



Activity 7: Effect of Environment on Plant Growth

The purpose of this activity is to demonstrate the effect of changes in the environment on the growth and fertility of landscape grasses and crop grasses such as wheat and rice. You will place the plants in environments such as high salinity, cold, heat, or drought and observe the different reactions of the plants to these conditions. Students will compare the growth of treated plants to that of the control plants which are grown under optimal conditions. You will then discuss the desirability of breeding new types of plants that are better able to withstand these changes if they occur in the general environment.

Objective

1. To plant, grow and maintain plants under different environmental treatment conditions.
2. To observe differences in plant growth between these treatments.
3. To compare the growth of treated plants with the growth of control plants.

Background

Breeding plants that are able to survive in different environmental conditions or when attacked by diseases or pests has occurred for thousands of years as people gradually domesticated the plants that they needed to use for food or as ornamentals. Generally this breeding involved identifying a trait in another related species that could then be used as the donor of this trait to the domestic plant they wished to improve. In traditional plant breeding, the two species would be crossed either naturally or artificially to produce a hybrid plant that contained genes from both parents. These hybrids were screened to see which of them contained the gene for the trait of interest. The selected hybrids were backcrossed to the domestic parent over many generations with selection at each step for the trait of interest. This ultimately results in a new plant that contains DNA mainly from the original domestic parent but also a small amount of DNA from the donor that encompasses the gene that we desired to transfer. As you can imagine this procedure is very expensive and time consuming and it is many years before a new plant is ready to be released to the general population. A second drawback with this procedure is that there is

inevitably some DNA present in the new plant that arose from the donor parent that may not be desirable.

A second method of breeding involves the use of biotechnology. Some of the laboratory techniques that you have learned in this program in the activities DNA extraction, restriction digestion, plant tissue culture, transformation and PCR are utilized in this type of breeding. The gene for the trait that we are interested in may be identified in either a related species or even a totally different genus or family. For instance a gene from a fish that is able to survive in very cold conditions has been bred into soybean so that the plants are now able to survive in much colder environments than the original soybean. The specific DNA that contains just the gene of interest can be cut out of the donor organism's DNA, cloned and prepared for insertion into the recipient plant. Transformation procedures are utilized to move the gene into the recipient and new plants are grown in tissue culture before moving into the greenhouse and the field. Selection methods are used during tissue culture to select only plants that have the new gene in their cells and thus these are the only ones that continue through the whole procedure. Once plants are mature enough to be planted in the field or greenhouse some of the more traditional methods of plant breeding are used to increase the number of plants, to test that the gene functions correctly under the relevant conditions, to determine if the new gene is stably inherited and, most importantly, to check that the new plants do not have a detrimental effect on either the environment or other plants and organisms.

Materials

- 25 germinated seeds of wheat or rice
- 16 10-cm (4") pots
- Potting soil/peat moss mixed 1:1
- 5 small containers to hold pots
- Aquarium or other clear container with lid or top
- Table salt (NaCl)
- Scale to weigh NaCl
- 1-liter measuring cylinder, volumetric flask or kitchen measuring jug
- Full spectrum grow-lights if necessary
- Use of refrigerator or cold room

Procedure

Note: The seeds must be germinated and planted at least 5 weeks before beginning the experiment. If you only want your students to see the results of the treatments, then you should begin at least 7 weeks before the class period is to occur.

1. Germinate 15-20 rice seeds by first washing in 10% bleach for 5 minutes and then rinsing 3 times in sterile distilled water. Place the seeds in a petri dish that has a filter paper in

the bottom. Moisten the filter paper with sterile distilled water until it is very wet and place in a warm area, preferably an incubator set at 28°C or in an area that is warmed by the sun. Seed should germinate within 5-7 days.

2. Plant one seed per 4" pot that is filled with potting soil mixed with equal parts of peat moss. Gently cover the seed until just the top part of the coleoptile (shoot) is visible. Water well and place in a well lit, warm environment such as an incubator or a warm windowsill (but protect from any cold drafts). Keep moist.
3. Make sure that the plants receive at least 12 hours of light per day. (If you are using rice seeds, once the new plants have begun to fully emerge after about 10 days, the pots can be placed into a container so that flood irrigation can begin. Flood irrigation involves filling the container with water until it is about 1/3 of the distance up the side of the pots. About once per week a small amount of fertilizer (follow the directions for potted plants for whichever fertilizer you choose) should be added to the water used to refill this container. The solution in the container should be completely changed every week before the fertilizer water is added and then topped up during the rest of the week). Wheat seedlings should be watered daily to maintain constant moisture in the soil, and fertilizer should be added about once per week.
4. When the plants are 5 weeks old you can begin the environmental stress demonstration.
5. Choose the ten best plants that are of approximately the same height and with the same number of leaves. The remaining plants can be discarded or kept for backup if something goes wrong with one of the treatments.
6. Two of the plants will be control plants that will continue to be flood irrigated, with fertilizer added once every week. Make sure that the plants have at least 12 hours of sunlight or artificial full spectrum "grow-lights" per day.
7. Separate the remaining eight plants into four groups so that there will be two plants for each treatment. Label two pots "drought", two pots "cold", two pots "saline" and the remaining two "heat". All pots should be placed in containers so that irrigation can continue during the treatment as necessary. Each treatment will last for 5-14 days, depending on the responses you have in your classroom situation.

