

CROSSING ARABIDOPSIS

Materials: Fine forceps (Miltex #5)
95% ethanol for sterilizing forceps
Magnifying glasses (for example "opti visors" from Donegan Optical Co. Kansas City, MO.)
"Female" plant - pistil donor, 4-6 weeks old.*
"Male" plant - pollen donor, 4-6 weeks old.*
* If the plants are transferred from high humidity growth condition to low humidity in a lab for crosses, they may wilt badly. Several hours exposure to the low humidity is usually sufficient for the plants to recover.

1. Begin with the "female" parent plant. Choose several young flower buds that are located at the top of the inflorescence on the main flowering stalk (Fig. 1). The newly emerging white petals should be barely visible in the most mature flower bud chosen (Fig. 2). Do not use any flower bud that has opened and potentially exposed its pistil to parental pollen or another pollen source. A bud at the correct stage will contain short immature stamens with anthers that are greenish-yellow in color (Fig. 3). Remove all other flower buds and flowers from the inflorescence.



Fig. 1

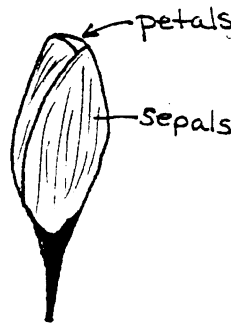


Fig. 2

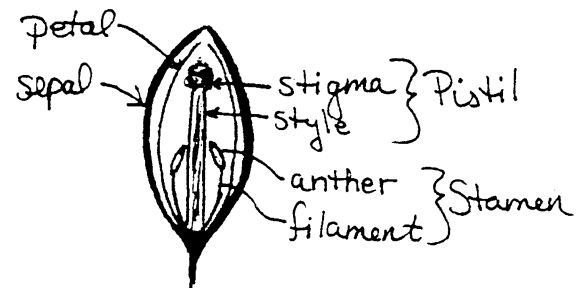


Fig. 3

Before dissecting the flower buds, sterilize the forceps and your fingers in 95% ethanol and air dry (to remove contaminating pollen). Next, remove the sepals, petals, and stamens from the flower buds. This is most easily accomplished by beginning with the tissue near the base of the flower bud. Generally, the most difficult step is the removal of the first sepal. Great care should be taken not to injure the pistil or flower stalk while dissecting the flower bud. When finished, the pistil should be free of sepals, petals, and stamens (Fig. 4).



Fig. 4

2. From the "male" parent plant choose a suitable pollen-donor flower. For example, for wild-type *Arabidopsis* choose a flower that has opened and has petals that are perpendicular to the main flower body (Fig. 5). Usually these flowers are in the process of releasing pollen. However, this is not always the case and therefore you should visually examine anthers from several flowers to identify the flowering stage associated with pollen release. Remove the flower from the flowering stalk and then remove the petals and sepals from the flower. There will be 6 stamens (2 short and 4 tall) in each flower. Remove several stamens and check their anthers for pollen. The pollen grains should be clearly visible when viewed under a dissecting microscope. The pollen releasing anthers will be grainy not smooth in appearance. When anthers brimming with pollen are identified, use them to pollinate the stigmas of the previously prepared pistils (Fig. 6). To maximize the probability of pollination, pollinate each pistil with several anthers.

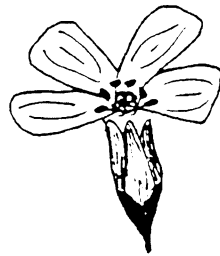


Fig. 5

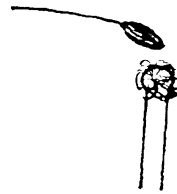


Fig. 6

3. When pollination is complete, cover the pistil with a small piece of plastic wrap (1cm x 1cm) to protect it from other pollen sources. Carefully fold the plastic wrap in half around the pistil (Fig. 7). Next, mark the pollinated pistil by applying a small piece of tape describing the cross on the corresponding flowering stalk (Fig. 7). Check the cross and remove the plastic wrap in 1 to 2 days. Following a successful pollination, the pistil will elongate as the seeds develop. When the silique is fully elongated and has dried to a golden-brown color, remove it from the plant. Take care not to shatter the silique and loose the seeds. Allow the seeds to dry for at least one week before planting. The seeds can also be chilled at 4° C. for several days following imbibition to increase the frequency of germination.

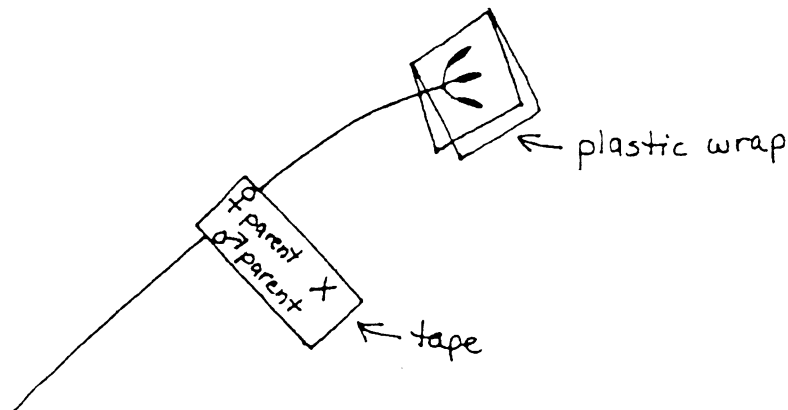


Fig. 7

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