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Pathological study of contemporary Islamic movements in the view of Imam Khomeini

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A B S T R A C T

Fighting oppression and denial of arrogance has been a fundamental idea of the struggle of Imam Khomeini and has always been emphasized. Islamic movements are always exposed to serious damages. The present study is entitles as "Pathological study of contemporary Islamic movements in the view of Imam Khomeini" and has reviewed and explained the damages of Islamic movements in the view of Imam Khomeini using the available sources in the library and also has addressed to gather information using digital resources. In general we can say that the damages of Islamic revolution in the view of Imam Khomeini include: (1) peace of mind due to the feel of victory is the first danger, (2) The story of the battle of Uhud and the advocates of strait seeking trophy is a symbolic example in this context, (3) Trusting the enemy is another great damage, (4) the damages of bringing elements on the job who have committed themselves to America and the west, (5) Another methods of the west to tire and desperate people, (6) For the west restoring the elasticity and dictatorship to the revolutionary countries is a principle that prescribes all these dirty ways and methods.

Introduction

Calotropis procera commonly known as usher is an important medical shrub of family, Asclepiadaceae. It is a spreading shrub or small tree 4 m, exuding copious milky sap when cut or broken; leaves opposite, grey-green, large up to 15 cm long and 10 cm broad, with a pointed tip, two rounded basal lobes and no leaf stalk; flowers waxy white, petal 5, purple –tipped and with a central purplish crown, carried in stalked clusters at the ends of the branches; fruit grey – green, inflated, 8 to 12 cm long, containing numerous seeds with tufts of long silky hairs at one end (Brandes, 2005). It

was used in traditional medicine as anticancer ((Hallbook et al., 2011; Mathur et al., 2009; Newman et al., 2008; Oliveira et al., 2007; Quereshi et al., 2005 and Quaquebeke et al., 2005). The latex of *Calotropis procera* has been used in traditional medicine to treat different inflammatory diseases.

The anti – inflammatory activity of latex protein (LP) has been well documented using different inflammatory model (Lima-filho et al., 2010), to ability to active macrophages –effectors cells in inflammatory and immune responses (Lima-Filho et al., 2010; Ramos et al., 2009; Kumar and Roy,2009;Seddek et al., 2009; Mathur et al., 2009), antifungal activity of leaves, root and stem barks extracts (Hassan et al., 2006).Though, Calotropis is considered to be important medicinal plant , studying of callus induction are very limited (Amuthapriya and Ravichandran, 2014; Pankaj et al .,2013; Sundaramet al ., 2011; Fabiano et al 2011).

The plant tissue culture technique would be useful for callus induction , multiplication , extraction of highly purified phytochemical form of Calotropisgigantea.

The aim of the present study, is to establish protocol for callus induction from hypocotyl leaf explant of *Calotropis procera*.

Materials and Methods

This study was carried out at plant tissue culture lab **,** department of biology and biotechnology, faculty of science and technology, AL Neelain university,Sudan.

Source of Plant Material

Healthy seeds of *Calotropis procera* used in this study were obtained from wild plant grown plant at Aljazeera region, Sudan (Figure 1).

Media preparation

Murashige and Skoog (MS) medium (Murashige and Skooge, 1962). 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 prior to 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 preparation

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 (15 mg) and dissolved in drop of 1N Na OH and the volume was made up to 15 ml with sterilized distilled water stored in a refrigerator as stock.

Culture Incubation Conditions

Cultures were maintained in a growth room at 25±2.0ºC under cool white fluorescent light (1000 lux) and (16 h light/8 h dark) photoperiod regime for micro propagation and callusing.

Sterilization

Sterilization of Equipment and Glassware's

All steps for *in vitro* culture were carried out inside a horizontal laminar air flow cabinet

with HEPA filters (Figure 2). The hood surface was wiped clean with cotton soaked in 70 % ethanol, sterilized by an ultraviolet light for overnight prior to use. All instruments, glassware's and other accessories were sterilized 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 Seed

Seed were washed under running tap water, then treated with 70% alcohol for 1min, rinsed three time with sterile distilled water, then sterilized for 15 min in 25% Clorox solution (containing 5.25% of sodium hypochlorite) with few drops of liquid soap (tween twenty). Finally explants were rinsed five times with sterile distilled water, put in sterilized filter paper to remove extra water, then cultured on culture jars. Finally incubated in incubation room.

In vitro culture

Establishment of Sterile Culture

Various steps are followed for in vitro micro plant establishing , the laminar airflow chamber was thoroughly cleaned with alcohol, under aseptic conditions, seeds were sterilized, inoculated on MS medium (6 seeds/jar).Finally the jars were sealed, labelled carefully ,incubated at incubation room under controlled conditions of temperature (25±2ºC) and light (16 hrs. light, 8 hrs. dark) to establish sterilized culture , the growth monitored weekly . Micro plants from *Calotropis procera*is obtain used as source of explants for callus induction.

