Indian Journal of Biotechnology
Vol 15, July 2016, pp 443-445

# Micropropagation of an important medicinal forest tree—Shyonaka

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*Received 3 November 2014; revised 2 June 2015; accepted 7 August 2015*

*Oroxylum indicum* (L.) Vent., vernacularly known as Shyonaka or Sonpatha, is a small to medium sized deciduous tree. Micropropagation of *O. indicum* was established from nodal explants cultured on MS medium supplemented with varied concentration of BA (6-benzaylaminopurine). Multiple shoot induction with the maximum 4 shoots with appropriate shoot length (5 cm) was generated on MS medium containing only BA (1.35 mg/L). On the other hand, the maximum root induction was achieved on half strength MS medium fortified with IBA (indole-3-butyric acid; 2.5 mg/L) and $AgNO_3$ (2 mg/L). The plantlets so generated were processed through hardening procedure for acclimatization and transfer to the field.

**Keywords:** Medicinal tree, micropropagation, *Oroxylum indicum*

*Oroxylum indicum* (L.) Vent. (Family: Bignoniaceae) is a traditional herbal medicine in many Asian countries and used as a cure of various diseases[1]. This plant is also one of the important ingredients in most commonly used ayurvedic formulation, such as, *Dusamula*, *Brahmi*, *Rasayana*, *Amratarista*, *Dantyarista*, *Dhanwantara*, *Ghrita*, *Narayana Taila etc*[2]. The root bark is used in fever, bronchitis, intestinal worms, leucoderma, asthma, inflammation and other troubles[1]. The roots are used for the treatment of tuberculosis and nasopharyngeal cancer[2]. The estimated demand of *O. indicum* in only Southern India is about 500 kg per annum[3]. Destructive and non-sustainable collection methods, low seed viability[4] and habitat destruction have posed serious threat to the survival and availability of this highly useful tree[5]. As a result, the existence of *O. indicum* in natural population has been categorized as vulnerable by the Government of India[4]. However, large-scale production is a pre-requisite to meet the pharmaceutical needs and also for the effective conservation of this valuable medicinal plant. To overcome the above-mentioned problems, tissue culture techniques can be applied to generate clonal propagules and to conserve, especially, those species in which either the underground parts or the whole plant is used in drug preparation. The aim of the present study was to establish an efficient and sustainable *in vitro* propagation protocol for *O. indicum*.

Mature fruits of *O. indicum* were collected in the month of April from Sabarkantha district, Gujarat. The mature seeds were treated with 0.5% bavistin for 5 min, followed by 0.05% mercuric chloride ($HgCl_2$) for 30 sec. The traces of reagents were removed by rinsing the seeds 4 times with sterile distilled water and inoculated on plain MS[6] medium. Nodal explants were isolated from *in vitro*-generated seedlings (60-d-old) (Fig. 1A) and implanted onto MS medium supplemented with various concentrations (0.67, 1.35 & 2.02 mg/L) of 6-benzylaminopurine (BA), 3% sucrose and solidified with 0.8% agar-agar (SRL; Mumbai, India). The pH of the medium was adjusted to 5.8 prior to adding agar. The medium was autoclaved at of 1.05 kg/cm$^2$ pressure and 121°C temperature (15 min). The cultures were incubated at

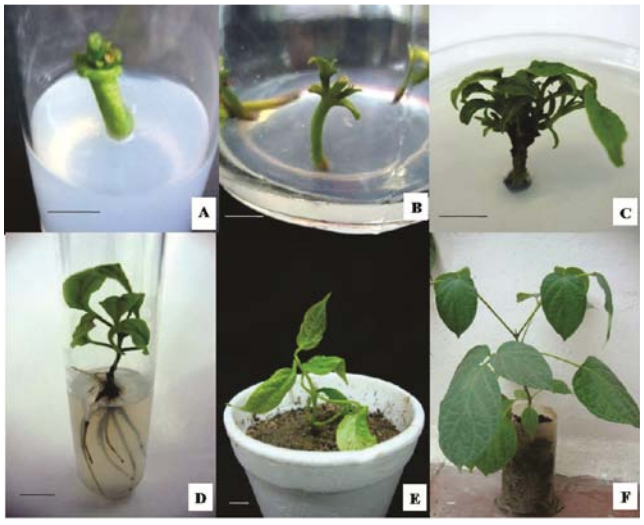


Fig. 1 (A-F)—Micropropagation of *O. indicum*: A, Nodal explants from *in vitro* grown plant; B, Sprouting of nodal bud on MS medium+BA (1.35 mg/L); C, Multiple shoots in 2nd subculture; D, Rooted shoots on ½ MS medium+IBA (2.5 mg/L)+$AgNO_3$ (2 mg/L); E, Hardening of plantlets; & F, Well grown plant. [Horizontal bars in each photograph is equal to 1 cm]

