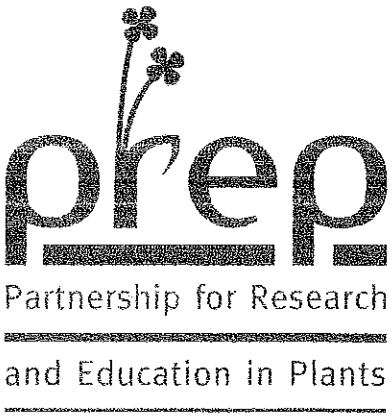

PREP Experiment Guide

Fralin Biotechnology Center
VIRGINIA POLYTECHNIC INSTITUTE & STATE UNIVERSITY
West Campus Drive, MC 0346, Blacksburg, VA 24061
ph: 540.231.6934 fax: 540.231.7126 email: biotech@vt.edu
http://www.prep.biotech.vt.edu/

Table of Contents

The students are encouraged to be creative in their experimental designs. The following suggestions are meant only as a guide to the students in designing conditions that cause an observable effect in the plants without killing them.

1. Light

1-1. Red light/Blue light – Plant photosynthetic pigments absorb primarily in the red and blue wavelengths. Not surprisingly, plants have sophisticated photo-sensing capabilities that allow them to detect even subtle changes in the wavelengths of light they receive. Both

© 2005, Fralin Biotechnology Center, Virginia Polytechnic Institute and State University

red and blue wavelength light have a particularly noticeable effect on how plants grow and develop.

Here are a few suggestions on creating colored light environments:

i. Use colored plastic wrap or Mylar to create colored light conditions.
ii. Use clear plastic wrap for the control plants.
iii. Be sure all light is filtered through the plastic, including grow lights, room lights and sunlight.
iv. Be sure air is still able to circulate around the plants.
v. Be sure to monitor the temperature around the plants. Temperatures above 28°C can be harmful to them. If this happens, consider putting the lights on a timer so that they are off for 8 hours of the day.

1-2. Dark germination – Dark-germinated plants exhibit a distinct set of characteristics including: lack of pigmentation, long narrow stems, and small undeveloped leaves. After sowing the seeds on the soil, place the pots in total darkness by putting them in box and placing the box in a drawer or cabinet. After 1-2 weeks, remove the plants from the darkness and make observations.

1-3. Phototropism – Germinate plants in darkness for 5 days and then expose them to a lateral unidirectional light source. To do this, cut a hole on one side of a box and use it to cover the plants. Place a clip-on light fixture or a small lamp near the hole on the side of the box. Observe plants from 5 to 24 hours later. See a movie of this phenomenon at http://sunflower.bio.indiana.edu/~rhangart/plantmotion/movements/tropism/tropisms.html

1-4. Light intensity - Explore the effects of varying light intensity (either more or less than the control lighting) by moving the plants closer or farther from the light fixtures, by adding or removing light bulbs, or by increasing or decreasing the wattage of the bulbs. Monitor the temperature to ensure that results are caused by changes in light, not temperature.

1-5. Photoperiod – If a timer is available, observe the effects of different photoperiods on the plants, such as 12 hours light/12 hours dark. The plants are not likely to bolt (produce stems with flowers) if they get less than 12 hours light. Be sure that plants grown in short photoperiod conditions don't receive light from any other sources during their dark period, for example, other growth lights, security lights on a timer, and overhead lights that may be turned on by other people in the building.

2. Temperature

Plants treated briefly with cold or heat are often able to survive subsequent more severe exposures. This process is called acclimation and is controlled by numerous plant genes.

2-1. Heat – When the plants are 2 weeks old, create 4 different conditions each using 1 pot of wild type and 1 pot of mutant plants. Since this involves 8 pots rather than 4, it may be helpful to have two groups of students perform this experiment.

i. Control – room temperature.
ii. Acclimation - 38°C for 90 minutes, then back to room temperature.

© 2005, Fralin Biotechnology Center, Virginia Polytechnic Institute and State University

iii. Acclimation plus heat shock – 38°C for 90 minutes, followed by 2 hours of 45-50°C, then back to room temperature.
iv. Heat shock – 45-50°C for 2 hours, and then back to room temperature.

