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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 2mgl^{-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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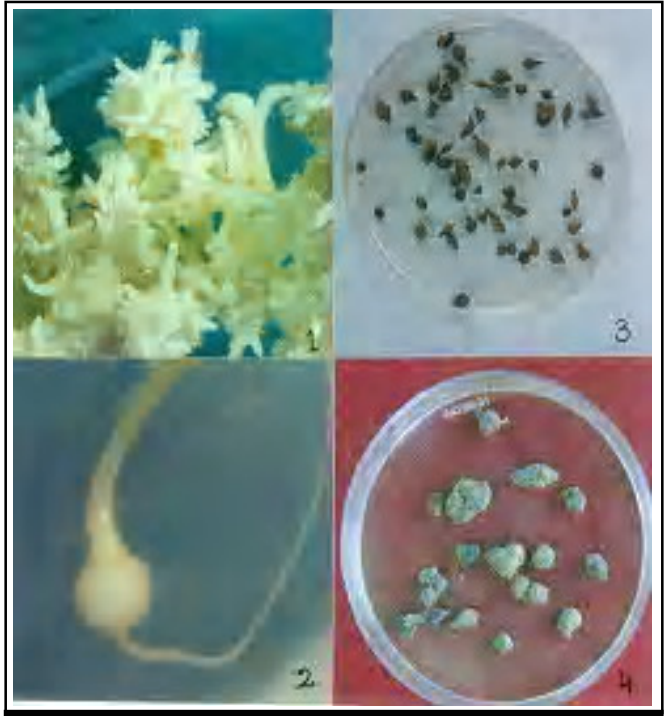

Fig. 1-4: Somatic embryo and tuber formation in *Bunium persicum* Boiss.

1. **Mature somatic embryos differentiated in dark at 7±1^{0}C for 6 weeks.**
2. **A microtuber developed on the root of a germinated somatic embryo after 6 weeks of culture.**
3. **Microtubers harvested from *in vitro* raised plantlets on a medium supplemented with 0.2mgl^{-1} Kn and 0.01mgl^{-1} IBA after 4 weeks of culture.**
4. **Microtubers after subculture on a medium supplemented with 8mg l^{-1} BAP and 0.02 mgl^{-1} TIBA after 6 weeks of culture.**

more effective than others for increasing microtuber induction (Ammirato,1982; Mantell and Huge, 1989). In the current studies, a range of different growth regulators was tested for their ability to increase the frequency of microtubers induction in *B. persicum* and to maximize the size of microtubers produced over a given time period (2 months). The frequencies of microtuberization obtained in somatic embryo plantlets grown on MS medium supplemented with growth regulators are presented in Table 1.

Of the different auxins tested 2,4-D inhibited tuber formation and induced callus formation whereas cytokinins BAP/Kn decreased the percentage of microtuberization at all concentrations evaluated compared to control. However, Kn in combination with IBA was found to be effective for tuberization and the size of tubers was enhanced as well. In general, microtubers were rough in surface texture and irregular in shape (Fig. 3). The promotive effective of auxins have also been reported previously by Ammirato (1982) and Sengupta *et al.* (1984).

Table 1 : Effect of growth regulators on tuber formation in *Bunium persicum* after 4 weeks of culture

Growth regulators (mgl^{-1})	No. of plantlets cultured	% plantlets with tubers	Tuber size (mm) (mean±s.e)
BAP			
0.0	74	70.2	2.2±0.2
0.1	68	44.1	2.3±0.2
0.2	64	42.1	2.2±0.2
Kn			
0.1	58	43.1	2.6±0.4
0.2	60	40.0	2.4±0.4
IBA (0.01)	65	31.2	2.0±0.1
NAA (0.01)	64	30.5	2.2±0.3
IAA (0.01)	68	28.0	2.4±0.2
Kn (0.2)+IBA (0.01)	76	71.0	3.0±0.4
Kn (0.2)+IBA (0.02)	69	68.1	2.1±0.4
Kn (0.2)+IBA (0.05)	70	42.8	2.3±0.3

Effect of temperature and photoperiod were evaluated which revealed that tuberization is suppressed, may be caused due to GA_3 concentration at high temperature (Hussay and Stacey, 1984). Maximum percentage of tuberization was found to occurs at a temperature of 15± 1^{0}C and 16/8 hour photoperiod (Table 2 and 3).

