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Somatic embryogenesis and plant regeneration from callus cultures of *Bunium persicum* Boiss

A. K. Wakhlu, Sangita Nagari, and K. S. Barna

Department of Biosciences, University of Jammu, Jammu-180004, India

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ABSTRACT

Callus was obtained from mericarps of Bunium persicum Boiss. on MS medium supplemented with 2.0 mg/l 2,4-D and 4.0 mg/l Kn. Small white clumps of compactly packed cells developed on the callus on a medium containing 1.0 mg/l 2,4-D and 0 mg/l Kn. These cell clumps differentiated numerous globular embryos on the same medium. Embryo maturation and germination was achieved on the basal as well as on 1 mg/l Kn supplemented medium. All regenerated plants examined were normal diploids with 2n=14.

ABBREVIATIONS

2,4-D, 2,4-dichlorophenoxyacetic acid; Kn, Kinetin.

INTRODUCTION

Bunium persicum Boiss. (Kala Zira), an commercially important umbellifer, is used as a prized spice for flavouring the dishes and as a carminative in ayurvedic medicines. The species grows wild on mountain slopes of Kashmir Himalayas at an altitude ranging from 1,829-3,353 m. Reports on its cultivation and improvement programmes are scarce (Bhartiya 1967, Kaith 1979, Kaith and Sharma 1982). Tissue culture techniques in conjunction with traditional breeding methods might prove useful for the improvement of this species. For the exploitation of in vitro techniques it is essential to optimize the conditions for whole plant regeneration. This paper presents the first report of somatic embryogenesis and plant regeneration from callus cultures of B. persicum.

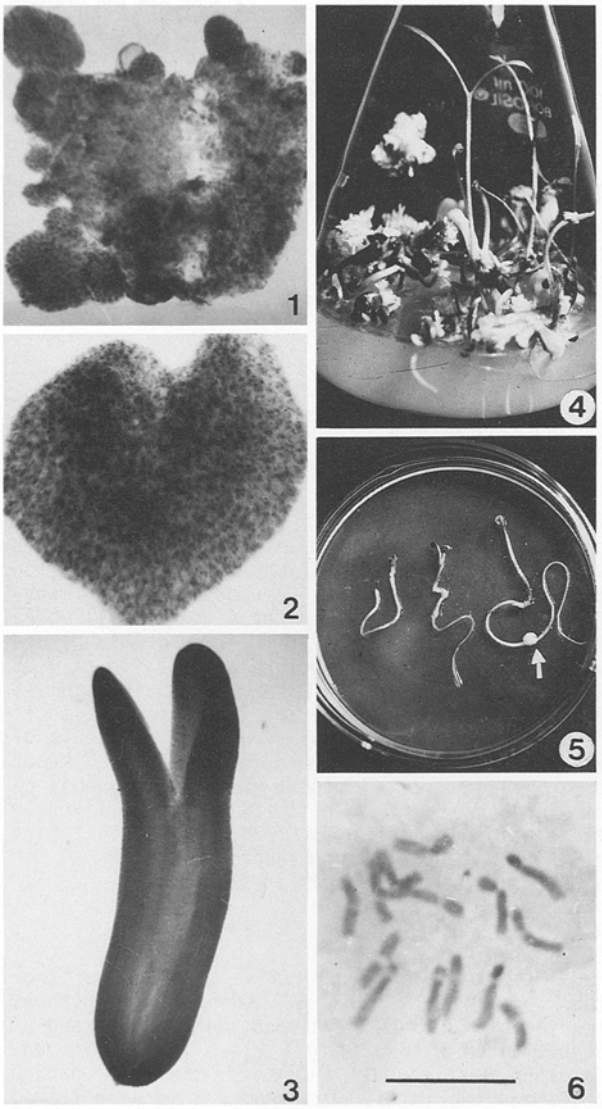
MATERIALS AND METHODS

Mericarps of Bunium persicum Boiss. (2n=14) were collected in April 1988 from a wild population located at Zainakote, Kashmir province, India. They were surface sterilized in 15% w/v calcium hypochlorite solution for 15 min and rinsed 4 times with autoclaved distilled water. The sterilized mericarps were cultured on MS (Murashige and Skoog 1962) basal medium containing 2.0 mg/l 2,4-D and 4.0 mg/l Kn for callus formation. Callus was subcultured on this medium at 4 week intervals. To induce somatic embryo formation, pieces of callus (300±5 mg fresh weight) from eight-week old cultures were placed on MS medium supplemented with 2,4-D (0,0.1,1.0,2.0,4.0 mg/l) and Kn (0,1.0 mg/l). Six callus pieces were used per treatment. The cultures were scored after 4 weeks. All media contained 3% w/v sucrose, 0.8% w/v agar (BDH) and were adjusted to 5.8 pH before autoclaving at 1.05 kg cm^{-2} for 17 min. Cultures were maintained at 25°C under a 16 h photoperiod (2500 lux). Mitotic chromosomes were examined from root tip cells of 10 randomly selected regenerated plants. Root tips were pretreated with a saturated aqueous p-dichlorobenzene solution for 3½ h at 8°C, fixed in 1:3 acetic alcohol for 24 h, hydrolysed in 1 part of 1N HCl : 9 parts of 1% aceto-orcein for 15 min at 60°C and squashed in 1% aceto-orcein.

RESULTS AND DISCUSSION

Friable pale-yellow callus was obtained from the explants after 8 weeks of culture. Embryogenic callus developed only on a medium supplemented with 1.0 mg/l 2,4-D and 0 mg/l Kn. Numerous small white clumps of compactly packed cells ranging in diameter from 2-3 mm were formed on the callus after 2 weeks. These clumps were composed of starch filled, richly cytoplasmic spherical and slightly elongated cells. A large number of globular embryos differentiated from each cell clump after 3 weeks (Fig.1). The globular embryos did not grow further in presence of 2,4-D and could be induced to form heart-shaped (Fig.2) and mature embryos (Fig.3) within 2-3 weeks after transfer to basal or 1 mg/l Kn supplemented medium. The inhibitory effect of 2,4-D on mature embryo formation has also been reported in other umbellifers such as Daucus carota (Halperin 1966, Jones 1974) and Apium graveolens (Williams and Collin 1976a). The number of mature embryos per callus piece on the basal medium was as high as 21 as compared to 10 on medium with Kn. Mature embryos were 2-3 mm long and had two cotyledons which contrasts with pseudomonocotyledonous condition found in zygotic embryos (Engstrand 1973). The embryos upon transfer to the basal or 1 mg/l Kn fortified medium germinated within 2-3 weeks and gave rise to rooted plantlets (Fig.4). A globose tuber (2-5 mm diameter) developed on the root of each plantlet (Fig.5). Every plantlet examined

Offprint requests to: A. K. Wakhlu



Figs.1-6. Somatic embryogenesis and plant regeneration in Bunium persicum. Fig.1. Globular embryos. Figs.2,3. Heart-shaped and mature embryos (x 400). Figs.4,5. Plantlets differentiated from embryos. Arrow shows a tuber. Fig.6. Root tip cell of a regenerant with 14 chromosomes (Bar = 10 um).

cytologically showed normal diploid chromosome number of 2n=14 (Fig.6). A similar situation has been demonstrated in celery plants regenerated through the process of somatic embryogenesis (Williams and Collin, 1976b). The morphology and performance of plants is being tested in the field conditions. The present study has shown that in B. persicum plant regeneration can be accomplished via somatic embryogenesis. This fact coupled with the chromosome stability of the regenerants can be exploited in the micropropagation of its plants.

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