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Category: Plant Sciences

Genetic and phenotypic comparison of four *Arabidopsis thaliana* strains when exposed to heavy metals, for future applications in agriculture.

A) Rationale

- Heavy metals in soil can affect the plants which grow in it. If the metal is a carcinogen, the carcinogenic effect can affect the plants and possibly the people eating them. In Taiwan, there was a higher incidence of oral cancer between 1982-2002, due to the large amount of factories which release pollutants which can affect the soil. Therefore affecting the plants which grow in the area and affecting the people who consume such plants (Su, C.-C., Lin, Y.-Y., Chang, T.-K., Chiang, C.-T., Chung, J.-A., Hsu, Y.-Y., Lian, I.-B., 2010).
- Contaminated soils are capable of causing damage to human and animal health, and food quality of plants grown in contaminated environments (Shams, Ekinci, Turan, Dursun, Kul, Yildirim., 2018).
- Plants that are commonly found in polluted areas may be contaminated with heavy metals in the atmosphere. Metals such as chromium, copper and nickel are shown to affect the growth and biomass of plants such as in the plant *Arabidopsis thaliana* (Li, W., Khan, M. A., Yamaguchi, S., Kamiya, Y., 2005).
- Testing common genotypes that grow naturally, determine which genotypes present the suitable characteristics to resist exposure from heavy metals.
- Genetically modifying the genotype of a certain plant that grows in domains that are affected by heavy metals with a mutant of a resisting plant may increase their growth rates (Wilke, C., 2019).
- Plants possess characteristics and mechanisms that grant the essential metal ions access to cellular compartments, while minimizing the damage that will be created by the nonessential ones (Keilig, K., Ludwig-Muller, J., 2009).
- The free metal ion concentration in the cytosol is need to be kept low in metal sensitive plants like *Arabidopsis thaliana* in order to maintain functional cell metabolism and function (Cho, M., Chardonnens, A. N., Diez, K. J., 2003).

B) Research Questions, Hypothesis, Expected Outcomes

- Which strains (Col-0, Ws-2, Santa Clara, Lov-1) of *Arabidopsis thaliana* are most resistant to the given heavy metals: copper sulfate, nickel sulfate and chromium sulfate?
- To determine the reasons why certain strains are more resistant to heavy metals than other strains by comparing the genomes of each strain for unique gene pathways/clusters.

- ➔ *Null Hypothesis (H_0)*- With the existing knowledge of the *Arabidopsis thaliana* plant, with the treatment of heavy metals, the Col-0, Lov-1, Ws-2 and Santa Clara genotypes will not be affected in any way.
- ➔ *Alternative Hypothesis (H_A)*- With the existing knowledge of the *Arabidopsis thaliana* plant, with the treatment of heavy metals, the Col-0, Lov-1, Ws-2 and Santa Clara genotypes will experience a negative or positive growth affect.

Expected Outcome: Considering that Col-0 is known as the wild type, it will be the most resistance to the heavy metals because it may be grown in an atmosphere with heavy metal exposures. Therefore having more heavy metal stressors.

C) Procedure:

I. Planting *Arabidopsis Thaliana*

1. Four trays with 12 pots in each should be prepared each pot representing a different heavy metal or the control.
2. Place cheesecloth (for 2.5 pots use 6x6 cm of cheesecloth) at the bottom of each pot to cover the whole.
3. Prepare a large tray with 3,782 g of soil and mix in 322.5 g (30 cap fulls) fertilizer with hands. Then soak the soil mixture in water to evenly distribute water.
4. Fill each of the planting pots with soil fertilizer mixture and flatten as pouring the soil. It is important for the soil to be flattened, but to compact will be hard for roots to grow.
5. In a bottle cap covered with wax paper place 15-20 Arabidopsis seeds of a strain and add 4 drops of water with a pipette.
6. Evenly administer the seeds to a pot of soil.
7. Repeat steps 5-6 for all 12 pots of each strain of seeds. Make sure to change wax paper when changing strain.
8. Place the pots in the respective trays and fill the tray with 1.5 cm of water and cover the pots with plastic wrap. Place the pots in a refrigerator for 2-4 days.
9. After cold treatment place the pots under the artificial light 15cm above. There must be 2 cm in the trays until the plants germinate. Germination takes around 3-5 days, make sure the water level is always at 2 cm. The plants germinate best at 20-22 degrees celsius
10. Continue adding water to the respective 2 cm, do not let the plants dry out.
11. A week after the first leaves have sprouted, gently water the pots by adding 5 milliliters of water to the top with a medicine dropper into each pot around the seedlings. Make sure that the water does not land directly on the leaf of the plant. Water every single day.

