In vitro shoot induction and callus induction of a medicinal tree *Oroxylum indicum* (Tattu) through tissue culture

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SUMMARY

In vitro shoot induction and callus induction of *Oroxylum indicum* (Tattu) was carried out by using apical and axillary bud and leaf midrib explants. A simple and reliable protocol was developed through apical and axillary bud and leaf midrib explants of *Oroxylum indicum* for multiple shoot induction and callus induction. Among the different types of growth regulators used for culture establishment BAP and 2, 4-D exhibited the best response for inducing multiple shoots and callus, respectively. Axillary bud showed significantly high shoot multiplication on MS medium with 2Mg/l BAP whereas leaf midrib explant was found to be more effective on MS medium with 4Mg/l 2, 4-D for callus induction.

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Key words : *Oroxylum indicum, In vitro,* Tissue culture

The plant *Oroxylum indicum* belonging to the family Bignoniaceae commonly known as Shivnak, Shyonak, Sonpatha or midnight horror, is a small deciduous, soft wooded tree with light grayish brown, soft and spongy bark that grows upright up to 20 meters high. It is also known as Indian Trumpet Flower and it's fear-provoking. The tree is often grown as an ornamental for its strange appearance. A deciduous medium sized tree, the pods, seeds, stem and root bark contain many flavones, weak acids and traces of alkaloids (Uddin *et al.*, 2003; Dalal and Rai, 2004). The plant contains flavonoids like chrysin, oroxylin and baicalein as active principles (Chen *et al*., 2003). Leaves are emollient and contain anthraquinone and aloe-emodin (Parrotta, 2001; Nakahara *et al.*, 2002). The leaf contains chrysin and baicalein. Other flavonoids, known for their anti-inflammatory and anti-allergy effects, are also present, though it may need to be used in high doses to get a response. Oroxindin has also been isolated from *Oroxylum indicum* (Nair, 1979).

Oroxylum indicum is widely used by the Indians for the treatment of various ailments. It has been categorized as vulnerable medicinal plant by the government of India (Ravi Kumar and Ved, 2000). In general, roots are used as astringent and for the treatment of tuberculosis (Bhattacharje, 2000). In India, roots are used in Ayurvedic preparation called "Dasamoola" *i.e.*, used as an astringent, anti-inflammatory, anti-helminthic, antibronchitic, antileucodermatic, anti-rheumatic, anti-anorexic and for treatment of leprosy and tuberculosis. *Oroxylum* root bark is the part used in Ayurvedic medicine, administered as an astringent, bitter tonic, stomachic, and anodyne. It is included in famous tonic formulations, such as Chyawanprash. The plant is also used in Asian folk medicine for the treatment of abdominal tumors (Soe and Myongure, 2004). It was also reported to possess anticancer properties (Lambertini *et al*., 2004; Costa-Lotufo *et al*., 2005).The seed extract exhibits antimicrobial, analgesic, anti-tussive and anti-inflammatory properties (Rasadah *et al.*, 1998).The fruits are used in treating bronchitis, leucoderma, helminthosis etc., (Parrotta, 2001; Dalal and Rai, 2004).

The uncontrolled collection and sale of large quantities of plant material from the forest leads to destruction of many forest plants.Cultivation of medicinal plants especially high value medicinal plants is creating new dimension in the field of agriculture. *Oroxylum indicum* is feared to become endangered soon. Hence there is a need for a scientific approach for propagation of medicinal plants.

Plant tissue culture offers unconventional technique for plant improvement. It has become an important tool

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for conservation and mass propagation of important tree species. Conventionally, *O. indicum* reproduces via viable seeds and roots, but the low percentage of seed viability and destructive collection of roots from trees, limits its natural propagation. Hence, alternative methods like *in vitro* techniques could be used to propagate this plant and thereby multiply elite genotypes. *In vitro* regeneration of this tree has been reported (Dalal and Rai, 2004). The present paper describes large-scale propagation of *O. indicum* through apical and axillary buds and leaf midrib through tissue culture technology.

