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In vitro Propagation of *Oroxylum indicum*-An Endangered Medicinal Tree

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Abstract: Seedling stem sections of *Oroxylum indicum* Vent. were cultured on Murashige and Skoog (MS) medium with or without growth regulators 6-benzylaminopurine and 1-naphthaleneacetic acid for proliferation and growth of shoots. Callus proliferation started after 2-weeks of initial culture on all the media supplemented with growth regulators, being maximum (>90%) in the treatment having 3.0 mg L^{-1} 6-benzyaminopurine and 0.5 mg L^{-1} 1-naphthaleneacetic acid. Subculture of seedling stem sections after 4-weeks on the shoot initiation medium led to multiple shoot induction during the next 4-weeks. Utmost, 70.8% explants initiated shoots at an average of 61.1 shoots per explant on the medium found best for callus proliferation. The most congenial rooting medium was half-strength MS medium plus 0.5 mg L^{-1} indole-3-butyric acid. The rooted plants were successfully transferred to soil, exhibiting a normal development.

Key words: *Oroxylum indicum*, *in vitro* culture, nodal segments, axillary bud, plant regeneration

INTRODUCTION

Trees of the *Oroxylum* (Bignoniaceae) genus are found in Indo-Malaysian region and China. One of the species, *Oroxylum indicum* Vent. is widely distributed in the deciduous and mixed miscellaneous forests of India. *O. indicum* vernacularly known as Shyonaka or Sonpatha is a small to medium sized deciduous tree with light grayish brown, soft, spongy bark; large pinnate, bipinnate or tripinnate ovate or elliptic leaves; lurid, purple, fleshy, foetid flowers and large, flat, sword shaped capsules full of many flat and papery thin seeds with broad silvery wings.

Most parts of *O. indicum* tree are used in Ayurvedic system of medicine. The root bark is well-known tonic and astringent useful in diarrhoea and dysentery. It is diaphoretic and used in rheumatism. Tender fruits are refreshing and stomachic and the seeds are purgative. The stem and root barks contain three flavon colouring matters, viz. oroxylin-A, baicalin and chrysin. Young shoots and unripe fruits are eaten as vegetables. The tree is also frequently lopped for fodder.

O. indicum is propagated naturally by seeds, which germinate in the beginning of the rainy season. Seedlings require moderate shade in the early stages. However, the seed set is poor and seed viability is low. Problems related with its natural propagation and indiscriminate exploitation for medicinal purpose have pushed *O. indicum* to the list of endangered plant species of India.

Various problems related with conventional propagation and high demand of planting material of medicinal and aromatic plants can be addressed by efficient and economical *in vitro* propagation in a short span. However, there is no report of micropropagation or tissue culture of the genus *Oroxylum*. During the present investigations, in an endeavor to develop micropropagation protocol, efficient plant regeneration has been obtained from callus cultures of nodal segments.

MATERIALS AND METHODS

Seeds of a selected *Oroxylum indicum* tree with high active flavon content were collected during December-January from the Medicinal Garden, JNKVV, Jabalpur. After removing the wings, seeds were washed with 70% ethanol for 1 min and subsequently surface sterilised with 1% sodium hypochlorite (w/v) solution containing 0.1% Tween 20 (w/v) for 15 min. Following rinsing with sterile distilled water, seeds were plated on Murashige and Skoog (1962) medium with 8 g L^{-1} agar in tissue culture containers for germination and seedling growth. Six-week old *in vitro* seedlings were used to isolate 1.0-1.5 cm nodal sections with one axillary bud for culture.

MS culture medium with or without growth regulators was used for micropropagation through seedling nodal sections (Table 1). Readymade powder MS basal medium and all other add-ons were procured from Himedia®, Mumbai. All the media were supplemented with 30 g L^{-1} sucrose as a carbon source. The pH of the media was adjusted to 5.8 prior to the addition of 8 g L^{-1} agar and autoclaved at 121°C for 20 min. The cultures were incubated in dark for seven days and subsequently maintained at 25±2°C with 16 h day light at an intensity of

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15-20 μmol $m^{-2}s^{-1}$ (PAR lamps OSRAM® Lumilux Plus and Plus Eco-Cool White). Each treatment consisted of ~50 explants and was replicated twice. The evaluated parameters are shown in Table 1. The data were analyzed statistically by ANOVA and the means were compared by the Tukey's test.

