Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 6, Issue, 2, pp.2714-2718, February, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

TINOSPORA CORDIFOLIA (WILLD.) MIERS EX HOOK. F & THOMS. (MENISPERMACEAE): RAPID *IN VITRO* PROPAGATION THROUGH SHOOT TIP EXPLANTS

Hemant Sharma*[1], B.D. Vashistha[2], Nidhan Singh[3] and Ravi Kumar[4]

[1]Department of Botany, Dayanand PG College, Hisar, Haryana, India
[2]Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India
[3]Department of Botany, IB (PG) College, Panipat, Haryana, India
[4]Department of Botany, SD (PG) College, Panipat, Haryana, India

ARTICLE INFO

Article History:

Received 5th, January, 2015
Received in revised form 12th, January, 2015
Accepted 6th, February, 2015
Published online 28th, February, 2015

Key words:

Shoot tips, *In vitro*, *Tinospora cordifolia*, Medicinal plant

ABSTRACT

Shoot tip explants were employed to develop a method of rapid *in vitro* multiplication of *Tinospora cordifolia*, an important plant for Ayurveda and medicine. Multiple shoot were induced on MS medium and woody plant medium supplemented with different concentrations of cytokinins (BAP and KIN). Maximum number of shoots was induced in woody plant medium supplemented with 2 mg/l BAP. *In vitro* rooting was achieved on half strength MS medium supplemented with IBA and IAA, with maximum rooting with 1.0 mg/l IBA. The shoots with well-developed roots were successfully transferred to field with 70 % survival.

INTRODUCTION

Tinospora cordifolia (Willd.) Miers ex Hook. f. & Thoms. (Menispermaceae) is a large, woody climber. The young stem is green, cylindrical and smooth. The mature stem has warts on surface, hanging aerial roots and peeling bark which is creamy-white. Leaves are petiolated, cordate and membranous (**Figure 1.A, B**). The flowers are small and yellow. The male flowers are clustered and female flowers are usually solitary. The fruits (drupes) are fleshy, ovoid, red, single seeded and pea-sized. The seeds are curved. Flowers grow during the summer and fruits during the winter.

It is popularly known as Giloy and is widely used in Ayurvedic system of medicine. The various herbal products of Giloy are available in Indian market to lower down the diabetes and to boost up the immune system of the body.

The conventional methods of propagation of *T. cordifolia* have limited potential for large scale production as this plant has poor seed producing capacity. Also, vegetative propagation by stem cuttings is delimited by unfavorable weather conditions, making it dependent on suitable climatic conditions for proper growth. As this plant is highly valuable for medicine and is always in great demand, rapid and reliable methods of propagation of this plant are much needed. Plant propagation by tissue culture is one of such approaches which allow mass multiplication and propagation under aseptic conditions. This technique can enhance its availability independent of the season and climatic conditions. Therefore, in the present work, efforts were made to develop protocols for rapid multiplication of *T. cordifolia* using shoot tip explants under *in vitro* conditions.

MATERIALS AND METHODS

Collection of Plant Material

Plants were collected from various locations in the campus of Kurukshetra University and few other places around Kurukshetra city, Haryana (India). Shoot tips were collected to be used as explants for micropropagation.

Sterilization of Explants

Explants were first washed under running water for half an hour, soaked in 0.1 % (v/v) Teepol® for 15 minutes and then washed with distilled water many times. Thereafter, explants were sterilized with 0.1 % (w/v) mercuric chloride solution for 2-3 minutes and were rinsed 3-4 times with sterilized double distilled water. For achieving full aseptic conditions, the explants were disinfected in 70 % (v/v) ethyl alcohol for about a minute.

*Corresponding author: **Hemant Sharma**
Department of Botany, Dayanand PG College, Hisar, Haryana, India

Culture Medium and Conditions

MS (Murashige and Skoog, 1962) and WPM (Llyod and McCown, 1980) with 3 % (w/v) sucrose, solidified with 0.8 % (w/v) Agar were used as media. The pH was adjusted to 5.8 by using 0.1 N NaOH or 0.1 N HCl before autoclaving the media at 121 ºC temperature and 15 psi pressure for 20 minutes. After culturing, temperature of 25 ± 2ºC and 16 hours photoperiod were maintained with 30 µmol m^{-2} s^{-1} irradiance provided by cool white fluorescent tubes (40 W-Philips, India).

