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Review Article

MICROPROPAGATION PROTOCOLS OF *TERMINALIA BELLIRICA* ROXB.

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ABSTRACT
Terminalia bellirica Roxb. is one the important medicinal plant, which needs to be propagated using in vitro techniques. This plant has a number of useful secondary metabolites like tannins, alkaloids, flavonoids, lignans etc., which can be used in treating a number of aliments. It is having a number of applications as antihelmintic, anti-inflammatory, antipyretic, antimicrobial agent. So with such significance there is a need to concentrate on the proper propagation of this plant. A review was attempt with micropropagation protocols, useful for the research personals.

Keywords: *Pharmacological activities, micropropagation, Terminalia bellirica*

INTRODUCTION
Terminalia bellirica Roxb. is a tall tree growing up to 37m above belongs to family Combretaceae. It has medicinal importance and often used in Ayurveda, Siddha and Unani preparations. It blossom in the moth of May and its kernels are sweet and narcotic. It have a number of medicinal properties like antifungal, antidiabetic, laxative, antihypertension, antipyretic, hypolipidimic, antihelmintic, anti-inflammatory cardiac disorders, skin diseases and antiemetic (Saha *et al.,* 2011; Saraswathi *et al.,* 2012). These secondary metabolites are present in the bark, leaf, fruits and seeds. All these properties are due to the presence of the secondary metabolites like glycosides, gallic acid, lignans, alkaloids, terpeinoids, flavonoids, tannins, ellagic acid, chebulagic acid, β sitoserol etc., (Saraphanchotiwitthaya *et al.,* 2008). World Health Organization also estimated that the more than 80% of developing and under developed countries depend on the medicinal plants for their treatments (Sharma *et al.,* 2005). Pharmacological properties of this plant will help the researchers to undertake the further advanced studies. So in the present paper the main focus is on its medicinal significance and its applications in the health of humans.

Phytochemical studies and chemical constituents
Phytochemical studies of this plant revealed the presence of many secondary metabolites with methanol, ethanol, acetone, ethylacetate, chloroform, petroleum ether and water extracts. Phytochemical constituents present in the bark, leaf, and fruit are tannins, gallic and ellagic acid, glucoside, gallotannic acid, resins, ellagic acid, lignins like, thannilignan, flavones, tannins, ellagic acid, chebulagic acid, phyllemblin, belleric acid, bellericosides, terpenoids, methyl gallate, luteolin, quercetine, kaempferol, proteins, carbohydrates, flavonoids, sitosterols, mannitol, galactopyranose, inositol, quinic acid etc. Phenolic substances have antioxidant nature and can be better extracted with the alcoholic solvents (Hazra *et al.,* 2010). Gallic acid is an antioxidant with anticancer activities and used in number of phytomedicines (Kaur *et al.,* 2015). Quinic acid is having nutritional efficiency and also has antioxidant activity (Badhani *et al.,* 2015). Anti-salmonella activity was stressed by Madani and Jain (2008). Antimicrobial activity of the fruit extract was also carried out by researchers like Devi *et al.,* (2014). Tannins might have involved in the protein synthesis inhibition. Phenols might have role in the fungus inhibition by leakage and wrinkling of the hyphae (Hung and Chung, 2003). *T. bellirica* extracts are having anti analgesic activities (Khan and Gilani, 2008; Sharma *et al.,* 2010). Sharma *et al.,* (2010) has used albino rats for their study using acetic acid induced writhing and hot plate test method in mice using the ethanolic fruit extracts. They have reported that acetic acid induced writhing was associated with

increased level of Prostaglandins and lipoxygenase products (Franzotti *et al.,* 2002). Flavonoids are thought to play a role in the inhibition of prostaglandin synthesis (Ramaswamy *et al.,* 1985). NSAIDS are involved in the inhibition of prostaglandins (Zeal, 1975).

In the present review we have given the complete plant tissue work details done till now, because of its medicinal significance there is the need to propagate it under in vitro conditions. Some of the researchers have attempted for the micropropagation of this plant.

