibiting effect of BA is reported by Krens and Jamar (1989) in *Beta vulgaris*. Initiation of the callus was perhaps due to the exogenous supply of BA, which disturbed the established polarity of organs leading to callus formation. Similar observations have been made in *Hemidesmus indicus* (Patnaik and Debta, 1996), *Gymnema sylvestre* (Reddy *et al*. 1998) and *Peganum harmala* (Goel *et al.* 2009). In contrast to the present result, Gururaj *et al*. (2007) produced multiple shoots from nodal explants of *T.cordifolia* on MS medium supplemented with higher doses of BA. This difference might be attributed to the genotypic difference of the plant materials used. The proliferated axillary shoots obtained so were further transferred to 5.0 µM BA supplemented MS medium. After 3rd subculture cycle all the leaves turned yellow and finally the shoots became leafless and stunted (Fig. 1c), which inhibited rejuvenation and survival of shoots. Mishra *et al*. (2008) also observed severe decline in shoot multiplication in *Bambus tulda,* which was overcome by incorporation of glutamine in the culture medium. Glutamine provides readily available source of nitrogen (Gamborg 1970) and its growth promoting nature is also corroborated for shoot multiplication in various plant species (Vasudevan *et al*. 2004, Saha *et al*. 2006). In the present study also the single shoot subcultured on to the medium supplemented with BA and glutamine significantly increased shoot multiplication with healthy and elongated shoots and a large number of cultures were saved after 3rd subculture passage. Among different combinations of BA and glutamine the medium containing 5 µM BA and 150 µM glutamine produced a maximum of four shoots per

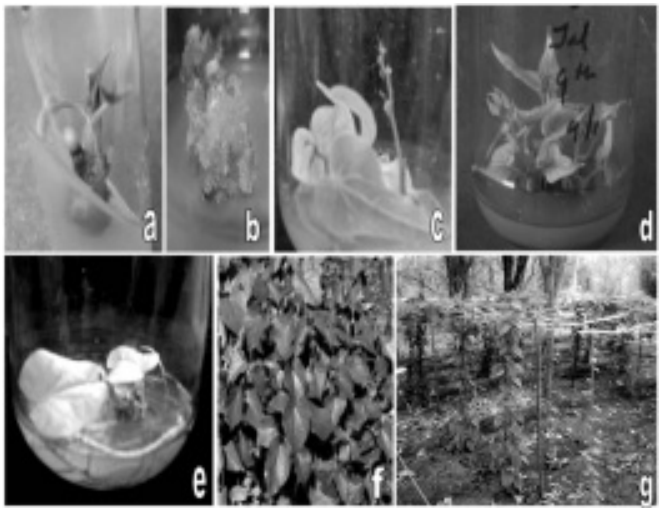

Fig. 1(a-g). ***In vitro*** **propagation of** ***Tinospora cordifolia*** **and establishment of plants in the field, (a) sprouting of axillary shoot from nodal segment explants, (b) profuse callus formation in the explants inoculated on** 15 µM **BA, (c) yellowing and leafless shoot after 3rd subculture, (d) multiple shoot proliferation on MS medium supplemented with 5 µM BA and 150 µM glutamine, (e) initiation of profuse rooting on ½ MS medium containing 0.5 µM IBA, (f) one month old hardened plants in polythene bags, (g) plants after 6 months of transplantation in the field.**

explants, which was significantly higher than all other combinations (Table 1; Fig. 1d). The added advantage of supplementation of glutamine is to get elongated shoots and reduce the additional step of elongation as described by Raghu *et al*. (2006).

Table 1. Effect of BA and glutamine on *in vitro* shoot multiplication of *T. cordifolia.*

BA (µM)	Gl (µM)			
	50.0	**100.0**	**150.0**	**Mean**
5.0	3.8	2.0	4.0	3.26
10.0	2.3	1.4	1.6	1.76
15.0	2.8	1.8	2.4	2.33
Mean	2.96	1.73	2.66	
$LSD_{(0.05)}$	BA and Gl = 0.75, BA x Gl = 1.30			

When elongated shoots of 2.5 - 3 cm were transferred to half strength MS medium variously supplemented with IBA, 100% shoots were rooted after 20 days on medium containing 0.5 µM IBA with a maximum of 6.75 cm root length (Table 2; Fig. 1e). Half strength MS medium in the present study facilitated callus free rhizogenesis as against the problem of profuse callus formation on full MS medium reported by Gururaj *et al*. (2007). Callus formation in full strength medium may be due to high nitrogen concentration, which tends to increase the level of endogenous cytokinin, thereby stimulating callus proliferation (Marks and Simpson 1994).

Table 2. Effect of different doses of IBA on *in vitro* rooting of *T. cordifolia.*

IBA (µM)	Rooting %	Root length (cm)
0	16.7 (23.9)	3.8
0.05	31.1 (43.1)	4.6
0.5	100.0 (90.0)	6.8
5.0	46.7 (56.9)	5.7
$LSD_{(0.05)}$	4.26	0.87

Values in parentheses are *arc sine* transformation

Based on our earlier published paper on *B. tulda* (Mishra *et al*. 2008) washing of *in vitro* raised plantlets of *Tinospora cordifolia* with 0.2% Bavistin helped to avoid bacterial contaminants, which resulted in fewer plantlets being infected during hardening (data not shown). The rooted and hardened plants were transplanted in polythene bags (Fig. 1f) under green house conditions for one month, followed by field transfer. Almost all the plantlets survived but exhibited slow growth up to 3 months and enhanced tremendously after 6 months of field transfer (Fig. 1g). By the protocol described here one can get 4 rooted plants from one explant in 102 days (in a three stage growth). The rapidity of multiplication and successful field establishment of *T. cordifolia* can be useful in conservation and propagation of elite plants for commercial exploitation.

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