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Research Article

An improved protocol for *In vitro* regeneration of *Ricinus communis* L.: A multipurpose plant

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Abstract

Ricinus communis L. (Euphorbiaceae) is a monotypic genus having economically great value. It is an oleaginous plant that can potentially be used as "bioindicator" owing to its large leaves. It has high medicinal value due to rich in secondary metabolites which are potential source of drugs. It shows anti-oxidant, anti-cancer, antimicrobial, hepatoprotective and many other medicinal properties. It has not only medicinal value but also great promises in the field of biodiesel production. Plant tissue culture is an important tool in plant biotechnology for an increase plant productivity and secondary metabolites production. Due to the geographical and seasonal variation and greater pressure from commercial sector there is need to conserve this plant species. The present piece of work deals with the callus induction and plant regeneration of *Ricinus communis* L. The maximum regeneration of plant were obtained on media supplemented with BAP in combination of TDZ by developed standard protocol. It was found most suitable for shoot multiplication from callus of apical node.

INTRODUCTION

Ricinus communis L. is a diverse and economically-important plant in the family Euphorbiaceae. It is spread throughout tropical regions and has vary greatly in its growth, habit and appearance. About 80% of world population is still dependent on traditional herbal medicines (Sharma *et al*., 2013). Approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant substances. *Ricinus communis* L. is also one of the highly medicinal plant. Leaves used as a remedy for skin diseases, diarrhea, urinary bladder infection(Naher K *et al*., 2012) and eye inflammation; fresh juice useful for treating jaundice. The root is useful in pains, fever, asthma and diseases of the rectum. Seeds oil of castor have unique chemical properties (Sujatha M, 1998) therefore commonly used for medicinal and industrial purposes. It is rich in secondary metabolites such as alkaloids (Jena J*et al*., 2012), flavonoids, saponins, glycosides(ricinoleic, isoricinoleic, stearic, dihydroxystearic acids, etc.) tannin and steroids which are potential source of drugs. Therefore it is widely used for treating and managing various diseases. Plant posses beneficial effects such as laxative, anti-oxidant, anti-ulcer, antidiabetic, anti-asthmatic, wound healing and has many other medicinal properties (Rana *et al*., 2012).

Ricinus communis L. has not only medicinal value but also great promises in the field of biodiesel production (Naz S *et.al*.,2011). The castor oil is mainly used in manufacture of various cosmetics, paints, transparent paper, printing-inks, lubricants, soaps, perfumery products, plastics, etc.(Ozturk *et al*., 2014).

Plants occupy a vital sector of health care system in India and represent a major national resource. Therefore, there is a need for conservation of diversity of medicinal plant by using the suitable strategy with most appropriate methods (Akshay KR *et al*., 2014).

Plant tissue culture is a biotechnological tool for an increase plant productivity and secondary metabolites production which are unique sources for development and synthesis of life saving drugs (Rafieian-Kopaei M., 2012). *Ricinus communis* is also rich in secondary metabolites(Sharma *et al.,* 2013); therefore it is potential source of drugs.

The *Ricinus communis* L. offers immense potential for improvement for disease and pest resistance, water and salt stress tolerant varieties through the application of tissue culture. Keep this thing in view present work was undertaken & focused to callus formation and shoot regeneration from nodal portion of *Ricinus communis* L.

MATERIAL AND METHODS

Source and Surface sterilization of explant:

Explants were collected from Botanical garden No.3, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and washed 2-3 times with tap water in laboratory. Surface sterilization of explant was carried out in the cabinet of laminar air flow. Explants were rinsed with sterile distilled water followed by 0.1% Mercuric chloride ($HgCl_2$) for 3 min. with continuous shaking then it was washed three times with sterilized distilled water. Finally all these explant were cut into small pieces and inoculated aseptically on MS medium supplemented with plant growth regulators.

Culture media and Growth conditions:

Full strength M.S medium (Murashige and Skoog, 1962) was used for callus induction and regeneration of *Ricinus communis*. It is supplement with 3% sucrose (Hi media Mumbai, India) ,0.3% Clerigar (Hi-media, Mumbai India) and different concentration of BAP (1.0 – 3.0mg/L) with combination of TDZ (0.5 and 1.0 mg/L) were used for callus induction. Polyvinylpyrolidone (PVP) was added for control the exudation of phenolic compound. The pH of the medium was adjusted to 5.8 before the addition of Clerigar. Culture bottles were filled with about 50 ml of the media and autoclaved at 15 lb pressure and 121℃ temperature for 15 min. The surface sterilized explants were incubated to callus induction medium. The cultures were incubated at 25± 2°C with 16 h photoperiod with the light intensity of 3000 lux under cool white florescent tubes. The humidity was adjusted to 65-70%. Each experiment was conducted in 5 replicates and repeated for 3 times. The color of callus, frequency of callus formation and shoot multiplication were observed and noted in the form of table after 30 days of culture.

