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IN-VITRO PROPAGATION OF *Tinospora cordifolia (WILD.) MIERS EX HOOK. F. THOMS.*

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Abstract- Medicinal plants are valuable sources of medicinal and many other pharmaceutical products. The conventional propagation method is the principal means of propagation and takes a long time for multiplication because of a low rate of fruit set, and/or poor germination and also sometimes clonal uniformity is not maintained through seeds. The plants used in the phyto-pharmaceutical preparations are obtained mainly from the natural growing areas. With the increase in the demand for the crude drugs, the plants are being overexploited, threatening the survival of many rare species. Also, many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation, and indiscriminate collection. Advanced biotechnological methods of culturing plant cells and tissues should provide new means for conserving and rapidly propagating valuable, rare, and endangered medicinal plants. The present investigations were carried out with a view to standardize an in-vitro culture technique for propagation of *Tinospora cordifolia* Hook. The Nodal and inter nodal segments from healthy grown plants were used as explants. For culturing Explants were cultured on standard Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl amino purine (BA), kinetin (Kn) and adenine (Ad) for primary shoot proliferation. The shoot proliferation (50 % single shoot,) was observed in MS medium containing BA 5.0 + Kn 1.0.For rooting of the micro shoots, half strength MS medium supplemented with 0.4mg/L Naphthalene acetic acid (NAA) exhibited best results with average rooting response of 40 %. After acclimatization and transplantation, 100% of the *in- vitro* derived plants were found healthy in ex vivo conditions .
Key words- *Tinospora cordifolia*, *in vitro* propagation

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Introduction

Guduchi [*Tinospora cordifolia* (Wild.) Miers ex Hook. F. Thoms] is large, glabrous, deciduous climbing shrub belonging to the family Menispermacaea. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300 m. The local names are Guduchi (Sanskrit), Amrita, Giloya (Hindi), Tinospora (English), Shindilkodi (Tamil), Citamedru (Malayalam). The stem of *Tinospora cordifolia* is rather succulent with long filiform flesh aerial roots from the branches. The bark is creamy white to grey, deeply left spirally, the space in between being spotted with large rosette like lenticels. The leaves are membranous and cordate. The flowers are small and yellow or greenish yellow in colour [1-5]. In auxiliary and terminal racemes or reacemose panicles, the male flowers are clustered and female are usually solitary. The drupes are ovoid, glossy, succulent, red and pea-sized. The seeds are curved. Fruits are fleshy and single seeded. Flowers grow during the summer and the fruits during the winter [6-10].
Guduchi is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic, and anti-diabetic [11-15]. The plant is used in ayurvedic, “Rasayanas” to improve the immune system and the body resistance against infections. The root of this plant is known for its anti-stress, anti-leprotic and anti-malarial activities [16-20].

Material and Methods

Source of explants

Plants of *T. cordifolia* were obtained from Ayurvedic Rasshala, a

commercial firm at Pune, engaged in the manufacture of herbal medicines, as well as from the experimental field station of the Bhabha Atomic Research Centre, Trombay, Mumbai. The plants were maintained in pots in the green house of our institute. The leaves, stems of these plants were used as explants for *in vitro* micro propagation.

Sterilization of explants

The stem and leaves of the plant *Tinospora cordifolia* were thoroughly washed with running water followed by washing with dettol and tween 80 (surfactant). They were subsequently washed with 0.1% $HgCL_2$ and repeatedly washed with sterile water and cultured on Murashige and skoog's (MS) medium (1962) with different PGR's at pH 5.8 (adjusted prior to autoclaving) supplemented with necessary macro-nutrients, micro-nutrients, an iron source, vitamins, 3% sucrose (as a carbon source) and 0.95% (w/v) Difco-Bacto agar as a gelling agent. These explants were also cultured in MS modified medium (1965).

