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In vitro callus induction studies on Abrus Precatorius L., A medicinal plant

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ABSTRACT

The present investigation deals with the high frequency callus induction studies on Abrus precatorius L., It belonging to family Fabaceae, an economically important multipurpose plant. The plant is scared with noval antidiabetic properties. The plant shows poor seed germination capacity (30%) due to hard seed coat and conventional vegetative regeneration methods are a complete failure. Therefore, the propagation of this plant by tissue culture techniques is an urgent need and well justified. Nodal intermodal and tendril explant were used for the callus induction studies. Axillary shoot proliferation on Murashige and Skoog's (MS) basal medium fortified with 2, 4 di chlorophenoxyacetic acid (2, 4-D) and Kinetin (Kin). In combination with auxins at different concentrations. The best callus induction was obtained with MS basal medium contain (2, 4-D 1.5 mg/l + Kin 0.5 mg/l) for node explants. Like in MS medium where (75%) of the inter node responded with (BAP 3 mg/l +kin 0.5 mg/l+ NAA 0.5 mg/l), at same time responsible with tendril leaf (84%) (2, 4-D 2.0 mg/l + Kin 1.0 mg/l). The results will facilitate the conservation and propagation of this important medicinal plant.

Keywords: *Fabaceae, In Vitro, callus induction recalcitrant, tissue culture.*

1. INTRODUCTION

Tissue culture is the process whereby small pieces of living tissue (explants) are isolated an organism and grown aseptically for indefinite periods on a nutrient medium under controlled condition. *In vitro* cultivation of plants is a necessary step in a large amount of experiments. Micro propagation, creation of virus free plants, genetic transformation, etc. (Georgieva *et al*., 1996). Nowadays five major areas of *in vitro* cell culture were currently applied and recognized as modern system for fundamental plant cell physiology aspects, generation of genetic modified fertile individuals, large-scale propagation of elite materials, preservation of endangered species, and metabolic engineering of fine chemicals. (Jhon *et al*., 2017).

A. precatorius, called Kundu Mani in Tamil and has been used in Siddha medicine for centuries. The white variety is used to prepare oil that is claimed to be an aphrodisiac (Mendes 1986). A tea is made from the leaves and used for fevers, coughs and colds. Seeds are poisonous and therefore are used after mitigation (Verma *et al.*, 2011). The Tamil Siddhars knew about the toxic effects in plants and suggested various methods which is called "suththi seythal" or purification. The protein is denatured when subjected to high temperatures which removes its toxicity (Dreisbach *et al*., 1987). The plant is also used in Ayurveda (Okoko *et al.*, 2010*)* and is said to promote hair growth. It is sometimes used as an ingredient in Indian hair products.

A. precatorius is best known for its seeds, which were used as beads and in percussion instruments, and which are toxic because of the presence of *abrin.* Ingestion of a single seed, well chewed, can be fatal to both adults and children. (Budavari 1989). The plant is native to India and grows in tropical and subtropical areas of the world. It has a tendency to become weedy and invasive where it has been introduced *A. precatorius* is a severely invasive plant in warm temperate to tropical regions, so that it has become effectively pan tropical in distribution. It had been widely introduced by humans the brightly colored and hard shelled seeds had been spread by birds.

The toxin *abrin* is a dimer consisting of two protein subunits, termed A and B. The B chain facilitates abrin's entry into a cell by bonding to certain transport proteins on cell membranes, which then transport the toxin into the cell. Once inside the cell, the A chain prevents protein synthesis by inactivating the 26 S subunit of the ribosome. One molecule of abrin will inactivate up to 1,500 ribosomes per second.

2. MATERIALS AND METHODS

In Vitro Propagation Protocols in *A. precatorious* L. were carried out through standard techniques at the Plant Tissue Culture Laboratory, Department of Botany National College, during the period (2016-2017).

Source and Choice of Plant materials

A. precatorious L.

One year old plants grown and maintained in the green house, Department of Botany National College, Inam Kulathur, keezlapatti, which was used as the source of explants.

Establishment of Cultures

Glassware and instruments

Borosil grade glassware's consisting of beakers, conical flasks, petridishes, standard flasks, pipettes, measuring cylinder etc. were used. Explants were cultured in wide neck Erlenmeyer's conical flask (100ml, 150ml, 50ml), culture tubes (150mm long and bottles ($20cm^3$, $60cm^3$).

