CALLUS AND CELL SUSPENSION CULTURE OF *CHELIDONIUM MAJUS L*

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ABSTRACT

ChelidoniummajusL has long history as a being useful for the treatment of many diseases in Asian and European countries. Aim of this study is to cultivatecallus and cell suspension culture*in vitro* using plant phytohormones. The proliferative capacity was tested on shoot and root explants, cultivated on *Murashige-Skoog* basal medium testing two auxins: 2,4-diclorphenoxiacetic acid (2.4D) and naphetelineacetic acid in combination with cytokinine: kinnetine (K). Calluses were developed on MS medium with 0.5 mg/l Kin, 0.5 mg/l IAA from root explants, as well when added with 0.5 mg/l NAA and 0.5 mg/l Kin from shoot explants. More biomassof cell suspension culture of shoot and root callus was accumulated on MS medium added with 0.1mg/l Kin.

KEY WORDS:auxin, cytokinine, microprogation, plant biotechnology

INTRODUCTION

*Chelidoniummajus*Lis only species of the tribe *Chelidonieae* of *Papeveraceae* family. The *papaveraceae*family is rich in specific alkaloids. 0.1-1% of alkaloids consists of benzylisoquinoline derivatives, which can be divided into three heterocyclic groups: benzophenantthridnes (e.g. chelidonine, sanguinarine, chelerythrine, nor chelidonine, isochelidonine, turkiyenine, among others), protoberberine (including coptisine, berberine,andstylopine)and asprotopine-type alkaloids (protopine, allocryptopine among others). Other types of alkaloids occur only in thraceamouts. At least 30 alkaloidal constituents have been identified in the drug. Coptisine is a main alkaloid (80-90%)in the aerial parts, while chelidonine is predominant in the underground parts of the plant. Dihydrocoptisine is considered as the predominant alkaloid in the fresh plant. Quantitativelysignificant are also known as sangguinarine, chelerythrine, berberine, stylopine, and chelidonine. The alkaloid level in the roots and rhizomes, which serve as the raw materials for commercial extracts, is relatively high up to 3%, according to some studies. Since chelidonine is the main alkaloid in these products, the labels should also indicate the qualitative composition of the main alkaloid fraction in addition to the total alkaloid content [11].Other substances structurally unrelated to the alkaloids were reported from the aerial parts, including several flavonoids, caffeic, ferulic and coumaric acids. Recently it was established that -four derivatives of hydroxycinnamicacids*Chelidoniummajus* L exhibit multiple biological actions, such as antiviral, antitumour, antibacterial and antifungal, as well anti-inflammatory effects. In the following part, these activities will be described with regard to the crude extract, the single alkaloids and the other

non-alkaloid components of the plants [8]. *In vitro* cultivation of chelidoniummajis L was initiated in order to evaluate the cell de-differentiationand re-differentiation as a conventionalalternative for plant biomass multiplication, the main source of bioactive compound with pharmaceutical value, anti-tumour activity [2-4]. A reliable highly efficient method for the regeneration of intact plants from *in vitro* cultures is essential for establishing a multiple micropropagation and genetic transformation protocol for*Chelidoniummajus* L. The plant regeneration of *Chelidoniummajus*Lsomatic embrogenesis using cytokinenes and auxins has been previous reported. (Kim et al.., 1999, vinterhalter and Vinterhalter, 2002, Woo et al.., 1996).

MATERIALS AND METHODS

Plant material

For preparing plant materials, seeds of *Chelidoniummajus* L were collected from mountain Bogd Khan in Mongoliaand stored at 4^0C. The seedssurface were sterilized with 70% (v/v) ethanol for 30s and 2% (v/v) sodium hypocholorite solution for 10 min, then rinsed three timesin sterilized water. Few seeds were placed on 25 mL of agar solidified culture medium Petri dishes. The basal medium consisted of MS salt and vitamin medium solidified with 0.7% (v/v) agar. The seeds were germinated in growth clamber at 25^0C under standard cool white fluorescent tubes with a flux rate of 35μmkl s^{-1} m^{-2} and 16-h photoperiod.

Induction of embryogenic callus

Shoots and roots of *C.majus* were cut into pieces approximately at size of 0.7x0.7cm, respectively, from plants grown *in vitro* that had been cut aseptically at the ends. Explants were placed on MS medium in 100x25 mm Petri dishes. The pH of the MS medium was adjusted to o 5.8 before adding phytoagar. For embryogenic callus and somatic embryo induction from the culture of shoots and roots the basal medium supplemented with different concentration 2.4D, IAA, Kin.

Table 1

Variants of MS medium for Callus induction

MS	mg/l		
	2,4D	IAA	KIN
1	1,5	0,0	1
2	0,0	1,0	1,0
3	0,5	0,0	0,5
4	0,0	0,5	0,5
5	0,0	2,0	1,5
6	2,0	0,0	2,0

The explants were cultured in each Petri dish for each treatment, 10 Petri dishes were sampled and data was combined. Cultures were maintained in a growth clamber in the in dark at 25^0C.

Induction of cell suspension culture

Cell suspension cultures of *Chelidoniummajus*L were prepared from long term callus cultures and maintained at Murashige and Skoog liquid medium with 0,1 mg/lkinetine and 2,0 mg/l naphtylacetic acid and 30 g/l sucrose. The cultures were maintained at 23^0C, 150 rmp, in light 650 lux (12h per day).

