Plastic Embedding and Sectioning of Plant Tissues

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[Abstract] Plastic (resin) embedding provides exclusively improvements to cellular definition compared to paraffin embedding. The combination of strongly cross-linking paraformaldehyde with glutaraldehyde and post fixed with OsO_4 is the fixative of choice for high-resolution light microscopy and electron microscopy. For this reason, this method is an ideal tool for visualizing plant cellular morphology and phenotype.

Materials and Reagents

1. Ethanol
2. Acetone
3. Na_2HPO_4
4. Gelatin capsules (Electron Microscopy Sciences, catalog number: 71012)
5. Glutaraldehyde (Fluka, catalog number: 49627)
6. Paraformaldehyde
7. OsO_4
8. Catalyst
9. Osmium (Fluka, catalog number: 75633)
10. Fixative solution (see Recipes)
11. Post-fixative solution (see Recipes)
12. $NaPO_4$ buffer (see Recipes)
13. Resin (LR white) (Fluka, catalog number: 62662) (see Recipes)

Equipment

1. Shaker
2. Incubator with temperature control
3. Light or electron microscopy

4. Vacuum
5. Glass blade or jeweler saw (Electron Microscopy Sciences, catalog number: 71012)

Procedure

1. Fixation: Cut and fix plant tissues in fixative solution for 2 h, then vacuum for 15 min without shaking. If the tissues did not sink, re-infiltrated for another 10 min. The tissues can be stored at 4 °C over night.
2. Rinse: Rinse the entire samples in 0.5 M $NaPO_4$ buffer, and then wash tissues in 0.5 M $NaPO_4$ buffer for 3 times; 15 min each at 4 °C. Add enough $NaPO_4$ buffer to cover the entire tissues.
3. Post-fix: Transfer samples to 1% Osmium (OsO_4) in 0.5 M $NaPO_4$ buffer for 1 h in the dark. The samples should be immersed in the post-fixative solution. The tissues can be kept for another hour in post-fixative solution until they all turned black.
4. Wash without changing container: 3 times in ddH_2O; 10 min each at 4 °C on a rotating shaker.
5. Dehydration without shaking: Dehydrate the tissues in a graded ethanol series as below:
   12.5% ethanol, 10 min
   25% ethanol, 10 min
   35% ethanol, 10 min
   50% ethanol, 10 min
   70% ethanol, 10 min
   80% ethanol, 10 min
   95% ethanol, 10 min
   100% ethanol, 10 min (twice)
   100% acetone, 10min (twice)
6. Infiltration: To damp the old solution and exchange the 100% ethanol with acetone-resin mixture as following steps:
   Acetone: resin (1:1) 1 h
   Acetone: resin (1:2) 1/2 h
   100% resin 1 h
   100% resin over night on shaker
7. Polymerize: Put samples in gelatin capsules filled with 100% fresh resin, leave samples at 50 °C for 60 h. Once the resin was solidified, the capsules can be kept at 4 °C for a few weeks.
8. Blocks can be trimmed and cut with glass blade or jewelers saw depending on what shapes were required for light or electron microscopy. To position the blocks depend on

the dimension (cross, transverse, longitudinal) section layout was required. The sections were picked up and floated out on 30% acetone on a warmer plate (42 °C) until dry.

Recipes

1. Fixative solution (make fresh with ddH_2O)
   3% Glutaraldehyde (10% stock)
   1.5% Paraformaldehyde (16% stock)
   1.6% 1 M $NaPO_4$
2. Post-fixative solution (make fresh)
   OsO_4 (4% stock) 2 ml
   1 M $NaPO_4$ 4 ml
   H_2O 2 ml
3. 1 M $NaPO_4$ buffer (pH 6.8)
   $NaH_2PO_4.H_2O$ 137.99 g/L
   Na_2HPO_4 141.97 g/L
4. Resin (LR white)
   Add 9.9 g catalyst to 500 ml LR white, shaken thoroughly.

Acknowledgments

This protocol is adapted from Alpers and Beckstead (1985) and Massover (2011).

References

1. Alpers, C. E. and Beckstead, J. H. (1985). Enzyme histochemistry in plastic-embedded sections of normal and diseased kidneys. *Am J Clin Pathol* 83(5): 605-612.
2. Massover, W. H. (2011). New and unconventional approaches for advancing resolution in biological transmission electron microscopy by improving macromolecular specimen preparation and preservation. *Micron* 42(2): 141-151.

