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RESEARCH ARTICLE

Induction and maintenance of callus cultures in *Bunium persicum* boiss

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SUMMARY

Best callus initiation was achieved on MS medium supplemented with 2 mgl^{-1} 2,4-D and 4 mg l^{-1} Kn. Petiole measuring 1cm was the most suitable explants for callus formation. 2,4-D was the most suitable auxin for callus growth. Maximum callus growth was achieved on MS medium supplemented with 2 mgl^{-1} 2,4-D and 4 mg l^{-1} Kn. The growth rate of callus on this medium registered a 17 fold increase in four weeks of culture.

Key Words **:** Callus initiation, *Bunium persicum* boiss, Callus cultures

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Bunium persicum (Apiaceae) is a native plant of limited zones of the west Asia and grows as herbaceous plant in dry temperate region of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh (1800-3300 m). Seed is used as a prized condiment for flavoring dishes and as a carminative in ayurvedic medicines. Volatile constituents of seed: γ-terpinene, cuminaldehyde , ρ-cymene and α-methyl-benzenemethanol are major compounds. Production of this plant is limited due to seed dormancy and several biotic stresses of which wilt diseases are the most serious. Only cold treatments are effective in seed germination. Other treatments such as gibberlic acid, cytokinin, potassium nitrate, washing and light treatments are not useful (Bonianpoor, 1995). Generally *Umbeliferae* species including *Bunium persicum* have antimicrobial properties (Shetty *et al.,* 1994). Potential genetic variability for conventional breeding is limited in *Bunium persicum In vitro* plant regeneration *via* somatic embryogenesis has been achieved from callus derived from mericarp (Wakhlu *et al.*, 1990). The present study was undertaken to establish protocol for callus formation so that the protocol can be useful for the production of various secondary metabolites from callus cultures.

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MATERIALS AND METHODS

Petiole excised from mature plants growing in Bhadarwah (1613 m altitude) Jammu, India were used as explants. They were surface sterilized in 70 per cent ethanol for 30 sec. followed by 0.1 per cent $HgCl_2$ for 2 min and rinsed 4-5 times with sterilized distilled water. MS medium fortified with varying concentrations of 2,4-D, IBA,NAA,BAP and Kn either singly or in combination were used.

Callus initiation was assessed visually using scale of 1-4 (small to largest). Small-"0" was given when no callus was formed. Callus index was calculated as:

$$\text{Callus index} = \frac{nXG}{N} x100$$

where n- total number of explants forming callus, G-average callus rating on explants and N- total number of explants cultured. Callus growth was determined by measuring fresh weight after 4 weeks of culture. The growth rate was expressed as the ratio of increase in fresh weight (FW) to initial FW (400 mg per callus piece). The effect of various concentrations of auxins and Kn used for assessing callus growth. Five callus pieces (400mg FW) per treatment were

used. The pH of all medium was adjusted to 5.8 prior to solidifying with 0.8 per cent agar and autoclaved at 15 psi for 15 min. All cultures were maintained at a temperature of 25±2^0 C and 50±5 per centrelative humidity under 16 h illumination of 30µ $Em^{-2}s^{-1}$ provided by Bajaj fluorescent tubes (40W). Each experiment was repeated at least once. Means were analyzed by analysis of variance (ANOVA) and compared with Duncan' multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The explants enlarged in size and turned yellowish in colour within 4-7 days of culturing. Callus initiation started from the cut ends of explants after 2 weeks of culture. Callus was readily formed on the medium containing 2mgl^{-1} 2,4-D and 4mgl^{-1} Kn (Table 1 and Fig. 1), as has also been reported in *Anethum graveolens* (Seghal,1978), *Daucus carrota* (Smith and Street,1974), *Foeniculum vulgare* (Hunault,1984) and *Trachyspermum ammi* (Jasrai *et al.,* 1992). The callus formation is reported to be influenced by the size of the explants (Hughes, 1981). In the present study best callus initiation occurred from 10 mm long explants as was indicated by the highest callus index (400). Poor calllogenic response of small (5mm long) and large explants (15mm long) may be because of an increase in the wound to intact cell ratio or ca be attributed to high level of cytokinins in them and in the medium become supra-optimal (Constabel, 1984). Compared with tuber slices and

Table 1 : Effect of 2,4-D and Kn on callus initiation from petiole explants of *Bunium persicum* after 4 weeks of culture

