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Harvesting Tobacco Seeds

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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Realizing Increased Photosynthetic Efficiency (RIPE)

ABSTRACT

Protocol for harvesting seeds from research tobacco plants.

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PROTOCOL CITATION

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KEYWORDS

Tobacco, greenhouse, harvesting, seeds, Nicotiana tabacu, Petite Havana, Samsun

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MATERIALS TEXT

- Pruning shears, Balkamp BK 5751193
- Seed tray, 25-3/4" L x 18" W x 3-11/16" Hgt. Green Tray with Handles or equivalent
- Weigh boat, large, Sterilin WB30317
- Storage vials, 20 mL Scintillation Vial VWR 66022-128
- Storage boxes, keep and reuse the cartons the storage vials come in.
- Biohazard bags, Scienceware®biohazard disposal bags B4408-100EA or equivalent.

SAFETY WARNINGS

Annual Worker Protection Standard training required to work in greenhouse. Contact UIUC ACES Plant Care Facility Coordinator to arrange training.

UIUC RIPE Greenhouse online training required to work in UIUC RIPE greenhouse. Contact UIUC RIPE Greenhouse Manager to arrange training.

BEFORE STARTING

If harvesting from transgenic plants, review all USDA-APHIS requirements for the RIPE APHIS permit for handling transgenic materials before beginning any experiment.

Pollinate plants at the appropriate time.

1 Inspect pollinating bag for any damage that could indicate cross-pollination. Discard any plants that look like the pollination bag may have been damaged.

2 Inspect the inflorescence. Seed development is complete when the plant begins to die back and the majority of the seed pods have turned brown.

Browning seed pods in an intact pollination bag.

3 Clip the stem of the plant just below the pollination bag. Be sure and include a plant identifier in or on the bag. Frequently the stake identifying the plant is moved into the pollination bag with the inflorescence that was just clipped

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from the plant.

4 Clean and sterilize a seed tray with 70% Ethanol. If working in an area used for transgenic plants, discard any seeds, plant material, or paper towel that may have seed on it in a biohazard bag.

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5 Place a new weigh boat into the seed tray.

The seed tray is for secondary containment only. Try to harvest the majority of the seeds into the weigh boat.

6 Place the inflorescence in the pollinating bag on to the seed tray and unwind the twist tie.

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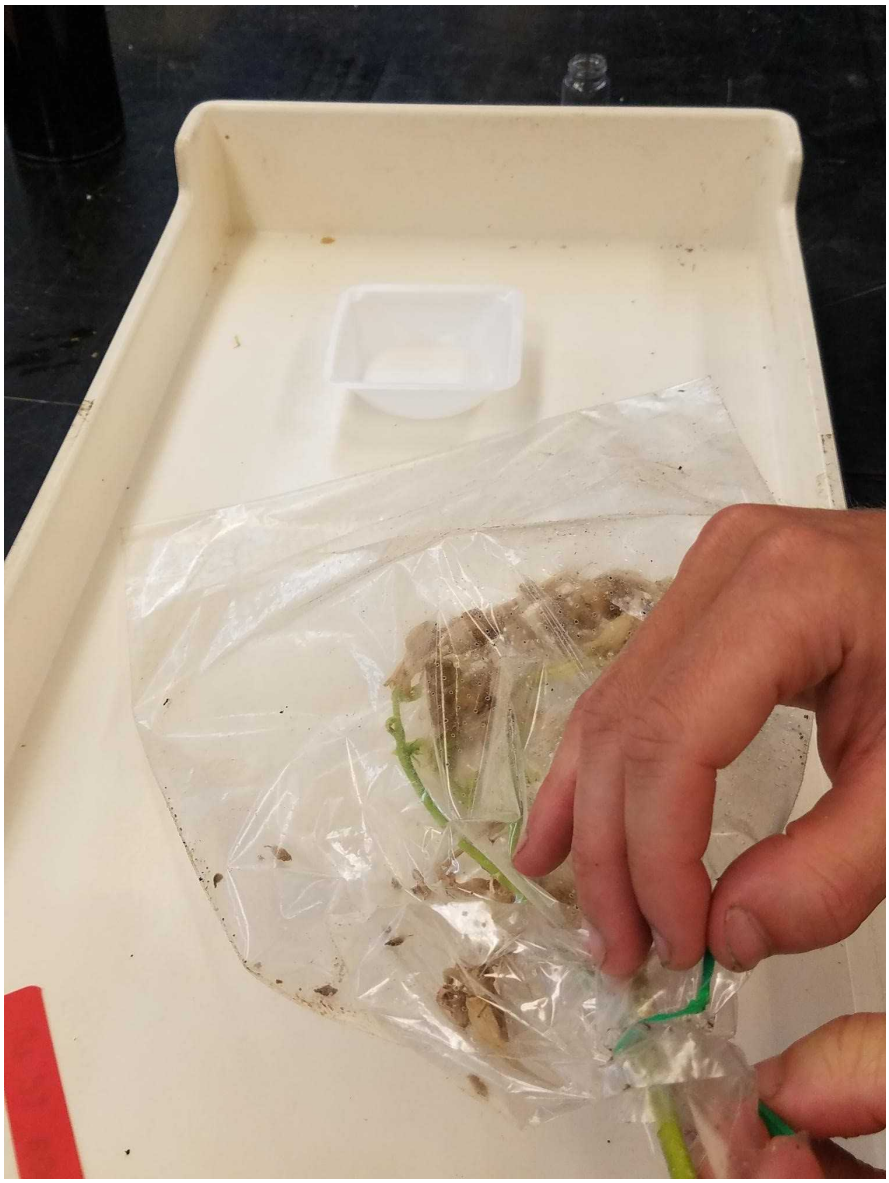

