I. Fixation and Embedding of *Arabidopsis* tissue

Solutions

Fixative Mix **FAA**	% in Mix	for 100 ml add
Ethanol 95%	50.0%	52.6 ml
Glacial Acetic Acid	5.0%	5 ml
37% Formaldehyde	3.7%	10 ml
Water	41.3%	32.4 ml

Infiltration Solution 10 ml "Basislösung Technovit" + 0.1g Härter I

Polymerisation Solution 15 ml of Infiltration solution + 1ml of Härter II

Protocol

Fixation

We have tried a variety of fixation procedures. For floral tissue, the best signals come from tissue fixed in FAA

- Place 10-15 ml of fixative into 20 ml vials.

- Cut a cluster of flowers at the apex of the floral stem that includes stages 1-14 and other selected organs, and immediately immerse in fixative. Cut so that 1-2 mm of floral stem is present. This helps to orient the tissue when sectioning. Cut off older flowers if you are not interested in these stages. If possible, cut tissue while immersed. Place as many pieces of tissue that will easily cover the bottom of a vial as a single layer of tissues.

- The tissue will float in the fixative. Incubate at room temperature until the tissue sinks to the bottom of the vial (fixation complete, 1-2 hours)

- Remove fixative and add 50% ethanol. Incubate at room temperature for 30 minutes. Repeat this step.

Dehydration

The tissue must be completely dehydrated before exposure to solvents from the Technovit "basis" solution otherwise, the water and solvents will form a white emulsion.

- Remove 50% ethanol and replace with 60% ethanol. Incubate for 30 minutes

- Repeat for the following ethanol solutions: 70%, 85%, 95%.

- Leave overnight in 95% ethanol

- Next day, remove as much as possible of 95% ethanol and replace with 100% ethanol. Incubate for 1 hour, not longer (e.g. overnight).

- Remove as much as possible of the 100% ethanol, replace with fresh 100% ethanol, and incubate for 30 minutes. (Repeat this step if it is impossible to remove virtually all of the solution in the 2 previous steps)

Clearing

The tissue must be permeated with the Technovit "basis" Solution because Härter I is not miscible in ethanol.

- Remove 100% ethanol and replace with 25% Technovit "basis" Solution:75% ethanol. Incubate at room temperature for 2 hours

- Repeat for the following Technovit "basis" Solution: ethanol solutions:

50% Technovit "basis" Solution:50% ethanol

75% Technovit "basis" Solution:25% ethanol

- Remove the 75% Technovit "basis" Solution and replace with 100% Technovit "basis" Solution. Incubate at room temperature for 1 day

Infiltration

Technovit "basis" Solution is used as an organic solvent for infiltration of "Härter I".

- replace 100% Technovit "basis" Solution with the **Infiltration Solution** and incubate overnight

Polymerization

Polymerization occurs when "Härter II" is added to the **Infiltration Solution.**

- Place **Polymerisation Solution** in either lids of microfuge tubes or in "small boats". Place tissue in it. Arrange the tissue into a regular array using a dissecting needle. Orient tissue pieces with the stems either straight up (for transverse sections) or lying on their side (for longitudinal sections). Tissue pieces must be at least 5 mm apart. Incubate overnight

Sectioning:

- Cut out blocks of embedded tissue and “glue” them onto histoblocks with Technovit 3040

- mount onto microtome blocks and section tissue at 10μm

- Cut ribbons into ~1.5 cm pieces. Float ribbon pieces on water for >1 minute. This step takes the compression out of the tissue

- Put slide in water just under the floating ribbon

- Bring slide up so as to catch the ribbon. Use a teflon-coated spatula to position the ribbon. Minimize the amount of water trapped between section and slide. Dry the slide before adding more ribbon pieces**.** Repeat for as many different ribbon pieces as you want to place on a given slide

- Incubate slide in a 60-70°C incubator until fully dry

- It is finally ready to be observed under the microscope