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Bedinger Lab Tomato: Pollen Staining Protocols
(September 22, 2010)

Emasculation

1. First, emasculate -1 stage tomato flowers (closed to slightly separating pale yellow petals. This is the stage of flower right before Bud Break, before any pollen is released from the anthers. The idea is that you are emasculating a flower that if left alone would open after 24hrs) by removing the anthers.

2. Mark the flowers by labeling with the female accession #, what it will be use for, and the date of pollination. Wait 24hrs.

3. Pollination is performed the next day by touching the stigma onto a surface (e.g. a 1.5 ml microfuge tube lid, or flower anthers) covered with pollen.
   Note: Pollen can be collected various ways, we mimic the vibration of bumble bee by using a hand-held tooth polisher to collect the pollen in a microfuge tube.

4. Pollinations are allowed to progress for 24 hours, unless specified otherwise (pollen tubes tend to be about 40% of the way down the style at 6 hours in a compatible/congruent pollination). Field flowers should be protected from pollinators by covering the inflorescence.

Fixing Pistils

1. After the desired amount of time post pollination, the remaining sepals, and petals are removed from around the pistil.

2. Using a scalpel or razor blade the pistil is cut at the base of the ovary just above the pedicel.

3. The excised pistil is placed in a 1.5 ml microfuge tube containing 0.5 ml (enough to completely submerge pistils) 3:1 95% EtOH: glacial acetic acid and left overnight or indefinitely.

Softening Pistils

1. Fix is removed by piptetting, and pistils are then submerged in 0.5 ml 5 M NaOH softening solution for 24 hours. Note: the tissue becomes extremely fragile and can be easily damaged after this stage. Also, the pistils tend to float right after adding NaOH so it good to gently shake the tube after a couple of hours and get the pistil to sink to the bottom.

Staining Pistils

1. After 24 hours the 5 M NaOH is carefully removed by pipetting (taking care not to disturb the pistil) and the pistil is gently washed 3-5 times with 0.5 ml ddH20 each time.

2. After the last wash the ddH2O is removed and replaced with 0.2 ml 0.001mg/ml ABF (Aniline Blue Fluorochrome) in 0.1M K_2HPO_4 pH 10 buffer.

a. Aniline Blue Fluorochrome was obtained from Biosupplies Australia (http://www.biosupplies.com.au). We prep a stock solution of 0.1mg/ml in ddH2O from the dry ABF they send. This is stored at +4° C. A further dilution of the stock solution to make a working stock solution is done using a 1:20 dilution of the stock solution into 0.1M K_2HPO_4. (In the past we have also used a 100X solution works best for us but you should try different amount that best work for your system).

3. Once the stain is added to the tubes, samples are immediately placed in the dark and allowed to sit for about 24hrs for best staining.

Mounting/Viewing Pistils

1. After incubation in ABF for 24 hours, pistils are removed and placed in a drop of 50% Glycerin on a microscope slide and covered with a cover slip.

2. View with a standard fluorescence microscope capable of exciting with a UV light source and DAPI emission filters to view the fluorescent signal from the tissue.

3. After viewing is complete, take nail polish and seal the edge of each cover slip. This stores the slide material so that we may return to it later.