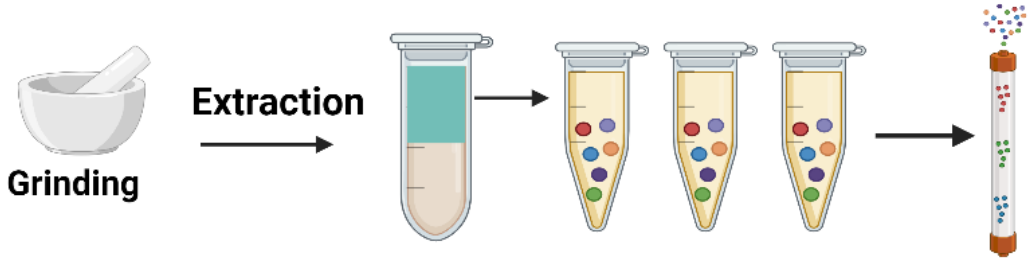
PRO-GRACE-workshop

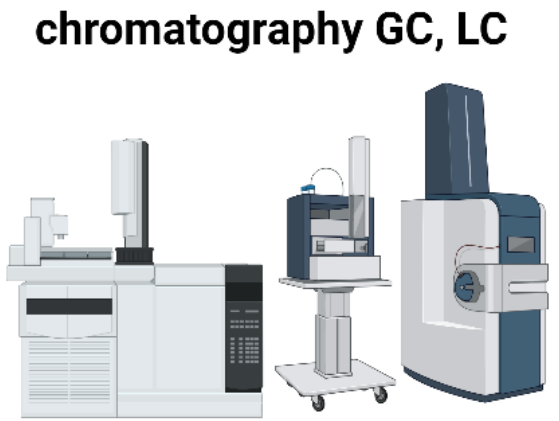
MPG

WORLDVEG

Introduction to metabolomics

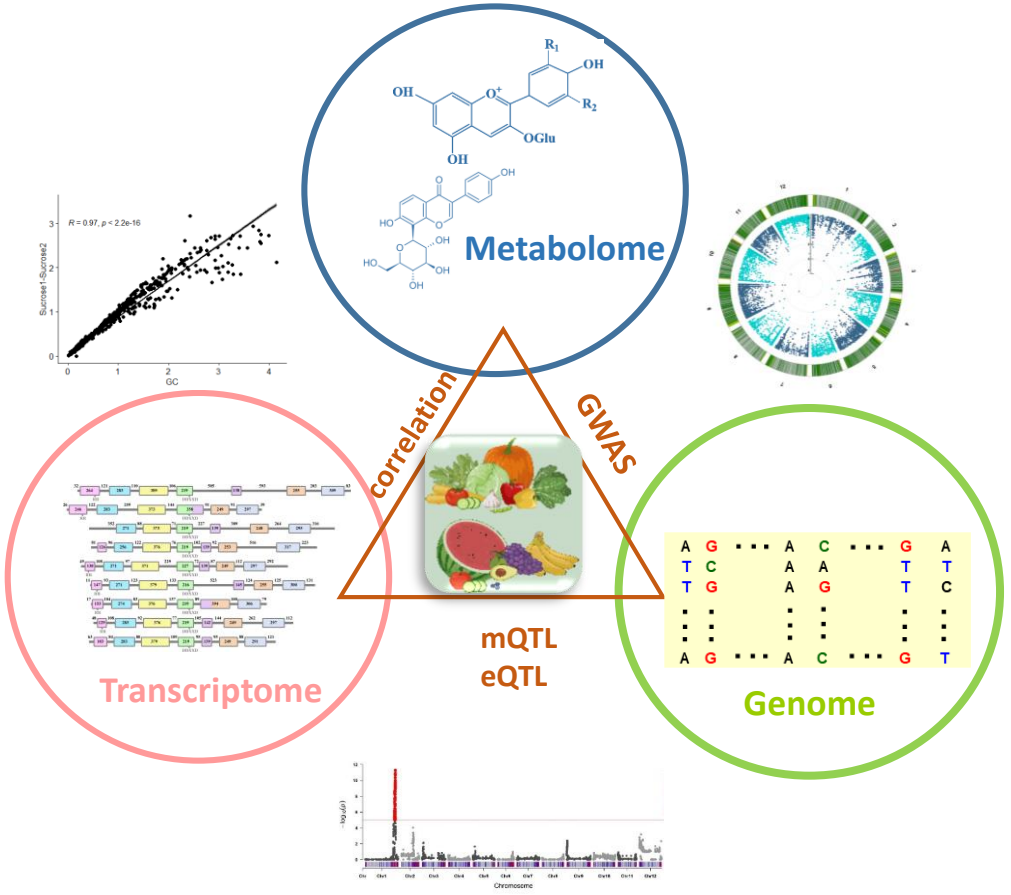
- Sample preparation
- Data acquisiton
- Data processing
- Metabolomics pipeline