Selection of explants

Initial experiments were conducted employing the shoot tip, nodal and internodal region, the leaf and the petiole of *T. cordifolia* as explants. Among the five explants, the nodal and internodal explants responded and showed slight callus formation. The other three explants failed to show any response in the basal medium. Hence further experiments were undertaken to test whether other media could prove effective in bringing about shooting or rooting response in the same explants. The media tested were: B5 or Gamborg et al. medium (1968) & Nitsch and Nitsch (N & N) medium (1969).

While nodal and internodal explants in B5 or Gamborg et al. medium (1968) showed callus formation, the other media proved to be completely in effective. Since in the above experiments it was not possible to bring about a shooting response in any of the media tested, further experiments were designed using MS modified medium (1965) supplemented with the growth promoters, Benzyladenine (BA), Kinetin (Kn) and adenine. In a similar set of experiments B5 or Gamborg et a1. Medium (1968) was employed and this was supplemented with the same growth regulators.

In the earlier experiments since only the nodal and internodal explants had shown a response by way of callus formation, in further experiments only these two explants were used. In MS modified medium supplemented with 5.0 mg/l BA + 1.0 mg/l Kn, 2-3 shoots were formed and 50% single shoots were formed. Because of the poor multiple shooting responses as compared to single shooting seen in the above noted experiments; it was decided to introduce slight modification in the growth media.

MS modified medium was then supplemented with growth regulators in a variety of combination were used viz. MS modified with 5.0 BA + 1.0 Kn mg/l, MS modified with 5.0 BA + 0.5 Kn mg/l ,MS modified with 5.0 BA + 1.0 Ad mg/l ,MS modified with 5.0 BA + 0.5 Ad mg/l ,Single auxins such as Indole-3-acetic acid (IAA), Indole-3 -butyric acid (IBA) and Naphthalene acetic acid (NAA) ranging from 0.2 to 0.8 mg/l nad compared with control. MS modified with 0.5 mg/l IBA + 5.0 mg/l BAP ,MS modified with 0.5 mg/l NAA + 5.0 mg/l Zeatin,MS modified with 0.5 mg// IAA + 5.0 mg/l Kinetin.

Establishment of callus cultures: For development of callus, also MS modified medium (1965) was used. The pH of the medium was adjusted to 5.7 with 1N NaOH or 1N HCL as required, before autoclaving. The MS modified medium (1965) was supplemented with the necessary hormones (2,4-D 3.0 mg/l and Kinetin 0.5 mg/l), the B5 medium (1968) and the MS modified medium (1965) was supplemented with 2,4-D of 3ppm, 5ppm and 7ppm concentration with coconut water, the Nitsch and Nitsch medium was supplemented with 2,4,5-T 2ppm, 4ppm, 5ppm, 7ppm concentration. Approximately 20 ml of medium was dispensed in each tube (length 15 cm; diameter 2 cm). The tubes were closed with autoclavable caps, autoclaved at a pressure of 15Kpa for 20 minutes. The tubes were kept tilted to prepare slants. The cultures were incubated at 25°C with a 16h light and 8h dark photoperiod using Gro-Lux fluorescent tubes. The relative humidity was maintained at 50-60%.

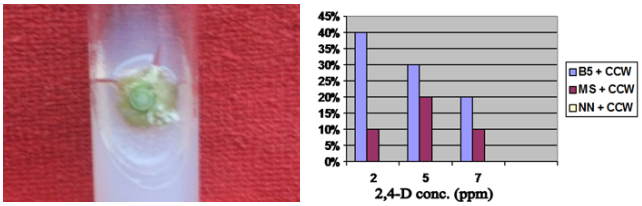
To avoid the browning of callus a variety of methods were tested. Initially, cotton and filter paper strips soaked in liquid medium were used as a bridge with the explants placed upon it so as to avoid its direct contact with the growth medium. Since this did not prove successful another different set up in which either activated charcoal or a combination of citric acid and ascorbic acid was also attempted, but this treatment was found to be lethal. But later PolyVinylPyrrolidine at concentration of 0.1% and 0.01 % was used in the medium, out of which 0.01 % PVP helped to reduce the browning of explants, but still explants were getting brown to black. Since none of the above methods were found to be suitable, in the present study therefore the callus cultures were subcultured every 15 days as against the usual period of 28-30 days. Thus, after every three sub-cultures spanning over 45 days, the callus exhibited considerably less browning. Each experiment was set up in five sets, each set containing 10 tubes.

