ASMS 2004

A METABOLOMIC APPROACH TO THE IDENTIFICATION OF TRAITS IN TOMATO INTROGRESSION LINES USING TOF-MS

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OVERVIEW

A non-targeted LC-TOF-MS based approach has been utilised to profile metabolites in aqueous extracts from ripe fruits from tomato introgression lines (ILs). The data generated has been processed using the Waters MarkerLynx Application Manager to detect, deconvolute and align the data into a single matrix of mass and retention time with associated intensities for all components. Principal components analysis (PCA) was then carried out within MarkerLynx or after export to SIMCA-P multivariate software (Umetrics AB, Sweden). The significant ions in the PCA loadings plot were identified from their exact mass measurements.

INTRODUCTION

As an alternative to candidate gene approaches to crop improvement, novel traits may be introduced into domesticated crop plants via cross-breeding with wild relatives. As well as increasing the genetic diversity of the crop, the wild species alleles can also lead to unexpected and beneficial traits. We are studying a population of introgression lines of *L. esculentum* containing defined chromosomal introgressions from the wild tomato species *L. pennellii*[1] (see Figure 1). Several quantitative trait loci (QTL) for agronomic traits such as total soluble solids, fruit weight, yield and colour have previously been identified.[1,2]

We are using metabolite profiling as part of our investigations into the phenotypes resulting from the chromosomal introgressions, with a view to mapping differences in metabolites to the introgressed regions. Data are presented from the two parent species and selected ILs. Examples of the parent species and fruit from the introgression lines are shown in Figures 2 and 3.

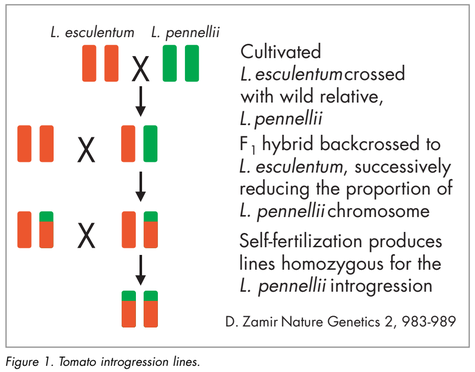

Figure 1. Tomato introgression lines.

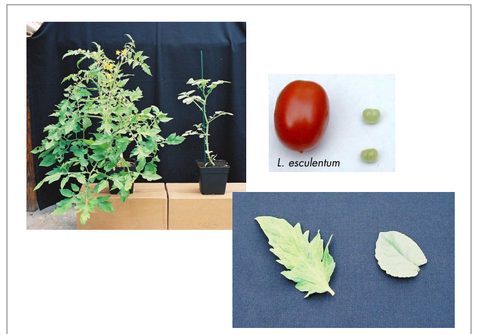

Figure 2. Parent Species.

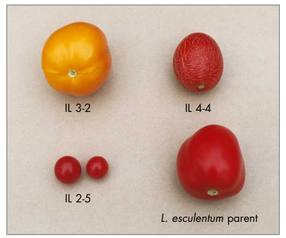

Figure 3. Introgression lines.

METHODS

Sample Preparation

Samples were taken from the pericarp of ripe fruit and extracted using the method shown in Figure 4. Only results from the aqueous extracts (polars) of the parent species and selected ILs are reported here.

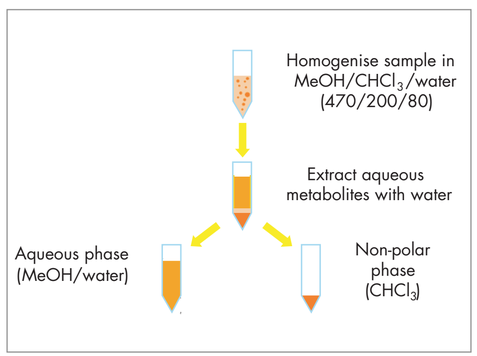

Figure 4. Schematic of extraction procedure.

Figure 5. LCT Premier with Waters 2795XC.

The samples were analysed on a Waters 2795XC coupled to a Waters Micromass LCT Premier (see Figure 5) using the following conditions:

HPLC Conditions:

HPLC:	Waters 2795XC
Column:	Waters Symmetry C18, 2.1x100mm, 3.5µm
Column temp:	40°C
Gradient:	0.1% aqueous formic acid to 95% MeCN
Injection volume:	10µL

MS Conditions:

MS:	Waters Micromass LCT Premier
Resolution:	>10,000 FWHM (W mode)
Ionisation mode:	Electrospray, Negative Ion
Sample Cone:	40V
Acquisition Rate:	0.4sec
Inter-scan Time:	0.1sec
Mode:	centroid with real-time lock mass correction

RESULTS

The resulting data were processed using the Waters MarkerLynx Application Manager.

