NMR-based plant metabolomics at Bordeaux Metabolome Facility: 3 short stories

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> Metabolomic pipeline

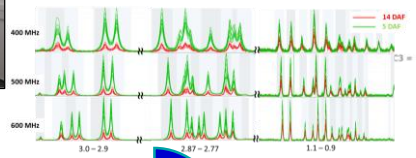
Metabolomic profiling & fingerprinting

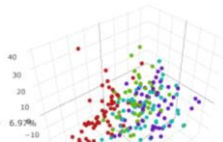
-1. **Design**

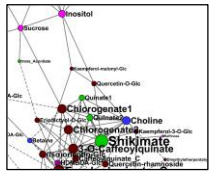
0. **Sampling** (dozens or hundreds of samples per experiment)

1. **Extraction(s)**

2. **Targeted** or **Untargeted** **biochemical analyses**







3. **Data pre-treatment**

4. **Data visualisation and mining**

5. **Data integration and fusion**

6. **Data & metadata sharing**

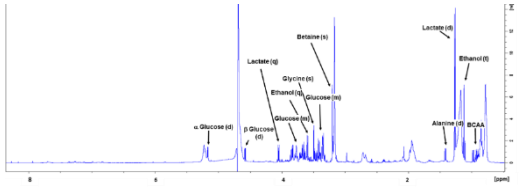
MetaboLights

Data INRAE

0-5. **Metadata**

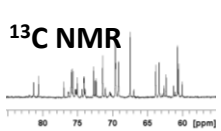
Sample	Metabolite1	Metabolite2	Metabolite...	...
S1	81024	25109	64349	
S2	83868	23798	62737	
S3	75038	19700	53531	
S4	32355	14219	22843	
S5	34559	11126	26513	
S6	24466	8831	19438	
S7	59931	13250	57774	
S8	63519	12885	55208	
...				

