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Research Article

IN VITRO MICROPROPAGATION OF MEDICINAL PLANT *OROXYLUM INDICUM -1*

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ABSTRACT

A simple and reliable protocol was developed through zygotic embryo culture and cotylidanary node explants of *Oroxylum indicum -1* for multiple shoot regeneration. Effect of 3 cytokinins; BAP, KN and TDZ, was studied. BAP at 5mg/L proved better than KN & TDZ with highest frequency of shoot initiation and maximum number of shoots initiated.For zygotic embryo culture seeds were surface sterilized with 0.1% $HgCl_2$, washed three times with sterile distilled water and were soaked for 24hrs. Seed coat was removed gently and isolated zygotic embryo (with cotyledons) and inoculated on ½ strength MS and MS basal medium with different levels of sucrose. Zygotic embryos were germinated within 7-8 days, cotylidanary nodes were germinated within 12 days of incubation.

KEY WORDS

Oroxylum indicum -1, Zygotic embryos, cotylidanary nodes, Micropropagation, Seed, incubation.

INTRODUCTION

Medicinal plants are important sources of providing health care in India as well as in the world. Extracts of many medicinal tree species have been used for long in ayurvedic preparations. The science of Ayurveda, Unani system of medicine and Homeopathy are based on plants or plant products. Even the modern allopathy depends on plants for several preparations in recent years with increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics. Interest in the use of plants and plant based drugs has revived throughout the world. Over 700 plant species are estimated to be used for medicinal purposes (Nadkarni, 1976). And about 80% of the rural people depend on herbal traditional medicine.

India is also a hub for the export of various medicinal plants. Today over 95% of the medicinal plants used by the industries in India are collected from forests and other natural resources (Lozoya, 1994). Less than 20 plant species are collected for industrial production is from wild populations. Over 70% of the plant collection involves destructive harvesting because of the use of parts like bark, root, stem, seed and whole plant.

All species of the digitalis plant contain useful cardiac glycosides that help to strengthen the

heart muscle. Lanoxin, which is used in the treatment of congestive heart failure, is made from grecian foxglove (*Digitalis lanata*).

The gradually increasing cases of human diseases all around the world, the incidents of antibioticresistant microbes have also increased to a great extent. Although pharmacological industries have produced a number of new antibiotics in the last three decades, the resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). It is now extremely important to find alternative treatments for microbial infections (João, *et al.,* 2004). The spread of multiple antibiotic resistant pathogenic bacteria has been recognized by the World Organization for Animal Health (OIE), the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a serious global human and animal health problem. The development of bacterial antibiotic resistance is an increasingly troublesome situation conducted in different countries to prove such efficiency of plant derived drugs (Almagboul, *et al.,* 1985; Evans, 1996; Ikram and Inamul, 1984; Izzo, *et al.,*1995; Kubo *et al.,* 1993; Shapoval, *et al.,* 1994; Sosua, 1991).

India is endowed with rich and diverse forest resources. Deforestation has resulted in a serious damage to biodiversity and gene resources (Nayar and Sastry, 1987). *Oroxylum indicum -1* tree is a native tree often grown as an ornamental for its strange appearance; it is distributed throughout the country up to an altitude of 1200m and found mainly in ravine and moist places in the forests (Bennet*et al*., 1992). Owing to the indiscriminate collection, over exploitation and uprooting of whole plants bearing roots, this valuable tree has become vulnerable in Karnataka and Andhra Pradesh and endangered in Kerala, Maharastra, M.P. and Chhatisgarh (Darshan and Ved, 2003; Jayramand Prasad, 2008) and is feared to become endangered soon in other states too.

It is an evergreen or partly deciduous tree to 5-10 m tall, and 15-20 cm in DBH. Young trees have a single stem and leaves are clustered at the top, making it look like a palm. The bark is thin grey-brown. The compound leaves are 60-170 cm long, with triangular-oval leaflets to 5 cm-13 cm long and 3-10 cm wide. The individual leaflets are 5-20 mm long and turn blue after drying. Inflorescences are cluster of flowers, 40-150 cm long, directed upwards from the tree crown. The funnel-shaped, fleshy and thick flowers, which open at night and have a bad smell, are 3-9 cm long and 1-10 cm wide. They have five wrinkled lobes and a light-yellow or red-purple colour. The fruit capsules grow upwards like the inflorescences and are dark-brown, linear and flat, 40-120 cm long, 5-9 cm wide, and 1 cm thick. The many white seeds are rounded and flat with a papery wing enclosing the 4-8 cm x 3-5 cm.