Results and Conclusion

Effect of media variation: Nodal and internodal explants excised from *T. cordifolia* were screened on different media such as: MS original medium (1962), MS modified medium (1965), B5 or Gamborg et al. medium (1968) and Nitsch and Nitsch medium (1969).

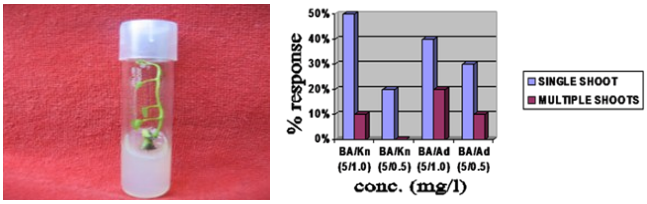
Slight swelling of the explants was observed in response to 2,4-D 3ppm, 5ppm and 7ppm with coconut water when used in MS modified medium, whereas same concentration of 2,4-D was supplemented in B5 medium showed callus response as compared to MS and N&N medium (Fig.1a, b). Nitsch and Nitsch medium supplemented with 2, 4, 5- D showed callus response, but started browning within 15 days.

Nodal and internodal explants showed very little shooting in B5 and N&N medium as compared to MS modified medium supplemented with growth hormones. Explants showed 5-6 multiple shoots in response to 5.0 BA + 1.0 Ad mg/l in MS modified medium. More number of single shoots was observed III response to 5.0 BA + 1.0 Kn mg/l (Fig. 2 a, b).



(a) (b)

Fig. 1 (a, b)- Response of explants to different media (M5, B5 AND N & N) supplemented with single plant growth hormone (Auxin) in terms of callus formation in %.

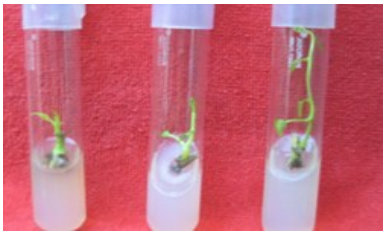


(a) (b)

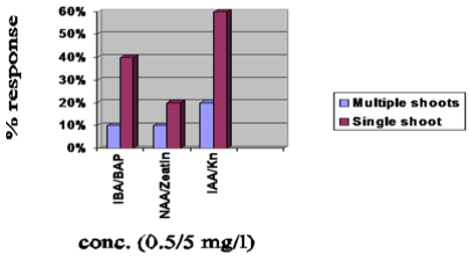
Fig. 2 (a, b)- Response of explants in MS medium supplemented with combination of growth hormones (Auxins and Cytokinins) in terms of multiple shoots and single shoot formation.

In the MS modified medium supplemented with different PGR's were also used. Medium supplemented with IAA/Kinetin 0.5/5.0 mg/l showed good response of multiple shoots as well as single shoots. Medium with IBAIBAP 0.5/5.0 mg/l and NAA/Zeatin 0.5/5.0 mg/l showed less response of multiple and single shoots (Fig. 3 a, b).

Among the four media tested, the best response in terms of multiple shooting, callusing as well as rooting was observed in MS modified medium with appropriate growth hormones. Response to different PGR's: As mentioned above since the explants showed the best response when cultured in MS modified medium (1965), in all further experiments the effect of different PGR's was tested using this medium. Further, only nodal and internodal explants of *T.cordifolia* were used since other explants failed to produce any response in earlier experiments.



