

## ***In vitro* Micropropagation of *Boswellia ovalifoliolata***

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A protocol for micropropagation of *Boswellia ovalifoliolata* Bal & Henry (Burseraceae) was developed using cotyledonary nodal explant on Murashige and Skoog modified medium (MS). A comparative study of micropropagation with 6-benzyladenine, kinetin and thidiazuron along with 1-naphthalene acetic acid (0.054  $\mu\text{M}$ ) was conducted. The highest shoot multiplication (7.1  $\pm$  0.2 shoots per node) was achieved in 50 d on MS supplemented with thidiazuron (2.72  $\mu\text{M}$ ). Excised shoot cuttings of 3.0 cm were placed on the MS basal medium supplemented with indole-3-acetic acid and indole-3-butyric acid alone and in combinations for rooting. Activated charcoal (100 mg l<sup>-1</sup>) and polyvinylpyrrolidone (40 mg l<sup>-1</sup>) were added to the medium to prevent browning of cultures. The regenerated plantlets have been successfully acclimatized and transferred to soil.

**Key words:** *Boswellia*, Cotyledonary, Regeneration

### **Introduction**

*Boswellia ovalifoliolata* Bal and Henry, a member of Burseraceae, is an endemic species (Ahmedullah and Nayar, 1986), which occurs at an altitudinal range of 250–600 m on Seshachalam hill ranges of Palakonda region of Eastern Ghats of India. It is a medium-sized tree and differs morphologically from the widely occurring *B. serrata* (Balakrishnan and Henry, 1961; Gamble, 1967). The extract of this plant is used for rheumatic pains by folklore and wood of this species is specially used in toy making industry besides its commercial importance as aromatic resin yielding

plant. Recently Reddy *et al.* (2003) found two new macrocyclic diaryl ether heptanoids, ovalifoliolatin A and B, in this species which show antibacterial activity. Conventional propagation is beset with problems like poor seed viability; tree species even otherwise are difficult to propagate through cuttings. *In vitro* techniques are therefore exploited for the conservation of valuable plants especially those, which are difficult to propagate by conventional means. Micropropagation of *B. serrata* has already been reported (Purohit *et al.*, 1995), but not *in vitro* propagation of *B. ovalifoliolata*. Based on medicinal and commercial importance, the present investigation was aimed at developing a suitable protocol for multiplication of this endemic tree *B. ovalifoliolata* using cotyledonary node as explant.

### **Materials and Methods**

Seeds of *Boswellia ovalifoliolata* were collected during April and May from a small population of trees and treated with 30% H<sub>2</sub>SO<sub>4</sub> for 5 min, washed thoroughly and soaked in distilled water for 1 h. The seeds were then suspended in Teepol for 15 min and washed thoroughly under running tap water. Subsequently they were surface sterilized with 0.1% (w/v) aqueous HgCl<sub>2</sub> for 5 min, agitated and washed with sterile distilled water. Sterilized seeds were inoculated on solidified 1/2 Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) with 3% sucrose. 1 cm segments of cotyledonary nodes were cut from 15-day-old seedlings and used as explant. For shoot proliferation and multiplication the medium was supplemented with either 1-naphthalene acetic acid (NAA) (0.054  $\mu\text{M}$ ) alone or with varying concentrations of 6-benzyladenine (BA) (1.11, 2.22, 4.44 and 8.88  $\mu\text{M}$ ), thidiazuron (TDZ) (1.82, 2.72, 3.63 and 4.54  $\mu\text{M}$ ) and kinetin (KN) (0.46, 1.15, 2.32 and 4.65  $\mu\text{M}$ ) and examined (Table I). Repeated subculturing was done every 25 d on the same medium for further elongation and multiplication.

From proliferated shoots, individual shoots were excised and cultured on MS medium supplemented with either indole-3-acetic acid (IAA) (2.8, 5.7, 11.4 and 17.1  $\mu\text{M}$ ) or indole-3-butyric acid (IBA) (2.4, 4.9, 9.8 and 14.7  $\mu\text{M}$ ) alone or in combination (Table II) for initiation and development of root growth. Antioxidants like charcoal (50 and

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**Abbreviations:** BA, N<sup>6</sup>-benzyladenine; KN, kinetin; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog; NAA, 1-naphthalene acetic acid; PVP, polyvinylpyrrolidone; TDZ, thidiazuron.

100 mg l<sup>-1</sup>) and polyvinylpyrrolidone (PVP) (20 and 40 mg l<sup>-1</sup>) were added to both shoot proliferation and rooting media. All media were supplemented with 3% (w/v) sucrose and pH value was adjusted to 5.8 ± 0.2 before gelling with 0.8% (w/v) agar. The gelled medium was dispensed into 150 mm × 25 mm rimless cultured tubes (15 ml/tube), plugged with non-absorbent cotton and autoclaved at 121 °C for 15 min at 0.103 MPa. The rooted shoots were removed from culture tubes, washed with distilled water and transplanted to 10 cm i.d. small plastic pots filled with autoclaved vermiculate. The containers were kept in a culture room (25 ± 2 °C) and watered with sterile MS salts solution for 20 d. Subsequently, the plantlets were transferred to 20 cm i.d. disposable plastic cups containing a mixture of soil and sand (1:1). For each experiment 20 explants were used and all experiments were repeated thrice. The effects of different treatments were quantified and the data was subjected to statistical analysis using standard error of mean.

