

Tobacco Protocol

***Nicotiana benthamiana* (Nb) Tobacco Seeding**

1. Fill a 288 plug tray with Pro-Mix PGX, being careful to break up any lumps in the mix.
2. Gently press the media into each plug to facilitate settling. Refill each plug with more soil.
3. Water the tray with the mist setting. As the media settles into each plug, air pockets form and burst, creating a hole in the surface of the media. Continue watering until no more air pockets form in the plugs.
4. Sow one seed in each plug by using scoop and spatula.
5. Place tray on bench in 10A with dome. Use clear RO water daily to mist soil when it is dry.
6. After one week, dome will be removed.
7. Continue to water during the second week as needed using RO water with 15-16-17 fertilizer at each watering. Plants should not get dry enough to wilt, but soil should be dry before watering. Edges may dry out faster. Over-watering and high rates of fertilizer will cause burning, leaf deformity, and tip death.

All other tobacco: e.g. *Nicotiana tabacum* (NN), SX, 266A, 277, 2005, Zanthi, etc.

1. To plant tobacco where uniformity is not necessary, fill a single 1801 pot with Pro-Mix PGX mix for each variety needed. (If more than one flat is needed, sow seed in plug trays instead.)
2. Water thoroughly.
3. Sow seed one pot at a time away from main planting to avoid cross contamination of the varieties.
4. Place on mist bench in 10A/Propagation House for 1 week.
5. Remove from mist and place on regular bench for the second week. Water as needed using RO water with 15-16-17 at each watering. Over-watering and high rates of fertilizer will cause burning, leaf deformity, and tip death.

Transplanting

This should be done about 2 weeks after sowing.

1. Place a set of 1801 deep pots into a holder tray and place that in a flat with holes.
2. Fill pots gently with Pro-Mix HP. Do not compact soil, Brush off any excess over tops of pots.
3. Moisten the soil until it has become fully saturated.
4. Add transplant to small hole in the center of each pot.
4. Water in gently with center setting on nozzle until root ball is covered with soil.

Watering

1. The plants are checked twice a day for watering needs. Plants kept too wet will become yellow and show signs of stress. Established plants should be allowed to dry down prior to adding more water.
2. The greenhouse staff will continue to water until the plants have flowered. If plants are able to be used past this age, please notify staff.

Fertilizer and treatments

Tobacco is fertilized three times per week with Jack's 15-16-17 @ 200 ppm N, and two times per week in the chambers with 15-16-17 @ 150 ppm. Unless the researcher requests not to use it, Paczol may be applied as a growth regulator on any tobacco other than Nb. Apply at rates of 5-7ml/2 gal water.

Plants for Seed

Staking- (types other than Nb)

1. Plants should be staked before they start to lean or grow curved.
2. When the plant reaches at least 6 inches high, a few leaves can be removed from the bottom of stem.
 - More water reaches the soil instead of running off leaves.
 - Soil will dry out more evenly and frequently.
 - Air circulation increases to help with disease or insect control.

Bagging- (Nb does not get bagged)

1. Before the first flower opens, it should be covered with a wax paper bag. This is done to prevent cross-pollination.
2. One of the bottom corners of bag can be folded up and paper-clipped. This helps the bag stay on, especially if the plant bends or is moved.
3. Top leaves of plant under the bud can be removed to help bag stay in place.
4. Once plants are ready to dry down, they can be spaced closer together.

Harvesting-

Most researchers usually only need 2-4 seed pods per plant. Check with individual researchers to see if they will want more than that.

1. After 2-4 pods become brown and dry, they can be cut off plant. If the researcher wants more seed, wait until the majority of pods are brown before cutting off.
2. Dried seeds are then put into the wax bag, along with the tag that was in the pot.
3. Remove plant and place in red bag to be autoclaved.

Cleaning-

1. For seeds that have already been harvested, cut the tip of the seed pod off. Dump the seeds into an envelope that has been properly marked.
2. For Nb seeds, the seed pods can remain on the plant. The pods will open as they dry from the base of the plant to the tips of the branches. To prevent seed loss, seeds should be collected as the pods open over the course of several weeks.

Once a pod is opened, it can be tipped into an envelope. The seeds will fall out easily if it is totally dry. The seed pod itself will stay attached to the plant.

Pest Management

The most common pests to watch for are thrips, whiteflies, and aphids, although the majority of the time, tobacco is usually pest-free. Unless otherwise specified, pesticides will be applied to control any pests present. Marathon may be put into each pot to help with potential insect problems. N. tabacum will get thrips more frequently than other varieties and may need to be sprayed more often.

Growing conditions:

Temp: 24-28 degrees Celsius

Humidity: 50-70%

Light: 200+ $\mu\text{mol}/\text{m}^2/\text{s}$

Photoperiod: Long day, 14 to 16 hours