

Planting Protocol

Before Planting:

1. Obtain the planting design sheets from the lab manager or produce them according to instructions and protocol "Create Planting Sheet".
2. Prepare germination plugs- one germination plug for every pot + 20% extra.
 - a. Moisten perhaps 400 ml. of germination mix with tap water. Make sure that the water is evenly mixed with the germination mix and absorbed.
 - b. Fill the wells of either 24-well or 96-well plates germination mix, pressing it in lightly.
 - c. Place the filled plates in the freezer.
3. Cut colored cotton thread into pieces ~ 1 cm. long. Place them in a small weigh boat in tap water. The planting design will require a specific number of thread colors and number of pieces of thread. The distinct colors can help to keep straight who is planted where.
4. Pot tags are placed in 164 ml. yellow "conetainers" 24/rack according to the planting design sheet. Pots are from Stuewe and Sons (www.stuewe.com)
5. A square of screen is pushed into the bottom of the pot and tamped down with a round aluminum rod; take care not to tamp it down to the point where the pot bursts or the top of the screen is below the holes in the pot.
6. Wet surface is poured into the pot and tamped with the white metal rods with white plastic tips; this eliminates the settling that would otherwise occur later in the experiment. Fill to within 6 cm. of the top after tamping. Place one scoop (1.5 ml.) of Nutricote fertilizer on top of the surface and fill and tamp again; after tamping, the top of the surface should be about 1 – 1.5 cm. below the top of the pot rim.
7. Place pot in a black plastic rack designed to fit into fiberglass ebb & flood bins, one bin for each tray. The trays are modified from Steuwe & Sons conetainer racks.
8. When all pots are placed, remove a tray of frozen germination plugs from the freezer. Once the outer edge of the plugs has started to melt,
 - a. Stab the plug with open fine-point forceps.
 - b. Squeeze slightly on the forceps and lift the plug from the well
 - c. Place the plug in the center of the pot so that the rim is even with the top of the plug.
 - d. Fill around the plug with moist surface.
9. Plant three seeds per pot according to the following protocol:
 - a. A few seeds are carefully knocked out of the microcentrifuge tube in which they are stored onto the creased piece of weighing paper.
 - b. A piece of thread is grasped near the middle with the tip of the forceps; one end of the thread is used to pick up a seed.
 - c. The seed is placed about 2/3 of the way toward the edge of the germination plug. The first seed is planted closest to the pot tag. The next 120 degrees clockwise, the last 240 degrees clockwise from the first.

They thus form a triangle with one seed at the apex that points toward the tag.

- d. Leave the thread in place with one end immediately adjacent to the seed.