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A NEW TWO DIMENSIONAL LIQUID PHASE SEPARATION TECHNIQUE TO ANALYSE AND COMPARE PROTEIN PROFILES FROM DIFFERENT PLANT TISSUES

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The addressing of complex biological questions through comparative proteomics is becoming increasing attractive as the rapidly expanding plant genomic and expressed sequence tags databases provide improved opportunities for protein identification. The evaluation of the proteome, the protein status of a cell type, tissue, organ and whole organism, is an alternative strategy to obtain the link from gene to phenotype. In particular, comparative proteomic analysis aims to characterise differences between protein profiles.

Complex plant protein expression analysis has typically been done using 2-D polyacrylamide gel electrophoresis. The use of liquid protein mapping, instead of gel mapping, to scan many samples for important or landmark proteins has proven to be a less laborious and more efficient method for characterising complex samples followed by selective, intact-protein analysis by MS.

A 2-D liquid phase technique was recently developed and tested on different protein samples from human (Yan et al., Anal. Chem. 75: 2299-2308, 2003) and bacterial cell cultures (Zheng et al., Biotechniques 35:1202-1212, 2003). However, no data are available on plant samples.

In this work we compared protein profiles from different plant tissues (leaves and roots of *Arabidopsis thaliana*) by using a new protein fractionation system, PF-2D (Beckman Coulter). This methodology produces 2-D maps of intact proteins based on protein pI and hydrophobicity. High-performance chromatofocusing (HPCF) produces liquid pI fractions at the first dimension separation, followed by high-resolution reversed-phase chromatography (HPRP) of each of the pI fractions as the second dimension. A purposely-developed software is then used to convert complex chromatograms of a large number of fractions into easily visualised 2-D maps, plotting pI versus retention time. This methodology was tested to compare protein samples obtained by using two different extraction buffers ($MgSO_4$-based and Urea-based extraction buffers). Differences were observed between protein profiles obtained from the two extractions methods. In particular, samples obtained with $MgS0_4$ extraction procedure showed a chromatogram of better resolution for both basic and acidic proteins, and a larger amount of proteins was observed in the gradient fractions.

Reproducibility of the protein profiles was also tested by performing different injections of the same sample (leaf and root protein lysates). Preliminary data confirmed the reproducibility of the protein patterns and the intensity of the bands observed. Detailed analysis of the chromatograms revealed that with this 2-D liquid phase technique, hundreds of individual protein peaks can be readily identified from a whole-cell lysate.

$MgSO_4$-based extraction method was then applied to identify differences between protein expression of *Arabidopsis thaliana* plants grown *in vitro* in standard conditions and on a potassium depleted medium. Comparison of protein profiles will be discussed.