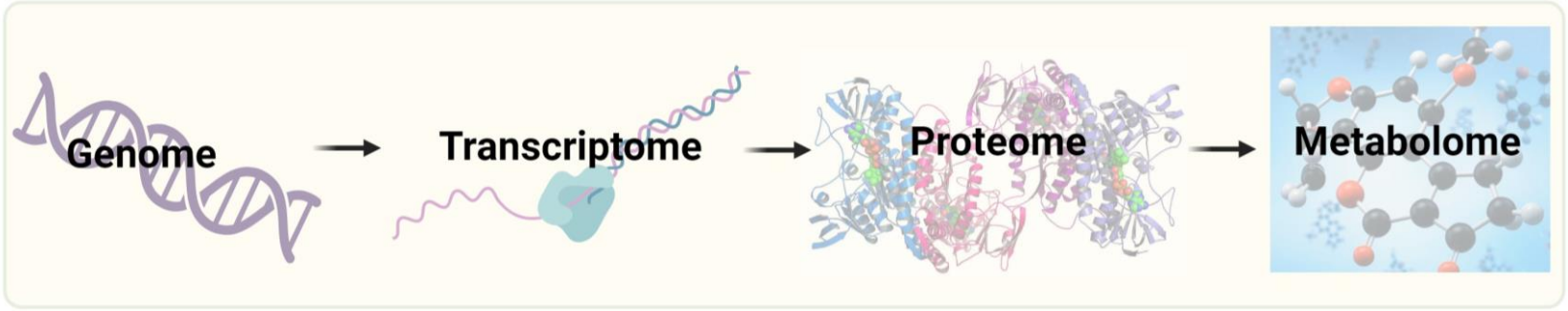




What is Metabolomics?

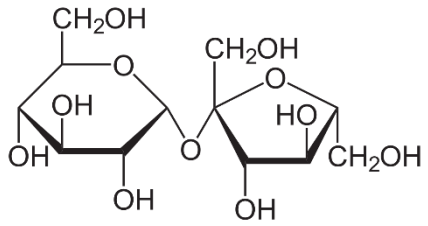
- **Metabolomics** is an approach of study that involves the comprehensive analysis of small molecules, known as metabolites, present in biological samples.
- The world of plant metabolites displays remarkable diversity, with over one million distinct chemical structures (Wurtzel and Kutchan 2016).

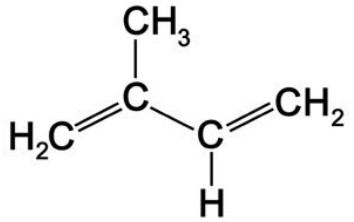


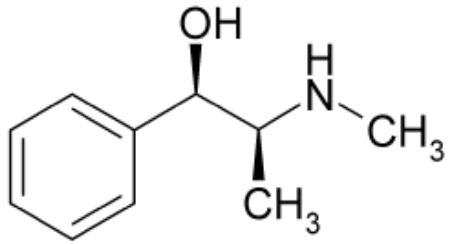


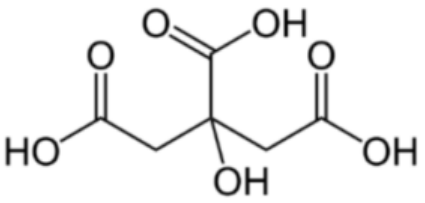
Plant Metabolism

- The world of plant metabolites displays remarkable diversity, with over one million distinct chemical structures (Wurtzel and Kutchan 2016).
- Currently, there has been a growing utilization of analytical technologies like metabolomics for the characterization of metabolites within biological specimens.
  - Sugars
  - Nucleotides
  - Organic acids
  - Ketones
  - Aldehydes
  - Amines
  - Amino acids
  - Small peptides
  - Lipids
  - Steroids
  - Terpenes
  - Alkaloids
  - Drugs (xenobiotics)



