Immunohistochemistry of cell wall protein in inflorescence stems of *Arabidopsis thaliana*.

Fixation, Embedding, and Sectioning of Inflorescence Stems of Arabidopsis thaliana

Arabidopsis plants were grown on rock wool until the primary inflorescence stem reached a length of 80 to 120 mm.

1) Cut the inflorescence stem into pieces about 2 to 3 mm in length each.
2) Fix the stem segments in 4% paraformaldehyde in phosphate-buffered saline (PBS).
3) Wash the segments in PBS.
4) Embed the segments in 5% (w/v) agar block-containing PBS buffer.
5) Section the agar-embedded stem segments transversely with a Vibratome Series 3000 Sectioning System (Technical Products International, Inc., St. Louis) using a razor blade. Transverse sections (50-µm thick) are collected in PBS.

Immunohistochemistry

To achieve high-sensitivity and high-resolution immunolocalization, we used the tyramide signal amplification (TSA) method based on the peroxidation of Alexa Fluor 488 tyramide, and detected the antigen according to the manufacturer's protocol (Tyramide Signal Amplification kits; Invitrogen-Molecular Probes). In the course of the peroxidation reaction, the highly reactive fluorescent radicals covalently couple to nucleophilic residues in the vicinity of the binding sites of a horseradish peroxidase antibody conjugate used as a secondary antibody.

1) Incubate the sections with 1-3% H_2O_2 in PBS for 60 minutes at room temperature (This procedure is employed for quenching endogenous peroxidase activity).
2) Incubate the sections with 1% blocking reagent for 60 minutes at room temperature.
3) Label the sections with a GRP-specific peptide antibody diluted* in 1% blocking reagent for 60 minutes at room temperature.
4) Rinse the sections three times with PBS.
5) Incubate the sections with the HRP conjugate working solution** for 30-60 minutes

at room temperature.

6) Rinse the sections three times with PBS.
7) Incubate the sections with the tyramide working solution*** for 5-10 minutes at room temperature.
8) Rinse the sections three times with PBS.
9) Mount the specimen on microscope slides using PBS/50% glycerol medium.
10) The samples were observed under a fluorescence binocular microscope (Leica MZ APO using a GFP filter).

* We used a GRP-specific peptide antibody against the less conserved C-terminal part of a cell-wall structural protein, Glycine Rich Protein (GRP). The antibody was used at a dilution of 1:20.

** Preparation of HRP conjugate working solution

1) Prepare the HRP conjugate stock solution by reconstituting the material provided in 200 μL of PBS.
2) Prepare a working solution of the HRP conjugate by diluting the stock solution 1:100 in blocking solution.

*** Preparation of Tyramide working solution

1) Prepare tyramide stock solution by dissolving the solid material provided in 150 μL of DMSO.
2) Prepare amplification buffer/0.0015% H_2O_2 by adding 30% hydrogen peroxide to amplification buffer.
3) Prepare a tyramide working solution by diluting the tyramide stock solution 1:100 in the amplification buffer/0.0015% H_2O_2 just prior to labeling.