7 Label a vial with the date, the plant identification, and any other relevant information such as transgene, species, generation, experiment, etc.

Do not assign the seed to the Master Seed Library at this time.

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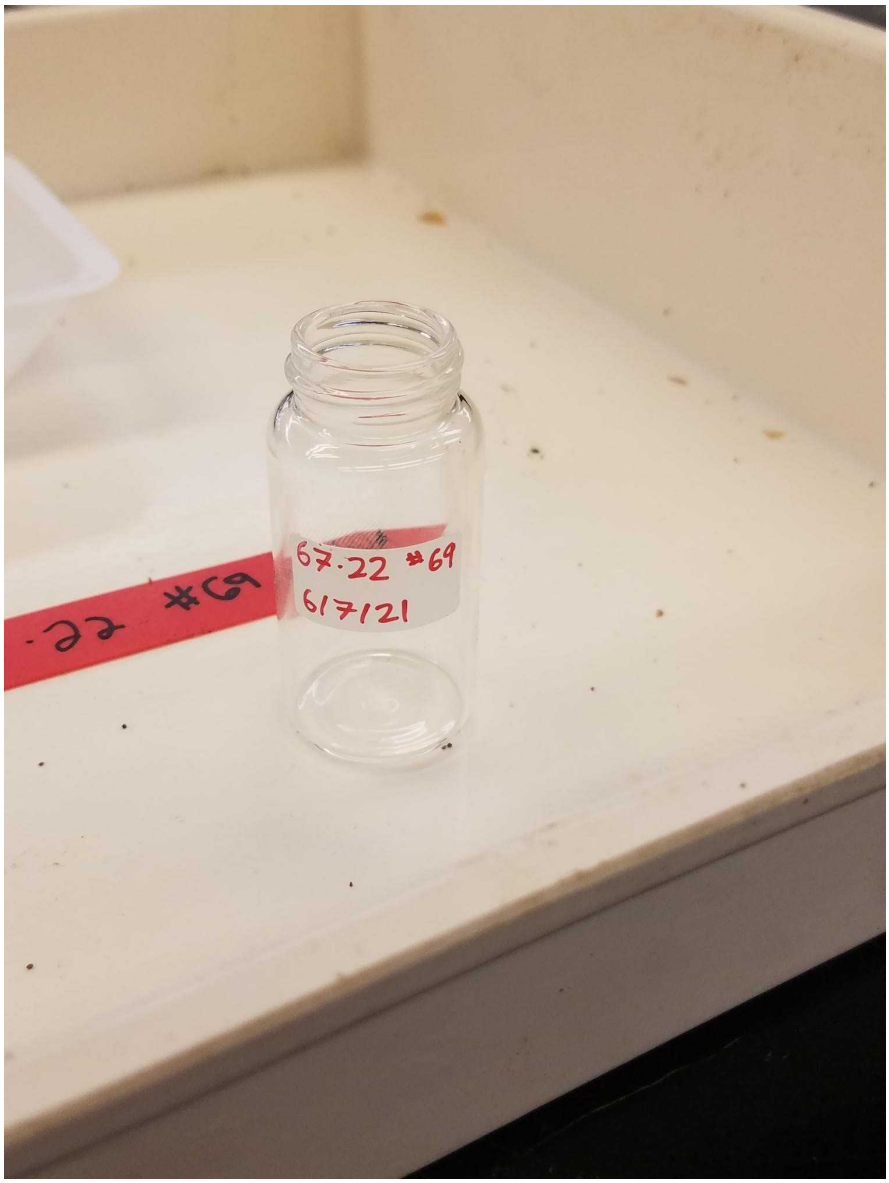

8 Pull out one stalk of florets. If the plants are transgenic, all plant pieces MUST remain in the seed tray. Even if plants are not transgenic, it is best lab practice to keep them in the seed tray to avoid contamination from foreign seeds.

