

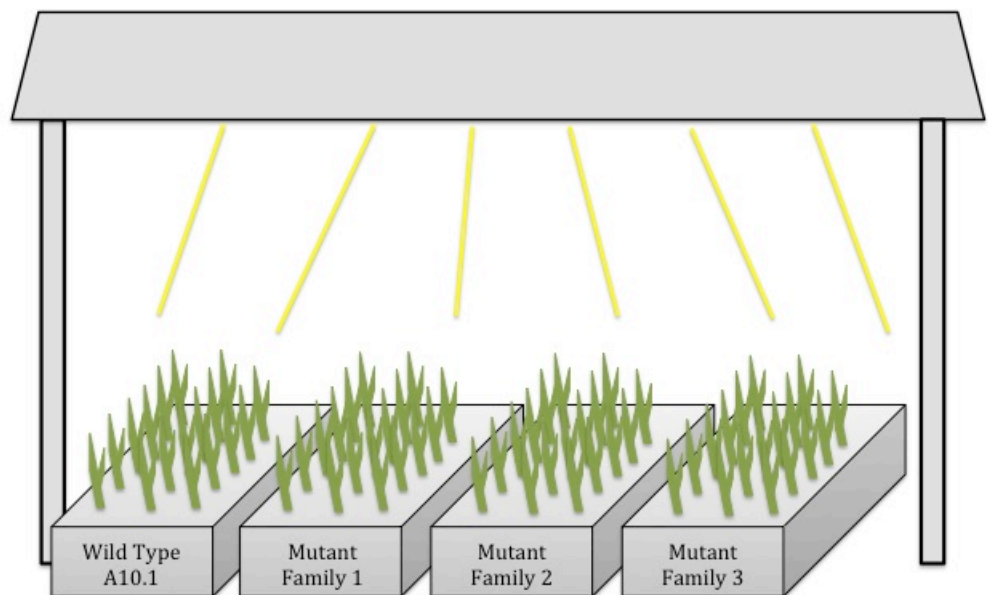
MUTANT MILLETS

Student Project Protocol Spring 2015



Part 1: Planting in soil trays

1. Fill clean pots with Metro Mix 360 potting soil. Place these in a tray leaving one space in the far right corner of the tray empty for watering.
2. Each tray will have plants from one family of *Setaria viridis*. You should always plant one tray with the wild type plants, A10.1. Additional trays should be planted with one mutant family each. Ask your teacher how your class will organize planting the trays.



An example of trays under a light bank

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- Using the large plant tags provided, label your pot with *Setaria viridis*, plant family number, generation (will start with an “M,” for mutant families only), the date of planting and your team/class name.

Example (wild type):

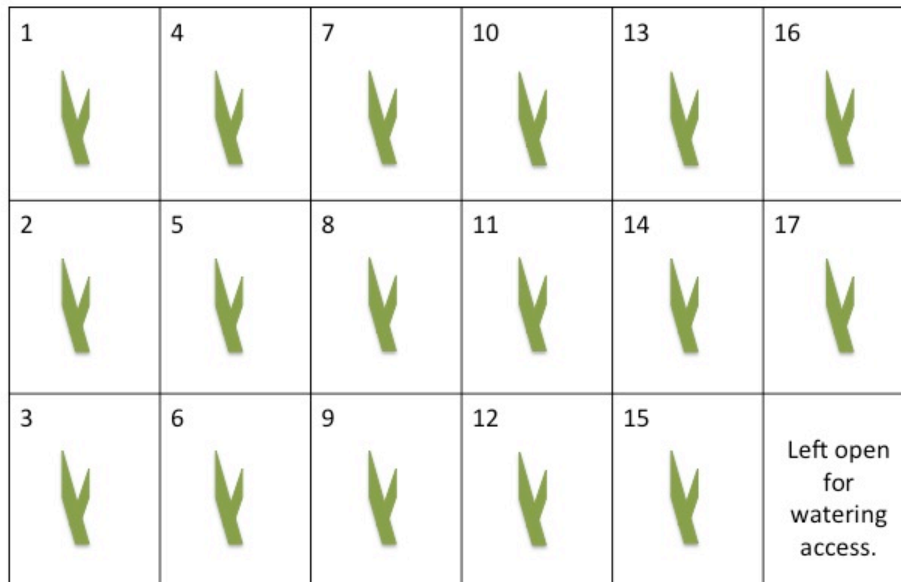
Setaria viridis A10.1 9/5/13 EP

Example (mutant family):

Setaria viridis 01200 M4 9/5/13 EP

- Using the small plant tags provided, label each pot 1-17, so you can track individual plants throughout the experiment.
- Moisten soil by misting with water from a spray bottle or using the “mist” setting on a hose. Hold the spray nozzle about 1 foot above the pot so soil does not fly out of the pots.
- Examine the seeds you are given. Be sure to only use the viable (black/grey) seeds, not the empty tan/white colored ones.
- Use forceps to plant **2 seeds** in the soil at a depth about equal to a pencil eraser. Using a plastic beaker, sprinkle a light layer of soil (about 0.5 mm) over the seeds.
- Mist the pots with water again. Be careful to hold the spray nozzle one foot above the pot so that you do not displace soil or the seeds.

