

# BIORETS Demonstration of Hands-on Laboratory Teaching Module

## Hydroponic Growth Systems and Plant Nutrient Deficiency

July 15, 2022, led by Yan Lu and Alex Kolstoe

### Learning objectives:

- (1) Understand the functions of different parts of a plant, such as roots, the stem, and leaves.
- (2) Observe and record nutrient deficiency symptoms in hydroponically-grown tomato plants.
- (3) Understand the importance of essential mineral nutrients to plant growth and development.

### Introduction

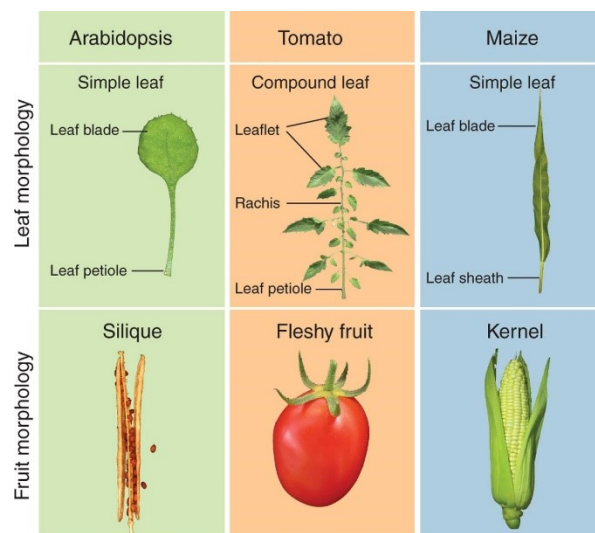
Most plants are made up of six basic parts: roots, stems, leaves, flowers, fruits, and seeds. **Roots** absorb water and mineral elements from the soil and anchor the plant to the ground. The **stem** supports the **shoot**, the above-ground portion of the plant, and transports water and mineral nutrients to leaves. The **leaves** undergo photosynthesis, which uses light energy to convert CO<sub>2</sub> in the air and water from the soil into glucose. Tomato plants have compound leaves (**Figure 1**). A compound leaf contains multiple leaflets which are distributed along the leaf rachis. **Flowers** and **seeds** allow plants to reproduce. **Fruits** help spread the seeds.

Plants require mineral elements for survival. Some elements (CHOPKINS Ca Mg), called **macronutrients**, are needed in large amounts (**Table 1**). Other elements (such as Fe, Cu, B, Mn, Zn, Mo, and Cl) are required in much lower quantities and are therefore called **micronutrients**.

Plants under mineral nutrient deficiency show unusual growth patterns and (dis)coloration, such as stunting, leaf yellowing, leaf death, and accumulation of the purple-colored pigment anthocyanin. These symptoms are known as **nutrient deficiency symptoms**.

**Nitrogen (N)** is an essential component of amino acids, proteins, nucleic acids, etc. Signs of nitrogen deficiency normally appear in the older leaves first and as the deficiency becomes more pronounced, proceed up the plant to the newer growth. The leaves lose their normal green coloration and become a pale yellow-green (**Figure 2**).

**Phosphorus (P)** is an essential component of sugar phosphates, nucleic acids, etc. Phosphorus deficiency produces many of the same effects as nitrogen deficiency, such as retarded growth, small leaves, and the premature loss of leaves. However, instead of the yellow coloration present in plants showing nitrogen deficiency, the leaves are dark bluish-green to purple (**Figure 2**).



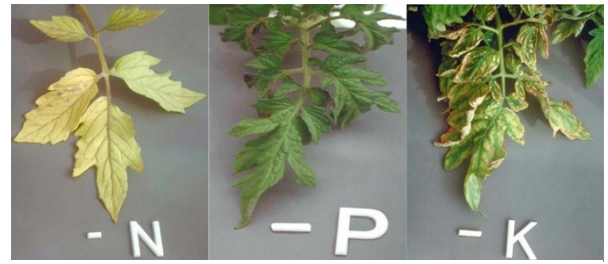
**Figure 1.** Comparison of Arabidopsis, tomato, and corn. Adapted from Ranjan et al. (2012).

