**Hydroponic Nutrient Project Protocol**

**version 5.0**

**University of Minnesota**

**Southwest Research and Outreach Center**

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**CAUTION: This procedure involves the use of chemical reagents and hypodermic needles. All appropriate safety precautions and procedures should be followed when exposed to or handling these materials. (Disclaimer).**

**Objective:** The purpose of this experiment is to introduce the concept of hydroponic culture of plants and to qualitatively and quantitatively measure the effect of induced nutrient deficiency symptoms for plant nutrients including: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), copper (Cu), zinc (Zn), Boron (B), Molybdenum (Mo), and Manganese (Mn).

There are five phases in this project; (1) seed germination, (2) seed elongation, (3) growth in full nutrient solution (4) growth in treatment solutions, and (5) observation and assessment. The duration of the experiment will be very flexible but one should plan for at least eight to twelve weeks. The longer the duration of the experiment, the more pronounced the negative effects of nutrient deficiency will be on plant growth and development. The protocol below is organized week-by-week along with an indication of the phase.

**WEEK 1**

**Seed germination**

Seed choice is important; avoid root/tuber plants such as beets, radish and potato. Recommended seed choices include: corn, soybean, tomato, pepper and cucumber.

Germinate more seedlings than you will actually need for the experiment because seed germination, depending on the age of the seed, can range from under 50 to over 90 percent. This will also allow you to choose the best looking seedlings at the next two steps to ensure you are starting the experiment with thriving healthy plants. To germinate the seed, lay out a few layers of paper towel and lightly wet with a spray or squirt bottle. Spread the seed out on the wetted paper towel keeping one to two inches between seeds. Apply a few layers of paper towel over the seed and lightly wet. Place in a unzipped plastic Ziploc bag (towels may be loosely rolled to fit several in a bag) and cover with an opaque material. The ultimate goal is to keep the seeds in an environment that is moist, dark and warm. Rewet towels when necessary. It is important to maintain the paper towel moist during seed germination to provide enough moisture to the germinating seedling. Seed will generally germinate in 2-5 days.

**WEEK 2**

**Seed elongation**

Once the seedlings have germinated and the main root system and first leaves are developing, the seedlings should be placed in paper towels, standing on end and stored in a container. Choose the healthiest and strongest looking seedlings to transplant, and transplant several more than you will need so you can again choose from the healthiest plants when it is time to transplant them into the nutrient solution.

To prepare the seedlings, lay out a few layers of paper towel and wet with a squirt bottle. Place seedlings at the top of the towel with shoots above the edge and roots lying on the towel, spaced apart about 1.5-2 inches. Lightly cover the seedlings as if to “sandwich” the roots but leaving the shoot still out the top of the towel, then wet the top paper towel. Gently roll the towel lengthwise so the shoots are still sticking out of the top and place in a container (a cottage cheese or yogurt container would work well, or you may use a 2.5 L container half-full of tap water). The water will wick from the end of the paper towel submerged in the container up to the top of the paper towel where the seed/root/shoot are located, keeping the seedling in constant supply of water The seedlings should be kept in the paper towel rolls and allowed to elongate for 1 to 2 weeks. Do not allow the container to become empty or the paper towels to dry out or the seedlings will die. Careful observation and rewetting will most likely be necessary.

**WEEK 4 - 7**

Once the root system of the seedlings becomes better developed and the shoot of the plant is approximately 2 inches, it is time to transplant the seedlings.

A warm, 70 F, well lit environment is the ideal place to set up your experiment.

**Full Nutrient solution preparation and addition**

Note: Use extreme caution when working with all chemical reagents. Have in an accessible/local file all chemical Material Safety Data Sheets (MSDS) and be familiar with hazards, safe handling procedures, reporting procedures, and first aid recommendations. In addition, follow all appropriate safety procedures (eye protection, gloves, etc) when using chemical reagents.