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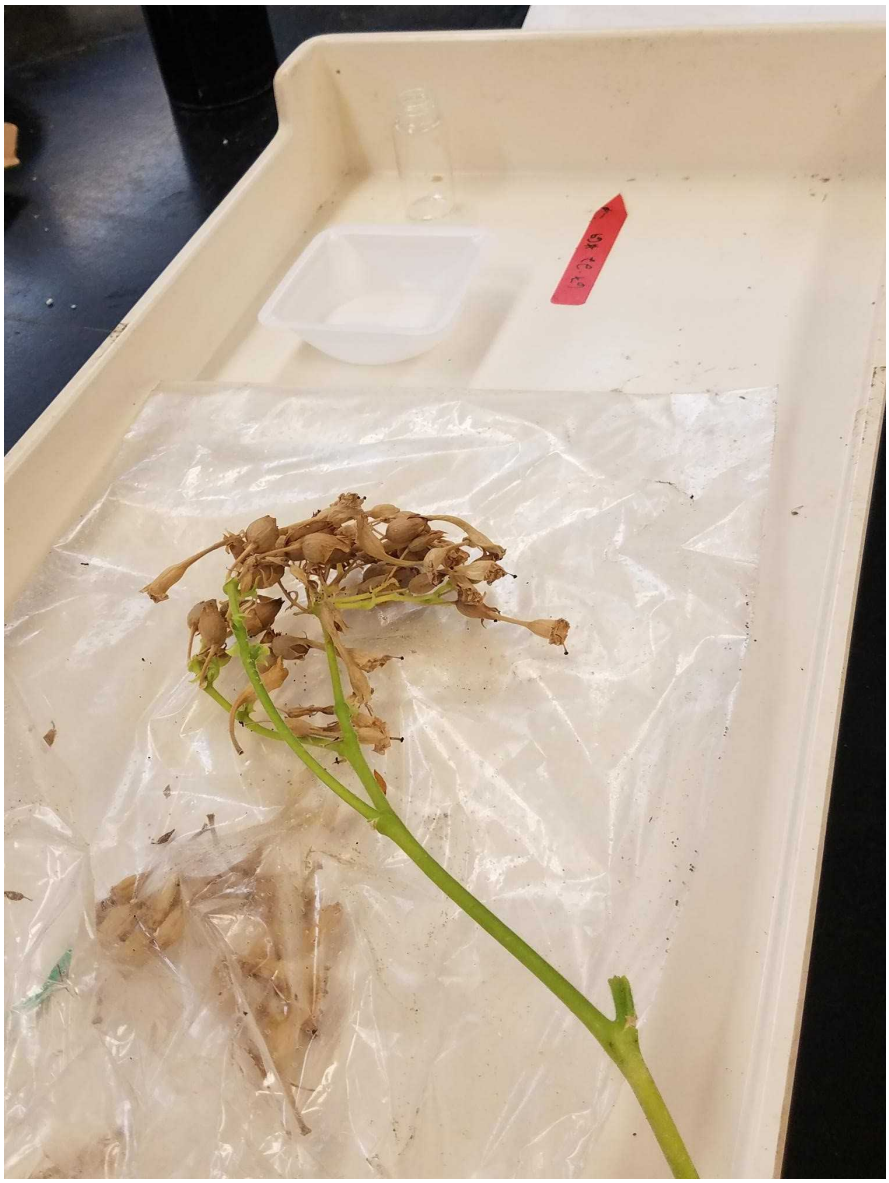

9 Pick a single seed pod off the floret, rip the top off, and dump seed into the weigh boat.

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10 Repeat step 9 with additional seed pods until a sufficient amount of seed has been collected.

11 Remove any chaff or plant matter from the seeds using tweezers. If the plant is transgenic, discard chaff and plant matter into the biohazard bin.

This step is required by the UIUC RIPE Greenhouse Aphis Permit for transgenic plants.

For non-transgenic plants, it is best lab practice to have clean seed for more even planting.

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12 Pour the cleaned, harvested seeds into the seed vial.

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13 Place the vial in the desiccator without a lid on for a minimum of 7 days.

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14 If plants are transgenic, discard any remaining seeds, plant parts, pollination bags, and weigh boats into the biohazard bin. Clean and sterilize all tools, counters, and hands with 70% ethanol. Discard any paper towels used to clean materials containing transgenic seeds into the biohazard bin.

If plants are not transgenic, thoroughly cleaning between harvesting of individual plants is still highly recommended to avoid seed cross-contamination.

15 After the seed has been allowed to dry for a minimum of 7 days, pull out the last seed box for that species according to the Master Seed Library (login required) from the seed cooler.

16 ⚠

Add seed vials sequentially to the last seed box. Assign numbers both sequentially and enter all seed vial data in the Master Seed Library. When a seed box is full, start a new tab for that species in the alphabetically sequential order and begin numbering vials numerically sequentially starting with the letter name of the seed box they are in and the number 1.

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If at any point, you are unsure about assigning the appropriate seed vial number, storage location, or modifying the Master Seed Library, STOP IMMEDIATELY and seek guidance from RIPE greenhouse management.

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