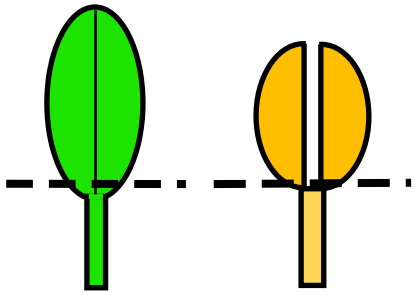
Fixation

Cotyledons and Leaves

Fixative*1
4% paraformaldehyde
1% glutaraldehyde
0.05M cacodylate buff. (pH7.4)

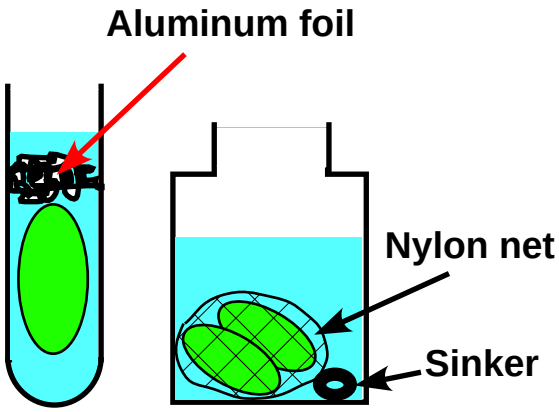
Washing buff.
0.05M cacodylate buff. (pH7.4)



*2 Cut slightly above the base of the petioles

*1 In case of etiolated cotyledons, use the fixative for dry seeds

Cut off the petioles (slightly above the base)*2 and soak the samples in fixative*3
↓
Vacuum infiltrate in fixative
(about -99kPa 10min → slowly return to normal pressure, 3 times)
↓
Cut the samples into 1-2mm square sections
↓
Fix for 2hours in fixative (room temp.)
↓
Wash twice with washing buff.



*3 When the samples float...

Dry seeds

Fixative
10% dimethylsulfoxide
4% paraformaldehyde
1% glutaraldehyde
0.05M cacodylate buff. (pH7.4)

Washing buff.
0.05M cacodylate buff. (pH7.4)

Cut the seeds in half and soak them in fixative

↓

Vacuum infiltrate

(about -99kPa 10min → slowly return to normal pressure, 3 times)

↓

Fix for 2hours in fixative (room temp.)

↓

Wash twice with washing buff.

Roots

Fixative

4% paraformaldehyde

1% glutaraldehyde

0.05M cacodylate buff. (pH7.4)

Washing buff.

0.05M cacodylate buff. (pH7.4)

Cut the samples into 1-2mm long sections and soak them in fixative

↓

Vacuum infiltrate

(about -99kPa 10min →slowly return to normal pressure, 3 times)

↓

Fix for 2hours in fixative (room temp.)

↓

Wash twice with washing buff.

Embedding

Dehydration and infiltration

Replace the buffer with 50% (v/v) DMFA (Dimethylformamide)

50% DMFA 4ºC 15min× 2
80% DMFA -20ºC 15min× 2
90% DMFA -20ºC 15min× 2
100% DMFA -20ºC 20min× 3
LR White : DMFA = 1:1 -20ºC 30min
LR White : DMFA = 2:1 -20ºC 30min
LR White -20ºC 30min
LR White -20ºC overnight

Polymerize

Fill the "Beem capsules" with LR White (accelerator added)
Put the samples in the capsules
Close the capsules without leaving any air inside*4

UV irradiation -20ºC 24hrs
UV irradiation 4ºC 1hr
UV irradiation room temp. 1-3hrs

***4 Add the resin until it overflows before closing the capsules**

Sectioning

Trim the polymerized blocks and make ultrathin sections using ultramicrotome
Mount the sections on nickel grids

Immunostaining

Phosphate buffer saline (PBS)
0.01M phosphate buff.– 0.85% NaCl

Blocking solution
1% BSA– 0.1% NaN_3 in PBS
(All antibodies are diluted with blocking solution)

Blocking

Room temp. 30min in blocking solution
Wash grids with PBS

1st antibody reaction

4ºC overnight in 1st antibody solution (in moisture chamber)
Wash grids with PBS

2nd antibody reaction

Room temp. 30min in gold-labeled 2nd antibody solution
Wash grids with DW

Electron staining

Uranium acetate 3min
Lead citrate 40sec

TEM observation