2-2. Cold – This experiment requires putting the plants in a refrigerator, which is dark, and therefore requires putting the control plants in a dark environment (such as a cabinet) at room temperature. Again, this experiment uses 4 sets of wild type and mutant plants and might be easier to perform with two groups.

i. Control – room temperature.
ii. Acclimation – 4 days @ 5 hours per day in the refrigerator (approximately 4°C) then back to room temperature. Be sure to record the actual temperature in the refrigerator.
iii. Acclimation plus cold shock – 4 days of 5 hours per day in the refrigerator at 4°C, followed by 2 hours in the freezer (-5°C to -20°C) then back to room temperature. Be sure to record the actual temperature of the freezer.
iv. Cold shock – 2 hours in the freezer and then back to room temperature.

3. Water

3-1. Drought – Drought experiments with *Arabidopsis* require vigilant attention because the plants must be checked every day. *Arabidopsis* can look perfectly healthy in apparently dry soil, but then wilt and die in 12 hours or less. For drought experiments, wait until the soil looks dry (the pot will feel light) and the plants are just starting to wilt, then water the plants.

3-2. Flood – Wild type *Arabidopsis* plants are quite tolerant of flood conditions. To produce flood conditions, saturate the soil by sitting the pots in a tray of water. The soil absorbs water through the holes in the bottom of the pots. Algae often grow on top of water-saturated soil, which can harm the plants. To reduce algal growth, water the plants with pH 4 water. Use sulfuric acid to lower the pH of water to 4. Be sure to water both the control and experimental plants with the acidic water.

3-3. pH – Examining the effects of exposing plants to acidic conditions is intriguing because of the phenomenon of acid rain. Acid depletes soil nutrients and liberates toxins like aluminum from soil particles, so the effects on the plants may be due to nutrient deficiency and toxin exposure rather than the low pH itself. Water experimental plants with water that has been acidified to pH 2 with sulfuric acid. Water control plants with tap water.

3-4. Osmotic stress – Excess solutes in the soil prevent the roots from absorbing water. Because the ions liberated from ionic solutes such as NaCl exert an additional stress to the plants, nonionic solutes such as mannitol are use to specifically examine the effects of osmotic stress. Use a pot to measure out enough soil for two pots (one wild type and one mutant) into a clean container, mix with 10 grams of mannitol and put the soil back into the pots.

© 2005, Fralin Biotechnology Center, Virginia Polytechnic Institute and State University

4. Soil

4-1. Excess nutrients – Explore the effects of adding excess nutrients on the plants by treating with water containing more than the normal amount found in fertilizer.

Nutrient(s)	Source	Concentration of stock solution	Amount in 1 liter fertilizer	5X	10X	20X
Nitrogen and Potassium	KNO_3	2M	2.5ml	12.5ml	25ml	50ml
Iron	Fe-EDTA	20mM	2.5ml	12.5ml	25ml	50ml
Potassium and Phosphorus	KH_2PO_4	1M	2.0ml	10ml	20ml	40ml
Magnesium and Sulfur	$MgSO_4$	2M	1.0ml	5ml	10ml	20ml
Calcium and Nitrogen	$Ca(NO_3)_2$	2M	1.0ml	5ml	10ml	20ml

4-2. Nutrient deficiency – Because soil contains nutrients, a different growth medium, like sand or water using a hydroponics set-up, must be used to examine the effects of nutrient deficiency on the plants. Grow control plants in a complete nutrient medium (includes calcium, magnesium, potassium, iron, nitrogen, phosphorus, sulfur, and trace nutrients) and grow experimental plants with one nutrient omitted.

4-3. Density – Soil that is at least 50% more dense (i.e., 50% more soil by weight in the same volume) causes a noticeable difference in *Arabidopsis* growth and development. Weight pots before filling them (2.5" pots ≈ 9.5 grams), then lightly pack the soil for the control pots and weigh them. Determine the soil weight by subtracting the empty pot weight from the filled pot weight. Multiply the soil weight by 1.5 and pack that mass of soil into the experimental pots.

4-4. Sandy soil – Examine the effects of sandy soil on plant growth by mixing a 50/50 blend of sand and soil for the experimental plants and using pure soil (the soil we provide contains no sand) for the control plants.

