Pollen Germination with Fast Plants

Pollen from Fast Plants can be stimulated to germinate in a sucrose-salt solution maintained as a hanging drop slide. Within 30 to 40 minutes, early pollen tubes can be observed under 40X magnification, growing from the pollen grains.

Materials

- table sugar and salts for stock solutions
- pipette
- clear film can lid
- 22 mm square #2 glass coverslip
- stamens
- Fast Plants flowers
- compound microscope

Prepare Stock Solutions

1. To prepare 1.2 M sucrose solution, dissolve 41 grams of sucrose (table sugar) in enough distilled water to make a final volume of 100 ml. This is equivalent to one level tablespoon in the volume that will fill one 35 mm film can.

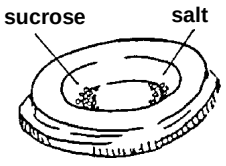
   Keep this solution refrigerated to avoid bacterial and/or fungal growth. Make up fresh solutions if any growth is observed.

2. Prepare a mineral salt solution by adding the following amounts of salts to distilled water to bring the final volume to one liter:

   - 0.417 g $Ca(NO_3)_2$ (calcium nitrate)
   - 0.200 g H_3BO_3 (boric acid)
   - 0.101 g KNO_3 (potassium nitrate)
   - 0.217 g $MgSO_4 \cdot 7H_2O$ (magnesium sulfate)

Procedure

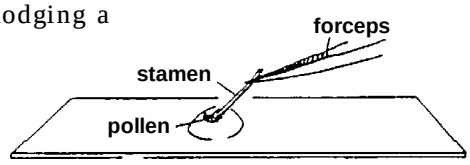
1. Place 2 drops of sucrose solution and 6 drops of salt solution into a clean, clear Fuji® film can lid.



2. Mix the liquid by sucking it into a pipette and squirting it back into the film can lid.

3. Use the pipette to place one drop of the mixture in the center of a 22 mm square #2 glass coverslip.

4. Pluck a stamen from a fresh Fast Plants flower and touch the anther to the drop, dislodging a small amount of pollen on the coverslip.



5. Carefully invert the coverslip so that the drop hangs from the underside and place the cover slip over the film can lid, which should contain some residue of solution or water to maintain a humid environment around the hanging drop.

   Note: After 30 to 40 minutes, short pollen tubes (early in development) will be visible. The full length pollen tubes will form in 50 minutes to one hour. Students may want to wait 24 hours and observe the tubes in the next class period. The tubes may be dry at this point, but they will still be visible. Keeping the coverslip inverted over the film can lid will help retain moisture. To allow time for students to observe the early pollen tubes within one class period, the teacher may prepare the stock solutions prior to the start of the class.

6. To observe the pollen tubes, carefully turn the coverslip over, so that the drop is again on top. Place the coverslip on the stage of a compound microscope and observe under 40X magnification or greater.

Department of Plant Pathology, 1630 Linden Drive, Madison, WI 53706 1-800-462-7417 wfp@fastplants.cals.wisc.edu

Observations and Questions

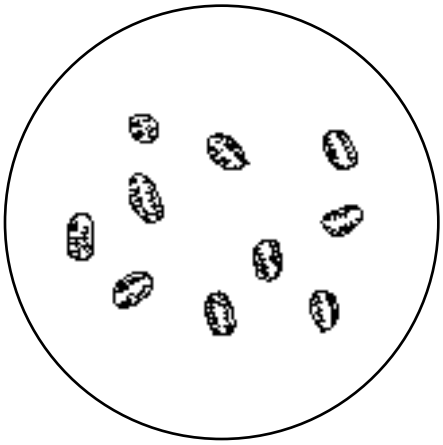
Observe and sketch your field of view at time = 0, after 30 to 40 minutes, after one hour and after 24 hours (see figures at right).

After one to 24 hours has passed, what do you observe?

- Approximately what percentage of pollen grains germinated?
- Draw some of the pollen tubes in your field of vision. Are some longer than others? What could cause this variation?

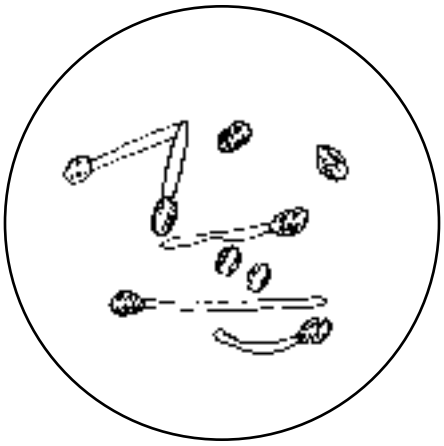
Extensions

- Place flowering Fast Plants in different environments. For example, put a pot of plants in a chamber of auto exhaust, cigarette smoke, rubber cement or glue fumes, ultraviolet radiation, etc. After a period of exposure, remove pollen and test it for germination. What do you see? Do you have viable pollen? Does the environment in which the plants are kept impact the rate of germination or the percentage of pollen that germinates?
- Try other brassica flower pollen. Do your results differ? What do you observe?
- Viable pollen reacts with MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide] to produce a magenta color. Mix one part of a 10 g/l MTT solution with five parts of a 60% sucrose solution. Place some Fast Plants pollen onto a microscope slide. Place a drop of solution onto the pollen. What happens? Is there a color change? Does the intensity of the stain depend on how "old" the pollen is?
- Fluorescein diacetate, a fluorescent stain, uses the integrity of the plasmalemma of the pollen grain as an indicator of viability. Mix a fluorescein diacetate solution by dissolving 2 mg in 1 ml acetone; this mixture is added drop by drop to 10 ml of 0.5M sucrose until the solution remains milky. Place a drop of the resulting 10^{-5} to 10^{-6}M fluorescein diacetate solution onto pollen grains on a microscope slide. Incubate the pollen for five minutes, then view under a fluorescence microscope. Viable pollen grains fluoresce bright yellow-green under near ultraviolet epiilluminance (350-400 nm).



Time = 0 minutes

Time = 30 minutes



Time = 1.5 to 24 hours