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Nuclear magnetic resonance reveals significant metabolite difference between wild type and cultivated watermelon roots

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Abstract

There is significant genetic variation between cultivated watermelon and the wild watermelon. This study is focused on exploring watermelon diversity by analyzing the complete metabolic profile of the cultivated watermelon and comparing it to the wild watermelon. Nuclear magnetic resonance-based profiling was used for metabolite identification. The roots of several wild type watermelons and one cultivar were selected. Different statistical tools were used to highlight the significant metabolite changes between the wild type watermelons and the cultivars. The significantly changing metabolites have been identified using multivariate loading plots. They were found in higher quantities in the wild watermelon (Asparagine, Valine, L-Glutamine, O-phosphocholine, Isoleucine, Arginine, Glutamate, Ethanolamine and Choline). Results also revealed the presence of some metabolites unique to the wild watermelon.

Goal of this project: To study the metabolomics approach to identify and quantify the phytochemicals in watermelon roots by quantitative ^{1}H NMR.

Overall Goal: By identifying the metabolites related to watermelon roots, we hope to study the effects that these compounds have on nematodes.

Background

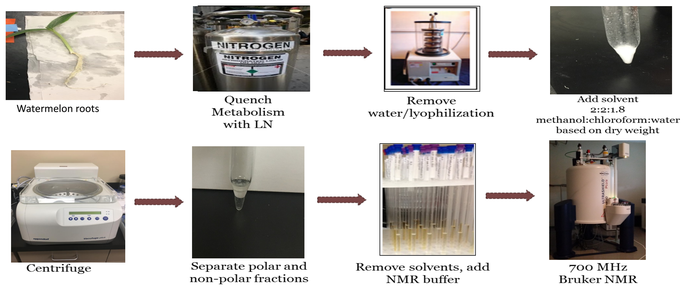
- Metabolomics is the study of changes in the metabolites contained within cells, tissues, and organisms
- Nuclear magnetic resonance has the ability to identify metabolites within watermelon roots
- Watermelon cultivar roots are susceptible to nematodes which will lead to a reduction of root volume as well as the roots efficiency to absorb water and nutrients.
- Wild watermelon, *Citrullus lanatus var. citroides (CLC)*, are tolerant to nematodes and have a very robust root system compared to the cultivars.

Sample Design

- Varieties of wild type watermelon roots
  - 1832
  - 1813
  - 1849
  - 2001
  - 1446
  - 1254
  - 1482

Figure 1. Charleston Gray

Materials and Methods



NMR Data Collection on 700 MHz Bruker:

- 1D ^{1}H spectrum for each sample = 1D metabolic profile

Metabolite Identification and Annotation:

Chenomx 700 MHz compound set
http://www.chenomx.com/

http://www.genome.jp/kegg/, http://www.bmrb.wisc.edu/, http://mmcd.nmrfam.wisc.edu/

http://www.metaboanalyst.ca/, http://www.bruker.com/, http://www.mathworks.com/products/matlab/

Results

Statistical	1446 vs CG	1482 vs CG	1813 vs CG	1832 vs CG	1849 vs CG	1254 vs CG	2001 vs CG
D_M	3.41	4.12	5.51	7.10	4.90	6.16	5.24
T_2	34.95	50.95	91.15	151.05	71.95	113.84	82.44
F-true	15.73	22.93	41.02	67.97	32.94	51.23	37.10
F-critical	4.96	4.96	4.96	4.96	4.96	4.96	4.96
Significance status	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 1. Mahalanobis distance (D_M) and F-test values. When $F_{true} > F_{critical}$ indicates a statistically significant separation between the groups.

