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,