Structural analyses of selected samples

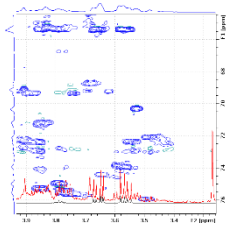


Metabolite identification

^{13}C NMR



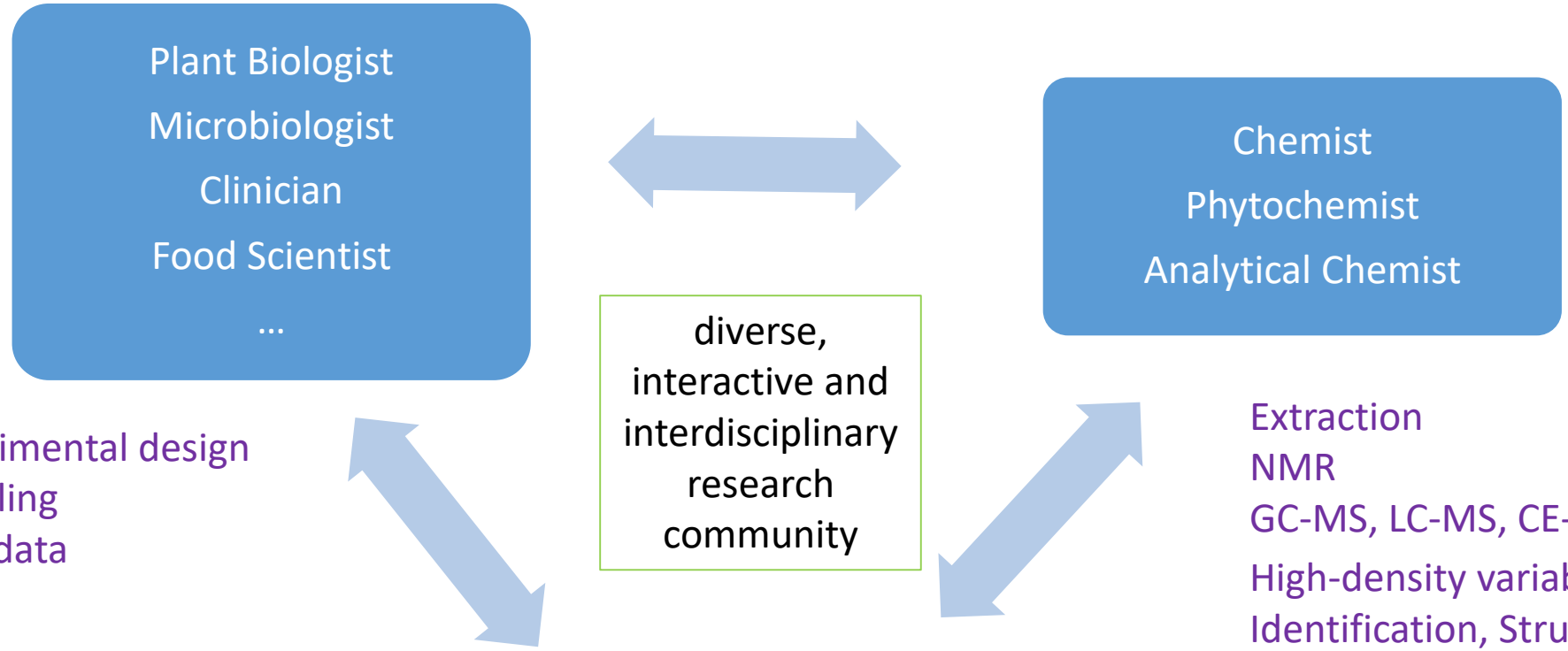
2D NMR



Databases (BMRB, SDBS, HMDB, ChenomX NMR Suite library…)
Commercial compounds

COSY, TOCSY, J-RES, HSQC, HMBC

Metabolomics ecosystem
Expertise and Savoir-faire

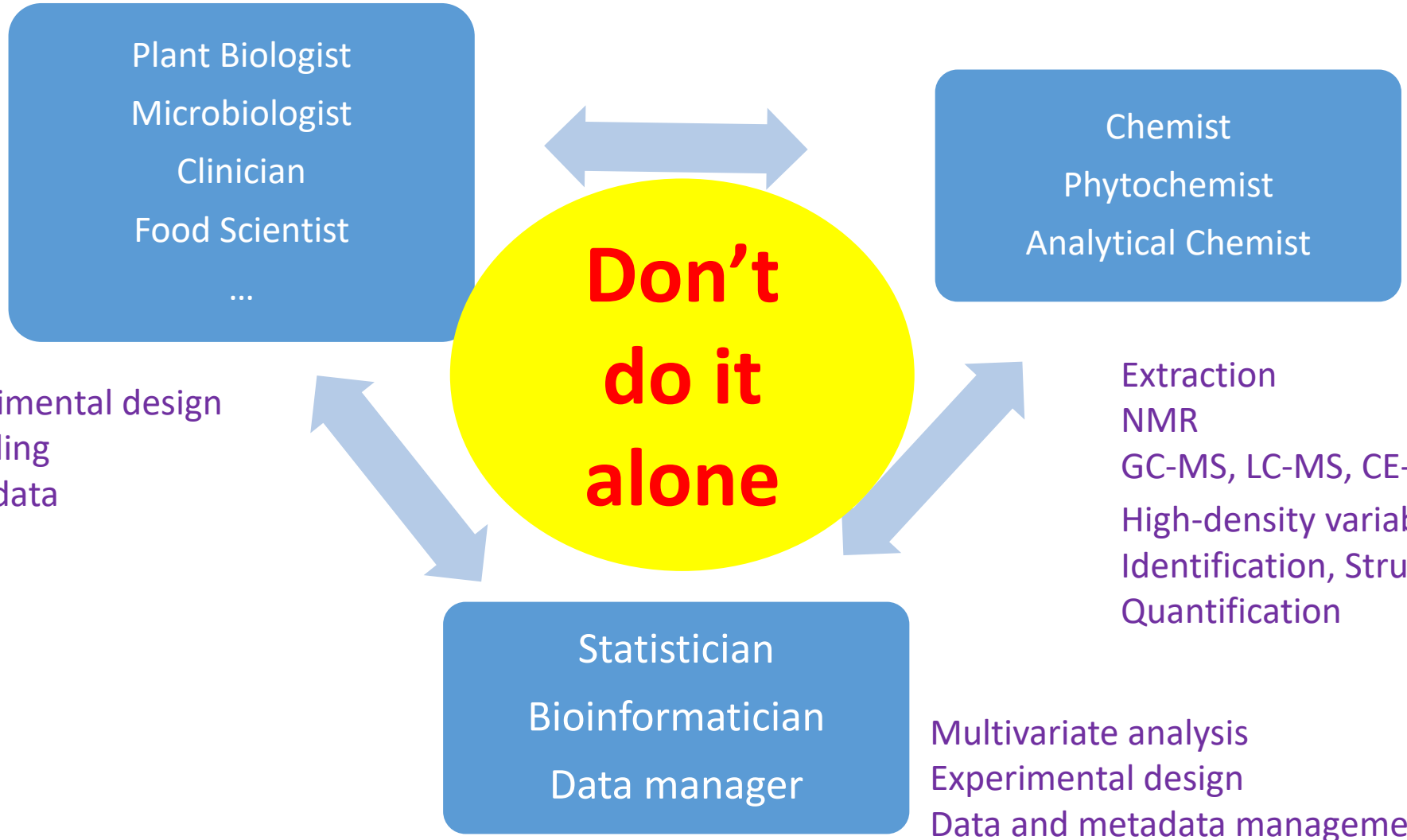


Experimental design
Sampling
Metadata

Extraction
NMR
GC-MS, LC-MS, CE-MS } Metabolic profiles
High-density variable generation
Identification, Structural analysis
Quantification

Multivariate analysis
Experimental design
Data and metadata management (FAIR)

Metabolomics ecosystem
Expertise and Savoir-faire



Developments and applications in NMR-based plant metabolite profiling

A selection of several developments and applications in NMR-based metabolite profiling of small molecules in plant extract performed with the 500 MHz spectrometer.

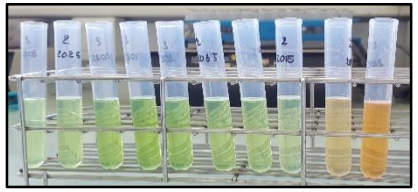
1 - Optimizing 1D ^{1}H-NMR profiling of plant samples

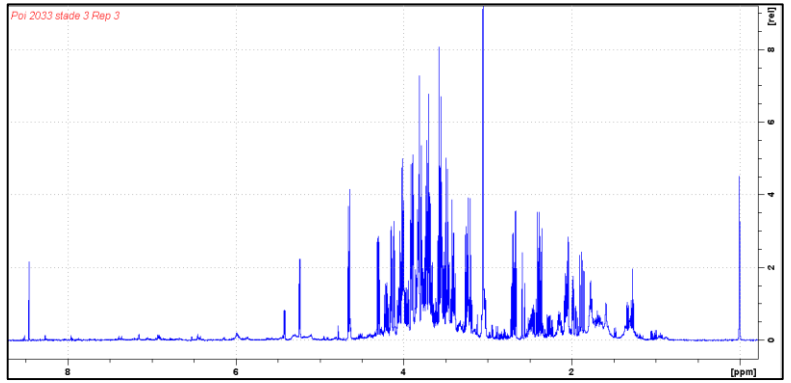
2- Optimizing 1D NMR-based metabolomics processing

