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CONSERVATION OF AN ENDANGERED ETHNOMEDICINALLY IMPORTANT FOREST TREE SPECIES *OROXYLUM INDICUM* THROUGH SOMATIC EMBRYOGENESIS

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ABSTRACT

In the present investigation, an attempt has been made to conserve the species *Oroxylum indicum* (L) Kurz an endangered ethnomedicinally important forest tree through somatic embryogenesis from cotyledonary leaf explants. Cotyledonary leaf explants were cultured on MS medium fortified with various concentrations of BAP (0.25-0.75 mg/L) in combination with 0.5 mg/L IAA/2, 4-D. Direct somatic embryogenesis was observed from all the concentrations and combinations of plant growth regulators (PGRs) used. Maximum percentage (95%) of response in inducing somatic embryos was found at 0.5 mg/L 2, 4-D+0.75 mg/L BAP. Maximum number of somatic embryoids/explant was also developed at the same concentration and combination of PGRs. More percentage of somatic embryo conversion into bipolar embryos was found at the same concentration of PGRs used. Highest percentage of bipolar somatic embryos was found to be germinated and converted in to plantlets at 0.2 mg/L IAA+0.25 mg/L BAP. These regenerated plantlets were acclimatized in the culture room. Thus, somatic embryogenesis and plantlet formation has been successfully achieved in *O. indicum.* This protocol can be used for developing synseeds, conservation and also multiplication of *true-to-type* of the species in a short period.

Keywords: *Oroxylum indicum*, Somatic Embryogenesis, Plantlet Formation

INTRODUCTION

Somatic embryogenesis is the potential area of research in tree Biotechnology. Generally trees are cross pollinated and it is difficult to get *true-to-type* of germplasm through natural breeding. Hence, there is a need to develop a protocol for induction of somatic embryogenesis in forest tree species in order to develop and conserve the elite germplasm consisting of *true-to-type*. In the present investigation we report on the conservation of an endangered medicinally important forest tree species *Oroxylum indicum* (L) Kurz through somatic embryogenesis from cotyledonary leaf explants.

The species *O. indicum* (Bignoniaceae), is commonly called as *Indian trumpet tree* or *Syonaka* or *Sonpatha,* a native of Indian subcontinent **[1]** and it is well known for its unique medicinal properties such as anti-oxidant **[2-4]**, anti-plasmodial, anti-inflammatory, diuretic, antiarthritic, antifungal and antibacterial activity **[6,7]**. Pharmacologically, it has been found to have astringent, antihelminthic, antibronchitic, antileucodermatic, antirheumatic, and antianorexic and many other medicinal properties **[8]**.The plant secondary metabolites present in all the parts of *O. indicum* **[9, 10]** showed immunostimulant, anti-microbial, anti-depressant, anti-snake venom **[11]** and anti-cancer activities **[12]**. Due to its medicinal importance it is being employed in famous ayurvedic formulations such as *Dashamoolam, Chyawanprasha* and *Amrutharishta*.

In view of the medicinal importance of the species *O. indicum*, it has been over exploited in different types of medical systems and as well as pharmaceutical industries. Hence, the species is on the verge of extinction and becoming an endangered. Problems related with its natural propagation and indiscriminate exploitation for medicinal purpose has pushed *O. indicum* to the list of Red Data. According to the report of task force on conservation and sustainable use of medicinal plants, Planning commission, Government of India (2000), the estimated demand of *O. indicum* in Southern India is 500 kg per annum **[13]**. Realizing the threat of extinction, there is a need to develop conservation strategies and rapid propagation protocol for *O. indicum*.

Considering the medicinal importance of the species *O. indicum* and the threat faced by them, the main objective of the present investigation has been undertaken to establish efficient and reproducible micropropagation protocols for large-scale propagation through somatic embryogenesis to satisfy the demand

in the market and also for reintroduction programme for conservation and restoration. As there is no report on *O. indicum* we have made an attempt to induce the somatic embryogenesis and plantlet formation in *O. indicum* to develop *true-to-type* of plants.

MATERIALS AND METHODS

Plant Material

For present investigation**,** *in vitro* grown healthy seedlings were selected. Cotyledonary leaves were excised, cut into 1cm^2 size under aseptic conditions and were used for induction of somatic embryogenesis.

Culture media and Culture conditions

The cotyledonary leaf explants (1.0 cm^2) were inoculated on MS **[14] (Murashige & Skoog, 1962)** medium supplemented with different concentrations of BAP in combination with auxins 0.5 mg/L IAA/2, 4-D.