**Table 1.** Tissue levels of essential elements required by most plants

Element	Chemical symbol	Concentration in dry matter (% or ppm) <sup>a</sup>
<b>Obtained from water or carbon dioxide</b>		
Hydrogen	H	6
Carbon	C	45
Oxygen	O	45
<b>Obtained from the soil</b>		
<b>Macronutrients</b>		
Nitrogen	N	1.5
Potassium	K	1.0
Calcium	Ca	0.5
Magnesium	Mg	0.2
Phosphorus	P	0.2
Sulfur	S	0.1
Silicon	Si	0.1
<b>Micronutrients</b>		
Chlorine	Cl	100
Iron	Fe	100
Boron	B	20
Manganese	Mn	50
Sodium	Na	10
Zinc	Zn	20
Copper	Cu	6
Nickel	Ni	0.1
Molybdenum	Mo	0.1

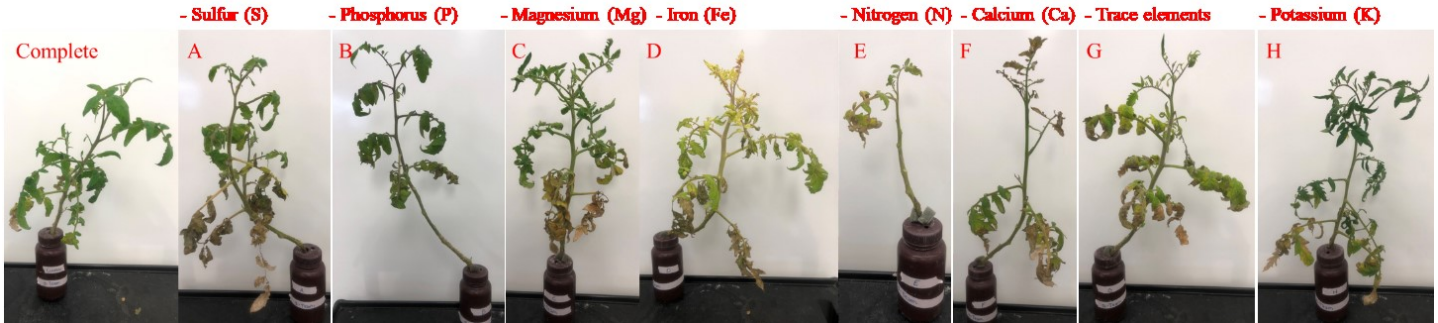
This table is adapted from Table 5.1 in the Plant Physiology textbook.

**Potassium (K)** is required as a cofactor for many enzymes. It regulates stomata opening which in turn regulates air flow into the leaf (i.e., gas exchange) and transpiration of water out of the leaf. Plants suffering from potassium deficiency grow very slowly and are usually stunted. The symptoms first occur on older leaves as a speckling along the margins. The edges of the leaf tend to turn yellow and then brown as the edges curl downward (**Figure 2**).



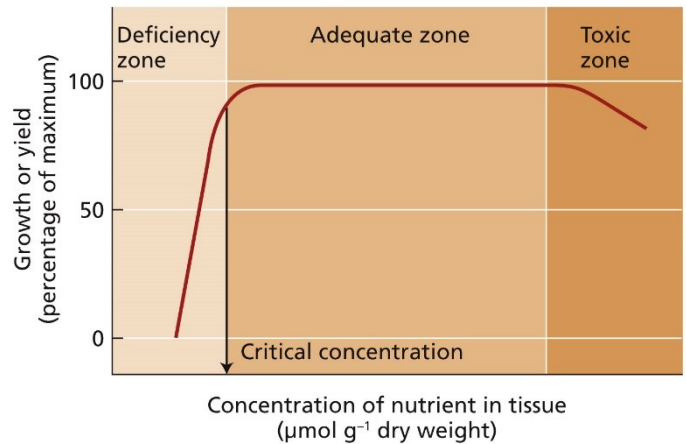
**Figure 2.** N, P, and K deficiency symptoms.

An inexpensive and empirical experiment can be set up to demonstrate the need for a particular nutrient; plants are grown hydroponically in various mineral-containing solutions that lack a particular element (**Figure 3**).



**Figure 3.** Hydroponic tomato plants that have been grown in various mineral-containing solutions lacking particular element(s) for 6 weeks. Photo credit: Yan Lu.