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25°C temperature under 16 h photoperiod provided by white fluorescent tubes (55 µmol/$m^2$/sec). Subcultures were made at 4 wk interval and results were recorded. Clusters of shoots were separated and transferred to ½ MS medium supplemented with $AgNO_3$ (2 mg/L) (Sigma-Aldrich) and various concentrations (0.5-2.5 mg/L) of IBA (Sigma-Aldrich) for root induction.

The generated plantlets were removed carefully, washed thoroughly with distilled water to remove agar, dipped in solution of fungicide (bavistin, 0.1% w/v) and planted in pots containing a mixture (soil, sand and farmyard manure; 1:1:1). The plantlets were irrigated with ¼ strength MS medium and processed for hardening[7]. The experimental layout was completely randomized. Each treatment had 10 replicates. All the data generated from this experiment were analyzed statistically with SPSS program (Version 19; SPSS Inc. Chicago, USA). Significance of treatment effects was determined using analysis of variance (ANOVA, $p \leq 0.05$), and comparison between mean values of treatments were made by Tukey's test.

The pre-treatment of seeds with bavistin before surface sterilization with $HgCl_2$ showed excellent results. It helped in reducing the rate of contamination and showed maximum establishment of seed germination (95%). Nodal explants showed 100% bud sprout response on a medium containing 1.35 mg/LBA (Table 1). Presence of BA in the medium promoted the lateral bud proliferation from nodal explants (Fig. 1B). Subcultures in the same medium yielded a cluster of shoots which were separated and used for further experiments (Fig. 1C). The explants cultured on MS medium supplemented with BA (0.67 & 2.02 mg/L) also demonstrated sprouting of buds but percentage of bud sprout and number of shoot multiples were not higher compared to the medium supplemented with 1.35 mg/L BA. Moreover, at higher concentrations of BA, though multiple-shoot production was observed but the shoots so formed were very slow in growth.

Multiplication stage was recycled many times to produce an unlimited number of shoots. In this study, six subcultures were made at 4 wk interval. In subculture-2, highest numbers of shoots (3-4) were obtained. After 2nd subculture the shoot multiplication rate demonstrated a declining trend (Fig. 2).

The maximum number of roots per shoot (4-7) with mean length 3.58 cm were obtained on half strength MS medium supplemented with IBA (2.5 mg/L) and $AgNO_3$ (2 mg/L) after 4 wk of culture (Table 2; Fig. 1D). Hundred percent shoots showed rooting in this medium. Length of roots and number of roots were increased with the incubation period of culture. The roots were whitish in colour and 1-6 cm long. Silver nitrate ($AgNO_3$) is an anti-ethylene compound.

Table 1—Influence of BA on bud sprout response from nodal explants of *O. indicum*

| Hormone conc. (mg/L) | Shoot multiples (mean±SE) | Response (%) |
|---|---|---|
| 0.67 | 3.00±0.47[a] | 100 |
| 1.35 | 4.30±0.55[b] | 100 |
| 2.02 | 2.10±0.6[a] | 100 |

SE: Standard error of the mean
Mean bearing the same superscripts letter within the column are not significantly (P≤0.05) different according to Tukey-HSD test

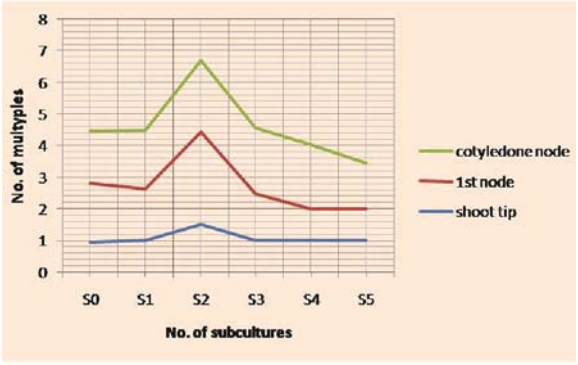


Fig. 2—Effect of subcultures on proliferation of *O. indicum* on MS medium+BA (1.35 mg/L).

