Novel software solutions for liquid chromatography v8:poster v2.qxd 06/06/2013 08:26 Page 1

Novel Software Solutions for LC-High Resolution MS Metabolite Profiling of Legumes Under Drought Stress

The EU FP7 five-year project ABSTRESS (www.abstress.eu), currently in its first year, is set to revolutionise the way in which new plant varieties are produced. Currently the yield of legume crops is severely undermined by both drought and fungal infection (*Fusarium oxysporum*).

This work describes the optimised methods currently being explored to accurately obtain and interpret metabolite profiles of stressed vs. non stressed legume plants to obtain information on resistance metabolomic profiles and describes the trial metabolomics analysis for the project. Precise data processing of the large complex data set is critical for achieving accurate and reliable results.

METHODS

Legume plants *Medicago truncatula* were grown under drought and control conditions in a controlled environment (n=3 per condition). After 8 days of drought stress, leaf samples were harvested and immediately frozen in liquid nitrogen before lyophilisation.

Freeze dried ground samples were extracted using methanol:water (1:1) before a further 4 fold dilution. Full scan accurate mass data was acquired by LC-HRMS in both positive and negative ionisation mode. Each biological sample was extracted in triplicate. Quality control (QC) material derived from a pooled control sample was extracted concurrently and acquired with the samples. Samples were acquired in a random order and a QC injection was included every 6 injections.

Data was evaluated using Progenesis CoMet (Nonlinear Dynamics). An in-house plant metabolite mass library was used to assist in identification of significant masses found.

RESULTS

After chromatographic alignment, feature detection, normalisation and deconvolution of adducts, 925 features were identified as significant ($P \leq 0.01$, mean mass fold change ≥ 2) by Progenesis CoMet in positive mode and 242 identified in negative mode between stressed and non-stressed plants. To aid identification isotope similarity (≥ 85%) and accurate mass checks (± 5 ppm) were performed using the software.

46 compounds have been tentatively identified that are up or down-regulated in leaves of *Medicago* due to drought stress. These include sugars, flavonols, amino acids, fatty acids, phenolic acids and polyamines. Compounds were tentatively identified by the CoMet software through multivariate statistics and database searching using Progenesis MetaScope.

LC-HRMS conditions

MS: Thermo Exactive (ThermoFisher Scientific) set at 50,000 resolution FWHM.

LC: Accela U-HPLC system (ThermoFisher Scientific).

Mobile phase A : 0.1% HCOOH in water.

Mobile B : 0.1 % HCOOH in acetonitrile.

LC column / gradient: ACE 3Q, 150 x 3 mm. 100% MPA for 5 minutes before increasing to 100% MPB over 15 minutes. Held for 10 minutes at 100% B before reverting back to 100% A and held at 100% for 2 minutes.

Injection volume: 10µl.**Flow rate:** 0.4 ml/min, **Column temp:** 25°C.

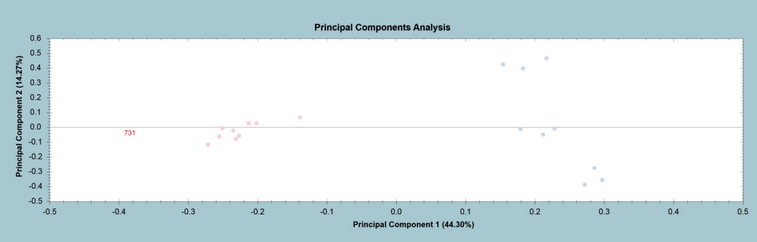

Figure 1. Principle Components Analysis from the experiment described. Drought (pink) vs control (blue) Medicago samples

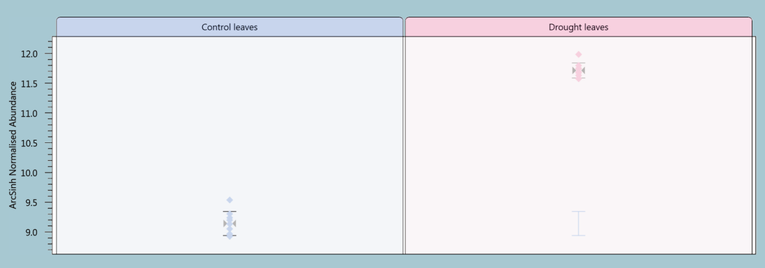

Figure 2. Abundance profile of proline, discovered as a significant metabolite between the 2 data sets (max fold change = 12.9, q = 3.3E-14, p = 5.6E-16)

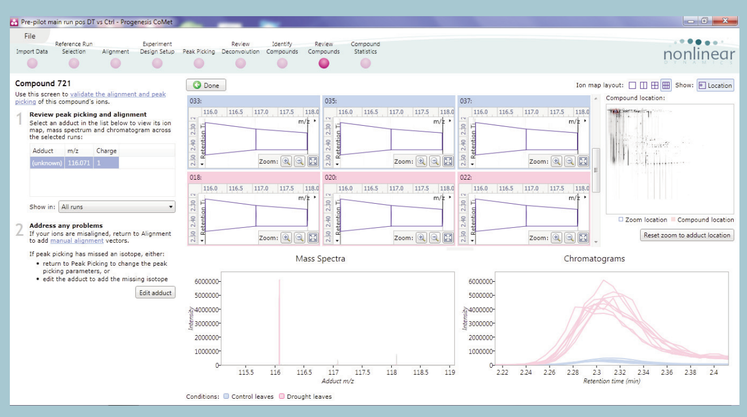

Figure 3. Validation of the identified significant compound proline. CoMet screenshot shows the ion maps, mass spectrum and chromatograms across all runs. Drought (pink) vs control (blue)

CONCLUSIONS

* Progenesis CoMet offers a user friendly, workflow based software solution for interpreting high resolution MS data. The software is precise, with a median %CV of < 10% over every feature in the pooled QC material (n=6).
* Over 1000 features from at least 16 chemical classes have been discovered to be significantly altered in *Medicago truncatula* under drought stress conditions using a LC-HRMS approach with Progenesis CoMet software for data manipulation. All compound identifications are tentative and will be confirmed using Nuclear Magnetic Resonance Spectroscopy as a complementary metabolomics technique.

The Food and Environment Research Agency

Data analysis performed using: Progenesis CoMet www.nonlinear.com

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