The main features of MarkerLynx are:

- peak detection and deconvolution.
- alignment of components across all samples within user defined mass and retention time windows.
- PCA analysis of data within MarkerLynx.
- an interactive browser.
- facile export of results table.

The MarkerLynx browser is shown in Figure 6 and includes:

- a list of the processed samples.
- a marker table of all the detected components (m/z and retention time pair) with associated normalised intensities across all the samples .
- a TIC trace for the currently highlighted sample.
- a trend plot which gives a graphical representation of the intensity of the selected component (m/z and retention time) across all samples. The trend plot shown is for m/z 115.0030 (fumaric acid) at 0.7min, which is raised in the *L. pennellii* samples.
- PCA scores and loadings plots designed to extract and display the variation in the data generated in the marker table.

The marker table was exported directly to SIMCA-P multivariate software (Umetrics AB, Sweden) for further analysis using the template within MarkerLynx. The 3-D PCA plot of the first three PCs is shown in Figure 7. This shows separation of the parent *L. pennellii* and *L. esculentum* from the ILs.

The primary metabolites responsible for the separation observed in the scores plot are tabulated in Figure 8. The organic acids and hexose sugars are potential markers of tomato fruit quality.

CONCLUSIONS

- The extended dynamic range of the LCT Premier allowed exact mass measurements to be generated with good accuracy for both trace level and high concentration components.
- The MarkerLynx Application Manager enabled the data to be reduced to a matrix of mass and retention time with associated intensities.
- PCA analysis indicated the ions responsible for the separation of the parent species from the ILs.
- Changes were observed in the levels of hexose sugars and organic acids, both of which are important determinants of tomato fruit quality.
- The use of an IL population will allow us to map changes in metabolites to areas of the genome responsible for the differences.

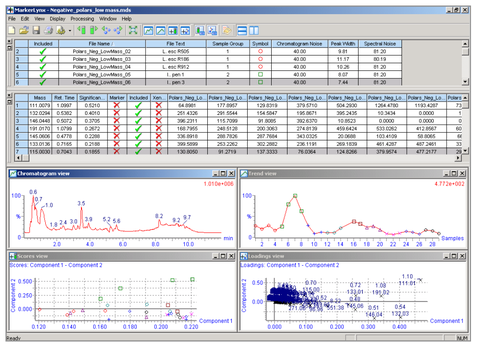

Figure 6. Interactive MarkerLynx report browser of parents and selected ILs.

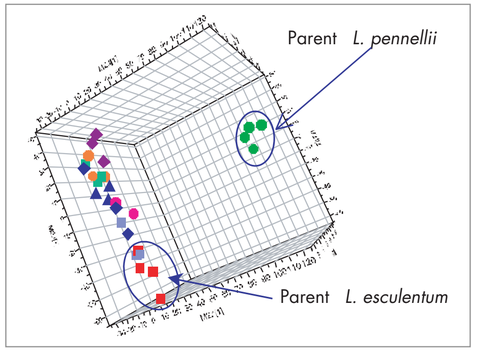

Figure 7. SIMCA-P 3D PCA scores plot of parents and selected ILs.

Measured Mass (m/z)	Error (mDa)	Elemental Composition	Postulated Identity
102.0554	-0.1	$C_4H_8NO_2$	amino-butyric acid
111.0079	-0.3	$C_5H_3O_3$	furoic acid
115.0030	-0.1	$C_4H_3O_4$	fumaric acid
128.0348	0.0	$C_5H_6NO_3$	pyroglutamic acid
131.0452	-0.5	$C_4H_7N_2O_3$	asparagine
132.0294	-0.3	$C_4H_6NO_4$	aspartic acid
133.0136	-0.1	$C_4H_5O_5$	malic acid
145.0606	-0.7	$C_5H_9N_2O_3$	glutamine
146.0448	-0.5	$C_5H_8NO_4$	glutamic acid
179.0549	-0.7	$C_6H_{11}O_6$	hexose

Figure 8. Table showing the principle ions identified as being responsible for the separation in the scores plot.

FURTHER WORK

- Analysis of all the data generated from both the aqueous and non-polar extracts is ongoing.
- HPLC methodology for improved separation of the polars and isomeric species is being developed.

REFERENCES

1. Eshed & Zamir (1995) Genetics 141, 1147-1162
2. Liu et al. (2003) Plant Biotechnology Journal 1, 195-207

May 2004 720000885EN LL-PDF