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In vitro organogenesis from *Tinospora cordifolia* (Willd.) Miers — A highly valuable medicinal plant

K. Mridula, S. Parthibhan, T. Senthil Kumar, M.V. Rao *

Department of Botany, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India

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ABSTRACT

Efficient *in vitro* regeneration protocols were established for *Tinospora cordifolia* through direct and indirect organogenesis, using cotyledon (C), young leaf (YL) and mature leaf (ML) explants. Highest response of 97.9–100.0% organogenic callus was induced on Murashige and Skoog (MS) medium containing indole-3-acetic acid (IAA) at 2.0 mg/L. Morphology of the callus varied from yellow friable to compact on auxin treatments. Shoot bud induction from callus was rapid on modified MS medium (mMS) containing IAA or 1-naphthalene acetic acid (NAA) with 6-benzyladenine (BA), kinetin (KN) and ascorbic acid (AA). Among the explants, cotyledons produced the highest shoot number (24.1 shoots) followed by YL (19.0) and ML (16.1) explants. Direct organogenesis was better on C than YL explant. Highest of 14.5 and 11.0 shoots were achieved on BA, KN, AA and IAA (0.5 mg/L) from C and YL explants, respectively. Best shoot length of 8.3 cm was achieved on MS medium containing gibberellic acid (GA_3) at 0.5 mg/L. All the shoots were rooted on MS medium at half strength macro salts (½ MS) with indole-3-butyric acid (IBA) 0.5 mg/L and NAA 0.5 mg/L. Rooted plantlets were successfully hardened under *in vitro* conditions and transferred to the glasshouse with 75% survival rate. This is the first report on successful organogenesis *via* callus and from different explants in *T. cordifolia*. The same protocols using different medium and plant growth regulators (PGRs) at different stages of organogenesis can be utilized for mass production to aid commercial needs and eco-restoration of this plant and to the related genera.

1. Introduction

The family Menispermaceae also known as the moonseed family consists of about 500 species under 70 genera (Ortiz et al., 2007). Several members of the family are known for their medicinal and toxic components such as, D-tubocurarine (a muscle relaxant), cycleanine (medicine for malaria), acetylcholinesterase inhibitors (potential drugs for Alzheimer's disease), curare (poison), *etc.* (Jahan et al., 2010). Some other species such as, *Cocculus carolinus*, *Cissampeols pareira*, and *Fibraurea tinctoria*, are valued for their ornamental, industrial and other economic values.

The genus *Tinospora* consists of 34 twining species from tropical regions, of which *Tinospora cordifolia* (Willd.) Miers is a large glabrous climbing shrub found throughout the tropical India ascending up to an altitude of 300 m (Fig. 1a). It is well known in Southern India by the following dialects as 'amrita' in Sanskrit and Hindi, 'amudam or chindle' in Tamil, 'tippeteege' in Telugu, 'amrutaballi' in Kannada and 'amrtyu' in Malayalam. The mature stem and roots of *T. cordifolia* are highly important in Ayurveda and tribal medicines to prepare Amrtaristam, Dhanvantharam tailam and Cheriya rasnadi kasayam. It is used to cure diabetes, fever, jaundice, diabetics, respiratory disorders, neurological disorders, rheumatism, and to help in functions of various organs (Albert, 2012; Sharma et al., 2015). Diverse bioactive components under the classes of alkaloids, diterpenoid, lactose, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds and polysaccharides are identified in *T. cordifolia* (Singh et al., 2003; Musliarakathbacker and Azadi, 2004).

This plant has been overexploited by the pharmaceutical companies and native people for traditional remedies. Nearly 90% of the raw materials of medicinal plants are acquired from the natural forests by drug manufacturing companies in India. Of around the 960 species of traded medicinal plants, 178 species are consumed over 100 metric tons annually (Sen and Chakraborty, 2017). The total annual requirement of raw materials is about >2000 tons, of which the demand for *T. cordifolia* was alone estimated to be 191.64 tons *per annum* for the period 2006–2011, with a steady growth rate of 5.70% *per annum* (Albert, 2012; Sen and Chakraborty, 2017). The increasing demand for the natural medicinal raw materials results in severe scarcity of the wild medicinal plants including *T. cordifolia*. Owing to its high demand, *T. cordifolia* has been listed among the highly prioritized medicinal plants in the agro-climatic zones of Rajasthan, Uttar Pradesh and Madhya Pradesh in India by the National Medicinal Plant Board, New Delhi.

Conventional propagation methods by seed germination and stem cuttings are hampered in this plant, due to poor seed viability, low germination and high susceptibility to infections. Alternatively, *in vitro*

* Corresponding author.
E-mail address: mvrao_456@yahoo.co.in (M.V. Rao).

