Plant-A-Plant Mineral Nutrition II Laboratory Guide

Task

Plant maize seedlings in aquariums and water them with various fertilizer concentrations deficient of a certain element. Record observations and determine the effects of various element deficiencies after 14-16 days. Harvest plants and compare the characteristics between treatments.

Prepare and Perform the Experiment

Materials and Tools (*per replicate*)

- ❑ 40 Maize seedlings
- ❑ Aquariums, approximately 5 liters (1 for each variable)
- ❑ Small ventilator (1 to be shared by two aquariums), aquarist hoses and disconnecting elements for them
- ❑ Aluminium foil
- ❑ Stock solutions (See Table 1)
- ❑ Distilled water (approximately 5 litres for each aquarium + about 2 litres for the preparation of each stock solution)
- ❑ Bleach (sodium hypochlorite)
- ❑ Pipettes
- ❑ Measuring cylinder
- ❑ Pencil and permanent marker
- ❑ Masking tape for labeling
- ❑ 1 beaker (1liter volume)
- ❑ 9 empty, clean plastic water bottles (0.5 liter) for stock solutions

** Note: At least <u>two</u> replicates are recommended for this experiment.

Preparation of stock solutions

The amount of the macro- and microelements that you will provide to the plants is very small and difficult to weigh with any accuracy. Therefore it is necessary to prepare stock solutions.

1. <u>Creation of Macroelement solutions:</u>
   a. Using the values in Table 1, dissolve each macroelement in a separate bottle containing 100 ml of distilled water.
   b. Keep the bottles containing dissolved macronutrients in a cool, dark place.
   c. Mark the bottles properly with the name of the chemical contained.

2. <u>Creation of Microelement solution:</u>
   a. Using the values in Table 1, add all microelements into a common bottle containing 100 ml of distilled water (Stock Solution A).
   b. It is then necessary to dilute Stock Solution A to 1:1000 (i.e. with a pipette add 1 ml of Solution A to 1 liter of distilled water) to create Stock Solution B.

** Please note that some chemicals (e.g. Fe citrate) need to be heated in order to dissolve. Work with your Chemistry instructor to determine how to best use these and other chemicals.

Table 1. Mass of Macro- and Microelements (grams/100 ml) for stock solutions.			
Macroelements	Mass of element for stock solutions (g/100 ml)	Microelements	Mass of element for stock solution A (g/100 ml)
$Ca(NO_3)_2 . 4H_2O$	29.6	H_3BO_4	0.72
KNO_3	12.7	$MnCL_2$	4.5
KH_2PO_4	3.4	$ZnSO_4$	0.08
$MgSO_4 . 7H_2O$	6.1	$(NH_4)2Mo_7O_{24}$	0.04
Fe Citrate	0.5	$CuSO_4$	0.02
$CaCl_2 . H_2O$	27.4		
K_2SO_4	10.9		

Preparation of aquariums

1. Cover aquarium walls with an opaque paintwork or covering (e.g. aluminium foil) to keep the roots away from light.

2. Wash aquariums with sodium hypochlorite (common household bleach) and then wash aquariums thoroughly with water.

3. Prepare the ventilation equipment.
   a. One fan can be used for two aquariums when using a disconnection tool (see Figure 1).
   b. Attach a hose to the bottom of each aquarium so that it goes the entire length of the aquarium (see Figure 1).
   c. Puncture the hose at various locations for equal ventilation.
   d. Test the ventilation system before planting by adding water to the aquarium reaching approximately the half full level and turning on the ventilator to make you sure that air goes through all the holes equally.

**Note: Maize can be grown in a hydroponic environment without ventilation, however it is necessary to change the solution after one week)

Figure 1. Ventilation and planting of the aquariums (left) and preparation of ventilation (right)

4. Fill each aquarium with distilled water to the top (be sure to record the exact volume of the aquarium).

5. Using a pipette add the stock solutions according to the amounts listed in Table 2.

***Note:* The amount listed in Table 2 is ml of solution per 1 liter of distilled water. Therefore if the aquarium holds 5 liters of distilled water, multiply the amounts in Table 2 by 5.

