

Plant metabolomics: Addressing the scientific concerns and uncertainties of genetic modification

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Background

The genetic modification of plants has been shown repeated to be successful at altering target (bio)chemical components. However, concerns over the specificity of these modifications, especially when undertaken in food crops, have fuelled the public GM debate. Subsequently the more general question has arisen of whether the existing, generally targeted, safety assessments applied to traditionally bred crops are sufficient or are new strategies required? Targeted analyses will, by definition, miss unexpected or unintended compositional changes and the application of 'catch all' analytical technologies such as LC-MSⁿ, GC-TOF-MSⁿ and NMR, *in toto* metabolomics technologies, have been proposed as the potential next wave of safety assessment approaches.

Potato, the fourth largest global food crop, has been the subject of many specific genetic modifications and served here as an excellent model to assess the relative efficiencies of metabolomic technologies in determining unintended effects, or deviations from substantial equivalence. Several transgenic lines and their tissue culture and vector-only controls were subject to metabolomics. In addition, and of equal importance, a broad range of potato varieties and landraces (lines which have not been subjected to controlled introgression of traits from a variety of wild species) were subject to metabolomics. The data from this allowed metabolite changes accompanying genetic modification, both intended and unintended, to be reported upon within a broader scale of germplasm biodiversity.

Approaches & Results

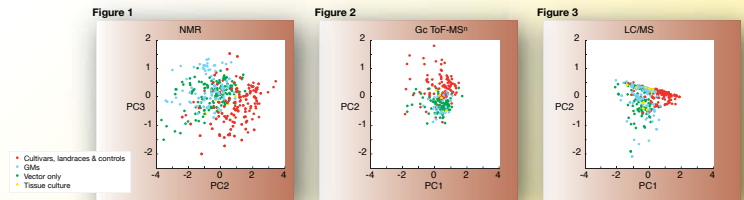
To get a better understanding of the incidence and characterisation of unintended effects a well characterised set of GMs (and their appropriate vector-only, tissue culture and wild type controls) were analysed alongside a wide range of non GM germplasm (varieties, landraces). Standard operating procedures were developed for field experimentation, harvest sampling and extraction and derivatisation¹. The extracts were analysed by LC-MSⁿ, GC-TOF-MS and NMR and that data subject to uni- and multivariate statistical analysis to identify specific differences and patterns in the data

GM lines Regulation	Gene	Comments
◊	35S-SAMDC	Extremely stunted phenotype
◊	Patatin-SAMDC	Tuber numbers increased
◊	GBSS prom.W2 gene	Waxy starch phenotype
◊	Mai 1 35S	Glycoprotein processing – stunted growth
◊	GBSS prom.SGT	50% reduction glycoalkaloids
◊	Fructokinase	Starch Sugar metabolism

Varieties and Landraces		
Cultivated "introgressed" tuberosum (Varieties)		Non-introgressed (landraces)
Desiree	Eden	Inca Sun
P.Dell	M.Piper	Mayan Gold
Record	P.Javelin	Lumpers
Sheelagh	Cara	Forty Fold
Stirling	P.Crown	Anyra
Torricon	Brodia	Brodick
Glenna	Barbara	PF Apple
Morag	G Wonder	91MT46E15(1)
		S. Phureja, diploid

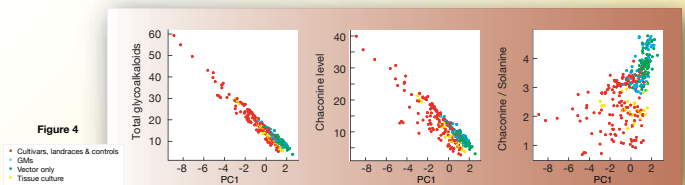
Unintended effects; Is it the GM or something more basic?

Broadly, much of the metabolite variation falls within the range covered by natural variation across the three 3 analytical approaches. For LC-MSⁿ there appears to be more of a division of the GM, vector only and tissue culture lines away from the range described by the varieties and landraces giving an indication of the chemistries being described here (Figs 1,2 &3)



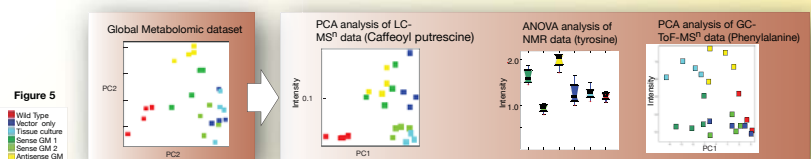
Plant Metabolomics: The discrete biological experimental level

Genetic modification and the apparently innocuous process of tissue culture both impact upon glycoalkaloid levels and the ratios of α -chaconine and α -solanine. Both processes decrease the total glycoalkaloid content whilst increasing the relative level of the reportedly more toxic α -chaconine. If this effect is repeated for other unrelated compounds it raises the question of how comparable plants are following repeated passage through tissue culture (Fig 4)



Plant Metabolomics: The discrete biological experimental level

Broad brush metabolomics can generate impenetrable datasets which often yield interesting data once refined to a defined experimental level. For example the sense and antisense modification of S-adenosylmethionine decarboxylase in potato produced both predictable and unintended effects with increases in methione, and tyrosine and caffeoyl putrescine respectively (Fig 5)



Conclusion

A combined metabolomic and statistical approach clearly functions as a high throughput method for establishing quantitative and qualitative chemical differences, in this case, with regard to unintended changes accompanying genetic modification.

Broadly speaking the majority of phytochemical variation accompanying GM was encompassed within natural biological variation

Acknowledgement

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Reference

Shepherd, T., Dobson, G., Marshall, R., Verrall, S.R., Conner, S., Griffiths, D.W., Stewart, D. & Davies, H.V. 2005. Profiling of metabolites and volatile flavour compounds from Solanum species using gas chromatography-mass spectrometry. Proceedings of the Third International Congress on Plant Metabolomics, Ames, Iowa, USA, June 2004