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Research Notes

Mucilage Production In Tissue Culture of *Plantago lanceolata*

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ABSTRACT

Leaf and root explants from 10 to 20 day– old seedlings of *Plantago lanceolata,* were cultured on MS, MSH, NT and B5 basal media with various concentrations of different plant growth regulators. The best callus induction was obtained with 0.4 mgl^{-1} 2,4-D in MSH I with 400 callus index. Callus growth was significantly stimulated (P=0.05)with 0.8 mgl^{-1} 2,4-D and 0.1 mgl^{-1} Kin in MSH II, with 799 mg fresh weight and 79 mg dry weight. A higher production of mucilage was obtained in MSH II. The mucilage content of different media varied from 10.40 to 14.73% dry weight. Perusal of the data reveals significant differences with regard to the total mucilage content. Callus has nearly 3 times and 1.5 times more mucilage than seeds and leaf and root parts respectively.

Keywords: Mucilage, *Plantago lanceolata*, Tissue culture.

INTRODUCTION

Mucilages are abundant in nature and present in many plant sources. The importance of various mucilages for pharmaceutical purposes has a long history and has increased considerably during the last few decades. This is not only the case for polysaccharides used as excipients that are essential for specific drug formulations, but also for a series of carbohydrate polymers that have proved to be most useful as physiologically active drugs [1]. Many interesting areas have been researched in the past,including their role in drug delivery, in wound treatment, in cancer therapy and in the diagnosis, prevention, and treatment of bacterial and viral diseases [5].

Mucilage commonly occurs in higher plants, although only two important reports on this class of natural products have so far been published by research workers studying plant cell cultures [3,5]. It has been reported that callus cultures from higher plants are relatively rich in mucilage which commonly makes up between 8-10% and 0.2-3.7% of dry weight in some plants [7,8]. This research was therefore, conducted to investigate callus culture of *P.lanceolata* for their mucilage content.

MATERIALS AND METHODS

Plant Materials

The species used in the present investigation is *P. lanceolata*, from the *Plantaginaceae* family. Mature seeds of this species were provided by the Isfahan Research Centre of Desert and Kavir Regions.

Initiation and Maintenance of Callus

The seeds were sterilized first with 96%

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V/V ethanol for 1 minute, then with 5% w/v Na-hypochlorite solution for 20 minutes, finally being washed five times with sterile distilled water. The sterilized seeds were aseptically germinated on an agar-solidified Murashing and Skoog (MS) basal medium and incubated at a temperature of 25±1°C. Leaf and root pieces (1.5cm) were excised from 10 to 20 day-old seedlings and used as explants in these experiments. MS, Hildebrandt and Schenk modified (MSH), Nagata and Takebe (NT), and Gamborg (B5) basal media (Table 1) were used [10, 13, 11 & 6].

Table 1. Composition of culture basal media.

Constituents	MS	NT	MSH	B5
		mgl^{-1}		
NH_4NO_3	1650	825	-	-
KNO_3	1900	900	2500	2500
$MgSO_4.7H_2O$	370	1.233	400	250
$NH_4H_2PO_4$	-	-	300	-
$(NH_4)_2SO_4$	-	-	-	134
KH_2PO_4	170	680	-	-
$NaH_2PO_4.H_2O$	-	-	-	150
$CaCl_2.2H_2O$	440	220	220	150
$MnSO_4.4H_2O$	22.3	22.3	22.3	10
H_3BO_3	6.2	6.2	6.2	3.0
$ZnSO_4.7H_2O$	8.6	8.6	8.6	3.0
KI	0.83	0.83	0.83	0.75
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$Na_2.MoO_4.2H_2O$	0.25	0.25	0.25	0.25
$CoCl_2.6H_2O$	0.025	-	0.025	0.025
$COSO_4.7H_2O$	-	0.03	-	-
$FeSO_4.7H_2O$	28.8	27.8	15	25.6
Na_2EDTA	37.3	37.3	20	34.3
Myo-Inositol	100	100	100	100
Thiamine HCl	0.1	0.1	5.0	10
Nicotinic acid	0.5	0.5	5.0	1.0
Pyridoxine HCl	0.5	0.5	0.5	1.0
Glycine	2.0	-	-	-
Sucrose	30000	20000	30000	20000

