International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 28-07-2021, Accepted: 06-08-2021, Published: 11-08-2021
Volume 6, Issue 4, 2021, Page No. 648-650

Efficient plant regeneration of *Terminalia bellirica*–A multipurpose woody medicinal plant

Pratima Rani Sardar
Department of Botany, Shivaji College, University of Delhi, New Delhi, India

Abstract
A high efficacy *in vitro* protocol for shoot regeneration was developed for Terminalia bellirica Roxb. - a valuable medicinal plant commonly known as Bahera. The influence of different media and cytokinin concentration had been assessed on the shoot production potential of the nodal explants derived from 30 d old seedlings. Among the various basal media tried, Murashige and Skoog's (MS) basal medium exhibited with a maximum average of 0.81±0.19 shoots per explant with an average shoot length of 0.58±0.12 cm. MS medium augmented with 10 µM N^6 Benzyladenine was the most effective in inducing multiple shoot proliferation in cent percent cultures with an average of 19.92±0.77 shoots per explant. High frequency (70.83 %) *in vitro* rooting was obtained on half strength MS + 15 µM NAA. Most of the plantlets survived in the field after gradual hardening in Soilrite and acclimatization in soil.

Keywords: *Terminalia bellirica*, N^6- benzyladenine, nodal explants, α-napthaleneacetic acid

Introduction
Terminalia bellirica known as Bahera is an important woody medicinal tree of Indian subcontinent. This species is distributed throughout the greater part of India, especially deciduous forests but not in the arid regions (Anonymous, 1992; Bhattacharjee *et al*., 2000) [1-2].
The fruit is used in Ayurvedic and Unani systems of medicine. It is given also as a brain tonic and is applied in cases of opthalmia. Besides it is useful in high blood pressure, piles, leprosy, diarrhoea, biliousness, headache and fever. The half-ripe fruit is considered to be purgative but the ripe fruit has the opposite property. The beleric fruit is one of the three constituents of the famous Indian preparation Triphala (Nadkarni, 1998) [3]. Leaves are used externally as a cover for sores and ulcers (Joshi, 2000) [4].
In vitro techniques would be beneficial in accelerating large scale propagation under pathogen free conditions and improvement of the plant using genetic engineering. Limited tissue culture work has been carried out on *Terminalia* species (Shyamal *et al*., 1988; Ramesh *et al*., 2005) [5-6]. The present investigation is the first report on *in vitro* propagation of *Terminalia bellirica* through 30 days old seedling derived explants and the multiplication rate achieved is high.

Materials and Methods
Plant Material
Mature seeds of *Terminalia bellirica* were procured from Madhya Pradesh, India. Thorough washing of seeds was done under running tap water for 20-25 min, and then soaked for overnight in water. The seeds were surface sterilized with 0.1% HgCl2 solution for 5 -10 min and then rinsed 4 or 5 times with sterile distilled water to inoculate. Seedlings 30 day old thus raised were employed as a source of explants.

Culture medium and culture conditions
Initially, four basal media namely MS (Murashige and Skoog 1962) [7]; Knop's; B5 (Gamborg *et al*., 1968) [8] and WPM (Lloyd and McCown 1980) [9] were employed to rear the seeds. For subsequent experiment five different basal media *viz*. MS, B5, SH (Schenk and Hilderbrandt 1972) [10], WPM and Knop's were used either alone or supplemented with N6-benzyladenine (BA).
The media were gelled with 0.8% agar (*Qualigens Fine Chemicals*, Mumbai, India) and 3% (w/v) sucrose (*DCM*, Daurala) and its pH were fixed to 5.8 using 0.1 M NaOH or 0.1 M HCl before autoclaving. Usually, 25 ml molten medium was poured in each culture tube 25x 150 mm (*Borosil Glass Works*, Mumbai, India) then plugged the test tube open end with non-absorbent cotton and sterilized these at 1.06 kg/cm2 pressure for 15 min.
All culture were incubated at 25±2° C, under uninterrupted light with a photosynthetic photo flux of approximately 450-460 µ W m^{-2} emitted from cool fluorescent tubes (Philips *India Ltd*., Kolkata, India).

Shoot regeneration
Primary shoots formed *in vitro* were cut into one-node pieces after excising the leaves. The nodal explants containing the axillary buds were cultured on MS supplemented with different concentration of N^6-benzyladenine, subsequent subcultures were done at 30d intervals.