II. Preparing the water and heavy metal mixture (All mixtures will be prepared by Mr. Gerald Wykes, the Chair of the Chemistry Department at NSHAHS)

1. All heavy metal mixture must be prepared in the hood and the sealed mixtures are completion kept in the hood.
2. Prepare the first heavy metal solution with 16 millimolars of copper sulfate and 1000mL of water. Add the 16 millimolars of copper sulfate and 500 mL of water into a 1000 mL volumetric. Place inside a ¾ inch metal bar and set the volume metric on a hotplate to dissolve the metal. After the metal was fully dissolved in the water, add another 500 mL of water and stir for a few seconds. After making the solution close the volumetric with its lid tightly and keep the solution inside hood.
3. The second solution should consist of 16 millimolars of nickel sulfate and 1000 mL of water. The 16 millimolars of metal and 500 mL of water should be added to a volumetric. Place inside a ¾ inch metal bar and set the beaker on a hotplate to dissolve the metal. After the metal is fully dissolved at another 500 mL of water and stir for a few seconds. Then close the volumetric tightly with its lid and keep inside hood.
4. The third solution will be prepared with 16 millimolars of chromium sulfate and 1000mL of water. Both the 500 mL of water and the 16 millimolars of chromium sulfate should be added into a volumetric. Put inside a ¾ inch metal bar and place the volumetric on a hotplate to dissolve the metal. After solution is fully dissolved add 500 mL and stir for a few seconds then seal tightly with lid. Leave the solution inside the hood.

III. Adding the heavy metal mixtures to the plants

1. The four trays are separated based on the type of heavy metal solution: chromium sulfate, copper sulfate, nickel sulfate and the control.
2. Each of the plants in the trays will be watered with 12.5 mL through a medicine dropper with the designated heavy metal.
3. The watering with the heavy metal solution will be performed three times per week, a total of five applications of the heavy metal solution will be administered.
4. After this time period, switch to watering the plants by dropping 5 mL everyday of water into all the plant pots.

Risk and Safety

Copper Sulfate;

Risk Assessment

- Biosafety Level 1
- Toxic if consumed or inhaled, if done so call a poison center. Do not eat, drink or smoke while handling chemicals. A chronic effect may be a possible mutagen. The targeted organs if ingested are the liver, kidneys, and blood.
- May cause skin and eye damage, corrosion or irritation. If in eyes rinse cautiously with water for several minutes. If contact lenses present, remove. If eye irritation continues get medical attention. If there is skin contact rinse with water for several minutes.
- Nonflammable and noncombustible but sulfur trioxide can be produced at temperatures above 653C.
- Storage: Keep container closed in hood at all times.

Supervision:

- Supervised by mentor by Dr. Lisa Runco PhD, and mentor Mr. Gerard Wykes, Chair of the Chemistry Department at NSHAHS.

Safety Precautions:

- PPE (Safety goggles, lab coats, goggles, eyewash and safety shower)

Disposal Method:

- The Town of North Hempstead- Solid Waste Management Authority will come for disposal.

Nickel Sulfate;

Risk Assessment

- Biosafety Level 1
- Harmful if consumed or inhaled. Causes skin irritation. May cause an allergic skin reaction. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Suspected of causing genetic defects. May cause cancer. May damage fertility or the unborn child. Causes damage to organs through prolonged or repeated exposure. Very toxic to aquatic life.
- If inhaled: Exit the laboratory for fresh air and rest at a comfortable breathing position.
- Eyes: Rinse eyes immediately and seek medical help
- Skin Contact: Wash with soap and water, take off and wash contaminated clothing.
- Safety precautions: PPE, local ventilation.

- Storage: Keep container closed in hood at all times.

Supervision:

- Supervised by mentor by Dr. Lisa Runco PhD, and mentor Mr. Gerard Wykes, Chair of the Chemistry Department.

Safety Precautions:

- PPE (Safety goggles, lab coats, goggles, eyewash and safety shower) and local precautions.

Disposal:

- The Town of North Hempstead- Solid Waste Management Authority will come for disposal.

Chromium Sulfate;

Risk Assessment

- Biosafety Level 1
- Harmful if consumed or inhaled. Causes skin irritation.
- Toxic if consumed or inhaled, if done so call a poison center. Do not eat, drink or smoke while handling chemicals.
- If chemical gets into eyes rinse cautiously with water for several minutes.
- If chemical gets on skin clean thoroughly with soap and water.
- If inhaled: move to area with fresh air and find comfortable breathing positions.
- Storage: Keep container closed in hood at all times.

Supervision:

- Supervised by mentor by Dr. Lisa Runco PhD, and mentor Mr. Gerard Wykes, Chair of the Chemistry Department at NSHAHS.

Safety Precautions:

- PPE (Safety goggles, lab coats, goggles, eyewash and safety shower)

Disposal Method:

- The Town of North Hempstead- Solid Waste Management Authority will come for disposal.

Data Analysis

- Measure the length of plants (from roots to tallest leaves) in millimeters. Begin measuring before heavy metal introduction to plants and everyday going forward. By measuring the plants we would be able to see the growth process and when the leaves start dying from exposure to the heavy metals. We would be able to conclude which of the three metals will have the greatest effect on the Col-0, Lov-1, Ws-2 and Santa Clara strains.
- Pictures were taken daily of each pot to capture the progress and process of growth and exposure.
- Count the number of leaves in each pot.
- Measurements will be placed in Google Sheets, averages and standard deviation will be calculated.
- Use spectrophotometry (Thermo Spectronic® 20 Genesys® Spectrophotometer) to measure the amount of chlorophyll A, B and carotenoids in each plant to determine the health of the plants. This will measure the effect of heavy metals on the health of plants.
    - Each leaf will be measured before being crushed
    - Each leaf from every plant will be crushed and and 3 mL of 80% acetone will be added
    - Measure spectrophotometrically. For chlorophyll A set the wavelength to 680 nm, for chlorophyll B set the wavelength to 450 nm and for the carotenoids set the wavelength to 510 nm.

D) Bibliography

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NO ADDENDUMS EXIST