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SOMATIC EMBRYOGENESIS AND SYNTHETIC SEED DEVELOPMENT IN *BUNIUM PERSICUM* B. FEDTSCH

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ABSTRACT

A protocol has been developed for propagation of Bunium persicum through somatic embryogenesis using explants from in vitro raised plantlets. Callus obtained from these explants regenerated embryos after 4- 5 weeks on MS basal medium and medium supplemented with growth regulators. Overlayering of callus by liquid MS medium fortified with 2,4-D (1mg/l) enhanced the frequency of induction of somatic embryos. MS basal medium was found best for embryo conversion.Synthetic seeds were also developed by encapsulating the embryos in calcium alginate. Germination of synthetic seeds was best achieved on MS medium fortified with and BAP 2mg/l.

Key Words: Callus, Overlayering, Somatic Embryos, Encapsulation, Synthetic Seeds, Acclimatization.

1. INTRODUCTION

B. persicum is a native of west Asia and is distributed in the mountainous regions of Iran, Turkmenistan, Tajikistan, Afghanistan, Pakistan and India (1). It belongs to the family Apiaceae and is commonly known as "Kala Zira" or "Black cumin". Seeds (schizocarp fruits) of this plant are used as medicine and spices (2). The plant propagates by seeds and because of excessive seed collection for commercial purposes, it has been reported to have becomes a rare in its natural habitat (3). Further two major problems encountered in the cultivation of this species are poor seed germination and long seed to seed cycle (4-5years). The competition for its seeds is so severe that instead of collecting the ripe seeds, the entire plant is removed even when the seeds are immature (4). The close field observations have revealed that propagation of this species in its natural habitats occurs rarely. Hence, micropropagation through somatic embryogenesis can be extremely useful to shorten the long sexual cycle and other problems like limited seed availability (5). The formation of somatic embryos in callus cultures, which was first reported in the Apiaceae member, carrot (*Daucus carota* L.), is a typical process of embryogenesis on the same medium or after transferring it to a second medium lacking plant growth regulator (PGR) supplements (6).The

present study describes a method for propagation of *B. persicum* through somatic embryogenesis from *in vitro* derived callus.

II MATERIALS AND METHODS

During the present study explants obtained from *in vitro* raised plants were used. The regenerated plantlets were taken out aseptically from the culture flasks under Laminar air flow hood with the help of sterilized forceps and placed into pre-autoclaved petriplates. The plantlets were cut into 3-5 segments to be used as explants. For callus production these explants were inoculated on MS basal medium or medium augmented with growth regulators. The callus produced was slightly meshed and spread over the agar containing MS basal medium and was finally overlayered by liquid MS medium (without agar) fortified with 2,4-D with the help of sterilized syringes. This increased the frequency of somatic embryogenesis. For encapsulation of somatic embryos, stocks of sodium alginate (3% w/v) and calcium chloride solution (100mM) were prepared. 3% sucrose was also added to the sodium alginate gel. The stocks were sterilized in an autoclave and kept under aseptic conditions till use. The embryos were carefully isolated from the culture flasks under laminar air flow hood with the help of sterilized forceps and then mixed with sodium alginate gel. The embryo alginate mixture was pippetted out and then dropped into $CaCl_2$ solution in which bead formation occurred by ion exchange reaction.

III RESULTS

In the present work, explants from *in vitro* raised plantlets were used for callus production. For somatic embryogenesis, callus was sub-cultured onto a fresh medium. Upon subculturing of callus on MS agar medium, somatic embryos were obtained with 23 ± 2.8 mean number of embryos after 43 average days of subculturing (Fig. 1a). However, when callus was subcultured on MS agar medium followed by liquid overlayering (Fig. 1b) with 2,4-D (MS medium without agar + 2,4-D 1 mg/l) the frequency of embryo induction increased (Fig. 1c) with 50 ± 17.05 mean number of embryos after 30 days of subculturing of callus.

Table 1: Effect of liquid overlayering on induction of somatic embryos

MS basal medium	Mean number of somatic embryos	Average number of days taken for induction
Without overlayering	23±2.8	43 days
Overlayering by 2,4-D (1mg/l)	50±17.05	30 days



Fig.1 a Fig. 1 b Fig. 1c

For conversion into plantlets, the somatic embryos were transferred to fresh medium (MS basal medium or medium without auxin). Conversion of somatic embryos was achieved on MS medium without PGRS as well as on medium supplemented with BAP. However the germination rate was best on medium free of growth regulators with 10.33 ± 0.6 mean number of embryos germinated (Fig. 2a) after 14 average number of days of subculturing. Embryos also germinated on medium containing different concentrations of BAP (1-5 mg/l). 2.6 ± 0.8 mean number of embryos (Fig. 2b) germinated on medium fortified with BAP (1mg/l) after 12 average number of days. Embryo germination was also achieved on medium containing BAP (2, 3, 4 and 5 mg/l) with 3.3 ± 0.8, 5.0 ± 0.5, 3.6 ± 0.6 and 5.6 ± 0.3 mean number of germinated embryos (Fig. 2c, 2d, 2e and 2f) respectively, after 13, 12, 11 and 7 average number of days respectively.

