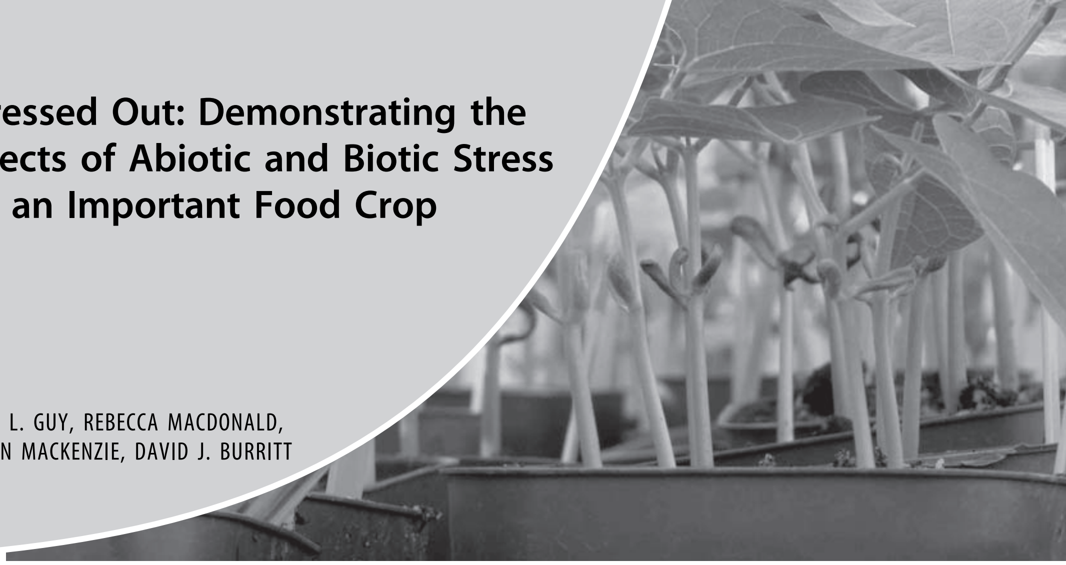
TIPS, TRICKS & TECHNIQUES

Stressed Out: Demonstrating the Effects of Abiotic and Biotic Stress on an Important Food Crop



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ABSTRACT

*In this inquiry activity, students use the genetic diversity of common beans (*Phaseolus vulgaris *L.) from their local garden center to investigate this species' resilience to salt stress and viral infection. Students use a number of simple to sophisticated measures of plant performance to gauge the impact of the stressors on plant growth and development. Students identify which cultivars may be useful in increasing future food security.*

Key Words: *formative learning; salinization; viruses; horticulture; food security.*

○ Introduction

We lack enough food to sustain the world's growing population (Ehrlich and Harte, 2015). Intensive agricultural practices developed since the early twentieth century created a number of problems including the emergence of new pests and diseases and the depletion, contamination, and salinization of soils and groundwater. Drought conditions increase soil salinity, which can worsen over time and affect crop yields (Fita et al., 2015). Prolonged irrigation during drought leads to soil salinization. Yields of most legume crops remain relatively low due to limited adaptability to a broad range of environmental conditions including salinization and due to susceptibility to pests and diseases. At least 26 different viruses infect common beans (Schwartz et al., 2005), and some of these cause serious disease and crop failures in many parts of the world. Improving crop adaptation to various stressors is a high priority for enhancing global crop productivity.

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This exercise uses the techniques of active learning to develop basic horticultural skills and to develop objective assessment of salt (abiotic) stress and disease (biotic) stress on an important food crop. For a good background, search online using the phrase "growing beans in the classroom" to access YouTube clips suitable for kindergarten and elementary students. Students discuss and determine which measures they will use (e.g., leaf number, plant height, appearance) to assess their plants' performance. Teachers can introduce additional stressors such as drought (withhold watering) and herbivory (remove leaves) to additional sets of plants or compare beans with more salt-tolerant species such as cucumber or beet.

○ Materials

- Packets of beans from a garden center or online supplier; one or two different types per group of 2–4 students. Optional for the seed contamination extension: a few pea seeds, a spoon full of small seeds from the kitchen (e.g., sesame, linseed, poppy).
- Table salt solutions of 150 mM (low salt: add 8.8 g to 1 L tap water) and 300 mM (high salt: 17.6 g in 1 L tap water) sodium chloride (NaCl), and tap water (control). Soils containing 150 mM and 300 mM NaCl are defined as soils of medium and high salinity, respectively (Brouwer et al., 1985).
- Soda bottles (2–3 L) to store solutions; measuring cylinders or jugs. Each student will need 1.2 L of each salt solution.
- 1–1.5 L plastic pots, pot labels, soil or potting mix, plastic rulers.
- Optional for the viral stressor extension: leaves from white clover plants growing in a well-established lawn.
- Cell phone cameras, photocopier or scanner.

○ Procedure

Day 0

Choose a selection of dwarf and runner beans (*Phaseolus vulgaris* L.) with different pod color and texture to maximize genetic diversity. Place seed in small containers. These containers can be "contaminated"

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(optional) with a few pea seeds and a pinch of small seeds. Give each student group two containers of beans (using two different lines with different seed colors or patterns is useful). Students inspect their seed batches for purity (nearly all will detect the pea seed contaminant, some will detect the small seeds). Each student group puts potting mix in 12 pots for the salinity experiment, or 24 pots if they are also doing the virus experiment. The students make sure all pots are filled evenly, and then make 4 holes about 1 inch deep in the soil of each pot. A bean seed is placed in each hole so that 6 or 12 pots have been planted with each line of bean. Each pot is labelled or numbered so that students know what line of bean is growing in it and which treatment it will receive. Popsicle sticks make good labels. Water the pots thoroughly and place outside or in a greenhouse. This activity can be scaled down so that each student group uses 6 or 12 pots, respectively. Water the pots daily and ensure that the soil remains damp.