Callus induction using in vitro micro plant

Three weeks-old micro plants obtained from *in vitro* culture above were used for callus induction. The explants were inoculated according to the following Steps: The laminar airflow chamber was thoroughly cleaned with alcohol, under aseptic conditions, cotyledon explants segment were cutting, aseptically placed horizontally on the solidified medium in jars containing 25 ml of MS medium with different concentrations of growth regulator for the initiation of callus, four explants per jar. The cultures were grown at 25°C ±2°C temperature, for about four weeks. Data recorded included: day of callus initiation, percentage of callus formation, type of callus, callus colours and callus degree.

For callus induction cotyledon explants segment(5-10) mm in length with cut end surface contact with culture medium were placed on MS medium supplemented with different concentration of plant growth regulator. Twotypes of auxins were used to assess their effect on callus induction. Explants were cultured in culture bottles (5x9 cm) containing 25 ml of culture medium . MS as basal media or supplemented with different concentration of 2, 4-D and NAA (0.0, 0.5, 1.0, 1.5, and 2.0 mg/l).

All cultures were incubated in a growth room at 25 ± 2 ºC under cool white fluorescent light (1000 lux) and 16 h light/8 h dark.

The final data was recorded including day callus initiation, callus percentage, callus colour, callus texture and callus degree (callus degree was evaluated on scale of (0-4) where 0 for no callus, while 1-4 for increasing callus formation till four time the size of the original explants).

Statistical Analysis

Data of callus induction including day of callus initiation, percentage of callus

formation, callus texture, callus colours and callus degree were recorded. Callus degree was statistically analysed using analysis of variance (ANOVA) and presented as average ± standard error (Snedecor *et al*., 1967).

Result and Discussion

Sterilization of Equipment and Glassware's

All steps for *in vitro* culture were carried out inside a horizontal laminar air flow cabinet with HEPA filters and using sterilized plant materials, equipment, glass ware. The hood surface was wiped clean with cotton soaked in 70 % ethanol, sterilized by an ultraviolet light for at least 15 min prior to use. All instruments, glassware's and other accessories were sterilized 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 Seeds

Seed werewashed under running tap water, then treatedwith 70% alcohol for 1min, rinsed three time with sterile distilled water, then sterilized for 15 min in25% Clorox solution (containing 5.25% of sodium hypochlorite) with few drops of liquid soap (tween twenty). Finally explants were rinsed five times with sterile distilled water and then incubated under 16 h/day photoperiod, light intensity of 1000 lux and temperature about 25ºC ±2 ºC. 100% sterilization rate with 95% survival was archived within ten day.

Establishment of Sterile Culture

Micro plants from *Calotropis procera* was obtained for callus induction as source of explants using MS medium by the various steps, (Figure 3).

Callus Induction

Table (1) explained callus induction from *Calotropis procera* cotyledon segmentexplant. Murashige and Skoog (MS) medium verifiedwith 2, 4 D explained 100% callusing (Figure 5 and 6)MSmedium verified with 1.5 mg/l 2, 4 achieved 100% of callus after ten day, 2,4-D at all concentration achieved 100% of callus. NAA display varied percentage of callus (4 5%-75%) . The highest callus degree was (3.75±0.1) obtained on the MS medium supplemented with 1.5 mg/l 2,4-D, followed by (3.05±0.2) , obtained on MS media supplemented with 2.0 mg/l 2,4-D, the texture of callus is watery and color is creamy . Maximum callus induce by NAA was (1.00 ±0.2) obtained on MS media supplemented with 1.5 mg/l NAA. . There was significant variation on callus response between the two hormones , on the callus percentage, callus color, degree of callus.

Healthy seed were sterilized for 15 min in 25% Clorox solution (containing 5.25% of sodium hypochlorite), 100% sterilization rate with 95% survival was archived within ten day.

This study agree with (Haque, 2009; Sundaram 2010), in the using of Clorox solution for sterilization of calotropis seed. In vitro grown micro plant use for callus induction.Hundred percentage callus was obtained on MS media supplemented by 2,4,D auxin at all level of concentration. Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D), enduce callus has been reported inTrigonella foenum-graecum. (Khadiga et al .,2014).