The pH of medium was adjusted to 5.8 with either 0.1N NaOH or 0.1N HCl and solidified with 0.8% (w/v) Difco-bactoagar (Hi-media, India) and autoclaved at 121ºC under 15 psi for 20 min. All the cultures were maintained in a culture room at 25±2ºC under 16/8hr a photoperiod provided with light intensity of 40 µmol m^{-2} s^{-1} supplied by cool white fluorescent tubes. For subculturing, the cultures were transferred on to fresh medium containing the same concentration and combination of plant growth regulators (PGRs).

For germination of somatic embryos, the bipolar stage embryos were shifted on to MSO and MS medium fortified with different concentrations of IAA (0.1-0.4 mg/L) + 0.25 mg/L BAP.

For histological studies, tissues were fixed in a mixture of formalin, acetic acid and alcohol, dehydrated in tertiary butyl alcohol series, embedded in paraffin wax (MP 58°C), sectioned with safranin and mounted on DPX.

Plantlet establishment

The plantlets developed through somatic embryogenesis were washed with sterile distilled water to remove remains of agar medium and transferred to plastic cups containing sterile vermiculite. These cups were covered with polythene bags to maintain the RH (90%) and kept in culture room for 4 weeks. Later these were shifted to pots containing sterile garden soil: compost (1:1) and maintained in the research field under shady place.

Data analysis

Data on somatic embryogenesis recorded for every 6 weeks of culture and 30 replicates were maintained for each treatment. Each experiment was repeated at least thrice and the data were analysed following the method of **Pillai & Sinha (1968) [15]**.

RESULTS AND DISCUSSION

Cotyledonary leaf explants were cultured on MS medium supplemented with various concentrations of BAP (0.25-0.75 mg/L) in combination with 0.5 mg/L IAA/2, 4-D **(Table 1)**. Somatic embryos were induced directly from cut ends of the cotyledonary leaf explants within 3 weeks of incubation in all the concentrations of BAP in combination with IAA/2, 4 D used **(Fig. I)**. The explants containing somatic embryoids were subcultured on the fresh medium fortified with same concentration and combination of PGRs for the maturation of somatic embryoids from globular to different forms (Heart and torpedo-shaped) **(Fig. I a - d)**. Whereas the morphogenic event was absent when the globular stage embryos cultured on MS medium without PGRs.

Maximum percentage (95%) of response in inducing somatic embryogenesis was found at 0.5 mg/L 2, 4-D + 0.75 mg/L BAP followed by 0.5 mg/L IAA+ 0.75 mg/L BAP . Maximum frequency of somatic embryo conversion was also noted at the same combination of growth regulators used **(Table 1)**. More number of somatic embryoids per explant was also recorded in the same concentration and combination of PGRs used, whereas at low concentrations of BAP, less percentage of somatic embryogenesis and a few number of somatic embryoids per explant were recorded in both the types of auxins used **(Table 1)**.

Table 1: Effect of IAA/2, 4-D + BAP on induction of somatic embryogenesis from cotyledonary Leaf explants in *O. indicum*

Concentration of PGRs (mg/L)	% of cultures with somatic embryogenesis	Average no. of embryoids/Explant ± (SE)[a]	% of somatic embryos conversion into bipolar ± (SE)[a]
IAA+ BAP			
0.5+ 0.25	58	13±0.01	10±0.02
0.5+ 0.50	68	35±0.16	25±0.01
0.5+ 0.75	89	58±0.70	48±0.16
2,4 D+ BAP			
0.5+ 0.25	63	23±0.16	08±0.01
0.5+ 0.50	78	45±0.27	43±0.03
0.5+ 0.75	95	63±0.18	78±0.21

[a]Mean±Standard Error; Data scored after 6 weeks

Table 2: Effect of growth regulators on germination of somatic embryos in *O. indicum*

PGRs (mg/L)	Percentage of germination
MSO	-
IAA+ BAP	51
0.1+0.25	62
0.2+0.25	43
0.3+0.25	37
0.4+0.25	