For callus formation, various concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D), α-naphthalenacetic acid (NAA), indol –3-acetic acid (IAA), 6-furfurylamino purine [kinetin (kin)] and 6-benzyladenine (BA) were used in the basal media (Table 2). The pH of the media was adjusted to 5.6 and media were solidified with 1% (w/v) agar (Difco) and by autoclaving at 1.06 kg/cm^2 for 20 minutes. For callus induction, four leaf and root explants were transferred into a petri-dish (100×20mm) with 25ml of the medium. A minimum of four replicates were raised per treatment. Observations were recorded after ten days. In order to check the effects of light, the explants were divided into two sets. One of these was exposed to 2000 lux light intensity provided by white fluorescent lamps (40 w) and incandescent lamps (100 w) with a 16 to 8 light/dark cycle and the other one was kept in the dark at 25±1°C.

Callus initiation was visually assessed on a scale of 1 to 4 (smallest to largest) with 1= less than 25%, 2=25- 50%, 3=50-75%, and 4= greater than 75%. The callus index was calculated as follows: [(n×G/N)]×100, where n= total number of callused explants, G=average callus rating on explants, and N=total number of cultured explants.

Callus growth was determined by measuring the fresh weight and dry weight of callus mean after five weeks of incubation (Table 2). A minimum of four replicates were used

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Table 2. Comparison between effects of basal media and plant growth regulators on callus growth.

Basal media	Plant growth regulators(mg^{-1}/l)				Callus growth (mean±S.d)[a]		
	2,4-D	NAA	Kin	6BA	mg fresh & dry weight		
MSH II	0.8	-	0.1	-	799± 128	79± 8	a
MSH III	1.2	-	0.1	-	646± 54	63± 9	b
B5 IV	0.5	2.5	-	1	633± 110	62± 9	b
B5 II	-	3.0	-	1	605± 34	60± 7	b
NT I	-	3.0	-	1	548± 44	54± 8	bc
B5 I	2.5	0.5	0.2	-	466± 59	45± 9	cd
B5 III	2.5	0.5	-	1	373± 49	39± 5	de
MS III	1.2	-	0.1	-	402± 95	39± 7	de
MS IV	2	-	0.1	-	379± 37	37± 7	def
MSH I	0.4	-	-	-	361± 33	35± 7	efg
MS I	0.4	-	-	-	325± 23	30± 5	efg
NT III	3.0	-	0.1	-	298± 37	29± 4	fg
NT IV	-	3.0	0.1	-	303± 25	29± 4	fg
MSH IV	2.0	-	0.1	-	293± 26	28± 3	fg
MS II	0.8	-	0.1	-	286± 62	26± 3	g
N.T II	2.5	0.5	0.2	-	261± 39	25± 4	g

[a] Duncan's multiple range test (Duncan's test was assessed only for dry weight data). Figures followed by the same letter within the group are not significanly different from each other.

per treatment for callus growth and formation. Fresh weight (FW) was obtained by weighting calli immediately after the removal of adhering agar. Dry weight (DW) was measured after drying the calli at 60°C for 24 hours. The calli were subcultured every four weeks.

Mucilage Content

Mucilage content was estimated by the modified technique of Kalyansundaram *et al.* [15]. For the extraction of crude mucilage content, eight week-old calli were analysed which were harvested, dried and ground. Ten millilitres of 0.1 N HCl were heated to boiling point in a 100 ml corning flask. The flask was then removed from the flame and a 400 mg (dry weight) test sample of calli for each extraction was added to it. Heating was resumed for five minutes and the flask was finally removed from the flame and the solution was filtered through layers of clean muslin cloth while still hot. In order to separate residual traces of mucilage, the ground calli were washed twice in 5mls of hot water and the solution obtained was filtered each time. The combined filtrate, containing the dissolved mucilage, was mixed with 60 ml of 95% ethanol, stirred and allowed to stand for five hours at 4°C. Finally, the supernatant liquid was decanted off and the beaker containing the precipitate was dried inside an oven maintained for 24 hours at 50°C. The weight of dry precipitate was taken to represent the total mucilage content [12]. Statistical data were assessed by the analysis of variance and Duncan's multiple range test [14].