Excess amounts of a nutrient prevent the roots from taking up water and may lead to nutrient toxicity within a plant (**Figure 4**). Prolonged treatments of fertilizers at high concentrations can result in salt burn symptoms, such as wilting of shoot tips. Using ¼-tsp of a general fertilizer per gallon of water has been found to be suitable for hydroponic tomato plants.



**Figure 4.** Relationship between growth and the nutrient concentration.

During the demo, BIORETS teachers will prepare a general fertilizer solution (20-20-20; **Figure 5**) and set up the hydroponics by suspending germinated tomato seedlings with their roots immersed in tap water and the fertilizer solution. Their growth and characteristics could be observed over several weeks. The consequences of plant **nutrient deficiency and toxicity** will be demonstrated by comparison between plants grown in tap water and plants grown in the fertilizer solution.



**Figure 5.** Schultz all-purpose plant food 20-20-20.

What does 20-20-20 mean? These numbers represent the three primary plant nutrients, nitrogen [N], phosphorus

[P], and potassium [K], in that order (**Figure 5**). Many all-purpose formulas contain 20% nitrogen, 20% phosphorus pentoxide [P<sub>2</sub>O<sub>5</sub>], and 20% soluble potash [K<sub>2</sub>O]). This ratio of nutrients has been found to be an excellent formula for feeding all plants, indoors and out.

### **Key Terms:**

Roots, shoot, stems, leaves, flowers, fruits, and seeds  
Simple leaf and compound leaf  
Leaf and leaflet  
Macronutrients and micronutrients  
Nutrient deficiency symptoms  
Nutrient deficiency and toxicity

### **Materials:**

- 6~8-weeks-old tomato seedlings of the same variety in disposable pots (2 seedlings/group). Tomato seedlings of this age work better than younger seedlings. Tomato seedlings could be also purchased from stores (e.g., Lowe's, Home Depot, Meijer, and Walmart) during spring time, e.g., May and early June. Disposable pots are easier to cut through and thus work better than non-disposable ones.
- Used 500-ml plastic water bottles (2 bottles/group). Regular bottles work better than those light-weighted ones; 250~500-ml brown plastic bottles do not require the use of aluminum foil and thus work even better.
- A roll of aluminum foil to block light into water bottles and minimize algal growth.
- Several trays to hold the 500-ml plastic water bottles (optional).
- Several rolls of tape.
- Several marker pens.
- All-purpose plant food/fertilizer 20-20-20 (e.g., 1.5-lb Schultz all-purpose plant food 20-20-20).
- A 1/4-tsp measuring spoon.
- A used 1-gallon plastic water jug (e.g., 1-gallon Meijer distilled water).
- Several plastic funnels to pour the fertilizer solution into water bottles.
- Several pairs of scissors.
- A sieve, a sink drainer, or a piece of nylon mesh to prevent the sink from clogging,
- A plastic scoop (optional).
- A large piece of sponge that could be cut into small pieces and used as foam seals.
- Several 100-ml graduated cylinders (1 cylinder/group).

### **Procedure:**

#### **Activity A: Prepare “water” and “fertilizer” bottles**

1. Each group takes 2 bottles. Wrap each water bottle with a piece of aluminum foil, and tape the foil together. Label one with “water” and the other with “fertilizer”. Label your group name on both bottles.
2. Fill the “water” bottle with tap water.
3. Measure ¼-tsp all-purpose plant food/fertilizer and place it to the bottle of a 1-gallon water jug. Fill the jug half-way and shake it vigorously to dissolve the fertilizer. Fill the jug all the way and shake again to mix the fertilizer solution. Share this jug of fertilizer solution with other groups. Repeat this step if needed.
4. Fill the “fertilizer” bottle with the fertilizer solution.

#### **Activity B: Prepare hydroponic tomato seedlings**

5. Each group selects 2 tomato seedlings of approximately equal size and height (**Figure 6**).
6. Cut the disposable pot with a pair of scissors and carefully remove the seedling from the pot (**Figure 6**).
7. Gently wash the soil from the root under running tap water (**Figure 6**). Make sure you don't clog the sink. This could be done by placing a sieve in the sink (or a sink drainer/a piece of nylon mesh in the sink drain hole) and periodically transferring soil particles collected in the sieve/drainers/mesh to a trash can.

