

Note: This is the complete lab exercise and instructor manual instructions from the Laboratory Manual that accompanies Biology of Plants, by Raven, Evert and Eichhorn.

The laboratory staff may have prepared the stock solutions, instructions for which are on pages 1 – 4 of this handout.

The instructions to set up the demonstration starts with **Exercise I – Mineral Nutrition**, on page four of this handout.

Inorganic Nutrients Required by Plants

GENERAL COMMENT

The observation time required for this topic is 30 to 45 minutes. A discussion of the observations requires about an additional 30 minutes. If time permits, it would be more instructive for the students to examine the cultures within a few days of initiation of the experiment and every 4 to 5 days thereafter, with the last observations made the day the topic is completed. It may be necessary to run the experiment for 3 to 4 weeks for some of the plants to show deficiency symptoms. This would most likely apply to effects localized on older leaves. At the end of this topic are the supplemental instructions the students need in order to set up their own control and nutrient-deficient cultures. The instructions may be photocopied and distributed to the students. Set up time takes about 45 to 60 minutes.

MATERIALS AND NOTES

Exercise I Mineral Nutrition

Note: Each nutrient-deficient culture need not be set up. Several of each deficiency symptom to be studied, along with several control cultures should be set up.

Note: The following materials are required if students set up cultures.

tomato (*Solanum lycopersicum*) AND/OR sunflower (*Helianthus annuus*) seedlings
(1 saucer of each per room)

Note: Some species of plants show particular deficiency symptoms better than others. The following examples may be helpful:

nitrogen deficiency—tobacco (*Nicotiana tabacum*)

phosphorus deficiency—tomato (*Solanum lycopersicum*)

potassium deficiency—soybean (*Glycine max*); tobacco (*Nicotiana tabacum*)

magnesium deficiency—sunflower (*Helianthus annuus*)

iron deficiency—tomato (*Solanum lycopersicum*)

sulfur deficiency—soybean (*Glycine max*)

calcium deficiency—sunflower (*Helianthus annuus*)

masking tape (1 or more rolls per room)

glass stirring rods (6 to 12 per room)

small spatulas to help remove seedlings from saucer (1 per saucer)

china markers (6 to 12 per room)

brown, wrapping paper **OR** cheesecloth to shade cultures in greenhouse

small bowls (6 to 12 per room)

graduated cylinders, 10-ml (12 per room)

Note: Clearly label each cylinder with the name of one stock solution and emphasize that the cylinders are not to be mixed up.

plastic tubing for aeration (if air outlets are available in greenhouse)

1-liter containers with wide mouths (glass **OR** plastic) (1 per plant)

Note: Wide-mouth jars with screw-top lids work well. Drill one 1-cm hole for the plant and a second 6-mm hole for an aeration tube, if air lines are available in the greenhouse. Rubber stoppers or corks may be used in place of screw-top lids; corks must be coated with wax to reduce evaporation.

foam plugs, glass wool, **OR** plugging cotton (nonabsorbent)

Note: Foam plugs should be larger than the holes in the lids. Use square or cylindrical plugs.

aluminum foil to cover bottles (1 or more rolls per room)

metric rulers (15 **AND** 100 cm long) (in greenhouse)

macronutrient stock solutions (1 of each per room)

micronutrient stock solution (1 per room)

chelated iron solution (1 per room)

MEDIA

Chelated Iron (Ethylenediaminetetraacetic Acid, Fe-EDTA)

Na ₂ EDTA	3.72 g
distilled water to make	1000 ml
ferrous sulfate (FeSO ₄ ·7H ₂ O)	2.78 g

Dissolve Na₂EDTA in distilled water; then add FeSO₄·7H₂O and heat to about 80°C. Store in refrigerator.

Shelf life: Four months at 5°C.

Macronutrient Stock Solutions

Chemical	Concentration
0.5M calcium nitrate (Ca(NO ₃) ₂ ·4H ₂ O) (MW = 236.16)	118.08 g/l
0.5M potassium nitrate (KNO ₃) (MW = 101.08)	50.55 g/l
0.2M magnesium sulfate (MgSO ₄ ·7H ₂ O) (MW = 246.50)	49.30 g/l
0.1M potassium phosphate, monobasic (KH ₂ PO ₄) (MW = 136.09)	13.61 g/l
1.0M sodium nitrate (NaNO ₃) (MW = 85.01)	85.01 g/l
0.5M calcium chloride (CaCl ₂) (MW = 147.03)	73.52 g/l
0.5M potassium chloride (KCl) (MW = 74.56)	37.28 g/l

(Macronutrient Stock Solutions continued)

0.2M magnesium chloride (MgCl ₂) (MW = 203.33)	40.67 g/l
0.2M sodium sulfate (Na ₂ SO ₄) (MW = 322.2)	64.44 g/l
0.1M sodium phosphate, monobasic (NaH ₂ PO ₄) (MW = 138.01)	13.8 g/l

Prepare each of the ten macronutrient solutions according to the molarity indicated for each chemical.