Rooting of shoots and plant acclimatization
For rooting, the regenerated shoots (3-3.5 cm in length) were cultured on half strength MS medium with 2% (w/v) sucrose either alone or supplemented with 0.5- 10 µM NAA (α-naphthaleneacetic acid).
The plantlets were removed from the semisolid medium, washed thoroughly with autoclaved water and dipped for 15 min in 0.1% (w/v) *Bavistin* (BASF, India Ltd; Mumbai). In the beginning they were transplanted in plastic pots with Soilrite for one month. The pots were covered with polythene sheets and maintained under controlled conditions (450-460 W cm^{-2}, 25±2° C, 55-65% RH). The plantlets were irrigated with 1/4th strength MS salt solution without sucrose

for one week and then with tap water. Later on, they were transferred to soil.

Observation of culture and data analysis

Cultures were observed on visual basis and using stereobinocular. The result presented are the mean of 2 replicates comprising 24 explants ± standard error (SE).

Results and Discussion

Influence of Medium

The nodal explants were inoculated on different media *viz.* MS, Knops, B5, SH and WPM on which shoot buds were induced within 10-12 days. In next 10 days these buds were differentiated into shoots and in addition simultaneous callus formation was noticed at lower cut end of the explant. A maximum of 43.75 % cultures differentiated shoots on MS basal medium with and maximum average of 0.81± 0.19 shoots per explant with an average shoot length of 0.58 ±0.12 cm. Furthermore, the shoots on MS basal medium appear to healthier, in contrast, the shoots on Knop's medium. The B5 and SH media showed some response only in terms of percentage responding but less in comparison to MS. Therefore, MS was found to be the best basal medium for shoot formation in *T. bellirica* and the superiority of MS due to high salt requirements of concentration over other salt formulation has been demonstrated in many other medicinal taxa such as *Dalbergia latifolia* (Swamy *et al.*, 1992) [11], *Pittosporum napulensis* (Dhar *et al.*,2000) [12].

Table 1: Morphogenic response of nodal explants of *T. bellerica* on five different basal media. Data were recorded after 30 Days.

Basal Media	% Explants developing shoots	Average number of shoots per explant	Average* shoot length
MS	43.75^{a}	0.81±0.19^{a}	0.58±0.12 ^{a}
B5	37.50^{b}	0.68±0.19^{b}	0.50±0.11 ^{ab}
SH	31.25^{c}	0.56±0.18^{c}	0.43±0.10 ^{b}
WPM	22.92^{cd}	0.41±0.16^{cd}	0.41± 0.10^{b}
Knop's	14.58^{d}	0.27±0.13^{d}	0.34±0.09^{c}

*Means±SE, n=24. Values in column followed by the same superscript are not significantly different as determined by SAS at p<0.05.

Influence of different concentration of N6-benzyladenine

Nodal explants were cultured on MS Medium supplemented with various concentrations (0.5, 1, 5, 10 and 20 µM) of BA**.** MS basal medium without phytohormones did not exhibited significant multiple shoot production**.** Nevertheless, application of BA to the MS medium facilitated significant shoot formation. The percentage responding cultures and the average shoot number increased with increase concentration of BA up to 10 µM. Among the various treatment tried, the best organogenic response (100%) in nodal explants was achieved on 10 µM BA, which exhibited maximum average of 19.92±0.77 shoots per explant (Figure 1). However average maximum shoot length (1.34±0.49 cm) of adventitious shoot was obtained on 1 µM BA. Besides, at higher concentration of BA, (20 µM) shoots become stunted due to shorter internodes. It was also noted that the optimum level of BA (10 µM) exhibited maximum shoot formation indirectly through callus and less through axil of explant.

Copious calluses were also formed at the bases of nodal explants on all BA concentrations tried. Similar observations were also found in other medicinal plants like *Cassia angustifolia* (Agrawal and Sardar 2003) [13] and *Withania somnifera* (Rani and Grover 1999) [14].

Table 2: Morphogenic response of nodal explants of *T. bellirica* on MS medium augmented with BA. Data were recorded after 30 Days.