The tree 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 has pushed *O. indicum* to the list of endangered plant species of India. Destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threat to the survival and availability of this highly useful tree [Yasodha *et a*l., 2004]. Mishra and Kotwal, [2010], also found that due to overexploitation several valuable medicinal plants are becoming rare in their natural

habitat. Mishra (2011) also found that besides over harvesting.

Kingdom :Plantae
Family :Bignoniaceae
Genus :*Oroxylum*
Species :***Oroxylum indicum -1***
Telugu Name :Manduka-parnamu

FIG: *Oroxylum indicum -1*

The species of *Oroxylum indicum -1* (L.)Kurz belongs to the family Bigoniaceae. It is commonly called as broken bones tree, midnight horror tree, tree of Damocles, in Sanskrit as syonaka, sonapatha and in Telugu as Madukaparni.

Oroxylum indicum -1 (L) Kurz is a deciduous forest tree species found in IndoMalaysian region and China, in India it is distributed in the deciduous and mixed miscellaneous forests. The tree of *Oroxylum indicum -1-1* reaches up to a height of 12m with reddish purple flowers. The tree is a night bloomer and flowers are adapted to natural pollination by bats (chirepterophily). They form enormous seed pods that hang down from bare branches those long fruits curve downward and resemble the wings of a large bird or dangling sickles or swords in the night. Each pod is 1m long and encloses a large number (100 – 350) of flat and winged seeds. *Oroxylum indicum -1* lives in relationship with the actinomycetes *Pseudonocardia oroxyli present* in the soil surrounding the roots.

It is a deciduous medium sized tree, the pods, seeds, stem and root bark contains many flavones, weak acids and traces of alkaloids (Uddin et al., 2003; Dalal and Rai, 2004). This tree possesses flavonoids viz. Baicalein used to check proliferation of human breast cancer cell line MDA-MB-435 (Lam bertin, E., Piva, R., Khan, M.T.H., Bianchi, N., Borgatti, M. and Gambari, R. 2004). Dichloromethane extracts of the stem bark and root possess antimicrobial, antifungal, anti-inflammatory and anticancerous properties (Ali *et al*. 1998; Lambertini *et al*. 2004).

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-1* in southern India is 500 kg per annum (Ravikumar and Ved,2000). Owing to the indiscriminate collection, over exploitation and uprooting of whole plants bearing roots, this valuable tree has become vulnerable in Karnataka and Andhra Pradesh and endangered in Kerala, Maharastra, M.P. and Chhatisgarh (Darshan and Ved, 2003; Jayaram and Prasad, 2008). Hence there is an urgent need for its mass multiplication and conservation under *in vitro* culture conditions. Plant tissue culture offers

unconventional techniques for plant improvement. It has become an important tool for conservation and mass propagation of important tree species. Conventionally, *O. indicum-1* 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 there by multiply elite genotypes. *In vitro* regeneration of this tree has been reported (Dalal and Rai, 2004).

Medicinal uses:

It is used in the cure of several ailments including bronchitis, jaundice, piles, small pox, leucoderma, cardiac disorders, scabies, enlarged spleen, helminthiasis, gastropathy, heamorrhoids and cholera (Pal and Jain, 1998; Kyo et al., 1998). The plant is used as a constituent of ayurvedic preparation Dashmularisht (Yasodha et al., 2004). This species also constitutes one of the ingredients in *Chyawanprasha* (Ghate 1999; Parle and Bansal 2006). Leaves are emollient and contain anthraquinone and aloe-emodin (Prrotta, 2001; Nakahara *et al*., 2002). The fruits are used in treating bronchitis, leucoderma, helminthiasis (Parrotta, 2001; Dalal and Rai, 2004). The seed extract exhibits antimicrobial, analgesic, anti-tussive and anti-inflammatory properties (Rasadah *et al*., 1998). In general roots are used as astringent and for the treatement of tuberculosis (Bhattacharje, 2000). Decoction of root bark is effective to cure nasopharyngeal cancer (Mao, 2002). In India roots are used in Ayurvedic preparation called "Dasamoola" considered to be an astringent, anti-inflammatory, antihelminthic, antileucodermetic,antirheumatic,antianorexic and for treatment of leprosy and snake bite (Manonmani *et al*., 1995).