8. Gently place the root and the lower part of the stem into a “water” or “fertilizer” bottle (**Figure 6**). If the seedling is too young/small, you may cut a small piece of sponge and place it between the stem and the bottle neck to support the seedling.
9. Place both hydroponic plants by a window (**Figure 6**). Fill the “water” and “fertilizer” bottles up to the neck line with appropriate liquids.



**Figure 6.** Setting up hydroponic tomato plants. Photo credit: Yan Lu.

### **Activity C: Watering and observing hydroponic tomato plants**

10. Re-fill the “water” and “fertilizer” bottles up to the neck line with a 100-ml graduated cylinder on Mondays, Wednesdays, and Fridays for 2~3 weeks. Make sure you rinse the graduated cylinder after you use it for the fertilizer solution.
11. (Optional) Record the exact volumes (in ml) of tap water and fertilizer solution on a watering log sheet on each watering day.
12. Observe and record the growth and characteristics of both hydroponic plants on a weekly basis for 2~3 weeks (e.g., 2~3 consecutive Fridays). **Take pictures of the plants, leaves, and flowers.** Visible differences in shoot height may be observed after 1 week. Visible differences in the coloration of old leaves may be observed after 2 weeks. Depending the starting age of the hydroponic plants, visible differences in flowering time may be observed after 2~3 weeks.

#### **13a. Leaf appearance.**

Observe the leaf appearance of both hydroponic plants. Are some leaves yellow or dead? Do some leaves have purple leaflets or veins? When do the plants start to display such leaf discoloration? Are the symptoms present on mature (old) or immature (young) leaves?

#### **13b. Leaf number.**

Count the total leaf number of both hydroponic plants. How many are yellow? How many are green? How many are dead? How many have purple leaflets or veins?

#### **13c. Shoot height.**

Measure in cm the height of both hydroponic plants. Is the plant in the fertilizer solution taller than that in tap water? When do the two plants start to display such difference?

#### **13d. Flowering time.**

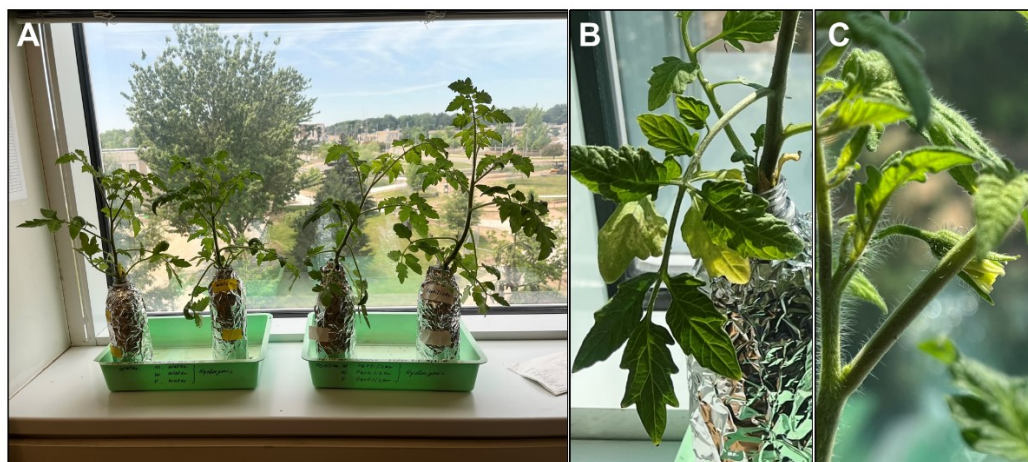
Observe the shoot tip of both hydroponic plants. Are there open flowers or flower buds? When do the plants start to have flower buds? When do the plants start to have open flowers?

**13e. Record your measurements and observations each week in the charts that follow.**

<b>Date</b>				
<b>Plant in water</b>	<b># of yellow old leaves</b>			
	<b># of dead old leaves</b>			
	<b># of green leaves</b>			
	<b># of leaves with purple leaflets or veins?</b>			
	<b># of yellow young leaves</b>			
	<b># of wilted young leaves</b>			
	<b>Shoot height (cm)</b>			
	<b># of flower buds</b>			
	<b># of open flowers</b>			
<b>Plant in fertilizer solution</b>	<b># of yellow old leaves</b>			
	<b># of dead old leaves</b>			
	<b># of green leaves</b>			
	<b># of leaves with purple leaflets or veins?</b>			
	<b># of yellow young leaves</b>			
	<b># of wilted young leaves</b>			
	<b>Shoot height (cm)</b>			
	<b># of flower buds</b>			
	<b># of open flowers</b>			