Data presented in Table 4 indicated that the mean tuber size and fresh weight of microtubers obtained when

Table 2 : Effect of temperature on tuber formation in *Bunium persicum* after 6 weeks of culture

Temperature (^{0}C)	No. of plantlets cultured	% plantlets with tubers	Tuber size (mm) (mean±s.e)
8±01	82	51.2	2.1±0.2
15±1	88	81.8	3.2±0.2
23±2	76	69.7	3.2±0.4

MS medium was supplemented with 0.2 mgl^{-1} Kn and 0.01 mgl^{-1} IBA

Table 3 : Effect of photoperiod (30uEm^{-2}s^{-1}) on tuber formation in *Bunium persicum* after 6 weeks of culture[1]

Photoperiod	No. of plantlets cultured	% plantlets with tubers	Tuber size (mm) (mean±s.e)
16h/8h	74	70.2	3.0±0.4
24h/0h	72	69.4	2.9±0.4
0h/24h	81	0.0	0.0

MS medium was supplemented with 0.2 mgl^{-1} Kn and 0.01 mgl^{-1} IBA

Table 4 : Effect of growth regulators on tuber growth in *Bunium persicum* after 6 weeks of culture

Growth regulators[1,2] (mgl^{-1})	Fresh weight (mg) (mean±s.e)	Tuber size (mm) (mean±s.e)
BAP (2)	3.7 ±0.6	80.4±1.2
BAP (4)	3.9±0.5	94.3±2.1
BAP (6)	4.0±0.2	112.0±2.1
BAP (8)	5.6±0.3	154.0±2.1
BAP (8) + TIBA(0.01)	6.4±0.2	174.2±2.1
BAP (8) + TIBA(0.02)	6.3±0.6	171.4±4.4
BAP (8) +NAA(0.01)	4.1±0.2	133.6±1.4
BAP (8) +NAA(0.02)	3.9±0.4	128.9±1.6
BAP (8) + IBA (0.01)	4.1±0.3	113.6±2.6
BAP (8) + IBA(0.02)	4.2±0.2	120.2±1.2

1 Concentration of growth regulators are given in parcenthesis
2 Tubers were incubated at a temperature of Mean initial size15^{0}C Mean initial tuber size and fresh weight were 2.9 mm (diameter) and 40 mg

BAP, TIBA, NAA, IBA were used. A significant increase in size and fresh weight of tubers than the control was obtained on a medium supplemented with 8 mgl^{-1} and 0.01 mgl^{-1}. Of the cytokinins tested BAP supported the highest microtuber fresh weights increasing to these to over 150 mg after 6 weeks of culture. When BAP incombination with auxins was present in the medium, microtubers ranging from 120 mg to 174 mg were obtained after the same time interval on medium supplemented with 8mgl^{-1} BAP and 0.01 mgl^{-1} TIBA (Fig. 4).

After harvesting from the culture tubes and washing in tap water, microtubers appear to undergone a period of dormancy. The microtubers were subjected to low temperature and dark photoperiod for breaking dormancy. Maximum number of tubers (69%) were found to sprout at a temperature of 8^{o}C and 24 h dark photoperiod (Table 5).

Table 5 : Effect of photoperiod and temperature on sprouting of tubers of *Bunium persicum* after 8 weeks of culture

Temperature o^{0}C	Number of tubers sprouted (%) photoperiod	
	16h/8h	0h/24h
8±1	54(40)	69(45)
15±1	29(45)	52(48)
22±2	00(45)	00(50)

About 100 sprouting tubers were transplanted to polyethylene bags containing moist sand and grown in a growth chamber maintained at a temperature of 15^{0}C and 16h photoperiod. Tubers developed 2-3 green leaves, which turned yellow and withered within 4-6 weeks. The hardened tubers were transplanted under field conditions where they showed poor survival rate (10%) and did not survive.

The possibility of producing microtubers *in vitro* from *B. persicum* on a consistent way will not only facilitate the introduction into the field of elite genotypes but also provide a system for immediate use in physiological and biochemical studies on ontology and development of *B. persicum* tubers.

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