Culture Establishment

Shoot tips about 0.5 cm in length were inoculated on MS and WP basal media, supplemented with different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of growth regulators (BAP and KIN). After 25 days, shoots were sub-cultured on same medium for multiplication. Percent response of explants, number of days required for shoot induction, number of shoots formed and shoot length were periodically recorded. Final data were collected and analyzed after 60 days of inoculation.

Rooting and Acclimatization

In vitro regenerated shoots were excised and planted on half strength MS medium supplemented with 1.0 mg/l IAA and IBA for rooting, after they attained a height of 2-3 cm. MS medium with double the usual concentration of sucrose was used for rooting. The rooted plantlets were gently pulled out of the medium, washed in running tap water and any remains of medium sticking to roots were carefully removed. The plantlets with well-developed roots were then transferred to small plastic pots having sterilized soil and sand mixture (1:1). For initial 15 days, the pots were covered with transparent polythene bags to maintain high humidity; then the bags were punctured for air circulation. Plants were watered with ¼ WP salt solutions on alternate days. After this, the pots with plantlets were transferred to Polyhouse for further hardening.

Statistical Analysis

Each experiment was repeated three times using 10 explants per treatment. All the data recorded till and after 60 days were subjected to analysis of variance (ANOVA) (P = 0.05) followed by Duncan Multiple Range Test (DMRT) (P = 0.05) to interpret the results.

RESULTS AND DISCUSSION

The cultures were raised on MS and WP basal Medium as well as with different concentrations of growth regulators. The observations, as recorded from various combinations, are being presented as follows.

MS and WP basal medium

Shoot tips did not show any response on MS basal medium. In contrast to MS basal medium, single shoot appeared in shoot tips on WP basal medium. Therefore, in *Tinospora cordifolia* MS or WP basal media alone (without growth regulators) were not much effective in inducing shoot buds. It was observed that in contrast to basal media, the media supplemented with cytokinins (BAP and KIN) induced multiple shoot formation. In other such studies also, on *Peganum harmala* (Saini and Jaiwal, 2000) and *Crataeva nurvala* (Walia *et al*., 2003) no shoot buds were found to be developed on MS basal medium.

MS and WP Medium supplemented with BAP and KIN:

Tables 1 & 2 show the effect of BAP and KIN on shoot tip explants. It was found that as the concentration of cytokinins increased from 0.5 mg/l to 2.0 mg/l, the response also increased and the 2 mg/l of BAP and KIN individually was optimum for shoot initiation and multiplication. At this concentration, percent response and number of shoots were maximum. Moreover, the time taken for bud initiation was also reduced. Further at higher concentration (4 mg/l), the response of explants was decreased.

From all these observations, it was concluded that explants cultured on MS medium took longer time for bud initiation as compared to WPM. Also, the percent response on MS medium was less than that on WPM. WPM proved better in terms of time of bud initiation, number of shoots and enhancement of shoot length in *Tinospora cordifolia* as well as many other woody plant species like *Prunus armenica* (Tornero *et al*., 2000), Blue berry (Tetsumura *et al*., 2008) and *Cinnamomum camphora* (Sharma and Vashistha, 2010).

