

**D203** Plant regeneration from mesophyll protoplasts of *Arabidopsis thaliana*

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Protoplasts were isolated from the leaf mesophyll tissue and cultured in liquid medium supplemented with 2.0 mg/L NAA, 0.5 mg/L BAP and 9% mannitol. Protoplast-derived microcolonies were formed after 4 weeks of culture in the dark at 27°C and transferred to CP callus propagation medium. Shoot were initiated from the green spots of the selected shoot forming calli cultured on MS regeneration medium supplemented with 0.05 mg/L IAA, 7.0 mg/L 2-iP and 30 g/L sucrose under continuous illumination for 4 weeks. Shoot regeneration frequency (calli regenerating at least one shoot) was more than 50%. Roots were induced from the regeneration shoot on MS medium without phytohormones. The regenerants were successfully transplanted into potting soil.

**D204** Characterization of Pathogenesis-Related Gene and Zinc Finger Protein cDNA Clone Obtained by Expressed Sequence Tags of *Pimpinella brachycarpa* Shoot-tip cDNA

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A cDNA library of *Pimpinella brachycarpa* shoot-tip was subjected random sequencing to isolate novel genes involved in shoot-tip development. Partial nucleotide sequences of 220 individual cDNA clones have been determined and compared with the Genbank database. This expressed sequence tag (EST) analysis identified 106 clones (48.18%) that had a significant homology to known genes in the Genbank database. Among these clones, two clones (*PbPRI* and *PbZFP1*) were characterized. *PbPRI* clone had an open reading frame of 462 bp which encoded a polypeptide of 154 amino acids (calculated molecular weight = 16.123 kD). Using adaptor-mediated nested PCR, 159 bp intron and 5' upstream region of *PbPRI* gene were isolated. *PbZFP1* clone was 1809 bp with an open reading frame of 1545 bases which encoded a polypeptide of 515 amino acids (calculated molecular weight = 57.553 kD) including two zinc finger motifs of the Cys<sub>2</sub>/His<sub>2</sub>. Expressed patterns of *PbPRI* and *PbZFP1* were examined by reverse transcriptase-polymerase chain reaction and Southern hybridization. *PbPRI* gene was highly expressed in leaves and petioles, but in roots. And *PbZFP1* clone was highly expressed in leaves, petioles, and flowers, whereas weakly expressed in roots.