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Effect of Explants and Plant Growth Regulators on Callus Induction in *Ricinus communis* L

Khadiga G. Abd Elaleem[*1], Magda Mohamed Ahmed[2] and Moawiya Khalid Mohammed Noor[1]
[1]Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, SUDAN
[2]Commission of Biotechnology and Genetic Engineering, National Centre for Research, P. O.Box 2404, Khartoum, SUDAN

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Abstract

The present research work aimed to shedding light on the effect of various plant growth regulator namely 2,4 dichlorophenoxy acetic acid (2,4-D) and α-Naphthalene Acetic Acid (NAA) on callus induction of ricinus commun. Callus culture were initiated from cotyledonary leaf and root segments explants from in vitro Ricinus communis L seedling . After four day of inoculation callusing was observed from cotyledonary leaf segment explants, after about one week from root segment explant. Auxins 2,4-D and NAA effecting in callus agitationbut 2.5 mg/L 2,4-D proved to be more effected for induction of callus in R. communis L. Individual treatment of NAA reveal low effectiveness for callus induction. Among all treatment media comprising 2,4 D (2.5 mg/l) showed superior response of callus degree (4 .0±0.0) with cotyledonary leaf segment explant and at this concentration, maximum amount of callus was observed whereas, root segment explants showed (2.7±0.19) degree of callus response in the same concentration . The callus from cotyledonary leaf segment explants was Light brown colour with compact nature in media fortified with 2,4 D while in media fortified with NAA callus was Light green colour with Friable nature. The callus from root segment explants was creamy to yellow colour with compact nature with the both tested auxin.

Keywords: 2, 4 dichlorophenoxy acetic acid (2,4-D), root segment, explant, callus induction.

Introduction

Castor (*Ricinus communis* L.), is an oil medicinal plant crop, dicotyledonous of the family *Euphorbiaceae* a Kumari *et al* [1].
Plant tissue culture is momentous technique in plant biotechnology, since we can produce large amount of callus and multiple shoot throughout this technique which use in phytochemical production[2-4].

There is many research about extraction of medicinal substance by regular extraction methods[5,6,7]. Extraction of medicinal component through callus produce high purified component. Callus have induced from different plant species using varied explant, media and growth regulator[8,9]. Callus induction from different medicinal plants have been founded under adequateness circumstances to enable creation of antimicrobials materials *in vitro*[10-12].

Callus induction from Ricinus plant have been established using different growth regulators, varied media and varied explants[1,13-18].

In this study, the aim was to setting up an affecting system for callus cultures from *in vitro* cotyledon leaf and root segment explant using MS media supplemented by two type of auxin namely 2,4 D and NAA.

Material and Methods

This study was carried out in the Laboratory of Plant Tissue Culture, department of Biotechnology and Biology, Faculty of Science and Biotechnology, AL Neelain University, Sudan.

Source of Plant Material: Seeds of castor (*Ricinus communis*) used in this study were obtained from the Khartoum state local market.

Sterilization of Equipment and Glassware's: All processes for *in vitro* culture were execute inside a sterilized horizontal cabinet with HEPA filters. The cabinet surface first sterilized by 70 % ethanol using wetted alcohol cotton, then sterilized by an ultraviolet light for at least 15 min prior to use. All tools, glass-ware and other adnexa were sanitized in autoclave at 121ºC with 15 psi for 15 min. Instruments like scalpel, forceps, and scissors were sterilized autoclaving and further by dipping in 70 % ethanol and flaming prior to use.

Sterilization of Plant Material: *Ricinus communis* seed was washed under running tap water until it cleaned, then treaded with 70% alcohol for 2 min and douched three times with disinfectant distiller water. Sterilized by shaken for 20 min with 20% colorx solution (containing 2.25 sodium hypochlorite) supplemented with few drop of liquid soap followed by three washes in disinfectant distiller water, disinfectant seeds were partially de coated and aseptically germinated in glass bottle

containing 30 ml media.

Media and Growth Regulator: Media: Murashige and Skoog (MS) medium[19]. Full strength was use in standard component, media were prepared by adding MS basal medium salt+ 3% sucrose and 7.0g\l agar. The pH of media was set to 5.8±0.02 before adding agar at concentration of 0.7% and then melted and dispensed in the tissue culture jar. These jars were then autoclaved at 121ºC for 15 minutes at 15 psi, and stored at incubation room.

Growth Regulators: Two type of auxin were used for callus induction, 2,4, - dichlorophenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA).

The powder of the appropriate auxins was weighed (100 mg) and dissolved in drop of 1N Na OH and the volume was winding up to 100 ml with sterilized dripped water stored in a refrigerator as stock.