Of the two cytokinins tried, BAP was more effective than KIN for percent response of explants, time taken for shoot-bud induction and number of shoots on both media. The superiority of BAP over other cytokinins has been reported in many other plant species like *Capparis decidua* (Tyagi and Kothari, 2001), *Rotula aquatica* (Sebastian *et al*., 2002), *Pterocarpus marsupium* (Chand and Singh, 2004), *Datura metel* (Khan *et al*., 2010), *Cinnamomum camphora* (Sharma and Vashistha, 2010) and *Pterocarpus santalinus* (Balaraju *et al*., 2011). However, shoot lengths were found to be more on KIN containing medium than on medium supplemented with BAP. Similar observations were made by Martin *et al*. (2005) in *Dendrobium* hybrids. In the present study, higher concentrations of BAP and KIN were inhibitory, as in other studies on *Celastrus paniculatus* (Nair and Seeni, 2001),

Table1 Effect of different concentrations of BAP and KIN on shoot initiation on shoot tip explants of *T. cordifolia* cultured on MS medium

Concentration (mg/l)	% response		Time taken for bud initiation (Days)		Average number of shoots		Average shoot length (cm)	
	BAP	KIN	BAP	KIN	BAP	KIN	BAP	KIN
0.0	-	-	-	-	0.0 ± 0.00^{e}	0.0 ± 0.00^{d}	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}
0.5	43.3	40.0	22	23	2.3 ± 0.13^{c}	1.0 ± 0.00^{c}	1.0 ± 0.05^{c}	1.7 ± 0.07^{c}
1.0	50.0	46.7	21	22	3.0 ± 0.15^{b}	1.2 ± 0.05^{b}	1.3 ± 0.05^{b}	2.0 ± 0.10^{b}
2.0	53.3	50.0	20	20	3.5 ± 0.15^{a}	1.7 ± 0.10^{a}	1.5 ± 0.09^{a}	2.4 ± 0.12^{a}
4.0	40.0	36.7	22	23	1.8 ± 0.10^{d}	1.0 ± 0.00^{c}	1.0 ± 0.05^{c}	1.5 ± 0.05^{d}

- Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.
- Means followed by the same letter within columns are not significantly different at *P* = 0.05 according to Duncan's Multiple Range Test.
- (-) No response.

Table 2 Effect of different concentrations of BAP and KIN on shoot initiation on shoot tip explants of T. cordifolia cultured on WPM.

Concentration (mg/l)	% response		Time taken for bud initiation (Days)		Average number of shoots		Average shoot length (cm)	
	BAP	KIN	BAP	KIN	BAP	KIN	BAP	KIN
0.0	20.0	20.0	25	25	1.0 ± 0.00^{e}	1.0 ± 0.00^{e}	0.5 ± 0.03^{e}	0.5 ± 0.03^{e}
0.5	46.7	43.3	20	20	3.6 ± 0.16^{c}	1.8 ± 0.09^{c}	2.2 ± 0.10^{c}	3.2 ± 0.13^{c}
1.0	53.3	50.0	19	20	4.1 ± 0.21^{b}	2.0 ± 0.11^{b}	2.7 ± 0.12^{b}	3.7 ± 0.14^{b}
2.0	60.0	53.3	19	20	4.8 ± 0.23^{a}	2.5 ± 0.13^{a}	3.1 ± 0.15^{a}	4.2 ± 0.16^{a}
4.0	46.7	40.0	20	21	3.0 ± 0.13^{d}	1.5 ± 0.09^{d}	1.3 ± 0.05^{d}	2.6 ± 0.12^{d}

- Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.
- Means followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's Multiple Range Test.

Table 3 Effect of different combinations of BAP and KIN on shoot initiation on shoot tip explants of *T. cordifolia* cultured on WPM

Growth regulators (mg/l)	% response	Time taken for bud initiation (Days)	Average number of shoots	Average shoot length (cm)
0.0 BAP + 0.0 KIN	20.0	25	1.0 ± 0.00^{g}	0.5 ± 0.03^{f}
2.0 BAP + 0.5 KIN	63.3	17	3.1 ± 0.15^{c}	2.2 ± 0.07^{d}
2.0 BAP + 1.0 KIN	70.0	15	4.5 ± 0.22^{a}	3.8 ± 0.13^{a}
2.0 BAP + 2.0 KIN	66.7	16	3.6 ± 0.18^{b}	2.2 ± 0.09^{d}
2.0 BAP + 4.0 KIN	60.0	18	2.6 ± 0.12^{e}	2.3 ± 0.10^{d}
0.5 BAP + 2.0 KIN	60.0	17	2.8 ± 0.13^{de}	2.7 ± 0.12^{c}
1.0 BAP + 2.0 KIN	66.7	17	3.0 ± 0.13^{cd}	3.3 ± 0.13^{b}
4.0 BAP + 2.0 KIN	56.7	18	2.2 ± 0.11^{f}	2.0 ± 0.07^{e}

- Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.
- Means followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's Multiple Range Test.