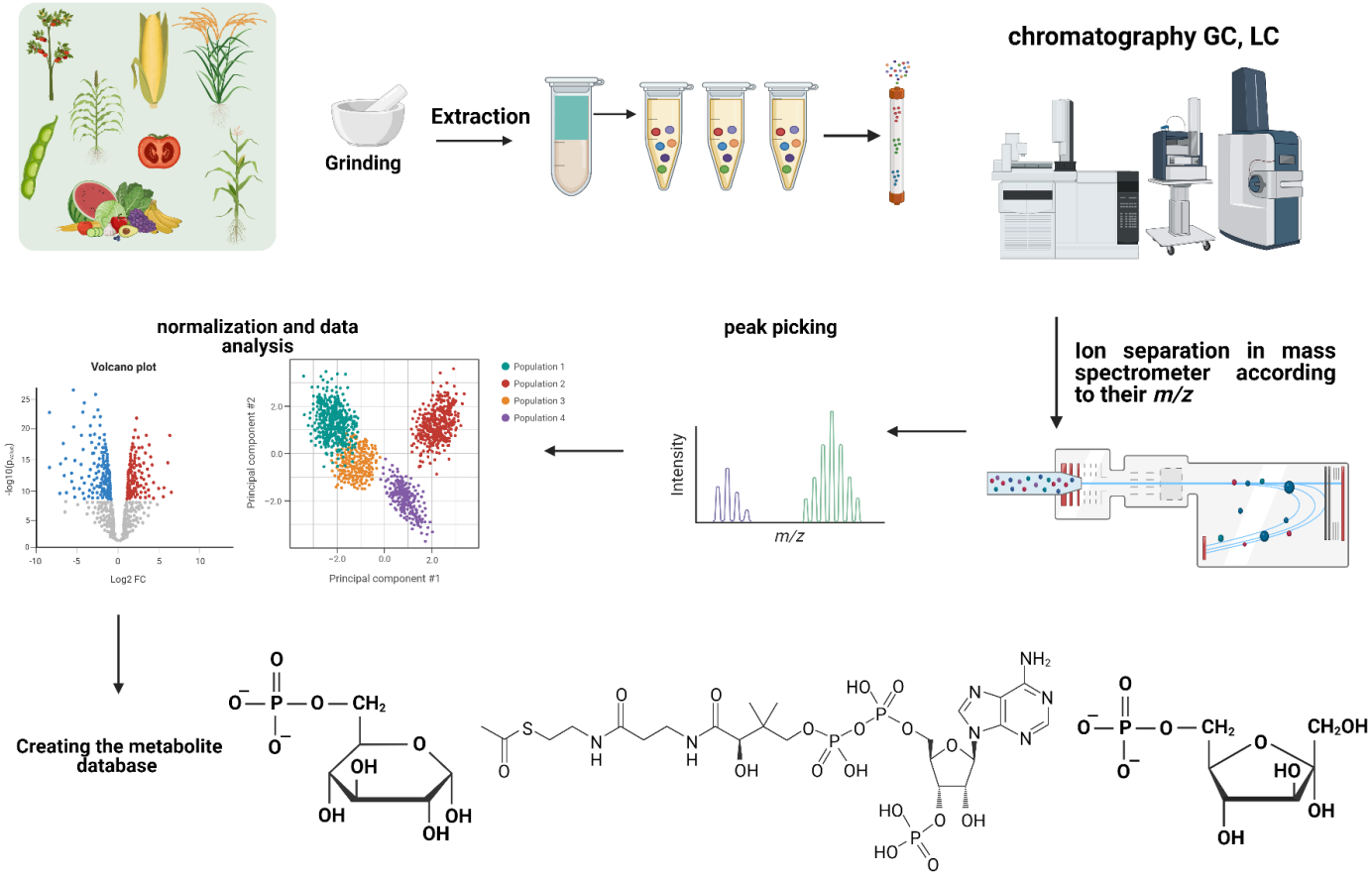






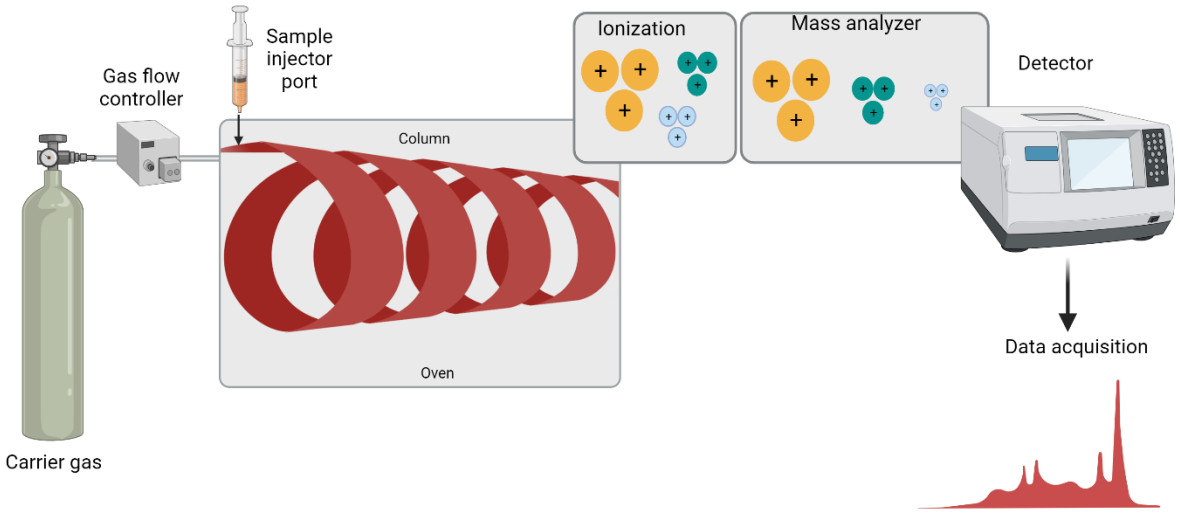
Schematic metabolomics workflow

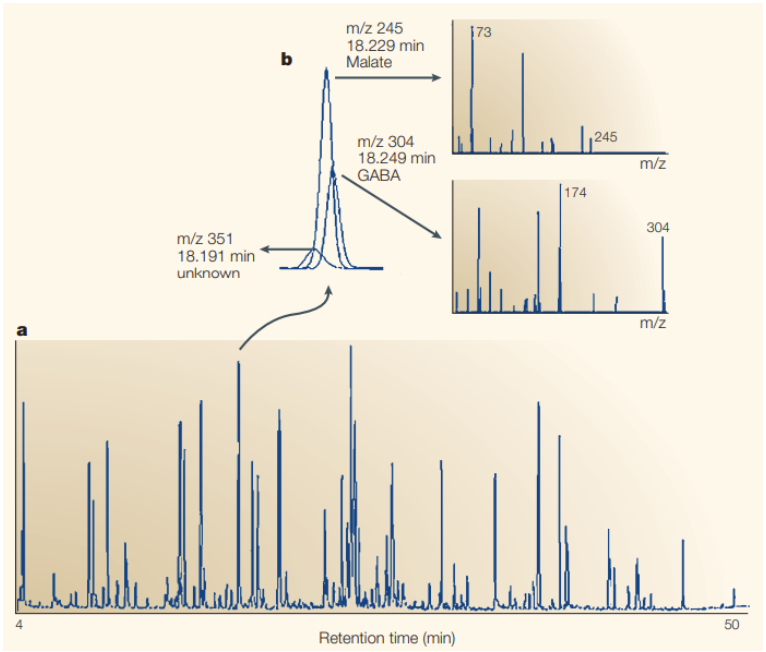
1. Experimental design and sampling
   - Preparation of freeze-dried plant material
   - Preparation of fresh frozen plant material
2. Metabolic profiling
   - Primary metabolites (GC-MS- gas chromatography)
   - Secondary metabolites (LC-MS- liquid chromatography)
   - Lipids (LC-MS)
3. Chromatogram processing and data normalization/analysis
4. Creating the metabolite databases



Metabolite profiling by gas chromatography mass spectrometry

- GC-MS technologies allow the identification and robust quantification of a few hundred metabolites within a single extract (Roessner, U. et al, 2004, Plant cell).