2,4-D (mgl^{-1})	Kn (mgl^{-1})	Callus index
0.0	0.0	0.0
1.0		13
2.0		40
3.0		47
4.0		53
1.0	0.5	120
2.0		220
3.0		200
4.0		140
1.0	1.0	160
2.0		300
3.0	3.0	260
4.0	1.0	107
1.0	2.0	220
2.0		347
3.0		180
4.0		120
1.0	4.0	260
2.0		400
3.0		160
4.0		100

Table 2 : Effect of growth regulators on callus growth of petiole derived callus in Bunium persicum after 4 weeks of culture

2,4-D (mgl^{-1})	Kn (mgl^{-1})	Increase in FW/ Culture (mg) (Mean+sd)	Increase in DW/ Culture (mg) (mean+sd)	Growth rate
0.0	0.0	2691±123.0^e	158±15.2	7
0.1		1656±121.3^g	94±12.5	4
1.0		1279±119.4^h	127±14.2	3
2.0		837±102.3^i	60±11.4	2
4.0		669±121.3^j	37±12.3	2
0.1	0.1	1580±122.1^g	138±13.2	4
1.0		1069±89.2^{hi}	150±12.4	3
2.0		612±58.3^j	70±11.2	2
4.0		552±54.6^j	29±8.9	1
0.1	1.0	2093±112.3^f	141±15.4	5
1.0		2298±121.5^ef	227±14.3	6
2.0		2435±125.3^e	104±11.0	6
4.0		1421±89.4^g	78±11.6	4
0.1	2.0	2591±102.6^e	125±12.5	6
1.0		3782±129.2^d	202±14.2	9
2.0		5544±130.1^b	218±10.5	14
4.0		4568±132.4^c	178±14.2	11
0.1	4.0	3737±129.2^d	213±14.4	9
1.0		5302±131.4^b	284±13.2	14
2.0		6940±119.2^a	286±14.6	17
4.0		3430±134.2^d	159±14.1	9

Means sharing the same letter are not significantly different from each other at 55 level by Duncan's new multiple range test

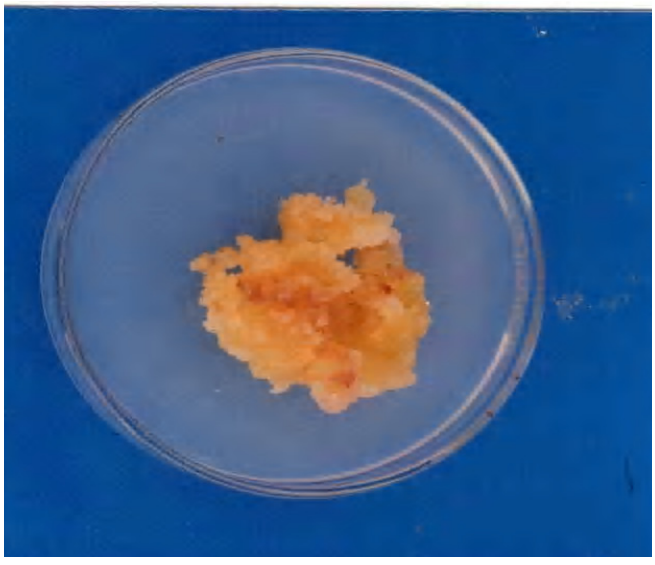

Fig. 1 : Callus formation on a medium supple mented with 2 mgl $^{-1}$ 2,4-D and 4 mg l $^{-1}$ Kn. after 4 weeks of culture

Table 3 : Effect of auxins on callus growth of petiole derived callus in *Bunium persicum* after 4 weeks of culture

Auxins* (mgl^{-1})	Increase in FW/ culture(mg) (mean±sd)	Increase in DW/ culture(mg) (mean+sd)
2,4 D	6889±122.3[b]	250±34.1[b]
NAA	3952±114.1[a]	167±31.2[a]
IBA	3838±109.2[a]	159±25.2[a]
IAA	3598±112.3[a]	143±28.4[a]

Source	df	SS	MS	f
Replication	4	6.56	1.64	
Treatment	3	26.24	12.08	12.98
Error	12	11.17	0.93	

