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Callus induction in *Oroxylum indicum* (L.) Kurz

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Abstract
The species *Oroxylum indicum* (L) Kurz (Bignoniaceae) is an endangered and important medicinal forest tree possessing various secondary metabolites with potential pharmaceutical properties. The present investigation reports the callus induction from leaf and cotyledonary leaf explants of *O. indicum.* The leaf and cotyledonary leaf explants were cultured on MS medium containing 30g/L sucrose supplemented with various types of auxins viz., IAA/IBA/NAA/2, 4-D to evaluate the efficiency of auxins for inducing the callus in *O. indicum.* Among the various auxins used for the present study, IBA and 2, 4-D were found to be potent for callus proliferation followed by IAA/NAA. Proliferation of callus was faster and very high yield of callusing mass was achieved within 4 weeks on IBA/2, 4-D followed by IAA/NAA. Among the explants tested cotyledonary leaf explants were highly efficient for inducing the callus production in *O. indicum.* Since, it is medicinally important and endangered species; the pharmaceutically valuable compounds can be isolated by using callus cultures throughout the year.

Keywords: *O. indicum*, Cotyledonary leaf, Callus induction, Secondary metabolites

1. Introduction
Plant tissue culture is found to be an attractive alternative approach to the traditional methods of plantations, as it offers a controlled supply of biochemicals independent of plant availability and more consistent product quality [1].
Secondary metabolites are produced by most of the plants in difficult environmental conditions, which aren't essential for growth, energy conservation or for primary metabolic pathways but are needed for the plant to interact with its environment and other organisms [2, 3, 4]. The evolving commercial importance of secondary metabolites in recent years resulted in a great interest particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology.
Plant cell and tissue culture technologies can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots, and meristems for multiplication and also for extraction of secondary metabolites through callus cultures. *In vitro* production of secondary metabolites in plant cell suspension cultures has been reported from various medicinal plants, and bioreactors are the key step for their commercial production. Cell suspension cultures are initiated from callus cultures and it is very useful to obtain commercially important secondary metabolites without sacrifying the whole plant.
Our present work has been carried out on *in vitro* callus induction from *Oroxylum indicum* (L) an endangered medicinally important forest tree species. The species *O. indicum* (Bignoniaceae) contains flavonoids like chrysin, oroxylene, baicalein as active principle [5]. Flavonoids namely, chrysin, oroxylin-a, scutellarin, baicalein, biochanin-a and ellagic acid are responsible for the anti-inflammatory, diuretic, anti-arthritic, anti-fungal and anti-bacterial activities [6, 7]. Baicalein is reported to possess an anti-inflammatory, anti-ulcer, antioxidant, hepatoprotective and immunomodulatory properties [6].
Phytochemical analysis of the plant extracts of *O. indicum* were tested to determine the presence of various phytochemicals and found to possess flavonoids, phenolics, alkaloids etc [8, 9]. Considering the medicinal importance of the species *O. indicum* the main objective of the present investigation is to establish an efficient protocol for *in vitro* callus induction which can be utilized for the production of secondary metabolites to satisfy the demand from the pharmaceutical industries. So, we have made an attempt to induce the callus from leaf and cotyledonary leaf explants of *O. indicum.*

2. Materials and methods
2.1 Plant material
For callus mediated induction, *in vitro* grown 2-3 weeks old healthy seedlings were selected. Cotyledonary and leaflet explants were excised carefully with the help of sterilized scalpel cut into small pieces of size 1-3 cm^2 and were inoculated on the medium for callus induction.

2.2 Culture media and Culture Conditions

The cotyledonary leaf and leaf explants were inoculated on MS medium containing 30 gm/L sucrose supplemented with various concentrations (1.0-5.0 mg/L) of auxins IAA/IBA/NAA/2, 4 D.

The p^H of the medium was adjusted to 5.7 with either 0.1 N HCl or 0.1 N NaOH prior to addition of 0.8% Difco-bacto agar. The medium was sterilized in an autoclave at 121ºC under 15lbs for 15-20 min and dispensed into different culture tubes. All the cultures were incubated at 25±2^{0}C under16 hrs photoperiod with photon flux density of 40-50 µmol m^{-2} s^{-1} provided with white fluorescent lights. The cultures were transferred onto fresh medium consisting of the same plant growth regulators (PGRs) for every 3 weeks.

2.3 Data analysis

Data on percentage of callusing and texture of callus were recorded for every 6 weeks of culture and 3 replicates were maintained for each treatment and each experiment was repeated at least thrice.