Shelf life: Six months at room temperature; one year at 5°C.

Micronutrient Stock Solution

potassium chloride (KCl)	373 mg
boric acid (H ₃ BO ₃)	155 mg
manganese sulfate (MnSO ₄ ·7H ₂ O)	67.8 mg
zinc sulfate (ZnSO ₄ ·7H ₂ O)	57.5 mg
cupric sulfate (CuSO ₄ ·5H ₂ O)	12.5 mg
ammonium molybdate [(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O]	1.84 mg

Add each of the above chemicals, one at a time, to about 500 ml distilled water. Dissolve one chemical before adding the next. After all six chemicals have been mixed, bring the volume up to 1000 ml with distilled water.

Shelf life: Six months at room temperature; one year at 5°C.

METHODS

Nutrient Culture Solutions

Component salts and amount of each stock solution required per 1000 ml of culture solution.

Solution	10 ml	10 ml	10 ml	10 ml	2 ml	10 ml
Complete	Ca(NO ₃) ₂	KNO ₃	KH ₂ PO ₄	MgSO ₄	Fe-EDTA	Micronutrient
Less Ca	NaNO ₃	KNO ₃	KH ₂ PO ₄	MgSO ₄	Fe-EDTA	Micronutrient
Less N	CaCl ₂	KCl	KH ₂ PO ₄	MgSO ₄	Fe-EDTA	Micronutrient
Less K	Ca(NO ₃) ₂	NaNO ₃ *	NaH ₂ PO ₄	MgSO ₄	Fe-EDTA	Micronutrient
Less Mg	Ca(NO ₃) ₂	KNO ₃	KH ₂ PO ₄	Na ₂ SO ₄	Fe-EDTA	Micronutrient
Less P	Ca(NO ₃) ₂	KNO ₃	KCl**	MgSO ₄	Fe-EDTA	Micronutrient
Less S	Ca(NO ₃) ₂	KNO ₃	KH ₂ PO ₄	MgCl ₂	Fe-EDTA	Micronutrient
Less Fe	Ca(NO ₃) ₂	KNO ₃	KH ₂ PO ₄	MgSO ₄	—	Micronutrient

*Add 5 ml only of the stock NaNO₃ solution. **Add 2 ml only of the stock KCl solution.

SUPPLEMENTARY INSTRUCTIONS

We suggest that the students either work in pairs, with each pair setting up one control and one nutrient-deficient culture, or work in groups of 4, with each group setting up one control and 3 or more different nutrient-deficient cultures. Other groupings can easily be designed. Use whichever works best for your situation. Every section, of course, should set up some of every nutrient-deficient culture you intend to demonstrate and some of each plant species provided.

The cultures are to be maintained until the plants have reached a stage of development at which they show fairly distinct deficiency symptoms. Most deficiencies will be exhibited in 2 to 4 weeks, but some may not be obvious until 4 to 6 weeks. At the completion of the experiment, we have found it helpful to select the most representative cultures of each deficiency and discuss the results as a group.

Setting up the Demonstration once the stock solutions have been prepared.

GENERAL COMMENT

Your instructor will assign you to work in groups of 2 or more to set up one or more cultures, each of which lacks an essential inorganic nutrient. You will also set up one culture containing all the chemical elements known to be essential; this culture is the control.

Before proceeding, read the information in your laboratory manual as well as the instructions provided below. It is important that you carefully follow the instructions. You and the other members of your group will be responsible for making periodic observations of your cultures for 2 to 4 or possibly more weeks. Some of your observations will have to be made during nonlaboratory hours.

When you have completed setting up your cultures, take your plants to the greenhouse. You will be responsible for adding distilled water to your cultures as necessary. It may be necessary for you to replace one or more of your plants after the first few days or to add extra Fe-EDTA. Extra seedlings and Fe-EDTA will be available in the greenhouse.