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Biological activity:

- Seeds, leaves, bark of stem and root are medicinally important due to the presence of flavonoids such as chrysin, Baicalein-7-0 glucoside, baicalein-7-diglucoside and tetunin, the 6-glucoside of Baicalin.
- In ayurvedic medicine it is administered as astringent useful in diarrhea and dysentery, bitter tonic to cure stomachache as anodyne to relieve body pains, edema, neuralgia, splenomegali, piles, jaundice and cardiac disorders.
- In Chinese herbal medicine the seeds are used as "muhudie" the tree butterfly.
- In Srilanka indigenous medicine, bark of the pecies is used as remedy for pains in joints or rheumatism.
- Isolates from root's bark showed significant reduction in gastric ulceration.
- Due to anti-oxidant activity it can reduce lipid peroxidation.

- The seeds showed high content of flavonoids, hence known to possess anti tumor potential.
- The extracts exhibit highest toxicity on all tumor cell lines and hence used relieve sore throat.
- They can act against persistent skin ulceration can also alleviate anxiety.
- Seed isolates can also induce conception in women.
- It is also used to cure smallpox, leucoderma, hermorrhoids.
- Oil extracted from dried seed is used in perfume industry.

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Tissue culture:

The theoreritical basis of tissue culture, in general was established as early as 1838-1839 in Schlieden and Schwann's cell theory. Haberlandet (1902) first attemted to prove this theory experimentally using monocotyledonous plant and though his attempt was unsuccessful; he elaborated the concept of *totipotency* that refers to the potential of an individual cell to regenerate into a whole plant. With the passage of time most of the pridictions in his paper of 1902 were confirmed experimentally and hence Haberlandet was regarded as "Father of Plant tissue Culture.

A large number of techniques developed over the last 150yrs have actually laid the foundation of the modern science in agriculture. Since, the last 50yrs development in agriculture has been greatly aided in general by the development of "Biotechnology" as mentioned earlier and "Plant Tissue Culture" and "Genetic Engineering" in particular (Brar and Khush, 1994).

The entire technology of "Plant Tissue Culture" is based on the ability of plant cells to be influenced by the surroundings and differentiate to give rise to a range of organs depending on the culture conditions. "Plant Tissue Culture" is the maintenance and propagation of plant parts as small as single cell, in axenic culture under controlled environmental conditions. In 1904, Hanning had initiated a new line of investigation, which later developed into an important applied area of *in vitro* techniques. Hanning (1904) cultured nearly mature embryos from seeds of several species of crucifers. Libacach (1925-1929) demonstrated the practical application of embryo culture in the field of breading.

For rapid propagation, hundreds and thousands of plants can be raised from small amount of callus cultured in a continuous process. But one of the serious drawbacks with this method is the production of genetically unstable plantlets. So Ball successfully raised transplantable whole plants of Lupines and Tropaeolum by culturing their shoot tips with a couple of leaf primordial.

Muir (1953) demonstrated that by transferring callus tissues of Tagetus erecta and Nicotiana tobacum to liquid medium and agitating the culture in a shaking machine, it was possible to break the tissue in to single cells and small cell aggregates. Later on Muir et al. (1954) also succeed in mechanically picking single cells from these shake culture as well as soft callus tissues, and making them devide by placing them individually on

separate filter papers resting on the top of a well established callus culture.

It is imperative that, viable strategies have to be taken to conserve the surviving population at least, the critically important medicinal species from further loss. Cultivation of medicinal plants is also difficult due to lack of standardized agronomic practices for the most species and unavailability of source plant materials. An alternative method is the micro propagation, which can produce large scale plantlets with in short time without any genetic variation (Sudha and Seeni, 1994). So, advanced biotechnological methods of culturing plant cells and tissues provided new means of conserving and rapidly propagating valuable, rare and endangered medicinal plants.