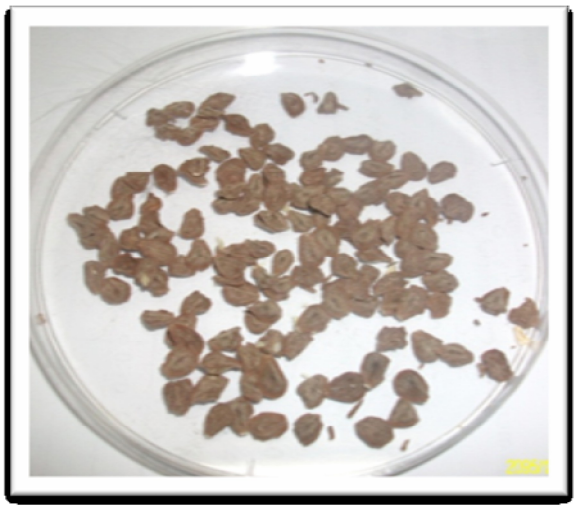

Figure.1: A) The seeds of *Calotropis procera (at left),* *B)* Plant of *Calotropis procera (at right)*

Figure (2) Callus induction Process

Figure (3)

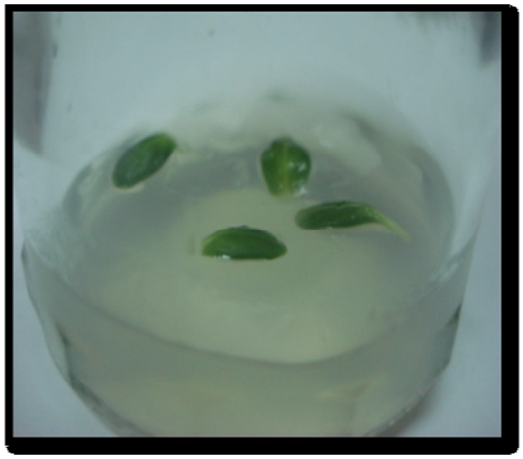

Figure (4)

Figure (3) Establishment of sterile culture microplant of *Calotropis procera* (on the left).
Figure (4) Cotyledon segment of *Calotropis procera*in MS media supplemented with auxin (on the right).

Figure (5)

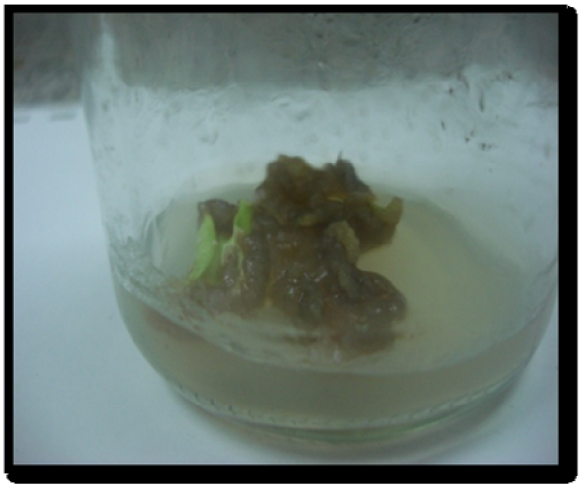

Figure (6)

Figure (5) Callus induction from *cotyledon segment explant of Calotropisprocera* on MS media supplemented with 2.0 mg/l 2, 4- D (on the left)
Figure (6) Callus induction from *cotyledon segment explant of Calotropisprocera* on MS media supplemented with 1.5 mg/l 2, 4- D (on the right)

Table .1 Effects MS media supplemented with different concentrations of 2, 4-D and NAA on callus induction from cotyledon leaf segment explant of the *Calotropis procera*

Growth regulators mg/l	Day of callusing	Percentage of callusing	Texture of callus	Callus colour	Degree of callus (Means ± SE)
2,4- D 0.0	-	0.0	-	-	0.00±0.0
2,4- D 0.5	15	100	Watery	Creamy	1.55±0.1
2,4- D 1.0	12	100	Watery	Creamy	2.35±0.2
2,4- D 1.5	10	100	Watery	Creamy	3.75±0. 1
2,4- D 2.0	16	100	Watery	Creamy	3.05±0.2
NAA 0.0	-	0.0	-	-	0.0 0±0.0
NAA 0.5	13	75	Friable	Green	0.75±0.1
NAA 1.0	10	45	Friable	Green	0.65±0.2
NAA 1.5	9	70	Friable	Green	1.00 ±0.2
NAA 2.0	8	55	Friable	Green	0.55±0.1

Callus was achieved from cotyledon leaf segments explant of *Calotropis procera* using in vitro microplant. Maximum callus degree was obtain by using 1.5 mg.l-12,4,D, followed by 2.0 mg /l 2,4,D in short period of time (10 and 16 day respectively). Callus induction from calotropis has been reported by (Sundaram, et al ., 2011). The effective of 2,4,D on callus induction with different plant species has been reported (Mutasimet al .,2010;Khadigaet al .,2014). This protocol can be apply for large scale production of medicinal phytochemical substance at highly pure state.

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