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In vitro tuberization and plant regeneration in *Bunium periscum* Bioss

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SUMMARY

The effect of plant growth regulators was investigated on microtuber induction and development in *in vitro* raised plant lets of *Bunium persicum*. Of the cytokinins and auxins when tested alone showed inhibitory effect on tuber formation. By contrast, the auxin IBA when used in combination with Kn showed the promotive effect on the induction and growth of the microtubers. Maximum number of plantlets with tuber were observed on a medium supplemented with 0.2 mgl^{-1} Kn and 0.01 mgl^{-1} IBA. Further maximum growth of microtubers on subculture was achieved on a medium supplemented with BAP and TIBA. When microtubers removed from cultures and planted in sterilized moist sand, sprouted (69%) after 8 weeks.

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Bunium persicum (Apiaceae) is a wild herbaceous plant growing in dry temperate region of Jammu & 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. The plant propagates by seeds and has been reported to have 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 vitro* plant regeneration *via* somatic embryogenesis has been achieved from callus derived from mericarp (Wakhlu *et al.*, 1990) but no further studies on development of microtubers have been carried out. Hence, the present study was undertaken to establish protocol for *in vitro* tuberization and plant regeneration from somatic embryo plantlets.

MATERIALS AND METHODS

Petiole explant (10 mm 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 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 subcultured at 4-week intervals. All media were supplemented with 3% sucrose, 0.8% agar and pH of medium was adjusted to 5.8 prior to autoclaving at 15lb/$inch^{-2}$ for 15 min. Plantlets were regenerated from somatic embryos according to the protocol described by Sharma (1995). A supply of plantlets was maintained by germination of somatic embryos under 16h photoperiod at 15^0C. The plantlets were tested for their tuber formation capacity by culturing on a medium supplemented with different combinations of growth regulators (BAP, Kn: 0.1-1.0 mgl^{-1}; IBA, NAA, IAA: 0.01-0.05 mgl^{-1}) and were incubated at different temperature and photoperiods. Tuber growth was studied by sub culturing tubers on a media supplemented with different concentrations of BAP (2-8 mgl^{-1}) alone or in combination with TIBA, NAA, IAA (0.01-0.02mgl^{-1}). The effect of temperature (5, 10, 15, 23^0C) and photoperiod (16h, 8h and dark) was tested on sprouting of tubers. Data was subjected to arscin 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 DISCUSSION

Somatic embryos were induced on a medium supplemented with 0.2mgl^{-1} NAA (Fig. 1) and germinated on amedium supplemented with 0.2mgl^{-1} Kn and 0.01 mgl^{-1} IBA (Sharma,1995). *In vitro* raised plantlets from somatic embryos were tested for microtuber formation (Fig. 2). Auxins and cytokinins are known effective inducers of microtuberization for a number of different yam species cultured *in vitro*, although certain types are

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