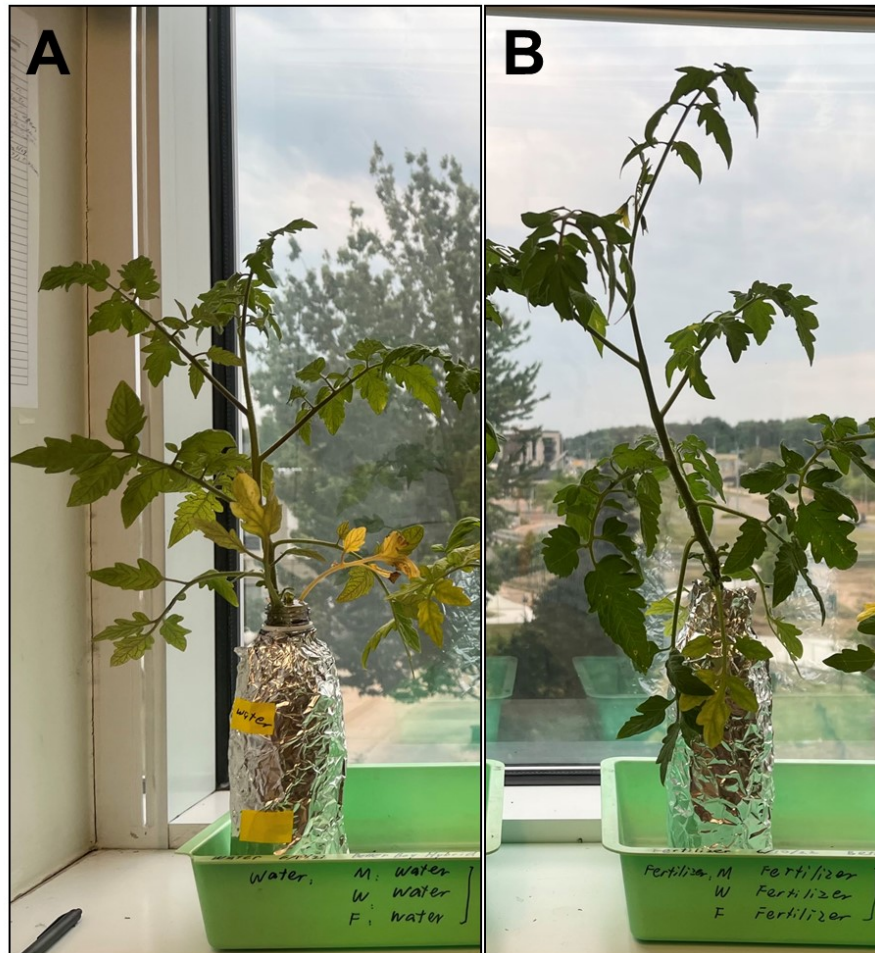
**Expected results:**

1. The hydroponic tomato plants grown in water may start to show stunting, yellowing of old leaves, and delayed flowering, ~10 days after the hydroponics is set up (**Figure 7**).



**Figure 7.** Hydroponic tomato plants ~10 days after the hydroponics was set up. A, the two tomato plants grown in water on the left started to show stunted growth. B, the tomato plants grown in water started to display yellowing of old leaves. C, the tomato plants grown in the fertilizer solution started to flower while those grown in water did not. Photo credit: Yan Lu.

2. The nutrient deficiency symptoms (e.g., stunting, yellowing of old leaves, and delayed flowering) of the tomato plants grown in water worsen after prolonged growth (**Figure 8**).



**Figure 8.** Hydroponic tomato plants ~19 days after the hydroponics was set up. A, a tomato plant grown in water. B, a tomato plant grown in the fertilizer solution. Photo credit: Yan Lu.

### Optional evaluation activities:

1. Ask students to draw a diagram of a plant and label the six basic parts.
2. Ask students to describe the function of each basic plant part.
3. Ask students to describe the difference between a simple leaf and a compound leaf.
4. Ask students to tell the differences between macronutrients and micronutrients.
5. Ask students to list several plant nutrient deficiency symptoms.
6. Ask students to describe the importance of essential mineral nutrients to plant growth and development.
7. At the beginning of the experiment, ask students whether they predict that the plant in tap water will grow slowly, have a shorter shoot height, have fewer leaves, display leaf yellowing and death earlier, and flower later than the plant in the fertilizer solution.
8. After the experiment is concluded, ask students whether the results are consistent with their predictions.