In recent years, biotechnology has emerged as a frontier branch of science increasingly being used in several areas. Biotechnological approaches are being employed to the production of secondary metabolites for pharmaceutical use. Plant tissue culture technique is being extensively used as cell cultures are the source material for the production and extraction of secondary metabolites (Constable and Tyler, 1994). Man has been exploiting these natural plant products for use in medicines, cosmetics, dyes, flavours and food.

So, plant tissue culture technology for a long time is contributing towards production of natural products via micropropagation, cell culture, scaled up bioreactors etc., (Akerele *et* al*.,* 1991). In fact, *in vitro* propagation and cryopreservation of medicinal plant help us to conserve biodiversity. Cryopreservation is reliable method for long term storage of the germ plasm of endangered species (Bramwell, 1990).

Tissue culture is thus proving to be useful in a variety of ways including plant propagation, raising and maintenance of high health status plants, Germplasm storage and a valuable technique in plant improvement. In plant improvement tissue culture may be used in the technique of gene insertion to improve plant stocks. Plant tissue culture has also been used in the production of secondary metabolites in plants. The production of flavours, sweeteners, natural colorants, as well as pharmaceuticals as achieved using tissue culture.

MATERIALS AND METHODS

Plant material:

Oroxylum indicum -1 (L) Kurz (seeds and cotyledonary nodes)

Chemicals:

The chemicals used during the course of the study were of analytical grade. Inorganic salts were obtained from Qualigens, Himedia, Merck, Loba and SD fine chemicals, INDIA. All vitamins and plant growth regulators were obtained from Loba Chemical co, INDIA. Agar Agar was obtained from Himedia and sucrose were obtained from Central Drug House (p) LTD, India.

Detergents

- Liquid soap (Cedepol)
- Tween-20

Surface sterilizing agents

- Mercuric chloride ($HgCl_2$)
- Hydrogen peroxide (30% H_2O_2)
- Sodium hypochloride (NaOCl)

Solvents

- Ethyl alcohol
- 1N NaOH
- IN HC1
- DMSO

Glassware

Test tubes (2.5 x 15 cm), petri plates (55 mm and 85 mm diameter), Erlenmeyer flask and beakers (100, 250, 500 and 1000 ml capacity), pipettes (2, 5 and 10 ml capacity) and measuring cylinders of all capacities (100 ml to 1000 ml were purchased from "Borosil"

INDIA. In addition glass screw capped bottles (6x15 cm) were also used for some experiments.

Plastic Ware

Micro tips (20-200 µl, 100-1000 µl and 500 to 5000 µl) were obtained from "Sigma Chemical Co" (USA) and plastic beakers of various capacities (50 ml to 1000 ml) were obtained from "Borosil" India.

Equipments used in plant tissue culture

- Laminar Air flow cabinet
- Autoclave
- Hot air oven
- Microoven
- pH meter
- Weighing balance
- Millipore unit
- Tissue culture racks
- Fluorescent tubes

Collection of plant material and maintenance

In the present investigation the selected plant *Oroxylum indicum-1* belonging to Bignoniaceae was collected from Srisalam forest region and was potted in garden of Departement of Botany, K.V.B Degree College, Kurnool, Andhra Pradesh. The seeds were inoculated on the MS basal medium. After the germination of the seedlings, the cotyledonary nodes, nodes and shoot tips were used as the explants for *in vitro* studies.

Washing and storage of glass ware

Major problem in tissue culture is contamination. Cleaning and maintenance oi glassware is the first step to begin the *in vitro* studies since tissue culture experiments conducted in a controlled and aseptic conditions, every article used in the experiments should be sterile.

Glassware is the main source for contamination. Hence it has to be thoroughly washed. The glassware was subjected to 40% chromic acid solution and sulfuric acid mixture for nearly 24 hours, washed thoroughly under running tap water. Later they were washed with Tween-20 solution and rinsed with tap water until soap traces were removed. Once again all the glassware was rinsed with distilled water and sterilized in hot air oven at 100°C for overnight after this the tubes were taken out and kept in closed area until use.

A separate cleaning technique was followed for contaminated vessels. Before cleaning the contaminated, vessels were decontaminated for twenty minutes at 15lbs pressure at 121°C in autoclave, in order to liquefy the agar and to kill any contaminants that may be present. The culture glassware is easier to wash after the spent medium has been liquified and removed. After scrubbing with a brush containing detergent Cedepol the glassware was rinsed repeatedly with tap water.