3- Case study in plant

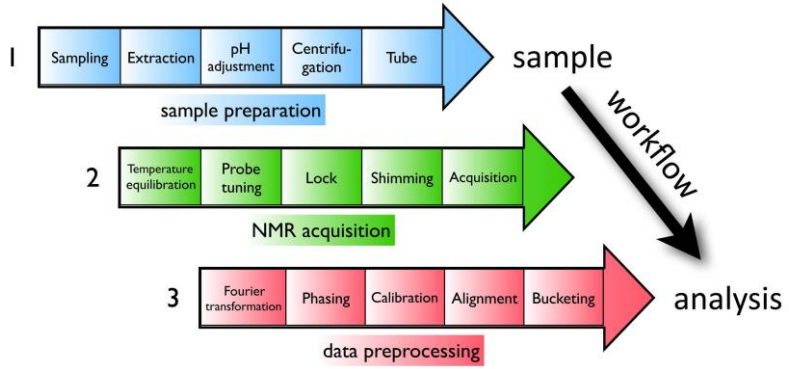
Selection of several developments and applications in NMR-based metabolite profiling

1 - Optimizing 1D ^{1}H-NMR profiling of plant samples: extract preparation, standardization





Optimizing 1D ^{1}H-NMR profiling of plant samples



- **increasing the through-put by using deuterated solvents to avoid extract lyophilisation step (1 week to 0.5 day)**
- **minimizing uncontrolled variability in plant ^{1}H-NMR profiling, by taking into account plant extract sample composition: pH and paramagnetic ion concentrations**

Plant solvent extraction:

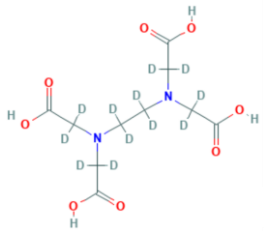
50/50 (v/v) MeOD – 90 mM K_2DPO_4/KD_2PO_4/ 11 mM EDTA-d_{12}, $pH_{apparent}$ 6.0

Plant extract pH adjustment:

adjustment to $pH_{apparent}$ 6.0 with NaOD by means of BTpH Unit (Bruker)

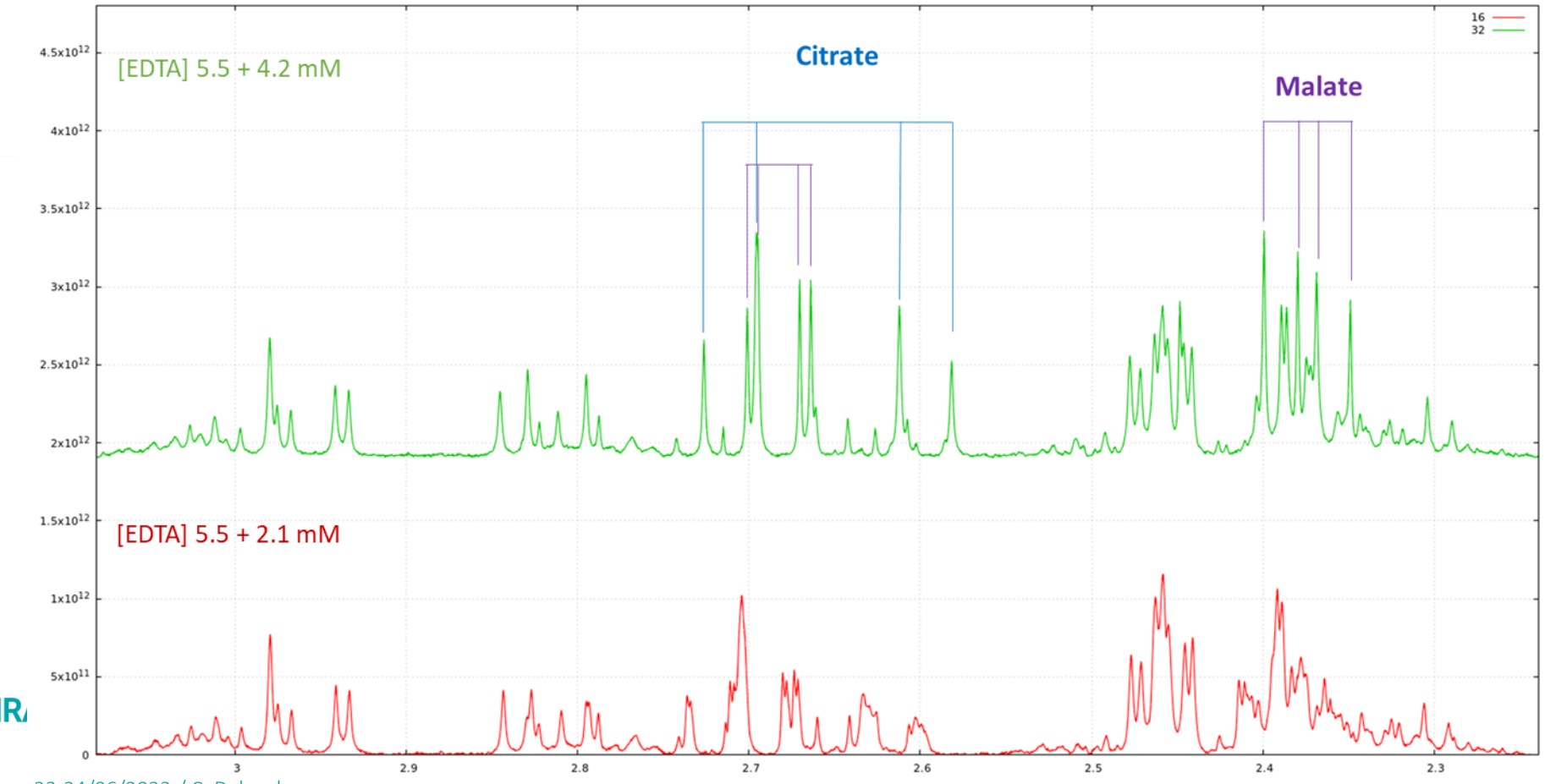
NMR visibility of major organic acids in wheat spikelet extract

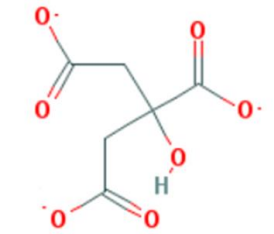
Effect of control of paramagnetic ions on ^{1}H NMR spectra at 500 MHz – Wheat spikelet extract
Citrate and Malate: two major organic acids in plant kingdom