All the glassware's were regularly cleaned to ensure free of contamination. Glassware's were thoroughly washed in running water using Labolene' detergent and finally rinsed with distilled water. The cleaned glassware's were placed in hot air oven at 100^oC to make them dry. The used culture vessels were autoclaved and washed following the earlier procedure after one use.

Accessories used like scalpels, forceps, spatula, needles, holder were made of stainless steel and were sterilized every time before use. The equipment and instruments used in the laboratory included Laminar Air Flow Chamber , Hot Air Oven, Refrigerator, Distillation unit, Electronic Monopan balance, Digital electronic pH Meter, Autoclave, Hotplate, Microscope, Rotary Microtome etc.

Basal Media

MS medium (Murashige and Skoog, 1962), B5 medium (Gamborg *et al*., 1968), with different constituents and concentrations were used.

Preparation of Culture Media

Standard procedures were followed for the preparation of media. The media Strength, plant growth regulators and other supplements used is depicted in the Stock solutions of major and minor salts, vitamins and growth regulators were prepared by dissolving required quantity of chemicals using distilled water. The stock solutions of nutrients were stored in amber colored bottles, vitamins in tightly lidded volumetric flasks and both will be kept in chilled conditions using refrigerator.

The stock solution in the required quantity were pipetted out into a standard flask containing distilled water. 3% sucrose and 100 mg L-1 myo-inositol, as per the specification of the media were added and dissolved in the media. All the plant growth regulators, additives for the different combinations were added before making up the media to the required volume. The pH of the medium was adjusted to 5.6 - 5.8 by using 0.1 N NaOH or 0.1 N HCL. Then 0.8% w/v difco bacterial grade agar (in the case of solid medium) was added to the medium and mixed well.

The medium was heated on a hot plate and stirred thoroughly, till it gets dissolved uniformly and the medium was poured into pre-sterilized culture vessels. From this 15 ml was taken in culture tubes (25 mm X 150 mm) and 50 ml was taken in flasks (250 ml). The culture tubes containing the medium were plugged tightly with non-absorbent cotton wool plugs and the flasks with autoclave lids and sealed with sealing film tightly.

Preparation of hormones

10 mg of NAA, 10 mg of BAP, 10 mg of 2, 4-D and 10 mg of kinetin were dissolved in 0.1N NaOH (0.3ml) and 0.1 N HCl (0.4 ml) respectively and diluted with distilled water to make up the volume to 100 ml and stored in the refrigerator. GA^3 hormone (10 mg in 100 ml) also prepared like 2, 4-D. These hormones were stable to be added before autoclaving the medium.

Sterilization

The sterilization of the culture medium was carried out in autoclave at 121^0C and 15 Lbs. pressure for 15 minutes. After sterilization, the culture tubes were stored in air conditioned culture room until further use.

All metal, glass instruments and other accessories used in inoculation chamber were wrapped in cotton blug and sterilized in an autoclave at 1.06 kg cm^2 pressure for 15-20 minutes at a temperature of 121^0 C. Scalpels , scissors, forceps etc. used were again dipped in alcohol and flamed on a spirit lamp at the time of use.

Explant Preparations and Surface Sterilization

The explants collected from the source plants were coarsely trimmed to a size of 3 cm and washed in running tap water for 5 minutes followed by washing in distilled water with a few drops of Labolene. After washing these explants in double distilled water, they were immersed in 0.1 % Mercuric Chloride solution and incubated for 5 - 20 minutes. The liquid was stirred by

swirling to give proper contact of chemical to the explant. The treated explants were washed in sterile distilled water for 3 – 4 times. After a final wash in sterile distilled water, the explants were spread on the pre-sterilized petridishes lined with sterile blotting paper inside a laminar airflow chamber. They were then trimmed finely to the appropriate size (1-1.5 cm).

Inoculation and Incubation

Single nodes (1.0-2.0 cm) and internodal segments (1.0-2.0 cms) were dissected out and all the inoculation operations were carried out under strict aseptic condition inside a Laminar Air Flow Chamber, which was made sterile by the incessant exposure of germicidal U.V. rays for half hour before use. All operations were carried out using pre-sterilized instruments and glassware. Explants were aseptically introduced into culture vessels in order to curtail contamination during drying and inoculation, only a few explants treated at a time.

