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MudPIT as a tool for the separation and quantification of proteins for GM crop safety assessments

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Background

Despite advances in gel-based approaches to proteomic analysis, the technology remains unsuitable for the rigorous safety assessment of GM plants. Limiting factors include the restrictive type of proteins that can be analysed (i.e. hydrophobic proteins are poorly detected), inadequate dynamic range and inaccurate quantitation. MudPIT is an attractive alternative. The procedure has been shown to detect low abundance and hydrophobic proteins and quantitative methodologies for MudPIT have been developed.

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Research Approach

The aim of the project was to assess the potential of MudPIT as a quantitative procedure to ascertain protein perturbations arising from genetic manipulation. The first stage of the project aimed to optimise front end procedures to maximise proteome coverage by MudPIT. The second stage of the project applied both so called‘non-chemical’ label-free methods, i.e., stable isotope labelled standards) and ‘chemical’ labelling methods (e.g. iTRAQ) to evaluate protein quantification of GM plant material. The unique collection of GM material and plants available at RHUL were used to fully evaluate and validate the procedure.

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Results

The MudPIT workflow was successfully developed for different mass spectrometry platforms: ESI Q-TOF, Orbitrap and Linear Ion traps and MALDI-TOF/TOF. A ‘non chemical’ label-free method was used to quantify iTRAQ labelled peptides using MS3. MudPIT was used with iTRAQ to gain quantitative information for 150 protein perturbations in non-GM and GM tomato cultivars developed and grown at RHUL using the Agilent 6520, QTOF. In the azygous cultivar, 7 proteins showed significant difference from the wild type; these were stress response proteins. In the GM

Psy1 sense cultivar, 60 proteins were found to be perturbed. 59 proteins were down-regulated and one protein was found to be significantly elevated in Psy1 sense: abscisic acid stress ripening inhibitor protein 1. The gene product phytoene synthase from the intended genetic alteration in Psy1 sense was notably absent from the iTRAQ quantitative protein profiles using the QTOF.

Research report

England, Northern Ireland and Wales

PDF
Gweld Protein-based investigation of use of mudpit as a tool for separation & quantification of proteins for GM crop safety assessment as PDF(Open in a new window) (6.23 MB)

England, Northern Ireland and Wales

PDF
Gweld Proteome changes in tomato lines research report as PDF(Open in a new window) (1.91 MB)