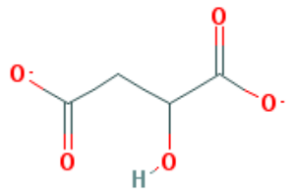
EDTA-*d12*

Chelation of Mn^{2+}, Cu^{2+}, Fe^{3+}





Citrate

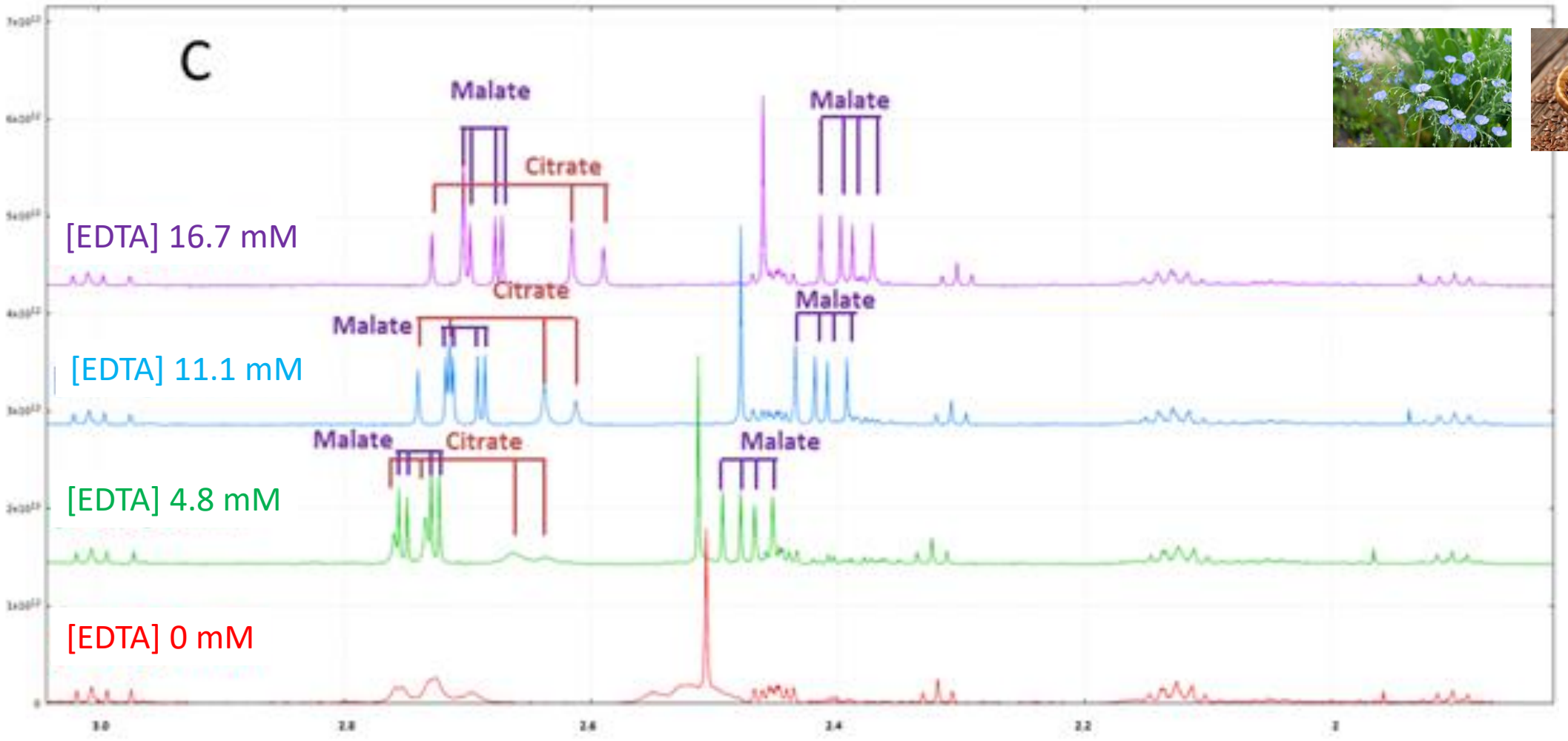


Malate

NMR visibility of major organic acids in flax root extracts

Effect of control of paramagnetic ions on ^{1}H NMR spectra at 600 MHz – Flax root extracts

