

Metabolite profiling of cuticular waxes from *Solanum* species

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Plant leaves are covered in a waxy layer which helps plants resist the effects of adverse environmental influences such as drought and disease (1). Such waxes consist of a complex mixture of mainly long-chain aliphatic compounds, including acids, alcohols, aldehydes, alkanes, ketones and esters in addition to compounds such as sterols and other terpenes.

Introduction and methods

We have been using metabolite profiling techniques to investigate biodiversity in *Solanum tuberosum* and the related species *S. phureja*, for example, by investigation of the metabolite content of potato tubers (2, 3). We have now extended our studies to include a survey of the cuticular wax chemistry of a wide range of cultivated potato varieties and landraces. Waxes and associated compounds are readily accessible in pure form, free of other inter and intra cellular contaminants, by dipping excised leaves in dichloromethane. Following silylation, individual wax components were analysed using a GC-(TOF)MS system.

Results and discussion

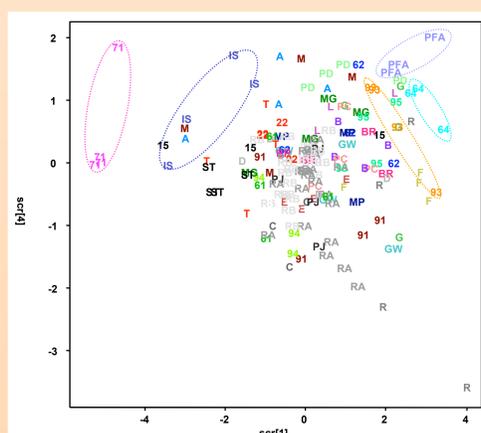
During biosynthesis of the major components in potato cuticular wax, acyl chains are first elongated in the synthesis *de novo* by fatty acid synthase FAS,

a process which occurs in plastids. Elongation involves stepwise addition of C_2 units, derived from malonate, to a short primer unit ($C_2 - C_6$). Depending on the specific primer used, even and odd carbon numbered chains are formed, which may be straight chain (*n*-) or have the *iso*- (*i*-) or *anteiso*- (*a*-) branched structures. A total of six structural variants are found in potato wax. Subsequent elongation of acyl chains to very long chain lengths is catalysed by an extra-plastidial enzyme complex, fatty acid elongase FAE. The acyl chains may then be released as fatty acids or may be subject to further chemical modification. Primary alcohols are formed by reduction of the acyl chain via an aldehyde intermediate. Alkanes, the major constituents of potato wax, are also formed by a process initially involving reduction of the acyl chain to an intermediary aldehyde. However, the aldehyde is then decarbonylated, losing CO, which accounts for the single carbon reduction in chain length of alkanes, in comparison with acids and primary alcohols.

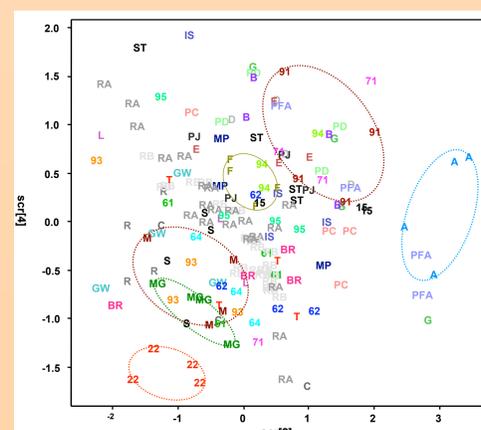
There are differences between some of the lines and cultivars in the distribution of the different structural classes found in alkanes present in potato cuticular waxes. Line 71 and cultivar IS both contain substantially lower proportions of both 2-Me and 3-Me isomers. These are both members of the species *S. phureja*. However, the third example of *S. phureja*, the cultivar MG, has a composition more closely related to the bulk of the examples of *S. tuberosum*. In contrast, the cultivar PFA and lines 93 and 64 have higher levels of the 2-Me odd carbon branched alkanes.

The chain length (CL) distributions of individual classes of wax component are known to be influenced by genetic and environmental factors. This is illustrated for the odd carbon *n*-alkanes ($C_{23}-C_{35}$), where individual cultivar and landraces show distinct separation on PCA. The most abundant homologues in most lines are C_{31} and C_{29} , however in lines 22, and cultivars MG and M, there is a shift in the CL distribution towards shorter chains, in comparison to line 91, with increased abundance of C_{27} and C_{25} . In line 22, $C_{27} > C_{29}$, and the distribution has become bimodal around C_{31} and C_{27} . The cultivar A has a narrower CL distribution around C_{29} and C_{31} than almost all other lines and cultivars.

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Distribution of alkane structural classes as % of total. Scr 1 v 4.



All cultivars. Odd alkane ($C_{23} - C_{35}$) chain length distribution. Scr 3 v 4.