Media preparation and sterilization

Nutritional requirements for optimal growth of a tissue *in vitro* may vary with the species. Even tissues from different parts of a plant may have different requirements Un-satisfactory growth (Murashige and Skoog, 1962). There is: no single medium that is suitable for all types of plant tissues and organs. In the present investigation universal medium MS (Murashige and Skoog, 1962) was used. The composition of three different media is represented in the table.

The principal component of most tissue culture media can be categorized into inorganic nutrients (Macro and Micro), organic nutrients (Vitamins and amino acids), carbon source, plant growth regulators and gelling agents. Other organic supplements such as casein hydrolysate and coconut milk were also used.

Table: 1 Composition of MS medium

Components	Murashige & Skoog (MS) (mg/ml)
$MgSO_4.7H_2O$	370
KH_2PO_4	170
$NaH_2PO_4.H_2O$	--
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
$(NH_4)_2SO_4$	--
$Ca(NO_3)_24H_2O$	--
K_2SO_4	--
Inositol	100
Sucrose	30000
H_3BO_3	6.2
KI	0.83
$Na_2MO_4.2H_2O$	0.25
$CoCl_2.2H_2O$	0.025
$MnSO_4.H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	27.85
Na_2EDTA	37.25
Thiamine HCl	0.5
Pyridoxine HCl	0.5
Nicotinic Acid	0.5
Glycine	2.0

Macro nutrients:

Required amounts of macronutrients were freshly weighed and were dissolved one by one in double distilled water and to this appropriate amount of stock solutions were added one by one. Otherwise stock solutions were prepared at 5x or 10x concentration of the final volume. A separate stock solution of calcium salts (calcium chloride or calcium nitrate) was prepared to avoid precipitation.

Micro nutrients:

The micronutrients were prepared as stocks and were stored in refrigerator. Stock solutions of micro nutrients stock-1 (40x), stock-II (40x) and vitamins and amino acid stock (40x) were prepared by dissolving the constituents individually in a 500 ml flask over a magnetic stirrer and finally made up to one liter and stored at 4°C. The stock solution of Iron (40x) was prepared by dissolving Na_2EDTA in 50ml boiling double distilled water and to this added $FeSO_4.7H_2O$ gradually. The mixture was kept on magnetic stirrer in hot condition for 1 hour until the colour of the solution changes to golden yellow. Volume was made up to 100 ml and stored in amber colored bottle at room temperature.

Stock solutions for plant growth regulators:

The stock solutions of different plant growth regulators such as auxins, cytokinins and gibberellins were prepared at 0.01% concentration using suitable solvent made up to 100 ml using sterilized double distilled water in a volumetric flask. Depending on the

requirement appropriate amount of PGR stock was added to the basal medium. The stock solutions once prepared were not used after three months.

Carbon source

All the plant tissue culture media required the presence of carbon as energy source. Sucrose was used as the carbon source in the plant tissue culture: 3% sucrose was used in case of multiple shoot regeneration and 2% sucrose was preferred in case of callus induction.

Gelling agent

For solidification bacteriological agar at 0.8% was used in almost all experiments, for rooting 0.6%. Agar was preferred.

Preparation of 1 liter medium

For preparation of 1 liter medium 500 ml of double distilled water was taken in clean conical flask. Macronutrients were added sequentially one after another followed by the addition of freshly weighed sucrose and 2.5 ml of each micronutrients stocks to get desired concentration. Required quantity of desired PGRs was added at this stage. After addition of all constituents of media, the pH was adjusted to 5.6 to 5.8 using 0.1N NaOH or 0.1 N HCl. Then medium was made up to 1 liter, agar was added as per requirement and medium was kept in micro wave oven to melt the gelling agent. It was then dispensed into test tubes (15 ml) or culture bottles (50 ml) and tightly fitted with screw caps. Then the medium was autoclaved at 121°C at 15 psi for 15 minutes. All the plant growth regulators used during the course of the present work were added before autoclaving the medium. After sterilization based on the requirements tubes were kept in slant or in vertical position.

