In vitro propogation of Ayurvedic important plant Tinospora cordifolia

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In vitro propagation of ayurvedic important plant *Tinospora cordifolia* (willd.) Miers

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Abstract

Tinospora cordifolia (Willd.) Miersis an important medicinal plant belongs to family Amaranthaceae found in India. The plant has medicinal properties like treat convalescence from severe illness, arthritis (or joint diseases), liver disease, eye diseases, urinary problems, anemia, cancer, diarrhea, and diabetes. It also helps to remove toxins from the body. The successful protocol for in vitro propagation has been achieved for the medicinal important plant T. cordifolia by using nodal and apical shoot tip segments as explants. In vitro plantlets raised on Murashige and Skoog (MS) medium containing 0.5–3.00 mg/l BAP in combination with 0.2-1.00 mg/l IAA, 3% sucrose, and 0.3% clerigar. After 21 days maximum percentage of shoot organogenesis was obtained on medium containing 2.0 mg/l BAP and 0.2 mg/l IAA. The regeneration protocol developed in this study provides an important method of micropropagation of this plant. Furthermore, this protocol may be used for a large scale production of its medicinally active compounds and genetic transformations for further improvement.

Keywords: In vitro, explant, propagation, Tinospora cordifolia.

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INTRODUCTION

Tinospora cordifolia is commonly known by the various names such as “Guduchi” and “Gulvel” etc. in Maharashtra, it is herbaceous vines belong to the family Menispermaceae. The plant is indigenous to the tropical areas of India, Myanmar as well as Sri Lanka. Morphologically the plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests. The leaves are heart shaped. The succulent bark is creamy white to grey in color, with deep clefts spotted with lenticels. It puts out long, slender aerial roots, and is often grown on mango or neem trees. Gulvel is a native plant from India, also known to be found in Far East, primarily in rainforests. It has stem about 6 cm in diameter, with light grey, papery bark. The leaves are 7.5-14 cm long, 9-17 cm broad, broadly ovate or orbicular, deeply heart shaped at the base. Tiny greenish yellow flowers occur in racemes 7-14 cm long. Flowers have 3+3 sepals in 2 layers, the outer ones are small, the inner large. Six stamens prominently protrude out (Sinha*et al*., 2004). The plant flowers during the summer and fruits during the winter. Gulvel prefers acidic, neutral or basic alkaline soil. It can grow in semi-shade or no shade, requiring moist soil. Gulvel grows easily without chemical fertilizers and use of pesticides. The herb has a long history in use by practitioners of Ayurveda known by its practitioners to treat arthritis (or joint diseases), liver disease, eye diseases, urinary problems, anemia, cancer, diarrhea, and diabetes. Also, help remove toxins from the body (Mishra *et al*., 2014). The plant is cultivated by stem cutting in the month of May-June and used in Tibetan medicine. The herb is known to have a sweet, bitter and acid taste. Extracted from the stem and root is a nutrient starch used to treat chronic diarrhea and dysentery. According to a legend,

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the herb is known locally as “giloy” or "heavenly elixir" kept the angels eternally young.

MATERIALS AND METHODS

All the experimental material (Explants) of T. cordifolia was collected from botanical garden Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Apical shoot tip and nodal segments were collected from previously grown plants and used as source of explants. These explants were surface sterilized with 0.3% (w/v) HgCl2 (RFCL Ltd, India) for four - six min. followed by washing with sterile distilled water 4-5 times. Explants were prepared (0.5 cm) and cultured on MS-media (Murashige and Skoog, 1962) contain 0.3% (w/v) clerigar (Himedia Pvt. Ltd, India) as a solidifying agent supplemented with plant growth regulators viz. IAA as an auxin and BAP as a cytokinine at different combinations were tried.

Culture condition

The present research work was carried out under controlled conditions. MS medium was fortified with 3% sucrose and 0.3% Clerigar. After the addition of phytohormones the pH was adjusted to 5.8 after that MS medium was sterilized in an autoclave under 15 psi pressures and 121° C temperature for 20 minutes. Sterilized medium was transferred into laminar air flow for inoculation. After inoculation culture vessels were transferred into culture room contains 25± 2°C temperatures for 3- 4 weeks with 16 hours of photoperiod and 70% relative humidity. Data was recorded after every week and analyze by five replicated with mean ± SE.

