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EFFECT OF GROWTH REGULATORS ON *IN VITRO* CALLUS CULTURE OF *RICINUS COMMUNIS* LINN.

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The potentialities of cell and tissue culture for the biosynthesis of plant constitutes were recognized in early 1960's. The introduction and development of this technique has allowed the study of those problems previously inaccessible. Unorganised cultures of medicinally useful plants were established and multiplied on MS medium supplemented with suitable combinations and concentrations of growth regulators (2,4-D and IAA) using leaves as explants. The combination of 2, 4 dichlorophenoxy acetic acid (2,4-D) 1.5 mg/l and Indole-3 acetic acid (IAA) 1.5 mg/l were found to be most effective for the callus culture of *Ricinus communis*.

Keywords : 2, 4 dichlorophenoxy acetic acid (2,4-D); Growth regulators; Indole-3 acetic acid (IAA);. *Ricinus communis*; Tissue culture; Unorganised cultures.

Introduction

Ricinus communis Linn. is native to Africa. The hot water extract of the dried root is taken orally to treat diarrhoea, stomach ulcers and stomachache. This plant is used to treat asthma, respiratory diseases and stomach cramps. The entire plant is taken orally as a purgative[1]. This plant is used as a mosquito repellant. The seeds are a commercial source of Lipase and are used by soap manufactures. The oil of leaf and root are used against various ailments. The oil is useful for skin diseases. The oil of seeds have tonic effect[2].

An efficient micropropagation of *R. communis* was reported by Mederos[3]. For this purpose, apical and axillary bud explants of two months old plantlets were cultured on modified MS medium. A novel plant regeneration protocol was established by Ahn[4]. Mature seed-derived cotyledon explants produced adventitious shoots when placed on Murashige and Skoog (MS) medium containing thidiazuron (TDZ).

Higher plants are important source of pharmaceutically important compounds, but it is difficult to maintain proper supply of these plants due to environmental conditions. Technical and economic problem in cultivation, indiscriminate use for medicinal purposes of these plants has posed problem of fast exhausting plant resources. This forced man to find out alternative sources for the production of pharmaceutically important metabolites and if possible in higher amounts and at lower costs. The supply of plant material is less than half of the demand[5]. The importance of tissue culture lies in fast multiplication rate and its ability to produce true to type plants[6]. The plant tissue culture system could be used to get a wide range of chemicals such as polysaccharides, glycosides, amino acids, proteins, enzymes, lipids, steroids and flavonoids which could be used in manufacturing of antibiotics, vitamins, hormones, food flavoring and coloring, insecticides, antiviral agents, perfumes and emulsifiers. Recently, plant tissue cultures have created unprecedented opportunities for genetic manipulations of plants. The enormous versatility of plants, biological and environmental problems, exploitation, technical and economic costs associated with the conventional cultivation has led to various developments in plant cell and tissue culture technology to endeavor the enhancement of secondary metabolites of pharmaceutical importance[7].

Ricinus communis of family *Euphorbiaceae* is one of the most important medicinal plants of our traditional system of Ayurvedic medicine and also listed in priorities of modern research and drug system. This plant has got tremendous importance in pharmaceutical industries due to presence of many bioactive compounds which are used to treat many diseases and disorders[8]. The present investigation is aimed at development of a protocol for *in vitro* callus culture of *Ricinus communis*.

Material and Method

Leaves from mature plant, growing in gardens of

Table 1. Effect of various concentration of 2, 4-D and IAA on callus formation of selected medicinal plant on MS medium.

Name of plant	Explant	Age of callus	Concentration of growth regulators (mg/l)		GI
			2, 4-D	IAA	
Ricinus communis	Leaf	6 week	1.5	1.5	8.65±0.264
		6 week	-	2	7.89±0.306
		6 week	-	1.5	6.90±0.233
		6 week	-	1	2.31±0.131
		6 week	2	-	5.02±0.253
		6 week	1.5	-	1.50±0.112
		6 week	1	-	1.1±0.129

GI= Growth index,
mg/l= milligram per litre.
Mean ± S.E.