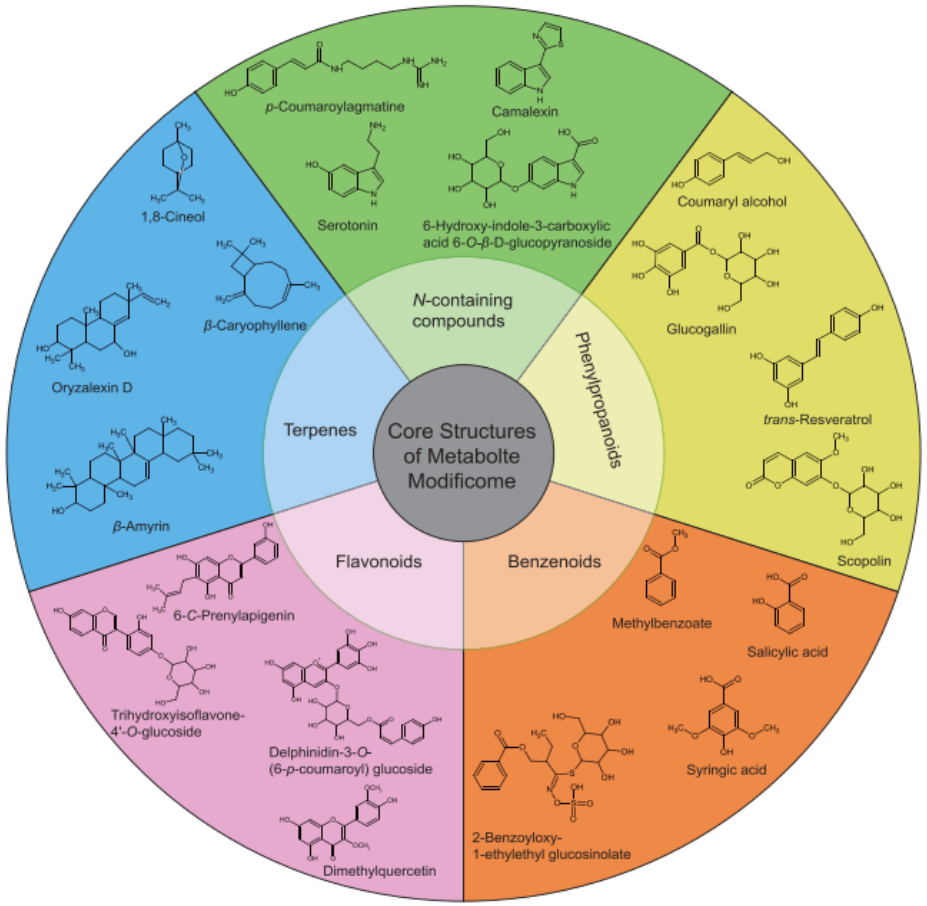


Fernie et al, 2004, Nature reviews molecular cell biology

- GC-MS is allow to measure the stable compounds at high temperature, volatile compounds.