The cultures were maintained in the culture room at $25\pm1C^0$ at under 16/8 hr. photoperiod of 2000 lux light intensity provided by white fluorescent tubes with 55%-60% relative humidity or in darkness, as per the treatment.8 replicates were made in each treatment and all trials were carried out three repeats.

Subculture

Cultures were regularly transferred to fresh medium or regeneration medium as per requirement after every 15-30 days.

Experiments & Observations

All experiments were conducted in 3 replicates twice and data on number of callus obtained through stem, node, and inter node, tendril leaf proliferation as callus induction.

Culture medium

The culture medium used to induce callus consisted of MS basal salts and vitamins supplemented with auxin (2, 4-D, IAA) alone or in combination with cytokinins (Kin, BAP).

Subculture

The Calli were subcultured at 15-30 days interval for proliferation.

Data collection and Presentation

Callusing Frequency

Cultures were scored for callus induction at the end of the fourth week (30 days) Frequency of callus induction was computed as the ratio of the number of explants responding to that of total number of explants cultured and the result was expressed as percentage as shown below.

$$\text{Frequency of response (\%)} = \frac{\text{Number of explants responding x 100}}{\text{Total no. of explants cultured}}$$

Nature Callus

Visual features of the callus developed were recorded and photographed.

Indirect Regeneration

Explants

Excised nodal segments, internodal segments, leaves (3.0-4.0cm) tender leaves (2^{nd} or 3_{rd}) from the apex and internodal segments (2.0-3.0cm) were used as explants for regeneration via callus.500mg of green compact calli was used as explants for indirect regeneration.

Culture medium

MS media was used as basal medium for callus induction Different combinations and concentrations of growth regulators such as cytokinins (BAP, Kin) and auxins (IAA, 2 4, D) were used.

Culture conditions

Cultures for callus induction were maintained in a light regine consisting of 16h light at a temperature of $25^{o} \pm 2^{0}$ C.

