RESEARCH PAPER

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Somatic embryogenesis in Kalazira (*Bunium persicum* Bioss.)

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In vitro multiplication of *Bunium persicum via* somatic embryogenesis is reported. The callus was derived from petiole explants on Murashige and Skoog medium containing 2mgl^{-1} 2,4-D and 2mgl^{-1} Kn differentiated globular embryos upon subculture to MS medium supplemented with different concentration of auxins. The globular embryos continued to multiply on a medium supplemented with 2,4-D but failed to mature. Further differentiation of globular embryos occurred when embryogenic callus was sub cultured to a medium lacking 2,4-D. However, the globular embryos formed on NAA and IBA supplemented medium differentiated into mature embryos on the same medium. The mature embryos were germinated on a medium supplemented with 0.2 mgl^{-1} Kn and 0.01 mgl^{-1} IBA at a temperature of 10-15^{0}C. The plantlets produced a small tuber on their roots which sprouted after 8 weeks.

Key words : *Bunium persicum,* Callus formation, Somatic embryogenesis.

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INTRODUCTION

Bunium persicum is an umbellifer growing as a wild species in dry temperate region of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh (1800-3300m). Seed is used as a prized condiment for flavoring dishes and as a carminative in ayurvedic medicines. The plant propagates by seeds and has been reported to becomes a rare in its natural habitat because of excessive seed collection for commercial purposes (Raina and Jamwal, 1990). Two major problems encountered in the cultivation of this species are poor seed germination and long seed to seed cycle (4-5years). In *Bunium persicum* somatic embryogenesis has been achieved from callus derived from mericarp (Wakhlu *et al*., 1990). The present study was undertaken to establish a protocol for high frequency somatic embryogenesis and plant regeneration from petiole explants.

RESEARCH METHODOLOGY

Petiole explants (10mm long) of *Bunium persicum* (2n=14) were collected from a wild population growing at Bhaderwah, Jammu, India. They were surface sterilized in 70% ethanol for 30 sec., followed by 0.1% (w/v) $HgCl_2$ for 2 min. and rinsed 4-5 times in sterile distilled water. The sterilized explants were cultured on MS medium (Murashige and Skoog,1962) supplemented with 2mg1^{-1} 2,4-D and 2mgl^{-1} Kn. Callus was sub cultured at 4-week intervals. All media were supplemented with 3% sucrose and 0.8% agar. pH of medium was adjusted to 5.8 prior to autoclaving at 15lb/inch^{-2} for 15 min. Eight week old yellow friable callus was transferred to medium supplemented with 2,4-D, NAA, IBA and IAA (0.1-2.0 mgl^{-1}) for induction of somatic embryos. Ten callus pieces per treatment (each 400mg FW) with two replicates were used. Different level of $AgNO_3$ (0.5- 10 mgl^{-1}), PEG (2-6%) and ABA (0.25-4.0mgl^{-1}) were tested for somatic embryo formation.

For the plantlet formation mature somatic embryos were transferred to hormone free medium or supplemented with Kn, IBA, GA_3, $AgNO_3$ and AC (Activated charcoal). All experiments were repeated at least once. Results on the induction of somatic embryogenesis, number of mature embryos per callus piece were recorded after 4 weeks of culture and data was subjected to arsine transformation for proportions before analysis and converted back to percentages for presentation in tables (Snedecor and Cochran, 1968) and compared by Duncan's new multiple range test (Duncan, 1955).

RESULTS AND ANALYSIS

The explants enlarged in size and turned pale yellowish in color within 4-7 days. Callus tissue emerged from the cut ends of explants after 2 weeks of culture. Callus formation was affected by growth regulators,

explant size and type (data not presented). Best callus initiation and growth was achieved from 10 mm long petiole explants on a medium supplemented with 2mgl^{-1} 2, 4-D and 2mgl^{-1} Kn. Eight week old friable callus developed nodules of merstemoids ranging 1-2 mm diameter over a period of 4-6 weeks after transfer on to a auxin rich medium that lacked cytokinin. These merstemoids were composed of closed packed thin walled, starch filled and densely cytoplasmic cells with a prominent nucleus. These merstemoids produced a large number of globular and mature embryos after 6 weeks of culture (Fig.1). The induction and maturation of somatic embryos was significantly influenced by auxin source and concentration (Table 1).