Culture incubation conditions: Cultures were incubated in incubation room at 25± 2ºC with a photo period of twenty four hour dark for seed germination and callus induction, at 1000 lux light intensity provided by cool white fluorescent lamp.

***In vitro* Culture:** Seed from Castor (*Ricinuscommunis*) were used for micropropagation on MS media . The laminar airflow chamber was thoroughly cleaned with alcohol, under aseptic conditions, seeds were sterilized, then inoculated in MS media 30 ml media in each jar (2 seed/jar), culture jars were sealed and labeled carefully and finally kept in the incubation room under controlled condition of temperature (25±2 ºC) and 24h dark, the growth was monitored weekly.

Callus induction: Three weeks old micro plants obtained from *in vitro* culture are used for callusing. MS media was used as a basal media or supplemented with different auxin for callus induction, two explants, namely cotyledon and root segment explant were incubated in a culture jar (5x9 cm) containing 25 ml of media fortified with different concentrations (0.0,1.0,1.5,2.0 and 2.5 mg/l) of auxin 2,4-D and NAA, four explants per jar. Cultures were preserve in a growth room at 25°C±2°C temperature under 24h dark photoperiod.

Callus induction observed regularly and day of initiation was recorded. The final data was recorded after four weeks including callus percentage, callus color, callus texture and callus degree (callus degree was evaluated on scale of (0-6) where 0 for no callus, while 1-6 for increasing callus formation till three time the size of the original explants).

Statistical Analysis: The data of influence of growth regulators on callus stimulation were include day of callusing, callus percentage, callus texture, callus color and callus degree were recorded callus degree was statistically analyzed using (ANOVA) analysis, explained as mean ± standard error of Snedeco*r et al*. 20.

Results and Discussion

The surface sterilized seeds exhibited100%, sterilization and 95% survival rate (figure-1).

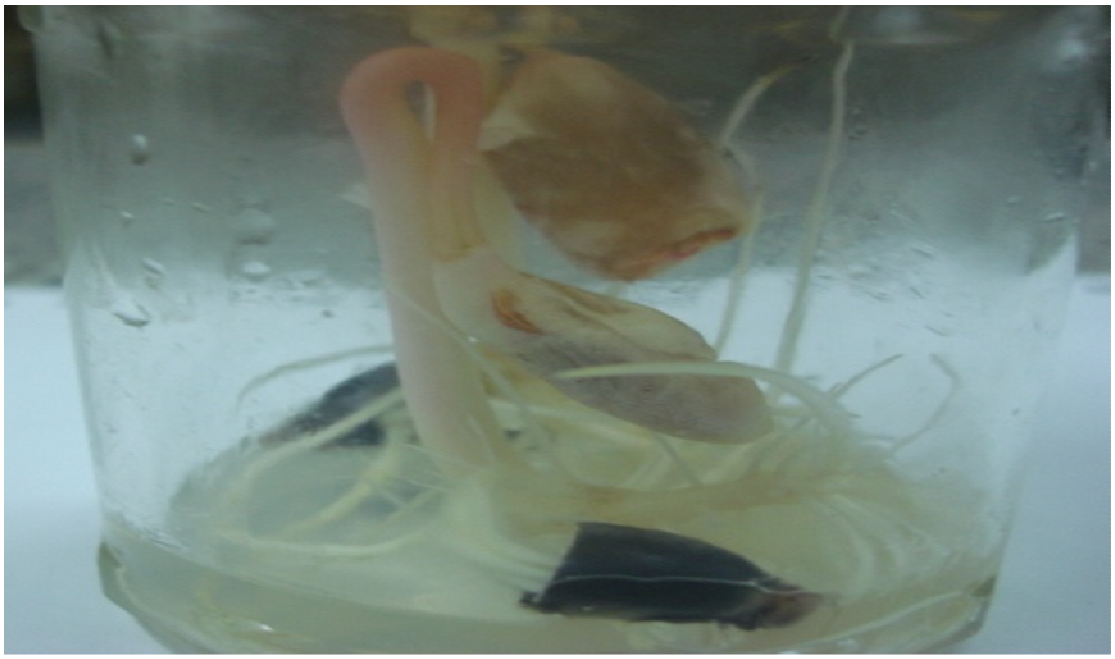

Figure-1
In vitro* micro plant of *Ricinus communis* Cultured *on MS media

For callus induction cotyledonary leaf and root segments explant prepared from *in vitro* micro propagation seedlings

then cultured on MS supplemented with 2,4-D and NAA at different concentration (0.0 mg/l - 2.5 mg/l), four explants per jar. MS media induced callus with all auxin type and concentration with the both explant. Data on day of callusing, callusing percentage, texture, color of callus and callus degree are presented in table-1.