5. Pollution

5-1. Salt – Examine the effects of both osmotic and ionic stresses on the plants by watering them with 4 to 8 grams of NaCl per liter of water. Grow the plants for two weeks and then start watering the experimental plants with the salt solution and the control plants with plain water, about 25-50 ml per pot 2-3 times per week. Be sure not to over water the plants or the salt solution will drain out the bottom of the pots.

5-2. Heavy metals – Heavy metals can accumulate in soil and interfere with plant growth and development. Lead, cadmium and mercury are too dangerous for classroom work. Copper, zinc and nickel are safer alternatives that are trace nutrients but toxic to plants at higher levels. Every year millions of tons of these heavy metals are released into the

© 2005, Fralin Biotechnology Center, Virginia Polytechnic Institute and State University

environment.

i. Copper – make a 8mM solution of copper sulfate by adding 499 mg of copper sulfate ($CuSO_4 \cdot 5H_2O$, FW=249.7) to 250 ml of water.
ii. Nickel – make a 8mM solution of nickel by adding 526 mg of nickel sulfate ($NiSO_4 \cdot 6H_2O$, FW=262.8) to 250 ml of water.
iii. Zinc – make a 8mM solution of zinc by adding 273 mg of zinc chloride ($ZnCl_2$, FW=136.3) or 288 mg of zinc sulfate ($ZnSO_4 \cdot 7H_2O$, FW=287.5) to 250 ml of water.

If PREP provides you with the heavy metals as 5X concentrate solutions in 50 ml conical tubes, add the contents of the tube to 200 ml of water to get the treatment concentrations. Each group needs 250 ml for their entire experiment. Two weeks after planting, apply the metals in 25 ml aliquots to each experimental pot every time the control plants are watered (5 applications total). After the metal solution is used up, water the plants with plain water.

6. Other options

6-1. Caffeine – Caffeine lowers calcium in plants and calcium is an essential mineral in cell signaling and cell wall synthesis. Make a 5mM solution of caffeine (add 0.48 grams to 500ml water) and apply 25 ml to the experimental plants each time the control plants are watered. If the plants are dry and need more than 25 ml, then add plain water but not so much that it washes the caffeine treatment out of the bottom of the pot. As usual, water the control plants with plain water.

6-2. Ethanol – *Arabidopsis* can normally tolerate up to 1% ethanol in their growth medium. Some mutants, however, are hypersensitive to ethanol. They look no different than wild type plants in the absence of ethanol, but if grown on even very low concentrations of ethanol they won't germinate. To determine whether the plants are affected by ethanol, germinate seeds on Petri dishes containing 0.1% ethanol (v/v). PREP will provide these Petri dishes.

6-3. Gravitropism – Gravitropism is a plants ability to orient itself with respect to gravity. Typically, shoots grow up and roots grow down. To determine whether plants respond normally to gravity, turn the experimental pots on their sides for 5 to 24 hours in total darkness. During this time the plants will reorient themselves so that their stems are vertical again. It is easiest to observe this response when the stems are about 2" to 4" tall. Control plants can be kept upright next to them in the dark to ensure that the effect is not due to absence of light. See a movie of this phenomenon at http://sunflower.bio.indiana.edu/~rhangart/plantmotion/movements/tropism/tropisms.html

6-4. Touch/Wind – Plants often respond to mechanical stimulation by changing their physical structure, for example, by developing thicker stems or not growing as tall. Explore the effects of mechanical stimulation by touching the plants or exposing them to wind.

i. Touch experiment – wait until the plants have 6-8 true leaves and then gently wiggle them by lightly placing four fingers on top of all the plants in the pot and rocking back and forth about $1/8^{th}$ of an inch in either direction 20 times. Do this

© 2005, Fralin Biotechnology Center, Virginia Polytechnic Institute and State University

3 times per week. When the stems start growing, slide the stems between your fingers and perform the same procedure.

ii. Wind experiment – a small fan works well for a wind experiment. Be sure that the plants don't dry out, that the wind blows evenly on all of the experimental plants, and that the wind does not blow on other plants. Expose the experimental plants to wind for 2 hours – 3 times per week. Check the plants regularly to see if they need water.

© 2005, Fralin Biotechnology Center. Virginia Polytechnic Institute and State University