All containers should be given the complete nutrient solution as soon as seedlings are transplanted and receive the full nutrient solution for four to six weeks. Prepare nutrient solutions from reagent grade compounds available from companies such as Fisher Scientific (Fisher.com). The complete solution includes nitrogen, phosphorus, potassium, iron, calcium, manganese, magnesium, zinc, boron, sulfur, copper, sodium, chloride and molybdenum. The complete nutrient solution should be prepared using the prescribed amount of pure dry chemical reagent plus the required amount of distilled/deionized water (Table 1). The reagents should be weighed out to three decimal places on an appropriate balance. It is recommended that reagents be prepared using volumetric flasks. However, graduated cylinders and beakers can be substituted. Alternatively, rather than purchasing reagents individually, which can be expensive, prepare nutrient solution A and B (GroMagnon Growth Nutrient Formulation) available from American Hydroponics. Part A and B mixed together represent the complete stock hydroponic nutrient solution concentrate. Substitute individual solutions of ammonium nitrate, sodium phosphate, and potassium chloride (muriate of potash) for part A in order to impose nitrogen, phosphorus and/or potassium treatments (see Table 1). A 2.5 liter polyethylene container should be used to conduct the experiment. Add about 2 liters of distilled/ deionized water to the container (ordinary distilled water is satisfactory for N, P, K, Ca, Mg, and Fe treatments). Add the designated amount of full nutrient solution to the 2.5 liter polyethylene container. The seedlings are now ready to be transplanted.

**Transplanting**

The lid should have two one-inch holes and one 1/4-inch hole pre-drilled in it. The seed and the entire root system should extend at least two inches below the lid and into the solution in the container once the lid is attached to the container. Choose the healthiest and strongest looking seedlings to transplant into the nutrient solution. To prepare the seedlings for the lids, wrap a rope of cotton or position several cotton balls around the seedling stem near and slightly above the seed. The cotton helps support the seedling and allows for stem expansion and growth during the experiment. Fit the wrapped seedling into the one-inch hole in the lid with enough cotton so it is secure enough that the seedling will not slip through. If the cotton gets wet, and there is not enough cotton to support the plant, the seedling will fall through the hole into the nutrient solution.

The configuration of the container lids allows for one or two plants of the same species or two different species to be grown in the same container. One advantage of growing one plant per container is that not only can “above-ground” nutrient deficiency symptoms (shape, size, color) be observed, but also “below-ground” symptoms (root growth).

 Light must be excluded from the container to prevent algal growth. Aluminum foil formed around the container and a piece on top of the container can be used to prevent algal growth.

**Aeration**

Caution: Use extreme care when working with needles! Know and follow all safety protocols, reporting procedures, and first aid recommendations.

Root aeration is critical for survival of hydroponically grown plants. A standard aquarium pump (100 gal.) can be used to aerate up to 40 containers. A 3/16th inch supply line is connected to the pump and positioned next to the containers. Aeration tubes are constructed using a 15-18 inch segment of 4 mm o.d. metric tygon tubing. The tubing is fastened to the flared end of a Becton Dickinson precision glide needle. A small amount of household glue is used to secure the tubing to the flared end of the needle. Care should be exercised when constructing the aeration tubes so as not to get adhesive in the end of the tubing or the tube will become blocked and the aeration tube will not work. Once the aeration tubes are ready for use, the tip of the needle is used to puncture the supply line. Place the open end of the tubing through the small hole in the lid and into the nutrient solution, so that it bubbles and creates water movement. It is helpful to tape the tubing to the lid at this point.

**Maintanence**

Daily maintenance of the experiment is required, from start to finish. A tremendous amount of effort is put into carrying out this experiment/demonstration and neglecting to address problems as they occur could result in failure of the activity.

**Daily.** It is important to check daily that the aeration system is functioning properly and that each aeration tube is operational. Minimizing light penetration into the containers is important to minimize algal growth. If algal growth occurs on the cotton support material it should be changed. It is critical to monitor water levels, and add distilled water as needed between weekly solution replacements (see below).

**Weekly.** The containers must be rinsed, cleaned and refilled with distilled/deionized water, and the nutrient solutions replenished on a weekly or bi-weekly basis. When refilling containers and replenishing the nutrient solutions, the container lids with growing plants can be temporarily placed on a clean bench but returned to the appropriate container as soon as possible, within 15-minutes. If cotton balls are checked for algae and changed when algae is initially detected and light penetration into the containers is limited then rinsing and cleaning of containers can be minimized or eliminated. When the distilled water is replenished it may be necessary to clean the containers with a brush or wash cloth because algae may begin to grow on the walls of the containers. The containers should always be kept free of algae.

**WEEK 8 -12**

**Introducing the treatment**

After the initial four to six week growth period in the full nutrient solution, treatments may be assigned to selected containers. Containers and lids should be clearly marked with a treatment number. It is also important to record the nutrient treatment (N, P, K) assigned to the container.