Table 2: Embryo conversion

Treatment	**Mean number of germinated embryos**	**Average Number of days taken for germination**
MS Basal	10.33±0.6	14
BAP(1mg/l)	2.6±0.8	12
BAP(2mg/l)	3.3±0.8	13
BAP(3mg/l)	5.0±0.5	12
BAP(4mg/l)	3.6±0.6	11
BAP(5mg/l)	5.6±0.3	7

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Fig. 2a

Fig. 2b

Fig. 2c

Fig. 2d

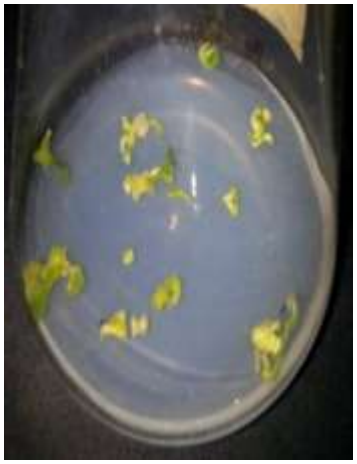

Fig. 2e

Fig. 2f

Synthetic seed production

Synthetic seeds were also developed by encapsulating the somatic embryos in calcium alginate (Fig. 3a). These synthetic seeds were then inoculated on MS basal medium as well as medium fortified with different concentrations of BAP. Synthetic seeds germinated on medium free of growth regulators as well as medium containing different concentrations of BAP (1-5 mg/l). On MS basal medium 1.0 ± 0.5 mean number of seeds germinated (Fig. 3b) after 10 average number of days. 2.0 ± 0.57 mean number of seeds germinated (Fig. 3c) on medium containing BAP at a concentration of 1 mg/l after 12 average number of days. Medium supplemented with BAP (2mg/l) proved to be best for synthetic seed germination with 2.6±0.3 mean number of seeds germinated (Fig. 3d) after 10 average number of

days. Synthetic seed germination was also achieved on other concentrations of BAP viz: 3, 4 and 5 mg/l used on which(1.6 ± 0.6, 1.3 ± 0.3 and 0.6 ± 0.3) mean number of embryos germinated (Fig. 3e, 3f and 3g), after 11, 13 and 15 average number of days respectively.

Table 3: Effect of BAP on synthetic seed germination

Treatment	Mean number of synthetic seeds germinated	Average number of days taken for germination
MS Basal	1.0 ±0.5	10
BAP (1mg/l)	2.0±0.57	12
BAP(2mg/l)	2.6±0.3	10
BAP(3mg/l)	1.6±0.6	11
BAP(4mg/l)	1.3±0.3	13
BAP(5mg/l)	0.6±0.3	15

Acclimatization/Hardening

For acclimatization, the *in vitro* raised plantlets were taken out of the culture flasks (Fig. 4a) with the help of sterilized forceps. The medium adhering to the basal portion of plantlets was washed with double distilled water. After washing they were transferred to jiffy pots containing vermicompost (Fig. 4b, 4c and 4d) and maintained under controlled conditions for hardening.

Fig. 4a

Fig. 4b

Fig. 4c

Fig. 4d

IV DISCUSSION

The results of this study showed that the frequency of induction of somatic embryos increases when the callus is overlayered by liquid medium containing 2, 4-D (1mg/l) which provides easy access of nutrients to callus. 2, 4-D has also been earlier reported to induce somatic embryogenesis in carrot by (7). (8) also obtained somatic embryos in *B. persicum* on MS medium containing 2,4-D (2mg/l), but they did not use the liquid medium for overlayering. Embryos showed germination when subcultured on hormone free MS medium as well as medium supplemented with different concentrations of BAP (1-5 mg/l), however the best germination rate was achieved on medium without PGRS. (9) also reported that somatic embryogenesis occured in *Ferula assafoetida* on hormone free MS medium after induction in a medium containing 2,4-D. (10) has recommended use of auxin free medium for embryo development. Medium supplemented with BAP (2mg/l) proved best for synthetic seed germination with 2.6±0.3 mean number of seeds germinated after 10 average number of days. 2.0±0.57 mean number of seeds germinated on medium containing BAP at a concentration of 1 mg/l after 12 average number of days. Synthetic seed germination was also achieved on other concentrations of BAP (3, 4 and 5 mg/l) used with (1.6 ± 0.6, 1.3 ± 0.3 and 0.6 ± 0.3) mean number of embryos germinated respectively, after 11, 13 and 15 average number of days respectively. On MS basal medium 1.0 ± 0.5 mean number of seeds germinated after 10 average number of days. Synthetic seed production has also been reported by (11) in case of *Centella asiatica*.

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