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Metabolomic Profile of the TOU-A Population

This quarter was spent within the Bergelson Lab continuing the metabolomic assay of of the TOU-A population of *Arabidopsis thailiana* from France. The 195 genotypes from the TOU-A popualtion were intially grown in the Summer of 2017 at the University of Chicago under standard 16 hour / 8 Hour day lighting cycles with one instance of fertilization occuring during the second week of growth. After growing for a total of 4 weeks pictures were taken of every plant in order to quantify the leaf area and presences of algae was also recorded. The plant material was then collected, only the leaves and the crown, and were placed into coin envelopes and flash frozen and were kept at 80 C.

In late November of 2017 the samples were then vaccumned dried within their coin envelopes and stored within a container drying material to prevent moisture from leaking into the envelopes. In December/January of 2017/2018 each coin envelope containing plant material was transfered to a 2 mL micro-centrifuge tube and cataloged. Some of the samples ended up bolting earlier than expected and had that noted within the dataset in order to control for it alongside the presences of algae which was seen in a few of the samples. The ImageJ Code, using the Fiji Distrubution, was then used in order to quantify the level of leaf area, excluding algae, using a custom script written by me in both Java and C (All images of the plants, along with the thresholded images, are stored on my personal UNIX workstation).

The next step of the project was to weight out 15 mg of dried plant material for metabolite anaylsis on the in house HPLC LC/MS. Only 1142 samples were weighed and placed into matrix tubes due to either the lack of plant growth in the greenhouse, or the lack of material. A few preliminary anaylses were performed on the TOU-141 genotype (replicates 1 – 6) which showed interesting peaks indicating the presence of Benzyl, Phenethyl (GSL), and 2OH-Phenethyl present within the plants. Interestingly, 2OH-Phenethyl has not been recorded in the literature for what its purpose is (a paper by Reichelt et al 2002 describes there being a 2-Phenethyl, but no paper I can find describes one with a hydroxyl group), but even 2-Phenethyl has been found only at low levels in previous analysis (Hogge et al. 1988). Due to the nature of 2-Phenethyl being a glucasinolate seeing 2OH-Phenethyl may indicate that it is a precursor to 2-Phenethyl and may be involved in the pathway. Previous samples run by other graduate students in the lab also showed faint 2-Phenethyl and 2OH-Phenethyl peaks in their chromatograms. Since only a total of 14 samples (including 6 TOU-141 samples) have been analyzed at this point it is hard to say if these chemicals will be found within all/most of the samples.

The Benzyl peak is a cruious one which Tim, Andy, and I are still unsure why it is present in the samples. We have thought that it might be a breakdown product in the plant, but we still do not have enough data to say. This particular chemical warrants more exploration and it may be elucidate by additonal samples being run in the future.

Next Steps

Since I have analyzed most of the images and recorded bolting and algal presences along with the samples being weighed the next steps will be to run all the samples in the HPLC LC/MS in order to quatifify the metabolite profiles of each sample. This is still being worked out with Tim as we determine what kinds of standards and controls we will be using on the maching, but they should be prepared and ready to run by the middle of next quarter.

References

Reichelt, Michael, Paul D. Brown, Bernd Schneider, Neil J. Oldham, Einar Stauber, Jim Tokuhisa, Daniel J. Kliebenstein, Thomas Mitchell-Olds, and Jonathan Gershenzon. "Benzoic acid glucosinolate esters and other glucosinolates from Arabidopsis thaliana." Phytochemistry 59, no. 6 (2002): 663-671.

Hogge, L. R., D. W. Reed, E. W. Underhill, and G. W. Haughn. "HPLC separation of glucosinolates from leaves and seeds of Arabidopsis thaliana and their identification using thermospray liquid chramatography/mass spectrometry." Journal of Chromatographic Science 26, no. 11 (1988): 551-556.