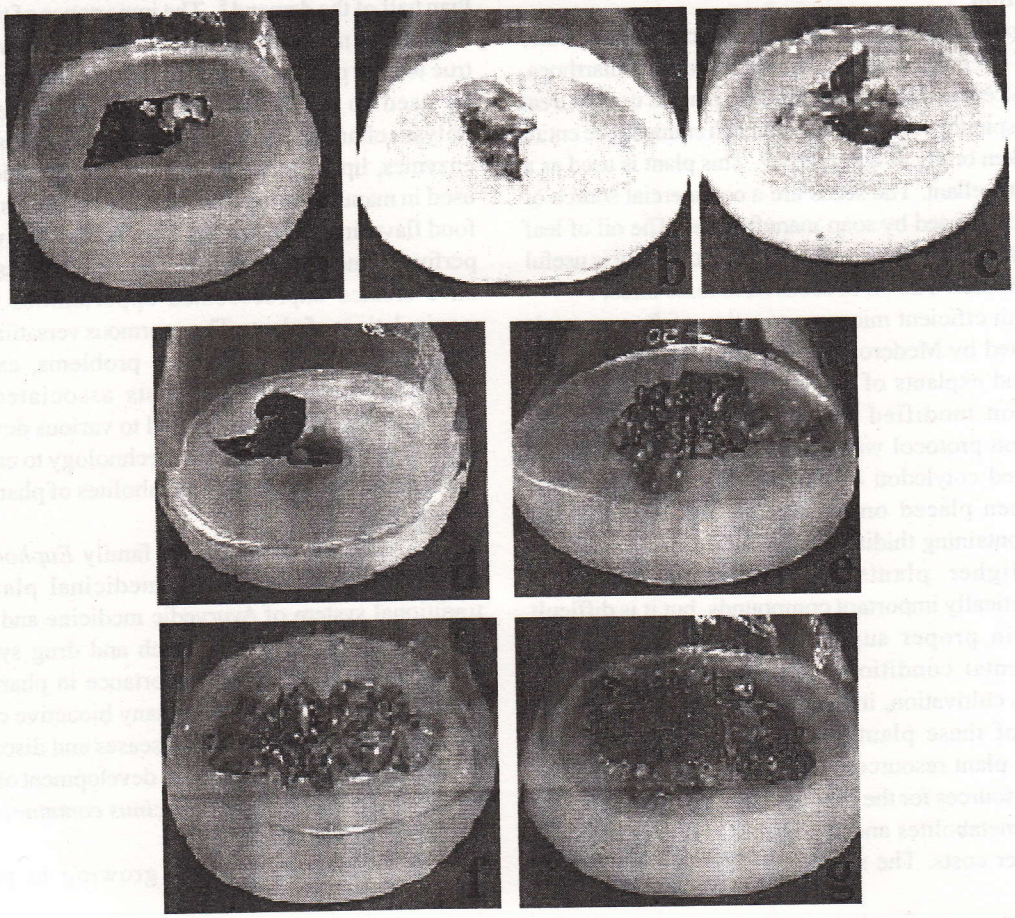

Fig.1 (a-g). Effect of various concentrations of 2, 4-D and IAA on callus formation of *Ricinus communis on MS Medium.*

Department of Botany, University of Rajasthan, Jaipur, were collected. The leaves were used as explants, washed for 30 minutes with 3.0% cedepol (commercial detergent) solution, surface sterilized with 0.1% $HgCl_2$ for 8 min. and finally rinsed thrice with sterile distilled water. About 1 cm segments of leaves were dried on sterile filter paper and cultured on MS medium (Murashige and Skoog, medium)[9] with 3.0% sucrose, 0.8% agar and 2,4 dichlorophenoxy acetic acid (2,4-D) (1 to 2 mg/l) and Indole-3 acetic acid (IAA) (1 to 2 mg/l) and pH of the medium was adjusted to 5.8 using 0.1 N NaOH before autoclaving. The cultures were grown under 24 h photoperiod with florescent light at 26±2°C. Calli produced were subcultured on previously described medium under similar physical condition. The growth indices (GI) of tissues were calculated at 6 weeks time intervals using formula-

$$GI = \frac{\text{Final dry weight of tissue - Initial dry weight of tissue}}{\text{Initial dry weight of tissue}}$$

Results and Discussion

The callus of *R. communis* was established successfully by using leaves as explants. Growth indices of tissues showed increase of callus of 6 weeks with different concentrations of growth regulators.

The morphogenetic response of the leaf explants varied with the plant hormones added to the MS medium. The leaf showed initiation of callus on MS medium supplemented with different concentrations of 2, 4-D in combination with IAA (Table 1).

In the present study best callus formation from the leaf (explant) of *R. communis* was achieved when cultured on medium with 2, 4 dichlorophenoxy acetic acid (2, 4-D) 1.5mg/l and Indole-3 acetic acid (IAA) 1.5 mg/l and maximum growth index was observed 8.65 in 6 weeks old callus of *R. communis* (Table 1, Fig. 1g).

Supplementation of 2,4-D alone (1.0 and 1.5 mg/l) showed poor response (GI 1.1; Fig. 1a and GI 1.5; Fig. 1b) as compared to 2 mg/l of 2,4-D (GI 5.02, Fig. 1c). However supplementation of IAA alone gave better results (GI 2.31 to 7.89; Fig. 1d-f).

For callus formation 2, 4-D and IAA, both are the most potent auxins that strongly antagonises development[10]. However, there are several reports of differentiation and organogenesis induced by these growth regulators [11].

On the basis of results it can be concluded that the concentrations of 2,4 dichlorophenoxy acetic acid (2, 4-D) 1.5mg/l and Indole-3 acetic acid (IAA) 1.5 mg/l were found to be most effective for the callus culture of *R. communis*.

Plant tissue culture has acquired large dimensions and has opened up new vistas as one of the most promising technique to study biosynthesis of natural plant products of commercial and medicinal importance [12].

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