**Shake the bottle well before adding Stock Solution B to mix the solution.

Table 2. Volume of stock solutions (ml per each 1 litre of distilled water in aquarium)

	Control	N deficiency	Fe deficiency	Ca deficiency
$Ca(NO_3)_2 . 4H_2O$	1	0	1	0
KNO_3	1	0	1	3
KH_2PO_4	1	1	1	1
$MgSO_4 . 7H_2O$	1	1	1	1
Fe citrate	0.5	0.5	0	0.5
$CaCl_2 . H_2O$	0	1	0	0
K_2SO_4	0	1	0	0
microelements (solution B)	0.5	0.5	0.5	0.5

Plant and Observe Seedlings

1. Cover the water level of each aquarium with a double layer of aluminium foil.

2. Make two rows of 5 pinholes (10 pinholes in total) into the foil equally for seedlings.

3. Plant 10 seedlings into each aquarium and label each plant with a number.

4. During cultivation check the volume of solution in aquariums. Refill the solution with distilled water so that the seedlings have access to water.

5. Grow the plants 14 -16 days at room temperature in a well lighted location (e.g. a window sill). Observe and record any differences between treatments.

Harvesting Plants and Evaluate Biomass

Materials and Tools (*per replicate*)

- ❑ Sink / washbasin with tap water
- ❑ 4 Plastic trays (it is possible to re-use the germination trays)
- ❑ Scissors (ideally fine surgical ones or nail scissors) or a razor blade
- ❑ Aluminium foil
- ❑ Permanent marker
- ❑ Pencil
- ❑ Laboratory scale (accuracy of 0.01 gram)
- ❑ Absorbent paper (paper towels, filter paper, etc)
- ❑ Data Sheets or Science Notebook

** Note: kiln or drying oven is also necessary

Harvest Procedure

1. Cut 48 squares from aluminium foil (approximately 15 x 15 cm)

2. Remove the plant from the top of the aquarium and place into a dish with water (plants must not dry out). Use a separate dish/tray for each treatment.

3. Compare the deficient plants with the control, record important observations and including: total height of plant and color of leaves.

4. Choose 6 of the 10 plants of each variable that have equal or similar height to weigh.

5. Dry plants with filter paper immediately before weighing as water droplets could distort the results.

6. Separate the root, grain and shoot with scissors.

7. Measure the length of the shoot and root. Record the values on your data sheet.

8. Place the individual plant parts into separate aluminium foil packets and label each with the plant number, nutrient, and plant part (i.e. 1 N roots). **Note: If your roots or shoots are too long you can cut them into smaller pieces. However be careful not to lose any pieces.

9. Make small holes in the foil packets with scissors or pins.

10. Weigh packets. Record the fresh weight on your data sheet.

11. Place the packets into kiln or oven at 90°C and dry them for 8 to 12 hours. It is also possible dry them at lower temperatures but for a longer time (e.g. 60°C for 2 to 3 days).

Report Results

1. Remove the foil packets from the kiln or oven (carefully as they will be hot) and weigh individually on the scale. Record values on your data sheet.

2. Calculate and record important values such as:
    a. Fresh weight of entire plant (weight root + weight shoot)
    b. Dry weight of entire plant (weight root + weight shoot)
    c. Water content in fresh plant biomass (%) for entire plant and for individual parts
    d. Increase in biomass (grams of dry weight, in %)
    e. Dry weight ratio of roots:shoot
    f. Length ratio of root:shoot

3. Graph interesting and/or important results.

Conclusions

1. Revise answers to questions posed at the beginning of the experiment in your science notebook or on Student Laboratory Questions sheet. Does the experimental outcome provide the answers or at least a clue?

2. Evaluate validity of your hypotheses. Were they supported or rejected? What was your evidence?

3. Did you encounter any issues/difficulties while performing the experiment? What were potential sources of error in the experiment? Are there ways the procedure could be improved?

4. Record any remaining questions about the experiment or its outcomes. How would you design an experiment to test one of these questions?

5. All scientists, once they have completed their investigation, share their findings with peers in their community. Follow the instructions provided by your teacher to share your work.