MATERIALS AND METHODS

Explants of *Oroxylum indicum* were collected from reserve forest area of Chandrapur city, Maharashtra., India. Leaves, apical and axillary bud of *Oroxylum indicum* were washed thoroughly in running tap water for 30 minutes to remove all adhering dust and phenols. Then they were treated with tween 20 and 0.1% mercuric chloride solution for 2-5 minutes. The explants were then thoroughly washed (4-5 washings) with sterilized distilled water to remove the traces of $HgCl_2$. Explants were inoculated under aseptic conditions on the sterile culture Murashige and Skoog medium in test tube supplemented with 3% sucrose, 0.8% agar and plant growth regulators; benzyl amino purine (BAP), Dichlorophenoxyacetic acid (2,4-D) and Adenine sulphate was tested individually and in combination. The pH of the media was adjusted to 5.8 before adding agar. Medium was dispensed in glass test tubes and was autoclaved at a pressure of 15 psi and a temperature of 121^0C for 15 min. All the cultures were maintained in an air conditioned culture room at a temperature of 25 ± 4^0C and a relative humidity of 75 - 80 per cent. The source of illumination consisted of 2.5 feet wide fluorescent tubes (40 watt) and incandescent bulb (25 watt). The intensity of illumination was 3500 lux at the level of cultures and a 12 hour light regime was followed by 12 hour darkness.

For the incubation of callus, provided a dark room with a temperature of 25 ± 2 and relative humidity at 45-55 per cent.

RESULTS AND DISCUSSION

The axillary bud, apical bud and leaf midrib were cultured on MS medium supplemented with different concentrations of growth hormones (Fig. 1, 2 and 3).

The apical and axillary bud explants were inoculated on MS Media with 9 defferent concentrations of BAP and Adenine sulphate, either alone BAP or combination of BAP and Adenine sulphate. Among the various treatments the effective results were obtained from the combination are given in Table 1. Within 6-8 days of inoculation explants turned green and showed establishment, of which best establishment was found in M_3 *i.e.* combination of MS + 2 Mg/l BAP followed by M_4 [MS + 3 Mg/l BAP] and M_6 [MS + 2 Mg/l BAP + 15 Mg/l Adenine sulphate]. Purohit *et al.* 2004 reported that the percentage of bud breakage in species *Wrightia tomentosa* was significantly higher on media supplemented with BAP (2.22 µM-8.86 µM)

Subculturing of established explants for multiplication on same respective medium induced multiple shoots in 7-8 days. As in Table 2, M_3 [MS + 2 Mg/L BAP] followed by M_4 [MS + 3 Mg/L BAP] and M_6 [MS + 2 Mg/L BAP + 15 Mg/L Adenine sulphate] showed highest multiple shoot induction. Upto 2-4 multiple shoots were observed from single inoculated explants. The proliferating buds were well defined pale green to greenish and 0.5-1 cm long with bulbous base. Ananthakrishnan *et al.* (1999), Ndoye *et al.* (2003) reported that supplementation of BAP alone is to be capable for production of multiple shoots efficiently in plants *Anacardium occidentale* and *Balanites aegypticaca.* Kathiravan *et al.* (1995) and Girija *et al.* (1999) concluded that concentration of BAP

Table 1 : Effect of different concentrations of plant growth regulators on *in vitro* shoot establishment

Sr. No.	Media treatments	MS Media	BAP (Mg/l)	Adenine Sulphate (Mg/l)	No. of explants inoculated	Percentage of explants establishing
1.	M_0	MS	-	-	20	40%
2.	M_1	MS	-	15	20	30%
3.	M_2	MS	1	-	20	80%
4.	M_3	MS	2	-	20	100%
5.	M_4	MS	3	-	20	95%
6.	M_5	MS	4	-	20	85%
7.	M_6	MS	2	15	20	95%
8.	M_7	MS	3	15	20	90%
9.	M_8	MS	4	15	20	90%