Treat as follows:

1. **DROUGHT** - Maintain these plants in the same environment as the control plants but do not add any water to the container for 3 days. This will allow the pots to dry out at which point the drought treatment will begin. Do not irrigate for a further 5 days. Irrigate normally for 2 days and then repeat the drought treatment for another 5 day cycle.
2. **COLD** - Place these plants in a refrigerator or other cold room (<10°C) for 10-14 days but continue to provide light, water and nutrients as for the control plants

3. **SALINE** - Make a 100 mM NaCl solution⁺ to use for irrigation. This should provide a solution that has a conductivity of approximately 10-12 dS/m). Irrigate the plants constantly with this solution for 14 days but be sure to keep all the other growth conditions the same as those of the control plants.
4. **HEAT** - Place the plant inside an aquarium or clear plastic container, cover and place on a warm windowsill or under grow lights. The temperature in the enclosed container needs to rise to above 35°C. Keep all other environmental conditions the same and be careful not to release too much heat from the container when you are changing the irrigation solution. Grow the plants in this environment for 14 days.
5. **CONTROL** - Keep these plants in the same environment as they were, making sure that there is still at least 12 hours of daylight and/or supplemental artificial light. The plants should remain flood irrigated and treated with fertilizer each week until the end of the experiment.

⁺You can have the class work out how to make this solution as part of a chemistry or mathematics lesson. A 1Molar solution contains 1 mole of the solute dissolved into 1 liter of water. 1 mole of NaCl contains 58.44 grams i.e. number of grams equivalent to the formula weight of NaCl. So, to make 100 mM we need to take 58.44/10 grams of NaCl. (100mM = 1M/10 or 1000mM/10). So you need to dissolve 5.844 grams of NaCl in water and then make the volume up to 1 liter for each liter of saline solution that your container will hold.

8. Have students record their observations. Note height of plants, number of new leaves, degree of wilting, color of leaves, and any other symptoms such as spots on leaves, browning of leaf tips, for example. When all the treatments are complete and observations noted, you may remove the plants from the pots, wash the roots carefully and measure the length of the roots.
9. Have students complete the activity sheet.

Some other questions to have students consider:

What do you think are important tools for improving our ability to feed the world?

Are there technologies that you think should not be used?

Find out how you could become a scientist who has the power to change children's lives by providing growers with the tools to grow nutritious food necessary for the healthy development of our children.

Observations:

1. Inspect all of the plants that your teacher has prepared for this demonstration and record your observations in the chart below. Record separate information for each of the two plants in each treatment and each of the controls

Treatment	Plant height		No. of leaves		Wilting? Yes/No		Color of leaves		Other stress symptoms	Root length	
	1	2	1	2	1	2	1	2		1	2
Control											
Drought											
Cold											
Saline											
Heat											

2. Compute the average for each category of measurements (plant height, number of leaves, root length) for each treatment and for the control.
3. The vigor of a plant is directly correlated with its ability to flower and to set viable seeds. If crops such as wheat and rice are forced to grow in areas that are affected by environmental changes such as increased heat, cold, or drought, what do you think will happen to the production of seeds and other plant parts for human and animal consumption?
4. Salinity of soils is increasing across many regions of the world, particularly in areas that are prone to drought and have low natural precipitation. As the limited water evaporates from the soil, any minerals contained in the water become more concentrated in the soil near the surface and thus affect crop growth. Similarly, as soils become poorer in quality we add more fertilizers to them and this also results in an increase in the salt level of the soil. Given your observations of plants grown in a saline environment, what do you think will happen to world food production as soil salinity changes?
5. Discuss how you think biotechnology could be used to increase food production throughout the world if our climate and hence the plant's growing environment are altered. Do you agree or disagree that the use of biotechnology is justified? Explain your answer and present alternative plans of action that you think could be used to solve these problems.