Preliminary Metabolomic Investigation of Saline-Stressed *Portulaca oleracea* using ^{1}H NMR

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Background

Portulaca oleracea, commonly known as Purslane, is a weedy medicinal plant which is also used fresh or cooked in many cultures. A wide range of medicinal properties have been attributed to Purslane, and many of its medicinal properties may be due to polysaccharides and/or polyunsaturated fatty acids, especially α-linolenic acid. We are interested in Purslane as a model for medicinal plants under stress such as drought, high temperatures and high salinity, the stressors expected as the climate warms.

Experimental Design

We grew two varieties of Purslane, a commercially available Tall Green variety (T-16) and a wild strain collected in Wisconsin (WI-9), under two conditions: tap water, and tap water containing 200 mM NaCl. Morphological parameters were measured as evidence of stress on fitness, and in other studies in our lab, we have measured antioxidants, betalain pigments and proline levels. All variables trended in the manner expected for salt-stressed plants. Here we focus on global change using a metabolomics approach. The diagram below summarizes the G x E (genotype by environment) experiments. The number above the slash is the total plants, the number below the slash the number used in the NMR study.

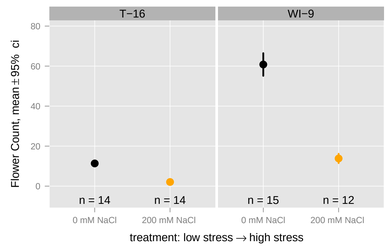
Extractions, Data Collection & Processing

Purslane leaves were ground in LN_2 and then extracted with 50% methanol in water. The extracts were filtered, concentrated to dryness, and dissolved in 600 μL 100 mM phosphate buffer at pH = 6.86 + 200 μL D_2O containing 1% TSP. After a final filtration, ^{1}H NMR spectra were collected using a Bruker Avance 500 MHz instrument equiped with a TBI probe. A 1D NOESY pulse sequence was used with water suppression. The spectra were aligned on TSP and regions containing the water peak and TSP were removed. Spectra were normalized to total area. All chemometric analyses used `ChemoSpec`, an `R` package.

Effect of Salinity on Fitness

The effect of salinity on reproductive fitness was demonstrated in several ways; here we show that flower production decreases notably under salinity stress.

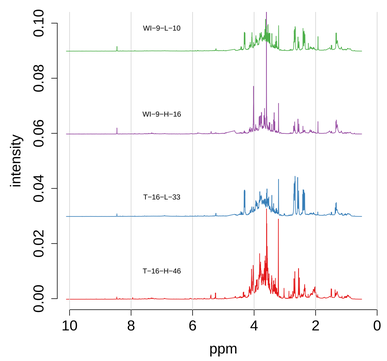
Fitness Under Salinity Stress



Sample Spectra

The figure below shows typical spectra from each G x E combination.

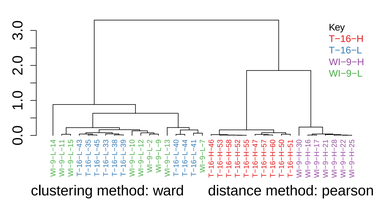
***P. oleracea* NMR Spectra**



Heirarchical Cluster Analysis

HCA on the raw spectra shows clustering of the no salt samples together, regardless of genotype. The high salt treatments also cluster together, but separation by genotype is evident.

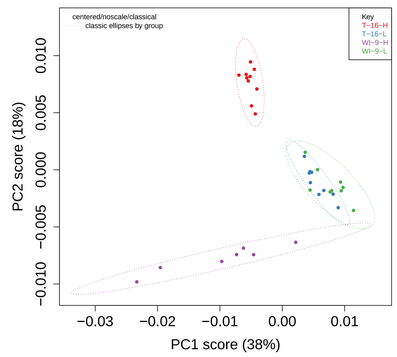
***P. oleracea* NMR Spectra : HCA Analysis**



Principal Components Analysis

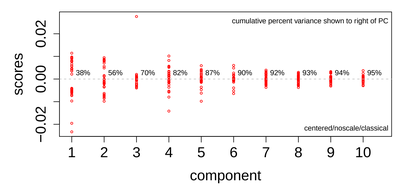
Principal components analysis on the unscaled spectra also shows clear separation into the groups indentifed by HCA. In particular, each genotype in the high salt treatment moves in a different direction along PC2.

***P. oleracea* NMR Spectra : PCA Score Plot**



As seen below, six principal components explain about 90% of the variation in the raw spectra. However, using just the first three PCs, MANOVA shows that all factors are significant.