For root initiation, shootlets were transferred to half-strength MS medium containing 0.1 or 0.5 mg L^{-1} IBA, 20 g L^{-1} sucrose and 7.5 g L^{-1} agar. Rooted plants were thoroughly washed with running tap water to remove the adhering agar and planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were kept at 30±2°C and 60±5% RH for 30 days in a glasshouse for acclimatization.

RESULTS AND DISCUSSION

It is a well-established fact that the cytokinin especially BA in the culture medium induces a high number of shoot buds. Cytokinins overcome apical dominance, release lateral buds from dormancy and promote shoot formation. Accordingly, to begin with seedling nodal explants of *Oroxylum indicum* with single axillary bud were inoculated on MS medium without growth regulators as a control, fortified with four levels of BA alone and three different combinations of BA and NAA to induce multiple shoots.

The initial response (after 10-15 days) of the nodal explants was similar in all the treatments. Basal periphery of the axillary bud present on seedling nodal explants became swollen leading to either professed callus proliferation (Fig. 1A) or shoot bud formation (Fig. 1B). These were multiple shoot buds engender due to precocious proliferation of axillary bud meristem. Buds proliferated precociously into shoots (Fig. 1C and D). During the next 3-5 weeks of culture, seedling nodal explants concomitantly gave rise to shoots from buds as well as profuse cream-brown callus. Developing shootlets appeared out of then non-growing callus mass after 7-8 weeks of initial culture (Fig. 1E). In no case *de novo* morphogenesis was observed from callus. Within next 4 weeks, subculturing led to the shoot elongation (Fig. 1F). Direct multiple shoot formation from basal periphery of axillary buds of seedling nodal sections without any intervening callus phase ensured production of true-to-type plants for clonal multiplication.

Callus proliferation was observed from seedling stem sections on all the initial culture media supplemented with growth regulators being maximum (>90%) on MS3BN, a combination of 3.0 mg L^{-1} BA and 0.5 mg L^{-1} NAA (Table 1). Morphogenic response was more evident after 60 days of culture with the onset of shoot emergence (Fig. 1D-E). To harvest maximum response, nodal explants with callus mass and emerging shoots were divided into small (approx 1 cm^2) pieces after every four weeks of shoot initiation and were subcultured again on the initial culture medium. Within next 4-weeks, subculturing led to multiple shoot induction and elongation (Fig. 1F).

Present investigation revealed that the presence of cytokinin in the culture media positively influence the *in vitro* propagation of *O. indicum* (Table 1). Callus initiation from seedling stem sections as well as shoot formation from such cultures increased significantly with the increase of BA concentrations upto an extent of 3.0 mg L^{-1}. Nonsignificant increase was observed for these parameters with an elevation of BA to 4.0 mg L^{-1} indicating 3.0 mg L^{-1} as the upper limit on BA concentration for *in vitro* propagation of *O. indicum*.

Addition of an auxin (NAA in this experiment) to BA further enhanced callus initiation and shoot formation. Utmost, 70.8% explants initiated shoots at an average of 61.1 shoots per explant on the medium containing 3.0 mg L^{-1} BA and 0.5 mg L^{-1} NAA (MS3BN) which was highest among all treatments. Morphologically normal shoots were produced on this medium suggesting that elevated concentrations of BA with NAA may improve upon regeneration to optimize the *in vitro* propagation of *O. indicum*.

Shootlets (2-3 cm long) obtained from repeated cultures were transferred to two different rooting media supplemented with 0.1 and 0.5 mg L^{-1} IBA. Better root initiation (68%) and development was observed on

Table 1: Effects of growth regulators on *in vitro* regeneration from seedling stem sections of *Oroxylum indicum*

	Growth regulators		Culture response		
Culture media	BA (mg L^{-1})	NAA (mg L^{-1})	Callus initiation† (percent explants)	Shoot initiating calli† (percent explants)	No. of shoots per explant‡
MS	-	-	-	-	-
MSB	1.0	-	37.6[a]	2.4[a]	2.2[a]
MS2B	2.0	-	52.8[b]	18.5[b]	8.6[b]
MS3B	3.0	-	60.1[c]	31.3[c]	30.6[d]
MS4B	4.0	-	65.8[e]	54.0[e]	33.0[d]
MSBN	1.0	0.5	72.0[d]	48.3[d]	18.4[c]
MS2BN	2.0	0.5	87.3[e]	53.8[d]	39.7[e]
MS3BN	3.0	0.5	90.6[e]	70.8[e]	61.1[f]