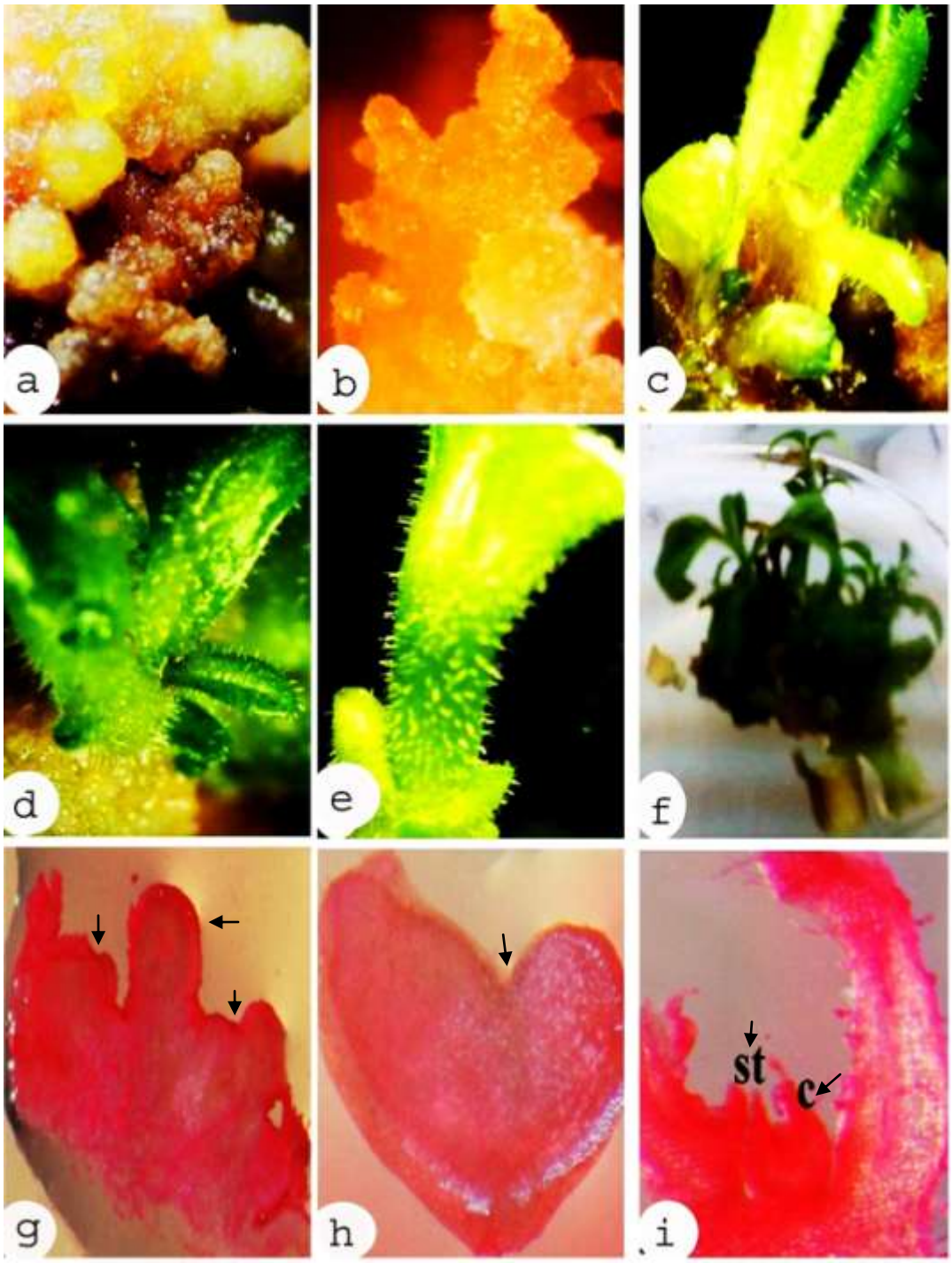

Fig. I: a-h: Somatic embryogenesis, plantlet development and histological studies in O. indicum a) Induction of somatic embryos on MS+ 0.5 mg/L 2, 4-D+0.75 mg/L BAP; b-c)Development of different stages of embryoids; d) A group of bipolar embryos; e) Cotyledonary stage embryo on MS+0.2 mg/L IAA+0.25 mg/L BAP; f) Germination of somatic embryo and note the elongation of shoot after germination; Histological sections; g) Globular embryo; h) Conversion of globular stage

into heart-shaped embryo with clear notch; i) Cotyledonary stage embryo.

