P 41 High Frequency Plant Regeneration from Petiole-derived Embryogenic Cell Cultures of *Hylomecon vernalis* Max.

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Objectives

Hylomecon vernalis Max. is a herbaceous medicinal plant belonging to the Papaveraceae. The whole body has medicinal potential and is also occasionally edible though it can be harmful if consumed in large quantities. Also it is one of the most charming and colorful poppies for the garden. However, despite its medicinal and ornamental potential scientific studies have not been reported on this species, yet. Only a few *in vitro* culture studies have been reported on systematically related species including production of sanguinarine in cell cultures and regeneration in pedicel explants and immature ovule-derived callus of *C. majus* var. *asiaticum* via somatic embryogenesis. Tissue culture techniques may provide an alternative mean for its mass multiplication and ex-situ conservation. There is, however, no recognized protocol for the tissue culture of this species. This study describes culture conditions for high frequency plant regeneration via somatic embryogenesis from cell suspension cultures of *H. vernalis*.

Materials and methods

1. Plant material: Petiole and leaf of *Hylomecon vernalis* Max. grown in the field were collected. Petioles and leaves were dissected with a forceps and scalpel. Petiole and leaf explants were placed onto callus induction medium in a Petri dishes. To examine the effect of 2,4-D on embryogenic callus formation, petiole and leaf explants were placed onto B5 medium supplemented with 0, 0.45, 1.36, 4.52, or 13.6 μM 2,4-D. Also, to increase the frequency of embryogenic callus formation, petiole and leaf explants were placed onto B5 medium containing 4.52 μM 2,4-D and 0, 0.44, 1.33, 2.22 and 4.44 μM BA. For the initiation of a suspension culture, the calluses derived from petiole were carefully disintegrated with sterile forceps and transferred to a 250 ml of flask containing 10 ml of liquid B5 medium supplemented with 4.52 μM 2,4-D (B51D). After two weeks of culture, 20 ml of liquid B51D was added. After two to three weeks culture, five ml of cell suspension culture were transferred to a 250 ml of flask containing 50 ml of liquid B51D medium.

Results and discussions

Culture conditions for high frequency plant regeneration via somatic embryogenesis in embryogenic cell suspension cultures of *H. vernalis* Max. are described. Petiole explants formed white embryogenic calluses at a frequency of 50% when cultured on B5 medium supplemented with 4.52 μM 2,4-D. The frequency of embryogenic calluses formation was slightly increased with an increasing concentration of 2,4-D up to 13.6 μM of 2,4-D, where the frequency reached 53%. Cell suspension cultures were established from embryogenic calluses using MS liquid medium containing 4.52 μM 2,4-D. Upon plating onto MS basal medium, cell aggregates from cell suspension cultures produced somatic embryos which then developed into plantlets.

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