Exercise I Mineral Nutrition

Procedure

- a. Obtain the number of 1-liter containers and lids necessary for setting up your cultures. Thoroughly cleanse the insides of the containers and the lids with tap water and detergent. Rinse them well with tap water and then rinse each piece several times with small amounts of distilled water.
- b. If the containers are not light-tight, cover their outsides with aluminum foil. Masking tape can be used to cover any tears. With masking tape and a china marker, label each container with your names, the date, your section number, and the description of the solution it is to contain.
- c. Now fill each container about half full with distilled water.
- d. Using the table on the next page, add the appropriate amounts of each stock solution. The graduated cylinders for measuring the stock solutions are labeled with the names of the solutions.

Each cylinder must be used only with the solution noted on the label. It is extremely important that you not introduce any of the element that is to be excluded to your nutrient solutions.

Add each stock solution one at a time with thorough mixing. Glass stirring rods are available for mixing.

- e. When all the nutrients have been added, bring the volume in each container up to about 1 cm below the brim with distilled water.

(Procedure continued)

Culture Media*

Stock solution	Complete	-N	-P	-Mg	-K	-Ca	-S	-Fe	Distilled water
Calcium nitrate [Ca(NO ₃) ₂ ·4H ₂ O]	10	—	10	10	10	—	10	10	—
Potassium nitrate (KNO ₃)	10	—	10	10	—	10	10	10	—
Potassium phosphate (KH ₂ PO ₄)	10	10	—	10	—	10	10	10	—
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	10	10	10	—	10	10	—	10	—
Chelated iron (Fe-EDTA)	2	2	2	2	2	2	2	—	—
Micronutrients (B, Mn, Cu, Zn, Mo, Cl)	10	10	10	10	10	10	10	10	—
Sodium nitrate (NaNO ₃)	—	—	—	—	5	10	10	—	—
Sodium sulfate (Na ₂ SO ₄)	—	—	—	10	—	—	—	—	—
Potassium chloride (KCl)	—	10	2	—	—	—	—	—	—
Calcium chloride (CaCl ₂)	—	10	—	—	—	—	—	—	—
Sodium phosphate (NaH ₂ PO ₄)	—	—	—	—	10	—	—	—	—
Magnesium chloride (MgCl ₂)	—	—	—	—	—	—	10	—	—

* Each number represents the amount of stock solution, in milliliters (ml), that is needed to prepare one liter of nutrient solution.

- f. From one of the saucers of tomatoes, sunflowers or other plants provided, select the number of seedlings you will need. Remove the plants carefully to minimize injury, especially to the roots. A spatula is provided to assist in removing the plants. Place the seedlings in a bowl of distilled water. Gently swish the seedlings back and forth to remove particulate matter from the roots, then support the seedlings on the rim of the bowl so that only the roots are in the water.

(Procedure continued)

- g.** Insert the lower part of the hypocotyl into the slit of a foam plug or wrap the hypocotyl with nonabsorbent cotton or glass wool. Carefully lower the roots through the hole in the lid of a culture container. The foam or other material should fit securely in the hole to give firm support to the plant. Only the roots of the seedling should be submerged in the culture solution when the lid is in place on the container. Plug any extra holes in the lids that will not be used.
- h.** Place the cultures in the greenhouse. If an aeration system is available, your instructor will show you how to assemble the plastic tubing to aerate your cultures. Shade the cultures with brown, wrapping paper or cheesecloth until they have recovered from injuries suffered during transplantation. This recovery period may last from 1 to 4 days; expose the plants to full sunlight as soon as possible. Replace any plants that die or look abnormal after the first several days in culture.
- i.** You will have to examine your cultures frequently for the next 2 to 4 or more weeks. Water lost by transpiration and evaporation must be replaced with distilled water. As the plants grow larger, transpiration will increase and the water will have to be replaced more frequently.
- j.** Also watch for indications of a general chlorosis (yellowing) of the seedlings. The chlorosis is probably due to iron deficiency, a problem common to seedlings. To correct the deficiency, add 2 ml of Fe-EDTA to each culture **except** the iron-deficient culture. Repeat the treatment every several days until the deficiency is corrected.
- k.** Compare your treated (nutrient-deficient) plants to your control plant, watching for the development of deficiency symptoms. The key to deficiency symptoms in your laboratory manual will help you describe these symptoms. You may want to measure the heights of your plants on a weekly basis.
- l.** Record all of your observations in Table 29-1 in your laboratory manual. In addition, you may find it helpful to make sketches of the plants showing deficiency symptoms.
- m.** If your group did not set up cultures of each deficiency symptom being considered in this experiment, examine cultures set up by your classmates. Unless your instructor requests it, do not measure the heights of the plants in these cultures. Record your observations.
- n.** Answer the questions in the laboratory manual.