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An example arrangement of pots in a tray

9. When the entire tray of pots has been planted, add water to the bottom of the tray, using the empty spot, so that the water level is just below the “ribs” on the bottom of the tray. You want the pots to have plenty of moisture for germination.
10. Cover the trays with the clear plastic clear lid until you see the germinating seedling emerge from the soil. The lid helps keep the humidity high to encourage germination. After the plants have emerged, loosen the lid at one end and slide the dome about 3 inches to *gradually* expose the plants to your classroom environment for one day.
11. The next day, remove the clear dome entirely. Save it for later use.
12. After you observe any plants emerging above the soil line, remove any extra plants, so that only one remains per pot. Should one pot have no seeds germinate, you may replant another group’s extra plant in the empty pot. Only one plant should grow to maturity in each pot.

Part 2: Plant Care

1. **IMPORTANT!** DO NOT overwater the planting trays as *Setaria* prefers pretty dry soil, and warm temperatures.

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- Add 1.5 L of water to the bottom of the tray, using the empty spot. You may want to briefly tip the tray slightly away from the empty spot, to allow the water to reach all pots on the other side of the tray.
 - After watering the tray, wait about one hour and then pour off any excess water into the sink. *Setaria* does not like to sit in standing water.
 - The soil should feel moist, but not dry or too moist/muddy. If the soil in one pot ever feels dry, you should slowly and gently water that pot from the top as well.
 - Coordinate with your teacher to determine a watering and fertilizing schedule, but know that your schedule may need to be adjusted depending on your classroom. Plan out with your classmates when the plants will be watered and who will do this. Make sure to account for weekends and holidays. If you are not sure if your plants need water, use the soil moisture meter. Insert the probe about halfway down the pot, about halfway between the plant and the side of the pot. *Setaria* prefers soil that reads about 2-3 on your soil moisture meter.
2. To fertilize, you should mix $\frac{1}{2}$ tsp of Jack's Classic Fertilizer per gallon of water (0.2 tsp per 1.5 L). Use this fertilized water to water the trays once every other week. Make sure your teacher approves your fertilizing schedule.

Part 3: Data Collection

1. After planting, check on your plants daily and record the date of germination. For the first week after the plants emerge, try to observe them daily. After that, observe your plants weekly. Refer to your student worksheet for what observations to make and what data to collect.
2. Share your observations and recorded data with your classmates to get a better picture of the characteristics of a *Setaria* population of plants versus your single plant. Are there any differences?

Part 4: Bagging your plants

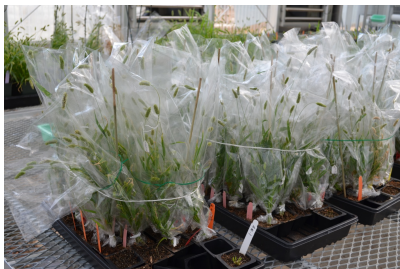
How do I know when to bag my plants?

*In general, plants can be bagged one to two weeks after flowering. Since flowering is not always easy to see in *Setaria viridis*, look for a few dark seeds*

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to appear on your main (tallest) panicle. When you see these seeds, it is time to bag your plants.

1. Hold a bread bag up to one of your plants. Take note of how long your bag will need to be to fit the plant inside. Leave enough room for the plant to grow about 3-4 more inches and cut the excess bag material from the open end.
2. Invert the bag over the plant, so that the closed end is on top. Each plant will be bagged separately. There will be extra space at the top of the bag so the plant has room to grow. Put the individual tags with family number and plant number into the bag with the plant so they do not get lost.
3. Use a twist tie to secure the bag around the plant near the soil line.
4. Sometimes the bags cause the plants to fall over. To fix this problem you can use a piece of string to wrap around a family of individually bagged plants. The string will hold all of the plants upright, and allow watering.



5. When your plants have matured and begin to undergo senescence (when they age to the point where the flag leaf (the uppermost leaf on the culm, or main stem) begin to yellow or brown), you should stop watering and let your plants dry down. When they are dry and mostly brown you are ready to collect seeds.

Part 5: Collection of Seeds

1. Cut the plant at its base beneath the twist tie.

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2. Roll the bag between your hands and use your fingers to break apart the panicles, to release the seeds.
3. Undo the twist tie and remove the plants from the bag. You will be left with a large bag with a lot of tiny seeds at the bottom. To make pouring the seeds into the envelope easier, you can cut a slit in the bag.
4. Place the seeds into a clean seed envelope for shipment back to DDPSC.
5. Place the seeds into a clean seed envelope and label *Setaria viridis*, family number, plant number, as well as the date the plant was planted (P) and seeds were collected (H, for harvested). If that plant displayed a mutant phenotype, write that on the envelope too. For mutant plants, include the generation. This should start with an M, and the number should be 1 greater than the seeds you planted, since this is the next generation. (i.e. if you planted M4 seeds, then on your seed packet of seeds collected from that plant, you would write M5). **THE SEEDS CANNOT BE USED IF YOU FORGET THIS STEP!!!** For your convenience, labels have been printed. All you need to do is stick the label to your seed envelope and write in the plant and harvest date as well as any mutant phenotypes displayed by that plant.

Example seed packets:

S. viridis 00317 M5 #2

Phen: *Light Green*

P: 1/21/15 H: 5/2/15

Part 6: Clean Up

When you are finished, all of your *Setaria* plants should be taken from the pots (soil and all) and placed into the red bag provided and taken back to the Donald Danforth Plant Science Center. These plants will be autoclaved before discarding. The pots and trays should be rinsed out, dried and boxed for return to the Danforth Center.