RESULTS AND DISCUSSION

Callus initiation invariably occurred from the cut portions of leaf and root after ten days of culturing. The best callus induction was obtained with 0.4 mg^{-1} 2,4-D in MSH I, 0.8 mgl^{-1} Kin in MSH II with the highest callus index (400), 1.2 mgl^{-1} 2,4-D and 0.1 mgl^{-1} Kin in MSH III, 3 mgl^{-1} NAA and 1 mgl^{-1} 6BA in NTI with 320 callus index, 2.5 mgl^{-1} 2, 4-D and 0.5 mgl^{-1} NAA and 0.2 mgl^{-1} Kin in B5 I with a 280 callus index and 2.5 mgl^{-1} 2,4-D, 0.5 mgl^{-1} Kin and 1 mgl^{-1} 6BA in B5 III with a 210 callus index respectively (Table 3). 2,4-D alone in MSH I and in combination with Kin in MSH II and

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Table 3. Effect of basal media and plant growth regulators on callus induction.

Culture media	Callus initiation[a]	Culture media	Callus initiation[a]
MS I	30	NT I	320
MS II	100	NT II	80
MS III	180	NT III	100
MS IV	180	NT IV	180
MSH I	400	B5 I	280
MSH II	400	B5 II	180
MSH III	320	B5 III	210
MSH IV	180	B5 IV	100

[a] Callus index = [(n×G)/N] ×100. where n = total number of callused explants, G = average callus rating of explants. N = total number of cultured explants.

MSH III, 3 mgl^{-1} NAA, $1mg^{-1}$ 6BA in NT I, 2.5 mg^{-1} 2,4-D $0.5mgl^{-1}$ NAA, 1 mgl^{-1} 6 BA in B5 III, 3 mgl^{-1} NAA, 0.1 mgl^{-1} Kin in NT IV stimulated the initiation of callus. Increasing 2,4-D from 0.4 mgl^{-1} in MSH I to 0.8 mgl^{-1} with 0.1 mgl^{-1} Kin in MSH II had no significant effect on callus index and 1.2 mgl^{-1} 2,4-D with 0.1 mgl^{-1} Kin in MSH III was inhibitory to callus initiation.

Callus growth in different media was significantly stimulated (P=0.05) by 0.8 mgl^{-1} 2,4-D and 0.1 mgl^{-1} Kin in MSH II with the highest callus growth (799 mg/F.W and 79 mg/D.W). 0.1 mgl^{-1} Kin in MSH III with 646 mg/F.W and 63 mg/D.W, 0.5 mgl^{-1} 2,4-D, 2.5 mgl^{-1} NAA and 1 mgl^{-1} 6BA in B5 IV with 633 mg/F.W and 62 mg/D.W, 3 mgl^{-1} NAA and 1 mgl^{-1} 6BA in B5 II with 605 mg/F.W and 60 mg/D.W respectively.

The best callus growth was obtained in MSH II and MSH III supplemented by 2,4-D and Kin. After these, were B5 IV medium containing 0.5 mgl^{-1} 2,4-D, 2.5 mgl^{-1} NAA and 1 mgl^{-1} 6BA and B5 II medium containing 3 mgl^{-1} NAA and 1 mgl^{-1} 6BA. Comparison between the different media using Duncan's test showed eight groups as follows: 1) MSH II, 2) MSH III, B5 IV and B5 II, 3) NT I, 4) B5 I, B5 III and MS III, 5) MS IV, 6) MSH I and MS I, 7) NT III, NT IV and MSH IV, and 8) MS II and NT II respectively (Table 2).