Sterilization of transfer area

Contamination is one of the main impediments in tissue culture studies. To overcome this problem laminar airflow chamber was used as a transfer area. Prior to commencing work the hands were washed with antiseptic soap followed by swabbing with ethanol the laminar airflow chamber was sprayed with ethanol and was swabbed with sterilized cotton. All requirements used in inoculation such as medium, forceps, scalpels, Petri plates, sterilized double distilled water, spirit lamp, cotton, spirit, surgical blades aluminum foils, cotton etc were transferred into the laminar airflow chamber and then the hood is closed, blower and fluorescent light was switched off and UV light was switched on for 30 minutes. UV light is Lethal to micro organism, it kills the organisms present in the laminar airflow chamber. UV light was switched off and airflow was switched on, the air flows with a velocity of 27 ± 3 m/sec Work in laminar airflow was started after 15 minutes of airflow, so as to remove the 03 which was built up in the chamber during radiation for 30 minutes. In order to minimize the contamination during inoculation hands and inoculating area was swabbed with alcohol frequently followed by flame sterilization of forceps and scalpel.

RESULTS & DISCUSSION

Zygotic embryo culture (seed culture):

Methodology

For zygotic embryo culture seeds were surface sterilized with 0.1% $Hgcl_2$, washed several times with sterile distilled water and were soaked for 24hrs. Seed coat was removed gently and isolated zygotic embryo (with cotyledons) and inoculated on ½ strength MS and MS basal medium with different levels of sucrose.