Table 4 Effect of different concentrations of auxins on rooting of *in vitro* regenerated shoots of *T. cordifolia* cultured on MS medium

Growth regulator	Concentrations (mg/l)	% root induction	Time taken for root induction (Days)	Average number of roots	Average root length (cm)
Control	0.0	-	-	0.0 ± 0.00	0.0 ± 0.00
1/2 MS + IAA	1.0	30.0	32	1.1 ± 0.06	1.5 ± 0.06
1/2 MS + IBA	1.0	40.0	29	1.5 ± 0.08	1.8 ± 0.10

- Values are means ± S.E. of three independent experiments, each consisted of 10 replicates per treatment. Data from 60 days old culture.
- (-) No response.

Entada phaseoloides (Rao and Vishnupriya, 2002), *Saussurea obvallata* (Joshi and Dhar, 2003), *Pterocarpus marsupium* (Anis *et al*., 2005) and *P. santalinus* (Balaraju *et al*., 2011).

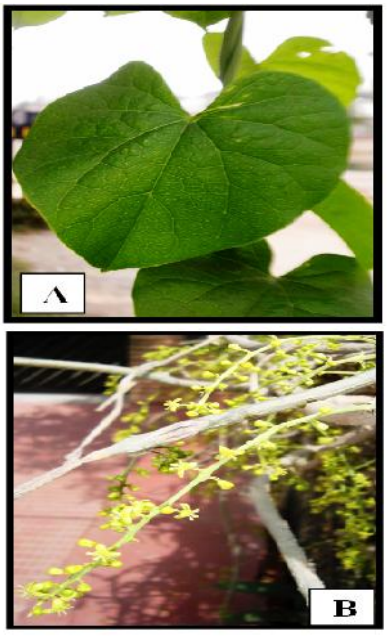
WP Medium supplemented with combination of BAP and KIN

The effects of combination of BAP and KIN were studied on shoot tip explants using WPM, this being found to show better results for this plant than MS medium (**Table 3**). As it was observed that BAP as well as KIN elicited maximum response at 2 mg/l, therefore, in one set BAP (2.0 mg/l) was combined with varying concentrations of KIN (0.5, 1.0, 2.0 and 4.0 mg/l). In another set, KIN at 2.0 mg/l was combined with different concentrations of BAP (0.5, 1.0, 2.0 and 4.0 mg/l). All the combinations, using both the reciprocal sets showed increase in percent response and reduced the time taken for bud initiation. Therefore, the combination of BAP and KIN were promotory for percent bud induction in shoot tip explants. It seems to be a function of the concentration of one hormone with respect to the other. It has been reported in many species including *Desmodium oojeinense* (Kumari and Shivanna, 2005) and *Aloe vera* (Thind *et al*., 2008). Still, of all the combinations tried in the present study, the combination with 2 mg/l BAP +1 mg/l KIN was optimum. Similar observation is reported for *Canavalia virosa* (Kathiravan and Ignacimuthu, 1999).

Rooting of in vitro developed shoots

No rooting was observed on half-strength MS basal medium until the medium was supplemented with auxins.

For rooting, IBA was found to be more effective than IAA and it induced healthy and elongated roots (**Table 4**). On similar lines, the promotive effects of IBA on rooting have also been reported in *Glycine max* (Srinivas *et al*., 2000), *Ceratomia siliqua* (Romano *et al*., 2002), *Cinnamomum camphora* (Sharma and Vashistha, 2010) and *Tinospora cordifolia* (Sharma and Vashistha, 2015).



