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Semi-thin section analysis

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Lin S, Miao Y, Su S, Xu J, Jin L, Sun D, Peng R, Huang L, Cao J (2019) Comprehensive analysis of Ogura cytoplasmic male sterility-related genes in turnip (*Brassica rapa* ssp. *rapifera*) using RNA sequencing analysis and bioinformatics. PLoS ONE 14(6): e0218029. doi: 10.1371/journal.pone.0218029

MATERIALS TEXT

2.5% glutaraldehyde in phosphate buffer (pH7.0), phosphate buffer (pH7.0), 1% OsO_4 in phosphate buffer (pH7.0), ethanol (50%, 70%, 80%, 90%, 95% and 100%), absolute acetone, Spurr resin, 0.5% toluidine blue.

1 Double fixation: The specimen was first fixed with 2.5% glutaraldehyde in phosphate buffer (pH7.0) for more than 4 hours; washed three times in the phosphate buffer; then postfixed with 1% OsO_4 in phosphate buffer (pH7.0) for 1 hour and washed three times in the phosphate buffer.

2 Dehydration: The specimen was first dehydrated by a graded series of ethanol (50%, 70%, 80%, 90%, 95% and 100%) for about 15 to 20 minutes at each step, transferred to absolute acetone for 20 minutes.

3 Infiltration: The specimen was placed in 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1 hour at room temperature, then transferred to 1:3 mixture of absolute acetone and the final resin mixture for 3 hours and to final Spurr resin mixture for overnight.

4 Embedding and semi-thin sectioning: Specimen was placed in capsules contained embedding medium and heated at 70°C for about 9 hours. Semi-thin sections (1 µm) were sliced under a LKB 11800 PYRAMITOME ultramicrotome (Stockholm, Sweden) and stained with 0.5% toluidine blue. Images of the anther cross-sections were obtained with a Leica DMLB fluorescence microscope (Leica Microsystems, Wetzlar, Germany).