RESULT AND DISCUSSION

Chelidoniummajus L. has gained considerable attention because of its increasing use as a medical plant. The objective of this work was to develop an efficient*in vitro*multification method for *Chelidoniummajus* L. shoot explants, which is an efficient unconventional alternative for drug yielding plant.

The explants surface were sterilized and cultured on MS media containing different combination of two types of auxins: 2,4diclorphenoxiacetic acid (2.4D) and indolyacetic acid (IAA) and a

cytokinine: kinetine (K) in contrations of 0.5mg/l and 1.0 mg/l.

The beginning of callus proliferation took a place differently, depending especially on type of explants, concentration, and combination of growth regulators.

After 10 days of inoculation, callusing of the cultured explants was observed at the cut ends. The callus covered the entire explants for 3 weeks. Primary callus culture was obtained from shoot explants8 cultured on MS supplemented with 0.5 mg/l IAA and 0.5 mg/l Kin. After about 4 weeks, after cultures initiation.

Table 2

Characterization of callus cultured on different media

Regulator mg/l				Characterization of callus					
№	2,4 D	IAA	KIN	Root explants			Shoot explants		
				Color, appearance	Size(мм^2) M±m	Weitht (mg) M±m	Color, appearance	Size(мм^2) M±m	Weit ht (mg) M± m
Control media (MS)				-	-	-	-	-	-
1.	1,5	0,0	1	Brown, solid	8,28±0,58	22,8±1,27	Greensofty	6,4±1,42	9,8± 0,55
2.	0,0	1,0	1,0	White, softy	10,1±062	26,5±2,55	Gray yellow softy	10,03±0,52	9,3± 1,54
3.	0,5	0,0	0,5	Whitesolid	8,56±097	25,1±2,55	Gray yellow solid	6,08±1,55	10,4 ±2,0 6
4.	0,0	0,5	0,5	Green solid	10,34±1,5	27,3±2,22	Gray yellow softy	4,28±1,3	7,7± 1,24
5.	0,0	2,0	1,5	Greensofty	9,39±1,96	24,4±0,81	Greensofty	4,24±1,7	7,1± 1,22
6.	2,0	0,0	2,0	Gray yellow solid	6,37±2,32	25,3±0,23	Gray yellowsofty	5,22±1,3	6.3± 0,42

Callus proliferation from the tissues of most dicotyledonous plants is usually thought to require the presence of both an auxin and a cytokinin in the growth medium[1]. Generally, equally balanced combination of cytokinin and auxin (medium number-4) is used for callus grown more used other different combination these regulators.27.3 mg callus from shoot explants from medium MS adding 0.5 mg/l IAA and 0.5 mg/l Kin and 10.4 mg callus from root explants cultured media adding 0.5 mg/l 2.4-D and 0.5 mg/l Kin.

thesemediums are shown to be suitable then the others. *Our results were similar with results of SmaradaVantu (2011) and Sook Young Lee (2011).*

The growth of the cells in a suspension culture was monitored by the measuring of the cell fresh or dry weight. Experiment was lasted for 35 days and results are summarized in table 3. The fast growth of biomass was observed during the first two weeks of sub cultivation due to cell division and cell enlargement after the 14th day, likelyafter startof the stationary phase of growth on the 21st day.

Table 3

Growth parameters ofsuspension culture of *chelidoniummajus* L.

№	Kin 0,1	NAA 2.0	Period of cultivation, day	Number of cell in 1 ml suspension, x10^6 M±m
1	0,1	2.0	3	4,50±0,36
			7	9,66±0,19
			10	12,78±0,21
			14	14,93±0,33
			17	21,55±0,36
			21	23,33±0,19
			24	20.93±0,21
			28	19,93±0,33

Table 4

Effect of grown regulators on biomass of cell suspension culture of *Chelidoniummajus*L

№	Media	K	NAA	Biomass of cell suspension			
				Cell biomass of shoot callus		Cell biomass of root callus	
				Fresh weight,mg M±m	Dry weight, mgM±m	Fresh weight,mg M±m	Dry weight, mg M±m
0.	MS	0.0	0.0	25	1,75	33	3.21
1.	MS	0,1	2.0	142.5	7.8	285	9.27
2.	MS	0,5	1.5	112.5	6.9	186	8.38
3.	MS	1,0	1.0	52	4.78	65.4	5.4
4.	MS	1,5	0.5	35	2.45	45	4.47
5.	MS	2,0	0.1	33	2.33	43	4.21

We have isolated 7.8 mg dry biomass from shoot cell suspension culture, 9.27 mg dry biomass from root cell suspension culture cultured on liquid MS medium added with 0,1mg/lKinand 2,0mg/lNAA.

CONCLUSIONS

1. Suitable medium for cultured callus were MS medium added with 0.5mg/l Kin and 0.5 mg/l IAA from shoot explants as well 0,5mg/l 2,4D and 0,5мг/л Kin from root explants, respectively.
2. In order To choose the sub cultivationperiod of cell suspension culture, the fast growth of biomass was observed during the first two weeks of subcultivationdue to cell division and cell enlargement after the 14th day likely after start of the stationary phase of growth on the 21st day
3. We have isolated 7.8 mg dry biomass from shoot cell suspension culture, 9.27 mg dry biomass from root cell suspension culture cultured on liquid MS medium added with 0,1 mg/l Kinand 2,0 mg/lNAA

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