RESULTS

• Cultivation of Arabidopsis plants under cold stress conditions, leads to reduced growth as illustrated in figure 1
• The delay in growth is also visible one week after re-sprouting the cold shocked plants to control conditions (20°C)

Quantitative Proteomic Analysis

• Approximately 5,000 individual peptide components were automatically detected from each LC-MS data set.
• Relative fold changes were calculated by normalising the data and comparing the cold shock data to the control sample, and also the 1 week recovery to its appropriate control.
• The majority of peptides, and hence, proteins detected in the sample remained static under cold shock conditions, however, several clearly underwent expression changes.
• COR6, a cold response kinogen, was seen to be up regulated during cold shock (approx 3.5 times).
• After recovery at 20°C, expression levels of COR6 drop to normal levels, as seen in Figure 2.

METHODS

Plant Growth and Harvesting

• 5 week old Arabidopsis thaliana plants were split into two groups.
• Cold stressed plants were kept at 6°C for one week while the control remained at 20°C.
• Rosette leaves from half the plants in each group were harvested and directly frozen in liquid nitrogen.
• The remaining plants were kept at 20°C for a further week before their rosette leaves were also harvested and frozen.

Protein Digestion

• Protein extracts were diluted to a protein concentration of 1.0% Rapigest® (Waters, Milford MA) with 30 mM Tris-HCl pH 8.5.
• Proteins were solubilised in 8M Urea, 2% CHAPS and 0.5% mercaptoethanol in acetone [1].
• Precipitated material was washed in acetone then dried in a vacuum centrifuge.
• Proteins were solubilised in 8M Urea, 2% CHAPS and 30mM Tris-HCl pH 8.5.

LC-MC-MS

• Liquid chromatography was carried out using a nanoACQUITY UPLC™ system (Waters, Milford MA) with a 75µm x 100 mm Atlantis dC18 3.0µm analytical column.
• Gradient: 0-40% acetonitrile containing 0.1% formic acid over 120mins.
• The Q-ToF Premier (Waters, Manchester, UK) was set to acquire LC-MC-MS data into two functions, alternating between low and elevated collision energy, as we have described previously [2].
• Proteins were identified and relative quantification was carried out using Waters Protein Expression System informatics.

Example Data - identification and regulation of COR6

• The identification of COR6 protein from 4 peptides using PLS2.2 is shown below, along with a list of the elevated-energy MS spectra, annotated with the amino acid sequence from the database.
• These 4 peptides amounted to 71% coverage of this small protein.

Peptide level analysis

• Data was also quantitatively compared at the peptide level, using the exact mass retention time (EMRT) route as described by Silva et al [4].
• Peptides from RNA binding protein were observed to be around 2.7 times up regulated after cold shock compared to the control (Figure 5).
• This compares to a 2.3 fold up-regulation observed by 2D gel based analysis of the same sample.

SUMMARY

• In this work we have used LC-MS to study protein expression changes in Arabidopsis thaliana.
• Under conditions of cold shock the majority of proteins identified remained unchanged, as might be expected.
• Changes in cold shock proteins were observed, notably in COR6, which underwent a 3.5 times up regulation after cold shock. These responses diminished after 1 week of recovery at 20°C.
• More subtle changes were characterised using the EMRT route, and revealed regulation changes in good agreement with gel based results.
• Future studies will look at a comprehensive study of cold shock response in Arabidopsis, using a 1D gel based separation in combination with an LC-MS strategy.

REFERENCES

1. Dernov et al (1986), Electrophoresis, 5, 52-54

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