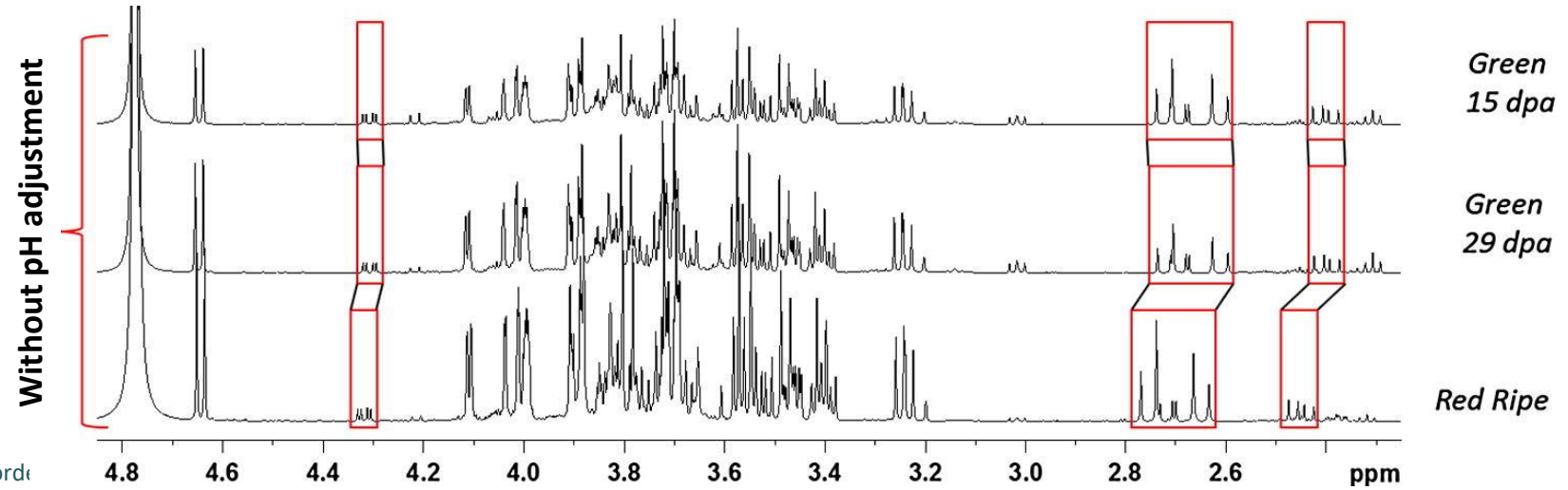
Without pH adjustment



JX Fontaine
R Molinié
F Mesnard

Influence of pH adjustment step on δ of major organic acids in tomato fruit extracts

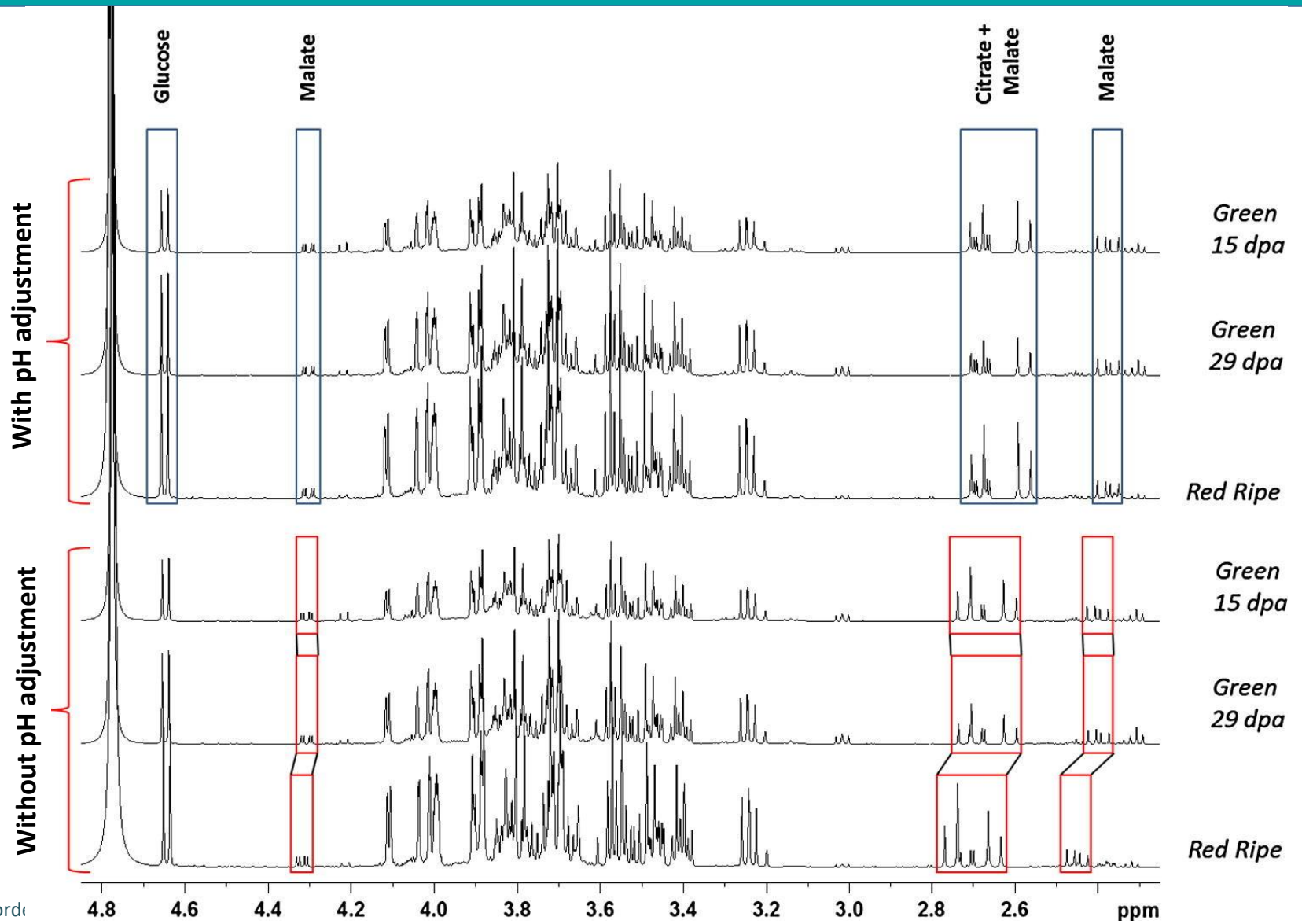
^{1}H NMR spectra @ 500 MHz Tomato fruit extracts



dpa: day post anthesis

Influence of pH adjustment step on δ of major organic acids in tomato fruit extracts

^{1}H NMR spectra @ 500 MHz Tomato fruit extracts



dpa: day post anthesis

pH range observed and EDTA-*d12* needed for plant tissue extracts

	Tissue	Number of plant samples (biological replicates)	Plant powder weight (mg DW)	Plant extract $pH_{apparent}$ before adjustment		$pH_{apparent}$ after adjustment	Volume of NaOD 1M added (µl)	Volume of DCl 1 M added (µl)	NMR tube
				Min	Max				EDTA-*d12* concentration (mM)
	**Grape berry skin (veraison)**	16	25 to 40	**5.64**	**6.12**	**6.00 +-0.02**	0.02 to 6.70	0.00 to 5.54	**11.1**
	White oak leaf	20		**4.86**	**5.96**				**10.2**
	**Wheat spikelet**	60		**6.39**	**6.63**				**9.4**
	**Tomato pericarp (unripe or ripe)**	36		**5.08**	**6.16**				**11.8**
	Wild tomato ripe fruit pericarp	24		**4.82**	**5.81**				**11.5**
	**Sweet pepper ripe fruit**	24		**5.83**	**6.36**				**11.5**
	Flax root	80		**5.70**	**6.79**				**16.0**

-> Variability of plant extract $pH_{apparent}$ among species, tissues and intra experiment.

