Better Living Through Stress: The Effect Of Environmental Stressors On Plant Metabolite Production

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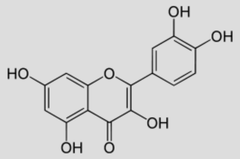
INTRODUCTION

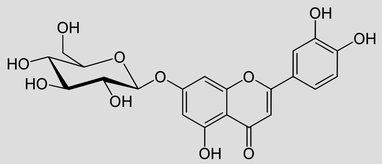
Metabolites are produced from metabolic reaction in response to stress. Plant metabolites are gaining interest, as they have been found to have health benefits in humans. We focused on flavonoids, which are anti-viral, anti-inflammatory, anti-bacterial, and potential anti-cancer properties when consumed. Our aim was to find suitable conditions to grow plants to produce the optimal amount of useful metabolites. We studied the trends in specific metabolite production when the parameters of pH and salinity of the water are varied in plants grown hydroponically. We measureed the quantity of flavonoids (quercetin and luteolin 7-glucoside/ cynaroside) produced in catnip, alfalfa, and green beans.

Quercetin



- Relieves allergies anti-inflammatory by preventing the production of pro-inflammatory mediators.

Luteolin 7-glucoside/ cynaroside



- Inhibits NFkB pathway (often active in cancer cells), which may prevent cancer.

Hydroponic set up in the United States with newly germinated alfalfa plants.

MATERIALS & METHODS

In this project, the following environmental parameters varied:

- **Salinity: 0, 50, 100, 200mM**
- **pH level: 5.5, 6, 6.5, 7, 7.5, 8 (created with mild acid/bases)**

Methodology

Collecting plant samples:

- Singapore: In order to grow of plants hydroponically, we 3D printed pots designed for hydroponics. The plants were grown hydroponically to ease the variation of pH and salinity. The seeds were planted on cotton wool for the duration of germination and plants growth.
- United States: The plants were grown hydroponically in nutrient-free soil, with an irrigation system supplying drips of water into the pots. The environmental parameters were varied through variation of the pH and salinity of the water fed to the plants.

Grinding of plant samples: The entire plant was then ground into powder or paste. The pounding of the samples increased the surface area for dissolving in our chosen solvent methanol for extraction of compounds. The samples were grinded when they were fresh to ensure that compounds in the sample were at their best states.
Dilution with 96% ethanol (10cm^3 per gram of plant): Add 96% ethanol to powdered leaf sample in the beaker (1g for 10ml of ethanol/ methanol) to dissolve the sample.
***Sonication*:** The beaker of the sample was placed sonicated for 60 minutes with water level filled up to the level of the sample in the beaker.
Filtration: The substance in the beaker was filtered and the filtrate solids were stored in a new clean dry falcon tube.
Evaporation of solute: After filtration, the remaining solution transferred into a bulb and evaporated in the following conditions of 100rpm, water bath: 50 degree Celsius, vacuum: 341 mbar. After evaporation, dichloromethane was added to the sample, and stored in a new clean dry falcon tube.
High Performance Liquid Chromatography (HPLC): A qualitative test was carried out to separate the metabolites of the plant through HPLC.
Nuclear Magnetic Resonance (NMR): A quantitative test was carried out on the selected metabolites (quercetin and luteolin 7-glucoside/cynaroside) using NMR.

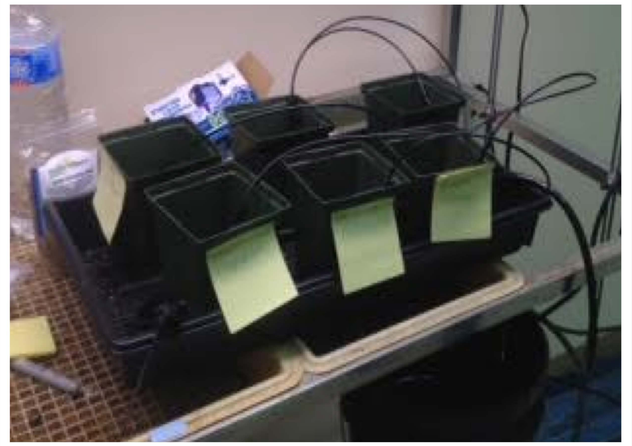
United States

Extraction: The sample's metabolites were extracted using the Bio Vision Plant Tissue Extraction Kit.

- 5µl of Protease Inhibitor Cocktail was added to 995µl of Plant Extraction Buffer and kept on ice until plant tissue was ready.
- 50 to 100 mg of fresh or frozen plant tissue was brought to room temperature, rinsed in DI H_2O, and ground into a paste with a mortar and pestle.
- This tissue was then placed in a 1.5 ml Homogenization Tube and mixed with 200 to 400 ml cold Plant Extraction Buffer and Proteate Inhibitor Cocktail.
- This mixture is then homogenized with the prestel provided in the kit by rotating the pestle 60 times.
- Then the tube was put on ice for 10 minutes.
- Next, the tube was centrifuged at 10,000 rmp per gram for 5 minutes.
- The supernatant was then collected.

NEXT STEPS

As this year comes to a close, the goal for the United States based research is to extract the metabolites from alfalfa grown under salinity stress once the Singapore side of the project has their High Performance Liquid Chromatography (HPLC) functioning and to then send those extracts to Singapore for analysis. Once analyzed, we hope to be able to disseminate our findings through a high school research journal.



Empty 3-D printed hydroponic pot without water or cotton wool for the Singapore lab set up.

Example of alfalfa sprouts after being rinsed before being ground into a paste for extraction of metabolites.

ACKNOWLEDGEMENTS / REFERENCES

Lopez-Lazaro, Miguel. January 2009. Distribution and Biological Activities of the Flavonoid Luteolin. Source: Mini Reviews in Medicinal Chemistry, Volume 9, Number 1, pp. 31-59(29) http://www.ingentaconnect.com/content/ben/mrmc/2009/00000009/00000001/art00004

T.P. Tim Cushnie, Andrew J. Lamb. February 2006. Antimicrobial activities of flavonoids. Source: International Journal of Antimicrobial Agents, Volume 27, Issue 2, Page 181 http://www.sciencedirect.com/science/article/pii/S0924857905002554

Anna Stochmal, Sonia Piacente, Cosimo Pizza ,Francesco De Riccardis, Rick Leitz, and Wieslaw Oleszek. February 2001. Alfalfa (Medicago sativa L.) Flavonoids. 1. Apigenin and Luteolin Glycosides from Aerial Parts Source: J. Agric. Food Chem. 49 (2), pp 753–758 http://pubs.acs.org/doi/abs/10.1021/jf000876p

Daniel Modnicki, Magdalena Tokar and Barbara Klimek. 2007. Flavonoids and phenolic acids of Nepeta cataria L. var. citriodora (Becker) Balb. (Lamiaceae) Source: Acta Poloniae Pharmaceutica - Drug Research, Vol. 64 No. 3 pp. 247-252 http://www.researchgate.net/publication/6144740_Flavonoids_and_phenolic_acids_of_Nepeta_cataria_L._var._citriodora_(Becker)_Balb._(Lamiaceae)

National Center for Biotechnology Information. PubChem Compound Database; CID=5280343, https://pubchem.ncbi.nlm.nih.gov/compound/5280343 (accessed Nov. 20, 2015)