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MICROPROPAGATION AND CALLOGENESIS OF A RECALCITRANT SPECIES *RICINUS COMMUNIS*

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Abstract

The present study deals with the micrpropagation and callogenesis of *Ricinus communis*, a medicinally important but recalcitrant plant species. Different media were evaluated for the micropropagation and callus induction from different explants of the plant. The best response was obtained on MS medium supplemented with 18µm TDZ, where percentage of multiple shoot formation was 90%. The callus induction from cotyledonary leaves were tested using different media, the best response (90%) was obtained on MS medium supplemented with 2.5mg/l BAP + 0.5mg/l NAA where whitish brown friable callus with maximum proliferation was produced.

Introduction

The castor oil plant *Ricinus communis* is a member of the family Euphorbiaceae. The evaluation of this plant family is relatively unexplored except the sole member of the genus *Ricinus* which is traditionally called as castor bean (Wedin *et al*., 1986). Castor plant is a tough annual that may grow upto 6 to 15 feet in one season when full sunlight, heat and adequate moisture conditions are available. It prefers hotter areas with temperature range of 20-26ºC with frost-free winters. It may live for many years and become quite woody and tree-like. The large, palmately lobed leaves may be over 20 inches (Khafagi, 2007).

Castor seed is the source of castor oil, which has a number of uses. Castor oil is a viscous, pale yellow, non volatile and non drying oil with a bland taste. It has good shelf life as compared to other vegetable oils. The seeds contain 40 to 60% oil that is rich in triglycerides mainly ricinolein a toxic alkaloid ricinine and very toxic albumen called ricin (Dejey, 1975; Zohary, 1987). The seed coat contains ricin, a poison which is present in lower concentrations throughout the plant (Despeyroux *et al*., 2000).

Assessment of the antibacterial (Khafagi, 1998), antifungal (Salvador *et al*., 2003; Shariff *et al*., 2006), antiviral and cytotoxicity (Sokmen, 2001) produced by *in vitro* cultures in contrast with their corresponding adult plants has also been documented. Castor oil is prescribed for infestation of intestinal worms. Infusion of the leaves was used as a remedy for rash, itch and eye inflammation. The decoction of roots is used for skin diseases, diarrhea and troubles of kidney and urinary bladder (Boulos, 1983). *In vitro* antiviral activity and hypoglycemic activity were also reported from leaf extracts (Ayensu, 1978; Khafagi, 2007).

Riccinus communis has not only medicinal value but it also has great promises in the field of biodiesel production. It is inexpensive and environment friendly (Ogunniya, 2006). There are different varieties of castor oil bean and on the average they contain 46-55% oil by weight (Ogunniya, 2006). Plant regeneration through tissue culture technique would be a feasible alternative for improving the quality and production of Castor oil plant. Micropropagation is the best method available for the production of high quality plants which are free of any disease and pests ensuring the maximum production potential of varieties. Micropropagation therefore, can be used to produce a large number of plants that are genetically identical to parent plant as well as to one another (Raven *et al*., 1999).

Production of secondary metabolite by plant tissue culture is a new horizon for the scientists for the discovery of new but useful compounds produced by natural plant populations in very small quantities or it is also possible that these compounds may not be produced by the adult plants which are available in cultures (Borris, 1996). Comparing metabolites accumulated *In vitro* in some plants varieties monotypic genus *Ricinus* L., may be valuable for future biotechnological application aiming at raising its *in vitro* secondary metabolite production (Raven *et al*., 1999).

Looking at the potential and promises of plant tissue culture technology efforts has been directed for implementing this technology to improve the productivity of these plants. Keeping this in view present investigation was undertaken and work was focused to callus formation and micropropagation from different explants.

Materials and Methods

The seeds used as primary explants were taken from Botanical garden of Lahore College for Women University Lahore, Pakistan. The collected material was washed several times with tap water and with common house hold detergent to remove all the dust and particles from the surface of explant. The seeds were washed carefully and surface sterilized with 0.1% Mercuric chloride for 15min. These explants were also surface sterilized with 30% sodium hypochlorite solution for 20 minutes. Then several rinses were given with autoclaved water till the removal of traces of Mercuric chloride.