RESULTS AND DISCUSSION

Surface sterilized explants were inoculated on MS medium supplemented with various concentrations of BAP (1.0-3.0mg/L) in combination with TDZ (0.5 and 1.0 mg/L). All combination of growth regulators were found more or less potent for induction of callus and plant regeneration (Table No.1). MS medium fortified with combination of BAP and TDZ showed development of callus and multiplication of shoot. Callus initiation was observed within a week of explant inoculation. MS medium supplemented with BAP (2.0mg/L) in combination of TDZ (0.5 mg/L) shown profused greenish color callus. Poor response for callus induction were observed on media supplemented with 1.5 mg/L BAP with 0.5 mg/L TDZ.

The maximum callus induction were observed on MS medium supplemented with BAP (2.0mg/L) and TDZ (1.0 mg/L) shown very profused light green color callus. As the concentration of BAP increases or decreases than 2.0 mg/L frequency of callus induction also decreases.

Multiplication of shoot were observed within 30 days of inoculation of the explant. High frequency of shoot multiplication were noted when developed shoots were further sub cultured on the same medium (Kumari. *et. al*., 2008).

In present experimental work it was observed that MS media supplemented with BAP in combination of TDZ more potent for callus induction from apical nodal explant of *Ricinus communis*. MS medium fortified with combination of BAP (2.0mg/L)+ TDZ (1.0 mg/L) were most suitable for maximum shoot multiplication from callus of apical node.Similar results for combination of BA with TDZ were obtained by Ahn Y. J. *et. al*.,2007. Root induction was observed on MS media supplemented with 2mg/L IAA alone.

Table No.1:- Effect of PGR's on Regeneration of *Ricinus communis* L.

Conc.of PGR's(mg/L)		Color of Callus	Frequency of Callus induction	Frequency of shoot multiplication	Mean ± S.E.
BAP	TDZ				
1.5	0.5	Greenish	+	+	7.4 ± 0.509
2.0	0.5	Greenish	++++	++	12.8 ± 0.583
2.5	0.5	Light green	+++	++	10.8 ± 0.374
1.5	1.0	Light green	+++	++	12.4 ± 0.509
2.0	1.0	Light green	+++++	+++++	21 ± 0.316
2.5	1.0	Greenish	++	+++	16.6 ± 0.6

Abb:- +: Poor; +++: Moderate; ++++: Profused; +++++: Very profused; BAP: 6- Benzyl amino purine; TDZ : Thidiazuron.

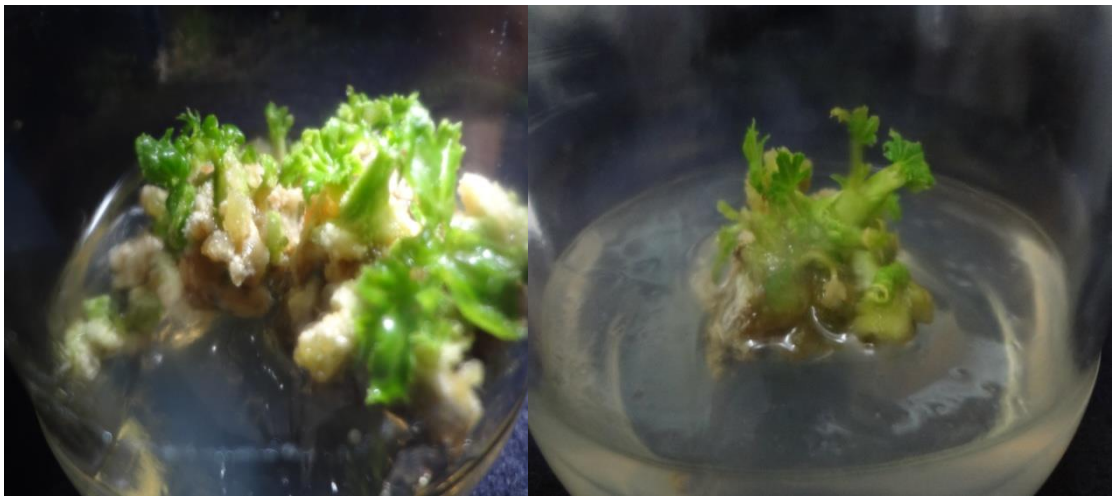

Photo Plate: Callus induction and Plant regeneration of *Ricinus communis* L.

CONCLUSION

The present piece of work deals with the callus induction and plant regeneration of *Ricinus communis* L have high medicinal as well as economical value. Therefore plant tissue culture methods would be beneficial in order to get standardized formulation from active compounds of plant both as qualitatively and quantitatively.

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