**Exploring grass morphology and mutant phenotypes using *Setaria viridis***

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**SUPPLEMENTAL MATERIALS**

**Activity 1: Continued**

**Procedure to compare seed phenotypes of the mutants versus wild type**

1. In *S. viridis*, seeds fall off when they are mature, so the plants must be bagged to catch the seeds. Look for a few dark seeds to appear on the tallest panicle to bag the plants, each plant is bagged separately. Invert a bread bag the bag over the plant, so that the closed end is on top and use a twist tie to secure the bag around the plant near the soil line.

 2. When plants have matured and begin to undergo senescence (the flag leaves [the uppermost leaf on the culm] begin to yellow or brown), stop watering and let the plants dry down. When they are dry and mostly brown collect seeds by rolling the bag between your hands breaking apart the panicles, to release the seeds into the bag.

 3. Place the seeds into a clean seed envelope and label *S.viridis*, family number, plant number, as well as the date the plant was planted and seeds were collected. 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, write M5).

**Activity 1: Assignment 3:**

To examine seed production among mutant families, students should discuss the questions below in small groups and record their responses in their lab notebooks:

1. Did the mutant plants in each family survive to produce seeds?
2. What do the seeds from each mutant plant look like when spread out on a piece of white paper? Are the seeds viable and filled (black-gray) or empty (white-tan)?
3. Estimate the percentage of seeds that are filled, empty.
4. Compare total numbers of seed per plant from the mutant plants with wild type plants, record whether they are less, the same, or more than the wild type.
5. Compared with seeds from a wild type plant; are seeds from the mutant plants smaller, the same size as wild type, or larger when laid out on graph paper? Students could also use an ocular micrometer to obtain more accurate estimates of seed size.

**Activity 3:**

**Assess the effect of environmental variables on *S. viridis* growth and development**

In this activity students will examine the effect of environmental variables such as light, photoperiod, temperature and humidity on plant growth. This activity requires students to grow mutant and wild type *S.viridis* plants in different environments such as a classroom and green house and is aligned with the following NGSS,

* MS-LS2-1 Ecosystems: Interactions, energy and dynamics
	+ SEP, Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms
* [HS-LS1-6, HS-LS1-7 From Molecules to Organisms: Structures and Processes](https://www.nextgenscience.org/pe/hs-ls1-6-molecules-organisms-structures-and-processes)

### SEP, [Constructing Explanations and Designing Solutions](http://www.nap.edu/openbook.php?record_id=13165&page=67)

### SEP, [Developing and Using Models](http://www.nap.edu/openbook.php?record_id=13165&page=56)

**Learning objectives**

1. Investigate the effect of photoperiod, light intensity, temperature and humidity on *S.viridis* growth and life cycle (phenology)

2. Compare and contrast the phenotypes and growth cycle progression (e.g. days to tillering, days to panicle emergence) of mutant and wild type plants grown in two different environments.

**Materials**

* Timer
* Thermometer
* Hygrometer
* Light Meter
* Trays of plants obtained as described in Activity 1
* Notebook

**Procedure**

Before planting and weekly throughout the life cycle of their *S.* *viridis* plants, students should record information on the variables listed below for each growth environment where the plants will be grown, for example, greenhouse and classroom.

* Average room temperature (oC), humidity (%)
* Light intensity provided to plants at soil level (in lux)
* Photoperiod
	+ How many hours a day the lights were on? off?
* Describe the watering and fertilizer application schedule.

Students should also collect data on the plant measurements listed in Table 2 for each growth environment and record any drastic changes in the plant growth conditions such as pest outbreaks.

**Table 1. Sample data collection sheet for plants in multiple environments**

|  |  |  |
| --- | --- | --- |
|   | Wild Type A10.1 | Mutant Family: |
| Event | Date | Days After Planting | Date | Days After Planting |
| Planting |   |   |   |   |
| Seedling emergence |   |   |   |   |
| Tillering |   |   |   |   |
| First panicle emergence |   |   |   |   |
| Seed formation |   |   |   |   |
| Plant senescence |   |   |   |   |
| Seed collection/harvesting |   |   |   |   |

 **Assignment 1:**

 Students compute the days to germination (DTG), days to tillering (DTT), days to panicle emergence (DPE) for each mutant family and the wild type for each growth environment.

* Students compare DTG, DTT and DPE for mutant and wild type plants grown in two different environments, and provide arguments for those differences.
* Students construct graphs showing DTG, DTT and DPE (x-axis) versus proportion of plants (%) for each mutant family and wild type.

**Table 2: Sample data from a school, showing differences in light intensity and effect on plant phenology for *S.viridis*.**

|  |  |
| --- | --- |
|  | **School** |
|   | **Classroom 1** | **Classroom 2** |
| Average Room Temp (oC) | 20.87 | 19.78 |
| Average Room Humidity | 36% | 37% |
| Average Light Intensity (Lux) | 2796 | 8862 |
| Photoperiod (hours ON) | 16 | 16 |
| % Seeds Germinated | 100 | 95 |
| Days Until Plantlets Appear (DTG) | 6 | 6 |
| Days until First Panicle Emerges (DPE) | 60 | 42 |
| Days Until Seed formation | 69 | 63 |