Assessing Diversity in Cassava through the Application of Metabolomics

Margit Drapal[a], Elisabete Carvalho[a], Laura Perez-Fons[a] Elliott Price[a], L. Augusto Becerra Lopez-Lavalle[b], Paul D. Fraser[a]

[a]School of Biological Sciences, Royal Holloway University of London, Egham, UK
[b]International Center for Tropical Agriculture, CIAT

Introduction

Cassava *(Manihot esculenta*) Crantz (Fig 1.) is one of the most important root crops worldwide, providing a staple food source for over 700 million people in Africa, Asia and South America (www.faostat.fao.org). Despite its widespread use better varieties with improved tolerance to biotic and abiotic stresses, enhanced nutritional properties, improved post-harvest properties and high yields/productivity are required urgently to address food security needs. Unlike other crops (e.g. tomato) the tools and resources necessary for the rapid and rational development of new varieties are not available in Cassava.

Fig. 1: Cassava plant

https://rauman.wikispaces.com/cassava

Recently, the sequencing of Cassava genomes has provided the impetus for genomic/marker based breeding.

Metabolomics plays a major role in augmenting modern breeding programs. This omic technique has the ability to determine small molecules (metabolites) which represent the end-point in cellular metabolism/regulation. In the present study metabolomic platforms have been established for Cassava and used to assess the biodiversity present in Cassava germplasm collections and elucidate underlying biochemical mechanisms associated with traits of interest.

Experimental

Plant Cultivation. A diversity panel was selected based on traits and geographical origin for analysis. Table 1 shows the diversity panel generated with coloured boxes representing lines also cultivated in the field.

Material preparation. *In vitro* plantlets were generated from the CIAT germplasm collection. A selection of lines were also cultivated in the field.

Sender Code	Characteristic trait	Sender Code	Characteristic trait
BRA1A	High carotene	ECU72	WF Resistance/Bacteriosis Susceptible
BRA488	High Cianide Content	GUA35	Low Sugar Content
COL113		PAN139	Trips Resistance
COL1505	Z01 nd Z04 adaptation	PAR36	High Amylose Content
COL1684	Z03 adaptation	PER 496	?
COL2017	High Sugar Content	PER183	High PPD Tolerance/Frog Skin disease/Low Carotene
COL22	PPD susceptible	TME3	CMD
COL2436	High Carotene/Trips Susceptible	TMS30555	CMD
COL638	Bacteriosis resistance	TMS60444	Transformation
CUB23	Waxy Potential	VEN25	Low Culinary Quality
CUB25	Low Amylose Content	VEN77	Drought tolerance
CUB74	High Culinary Quality/Z06 adaptation		

Extraction. Fresh material was freeze-dried and ground then an aliquot extracted with Methanol/water (1:1) and chloroform to create a polar and non-polar phase.

Analytical platforms. LC-MS analysis (ESI negative mode, gradient with acidified water and acetonitrile). GC-MS metabolite profiling was performed following sample derivatisation. Targeted LC-PDA/MS was carried out on specific classes of metabolites such as carotenoids.

Results

1. Workflow development. The workflow implemented for the analysis of the Cassava metabolome is provided in Figure 2. Standardised *in vitro* cultivation of diverse accessions was performed and metabolite profiles obtained from just 10 mg/DW. Solvent extraction was performed to obtain a polar and non-polar fraction. LC-MS was used in an untargeted mode to obtain chemical fingerprints of the tissues (accessions). Over 9000 molecular features were obtained. PCA of these data enabled the separation of the accessions and differentiating ions to be detected.