BA (µM)	% Explants developing shoots	Average* number of shoots per explant	Average* shoot length (cm)
0.5	50.00^{c}	2.96±0.67^{d}	0.86±0.34^{b}
1.0	72.92^{b}	7.96±1.04^{c}	1.34±0.49^{a}
5.0	77.08^{b}	14.90±1.76^{b}	1.15±0.52^{ab}
10.0	100.00^{a}	19.92±0.77^{a}	0.88±0.18^{b}
20.0	72.92^{b}	6.98±0.90 ^{c}	0.71±0.24^{b}

*Mean±SE.n=24 Values in colomn followed by the same superscript are not significantly different as determined by SAS at p<0.05.

Fig 1: Morphogenic response *of Terminalia bellirica* nodal explants. (a) Single axillary shoot developed on basal medium after 30 d of culture. (b) Differentiation of multiple shoots on 10 µM BA after 4 weeks of inoculation.

Rooting of *in vitro* raised shoots and transfer to field

The ultimate success of micropropagation depends on the rooting percentage and survival of the plantlets in field conditions. For rooting regenerated shoots (2 cm) were transferred to half strength MS medium containing different concentration of NAA. A maximum of 70.83% *in vitro* rooting was observed on half strength MS + 15 µM NAA.

The plantlets were taken out after a month from the test tubes. Their roots were thoroughly washed with water and remove any traces of agar adhering to their surface. After this they were transferred to jars containing sterilized *Soilrite* for acclimatization. Since the plant grown in cultures lack a well-developed epicuticular wax layer (Sutter and Langhans 1979; Brainerd) [15-16], so one of the important requirement of transplantation stage is to maintain a high humidity around the plants at the initial stage. When transferred to field these plantlets survived and their morphological characters are similar to those of wild plants.

Acknowledgements
The author is thankful to Prof. Veena Agrawal for her guidance and support.

References
1. Anonymous. The Wealth of India, Raw Materials, CSIR, New Delhi,1992:3:354-363.
2. Bhattacharjee SK. Handbook of Medicinal Plants. Pointer Pub, 2000, 75-76.
3. Nadkarni KM. Indian Plants and drugs with their medicinal properties and uses, Asiatic Pub. House, Delhi, 1998, 392-395.
4. Joshi SG. Medicinal Plants. Oxford & IBH Pub. Co. Pvt Ltd, 2000, 142-143.
5. Shyamal KR, Prabir KP, Assish KD. Propagation of a timber tree, *Terminalia bellirica* Roxb. By tissue culture. Bangladesh J. Bot,1988:16:125-130.
6. Ramesh M, Umate P, Rao V, Sadan. Micropropagation of *Terminalia bellirica* Roxb. - A sericulture and medicinal plant. *In Vitro* Cell. Dev. Biol. – Plant,2005:41:320-323.
7. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant,1962:15:473-497.
8. Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res,1968:50:151-158.
9. Lloyd G, McCown B. Commercially feasible micropropogation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Proc. Int. Pl. Prop. Soc,1980:30:421-427.
10. Schenk RU, Hilderbrandt AC. Medium & techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot,1972:50:199-204.
11. Swamy BVR, Himabindu K, Sita GL. *In vitro* micropropagation of elite rosewood (*Dalbergia latifolia* Roxb.). Pl. Cell. Rep,1992:11:126-131.
12. Dhar U, Upreti J, Bhatt ID. Micropropgation of *Pittosporum napaulensis* (DC) Rehder and Wilson-a rare endemic Himalayan medicinal tree. Pl. Cell. Tiss. Org. Cult,2000:63:231-235.
13. Agrawal V, Sardar PR. *In vitro* organogenesis and histomorphological investigations in Senna (*Cassia angustifolia*) - a medicinally valuable shrub. Physiol. Mol. Biol. Plants,2003:9:131-140.
14. Rani G, Grover IS. *In vitro* callus induction and regeneration studies in *Withania somnifera*. Pl. Cell. Tiss. Org. Cult,1999:57:23-27.
15. Sutter E, Langhans RW. Epicuticular wax formation on carnation plantlets regenerated from shoot tip culture. J. Amer. Soc. Hort. Sci,1979:104:493-496.
16. Brainerd KEIH, Fuchigami S, Kwaitkowski, Clark CS. Leaf anatomy & water stress of aseptically cultured “Pixy” plum grown under different environments. Hort. Sci,1981:16:173-175.