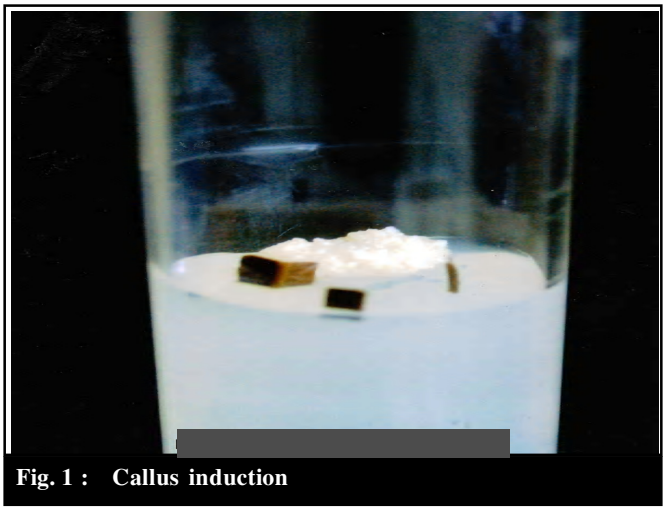

Fig. 1 : Callus induction

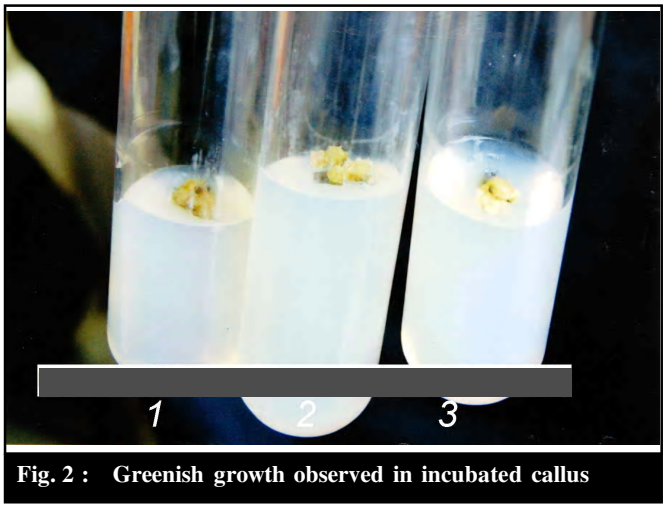

Fig. 2 : Greenish growth observed in incubated callus

Table 2 : Effect of different concentrations of plant growth regulators on *in vitro* shoot proliferation from shoot tip explants of *Oroxylum indicum*

Sr. No.	Media treatments	MS Media	BAP (Mg/l)	Adenine Sulphate (Mg/l)	No. of explants inoculated	No. of shoots per explants
1.	M_0	MS	-	-	20	-
2.	M_1	MS	-	15	20	-
3.	M_2	MS	1	-	20	1-2
4.	M_3	MS	2	-	20	3-4
5.	M_4	MS	3	-	20	2-3
6.	M_5	MS	4	-	20	1-2
7.	M_6	MS	2	15	20	2-3
8.	M_7	MS	3	15	20	1-2
9.	M_8	MS	4	15	20	1-2

Table 3 : Effect of different concentrations of plant growth regulators on in vitro callus induction from leaf midrib explants of *Oroxylum indicum*

Sr. No.	Media treatments	MS Media	2-4D(Mg/l)	Callus growth
1.	M_0	MS	-	-
2.	M_1	MS	1	+
3.	M_2	MS	2	++
4.	M_3	MS	3	++
5.	M_4	MS	4	++

where, - No growth, + Normal growth, ++ Good growth

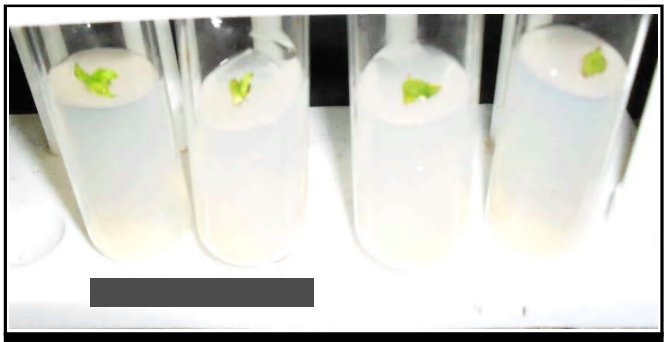

Fig. 3 : Shoot induction

4.43µM evoked shoot proliferation in *Crossandra* and Citrus. Gokhale *et al* 2009 reported that axillary bud of *oroxylum indicum* showed high frequency of shoot multiplication on MS medium with 4.43µM BAP.

Leaf internodal disc and leaf midrib were inoculated for callus induction on MS media supplemented with different concentrations of 2-4D. As per Table 3, C_4[MS + 4Mg/l 2,4-D] followed by C_3 [MS + 3 Mg/l 2,4-D] and C_2 [MS + 2Mg/l 2,4-D] showed callus induction in 23-25 days.

In conclusion, a simple efficient and high fidelity protocol for mass propagation of *Oroxylum indicum* from apical and axillary bud and leaf midrib explants has been established. Using this protocol it is possible to produce viable, uniform and healthy plants with maximum survival rate.

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