Arabidopsis Pollen Tube Aniline Blue Staining

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[Abstract] The aim of this experiment is to track pollen tube growth *in vivo* in female tissues after pollination. This can be used to phenotype pollen germination, tube growth and guidance, as well as reception.

Materials and Reagents

A. Stock solutions:
   1. Acetic acid
   2. Ethanol
   3. NaOH
   4. K_2HPO_4
   5. KH_2PO_4
   6. Aniline blue (Thermo Fisher Scientific)
   7. Glycerol

B. Working solutions:
   1. 10% acetic acid in EtOH (fixative)
   2. 0.01% aniline blue in KPO_4 buffer (dye)
   3. KPO_4 buffer made with 50% glycerol (mounting media)
   4. KPO_4 buffer (see Recipes)

Equipment

1. Microscope with UV light

Procedure

1. Submerge pistil tissue in 250 µl acetic acid and fix it for 1.5 h or more in an Eppendorf tube. Tissue can be fixed overnight if necessary.
2. Soften tissue by submerging it in 1 M NaOH overnight.
3. Wash 3 times with KPO_4 buffer (tissue is fragile at this stage).
4. Stain with 200 µl aniline blue for 5-10 min or as long as 10 h.

5. Transfer to a slide, add mounting media and observe under UV. Squash if necessary.

Recipes

1. 50 mM KPO_4 buffer (pH 7.5)
   4.17 ml 1 M K_2HPO_4
   0.83 ml 1 M KH_2PO_4
   995 ml H_2O

References

1. Lu, Y., Chanroj, S., Zulkifli, L., Johnson, M. A., Uozumi, N., Cheung, A. and Sze, H. (2011). Pollen tubes lacking a pair of K^+ transporters fail to target ovules in *Arabidopsis*. *Plant Cell* 23(1): 81-93.
2. Modified from the online protocol on Dr. Daphne Preusss's lab (Univ of Chicago).