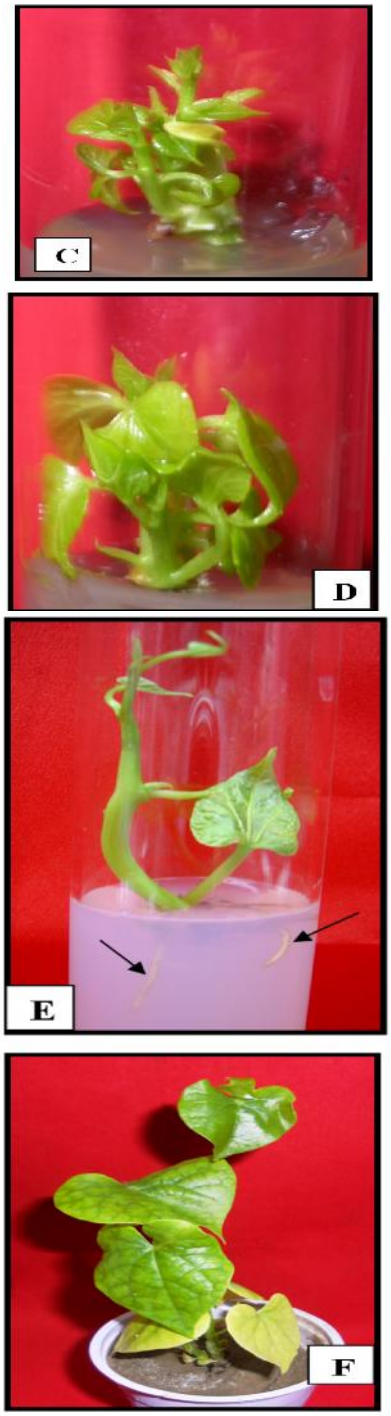


Figure 1 *Tinospora cordifolia*: **A.** leaf; **B.** flowering branch; **C.** *in vitro* shoot formation on MS + 2 mg/l BAP; **D.** *in vitro* shoot formation on WPM + 2 mg/l BAP. **E.** *in vitro* rooting in MS + 1 mg/l IBA; **F.** *in vitro* regenerated plant in plastic cup.

Hardening and transfer of plantlets to the field

Shoots with well-developed roots were transferred to plastic cups containing sterilized soil and sand mixture in equal ratio **(Figure 3.F)**, humidity was maintained for initial 15 days with the help of polythene bags. Thereafter, these pots were exposed to natural conditions for 3-4 hours every day, increasing the period of exposure every week. The plants were shifted to semi-natural environment of the polyhouse after about a month, where they grew almost normally with 70% survival rate. After one month in polyhouse, these plants became capable of growing in natural environment of the field and were transferred to the field, where they survived very well and grew normally.