Seeds of *Ricinus communis* were cultured on MS basal medium (Murashige & Skoog, 1962) and the cultured explants were observed after inoculation and the data was recorded about the contamination, percentage of germination and number or frequency of regenerated plants per explants after given culture period. Then from germinated seeds, shoot tips and cotyledonary leaves were used as secondary explants for micropropagation and callogenesis respectively.

For micropropagation, shoot tips from *In vitro* grown seedlings were inoculated on MS basal medium supplemented with different concenterations of BAP and TDZ alone and also in their combinations. Cotyledonary leaves were also cultured on MS basal medium supplemented with different concentrations and combinations of 2,4-D and cytokinins to select the most suitable medium for optimum callus induction and proliferation.

Optimum temperature required for culture environment was maintained at 21 ± 2^{o}C. The cultures were incubated at 16-18 hrs light period (from cool white florescent tubes) and 6-8 hrs dark with light intensity of 2000-3000 lux.

A completely randomized design with 5 replicates was used for the experiment. Analysis of Variance (ANOVA) was applied on the data by using Costat V.63: Statistical Software (Cohort Software, Berkley, California). The mean values were compared with the least significant difference (LSD). This was done with the help of Duncan's new multiple range tests at 5% level of significance.

Results and Discussions

Seeds of *Ricinus communis* cultured on MS basal medium germinated within 9-12 days of inoculation (Fig. 1). The shoot tips and cotyledonary leaves of these seedlings were used as secondary explants for micropropagation and callogenesis. For micropropagation of the *Ricinus communis*, shoot tips of 2cm each, were cultured on the MS media with different concentrations of BAP and TDZ (Table 1). Combination of BAP and TDZ were also applied to see their effect. Results were analyzed on the basis of time for shoot formation and percentage for shoot formation. Medium MS+ BAP 3 mg/l+TDZ-1mg/l showed the best response after 12±0.632 days (Figs. 2 & 3). Maximum response 90% was shown by medium MS+TDZ 18µmol and 80 % in MS+ BAP 2 mg/l. MS+ BAP 2 mg/l appears to be the better medium for the micropropagation of *Riccinus communis* as compared to other media combinations applied (Figs. 3 & 4). A lot of work is still required for the development of a better media for the micropropagation of the castor bean plant.

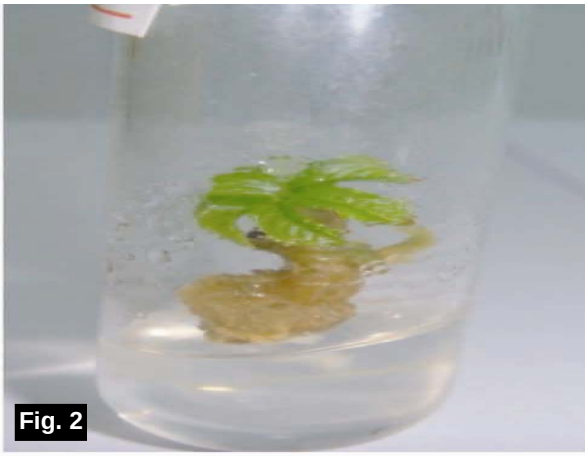
Table 1. Effect of different concentrations of BAP, TDZ and their combinations on micropropagation of *Ricinus communis*.