(a)



(b)

Fig. 3 (a,b)- Response of explants to synergistic effect of plant growth hormones in terms of multiple shoot formation (%)

Effect of IAA- The response of nodal and internodal explants to the presence of IAA alone in the growth medium was tested at four concentrations along with control 0.2, 0.4, 0.6 and 0.8 mg/l. While there was more response of rooting in 0.2 and 0.8 mg/l concentration, whereas in other concentration there was less response of rooting. In 0.8 mg/l concentration slight callus was also observed (Fig. 4 a, b).

Effect of IBA- Nodal and internodal explants of T. cordifolia were cultured in the same medium but supplemented with NAA singly as control 0.2, 0.4, 0.6 and 0.8 mg/1. In media with 0.4 and 0.6 mg/1 concentration showed more rooting response and also in 0.4 mg/l concentration slight swelling of explants as well as shoot was observed. Shooting was also in the media without IBA, which is a control (Fig. 5 a, b).

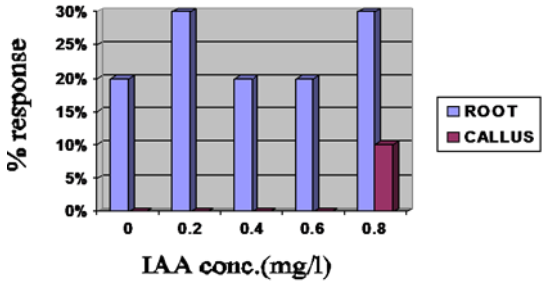
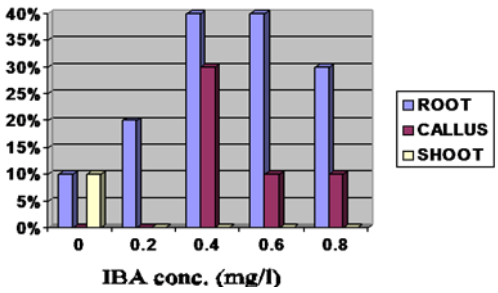


Fig. 4- Response of explants to single plant, growth hormone supplemented with different concentration. (%).

(a)

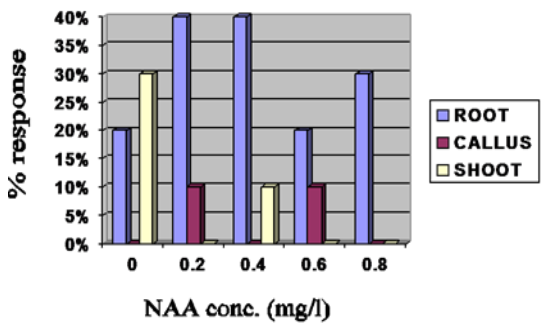


(b)

Fig. 5 (a, b)- Response of explants to single plant growth hormone (IBA) supplemented with different concentration (in %)

Effect of NAA- The auxin NAA was used singly at concentration 0.2, 0.4, 0.6 and 0.8 mg/l and with control. Slight callusing was observed in response to 0.2 and 0.6 mg/l in the medium. But at 0.2 mg/l and 0.4 mg/l concentration rooting response was more than any other concentration in the medium. Medium without NAA showed good single shooting and rooting (Fig. 6 a, b).

(a)



(b)

Fig. 6 (a, b)- Response of explants to single plant growth (NAA) hormone supplemented with different concentration (in %).

In summary, the ideal medium for raising in vitro cultures of T. cordifolia in MS modified medium (I965) supplemented with appropriate growth hormones. Multiple shoots could be developed from the nodal and internodal explants in response to the presence of BA + Kn+ Ad in the growth medium. However the later combination of growth hormones (0.5 mg/l IAA + 5.0 rng/l Kinetin) appeared to be somewhat more effective. NAA and IBA were apparently more suitable than IAA for inducing rooting response. While both the nodal and internodal explants developed roots in response to NAA (0.2 mg/l & 0.4 mg/l) treatment, only the internodal explants, responded to IAA treatment.

For callus formation a growth hormone 2, 4-D 3ppm supplemented in B5 medium proved to be ideal. In fact, a considerable amount of callus was formed within a short period of 18 days.

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