No callus was obtained on MS media without growth regulators, generally MS media supplemented with various concentration of auxins (2, 4 D or NAA) originated 100% callus with in all concentration with the both explant, the root and cotyledonary leaf segment explants were taken from the three week old well established seedlings. After four day of culture maintain, callusing was viewed from cotyledon leaf segment explants, after about one week from root segment explant (figure-2). The cotyledon leaf explants viewed best response for callus inducement, this finding is in agreement with Kumari et al[1].

The highest callus degree (4 .0±0.0) was obtained in MS media fortifiedwith 2.5 mg/l 2,4, D by cotyledon explant (figure-3) during shortest period of time (4 day), , followed by (3.9±0.06) induced in MS media fortified with 1.5 mg/l 2,4, D with the same explant during (5 days). In concise with this result, in different plants, Potato plant, high quantitativeness of auxins (2,4-D) alone or in combination with cytokinins (BA or TDZ) at low concentration, was numerously used for callus induction by khadiga *et al* [21] and Mutasim *et al* [22], in trigonella plant by Khadiga *et al* [12], in *Abutilon indicum* L. plant by Rout*et al* [23].

On the other hand, maximum callus degree achieved when NAA used is (3.4±0.13), obtained in MS media fortified with 2.0 mg/L during 7 day by cotyledon explant, callus texture is friable and the color of callus is light green (figure-4).

Conclusion

In conclusion, call us was achieved from cotyledonary leaf and root segments explant of *Ricinus communis*, in vitro microplant. Maximum callus degree were induced by using 2.5 mg.l-12,4,D in short period of time . This protocol has capability for large latitude production of phytochemical and implementation plant rearing study programs.

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Table-1
Effects of different concentrations of 2, 4-D and NAA on callus induction from cotyledonand root segments explant culture on MS Media of the *Ricinus communis* L

Growth regulators mg/l	Explants	Day of callusing	%of callusing	Texture of callus	Callus color	Degree of callus
2,4 D 0.0	Cotyledon	0	0	0	0	0.0±0.0[j]
2,4-D 0.5	Cotyledon	5	100%	Compact	Light brown	3.1±0.15
2,4-D 1	Cotyledon	5	100%	Compact	Light brown	2.9±0.17
2,4-D 1.5	Cotyledon	5	100%	Compact	Light brown	3.9±0.06
2,4-D 2	Cotyledon	5	100%	Compact	Light brown	3.3±0.12
2,4-D 2.5	Cotyledon	4	100%	Compact	Light brown	4 .0±0.0
NAA 0.0	Cotyledon	0	0	0	0	0
NAA 0.5	Cotyledon	7	100%+Root	Friable	Light green	2.4±0.26
NAA 1	Cotyledon	7	100%+Root	Friable	Light green	2.5±0.21
NAA 1.5	Cotyledon	6	100%+Root	Friable	Light green	2.9±0.18
NAA 2	Cotyledon	7	100%+Root	Friable	Light green	3.4±0.13
NAA 2.5	Cotyledon	6	100%+Root	Friable	Light green	1.4±0.26
2,4-D 0.0	Root	0	0	0	0	0
2,4-D 0.5	Root	10	100%	Compact	creamy to yellow	1.4±0.15
2,4-D 1	Root	9	100%	Compact	creamy to yellow	2.1±0.27
2,4-D 1.5	Root	8	100%	Compact	creamy to yellow	2.6±0.18
2,4-D 2	Root	8	100%	Compact	creamy to yellow	2.7±0.19
2,4-D 2.5	Root	10	100%	Compact	creamy to yellow	2.7±0.19
NAA 0.0	Root	0	0	0	0	0
NAA 0.5	Root	10	100%+Root	Compact	creamy to yellow	1.2±0.09
NAA 1	Root	10	100%+Root	Compact	creamy to yellow	1.5±0.11
NAA 1.5	Root	9	100%+Root	Compact	creamy to yellow	1.5±0.11
NAA 2	Root	8	100%+Root	Compact	creamy to yellow	2.1±0.19
NAA 2.5	Root	8	100%+Root	Compact	creamy to yellow	2.4±0.15