***P. oleracea* NMR Spectra : Scores by PC**



MANOVA on PCs 1 to 3

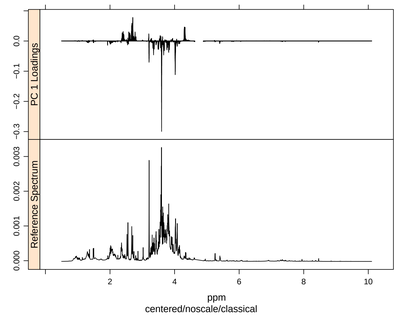
	Df	Pillai	approx F	num Df	den Df	Pr(>F)
(Intercept)	1	0.00	0.00	3	29	1.0000
genotype	1	0.90	87.13	3	29	0.0000
treatment	1	0.80	37.70	3	29	0.0000
genotype:treatment	1	0.90	86.89	3	29	0.0000
Residuals	31					

Identifying Peaks of Interest

Having demonstrated a significant separation of samples based upon the G x E condition, the next step is to investigate which compounds are up or down regulated and likely responsible for the decreased fitness observed under salt stress. In turn, this requires finding the peaks that drive the PCA separation. Examination of PC1 loadings (next figure) shows that much of the sample descrimination is driven by peaks in the δ 2.5 - 4.5 range.

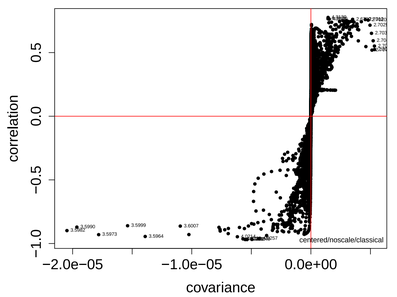
Peaks of Interest, con't

Portulaca oleracea NMR Spectra: Loadings Plot



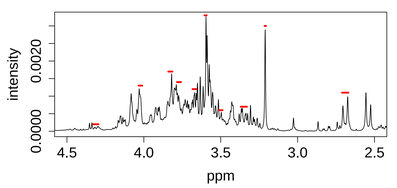
Another valuable approach to finding significant peaks is the S-plot, shown below. The peaks in the extreme corners, where a high correlation suggests a reliable marker, and high covariance means good signal-to-noise, are of greatest interest.

***P. oleracea* NMR Spectra : S – Plot**



We can combine these different approaches by selecting the peaks with the most extreme loading values, and then among those, the peaks which are found in the extremes of the S-plot. In particular, we chose loadings > $\pm$ 0.02 and the most extreme 20% of peaks in the S-plot. This narrows the results down to shift ranges for focused investigation (highlighted in the figure below).

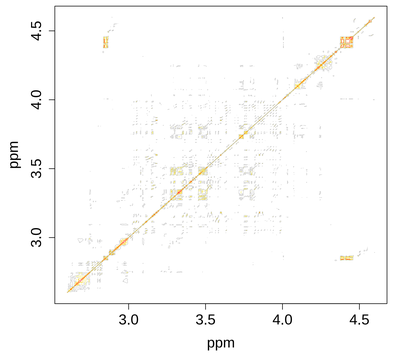
***P. oleracea* NMR Peaks of Interest**



Compounds & Pathways of Interest

In addition to identifying the peaks that appear to be the most important markers generally, we need to identify which compounds correspond to these peaks. Nicholson's STOCSY (Statistical Total Correlation Spectroscopy) is an important tool in this regard. The figure below shows the STOCSY plot for the busiest region of the spectrum. This plot is interpreted much as any 2D NMR plot is interpreted. Strong cross peaks are indicative of peaks from the same compound and can be followed to make a tentative identification. Somewhat less strong cross peaks are expected from different compounds which belong to the same metabolic pathway and are up or down-regulated in a coordinated manner.

***P. oleracea* NMR Spectra : STOCSY Plot**



Next Steps

We are currently studying the STOCSY, loadings and S-plot results to identify which specific compounds are involved in the response to saline stress in Purslane.

References

ChemoSpec: Exploratory Chemometrics for Spectroscopy, Bryan A. Hanson (2013) R package version 1.61-0

S. L. Robinette, J. C. Lindon & J. K. Nicholson "Statistical Spectroscopic Tools for Biomarker Discovery and Systems Medicine" *Analytical Chemistry* vol. 85, pg. 5297 (2013) and references therein.

Acknowledgements

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