*Medium was supplemented with 2 mgl^{-1}auxins and 4 mgl^{-1} Kn. Means sharing the same letter are not significantly different from each other at 55 level by Duncan's new multiple range test

intermode, petiole explants exhibited better callus forming ability (Data not presented). Similar results has earlier been reported in *Coriandrum sativum* (Kumar *et al.,* 1982), *Foeniculum vulgare* (Hunault , 1981) and *Rosa hybrid* (Khosh-Khaui and Sink, 1982). Callus growth was slow on the medium containing only 2,4-D, addition of Kn in the medium increased the callus growth (Table 2). Callus showed vigorous growth in the presence of 2mgl^{-1} 2,4-D and 4mgl^{-1} Kn (FW: 6940 mg; DW: 286 mg). High level of 2,4-D decline callus growth. Among the four auxins tested (NAA, IBA, IAA and 2,4-D), 2,4-D was found to be more suitable for callus growth (Table 3). A 17 fold increase in fresh weight and dry weight of callus occurred on this medium within 4 weeks (Table 4). The growth rate of callus decline after 4 weeks. A similar growth pattern has been previously reported in callus cultures of Plantago ovata (Wakhlu and Barna, 1989) and Theobroma cacao (Tsai and Kinsella, 1981). The present protocol can be used by those who would like to exploit the callus of *Bunium persicum* for the production og secondary metabolites.

Table 4 : Effect of incubation period on callus growth of petiole derived callus in *Bunium persicum* after 4 weeks of culture

Culture* period wee	Increase in FW/ culture(mg) (mean±sd)	Increase in DW/ culture(mg) (mean+sd)
1.	419±22.3[d]	36±10.2[d]
2.	1643±24.1[c]	86±11.2[a]
3.	4340±29.2[b]	135±25.2[c]
4.	6889±42.3[a]	250±28.4[a]
5.	4690±28.3[b]	210±15.9[b]

Source	df	SS	MS	f
Replication	4	1.97	0.45	
Treatment	4	30.82	32.70	47.39
Error	16	11.17	0.93	

Medium was supplemented with 2 mgl^{-1}auxins and 4 mgl^{-1} Kn. Means sharing the same letter are not significantly different from each other at 55 level by Duncan's new multiple range test

REFERENCES

Bonianpoor A. (1995). Investigation of sexual propagation of *Bunium persicum* and *Cuminum cyminum* and cumin callus induction. M.Sc. Thesis, Agricultural College, Shiraz, University of IRAN.

Constabel, F. (1984). Cell culture: Induction and maintenance. In: I.K. Vasil(ed) *Cell culture and somatic cell genetics of plants*. Vol.**1** Academic Press, INC. London pp. 27-35.

Duncan, D.B. (1955). Multiple range and multiple F – tests. *Biometric,* **11**: 1-42.

Hughes, K.W. (1981). Ornamental species. In: B.V.Conger(Ed.) *Clonining agricultural plants via in vitro technique*. CRC, Press, Florida. pp. 5-50.

Hunault, H.(1981). *Physiologia vegtale-La in vitro des tissues de (Foeniculum vulgare Millert) Premiers observations sur et. Descals*. C.R. Acad. Sc. Paris t. 293, pp. 553-558.

Hunault, H.(1984). *In vitro* culture of fennel tissue (*Foeniculum vulgare* Miller) from cell suspension to mature plant. *Scientia Hort.,* **22**: 55-65.

Jasrai, Y. T., Barot, S.M. and Mehta, A.R. (1992). Plant regeneration through somatic embryogenesis in hypocotyls explants of *Trachyspermum ammi*(L.) Sprague. *Plant Cell Tiss. & Org. Cult.,* **29**: 57-60.

Khos-Khui, M. and Sink, K.C. (1982). Callus induction and culture of Rosa. *Scientia Hort.,* **17**: 361-370.

Sehgal, C.B. (1978). Differentiation of shoot bud and embryiods and inflorescence of *Anethum graveolens* in culture. *Phytomorphology,* **28**: 291-297.

Shetty, R.S., Singhal, R.S. and Kulkarni, P.R. (1994). Antimicrobial properties of cumin. *J. Microbial Biotech.,***10**:232-233.

Smith, S.M. and Street, H.E. (1974). The decline of embryogenic potential as callus and suspension cultures of carrot (*Daucus carota* L.) are serially subcultured. *Ann. Bot.,* **38**: 223-241.

Tsai, C.H. and Kinsella, J.E. (1981). Initiation and growth of callus and suspension of *Theobroma cacao* L. *Ann. Bot.,* **48**: 549-557.

Wakhlu, A.K. and Barna, K.S.(1989). Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk. cv. GI-2. *Plant Cell Tiss. Org. Cult.,* **17**: 235-241.

Wakhlu, A.K., Nagari, S. and Barna, K.S. (1990). Somatic embryogensis and plant regeneration from callus cultures of *Bunium persicum* Bioss. *Plant Cell Rep*., **9**:137-138.

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