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Isolation and assay of polysaccharide from somatic embryos of *Angelica gigas* Nakai cultured in bioreactors

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Objectives

1. To establish a system for high frequency plant regeneration system via somatic embryogenesis of *Angelica gigas* using bioreactor.
2. The comparison of polysaccharide content of cultivated roots and bioreactor cultured somatic embryos.

Materials and Methods

1. Material: Somatic embryos of *Angelica gigas*
2. Methods:
   - After seedlings in vitro, excised cotyledons and hypocotyls were placed on MS medium supplemented with 0.5, 1.0, 3.0 mg/L 2,4-D. Embryogenic calli were induced from the excised tissue and they developed into somatic embryos in 10 L bioreactor liquid medium. Somatic embryos were cultured in liquid MS basal medium with 3% sucrose and B5 basal medium with 3% glucose for plant regeneration.
   - Extraction and assay of polysaccharide:Polysaccharides were extracted in 32 mL of 80% ethanol from early somatic embryos (entrapped 300 m sieve), plantlets (entrapped 20 mm sieve) and cultivated root dried for 20 hrs at 60℃. Polysaccharide (with a potent Immunostimulating activity) content was analyzed by phenol-sulfuric acid methods.

Results and Discussion

Embryogenic calli were induced from hypocotyls and cotyledons on MS solid medium supplemented with 0.5-3.0 mg/L 2,4-D. The frequency of embryogenic calli was better in the explants of cotyledons than those of hypocotyls. Embryogenic cells were maintained on MS medium supplemented with 1.0 mg/L 2,4-D. They were transferred to 250 mL erlenmeyer flask containing 50 mL liquid medium and cultured by suspension culture during 2 weeks. Early somatic embryos produced from embryogenic cells in shake-flask culture were transferred to 10 L bioreactors. After 4 weeks of culture, somatic embryos and plantlets were obtained in 10 L bioreactors. Somatic embryos were more developed in MS medium with 3% sucrose than B5 medium with 3% glucose. The polysaccharide content of cultivated roots, somatic embryos and plantlets cultured in B5 medium, somatic embryos and plantlets cultured in MS medium was 18.13, 17.03, 17.33, 16.21, 16.47 mg/g DW, respectively.

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