## High Frequency Plant Regeneration from Leaf-derived Embryogenic Cell Suspension Cultures of *Pinellia tripartita* (Blume) Schott.

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## **Objectives**

Pinellia tripartita (Blume) Schott is herbaceous medicinal plant belonging to the Araceae. The fresh root of pinellia is extremely acrid and contains toxins (presence of calcium oxylate), these are destroyed by drying or cooking. The chemical composition of pinellia is not well established, with small amounts of alkaloids being identified, including traces of ephedrine. A glycoprotein fraction was reported to have notable antiemetic effects (Kurata and Tai, 1998). However, despite its medicinal importance scientific studies of Pinellia tripartita have not been reported, yet. Also natural habitats of this plant decreased gradually because of industralization. Tissue culture techniques may provide an alternative mean for its mass multiplication and ex-situ conservation. This study describes culture conditions for high frequency plant regeneration via somatic embryogenesis from cell suspension cultures of Pinellia tripartita.

## Materials and methods

Plant material: Mature plants of *Pinellia tripartita* (Blume) Schott grown in the field from Jook-do 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 somatic embryo formation, petiole and leaf explants were placed onto MS medium supplemented with 0, 0.45, 1.36, 4.52, or 13.6  $\mu$ M 2,4-

D. To increase the frequency of somatic embryo formation, the effect of combination treatment (NAA and BA) was examined. Petiole and leaf explants were placed onto MS medium containing 8.8  $\mu$ M BA and several concentration of NAA. Also, petiole and leaf explants were placed onto MS medium containing 10.74  $\mu$ M NAA and several concentration of BA.

## Results and discussions

Culture conditions for high frequency plant regeneration via somatic embryogenesis in cell suspension cultures of Pinellia tripartita are described. Leaf explants formed white nodular structures without callus formation at a frequency of 36.8% when cultured on MS medium supplemented with 10.74  $\mu$ M NAA and 1.33  $\mu$ M BA. However, the frequency of somatic embryo formation was slightly decreased with an increasing concentration of BA up to 4.44  $\mu$ M, where the frequency reached 26.7%. In 2,4-D alone treatment, no somatic embryo formation was observed with an increasing concentration of 2,4-D up to 13.6 µM, regardless of kinds of explant and concentration of 2,4-D. Leaf and petiole-derived embryogenic calluses were subcultured at four-weeks interval, respectively. And cell suspension cultures were established from the calluses using MS liquid medium containing 4.52  $\mu$ M 2,4-D. Upon plating onto MS basal medium, cell aggregates from cell suspension cultures produced somatic embryos which then developed into plantlets. Regenerated plantlets were transplanted to potting soil and grown to maturity in a growth chamber.