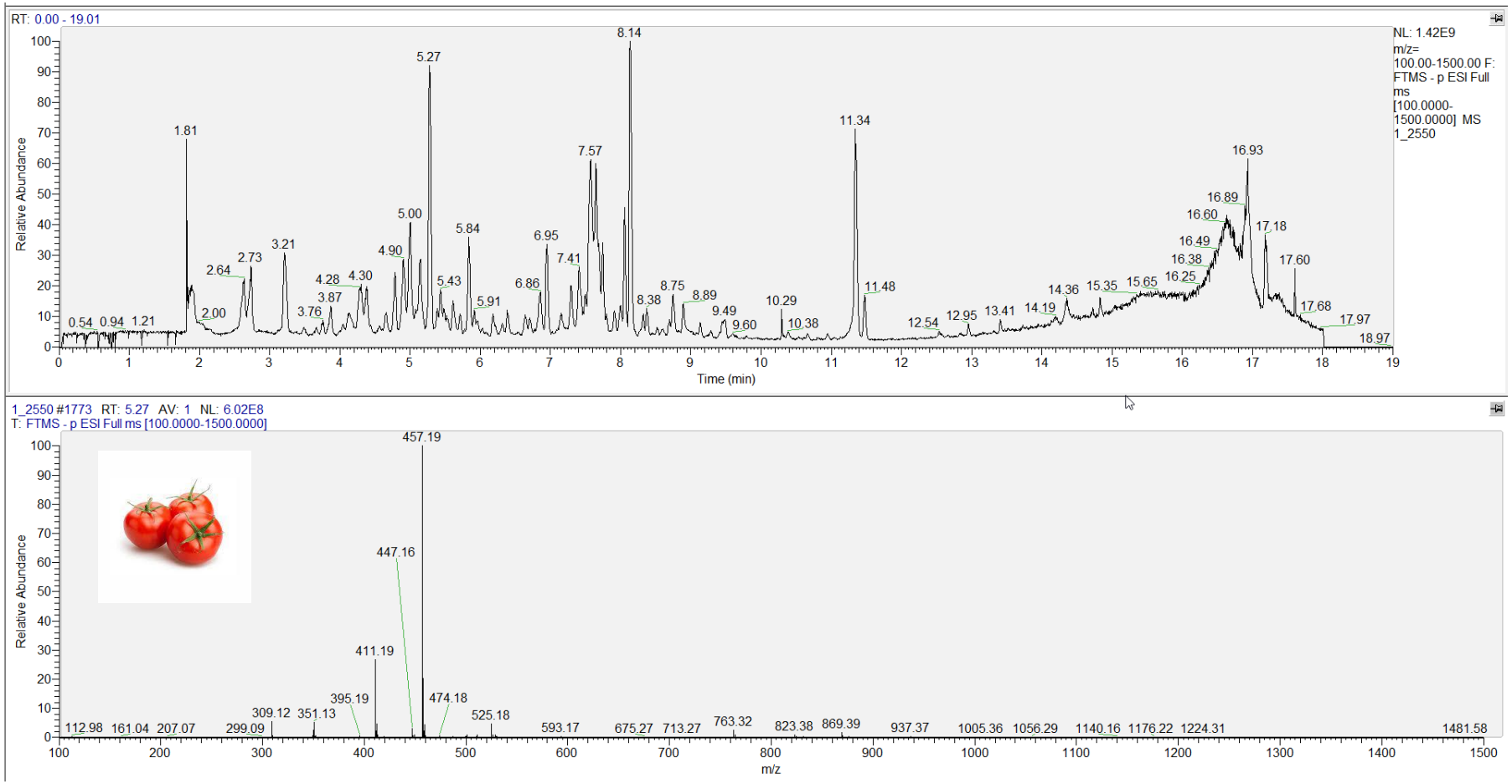
Metabolite profiling by liquid chromatography mass spectrometry

- Comparing to GC-MS, LC–MS can be adapted to a wider array of molecules, including a range of secondary metabolites such as alkaloids, flavonoids, glucosinolates, isoprenes, oxylipins, phenylpropanoids, pigments and saponins (Aharoni, A. et al, 2003, Plant Cell).
- LC-MS allows to measure unstable and stable compounds at high temperature, non-volatile compounds, high molecular weight.



Wang et al, 2019, Molecular plant

Chromatogram processing and identification



- Library matching and quantification of the metabolites.
- Data analysis and creating the metabolomics datasets.

Activity 1

Refinement and demonstration of phytosanitary methods for surveillance during PGR ex situ and in situ management and phytosanitation of contaminated unique material

(D3.5)

- Make available crop biodiversity while avoiding the spread of quarantine pathogens
- Comply with phytosanitary regulations