Table 2—Root induction in *in vitro* generated shoots of *O. indicum*

| Medium strength | IBA (mg/L) | $AgNO_3$ (mg/L) | No. of roots per shoot (mean±SE) | Root length mean±SE) (cm) | Response % |
|---|---|---|---|---|---|
| ½ MS | 0.5 | 2 | 0[a] | 0[a] | 0 |
| | 1 | 2 | 1[b] ± 0.89 | 5.24[d] ±1.24 | 67 |
| | 1.5 | 2 | 1[b] ±0.44 | 1.1[b] ±0.43 | 33 |
| | 2 | 2 | 1[b] ±0.44 | 2.8[c] ±1.12 | 33 |
| | 2.5 | 2 | 5.8[c] ±0.95 | 3.58[c] ±1.09 | 100 |

SE: Standard error of the mean
Mean bearing the same superscripts letter within the column are not significantly (P≤ 0.05) different according to Tukey-HSD test

Addition of silver nitrate in tissue culture medium inhibited secretion of phenolic compounds from explant, protected the medium from bacterial contamination and allowed the improvement of regeneration process[8].

All rooted plants were successfully hardened in the greenhouse. In the present study 20% survival rate was recorded. The plants established in pots were similar to the mother stock in terms of morphological characteristics (Fig. 1F). Earlier studies on *in vitro* propagation of *O. indicum* has been reported that maximum number of shoots (11 shoots/explant) obtained on MS medium fortified with BA (8.87 μM) and IAA (2.85 μM) from embryonic axis[9]. However, this protocol had the problem of phenolic exudates, which could not be resolved. In other studies[10,11], multiple shoot multiplication in *O. indicum* was also obtained through indirect orgenogenesis. Thus, the present protocol can be used for mass multiplication through direct orgenogenesis (nodal culture) for large-scale cultivation of this important and most sought after medicinal plant.

## References


1 Gokhale M & Bansal Y K, Somaclonal variation in *Oroxylum indicum* (L.) Vent.—An endangered tree species, *J Phytol*, 2 (2010) 1-7.

2 Gohil P, Zaveri M & Jain S, Immunomodulatory activity of *n*-butanol extract of *Oroxylum indicum*, *Pharm Biol*, 46 (2008) 914-919.

3 Jayaram K & Prasad M N V, Genetic diversity in *Oroxylum indicum* (L.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker, *Afr J Biotechnol*, 7 (2008) 254-262.

4 Ravikumar K, Ved D K, Vijaya Sankar R & Udayan P S, 100 red listed medicinal plants of conservation concern in Southern India (Foundation for Revitalisation of Local Health Traditions, Bangalore, India) 2000.

5 Yasodha R, Ghosh M, Barthwal S & Gurumurthi K, Importance of biotechnological research in tree species of Dashmula, *Indian For*, 130 (2004) 79-88.

6 Murashige T & Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol Plant*, 15 (1962) 473-497.

7 Jasrai Y T, Kannan R V, Ramakanthan A & George M M, *Ex vitro* survival of *in vitro* derived banana plants without greenhouse facilities, *Plant Tissue Cult*, 9 (1999) 127-132.

8 Cristea T O, Leonte C, Brezeanu C, Brezeanu M, Ambarus S *et al* Effect of $AgNO_3$ on androgenesis of *Brassica oleracea* L. anthers cultivated *in vitro*, *Afr J Biotechnol*, 11 (2012) 13788-13795.

9 Dalal N V & Rai V R, *In vitro* propagation of *Oroxylum indicum* Vent. a medicinally important forest tree, *J For Res*, 9 (2004) 61-65.

10 Parveen S, Khan M O, Austin S E, Croft S L, Yardley V *et al*, Antitrypanosomal, antileishmanial, and antimalarial activities of quaternary arylalkylammonium 2-amino-4-chlorophenyl phenyl sulfides, a new class of trypanothione reductase inhibitor, and of *N*-acyl derivatives of 2-amino-4-chlorophenyl phenyl sulfide, *J Med Chem*, 48 (2005) 8087-8097.

11 Gokhale M & Bansal Y K, Indirect organogenesis in *Oroxylum indicum* (L.) Vent., *Indian For*, 136 (2010) 804-811.