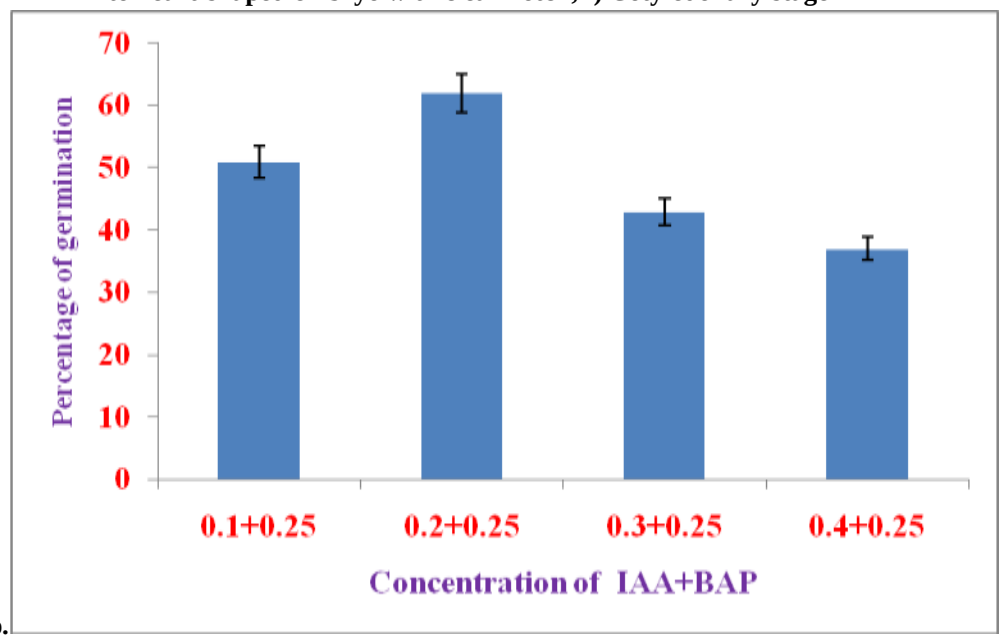

Fig. II: Effect of growth regulators on germination of somatic embryos in *O. indicum*

Among the various auxins used, 2, 4-D was identified as the best PGR for induction of somatic embryogenesis and somatic embryo maturation up to torpedo-shaped. Histological sections of explants with somatic embryos revealed a globular, heart and cotyledonary stage embryos formation **(Fig. I g-i)**. The explants with bipolar embryos were cultured on the same concentration and combination of PGRs for several times, but bipolar embryoids didn't germinate further.

Germination of somatic embryos

The bipolar somatic embryoids were shifted onto MSO and also MS medium fortified with different concentrations (0.1-0.4mg/L) of IAA+0.25mg/L BAP **(Table 2)**. The torpedo-shaped embryos were germinated in all the concentrations of IAA in combination with BAP used **(Fig. I d-f)**. Maximum percentage of bipolar embryos were germinated and converted into plantlets at 0.2 mg/L IAA + 0.25 mg/BAP (62%) followed by 0.3 mg/L IAA + 0.25 mg/L BAP (43%) (Fig. II), whereas these bipolar embryos didn't respond on MSO.

Plantlet establishment

The plantlets developed through somatic embryogenesis were acclimatized in culture room for 4 weeks. Afterwards these were shifted to plastic pots containing garden soil: compost (1:1) and maintained in the research field under shady place. The plantlets survival percentage was found to be 59%. The plants

developed through somatic embryogenesis were similar to donor plants in morphology.

In the present investigations, the combination of both auxin and cytokinins induced the somatic embryogenesis directly from the explants. The PGRs concentration and combination of 2, 4-D + BAP elicited the maximum number of somatic embryos induction in all the concentrations of BAP compared to IAA in *O. indicum.*

Similarly, the requirement of cytokinin in addition to auxin was observed in *Sapindus trifoliatus* **[16]**; *Terminalia arjuna* **[17]** and *Psorolia corylifolia* **[18]**. According to **Zimmerman (1993) [19]** new gene products are needed for the progression from the globular to the heart-stage and these new products are synthesized only when an exogenous auxin is removed. Bozhkov and **Von Arnold (2002) [20]** have also observed the induction of somatic embryogenesis after withdrawal of growth regulators in Norway spruce. But, we found the requirement of auxin and cytokinin combination for inducing somatic embryogenesis in *O. indicum.* After withdrawal of PGRs, the globular stage somatic embryos didn't mature further in *O. indicum*.

In the present investigations IAA/ 2, 4-D in combination with BAP found to be more effective for induction of somatic embryogenesis in *O. indicum*. According to our observations, 2, 4-D+BAP combination showed superiority in inducing somatic embryogenesis and also maximum percentage of embryo conversion into bipolar stage embryos in *O. indicum.* But BAP with NAA was found to be superior for inducing high percentage of somatic embryogenesis in *Givotia rottleformis* **[21]** and their subsequent germination at 0.1 mg IAA + 0.25 mg/L BAP. Thus, a combination of IAA/2, 4-D+BAP found to be necessary for maturation and germination. Conversion of somatic embryos and also maturation are dependent on many extrinsic and intrinsic factors **[22]**. A major factor for somatic embryogenesis is the nature of growth regulator present in the medium. The type of auxin or auxin in combination with cytokinin provided in the medium greatly influences the frequency of somatic embryogenesis and plantlet formation.

We have successfully achieved the plantlet regeneration via somatic embryogenesis. Thus, this technique is more effective for multiplication of *true-to-type* of plants in an endangered forest tree species like *O. indicum*. The present protocol is useful for large-scale production of plants, conservation of germplasm, gene transfer experiments and also for the production of synseeds in an

endangered and ethno medicinally important forest tree species *O. indicum.*

CONCLUSION

In conclusion, the results presented here describe an efficient protocol for *in vitro* multiplication of an endangered medicinal forest tree *Oroxylum indicum* through somatic embryogenesis from cotyledonary leaf explants. This protocol can be used for its conservation and rapid multiplication.

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