[a-e]Means followed by the same letter are not significantly different ($p<0.05$) using Tukey's test. †Evaluation was made after 60 days in culture. ‡Evaluation was made after 90 days in culture

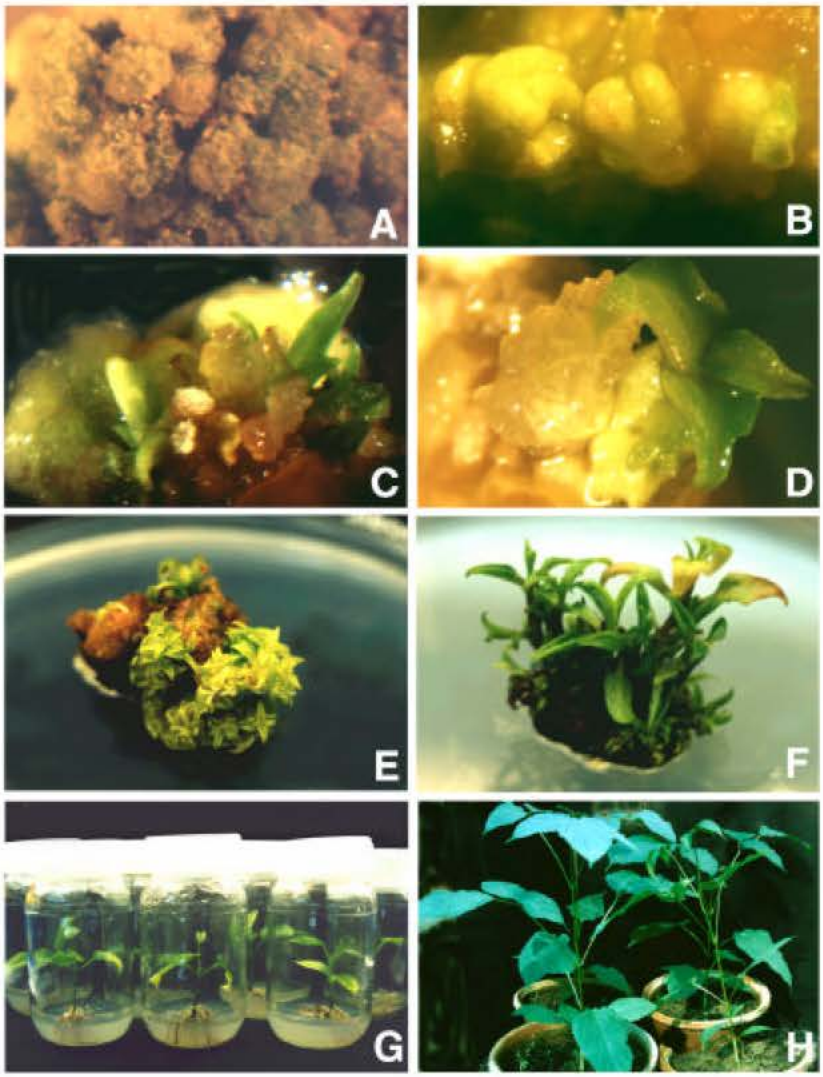

Fig. 1: *In vitro* propagation of *Oroxylum indicum*. (A) Morphogenic callus initiation from seedling stem sections; (B) Shoot buds on callus culture; (C-D) Shoot bud proliferation; (E) Multiple shoot initiation from seedling stem section culture; (F) Shoot elongation from subcultured callus; (G) Rooted plants in culture and (H) Regenerated plants ready for field transfer after hardening

½ MS with 0.5 mg L^{-1} IBA (Fig. 1G) as compared to medium with 0.1 mg L^{-1} IBA. All the regenerants acclimatized well in the green house and then in outdoor conditions (Fig. 1H). More than 400 plants transferred to the field showed high homogeneity in growth and general morphological features suggesting absence of major somaclonal variations.

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