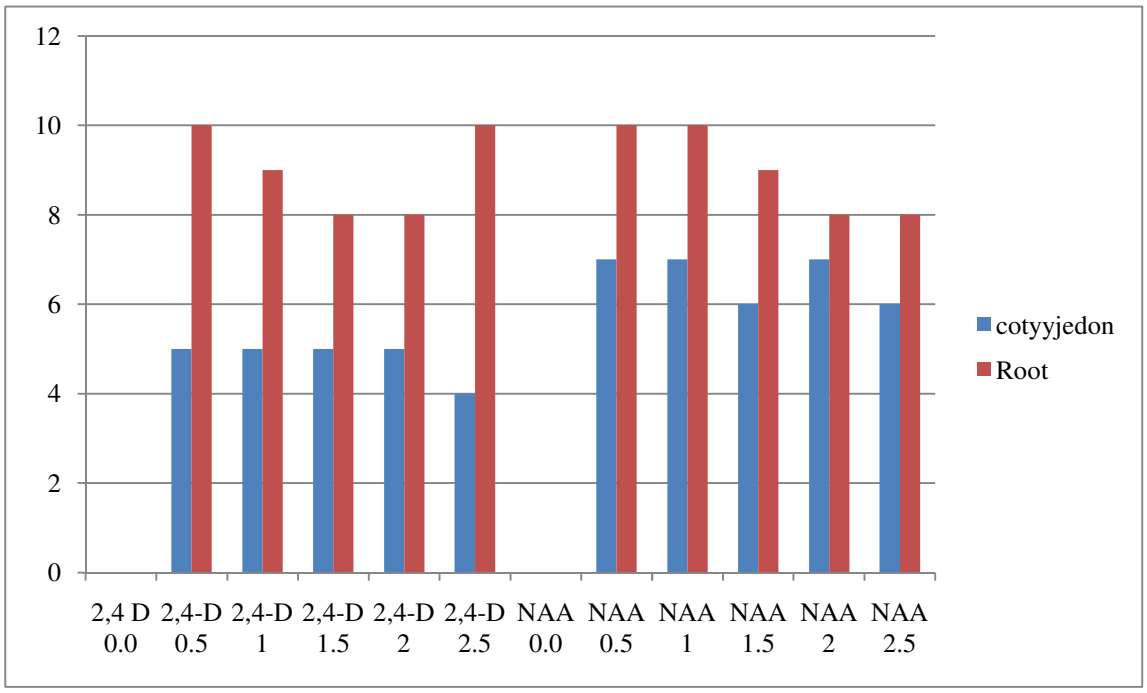

Figure-2
Effects of different concentrations of 2, 4-D and NAA on day of callus induction from cotyledon and root segments explant culture on MS Media of the *Ricinus communis* L