NAA (0.2mgl^{-1}) was the most suitable of the auxin tested for somatic embryogenesis (Fig.2). This agrees with the results from other umbelliferous species such as *Anethum graveloens* (Steward *et al.*, 1970), *Bupleurm falactum*(Wang and Hauang,1982) and *Carum carvi* (Krens *et al.*, 1997). However, the callus formation on 2,4-D supplemented medium was not able to differentiate embryos. But the callus upon subculture on the basal medium differentiate the mature embryos. This inhibitory effect of 2,4-D on maturation of somatic embryos has also been reported in *Coriandrum ativum* (Kim *et al.*, 1996), *Foeniculum vulgare* (Hunault, 1984) and *Apium graveolens* (Ortan,1984). The addition of $AgNO_3$, PEG, ABA did not improve the somatic embryo formation (Data not presented). The cultures with differentiated somatic embryos were incubated in dark at 7±1^{0}C for maturation for 6-8 weeks (Fig. 2).

Somatic embryos were 3-4 mm long and characterized by the presence of two well developed cotyledons and an elongated hypocotyls-root axis (Fig. 3). The optimum medium for somatic embryo germination was supplemented with 0.2mgl^{-1} Kn and 0.01 mgl^{-1} IBA (Table 2).

The plantlets did not developed plumule, however,

Table 1: Effect of auxins on somatic embryogenesis from petiole derived callus in *Bunium periscum* after 4 weeks of culture

Auxins (mgl^{-1})	Calli showing embryogenesis (%)	Somatic embryos / culture (mean ±s.d)
2,4-D		
0.1	50.8±2.8^{d}	-
0.2	60.4±5.4^{e}	-
0.5	60.8±4.8^{e}	-
1.0	60.8±4.4^{e}	-
NAA		
0.1	73.6±3.7^{f}	10.4±1.3^{d}
0.2	86.6±4.4^{g}	24.8±3.4^{f}
0.5	76.2±3.4^{f}	20.2±2.0^{e}
1.0	74.5±4.4^{f}	20.4±3.2e
IBA		
0.1	20.2±2.4^{a}	8.2±1.0^{c}
0.2	35.3±3.6^{bc}	8.4±1.0c
0.5	40.5±3.2^{c}	6.2±1.0^{b}
1.0	40.4±2.1^{c}	6.4±1.0^{b}
IAA		
0.1	-	-
0.2	-	-
0.5	30.4±2.0^{b}	2.1±1.0^{a}
1.0	30.0±3.0^{b}	2.2±1.0^{a}

Means within column followed by the same superscript are not significantly different from each other at 5% level

Figs 1-4: Somatic embryogenesis in *Bunium persicum* Boiss.

1. Globular and heart shaped somatic embryos after 6 weeks of culture
2. Mature somatic embryos on incubation in dark at 7±1°C for 6 weeks
3. A dicot somatic embryo with well developed cotyledons and hypocotyls axis
4. A germinating somatic embryo with a tuber on the root after 4 weeks of culture

Table 2: Effect of different media on somatic embryo germination after 4 weeks of culture

Medium	% embryo germination
MS basal medium	89.3^{c}
BAP(0.2)+IBA(0.01)	70.6^{b}
Kn (0.2) +IBA(0.01)	95.0^{d}
$AgNO_3$ (0.2)	42.2^{a}
AC (0.2%)	42.2^{a}

Means within column followed by the same superscript are not significantly different from each other at 5% level

produced a small tuber (2-3 mm diameter) on their roots (Fig. 4). The plantlets with well developed tubers were incubated at 15±2^{0}C. The tubers sprouted to produce 2-3 leaves. Thus plants produced kept under observation for hardening and field transplantation.

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