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HIGH FREQUENCY CALLUS INDUCTION AND SHOOT MULTIPICATION IN RICINUS COMMUNIS L.

Botany

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ABSTRACT

Context: *Ricinus communis* L. a monotypic genus of family Euphorbiaceae. It has high medicinal value due to rich in secondary metabolites which are potential source of drugs. It shows anti-cancer, anti-oxidant, hepatoprotective, antimicrobial and many other medicinal properties. Plant tissue culture is an important tool in plant biotechnology for an increase plant productivity and secondary metabolites production.

Results: The maximum callus induction was obtained on MS medium supplemented with 2,4-D(0.1mg/L), BAP (2.0mg/L) and KIN (2.0mg/L) alone from cotyledonary leaf. The combination of 2.5mg/L BAP+ 0.5mg/L NAA was found most potent for shoot multiplication from cotyledonary node.

Conclusion: The present study deals with the callus induction and plant regeneration of Ricinus communis L one of the highly medicinal plant. Therefore alternative propagation methods would be beneficial in order to get standardized formulation from active compounds both qualitatively and quantitatively.

KEYWORDS

Callus induction, *Ricinus communis*, Cotyledonary leaf.

Introduction

Ricinus communis L. is commonly known as Castor oil plant; which is one of the important medicinal plant of family Euphorbiaceae. It is spread throughout tropical, subtropical regions and also cultivated for its oil seeds. The plant varies in its growth and appearance.

According to the WHO about 80% of world population is still rely on traditional herbal medicines (Sharma *et al*.,2013). *Ricinus communis* is rich in secondary metabolites such as alkaloids (ricinine and N-demethylricinine) (Jena *et. al*., 2012), glycosides, tannins, flavonoids, saponins, phenolics and steroids which are potential sources of drugs. Therefore it is widely used for treating and managing various ailments and diseases. Leaves used as a remedy for skin diseases, diarrhea and fresh juice is useful for treating jaundice (K.M. Preeti, 2014). The root is useful in diseases of the rectum. Seeds oil of castor have unique chemical properties (Sujatha M.,1998) therefore commonly used for medicinal and industrial purposes. Castor oil is prescribed for infestation of intestinal worms. Plant possess beneficial effects such as anti-oxidant, antidiabetic (Rana *et.al.,* 2012), hepatoprotective, anti-fertility, anticancer, wound healing and many other medicinal properties. also has great promises in the field of biodiesel production (Naz S. *et. al*., 2011) which is eco-friendly.

The plant tissue culture is an important tool in plant biotechnology that consent 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). It facilitates the rapid propagation in controlled conditions which enhance the secondary constituents present in plant. Looking towards the importance of plant; tissue culture method for propagation would be beneficial in order to get standardized formulation from active compounds both qualitatively and quantitatively. The present work was undertaken & focused to callus induction and shoot multiplication by using different explant of *Ricinus communis*.

Material and Methods

Source of explants:

The plant material have collected from different localities of Dist. Aurangabad and authenticated from Herbarium "BAMU", Aurangabad. Seeds of Castor plant have sown in the earthen pots to get explants in Botanical Garden No.3, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Cotyledonary leaves and node were used as explants from 5-7 days old plantlets.

Surface sterilization of explant:

Explants were collected 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 washed with sterile distilled water followed by treatment with 0.1% Mercuric chloride ($HgCl_2$) for 2-3 min. with continuous shaking in front of spirit lamp and again washed with distilled water to remove the traces of mercuric chloride. Finally all these explants were excised into small pieces (1cm×1cm) and inoculated aseptically on MS medium supplemented with plant growth regulators.

Culture media and Growth conditions:

MS media (Murashige and Skoog.,1962) was used for callus induction and regeneration of *Ricinus communis*. It is supplemented with 3% sucrose (Hi media, Mumbai, India), 2.5gm/L Clerigel (Hi-media, Mumbai, India) and various concentration of Dichlorophenoxy acetic acid (2,4-D) , **6-**Benzyl Amino Purine (BAP), Kinetin (KIN) alone and in combination with auxins such as Naphthalene acetic acid (NAA), Indole-3-Butryic acid (IBA). Polyvinylpyrolidone (PVP) was added for control the exudation of phenolic compound. The pH of the medium was adjusted to 5.8 by 0.1N NaOH or 0.1N HCl before the addition of Clerigel. About 50 ml of media was poured in culture bottles and autoclaved at 15 lb pressure and 121C temperature for 15 min.

Surface sterilized explants were inoculated on this medium aseptically. These cultured bottles were transferred to culture room and 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 Number of days, frequency of callus formation and color of callus were observed and noted in the form of table after 30 days of culture.

Results and Discussion

Effect of different Cytokinins and Auxins on callus induction:

During the present research work maximum rate of callus induction and shoot proliferation was recorded after four weeks old cultures. Maximum callus induction was recorded on MS medium supplemented with 2,4-D, BAP and KIN alone and combination of BAP+ NAA and IBA while using cotyledonary leaf and cotyledonary node as an explant.

Effect of BAP on callus induction:

After the inoculation of explant on MS medium fortified with different concentration (1.0, 2.0….5.0 mg/L) of BAP alone callus initiation observed within 15 days. When MS medium supplemented with 2.0 mg/l BAP shown very profuse callus from cotyledonary leaf. In case of nodal explant MS medium fortified with 3.0 mg/L BAP induced profuse callus. As increase in the concentration of BAP (3mg/l) frequency of callus induction decreases.

Effect of KIN on callus induction:
MS medium fortified with different concentration of KIN (1.0, 2.0…..5.0 mg/l) shows more or less callus induction. Best results obtained on 2.0 mg/l KIN by using all explants but while using cotyledonary leaf explant induced very profuse whitish green callus and in case of the node explant resulted in profuse whitish callus.

