Arabidopsis Pollen Tube Germination Protocol

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[Abstract] This method uses a PEG-supplemented liquid solution to germinate separated *Aradidopsis* pollen. It thus eliminated the need for humidity control.

Materials and Reagents

A. Plant growth

1. Stratify surface-sterilized seeds on 1x Johnson's medium at 4 ˚C for at least 3 days to synchronize the flowering time. Transfer the plates to growth chamber with sufficient illumination (*e.g.* 12-16 h light/day, growth chamber on the 2nd or 3rd floor is OK).
2. 1 week seedlings are transferred to soil pots. I usually grow up to 8 seedlings per standard pot. Mutant and wildtype should be put side by side to avoid position effect in the growth chamber.
3. Water the plants twice a week (Tuesday and Friday, for example), and apply 0.5x Johnson's medium once every other week. The plants may begin to flower 3-4 weeks after transferring. The fresh flowers on the first inflorescence will be good for pollen germination experiments (*note: The time to pick flowers is very critical*).

B. Preparation of germination plates

Prepare germination medium plates as following (Fan *et al.,* 2001)

	Stock	Final Conc.	V stock/40 ml
MES-Tris (pH 5.8 adjusted with Tris base)	200 mM	5 mM	1 ml
KCl	1 M	1 mM	40 µl
$MgSO_4$	0.5 M	0.8 mM	64 µl
Boric acid	100 mM	1.5 mM	600 µl
$CaCl_2$	0.5 M	10 mM	800 µl
Sucrose		5% w/v	2 g
PEG4000		15% w/v	6 g

Note:

a. *Mix well when add $MgSO_4$ and $CaCl_2$ to avoid precipitation.*
b. *5 mM Ca might be better than 10 mM sometimes.*
c. *You can make pollen germination medium (PGM) without Ca, use it to prepare the pollen resuspension. The unwanted germination before time 0 can be avoided this way.*

Equipment

1. Incubator with humidity control or saturated humidity
2. Nikon microscope
3. Scion Image (free download from NCBI website)
4. 8-well chamber (Lab-Tek International, catalog number: 155411 or VWR International, catalog number: 43300-774)

Procedure

1. Collect 20-50 freshly opened flowers (stage 13-15, in which the long filaments are just level with stigma and petals) in 1.5 ml tube, let dry on RT for 0.5 h (tube cap opened).
   Note: You can remove all open flowers from the plant 16-24 h before the pollen experiment. By this way just simply pick all flowers without spending time on identifying right stage (warning: wounding response may happen). Flowers picked in the morning are better than in afternoon.
2. Add 1 ml germination medium to submerge the flowers. Vortex at maximal speed for 1 min.
3. Concentrate the pollens by 500 *x g* for 5 min, at RT centrifuge (3,000 rpm on mini-centrifuge). Carefully remove the supernatant and floating flower residues, resuspend the pollen pellet in 1 ml germination medium (with Ca if you used –Ca PGM previously) by vortex.
4. Use 10 µl suspension for pollen amount estimation and purity check under light microscope (typical yield of 10 µl from 20 flowers is somewhere 2,000-5,000 grains).
5. Germinate the pollen grains at 25-28 °C for 6 h or over night in the chambers of a chambered coverlip (200 µl pollen suspension/ 8-well chamber); no agitation.
6. Take photos of the germinated tubes using 10x lens on Nikon microscope (inverted is better). Since the tube is transparent, phase contrast will give good pictures.

7. Analyze the tube germination (rate and length) using Scion Image. A normal distribution is expected for each population of pollen tube length, the P value should be less than 0.05 for student's test.

References

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