Experimental results

Present research work was initiated on in vitro propagation of T. cordifolia by testing MS medium supplemented with different concentration and combination of growth hormones. During the work apical shoot tip and nodal segments were introduced on MS medium alone with BAP, KIN in combination or either alone IAA and NAA, all the combination rising the shoot proliferation were recorded in present study but it required optimum concentration of PGRs. When the shoot tip explant was inoculated on MS medium supplemented with BAP at 2.0 mg/l either alone or in combination with IAA at 0.2 mg/l derived highest percentage of shoots proliferation were recorded. For the nodal segment MS medium + BAP 3.0 mg/l + IAA 0.2mg/l shown highest percentage of shoot proliferation as compared to any other concentration and combination of BAP + IAA (table). Growth hormones like BAP, at 0.5 to 3.0 mg/l were tested with MS Medium to induce shoot multiplication as well as rising the calli were noticed. Among all the concentrations and combinations of growth hormones used 2.0 and 3.0 mg/l BAP + 0.2 -1.0 mg/l IAA showed better result for multiple shoot induction. Callus obtained from shoot tip as an explant was compact, greenish and cream colored which was inoculated with 0.5–3.0 mg/l BAP in combination with 0.2 - 1.0 mg/l IAA (Table 1). The average percentage of shoots regenerated derived in MS medium + 1.0mg/1 KIN or 2.0 mg/l + 0.2 mg/1 IAA. Maximum percentage of induction callus was recorded on MS medium supplemented with 3.0 mg/l BAP + 0.2 mg/l IAA as compared to any concentration and combination of MS medium supplemented with KIN alone with IAA. Both the explants were inoculated on MS medium containing BAP, KIN either alone or in combination with IAA at optimum concentration all the culture were raising the multiple shoots as well as significantly increasing the shoot length (μ ± SE). The maximum percent of shoot length were measured on MS medium + BAP 2.0 + IAA 0.2 mg/l from the shoot tip explant. For the nodal segment maximum percent of shoot length were recorded on MS medium supplemented with BAP 2.0 mg/l in combination with 0.2 mg/l IAA. On the table summarizing that BAP was most significantly affected on rising of shoot length of in vitro culture as compared to any other combination or concentration of KIN either alone or in combination of IAA.

Table 1: Effect of PGR on Shoot multiplication of *T. cordifolia*

Source of explant	PGRs	Concentration mg/l	Frequency of callus	Induction of shoot %	Mean ± SE Shoot length (in cm)
Shoot tip	BAP + IAA	1.0 + 0.2	+	30	3.7± 0.24
	BAP + IAA	2.0 + 0.2	+++	60	4.2 ± 0.15
	BAP + IAA	3.0 + 0.2	++++	50	3.5 ± 0.20
	KIN + IAA	0.5 + 0.2	-	20	2.2 ± 0.16
	KIN + IAA	1.0 + 0.2	++	30	2.5 ± 0.19
	KIN + IAA	2.0 + 0.2	+++	20	2.4 ± 0.18
Nodal segment	BAP + IAA	1.0 + 0.2	-	40	3.8 ± 0.26
	BAP + IAA	2.0 + 0.2	++	65	4.5 ± 0.14
	BAP + IAA	3.0 + 0.2	+++	50	4.1 ± 0.15
	KIN + IAA	0.5 + 0.2	-	20	3.7 ± 0.24
	KIN + IAA	1.0 + 0.2	++	40	3.9 ± 0.27
	KIN + IAA	2.0 + 0.2	++	45	4.2 ± 0.15

Highest percentage of shoot induction per explant (60 and 65) was recorded on BAP + IAA and KIN + IAA by introducing shoot and nodal segment explant respectively. Present result summarized by Singh *et al*., 2009 on in vitro multiplication of T. cordifolia on MS medium + BAP 1.0 mg/l + NAA and KIN 1.5 mg/l Combination with NAA at low concentration. Kumari in 2012 also reported rapid multiplication in T. Cordifolia through nodal explants on MS medium containing various concentration of growth regulators such as BAP (1- 2 mg/l) and Kinetin (1- 2 mg/l) respectively. Recently Sivakumar*et al*., in 2012 reported in vitro micropropagation of T. cordifolia on MS medium supplemented with 4.36 µM KIN produces 2.32 ± 0.1 cm shoot lengths with 1.8 ± 0.1 numbers of shoot per explant with 70% response.

Figure 1: a) Callus along with shoot initiation **b)** Multiple shoots

CONCLUSION

Present research work was concluded that in vitro propagation of T. cordifolia required optimum concentration of plant phytohormones as well as MS medium was the best medium for rapid growth of in vitro plantlet and induction of various types callus. Most suitable combination and concentration of BAP and IAA was 2.0 mg/l and 0.2 mg/l was recorded respectively.

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