Solanum tuberosum

Cultivars:

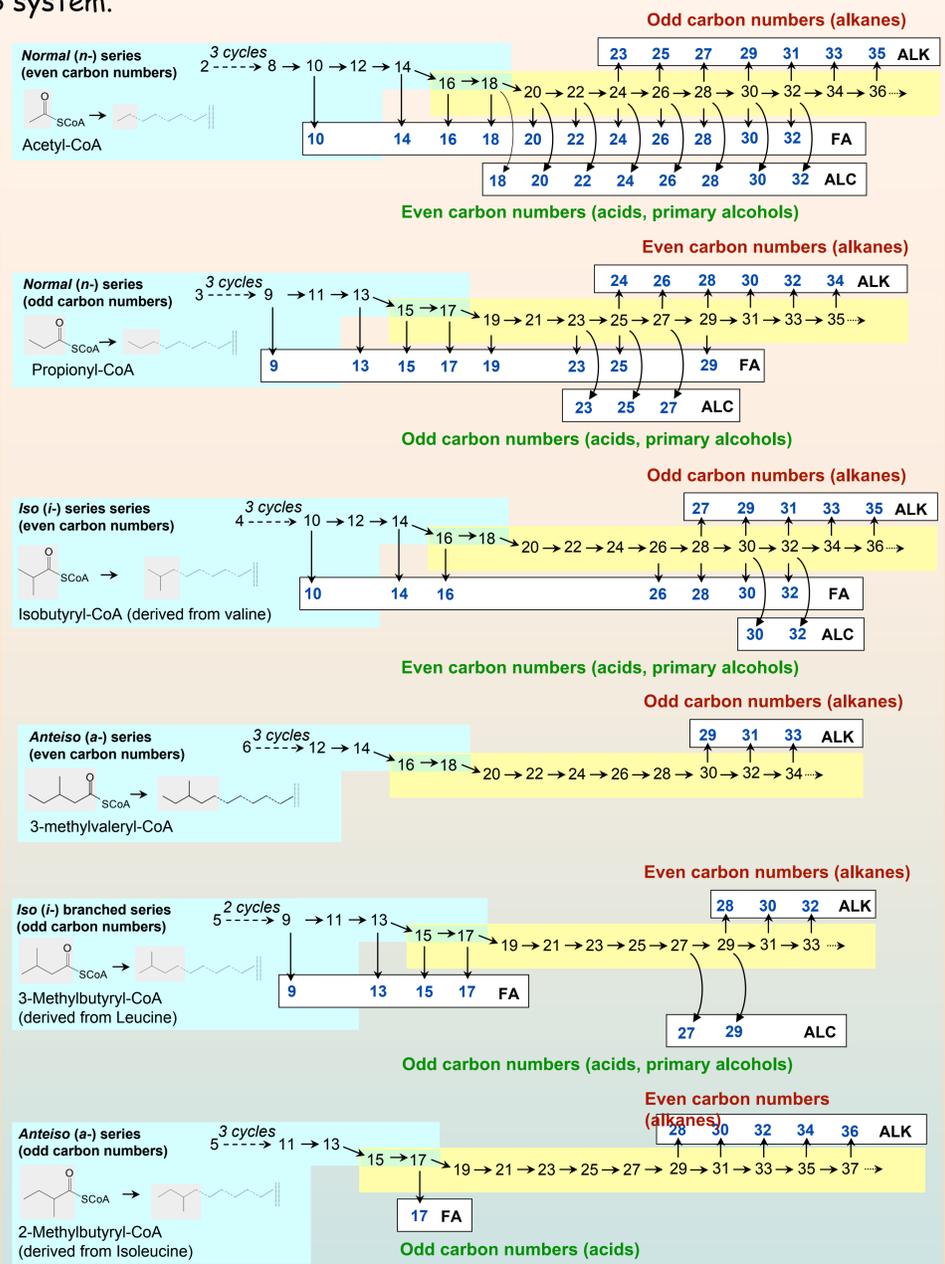
(A): Anya	(G): Glenna	(PJ): Pentland Javelin
(B): Barbara	(GW): Golden Wonder	(PFA): Pink Fir Apple
(BR): Brodick	(L): Lumpers	(R): Record,
(C): Cara	(MP): Maris Piper	(S): Shelagh,
(D): Desiree	(M): Morag	(ST): Stirling
(E): Eden	(PC): Pentland Crown	(T): Torrion
(F): Fortyfold	(PD): Pentland Dell,	(91): 91 MT 46 E15

Landraces:

(22): TBR 3302 (2)	(94): TBR 3369 (4)	(62): TBR 5646 (2)
(91): TBR 3369 (1)	(95): TBR 3369 (5)	(64): TBR 5646 (4)
(93): TBR 3369 (3)	(61): TBR 5646 (1)	

Solanum phureja

(IS): Inca Sun,	(MG): Mayan Gold	(71): PHU 4637 (1)
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Biosynthetic pathways showing formation of the major components in potato cuticular wax.

Products of fatty acid synthase FAS: Alkane, fatty acid and primary alcohol homologues found in potato cuticular wax
Products of fatty acid elongase FAE

Conclusions

Metabolite profiling techniques are proving to be powerful tools for investigation of the complex biochemistry underlying the formation of cuticular waxes in *Solanum* species.

References:

- Shepherd, T. & Griffiths, D. W. (2006). The effects of stress on plant cuticular waxes. *New Phytologist* 171: 469-499.
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- Shepherd, T., Dobson, G., Verrall, S. R., Connor, S., Griffiths, D. W., McNicol, J. W., Davies, H. V. & Stewart D. (2007). Potato metabolomics by GC-MS: what are the limiting factors? *Metabolomics*, in press.