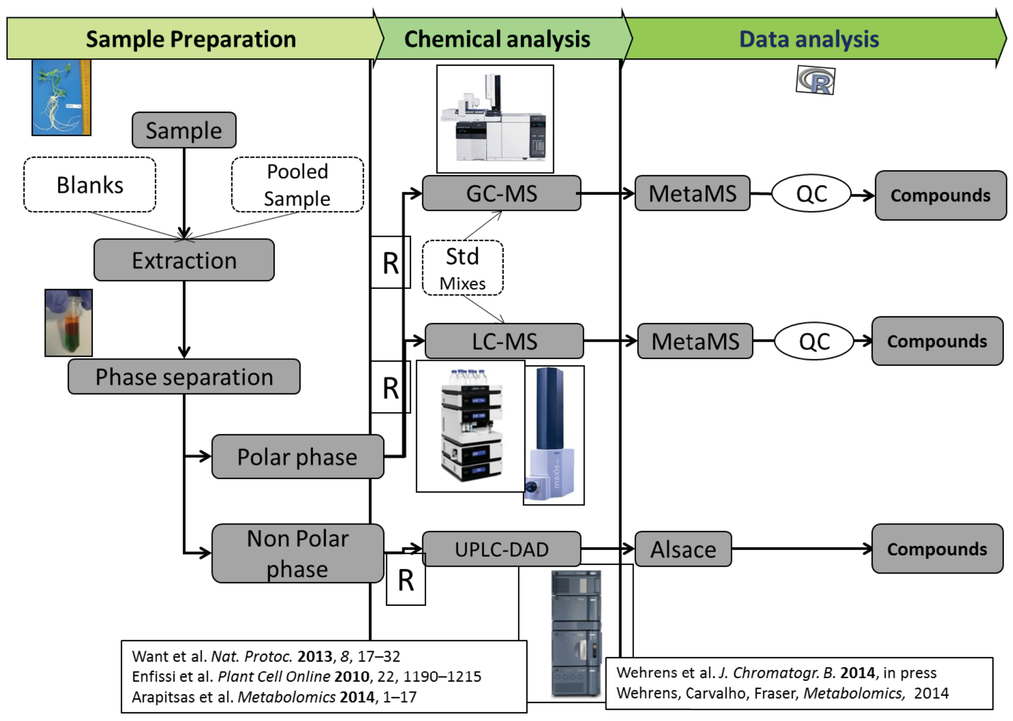


Figure 2. Displaying the workflow implemented for the analysis of the Cassava metabolome.

GC-MS metabolite profiling of the polar and non-polar fractions following derivatisation enabled the construction of customised searchable libraries in a n AMDIS format. The system was able to identify over 100 metabolites in an unambiguous manner. The classes of compounds detected with good sensitivity included amino acids, sugars, alcohols, phosphates, fatty acids, organic acids, terpenoids and phenolics.

2. Analysis of the diversity panel. The diversity panel displayed in Table 1 were analysed using UPLC, LC-MS and GC-MS. The data was combined in one matrix and subjected to Principal Component Analysis as shown in Figure 3. The accessions separated in a reproducible manner . In addition to traits the geographical origin appears to play an important role in determining chemical composition.

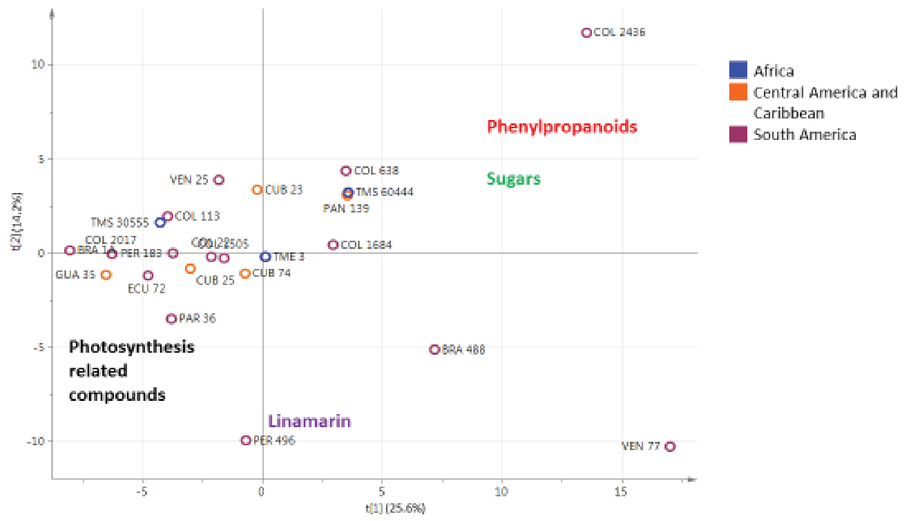
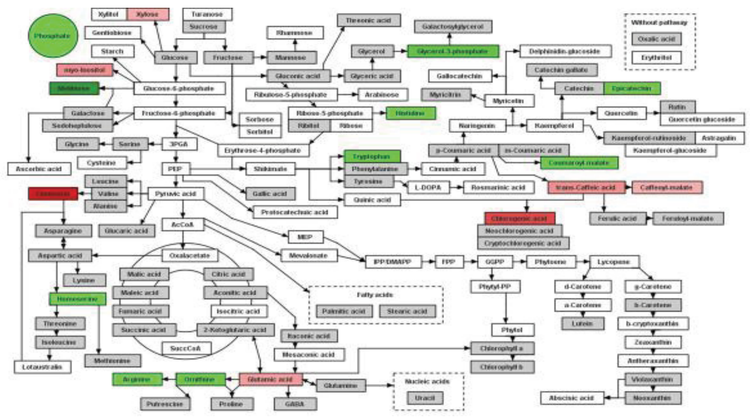


Figure 3. PCA analysis of Cassava diversity panel using unambiguously identified metabolites across LC-MS, GC-MS and UPLC-PDA platforms

3. Data visualisation over a cassava biochemical network. Using data for known metabolites a biochemical network was constructed (Figure 4). Metabolite changes between different accessions can be painted over the network.



A comparison between an African and South American Accession.

Increased

decrease

Figure 4. Biochemical network constructed from the metabolomics data.

Conclusion

- Robust metabolomics platforms have been established for Cassava. These include “untargeted and targeted”.
- The procedures have been validated using a diversity panel.
- PCA of the panel displayed separation not only on the basis of traits but also geographical origin.
- Comparison between the African, central American and South America accessions suggested the most influential progenitor of the separation were altered secondary metabolites levels.

Acknowledgements

The project is funded through the RTB-CGIAR Consortium. The *in vitro* and field plant material was kindly provided Dr L. Augusto Becerra Lopez-Lavalle team members at CIAT.