## Results and Discussion

The seeds of *Boswellia ovalifoliolata* showed 2% germination. Acid pretreatment was given to augment the process of germination. Gentle acid treatment followed by soaking in distilled water for 1 h at room temperature led to 10% germination. On MS basal medium the explants remained fresh and green for about 10 d but failed to show any response till 25 d and the cultures finally succumbed after dehydration. Addition of low levels

of NAA (0.054 µM) and various concentrations of BA, KN or TDZ resulted in shoot induction. Depending on the concentrations of cytokinin used, the response in shoot induction varied. TDZ with NAA proved most effective for multiple shoot induction and subsequent growth (Table I). Similar results have been observed in various other plants (Lu, 1993).

Three shoots were produced in MS medium supplemented with 4.44 µM BA and 0.054 µM NAA with 70% response after 25 d. At the same time 2.72 µM TDZ along with 0.054 µM NAA resulted five shoots per node with 90% efficiency. Increasing or decreasing concentration of TDZ along with NAA decreased the number of shoots. The potential of TDZ alone and along with NAA in regeneration studies has been proved in a wide range of plants earlier (Faure *et al.*, 1998; Magioli *et al.*, 1998; Malik and Saxena, 1992). Only 50% of the cultures were responded with KN (2.32 µM) along with NAA (0.054 µM) and resulted four shoots after 25 d. With respect to shoot length BA or KN along with NAA was better than TDZ combination (Table I). The regenerated shoots were primarily albino but later turned reddish in around 25 d. Callusing also occurred with various levels of cytokinins. Callus during every subculture showed browning adversely affecting the growth. Adding charcoal and PVP to media could solve this problem. 100 mg l<sup>-1</sup> activated charcoal and 40 mg l<sup>-1</sup> PVP proved best for regeneration. Similar browning was observed in cultures of *B. serrata* (Purohit *et al.*, 1995).

Table I. Effect of NAA, BA, TDZ and KN on shoot multiplication and shoot length of *B. ovalifoliolata*.

Hormones [µM]				Cultures showing response (%)	Mean number of shoots/node ± S.E. 25 d	Mean shoot length [cm] ± S.E. 25 d	Mean number of shoots/node ± S.E. 50 d	Mean shoot length [cm] ± S.E. 50 d
NAA	BA	TDZ	KN					
0.054	1.11	–	–	55.0	2.0 ± 0.5	1.6 ± 0.2	2.5 ± 0.2	2.1 ± 0.1
0.054	2.22	–	–	61.6	2.3 ± 0.4	1.8 ± 0.5	2.4 ± 0.4	2.7 ± 0.2
0.054	4.44	–	–	70.0	3.2 ± 0.4	2.0 ± 0.3	4.8 ± 0.6	3.0 ± 0.2
0.054	8.88	–	–	23.3	2.0 ± 0.1	1.5 ± 0.2	3.0 ± 0.6	2.0 ± 0.7
0.054	–	1.82	–	63.3	3.6 ± 0.2	1.1 ± 0.4	3.8 ± 0.4	1.2 ± 0.4
0.054	–	2.72	–	90.0	5.2 ± 0.3	1.0 ± 0.2	7.1 ± 0.2	1.9 ± 0.1
0.054	–	3.63	–	76.6	5.0 ± 0.2	1.1 ± 0.3	6.1 ± 0.4	2.0 ± 0.2
0.054	–	4.54	–	40.0	3.1 ± 0.2	0.9 ± 0.2	4.2 ± 0.2	1.1 ± 0.7
0.054	–	–	0.46	33.3	3.6 ± 0.4	0.9 ± 0.6	3.1 ± 0.6	2.2 ± 0.2
0.054	–	–	1.15	46.6	3.8 ± 0.6	1.3 ± 0.2	4.2 ± 0.1	2.6 ± 0.1
0.054	–	–	2.32	50.0	4.2 ± 0.7	1.8 ± 0.4	5.4 ± 0.2	2.2 ± 0.4
0.054	–	–	4.65	21.6	3.5 ± 0.8	1.1 ± 0.2	3.1 ± 0.5	2.2 ± 0.4

Mean of 3 replicates of 20 explants ± standard error (S.E.).

Auxin concentration [μM]		Cultures showing response (%)	Mean number roots per shoot ± S.E.	Mean root length [cm] ± S.E.
IAA	2.8	51.3 ± 0.6	2.0 ± 0.1	1.6 ± 0.1
	5.7	52.4 ± 1.2	2.3 ± 0.6	2.8 ± 0.6
	11.4	33.6 ± 1.5	1.2 ± 0.4	1.1 ± 0.5
	17.1	0	0	0
IBA	2.4	52.2 ± 1.3	2.3 ± 0.2	1.8 ± 0.2
	4.9	56.8 ± 1.4	3.3 ± 0.6	2.2 ± 0.8
	9.8	38.2 ± 1.6	2.2 ± 0.4	1.4 ± 0.1
	14.7	0	0	0
IAA + IBA	2.8 + 2.4 5.7 + 4.9	52.2 ± 0.8 0	2.6 ± 0.3 0	1.8 ± 0.6 0

Table II. Effect of auxins on rooting of *in vitro* produced shoots of *B. ovalifoliolata* after 50 days.

Mean of 3 replicates of 20 explants ± standard error (S.E.).

The shoots that developed *in vitro* failed to form roots on MS basal medium containing NAA. Root initiation was achieved on IAA and IBA after 20 d. IBA at 4.9 μM proved good for rooting (Table II). The rooted shoots were transferred after 30 d auxin-free medium for subsequent growth period. After acclimatization the plantlets were subjected to field trial. The plants had normal leaves without any morphological variation.

The present work demonstrates a simple and successful procedure for rapid propagation of *B.*

*ovalifoliolata* through cotyledonary node explants. This method could be explored for mass production and conservation of economical and commercial importance of this endemic species.

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