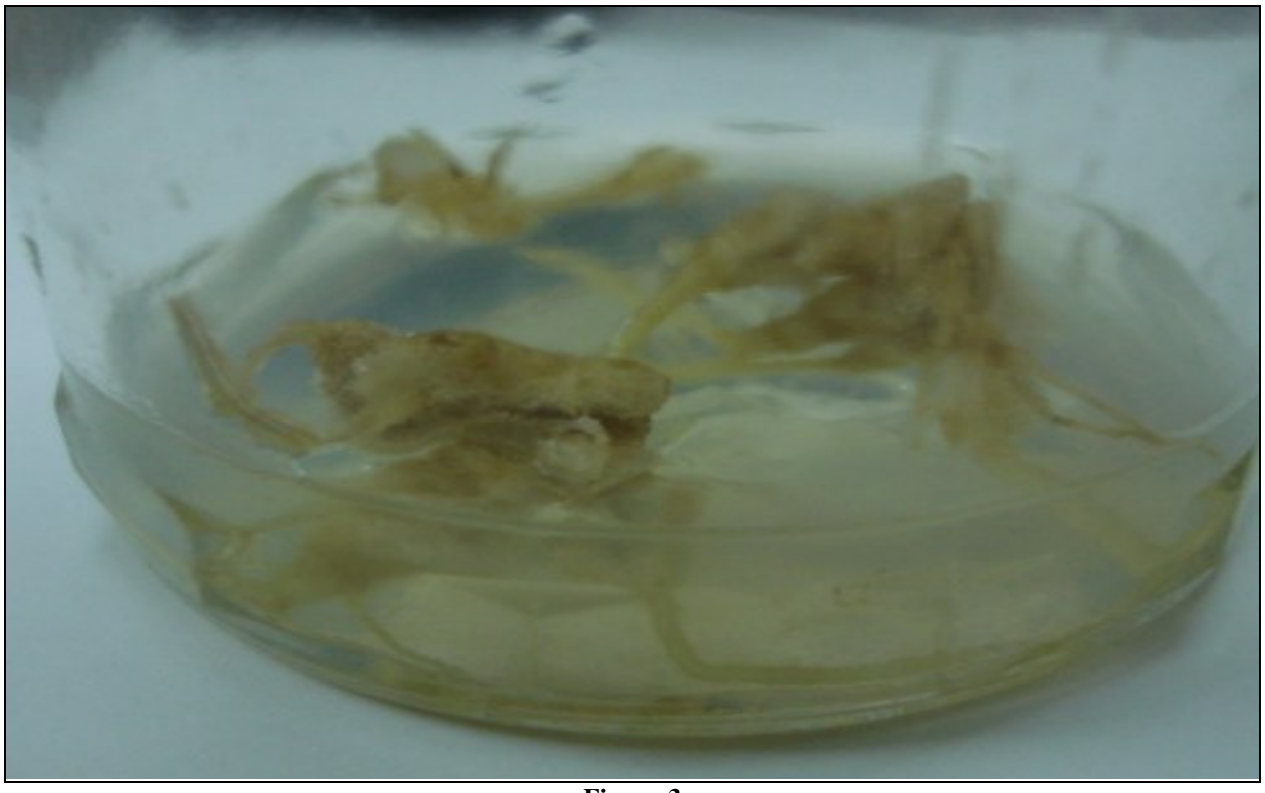

Figure-3
Callus formation from root segment explant in MS medium supplemented with 2.5 mg/l 2,4-D after four weeks of culture

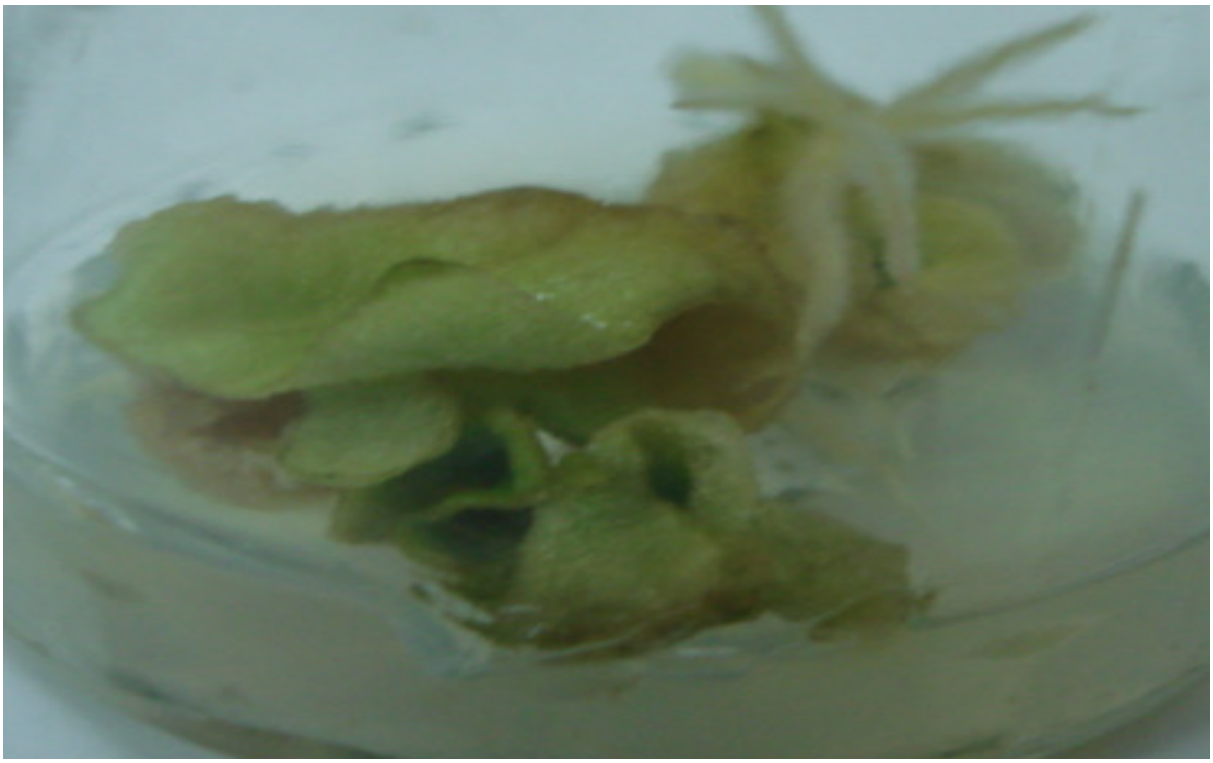

Figure-4
Callus formation from cotyledonary leaf segment explant in MS medium supplemented with 2.0 mg/l NAA after four weeks of culture

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