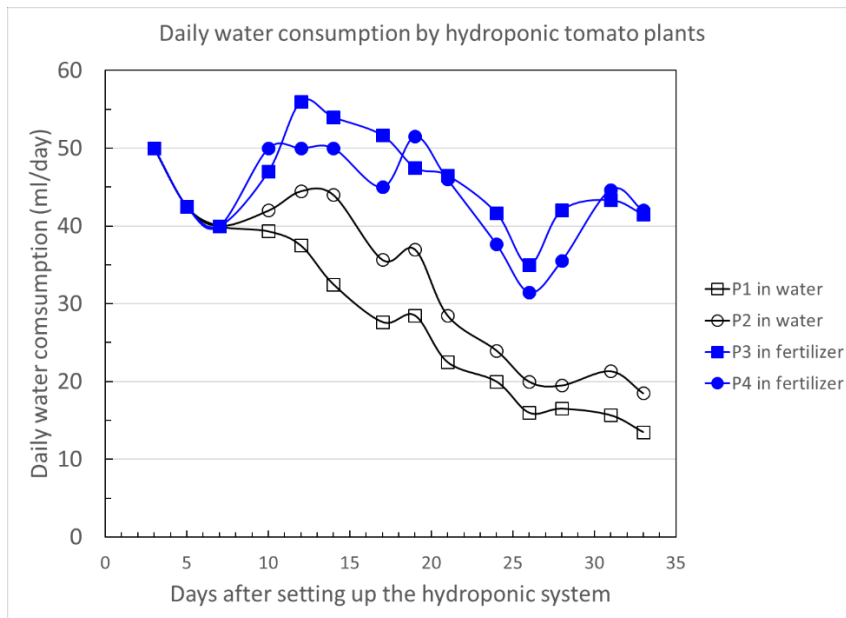
**Potential modifications:**

1. Include three treatments in the experiment: tap water, 1/4-tsp fertilizer/gallon of tap water, 1-tsp fertilizer/gallon of tap water.
2. Include soil-grown tomato plants in the experiment. Although the tomato plant supplied with water grows slower than the one supplied with the fertilizer solution, the plant does not display other visible nutrient deficiency symptoms such as leaf yellowing (**Figure 7**).



**Figure 7.** Soil-grown tomato plants. Picture on the left: soil-grown tomato plants on the day they are placed by a window. Picture on the right: soil-grown tomato plants 17 days after being placed by a window. Photo credit: Yan Lu.

3. Have students calculate and plot daily water consumption (i.e., transpiration rate) for both hydroponic plants with Google Sheets (**Figure 8**).



**Figure 8.** Plotting daily water consumption by hydroponic tomato plants. Graphing credit: Yan Lu.

4. Explain phototropism (the orientation of a plant or other organism in response to light) to students if some plants display growth towards the sunlight (**Figure 9**).



**Figure 9.** Tomato seedlings germinated by a window tend to be tall and slender and display growth towards the sunlight. Photo credit: Yan Lu.

#### **Potential applications in our daily lives:**

1. Understanding the importance of fertilizer usage to crop growth and yield.
2. Appropriate fertilizer usage in home gardening.
3. Understanding the adverse effects of fertilizer overuse.

#### **Additional notes:**

1. This experiment could be easily adapted to a middle or high school biology class during the fourth marking period of a school year.
2. If you plan to germinate tomato seeds on your own, you may purchase a pack of tomato seeds from stores (e.g., Lowe's, Home Depot, Meijer, and Walmart; **Figure 10**). Start the seeds indoors in a warm, well-lighted area 6-8 weeks before setting up the hydroponics. Sow seeds  $\frac{1}{4}$  inch deep into disposable pots filled with soil mix. Keep moist by covering the tray of pots with a clear dome or a piece of plastic wrap (**Figure 11**). Seedlings emerge in 7-10 days at 70-80°F. If the classroom window does not supply sufficient natural light, you may provide additional light fixture on a timer (**Figure 11**).