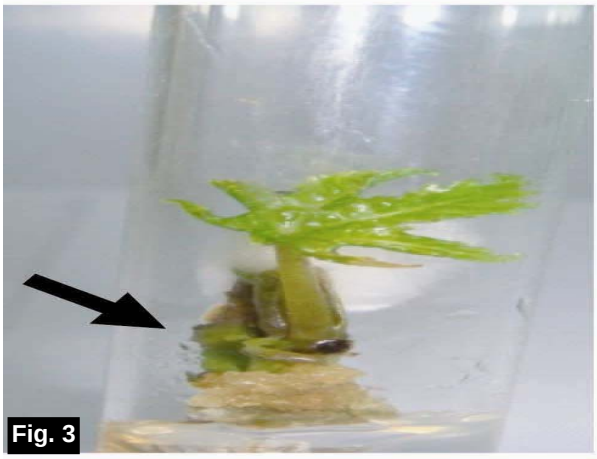
Media	Time for shoot formation (days)	Percentage of shoot formation (% age)
BAP 1.0mg/l	28 ± 0.392	30
BAP 1.5mg/l	20 ± 0.6321	40
BAP 2.0mg/l	14 ± 0.489	80
BAP 3.0mg/l	18 ± 0.848	65
TDZ 1µmol	30 ± 0.489	40
TDZ 8µmol	27 ± 0.632	50
TDZ 10µmol	24 ± 0.489	60
TDZ 18µmol	16 ± 0.692	90
BAP1mg/l + TDZ 1mg/l	20 ± 0.429	40
BAP2mg/l + TDZ 1mg/l	18 ± 0.848	50
BAP2mg/l + TDZ 1mg/l	15 ± 0.4	65
BAP3mg/l+ TDZ 1mg/l	12 ± 0.632	75
LSD	2.177	

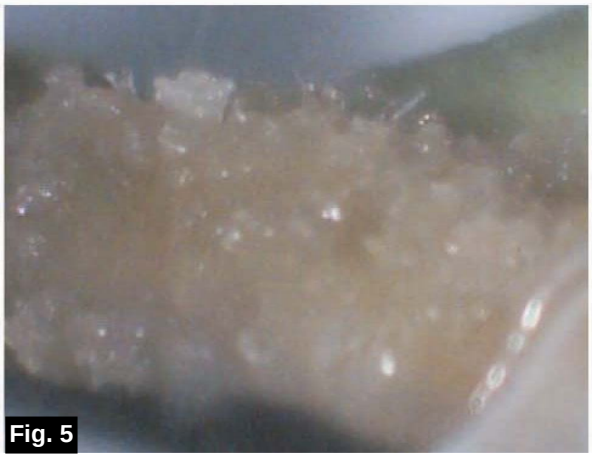
No. of test tubes cultured = 10.
Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ significantly at p=0.05 according to Duncan's new Multiple Range Test

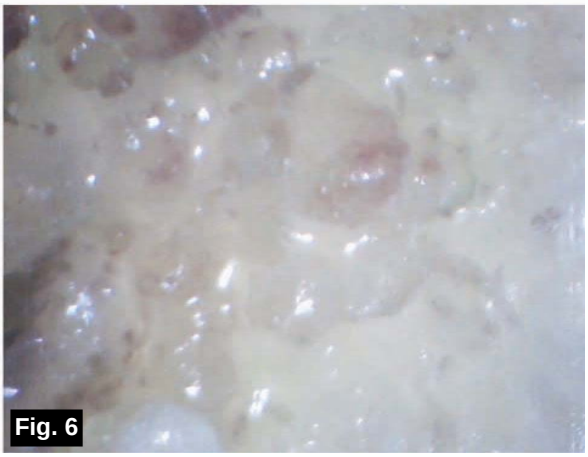
Mederos & Schobert (1995) reported the micropropagation of *Ricinus communis* by using apical and axillary buds of two months old plant. They observed the best growth of shoots on MS medium supplemented with 1000 mMol BAP and highest number of roots were observed in MS medium supplemented with 100 mMol IBA. In the present experiment both roots and shoots were induced in the same medium. Sujatha & Reddy (1998) worked on micropropagation of *Ricinus communis* with MS media supplemented with different concentrations of TDZ, Zeatin, Kinetin and BA. They were successful to get micro shoots with 1-10 mg/l TDZ and roots with 0.5mg/l BA. Ahn *et al*., (2007) developed a protocol for the development of *Ricinus* plants from hypocotyls by treating with TDZ and BA. Ahn & Grace, (2008) reported micropropagation of castor oil bean by using cotyledon as explants. Multiple shoots were originated on MS medium containing 5µmol TDZ. Then again MS+ 5µmol IBA was used for root induction.