Solutions should be mixed according to Table 1, and treatments prepared according to the guidelines in Table 2. Plants should grow in treatment solutions for a minimum of two to four weeks. Four weeks is recommended to ensure full development of deficiency symptoms. One possible treatment design could consist of five treatments including: a nitrogen deficient treatment (zero N), a phosphorus deficient treatment (zero P), a potassium deficient treatment (zero K), a zero nutrient control (just distilled water), and a full treatment (complete solution). Although this is one option, any of the nutrients found in the complete solution could be used as a treatment. Alternatively, various other combinations of treatments may be imposed. Factors that may be considered as possible treatments beyond nutrients include: light, water, solution pH, and temperature. For example, a complete nutrient solution could be use throughout the experiment but plants could be subjected to varying degrees of shading (0-100%).

**Maintenance**

**Daily.** Again, daily maintenance is imperative. Plants and aeration tubes should be checked every day. Continue to minimize light penetration into the containers to avoid algal growth. Change the cotton as needed.

**Weekly.** The containers must be rinsed, cleaned and refilled with distilled/deionized water, and the proper treatment solutions replenished on a weekly basis. When refilling containers and replenishing the nutrient solutions, the container lids with growing plants can be temporarily placed on a bench but returned to the appropriate container as soon as possible, within 15-minutes. If cotton balls are checked for algae and changed when algae is initially detected and light penetration into the containers is limited then rinsing and cleaning of containers can be minimized or eliminated. It may be necessary to clean the containers with a brush or wash cloth because of algae growth on the walls of the containers before adding the fresh solution. The containers should always be kept free of algae.

**WEEK 12**

**Observation and assessment**

Quantitative and qualitative observations can be documented during and/or at the end of the experiment/demonstration. Quantitative observations are those characteristics of a process or object that can be precisely measured: length, height, diameter, area, volume, mass, speed, time, temperature, cost, age, etc… In contrast, qualitative observations are often categorized into patterns; observations are generally descriptive accounts of a process or object’s characteristics. Examples of qualitative data can include: observation and reflection field/laboratory notes, appearance, color, texture, smell, taste, pictures, and other materials. An example form is included at the end of this document which can be used for quantitative and qualitative observation and assessment.

Examples of quantitative measurements that could be used for this experiment/demonstration include evidence of stunted growth (plant height, stem diameter, number of growth points, root system length, plant and/or root mass, number of leaves), percent of plant displaying symptoms (leaf count, healthy versus unhealthy leaves), other direct measurements (tissue analysis, yield).

Examples of qualitative measurements that could be used for this experiment/demonstration include leaf discoloration [yellow/white (chlorosis), brown/tan (necrosis), pale green, red, purple], location where symptoms occur (new or old leaves, leaf margins, between leaf veins, spots, stripes), leaf shape (misshaped, curled, folded, wilted or bent). These measurements could be expressed in written notes, illustrations, or even photographs.

The assessment component of this experiment/demonstration could consist of a written report and/or group discussion. For example, a group discussion could be organized by a particular treatment or a specific plant species. Once the groups are formed, the data sheets can be used to facilitate the discussion by using leading questions in order to determine it the students learned how to properly identify nutrient deficiency symptoms. The students could be initially asked to identify which was the best treatment and which was the worst and why. Then the instructor would describe the main characteristics of nutrient deficiencies, for example, nitrogen, phosphorous, and/or potassium deficiency, if these were the chosen treatments. As a follow up the attention would then turn back to the plants as which time the students would be asked to identify the appropriate treatments.

Disclaimer:

* The University of Minnesota does not assume liability for injuries incurred as a result of following this protocol.
* The University of Minnesota does not imply that methodologies and suggestions outlined are the only applicable ones.
* Mention of products/companies is not intended to reflect endorsement.
* It is the responsibility of the science teachers and/or school administrators to use/observe safety policies and follow appropriate safety procedures.
* It is the responsibility of the science teachers and/or school administrators to instruct students to use/observe safety policies and follow appropriate safety procedures in order to make it safer in the science laboratory.

