Can metabolomics identify unintended effects in transgenic potato tubers?
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Introduction
It is imperative that the public’s concerns regarding the safety and value of genetically modified foods be addressed, if these are to be commercially successful. One issue under debate is whether genetic modification is accompanied by unexpected metabolic perturbations. This study aims to assess the capability of GC/MS and LC/MS approaches at screening for such unintended effects and establishing “substantial equivalence”. The data described is from a replicated field trial designed to investigate the effects of transgenesis, empty vector insertion, and the tissue culture procedures within the common cultivar, Desiree.

Plant Materials

<table>
<thead>
<tr>
<th>Line Number</th>
<th>Type</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>Danne Wild Type</td>
<td>None</td>
</tr>
<tr>
<td>57</td>
<td>Desiree, LL403</td>
<td>Natural-grown controls</td>
</tr>
<tr>
<td>58</td>
<td>Desiree, LL403</td>
<td>Transgenic with empty plasmid vector</td>
</tr>
<tr>
<td>59</td>
<td>Desiree, LL403</td>
<td>Transgenic with empty plasmid vector with CAMV poly A tail</td>
</tr>
<tr>
<td>61</td>
<td>Desiree, LL403</td>
<td>Transgenic with empty plasmid vector with SAUR gene</td>
</tr>
<tr>
<td>62</td>
<td>Desiree, LL403</td>
<td>Transgenic with empty plasmid vector with pBA1 vector</td>
</tr>
</tbody>
</table>

Sample taken from cultivar Desiree grown in a separate plot in a replicated field trial (2010 is out of the range for each line, resulting in a total of 12 samples for each line).

Methods and data reduction

- Freeze-dried potato
- LCMS Analysis
  - Sequential extraction of plant material with methanol, distilled H2O and CHC13
  - Separation of polar and non-polar fractions
  - Polar fraction: Oximation
  - Non-polar fraction: Transesterification
- GCMS analysis
  - Derivatization
- GCMS TOF analysis

Data reduction has been carried out using Xenlib™ and employing a number of user-created processing methods. In addition, AMDIS has aided the visualisation of mass-co-eluting components. From the 750 components identified by our methods, we used 178 in this analysis.

Results

Visual display of results is produced by Principal Components Analysis (PCA) using Genstat and ExcelSTAT.
Score plots showed separation of treatments (Figure 1).
Different treatments had varying effects on individual compounds (Figure 2).
PCA analysis of the LC/MS data (including Pentland Javelin samples) showed a greater variation was present between the two land races than between the plants of different treatments (Figure 3).

Conclusions
- An untargeted (metabolomics) approach is capable of rapidly providing significantly large data on large sample numbers.
- Traditional multi-variate approaches are able to identify variation within large sample sets and begin to address “substantial equivalence” in transgenic materials.
- Data indicate numerous perturbations that result from stable transgenesis, yet also show comparable quantitative perturbations from insertion of empty vectors as well as in vitro culture.
- Preliminary data would indicate that the scale of variation observed by treatment (in vivo manipulation, empty vector or transgene insertion) is less than that present within cultivars and indeed, subspecies of Solanum tuberosum i.e. natural variation was greater than that following genetic modification.

Acknowledgements

The authors acknowledge funding for this research from the Food Standards Agency and the Scottish Executive Environmental Rural Affairs Department.