Mucilage production in different media is shown in Table 4. The best mucilage production was obtained in MSH II, B5 I, NT II and NT I. Sixteen media are ranked with respect to mucilage production using Duncan's test into seven groups respectively as follows: 1) MSH II, 2) B5 I, 3) NT II and NT I, 4) MSH IV, MS I, MSH III, NT III, MSH I, MS II and MS III, 5) B5 II and MS IV, 6) NT IV, and 7) B5 IV and B5 III.

There were no significant differences in callus formation, growth and mucilage content when callus was kept in a light-dark cycle or in darkness and in the callus derived from leaf and root. Leaf and root mucilage content in the intact plant of *P. lanceolata* was 10% G/G D.W [2] and in seeds was 5% G/G/D.W [8] while in the callus in this investigation in the MSH II medium it was 14.75% G/G D.W. Perusal of the figures reveal significant differences with regard to the total mucilage content. Callus has nearly 3 times and 1.5 times more mucilage than seeds and leaf and root parts respectively. It

Table 4. Mucilage content in different media

Culture media	Mucilage content (mean ± sd)[a] (%G/G) dry weight		culture media	Mucilage content (mean ± s.d.)[a] (%G/G) dry weight	
MSH II	14.73±0.65	a	MSH I	12.62±1.47	bcd
B5 I	13.58±0.47	b	MS II	12.50±1.23	bcd
NT II	13.35±1.91	bc	MS III	12.42±0.88	bcd
NT I	13.04±0.99	bc	B5 II	12.35±0.89	cd
MSH IV	12.85±0.47	bcd	MS IV	12.25±1.69	cd
MS I	12.83±1.56	bcd	NT IV	11.80±1.14	d
MSH III	12.75±0.96	bcd	B5 IV	10.48±1.11	e
NT III	12.65±3.8	bcd	B5 III	10.40±0.78	e

[a]Duncan's multiple rang test. Figures followed by the same letter within the group are not significantly different from each other.

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can be therefore used for *in vitro* culture since it is more suitable than the other species for the purpose of mucilage production through tissue culture [4].

Finally, regarding the "specially useful traits" of mucilage compounds in *Plantago lanceolata* [1] and " low mucilage production " in the natural organs of this species (both in comparison with other species of *Plantago*) [2,12], the results reported in this paper require that sufficient mucilage is produced from *Plantago lanceolata* through an appropriate series of tissue culture methods.

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تولید موسیلاژ در کشت بافت بارهنگ نیزه‌ای *(Plantago Lanceolata)*

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چکیده

قطعات جداکشت برگ و ریشه دانه رست‌های ده تا بیست روزه گیاه بارهنگ نیزه‌ای *(Plantago Laceolata)*، روی محیط‌های کشت پایه B5, NT, MSH, MS با غلظت‌های مختلفی از تنظیم کننده‌های رشدکشت گردیدند. بهترین محیط کشت ازنظر القاء کالوس درمحیط کشت MSH I محتوی ۰/٤ میلی‌گرم در لیتر ۲ و ٤- دی با اندیس کالوس ٤۰۰ می‌باشد. رشد کالوس بطور معنی‌داری (P =۰/۰۵) با ۰/۸ میلی‌گرم در لیتر ۲، ٤- دی و از میلیگرم در لیتر کینتین در محیط کشت MSH II ، با ۷۹۹ میلیگرم وزن تر و ۷۹ میلی‌گرم وزن خشک انجام گردید. بیشترین مقدار موسیلاژ در محیط کشت MSH II ،تولید شد. مقدار موسیلاژ در محیط‌های کشت مختلف بین ۱۰/٤۰ درصد تا ۱٤/۷۳ درصد وزن خشک متغییر بود. بررسی یافته‌ها در ارتباط با مقدار موسیلاژ تفاوت معنی‌داری را نشان دادند. مقدار موسیلاژ کالوس تقریبا" ۳ برابر بیشتر از بذر و ۱/۵ برابر بیشتر از ریشه و برگ می‌باشد.

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