The experiment can be continued for several weeks or terminated after 21 days.

Day 7

Students check the pots for germination (usually 6–10 days). Students count and record the number of germinated seeds in each pot, and note whether the seed coat has fallen off to expose the cotyledons and the shoot tip.

Day 14

Students measure the height of each plant (ground to apical shoot tip), and record the growth stage of plants (see Schwartz et al., 2005). The two heart-shaped primary leaves will have expanded by this stage. Students photograph their plants. Students apply 100 mL of tap water to the control pots, 100 mL of 150 mM NaCl to the low-salt pots, and 100 mL of 300 mM NaCl to the high-salt pots.

Day 21

Students measure the height of each plant (ground to apical shoot tip), and record the growth stage of plants. The first and second divided leaves will be expanding. Note any changes in leaf color or shape caused by the salt treatments. Students photograph their plants. Students apply 100 mL of tap water to the control pots, 100 mL of 150 mM NaCl to the low-salt pots, and 100 mL of 300 mM NaCl to the high-salt pots.

Day 28

Differences should have appeared (Figure 1). The salt-treated plants will be smaller and shorter. Students do the same measurements and observations and apply tap water and the salt solutions as for day 21.

Day 35

Students repeat measurements and apply the treatments.

At the end of the experiment cut off some of the plants that typify each treatment and use a photocopier or scanner to produce good-quality images (200–400 dpi is fine). This is a good way to record differences in size, shape, color, and texture caused by the treatments. Figures 2 and 3 give examples of how students can present and analyze their data using Microsoft Excel. Each group can discuss why their data are similar or different to other groups using the same or different cultivars of beans.

Figure 1. Top Crop beans treated with 0, 150, and 300 mM NaCl on day 28 of the experiment.

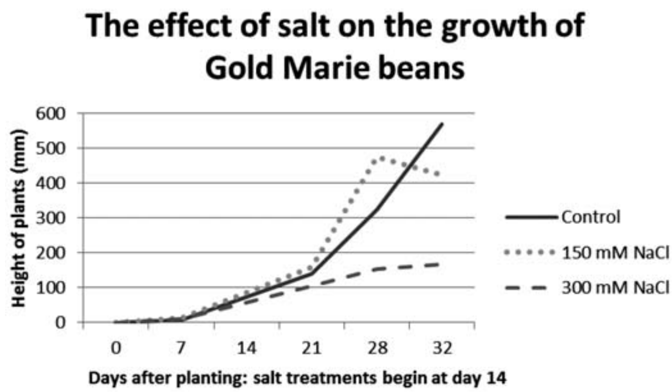

Figure 2. The effect of an abiotic stressor (salt) on the growth of Gold Marie beans.

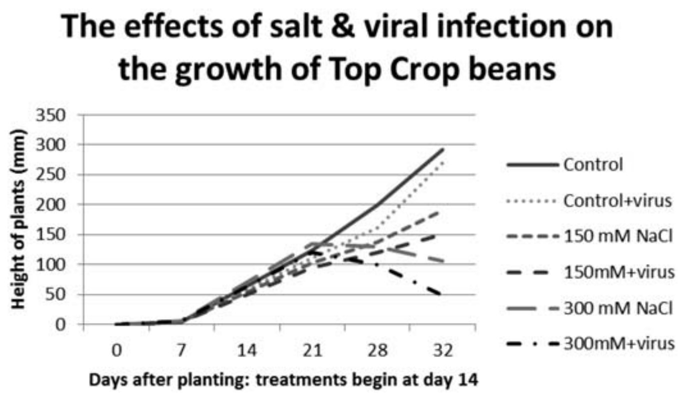

Figure 3. Single and combined effects of an abiotic (salt) and a biotic (virus) stressor on the growth of Top Crop beans.

Extensions

The Contamination Option

Contamination of seed lots with weed species costs agricultural systems millions of dollars per annum and is a major avenue for the introduction of unwanted species into new countries around the world (Gbèhounou, 2013). Spiking the students' seed lots with unwanted seeds introduces them to the need for seed certification and inspection services.

Biotic Stressor: The Viral Option

On day 14 collect 5–6 leaves from white clover (*Trifolium repens* L.) plants growing in a lawn. These will invariably be infected with *White clover mosaic virus*, which is transmitted when sap from infected plants enters small wounds on healthy plants (Wilson, 2014, p. 72). Crush leaves in a little tap water with a mortar and pestle (if available), dip a Q-tip in the liquid sap extract, and rub it on the heart-shaped primary leaves of your second block of 12 pots of beans (6 pots in the scaled-down version). Yellow or brown spots or lesions will appear on the inoculated leaves 7–10 days later. After two weeks the virus will move to the upper leaves and cause vein yellowing and/or yellow speckling of the leaves. The symptoms will vary in different lines of beans.

In some bean cultivars, viral infection will produce a noticeable depression in plant growth for all treatments, whereas in resistant cultivars it will have negligible effects.

For our undergraduate university course, the basic measurements are supplemented by taking chlorophyll content measurements using a chlorophyll meter, and Fv/Fm and performance index (OJIP) using a chlorophyll fluorescence meter. The usefulness of these measurements is critically evaluated against the basic measurements already outlined.

Conclusions

This activity develops skills to set up and run a simple multifactorial experiment that introduces students to the pressing problem of declining crop productivity caused by salinization and disease. Students collect quantitative and qualitative data, and determine which measurements or observations are good for assessing the performance of their bean cultivars under abiotic and biotic stress.

Acknowledgment

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