Molecular dissection of sensory traits in the potato tuber

Wayne Morris, Laurence Ducreux, Pete Hedley, Glenn Bryan, Heather Ross and Mark Taylor
SCRI, Invergowrie, Dundee, DD2 5DA, Scotland, UK
E-mail Wayne.Morris@scri.ac.uk Tel: +44 (0)1382 562731 Fax: +44 (0)1382 562426

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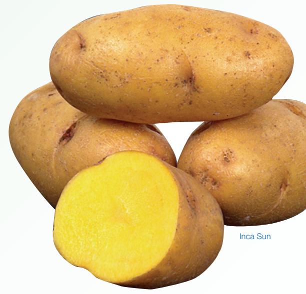


Inca Sun

Introduction

Sensory traits such as flavour and texture are becoming increasingly important factors in consumer preference trials. In fact, a recent survey reported that “after cost the most important driver for consumer food purchase is flavour” Connecting with Consumers, ICD, 2005. In order to make improvements in germplasm it is important to increase our understanding of the molecular basis of these traits.

Background

Our tuber flavour and texture studies were based on the comparison of *Solanum tuberosum* group Phureja tubers with *Solanum tuberosum* group Tuberosum tubers. Phureja tubers not only consistently score higher in professional sensory evaluation panels but they also tend to cook more quickly than Tuberosum tubers. This led us to make comparisons of volatile and matrix associated metabolites from boiled tubers from a range of Phureja and Tuberosum cultivars and investigate differences in tuber texture.

Aims of the project

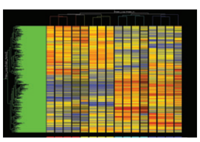
By comparing different potato germplasm, we wish to gain insights into the factors that contribute to tuber flavour and texture.

We wish to exploit this knowledge to understand the metabolic pathways responsible for these traits in order to pinpoint target genes.

Results

Microarray analysis

A recently developed 44,000-element potato microarray[1] was used to identify tuber gene expression profiles that correspond to differences in tuber flavour and texture. Gene expression was compared in two Phureja cultivars and two Tuberosum cultivars; 309 genes were significantly and consistently up-regulated in Phureja whereas 555 genes were down-regulated.



Statistically significant genes showing groupings of the 4 genotypes.

Volatile taste metabolites

A clear difference in the cooked tuber flavour volatile profile is the higher level of a sesquiterpene compound called alpha-copaene in Phureja compared with Tuberosum. A sesquiterpene synthase gene was identified as being more highly expressed in Phureja tubers and this result was confirmed by RT-PCR analysis.

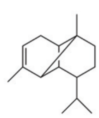


Figure 1. Chemical structure of alpha-copaene.

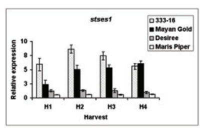


Figure 2. Sesquiterpene synthase (*stses1*) gene expression profile in Phureja (333-16, Mayan Gold) and Tuberosum (Desiree, Maris Piper) cultivars during tuberisation (harvest stages H1 to H4) as determined by semi-quantitative RT-PCR analysis. Values are the means of three replicates and error bars represent standard error of the mean.

The corresponding full length cDNA was isolated and over expressed in *E. coli* and the reaction products, when fed with farnesyl pyrophosphate precursor, were analysed by GC-MS. There was only one peak in the total ion GC-MS trace and the mass spectrum and retention time confirmed that this was α-copaene.

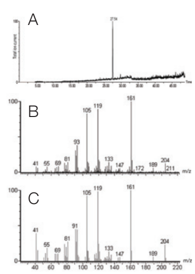
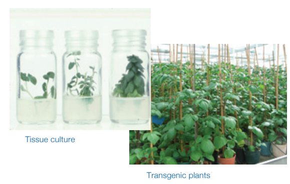


Figure 3. GC-MS analysis of the products produced by the putative sesquiterpene synthase. A- Total ion current trace. B- Mass spectra of peak at retention time 27.54 min. C- Adam's library spectrum for alpha-copaene.

Tuber-specific over-expression of the cloned sesquiterpene synthase cDNA in Tuberosum leads to the accumulation of alpha-copaene.



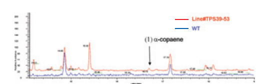


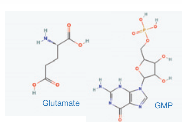
Figure 4. GC-MS analysis of extracts of wild type and sesquiterpene synthase transgenic potato lines.