-> No universal EDTA concentration established for all plant tissues

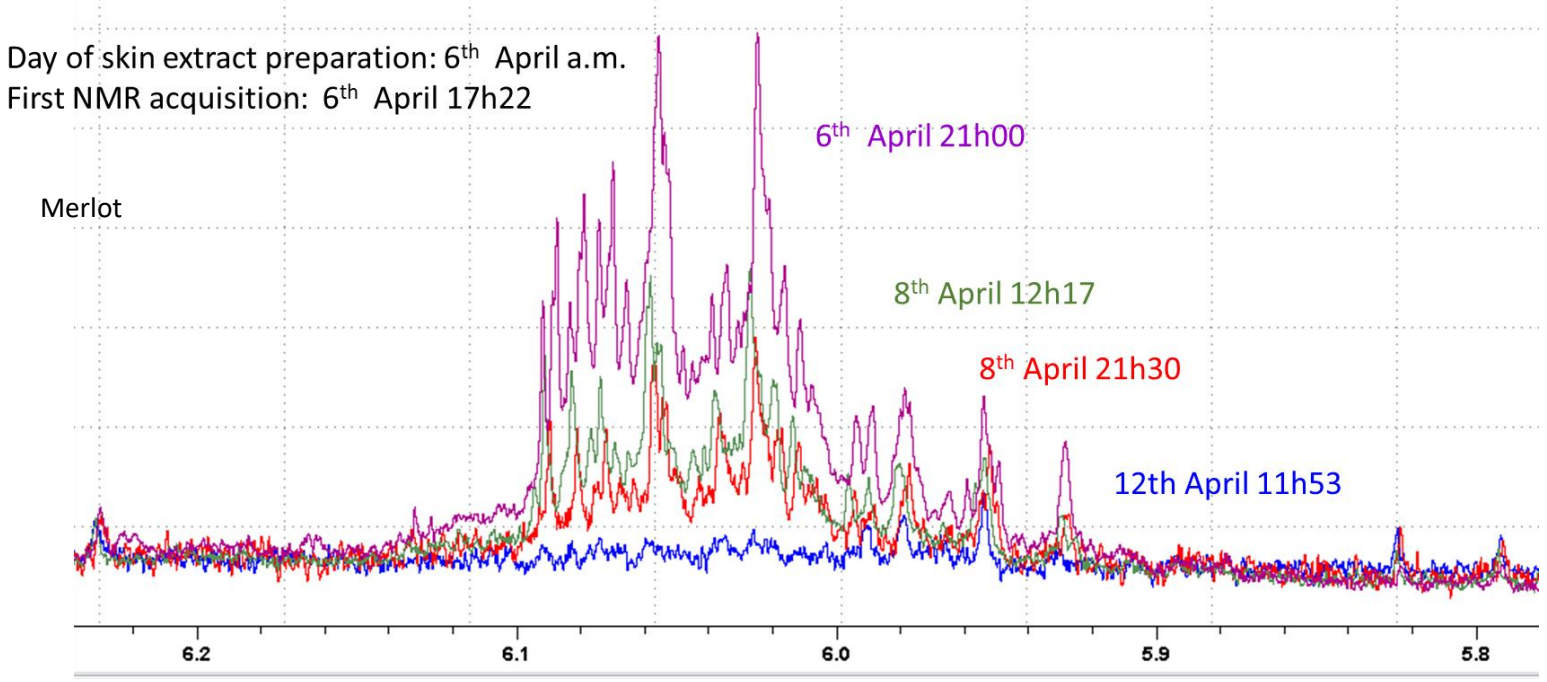
-> EDTA optimal concentration should be determined experimentally for each specific plant tissues from an organ at a given developmental stage.

Adapted from Deborde *et al.*, **Optimizing 1D ^{1}H-NMR profiling of plant samples for high-throughput analysis: extract preparation, standardization, automation and spectra processing.** *Metabolomics 2019, 15:28*

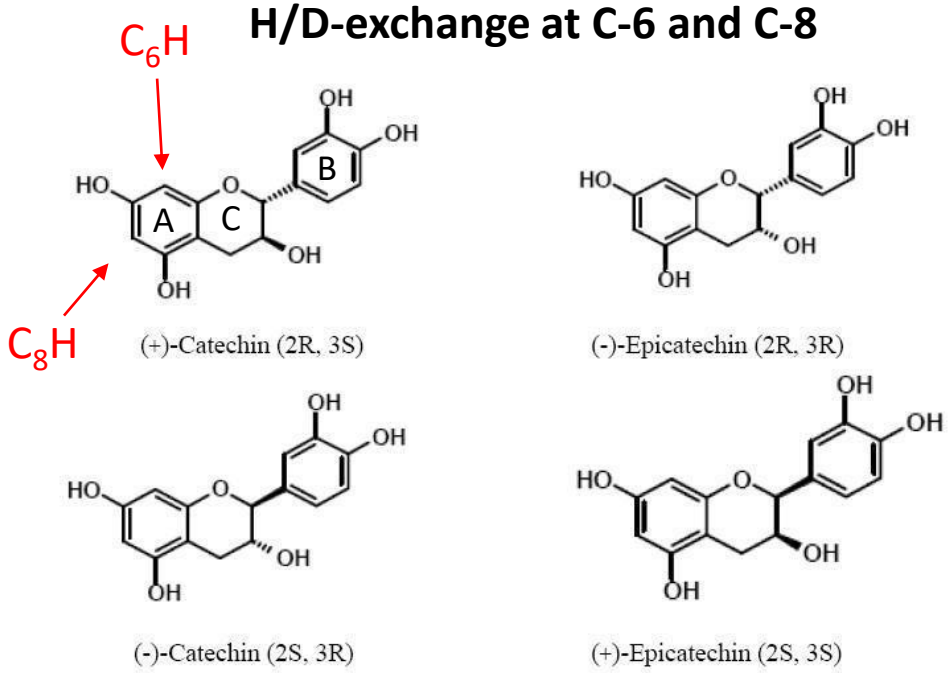
Stability of plant tissue extracts
ex: Grape Berry skin extract