References

1. Anis, M., Husain, M.K. and Shahzad, A. 2005. *In vitro* plantlet regeneration of *Pterocarpus marsupium* Roxb. an endangered leguminous tree. *Curr. Sci.*, 88: 861-863.
2. Balaraju, K., Agastian, P., Ignacimuthu, S. and Park, K. 2011. A rapid *in vitro* propagation of red sanders (*Pterocarpus santalinus* L.) using shoot tip explants. *Acta Physiol. Plant.*, 33: 2501-2510.
3. Chand, S. and Singh A.K. 2004. *In vitro* shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree, *Pterocarpus marsupium* Roxb. *In Vitro Cell. Dev. Biol.-Plant*, 40: 464-466.
4. Joshi, M., and Dhar, U. 2003. *In vitro* propagation of *Saussurea obvallata* (D.C.) Edgew- an endangered ethno religious medicinal herb of Himalaya. *Plant Cell Rep.*, 21: 933-939.
5. Kathiravan, K. and Ignacimuthu S. 1999. Micropropagation of *Canavalia virosa* (Roxb.) Wight and Arn. a medicinal plant. *Phytomorphology*, 49: 61-66.
6. Khan, S., Tyagi, P., Kachhwaha, S. and Kothari, S.L. 2010. High frequency plant regeneration and detection of genetic variation among micropropagated plants of *Datura metel* L. *Phytomorphology*, 60: 1-8.
7. Kumari, M.M.V. and Shivanna, M.B. 2005. Callus mediated regeneration of *Desmodium oojeinense* Roxb. *Phytomorphology*, 55: 171-177.
8. Lloyd, G.B. and McCown, B.H. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use of shoot-tip culture. *Proc. Int. Plant Prop. Soc.*, 30: 421-427.
9. Martin, K.P., Geevarghese, J., Joseph, D. and Madarsery, J. 2005. *In vitro* propagation of *Dendrobium* hybrids using flower stalk node explants. *Indian J. Exp. Biol.*, 43: 280-285.
10. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473-497.
11. Nair, L.G. and Seeni, S. 2001. Rapid *in vitro* multiplication and restoration of *Celastrus paniculatus* Willd. sub sp. *paniculatus* (Celastraceae), a medicinal woody climber. *Indian J. Exp. Biol.*, 39: 697-704.
12. Rao, J.V.S. and Vishnupriya, K.S. 2002. *In vitro* response of embryo axis of *Entada phaseoloides* Merrill. *Phytomorphology*, 52: 97-102.
13. Romano, A., Barros, S. and Martinus-Lucao, M.A. 2002. Micropropagation of the Mediterranean tree *Ceratomia siliqua*. *Plant Cell Tiss. Org. Cult.*, 68: 35-41.
14. Saini, R. and Jaiwal, P.K. 2000. *In vitro* multiplication of *Peganum harmala*- an important medicinal plant. *Indian J. Exp. Biol.*, 38: 499-503.
15. Sebastian, D.P., Benzamin, S. and Hariharan, M. 2002. Micropropagation of *Rotula aquatica* Lour- an important woody medicinal plant. *Phytomorphology*, 52: 137-144.
16. Sharma, H. and Vashistha, B.D. 2010. *In vitro* propagation of *Cinnamomum camphora* (L.) Nees & Eberm using shoot tip explants. *Ann. Biol.*, 26: 109-114.

17. Sharma, H. and Vashistha, B.D. 2015. *In vitro* plant regeneration through callus in Giloy (*Tinospora cordifolia* (Willd.) Miers ex Hook. f & Thoms.). Indian J Science, 12(34): 59-68.
18. Srinivas, B., Rao, D. and Ramulu, C.H.A. 2000. Culture conditions affecting adventitious shoot development from cotyledons of *Glycine max* cv. PK-472. *Phytomorphology*, 50: 281-286.
19. Tetsumura, T., Matsumoto, Y., Sato, M., Honsho, C., Yamashita, K., Komatsu, H., Sugimoto, Y. and Kunitake, H. 2008. Evaluation of basal media for micropropagation of four highbush blueberry cultivars. *Sci. Hortic.*, 119: 72-74.
20. Thind, S.K., Jain, N. and Gosal, S.S. 2008. Micropropagation of *Aloe vera* L. and estimation of potentially active secondary constituents. *Phytomorphology*, 58: 65-71.
21. Tornero, O.P., Lopez, J.M, Egea, J. and Burgos, L. 2000. Effect of basal medium and growth regulators on the *in vitro* propagation of apricot (*Prunus armenica* L.) cv. Canino. *J. Hort. Sci. Biotechnol.*, 75: 283-286.
22. Tyagi, P. and Kothari, S.L. 2001. Continuous shoot production for micropropagation of *Capparis decidua*- A tree of arid agroforestry system. *J. Indian Bot. Soc.*, 8: 5-8
23. Walia, N., Sinha, S. and Babbar, S.B. 2003. Micropropagation of *Crataeva nurvala*. *Biol. Plant*, 46: 181-185.

How to cite this article:

Hemant Sharma et al. Tinospora cordifolia (willd.) miers ex hook. f & thoms. (menispermaceae): rapid in vitro propagation through shoot tip explants. *International Journal of Recent Scientific Research Vol. 6, Issue, 2, pp.2714-2718, February,*

****