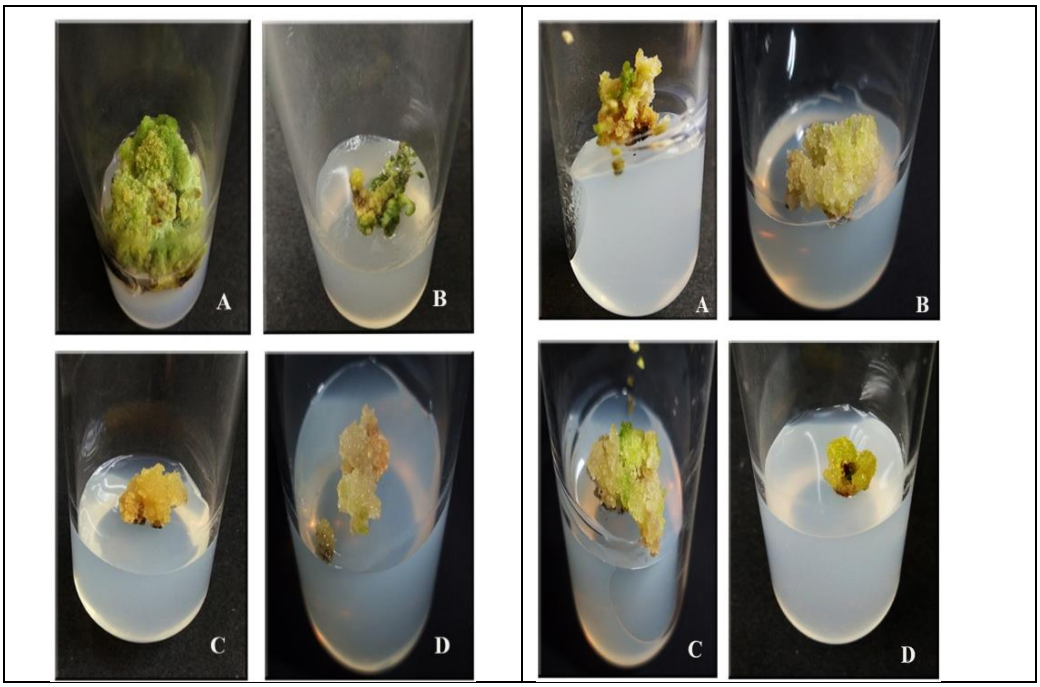
3. RESULTS AND DISCUSSION

The current study resulted in compact green callus formation from leaf explants culture either alone or in combination with {2, 4-D 1.5 mg/l+ KIN 0.5 (Table 1)}. Maximum number of callus obtained from 2, 4-D and Kin (83%), likewise MS medium supplemented with various combinations and concentrations of (BAP 3 mg/l +kin 0.5 mg/l+ NAA 0.5 mg/l) (75%) callus formation and (2, 4-D 2.0 mg/l + Kin 1.0 mg/l) (84%) took place on MS fortified with (2, 4-D 2.0 mg/l with Kin 1.0 mg/l, BAP 1 mg/l +Kin 1.0mg/l) after two successive subcultures. In this combination light yellowish green and nodular callus developed (Plate - 3). Callus was also induced in 2, 4-D and Kin supplemented medium. However 2, 4-D was found to be more effective than Kin for callus induction (Plate 2, figure A). According to (Preece *et al*., 1991), callus forms frequently at the basal cut ends of nodal explants on cytokinin enriched medium in species exhibiting strong apical dominance.

DISCUSSION

In the present investigation, micropropagation protocol of *A. precatorius* from node explants derived from about three month old plant was established. During the present investigation, effect of two cytokinins, auxin 2, 4-D and Kin were studied for nodal explant of *A. precatorius*. Axillary shoot proliferation from nodal segment containing axillary bud of *A. precatorius* compared to medium supplemented with BAP, Kin, and NAA. This result is contrasting with reports on leguminous species *Clitoria ternatea* L. and Gyana Ranjan Rout 2004, where BAP and Kin gave maximum callus proliferation.

The findings are in agreement with earlier reports on many woody tree species including *Onobrichis Altissima* Gross and *Abrus precatorius* L. (Aliheydar Kamalvand *et al*., 2013 & Animesh Biswas *et al*., 2007).



2.Micropropagation of *A. precatorius* from node explants (**A**) Compact Dark Green Callus, (**B**) Axillary shoot proliferation from nodal segment containing axillary bud of *A. precatorius* Green and light Green Callus. The above mentioned (C&D) were get sub cultured from (A&B).

Fig 3. Micropropagation of *A. precatorius* from Inter node explants (**A**) Friable Compact Green Callus. (**B**) Axillary shoot proliferation from nodal segment containing axillary bud of *A. precatorius* friable loose light Green Callus The above mentioned (C&D) were get sub cultured from (A&B).

Table 1. Effect of different concentrations and combinations of growth regulators on MS for callus induction in *A. precatorius* from nodal explants

Explant sources	Plant growth regulators (mg/L)				Percentage of Callus	Texture of Callus	Colour of Callus
	BAP	2,4-D	KIN	NAA			
Node	-	1.5	0.5	-	83.53±3.75	Compact	Dark green
	-	2.0	1.0		71.13±6.61	Friable & loose	Yellow & Green callus
	-	0.5	-	0.8	61.2±2.32	Loose	Light yellow colour
	2.0	1.0	-	-	49.56±4.86	-	-
	2.5	1.5	-	-	33.1±15.42	dry	-
Inter node	3.0	-	0.5	0.5	75.53±5.25	Friable & loose	Green & light green
	3.5		1.0	0.1	69.73±1.45	Friable compact	Yellowish colour
	5.0			0.5	23.7±2.60	Light Green	Light green &yellowish colour
	0.4	-	-	0.2	0.00	-	-
	3.0	2.0	-	0.1	0.00	-	-
Leaf	-	-	0.8	0.8	54.83±5.00	Green & light green	Light green
	-	-	1.0	0.1	84.36±4.02	Nodular callus	Greenish colour
	-	-	0.5	0.2	0.00	-	-
		0.6	-	2.0	0.00	-	-
	0.8	-	-	-	0.00	-	-

4. CONCLUSION

From the above study, it was concluded that shoot tip, cotyledonary node and inter node explants are suitable for clonal propagation of *A. precatorius*. Cotyledonary node explants may be used for their higher rate of callus induction. The protocol described in the present study is reproducible and can be used in future for further developments of the crop.

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