Micropropagation Protocols

Sterilization of the explants: First attempt of micropropagation was done by Ramesh *et al.,* (2005). They used seeds and grown them under the green house conditions and taken the nodal segments for propagation. They have washed these under tap water for 30 min and then treated with 0.1% $HgCl_2$ for 5 min, followed by rinsing for 4 times in sterile water. The seedling nodes were later inoculated on to MS medium (Murashige and Skoog, 1962). Medium containing 13.3µM BA (6-benzyladenine) showed the highest shoot length (1.9±0.2cm) in the primary culture. Percentage and average shoot lengths differed with Kn (Kinetin) (18.6 µM) and BA (13.3µM). When tested individually, even though the number of shoots formed was same in both the concentrations. When subcultured on to the fresh medium containing BA (6.6 µM) shoot number was increased to 3.1±0.1. For rooting of the shoots they have placed the shoots of mean length 2.9 on two media tested, modified Gamborg's (B5) medium (Gamborg *et al.,* 1968) or Woody Plant Medium (WPM) (Lloyd and Mc Cown, 1980), both supplemented with 4.9 µM indole-3-butyric acid (IBA) among these WPM containing medium produced 1.9±0.9 roots.

Rathore and his coworkers (2008) attempted for the in vitro propagation of seedling explants. They sterilized with 0.15% $HgCl_2$ for 15 min and rinsed 5-6 times with sterilized distill water. They have reported BAP (6-benzylaminopurine) 1.5mg/l containing MS medium best for culture initiation. The number of shoots increased with sub culturing on to the same medium after every 21 days, with a maximum of 10.6 shoots. IBA (0.1mg/l) alone initiated better rooting and these plantlets were acclimatized in the mist house.

Suthar *et al.,* (2009) attempted for the shoot multiplication using the cotyledonary nodes and epicotyledonary node explants obtained from seedling (15 days). They sterilized using 0.15% w/v $HgCl_2$ for 10 min and washed with sterilized water 4-5 times. They cultured these explants on SH medium (Schenk and Hildebrandt, 1972) with 1.5mg/l BAP, later they transferred these shoots after 21 days on to MS medium with same concentration of BAP. The shoot developed were 7.7 per cluster. They stressed the role of CO_2 enriched environment ($40gm^3$) and sucrose free medium for obtaining the shoots.

Sahu and Koche (2013), used nodal explants of mature tree and washed with 0.2% lebolin followed by treatment with $HgCl_2$ (0.2% w/v). Later they placed these shoots on the MS medium containing BAP (0.5mg/l) with 3.0±0.42 shoots. Then they transferred micro nodes of the shoots elongated on explants containing BAP (0.25mg/l) + NAA (0.25mg/l) (α-naphthaleneacetic acid), where the shoot number increased to 6.5±0.62. For rooting they used 2-3 cm containing micro shoots and given pulse treatment. When the shoots were placed in the 1/2MS basal medium containing 0.25 mg/l IBA for 72 h. Number of roots obtained were 2.2±0.54. Rooted shoots were washed and placed in soilrite or sand soil mixture and growth in the green house during acclimatization.

Dangi *et al.,* (2014) attempted for the micropropagation using the nodes of mature tree. They have washed with 20% (v/v) Extran and treated with HgCl2 (0.1% w/v) for 7 min finally rinsed with distilled water thrice. They used MS medium containing BAP (8.8 µM) and reported 2.5± 0.2 shoots. Later transferred the shoots on to BAP (8.8 µM) + NAA (2.6 µM) in addition to antioxidants. For shoot elongation used BA (4.4 µM) + Phloroglucinol (PG) (3.9 µM). For rooting they used IBA (2.5 µM). They have also attempted for the genetic fidelity test using intersimple sequence repeats (ISSR) and randomly amplified polymorphic DNA (RAPD) markers. They have reported the regenerated plantlets to be true to type clones. They have use three different antioxidants like PVP (polyvinylpyrrolidone) (50mg/l), activate charcoal (500mg/l) and ascorbic acid (50mg/l) alone or in combinations to prevent phenolic oxidation.

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