Cotyledonary leaves from *in vitro* grown seedlings of *Ricinus communis* were cultured on MS medium supplemented with different concentrations and combinations of 2,4-D, BAP, NAA and kinetin. Different parameters of explants cultured, i.e., days for callus formation, test tubes showing callus formation, percentage (%) of callus formation, were observed. It is evident from Table 2 that the time for callus formation ranged from 10 to 30 days of inoculation. In treatment from the surface of explant. The seeds were MS+BAP-2.5mg/l+NAA-0.5mg/l, callus was initiated in 10 days. The percentage of callus initiation was 90% on this medium. Then characteristics of callus formed were compared and as clear from the Table 2 that MS+BAP-2.5mg/l+NAA-0.5mg/l was the best media for callus initiation of *Ricinus communis* as it produced brownish white friable callus (Fig. 5) and by addition of 2,4-D white friable callus was produced (Fig. 6).









Micropropagation of *Ricinus communis*
Fig. 1. Seed germination of *Ricinus communis* in basal medium.
Fig. 2. Shoot formation in MS medium+BAP 3 mg/l+ TDZ 1mg/l.
Fig. 3. Multiple shoot formation in MS medium+ TDZ 18µmol as shown by arrow.
Fig. 4. Micropropagation in MS medium+ BAP1mg/l.
Fig. 5. Callus formation in MS medium+BAP 2.5mg/l+ NAA 0.5mg/l.
Fig. 6. White callus in MS medium+2,4-D 3mg/l+ BAP 1mg/l+ NAA 1mg/l.

Kumari *et al*., (2008) demonstrated the effect of growth regulators on the callus formation of *Ricinus communis*. They were successful to obtain green organogenic callus of *Ricinus communis* on medium containing MS salts, B5 vitamins, 2mg/l BA and 0.8 mg/l NAA. They also achieved multiple shoot formation from that callus. They again used MS salts, B5 vitamins with 2.5mg/l TDZ, 0.4 mg/l NAA and 15 mg/l Glutamine for multiple shoot formation. For rooting they added 0.3mg/l IBA and 0.6 mg/l silver nitrate with MS salts and B5

vitamins. Khafagi, (2007) compared the callus induction, seed germination and seedling growth of two species of *Ricinus communis* and found that large seeded variety was better in producing callus and for seedling growth.

So keeping in view the medicinal significance of *Ricinus communis* and its recalcitrant nature, this study is especially helpful to micro propagate the plant on commercial level. Its callus is going to be a new horizon for scientists interested in the production of secondary metabolites from callus cultures and cell suspension cultures.

Table 2. Effect of different concentrations of 2,4-D, BAP, NAA and Kinetin on callogenesis of *Ricinus communis*.

Media	Conc. mg/l	Time for callus initiation (days)	Percentage of callus initiation (%age)	Characteristics of callus	
				Color	Texture
2,4-D + BAP + NAA	0.5+0.5+0.5	$20^{b} \pm 0.489$	40	Light brown	Less crystalline
	1+0.5+0.5	$18^{bc} \pm 0.848$	50	Light brown	Less crystalline
	2+1+1	$14^{cde} \pm 0.632$	60	White	Less crystalline
	3+1+1	12 ± 0.566	80	Brown	Crystalline
2,4-D + BAP + Kin	0.5+0.3+0.3	$20^{b} \pm 0.848$	40	White	Foamy
	1+0.5+0.5	$15^{cde} \pm 0.4$	85	White	Less crystalline
	1.5+0.5+0.5	$24^{a} \pm 0.632$	60	White	Less crystalline
	2+0.5+0.5	$30^{a} \pm 0.489$	50	White	Foamy
BAP + NAA	0.5+0.5	$20^{b} \pm 0.489$	30	White	Less crystalline
	1.5+0.5	$16^{bcd} \pm 0.692$	50	Brownish white	Friable
	2+0.5	$12^{ef} \pm 0.632$	60	White	Friable
	2.5+0.5	$10^{f} \pm 0.632$	90	White	Friable
LSD		1.91			

No. of test tubes cultured = 10.

Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ significantly at p=0.05 according to Duncan's new Multiple Range Test

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