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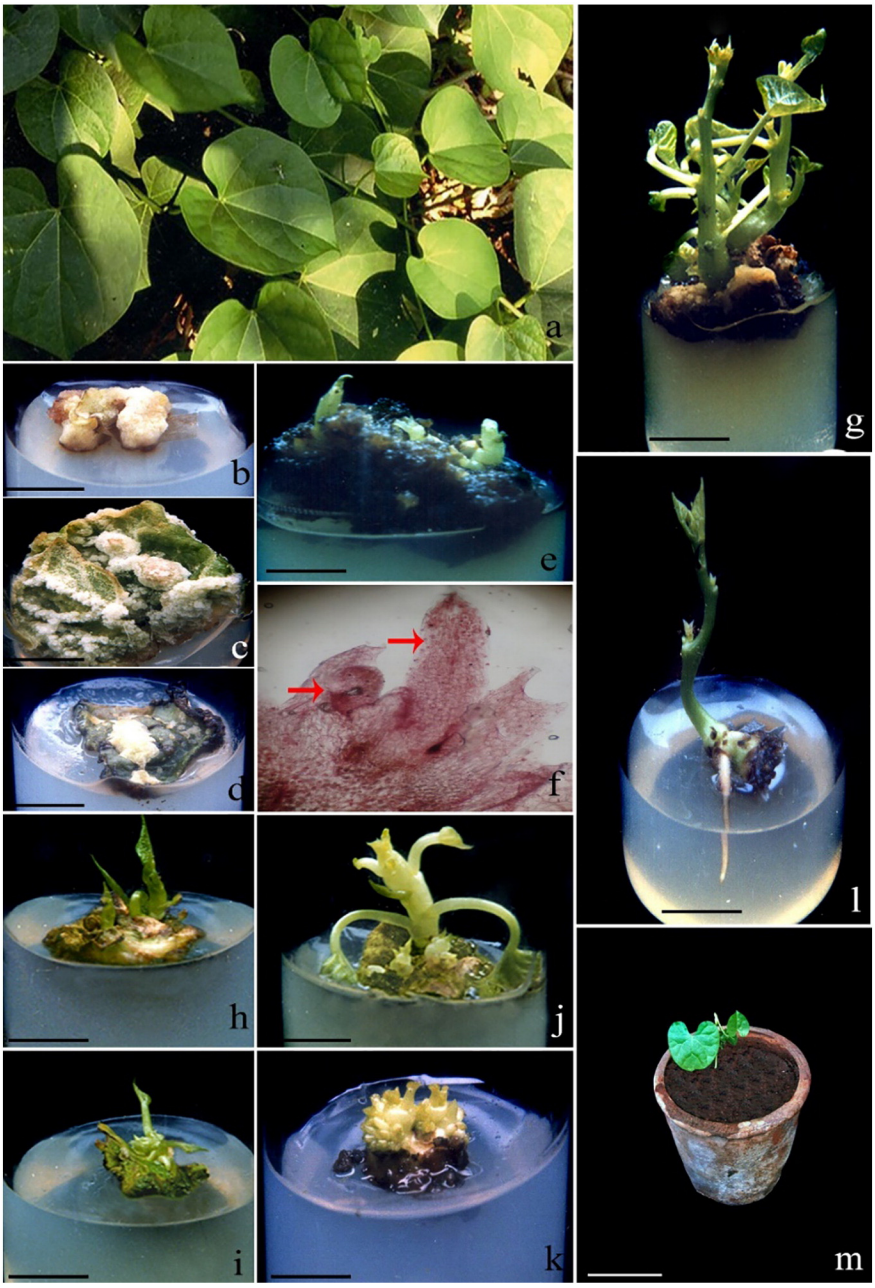

Fig. 1. Indirect and direct organogenesis from cotyledon (C), young leaf (YL) and mature leaf (ML) explants of *Tinospora cordifolia*. a. Plant; b–d. Callus induction from C, YL and ML explants, respectively; e. Callus proliferation from YL explant; f. Hand-made section of callus showing shoot bud induction; g. Shoot bud induction and multiplication from C explant; h & i. Direct multiple shoot bud induction from C and YL explants; j & k. Multiple shoot proliferation from C and YL explants; l. Elongated shoot rooted of shoot; m. Hardened plantlet. Bars: b–d, g, h–j = 10 mm; e, f, k, l = 8 mm; m = 15 cm.

propagation methods through direct and callus mediated shoot induction can produce plantlets in large quantities with true to the type and healthy stocks in a short span of time. Moreover, the standardization of callus production and cell culture protocols could be an effective alternative for the bioactive compound production and minimize the pressure on the wild population. The present study was aimed to develop efficient reproducible protocols for mass multiplication on *T. cordifolia via.* callus and direct organogenesis.

2. Materials and methods

2.1. Explant source and sterilization

Young and mature leaves (YL and ML) and internodal (IN) segments collected from 3-years-old field grown plants were used as explants. Cotyledons (C) were obtained from seedlings germinated *in vitro* on MS basal medium at full strength. The *in vivo* plant segments were washed under running tap water for 3 min with few drops of soap solution (Teepol, India) and then surface sterilized with 70% alcohol for 30 s, followed by 0.1% (*w*/*v*) $HgCl_2$ for 2–3 min (based on explant type), and finally washed with sterile distilled water for 3–5 times. The sterilized explants were prepared by cut into pieces of 1.0–1.5 cm in size and slightly wounded. All the explants were cultured horizontally by placing the wounds in contact with the medium.

2.2. Basal media and culture conditions

Murashige and Skoog (1962) medium at full macro strength (MS), half macro strength (½ MS) and modified with B5 vitamins (mMS)