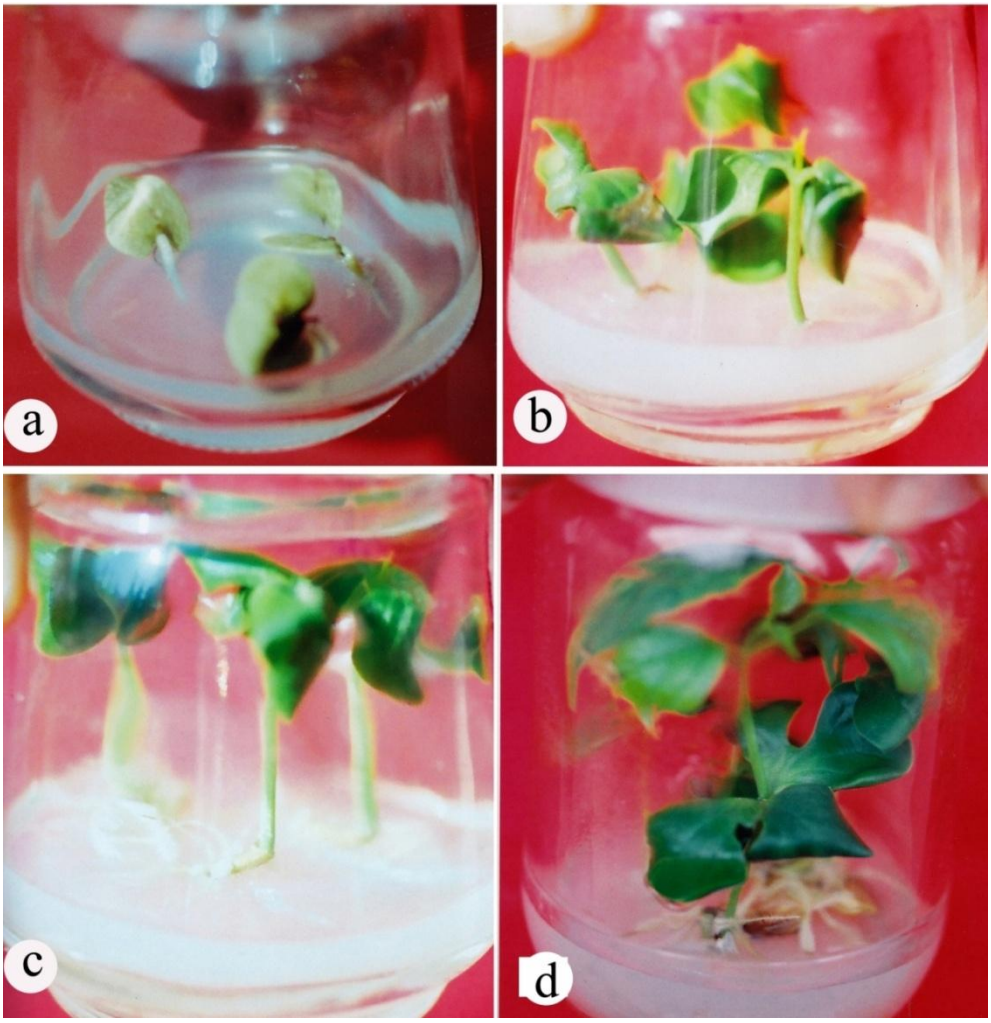

Fig: 1 *in vitro* seed germination of *Oroxylum indicum -1*

a) Germination of seed
b) Seedling
c) & d) seedling with health root & shoot system.

Table: 2 Effect of different type of MS media on zygotic embryo germination in *O.indicum-1*

Type of medium	Germination (%)	Days for germination	Morphology of seedling
MS+30g/L Sucrose	100	8	Healthy elongated shoot &root
MS+20g/L Sucrose	80	7	Dwarf roots
MS+15g/L Sucrose	60	7	Branched roots
½ MS+30g/L Sucrose	80	6	Branched roots
½ MS+20g/L Sucrose	60	7	Weak
½ MS+15g/L Sucrose	20	6	Weak

RESULT

Zygotic embryos were germinated within 7-8 days of incubation. Healthy seedlings with absolute percentage of germination was observed on MS medium containing 30g/L sucrose in comparison to all other concentrations sucrose levels used. Where as the percentage of germination was found to

be decreased at all the levels of sucrose on ½ strength MS medium.

Micro propagation:

Cotyledonary node culture

Methodology:

For cotyledonary node culture; the explants were used from *in vitro* grown seedlings. Healthy explants were selected. The cotyledonary nodes were trimmed and inoculated on MS medium supplemented with different concentrations of BAP.

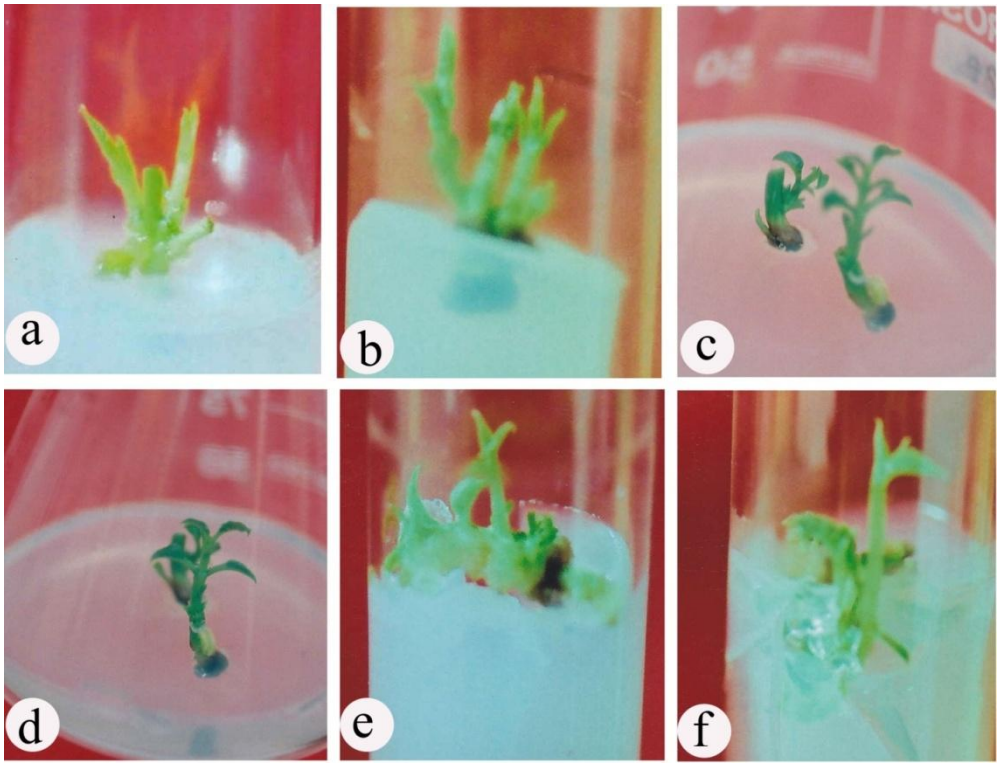

Fig: 2 cotyledonary nodal culture in *Oroxylum indicum -1.*

A) Shoot initiation from axils of cotyledonary node.