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| **Table 1. Stock Nutrient Solutions. Adapted from Soil Science/Agronomy/Horticulture 326 Laboratory Manual University of Wisconsin, Madison.** |
| **Salt** |  |  | **Formula weight** | **Nutrient content** | **Stock solution** | **Nutrient conc.** | **Nutrient solution** | **Nutrient conc.** |
| **name** | **formula** | **element** | **g** | **% g/L** | **g/L** | **mg/L** | **mL stock/L** |  **mg/L** |
| **Ammonium nitrate** | **NH4 NO3** | **N** | **80.04** | **35.0 N** | **35.7** | **12,500 N** | **5** | **62.5 N** |
|  |  |  |  |  |  |  |  |  |
| **Sodium phosphate, dibasic heptahydrate** | **Na2HPO4 . 7 H2O** | **P** | **268.07** | **11.6 P** | **12** | **3,100 P** | **5** | **15.5 P** |
|  |  |  |  |  |  |  |  |  |
| **Potassium chloride** | **KCl** | **K** | **74.55** | **52.4 K** | **28.6** | **15,000 K** | **5** | **75.0 K** |
|  |  |  |  |  |  |  |  |  |
| **Magnesium sulfate heptahydrate** | **MgSO4 . 7 H2O** | **Mg, S** | **246.50** | **9.9 Mg****13.0 S** | **49.3** | **4,900 Mg****6,400 S** | **5** | **24.5 Mg****32.0 S** |
|  |  |  |  |  |  |  |  |  |
| **Calcium chloride dihydrate** | **CaCl2 . 2 H2O** | **Ca** | **147.02** | **27.3 Ca** | **36.8** | **10,000 Ca** | **5** | **50.0 Ca** |
|  |  |  |  |  |  |  |  |  |
| **Manganese chloride tetrahydrate** | **MnCl2 . 4 H2O** | **Mn** | **197.91** | **27.8 Mn** | **0.2715** | **75 Mn** | **5** | **0.38 Mn** |
|  |  |  |  |  |  |  |  |  |
| **Copper (II) Chloride dihydrate** | **CuCl2 . 2 H2O** | **Cu** | **170.48** | **37.3 Cu** | **0.0161** | **6 Cu** | **5** | **0.03 Cu** |
|  |  |  |  |  |  |  |  |  |
| **Zinc Chloride** | **ZnCl2 (95%)** | **Zn** | **136.29** | **45.6 Zn** | **0.066** | **30 Zn** | **5** | **0.15 Zn** |
|  |  |  |  |  |  |  |  |  |
| **Boric acid** | **H3BO3** | **B** | **61.83** | **17.5 B** | **0.169** | **30 B** | **5** | **0.15 B** |
|  |  |  |  |  |  |  |  |  |
| **Molybdenum (VI) acid monohydrate** | **H2MoO4 . H2O** | **Mo** | **179.97** | **53.3 Mo** | **0.0019** | **1 Mo** | **5** | **0.005 Mo** |
|  |  |  |  |  |  |  |  |  |
| **†Ferric Chloride hexahydrate** | **FeCl3 . 6 H2O** | **Fe** |  |  |  |  |  |  |
| † The stock iron solution consists of 1.965 g DTPA (diethylentriamene pentaacetate), 1.35 g FeCl3 . 6 H2O, and 0.60 g NaOH (sodium hydroxide). Dissolve the FeCl3 . 6 H2O in 250 mL distilled/deionized water. Add the NaOH to the DTPA in 500 mL distilled/deionized water and stirring until dissolved. Then slowly add, while stirring, the FeCl3 . 6 H2O solution. Dilute to 1 liter and store in a dark container, preferably brown. |

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| **Table 2. Preparation of dilute nutrient solutions from stock solutions. Milliliters of stock solution per 2 liters of distilled/deionoized water** |
| **Stock solution** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **formula** | **complete** | **N** | **P** | **K** | **Ca** | **Mg** | **S** | **Mn** | **Cu** | **Zn** | **B** | **Mo** | **Fe** |
| **NH4 NO3** | **10** | **-** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **Na2HPO4 . 7 H2O** | **10** | **10** | **-** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **KCl** | **10** | **10** | **10** | **-** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **MgSO4 . 7 H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **CaCl2 . 2 H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **MnCl2 . 4 H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **CuCl2 . 2 H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **ZnCl2 (95%)** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **H3BO3** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **H2MoO4 . H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |
| **FeCl3 . 6 H2O** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** | **10** |

***Plant characterization***

Treatment: 1 2 3 4 5

Crop :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Quantitative

 Height: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Girth: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Number of leaves

 Healthy \_\_\_\_\_\_\_\_\_\_\_\_

 Unhealthy \_\_\_\_\_\_\_\_\_\_

 Growth points \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Qualitative

 What

Draw a diagram of the plant or a leaf showing characteristic nutrient deficiency symptoms in the box above.

 Where

Crop :\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Quantitative

 Height: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Girth: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Number of leaves

 Healthy \_\_\_\_\_\_\_\_\_\_\_\_

 Unhealthy \_\_\_\_\_\_\_\_\_\_

 Growth points \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Qualitative

 What

Draw a diagram of the plant or a leaf showing characteristic nutrient deficiency symptoms in the box above.

 Where