Effect of 2,4-D on callus induction:
The effect of different concentration (0.05-0.25mg/l) of 2,4-D on callus induction shown in table no.1.Maximum induction of callus obtained on MS medium supplemented with (0.1mg/l) 2,4-D by using cotyledonary leaf as an explant. During present investigation frequency of callus induction was decreased with increases 2,4-D concentration.

Table 1: Effect of Different concentration of PGR's on Callus induction of *Ricinus communis* L

Explant	PGR' Conc. BAP - mg/L	Frequency of Callus	Color of Callus	PGR's Conc. KIN- mg/L	Frequency of Callus	Color of Callus	PGR's Conc. 2,4D - mg/L	Frequency of Callus	Color of Callus
Cotyledonary Leaf	1.0	+++	Green	1.0	+	Whitish green	0.05	+	Creamish
	2.0	++++		2.0	++++		0.1	++++	
	3.0	+++		3.0	+++		0.15	+++	
	4.0	+		4.0	+		0.2	+	
	5.0	+		5.0	+		0.25	+	
Node	1.0	+	Whitish	1.0	+++	Greenish	0.05	+	Creamish
	2.0	+++		2.0	++++		0.1	+++	
	3.0	++++		3.0	+++		0.15	+++	
	4.0	+		4.0	+		0.2	+	
	5.0	+		5.0	+		0.25	+	

Abb: - : No callus; + : Weak; +++ : Moderate; ++++ : Profuse; +++++ : Very profuse callus.

Shoot development and regeneration from callus:
In present piece of work MS medium enriched with different concentration of auxins and cytokinins were used for the regeneration and multiplication of shoot from cotyledonary node. During the experimental investigation all the concentration (1.0…..3.0mg/L) of BAP with NAA (0.5mg/L) and IBA (0.5mg/L) were observed more or less capable for shoot development and multiplication (Table No.2) from callus of cotyledonary node.

MS medium fortified with combination of BAP + NAA shown maximum induction of callus within 15 days of explant inoculation. MS medium supplemented with combination of BAP (2.5mg/L) and NAA (0.5mg/L) shown very profused whitish brown callus and multiplication of shoot 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 (Naz S. *et al.,* 2011).

MS media fortified with BAP (2mg/L) + IBA (0.5mg/L) and BAP (2.5mg/L)+ IBA (0.5mg/L) induced moderate light green color of callus but frequency of shoot multiplication was higher on MS medium fortified with BAP (2mg/L)+ IBA (0.5mg/L). Similar results were obtained by Goyal and Bhadauria (2007) in *Emblica officinalis* when nodal explant cultured on MS media shown minimum callus formation with maximum proliferation of shoots.

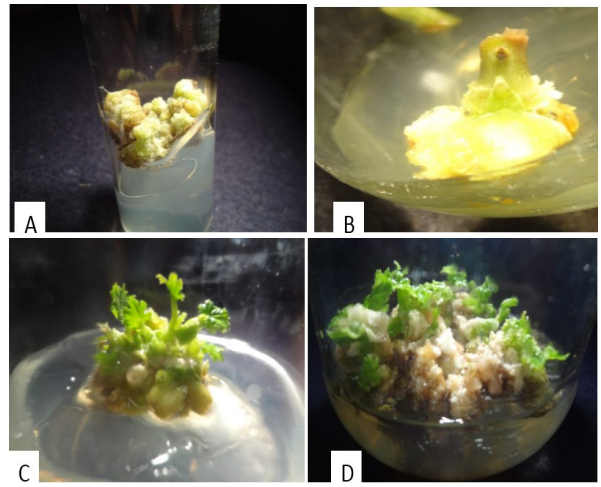
The well developed shoots were sub-cultured on MS medium enriched with 1.0mg/L of IAA for rhizogenesis.

Table No.2 :- Effect of PGR's on Shoot multiplication from cotyledonary node of *Ricinus communis* L.

Concentration of PGR's (mg/L)			Frequency of callus induction	Frequency of shoot multiplication	Mean±SE
NAA	IBA	BAP			
0.5	-	1.0	+	+	4±0.374
0.5	-	1.5	+++	+	7±0.509
0.5	-	2.0	+++++	++++	14±1.067
0.5	-	2.5	+++++	+++++	17±1.280
0.5	-	3.0	+++	+++	11±0.860
-	0.5	1.0	+	+	3±0.509
-	0.5	1.5	+	+	7±0.678
-	0.5	2.0	+++	++++	15±1.428
-	0.5	2.5	+++	+++	12±1.140
-	0.5	3.0	+	+	8±1.208

+ : Weak; +++ : Moderate; ++++ : Profuse; +++++ : Very profuse.

Photo Plate:1. Effect of PGR's on Callus induction and Shoot multiplication from different explants of Ricinus communis L.



A=Callus from leaf, B= callus from node, C & D= Multiple shoots from node

In present experimental investigation it was observed that MS media supplemented with BAP, KIN and 2,4-D alone more potent for callus induction from different explants of Ricinus communis. MS medium fortified with combination of BAP(2.5mg/l)+ NAA(0.5mg/l) were most suitable for maximum shoot multiplication from callus of cotyledonary node. Similar results were obtained by earlier workers. Mohajer et al.,(2012) reported that from obtained mean results BAP and NAA were more effective than BAP and IBA. on different explants of *Onobrychis sativa.*

Conclusion
The present study deals with the callus induction and plant regeneration of Ricinus communis L. one of the highly medicinal plant. Therefore alternative propagation methods would be beneficial in order to get standardized formulation from active compounds both qualitatively and quantitatively.

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