Metabolomic analysis of the potato tuber life cycle

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The potato tuber is a valuable model system for studying sink organ formation and storage organ metabolism. With the emergence of modern platform "omics" technologies (transcriptomics, proteomics and metabolomics) approaches are being developed to facilitate a much deeper understanding of the tuber life cycle, from tuber initiation through development and storage and into the sprouting phase. "Omics" approaches, linked to appropriate phenotyping, will allow a clearer definition of temporal changes in trait development and the identification of genes, proteins and metabolites driving these events. They will also facilitate a fuller understanding of the drivers of genotypic variation in commercially important phenotypes.

We have used a range of metabolite profiling approaches to understand better the complexity of events at the metabolite level during the potato tuber life cycle. Through collaboration with BioSS the complex data sets have been analysed using multivariate techniques such as principal component analysis (PCA) and, where applicable, for individual compounds analysis of variance (ANOVA).

Glasshouse grown plants of *Solanum tuberosum* cv. Desirée were harvested sequentially over about 5 months to obtain tubers at predefined developmental stages. Tuber sub-samples were freeze-dried and milled prior to analysis by gas chromatography–mass spectrometry (GC–MS).

Analysis of polar constituents using GC-MS was able to discriminate between most of the life cycle stages examined and in particular between the developing and mature tubers (Fig. 1). There were smaller differences between mature and sprouting tubers using these co-ordinates. However, separation of mature and sprouting tubers was possible using other principal components which accounted for a lower percentage of the variance. Cold stored tubers (5 °C) were easily separated from those stored at room temperature and this was primarily driven by differences in sugar content. The fact that stage 5a (excised developing tubers) could be separated from 5b (tubers maintained attached to the mother plant) was particularly interesting as was the fact that excised tubers clustered more closely to the naturally senesced tubers. This indicates that the excision of developing tubers from the mother plant stimulates the tuber maturation process and is in line with our previous studies which showed that excised tubers undergo a rapid sink to source transition with a reduced capacity to synthesise starch. As with transcriptional changes during the life cycle (Kloosterman *et al*., 2005) metabolites could be grouped depending on the pattern

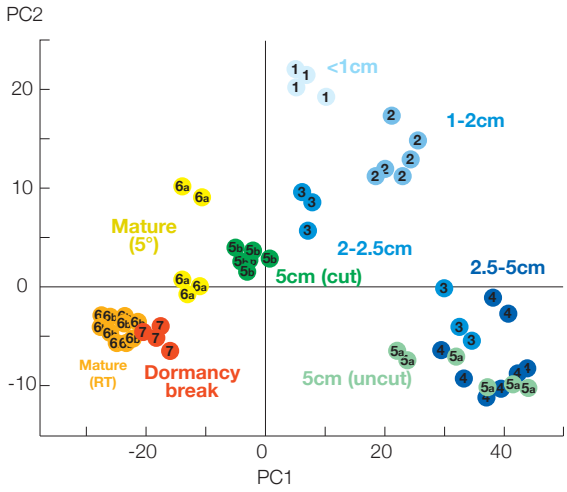

Figure 1 Principal component analysis of 90 polar metabolites identified by GC-MS for tuber life cycle stages 1-7. Samples are labelled according to developmental stage.

Life cycle stages for metabolomics

- 1 Small developing tubers (<1 cm diameter);
- 2 Small developing tubers (1-2 cm diameter);
- 3 Small developing tubers (2-2.5 cm diameter);
- 4 Small developing tubers (2.5–5 cm diameter);
- 5a Larger developing tubers (5-6 cm diameter; excised from plant and stored at 10°C for 3 days);
- 5b Larger developing tubers (5-6 cm diameter; not excised but sampled at the same time as 4a);
- 6a Mature tubers stored at 5°C for 4 weeks;
- 6b Mature tubers stored at room temperature for 4 weeks;
- 7 Mature tubers (dormancy broken at 5°C followed by 2 weeks at 10°C; sprouts ca 1 cm long).

of change observed and the specific stages in tuber development/maturation when significant changes became manifest. While it is clear is that metabolites change with development the fact that mature dormant and non-dormant tubers appear to be relatively uniform in their metabolite profile within a cultivar gives some confidence for experiments which use mature tubers to assess, for example, the extent of natural variation or the impact of specific transgenes on composition, i.e. there appears to be a window of relative stability in the metabolome.

Reference

Kloosterman, B., Vorst, O., Hall, R.D., Visser, R.G.F. & Bachem, C.W. 2005. Tuber on a chip: differential gene expression during potato tuber development. *Plant Biotechnology Journal* **3,** 505-519.

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