3. Results & Discussion

The cotyledonary leaf and leaf explants were cultured on MS medium supplemented with different concentrations of auxins IAA/IBA/NAA/2, 4-D alone to evaluate the efficiency of auxin and the explant for inducing the callus in *O. indicum*. The explants cultured on MS medium without PGRs didn't respond.

Leaf culture

The results on callus induction from leaf explants on MS medium fortified with various concentrations (1.0-5.0mg/L) of auxins IAA/IBA/NAA/2, 4-D individually are presented in Table-1 and shown in Fig-1. Among the different auxins used, IBA was found maximum percentage (90%) of callusing efficiency at 2.0 mg/L IBA, followed by 2.0 mg/L IAA (89%), 3.0 mg/L 2, 4-D (80%) and 2.0 mg/L NAA. It is interesting to note that nodular callusing was observed at all levels of IBA used (Fig-1, c) except at 1.0 and 5.0 mg/L IBA. The texture of the callus was found to be varied based on the concentration of PGRs used.

Cotyledonary leaf culture

The cotyledonary leaf explants were cultured on MS medium fortified with various concentrations (1.0-5.0 mg/L) of auxins viz., IAA/IBA/NAA/2, 4-D individually are presented in Table-2 and shown in Fig-2. Callus induction was observed after 10 days of culture in all the concentrations of PGRs used. Maximum percentage (75%) of callusing response was found at 3.0 mg/L 2, 4-D/IBA followed by 2.0 mg/L IAA/NAA whereas more amount of callus formation was observed at 2.0/3.0 mg/L 2, 4 D/IAA/IBA/NAA. Less percentage of response and low amount of callusing ability was recorded at low and high concentrations of 2, 4-D/IAA/IBA/NAA used. The texture of the callus was found variable depending upon the concentration and the type of PGR used.

Callusing response was found to be varied from auxin to auxin and also with explant to explant. Morphogenic response (morphology and texture) of callus was found to be different in leaf and cotyledonary leaf explants cultured on MS medium fortified with different PGRs in *O. indicum.* Callusing response was higher in cotyledonary leaf explants in all the concentrations of auxins used except at high and low concentrations of PGRs. Maximum frequency of responding cultures and high amount of callusing were recorded at 2.0/3.0 mg/L auxins tested.

The auxins IBA and 2, 4-D induced high amount of callus in both the explants studied in comparison to NAA/IAA. Similar results were also reported by Praveen *et al* (2001) in *Strychnos potatorum* and *Terminalia alata* by Lakshman (2006) [10, 11].

For development of callus from any explant, the supply of exogenous auxins is essential [12] but depending upon the species callusing ability was enhanced by adding low level of cytokinins along with high levels of auxins. These PGRs act synergistically for promoting callus induction at particular level of concentrations. Similar findings were also found in tree species *Strychnos potatorum* on MS medium fortified with 2, 4-D in combination with BAP/Kn [10].

Callus produced from different explants showed variability in texture, form and coloration. This difference is dependent upon the responses of plant tissue to various PGRs present in the media. Thus successful callus induction depends upon the various factors such as composition of the nutrient medium, hormonal balance besides the type, age and genotype of the explants [13, 14].

Since the species contains many therapeutically important plant secondary metabolites, the callus induction in *O. indicum* plays a vital role in the production of bioactive molecules. Thus, the protocol developed for callus induction can be used for isolation of pharmaceutically important compounds from the callus/cell suspension cultures in *O. indicum.* The use of *in vitro* plant cell culture for the production of chemicals and pharmaceuticals had made great strides building on advances in plant science. Still it needs lot of research to know the biosynthetic pathways of desired phytochemicals present in medicinal plants.

Table 1: Callus induction from leaf explants on MS medium supplemented with IAA/IBA/NAA/ 2, 4 D in *O. indicum.*

Concentration of PGR (mg/L)	Percentage of response	Callusing	Morphology of callus
2,4-D			
1.0	62	++	White-compact
2.0	75	+++	White-nodular
3.0	80	+++	Green-compact
4.0	52	++	Green-nodular
5.0	40	++	White yellow-compact
IAA			
1.0	50	+	Light green-compact
2.0	89	+++	Light yellow-nodular
3.0	60	+++	Green-nodular
4.0	47	++	Green-nodular
5.0	42	++	White-compact
IBA			
1.0	60	+++	White yellow-compact

2.0	90	+++	Light green-nodular
3.0	70	+++	Light green-nodular
4.0	52	++	Yellow green-nodular
5.0	50	++	Light yellow-compact
NAA			
1.0	55	+	White-compact
2.0	79	+++	Yellow green-nodular
3.0	65	+++	Green-friable
4.0	45	++	Light green-compact
5.0	39	++	White-compact