B, c, d, e&f multiple shoots production from cotyledonary node.

Table: 3 Effect of BAP on cotyledonary node culture in *O.indicum-1.*

Concentration of PGR(mg/L)	% of response	Average no.of shoots/explants	Average length of shoot (In cm)
1.0	30	4	2.1
2.0	40	6	2.8
3.0	60	6	3.2
4.0	80	8	3.5
5.0	100	20-25	4

Table: 4 Effect of KN on cotyledonary node culture in *O.indicum.*

Concentration of PGR(mg/L)	% of response	Average no.of shoots/explants	Average length of shoot (In cm)
1.0	20	3	2.2
2.0	30	5	2.9
3.0	45	6	3.0
4.0	70	8	3.2
5.0	90	15-20	3.5

Table: 5 Effect of TDZ on cotyledonary node culture in *O.indicum-1.*

Concentration of PGR(mg/L)	% of response	Average no.of shoots/explants	Average length of shoot (In cm)
1.0	10	2	1.8
2.0	20	3	2.3
3.0	40	5	2.8
4.0	60	7	3.0
5.0	70	12-15	3.3

RESULTS

The cotyledonary node explants were cultured on MS medium supplemented with different concentrations of BAP, KN and TDZ. More number of multiple shoots with maximum percentage of response was observed on MS +30gm/L Sucrose supplemented with 4.0-5.0 mg/L BAP within 12 days of incubation.

DISCUSSION

Among the various cytokinins used for the shoot tip culture BAP (Benzyl amino purine) was found to be the best medium, not only for the production of multiple shoots but also for the elongation of shoots.

In our present investigation of *in vitro* micro propagation studies in an endangered medicinal plant *Oroxylum indicum -1*, different *in vitro* culture techniques have been followed to establish an *in vitro* regeneration and mass multiplication protocol for the propagation and conservation of this endangered medicinally important tree species.

Among the various *in vitro* culture techniques employed such as *in vitro* regeneration through zygotic embryo culture (seed culture) and micro propagation through cotyledonary nodes have been used. For culturing various explants *in vitro,* different plant growth regulators, cytokinins in particular, have been used to test their effect on response of various explants. Each kind of explant has been cultured on MS (Murashige & Skoog's) supplemented with different cytokinins such as BAP (Benzyl Amino Purine), KN (Kinetin) and TDZ (Thiodiazuron) with varying concentrations ranging from 1.0 -5.0mg/L . When cotyledonary nodal explants were cultured on MS medium fortified with 1-5mg/L BAP, KN and TDZ individually, highest percentage of response with maximum number of multiple shoots (15-20) was found to be on MS medium fortified with 5mg/L BAP and KN, the no.of multiple shoots got increased on successive subcultures on to the same medium i.e on second subculture multiple shoot number was (30) and more than 40 shoots were resulted on third subculture.

Cotyledonary nodal explants responded well on all cytokinins with at least 3-4 shoots on each concentration of PGR used, while the seeds when cultured on MS and ½ strength MS medium supplemented with various concentrations of sucrose i.e MS medium containing 30g/L, 20g/L and 15g/L sucrose; ½ strength MS medium containing 30g/L, 20g/L and 15g/L sucrose, the best suitable medium for the germination of seed with healthy seedling bearing roots was found to be MS medium containing 30g/L sucrose while on other MS medium containing sucrose combinations, the time taken for the germination and nature of seedling was not satisfactory.

Thus through present study of *in vitro* micropropagation studies in *Oroxylum indicum -1,* we have established efficient protocols for propagation, conservation and mass multiplication of endangered species of *Oroxylum indicum -1* through seed culture and cotyledonary nodes.

CONCLUSION

Thus it can be concluded that through *In vitro* culture technology by culturing different types of explants such as zygotic embryos and cotyledonary nodes on various concentrations of PGR's particularly on cytokinins, protocols for *invitro* mass multiplication of an endangered medicinal forest tree *Oroxylum indicum -1* have been standardized which can be used for its propagation and conservation.

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