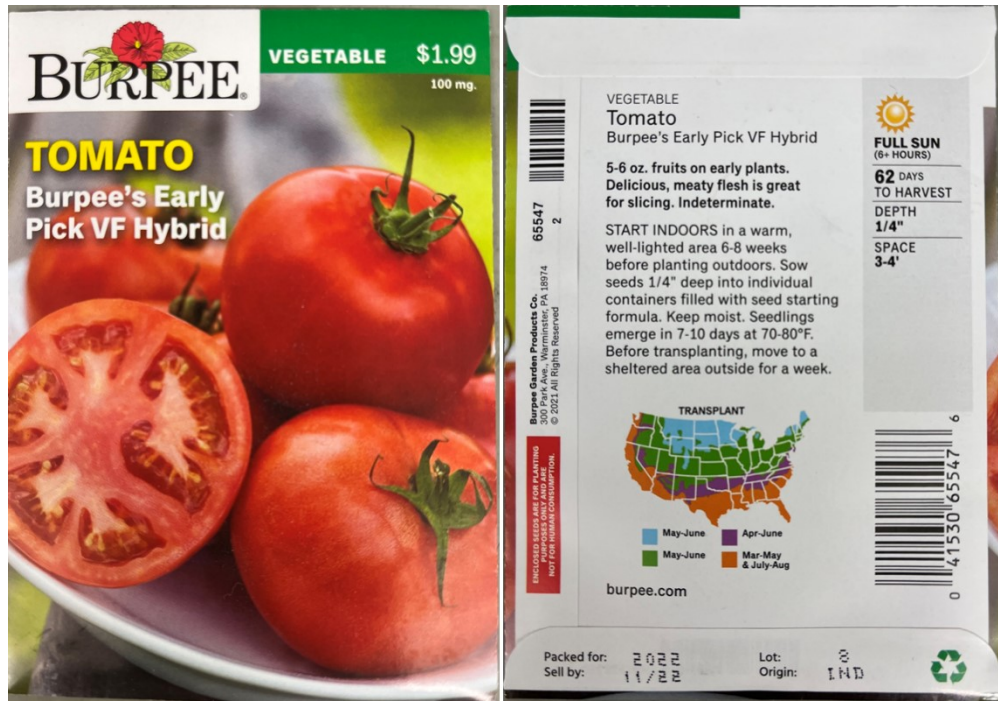


Figure 10. Pack of tomato seeds.

- Tomato seedlings germinated in a growth chamber ~9 days ago are too young for the hydroponic system. The two narrow-shaped “leaves” are cotyledons, not true leaves (Figure 11).



Figure 11. Start tomato seeds indoors. Photo credit: Yan Lu.

#### References:

- Lu Y. (2022) WMU BIOS 3190 Plant Physiology Laboratory Manual, pp. 39-47.
- Ranjan A., Ichihashi Y., and Sinha NR. (2012) The tomato genome: implications for plant breeding, genomics and evolution. *Genome Biol* 13: 167
- Taiz L. and Zeiger E. (2015) *Plant Physiology and Development*, Ed 6, Sinauer Associates Inc., pp. 119-142.

## Thirteen Mineral Nutrients

**Nitrogen:** An essential component of amino acids, and therefore all proteins. An essential component of nucleic acids; and therefore, needed for all cell division and reproduction. Enzymes are specialized proteins and serve to lower energy requirements to perform many tasks inside plants. Nitrogen is contained in all enzymes essential for all plant functions.

**Phosphorus:** A component of the compound within plants that supplies the energy to grow and maintain the plant. Part of cell membranes, the structures that selectively keep out unwanted compounds and allow in those compounds that are needed for the plant cells to function correctly. A component of DNA and its relatives, needed for cell division and for reproduction.

**Potassium:** Activates certain enzymes, regulates stomata opening which in turn regulates air flow into the leaf and transpiration of water out of the leaf. It acts to balance charge between negatively and positively charged ions within plant cells. It regulates turgor pressure that helps to protect plant cells from disease invasion. In certain plants, potassium can be replaced by sodium.

**Sulfur:** Sulfur is part of certain amino acids and all proteins. It acts as an enzyme activator and coenzyme (compound which is not part of the enzyme, but is needed in close coordination with the enzyme for certain specialized functions to operate correctly).