+=low; ++=moderate; +++=high amount of callus induction

Table 2: Callus induction from cotyledonary leaf explants on MS medium supplemented with IAA/IBA/NAA/2, 4-D in *O. indicum.*

Concentration of PGR (mg/L)	Percentage of response	Callusing	Morphology of callus
2,4-D			
1.0	50	++	White-compact
2.0	65	+++	Green-compact
3.0	75	+++	Green-nodular
4.0	40	++	White-compact
5.0	35	++	White-compact
IAA			
1.0	40	++	White-compact
2.0	70	+++	Green-nodular
3.0	50	+++	Green-nodular
4.0	45	++	Green-compact
5.0	38	++	White-compact
IBA			
1.0	45	++	Light green-nodular
2.0	75	+++	Green-nodular
3.0	70	+++	Green-nodular
4.0	42	++	White-compact
5.0	40	++	White-compact
NAA			
1.0	45	++	White-compact
2.0	70	+++	Green-friable
3.0	62	+++	Yellow-friable
4.0	38	++	Green-compact
5.0	32	++	White-compact

+=low; ++=moderate; +++=high amount of callus induction.

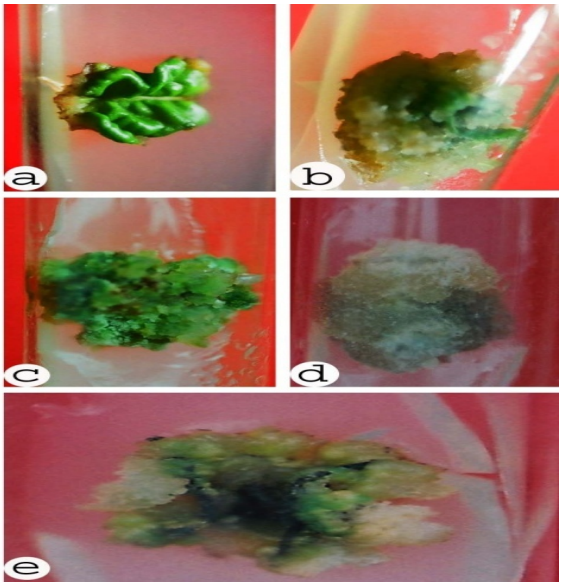

Fig 1: Callus induction from leaf of *O. indicum*

a) Callus formation at 3.0mg/L 2, 4-D; b) Proliferation of callus at 2.0 mg/L IAA; c) Formation of green-nodular callus at 2.0 mg/L IBA; d) Formation of yellow green-friable callus at 3.0mg/L NAA; e) formation of yellow green-nodular callus at 2.0 mg/L IBA.

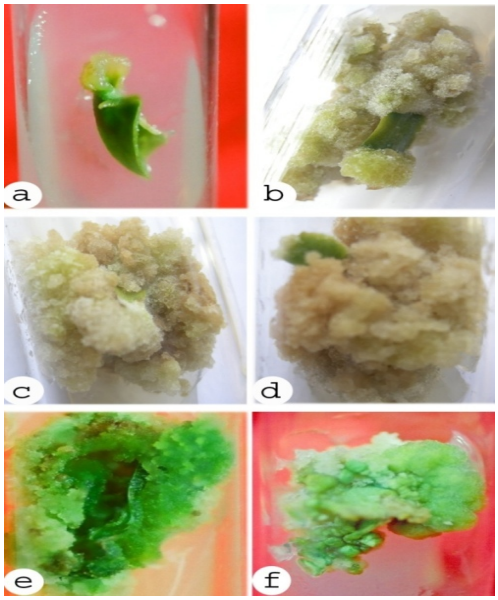

Fig 2: Callus induction from cotyledonary leaf explants of *O. indicum*

a & b) Callus induction at 2.0 mg/L 2, 4-D after 2 and 6 weeks respectively; c) Formation of white-compact callus at 4.0mg/L IBA; d) Development of high amount of friable callus at 3.0 mg/L NAA; e) Green friable callus at 3.0 mg/L

NAA; f) Formation of green nodular callus at 2.0 mg/L 2, 4-D/IBA.

4. Conclusions

It is evident from our results that among the auxins, IBA and 2, 4-D were found to be potent for callus development followed by IAA/NAA. Proliferation of callus was faster and very high yield of callusing mass was achieved within 4 weeks on IBA/2, 4-D followed by IAA/NAA. Among the explants tested cotyledonary leaf explants were highly efficient for inducing the callus compared to leaf explants in *O. indicum.*

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