Non-volatile taste compounds

The non-volatile matrix associated umami compounds enhance flavour and mouth feel. The major umami compounds present in potato tubers are the amino acids, glutamate and aspartate and the 5’ ribonucleotides, GMP and AMP.

These compounds were measured in tuber samples during the cooking process[2]. Tubers were sampled at several time points during the growing season. The levels of both glutamate, aspartate and 5’-ribonucleotides were significantly higher in mature tubers of two Phureja cultivars compared with two Tuberosum cultivars. The umami taste intensity increases exponentially when glutamate interacts with 5’-ribonucleotides. The synergistic effect between certain free amino acids and 5’-ribonucleotides can be measured using an equivalent umami calculation (EUC). EUC values are significantly higher in Phureja cultivars.

Correlation of sensory evaluation scores with equivalent umami concentration of potato shows a positive relationship. Sensory scores were carried out by a trained sensory evaluation panel.



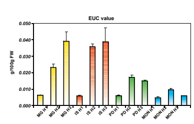


Figure 5. Effect of tuber developmental stage on equivalent umami concentrations (EUC) in cooked potato cultivars Mayan Gold (MG), Inca Sun (IS), Pentland Dell (PD) and Montrose (MON). H1, harvest 1; H2, harvest 2; H3, harvest 3. Error bars represent the SEM (n=3).

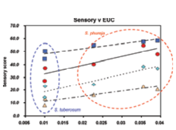


Figure 6. *S. tuberosum* cultivars Maris Piper (MP) and Record were compared with *S. phureja* clones DB333-16 and DB257-28, and cultivar Mayan Gold (MG). Square, flavour intensity; circle, acceptability; diamond, flavour sweet; triangle, flavour creamy.

Texture related gene expression analysis

Major differences in the expression levels of genes involved in cell wall biosynthesis (and potentially texture) were also identified by microarray analysis including genes encoding pectin methylesterase and pectin acetylesterase. Quantitative PCR assays were performed to confirm the microarray expression patterns.

Enzyme activity of pectin methylesterase was measured using an in-gel enzyme assay. PME activity was consistently higher in Tuberosum compared with Phureja.

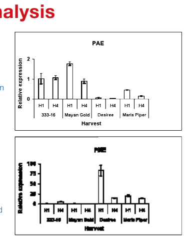


Figure 7. Expression profiles of pectin methylesterase (PME) and pectin acetylesterase (PAE) in Phureja (333-16, Mayan Gold) and Tuberosum (Desiree, Maris Piper) cultivars during tuberisation (harvest stages H1 to H4) as determined by quantitative RT-PCR. Values are the means of three replicates and error bars represent standard error of the mean.

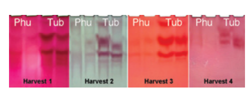


Figure 8. PME activity gels. Gels were loaded with equal protein and incubated with apple pectin prior to staining with Ruthenium red (binds unmethylated pectin). Darker bands indicate higher methyl esterase activity. Phu, Phureja; Tub, Tuberosum.

Work in progress

Analysis of gene function using transgenic plants.

Gene mapping and development of molecular markers for these traits.

Further mining of microarray data.

References

1) Morris W.L., Ross H.A., Ducreux L.J.M., Bradshaw J.E., Bryan G.B., and Taylor M.A. 2007. Umami compounds are a determinant of the flavor of potato (Solanum tuberosum L.). *Journal of Agricultural and Food Chemistry* 55, 9627-9633.

2) Kloosterman B., De Koeyer D., Griffiths R., Flinn B., Steuernagel B., Scholz U., Sonnewald S., Sonnewald U., Bryan G.J., Bánfalvi Z., Hammond J.P., Geigenberger P., Nielsen K.L., Visser R.G.F. & Bachem C.W.B. 2008. The potato transcriptome: a new look at transcriptional changes during tuber development using the POCI array. *Comparative and Functional Genomics*. DOI 10.1007/s10142-008-0083-x.

Discussion

Significant and consistent differences in both non-volatile and volatile components were detected and we hypothesise that these compounds underpin the preferred flavour of Phureja. We are currently aiming to understand the metabolic pathways by which these compounds are made in order to pinpoint target genes.

In addition to using transgenic models, it will also be of interest to use a genetic approach to identify key regulatory genes involved in potato tuber quality. Mapping populations (including a Phureja × Tuberosum cross) that may help in the identification of quantitative trait loci (QTL) associated with potato flavour and texture) have been generated (G. Bryan et al., unpublished data). Co-localisation of trait QTL (flavour and texture) with the map locations of candidate genes will help to identify the key genes that contribute to the trait.

Acknowledgements
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