**Calcium:** Calcium is part of cell walls and regulates cell wall construction. Cell walls give plant cells their structural strength. Calcium also enhances uptake of negatively charged ions such as nitrate, sulfate, borate and molybdate. It balances charge from organic ions produced through metabolism by the plant. Some enzymes are regulated by Ca calmodulin.

**Magnesium:** A central element within the chlorophyll molecule. It is an important cofactor for the production of ATP, the compound that is the energy transfer tool in plant cells.

**Boron:** Boron is important in sugar transport within the plant. It has a role in cell division and is required for the production of certain amino acids although it is not a part of any amino acid.

**Molybdenum:** Needed for the reduction of absorbed nitrates into ammonia prior to incorporation into an amino acid. It performs this function as a part of the enzyme nitrate reductase. In addition to direct plant functions, molybdenum is also essential for nitrogen fixation by nitrogen-fixing bacteria in legumes. Responses of legumes to molybdenum application are mainly due to the need by these symbiotic bacteria.

**Iron:** Component of the many enzymes and light energy transferring compounds involved in photosynthesis.

**Zinc:** Component of many enzymes and essential for plant hormone balance, especially auxin activity.

**Manganese:** Cofactor in many plant reactions, it is essential for chloroplast production.

**Copper:** Component of enzymes involved in photosynthesis.

**Chlorine:** Used in the form of chloride ion, it is useful as a charge-balancing ion and for turgor regulation and keeping plant cells relatively free of infection. It is essential for photosynthesis.

## Symptoms of Mineral Deficiency – Supplementary Notes

### ***Nitrogen***

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Signs of nitrogen deficiency normally ***appear in the older leaves first*** and as the deficiency becomes more pronounced, proceed up the plant to the newer growth. The ***leaves lose their normal green coloration and become a pale yellow-green***. When grown in a nitrogen-deficient nutrient solution the entire plant may become a pale yellow and premature leaf-fall occur. The young leaves remain small and the growth of the entire plant, including the root system, is very poor. The ***lack of lateral branching gives the plant a spindly appearance***.

### ***Phosphorus***

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Phosphorus deficiency produces many of the same effects as nitrogen deficiency: ***retarded growth, spindly appearance, small leaves*** and the premature loss of leaves. However, instead of the yellow coloration present in plants showing nitrogen deficiency, the ***coloration of the leaves is dark bluish-green to purple***. This is especially true of tomato plants in later stages, thin, dry, brown spots may occur on the leaves.

### ***Potassium***

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Plants suffering from potassium deficiency grow very slowly and are usually stunted. The ***symptoms first occur on older leaves as a speckling along the margins***. The edges of the leaf tend to turn yellow and then brown as the edges curl downward. ***New leaves have a distorted, crinkled appearance*** and spots may develop rapidly. These spots usually turn brown and are frequently dry and brittle. The leaf color around the margins and between the veins fades to a pale grey. The stems of the plants are woody and the poorly developed roots often turn brown.

### ***Magnesium***

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Although symptoms of magnesium deficiency vary among species of plants, the ***symptoms normally appear on the older leaves first*** and progress toward the younger growth as the deficiency becomes more severe. ***Interveinal chlorosis is quite common***, although some plants may show yellow or brown spotting of the leaves. The entire leaf may turn brown and fall from the plant. The leaves become brittle and tend to curve upward along the margins.

### ***Calcium***

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Calcium deficiency ***appears first in the young leaves*** of the plant. The ***leaves turn yellow or brown and the edges may begin to curl***. The upper leaves and stems may develop brown spots. The plants are weak and the ***stems often lack turgor*** and are unable to support the plant. The roots are poorly developed, being short with many branches and often becoming brown. The terminal buds often die.

### ***Sulfur***

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The symptoms of sulfur deficiency resemble those of nitrogen deficiency. The first sign is usually ***chlorosis of the older foliage***. Even though there is ***considerable increase in the length of the roots and stems, neither exhibits much increase in diameter***. Stems are very woody and hard, and growth of the plant is upright.

### ***Iron***

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Iron deficiency symptoms ***occur first in the youngest leaves*** of the plant. They show a mottling or ***interveinal yellowing which progresses until they become completely pale yellow to almost white***. The stems near the growing tip of the plant become yellowish-green and growth of shoots is very poor if the deficiency persists.