Pair-wise analysis between wild type and Charleston Gray

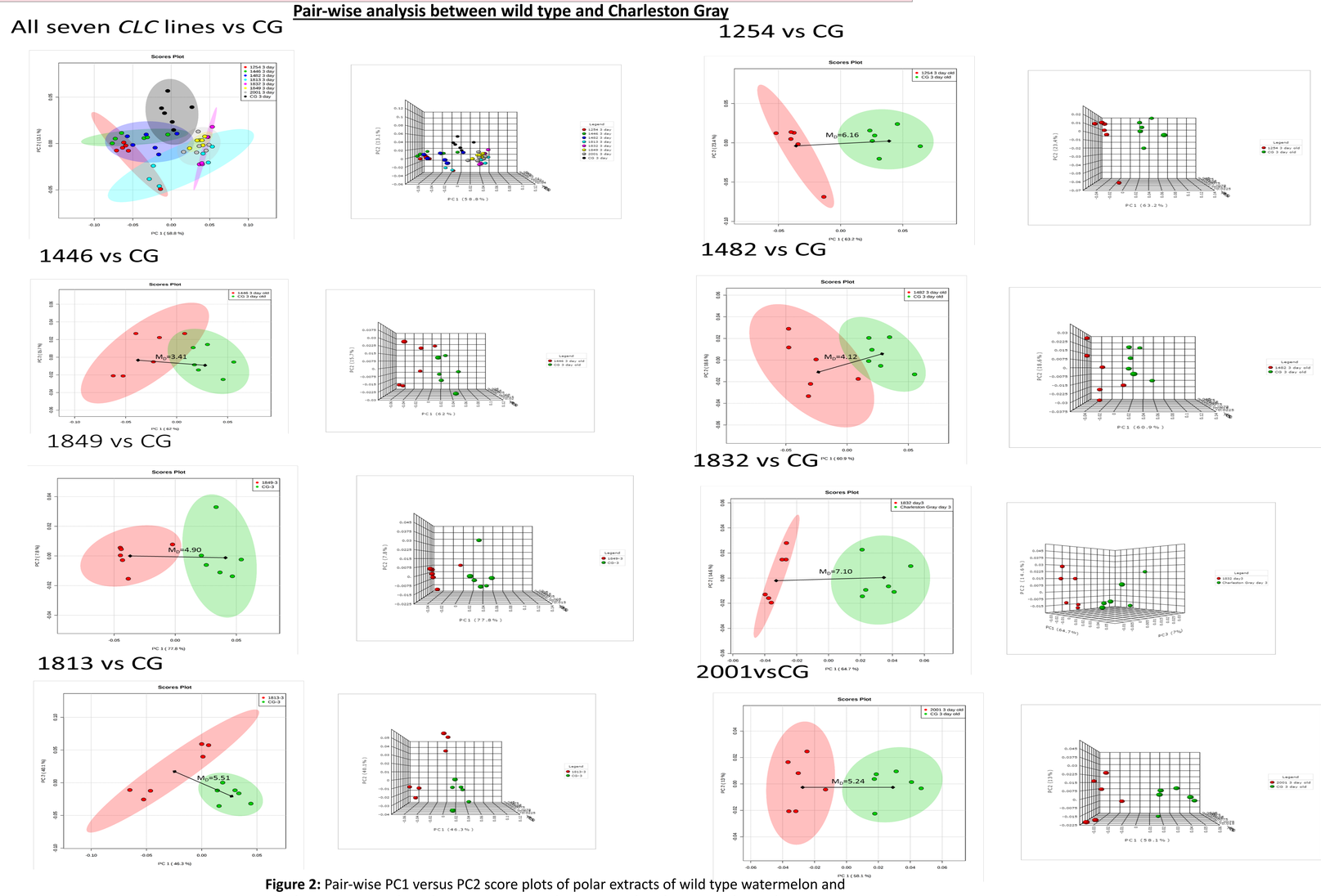


Figure 2: Pair-wise PC1 versus PC2 score plots of polar extracts of wild type watermelon and Charleston Gray. Each oval represents a sample group, and each point in the oval represents a single sample.The ovals in the square plots represent 95% confidence intervals.

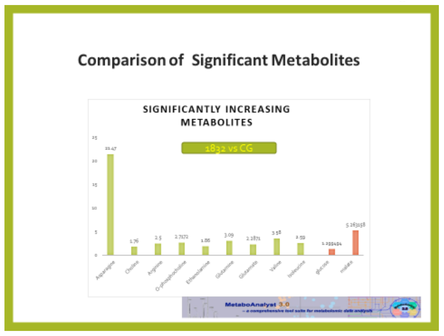


Figure 3: Comparison of significantly increasing metabolites in 1832 (green) and Charleston Gray (red).

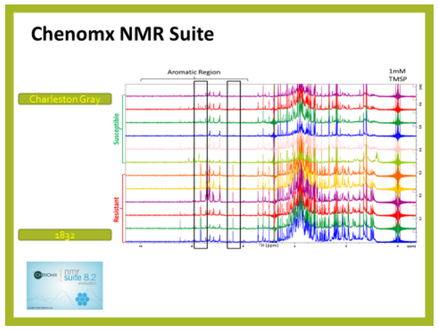
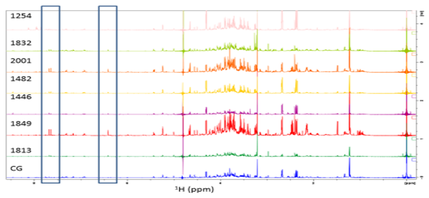


Figure 4: Metabolomic chromatogram comparison between six samples of Charleston Gray and other six samples of *Citrullus lanatus var. citroides* line 1832.

Discussion

- Hoque et al. (2014) showed that some amino acids, especially DL- phenylalanine, L-proline and L-histidine, promote plant growth and reduce galling incidence.
- Leonetti et al. (2010) suggest that nitric oxide (L-arginine is used as a substrate) and hydrogen peroxide play an important role in tomato plants' defense against the root-knot nematodes.
- A study performed by Sayed and Thomason, 1988 showed the effectiveness of ascorbic acid, thiamine, L-arginine and L-glutamic acid on egg hatch, development, and reproduction of *Meloidogyne incognita*
- The 1D proton spectrum comparison between *CLC* and *CLL* revealed the presence of unkno[illegible]gion of citroides



Conclusion

- This study was successful in identifying several metabolites present in significant higher concentrations in *CLC.*
- Asparagine and Valine metabolites were the most important ones because of their higher concentrations.
- Some "*unknown*" metabolites were present in *CLC* in significant higher concentrations
- Further studies would be performed to identify the "*unknown*" metabolites
- This study is the first (to our knowledge) to describe the methodology of metabolite extraction from watermelon roots. It uncovers the full roots' metabolomic profile of the cultivated watermelon (*CLL*) and of the wild watermelon (*CLC*), while also highlighting the metabolic differences between the two.

Future works

- Identify and quantify the unknown spectral peaks using LC-MS
- Establish the metabolic pathway of the unknown compounds
- Based on the obtained results, link the presence of the unknown compounds with nematodes resistance

Acknowledgments

- Claflin University Departments of Biology and Chemistry
- Research reported in this publication was partially supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R25GM113740. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Heath.

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