https://www.abiopep.com/

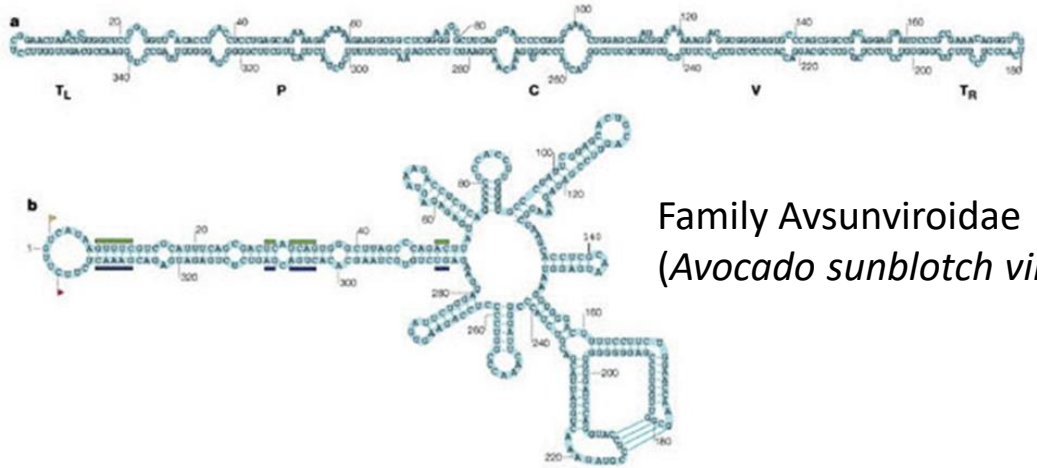
Optimize seed treatment to prevent contamination with seed-borne pathogens

1) Compile information on phytosanitary seed treatment for plant pathogens
- focus on virus and viroids

2) Testing current methods used to remove viroids from solanaceaous seeds:
- Different concentrations and duration of hydrochloric acid and trisodium phosphate
- Heat treatment

Viroids:
- Circular, single-stranded RNA
- Not protein encoding
- Rolling-circle replication in host nucleus or chloroplast

Family Pospiviroidae (*Potato spindle tuber viroid*)



Family Avsunviroidae (*Avocado sunblotch viroid*)

Elimination of *Potato spindle tuber viroid* and *Columnea latent viroid* from tomato seed

Treatment		Viroid testing (positive No./ sub-sample No.)					
		Replicate I		Replicate II		Replicate III	
		Pospi1F/ Pospi1R[1]	CLVd[2]	Pospi1F/ Pospi1R	CLVd	Pospi1F/ Pospi1R	CLVd
1	Not treated	5/5[3]	2/5	5/5	2/5	5/5	2/5
2	0.5 N HCl[4] x 15 min + 10% TSP[5] x 1 hr	0/5	0/5	4/5	0/5	1/5	0/5
3	20% Clorox[6] x 15 min + 80˚C x 24 hrs	2/5	0/5	3/5	0/5	3/5	0/5
4	0.5 N HCl x 15 min + 10% TSP x 1 hr + 80˚C x 24 hrs	0/5	0/5	1/5	0/5	0/5	0/5

[1] Pospi1F/Pospi1R primer set (Verhoeven et al., 2004); [2] TARI Taiwan

[3] each sub-sample contains about 400 seeds; [4] HCl: Hydrochloric acid; [5] TSP: Trisodium phosphate; [6] Clorox: domestic bleach

Optimize identification methods for seed-borne pathogens

- Test and compare sampling and detection methods for seed-borne pathogens with a focus on virus and viroids of Solanaceae

Target pathogens:

- *Tomato brown rugose fruit virus* (ToBRFV)
- *Pepper mild mottle virus* (PMMoV)
- *Tomato mottle mosaic virus* (ToMMV)
- *Pepino mosaic virus* (PepMV)
- *Cucumber green mottle mosaic virus* (CGMMV)
- Other new emerging pathogens

- Test detection method (RT-PCR, PCR, RT-LAMP, LAMP) on at least 100 Solanaceous seed lots (infected/treated/control)
- Develop and publish a Standard Operation Procedure (SOP) for optimized pathogen screening in seed lots

Outputs

- Seed treatment protocol compilation
- Best combinations of seed treatments to eliminate seed borne viroids and viruses without harming seed viability
- SOP for phytosanitary seed treatment developed and shared
- Seed treatment manual for training purposes
- Efficient methods to detect quarantine seed-borne pathogens such as viroids and viruses (at least 5 target pathogens)
- Methods validated on >100 seed samples and ready for routine application
- Strategy to improve the phytosanitary status of genebank collections

Outcome

Improved availability of unique Solanaceae genetic resources from ex situ and in situ collections for research and breeding