On-going project on Grape Berry under heat stress

M. Cariou, M2 student



H/D-exchange at C-6 and C-8



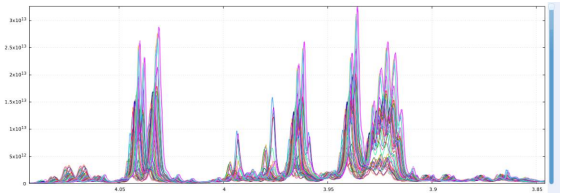
-> catechin and epicatechin exhibit competitive deprotonation on B and A ring leading to a mixture of different monophenolates*.

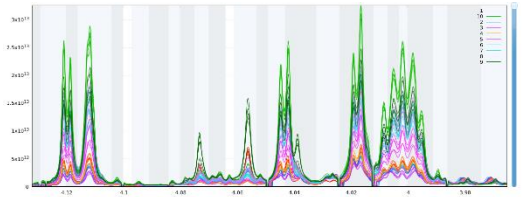
-> Plant extract stability should be checked experimentally for each specific plant tissues and at a given developmental stage – **impact on bucketing for untargeted approach.**

* Kiehlmann et al., **Iodination and deuteration of catechin derivatives.** *Can. J. Chem. 1988, 66, 2431–2439.*
Cren-Olivé *et al.*, **Catechin and epicatechin deprotonation followed by 13C NMR.** *Tetrahedron Letters 2002, 43:25*

Selection of several developments and applications in NMR-based metabolite profiling

2- Optimizing 1D NMR-based metabolomics processing





Variables

(spectral region area)

$$M = \begin{pmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{m,1} & a_{m,2} & \cdots & a_{m,n} \end{pmatrix}$$

Samples

Data matrix

1D Spectra Visualisation and Processing tool

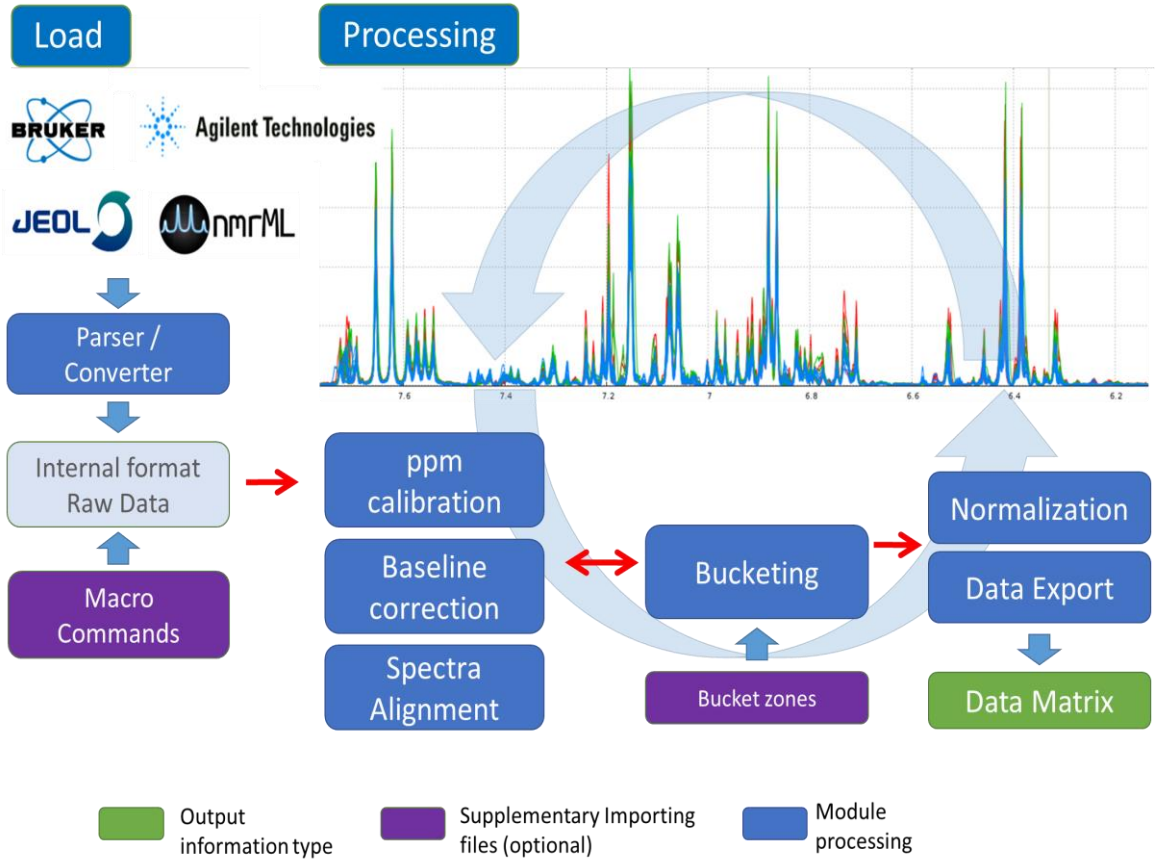
NMRProcFlow

nmrprocflow.org

Spectra Visualisation according to factors, *i.e.* tissue, organ, time, stress…

and Processing Interactive processing

Variable Size and Intelligent Bucketing



- baseline correction
- chemical shift calibration
- resonance alignment

Sample spectral series:

Small < 50

Average 50 – 200

Large > 200

Jacob *et al.*, Metabolomics, 2017
doi:10.1007/s11306-017-1178-y

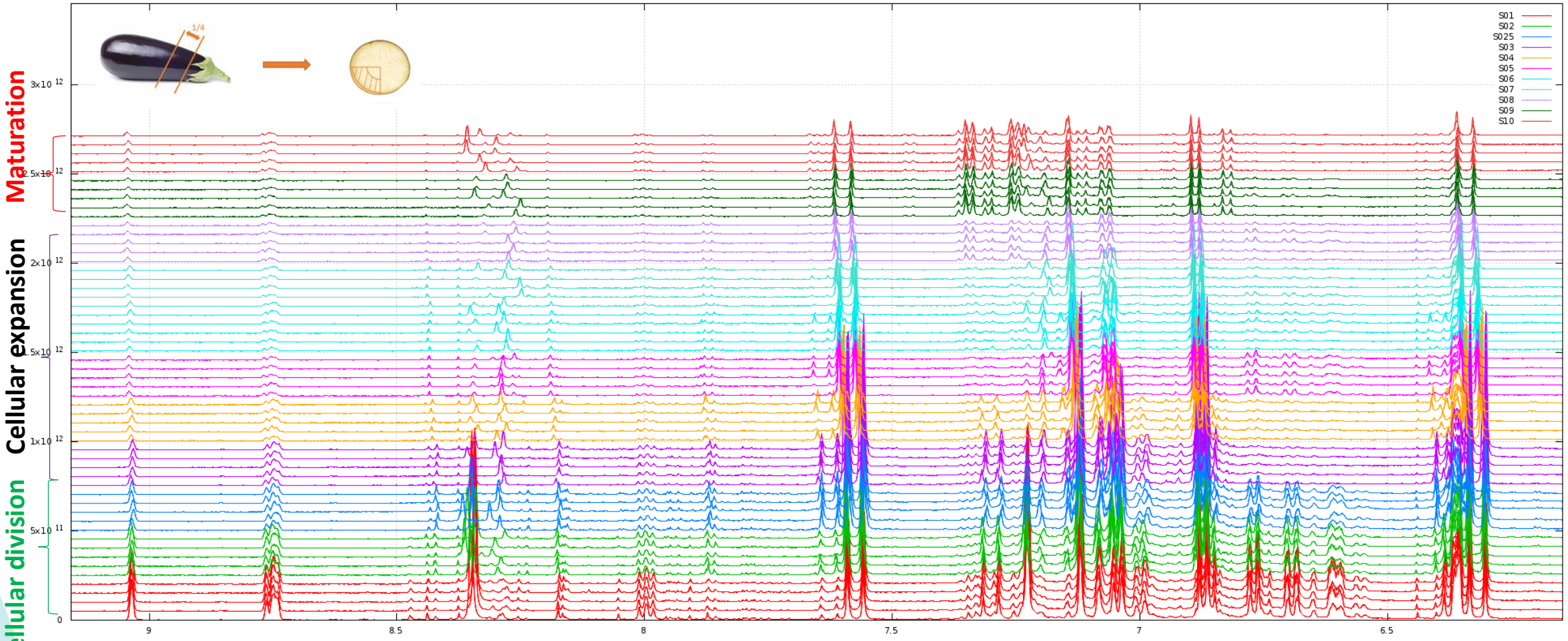
METABOHUB

1D Spectra Visualisation

51 samples of Eggplant pericarp extracts
11 developmental stages

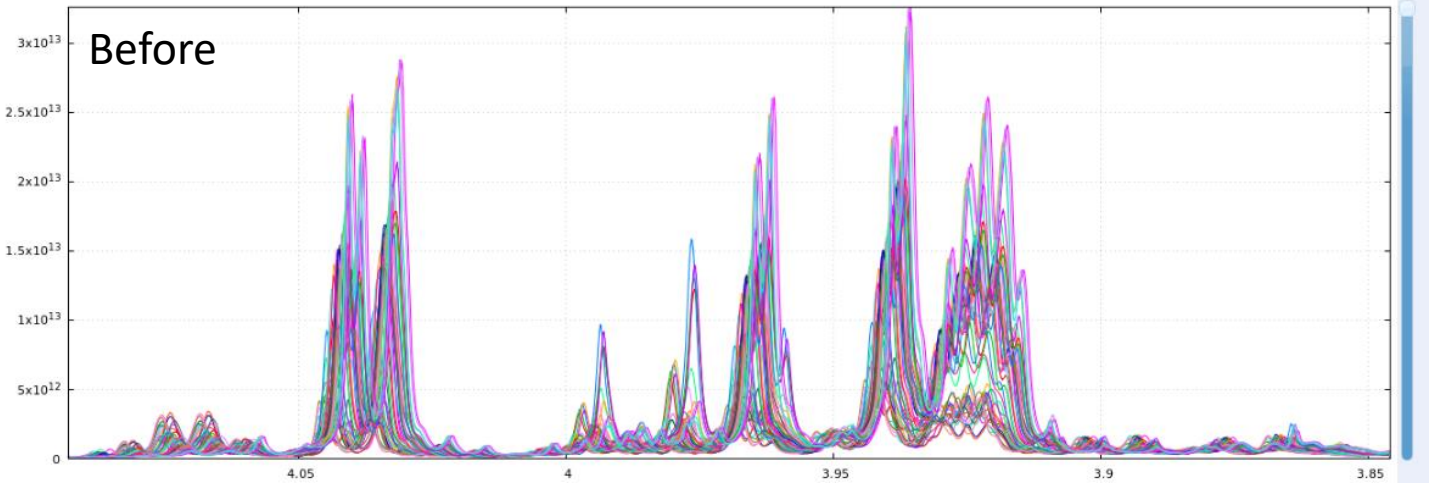
according to developmental stages

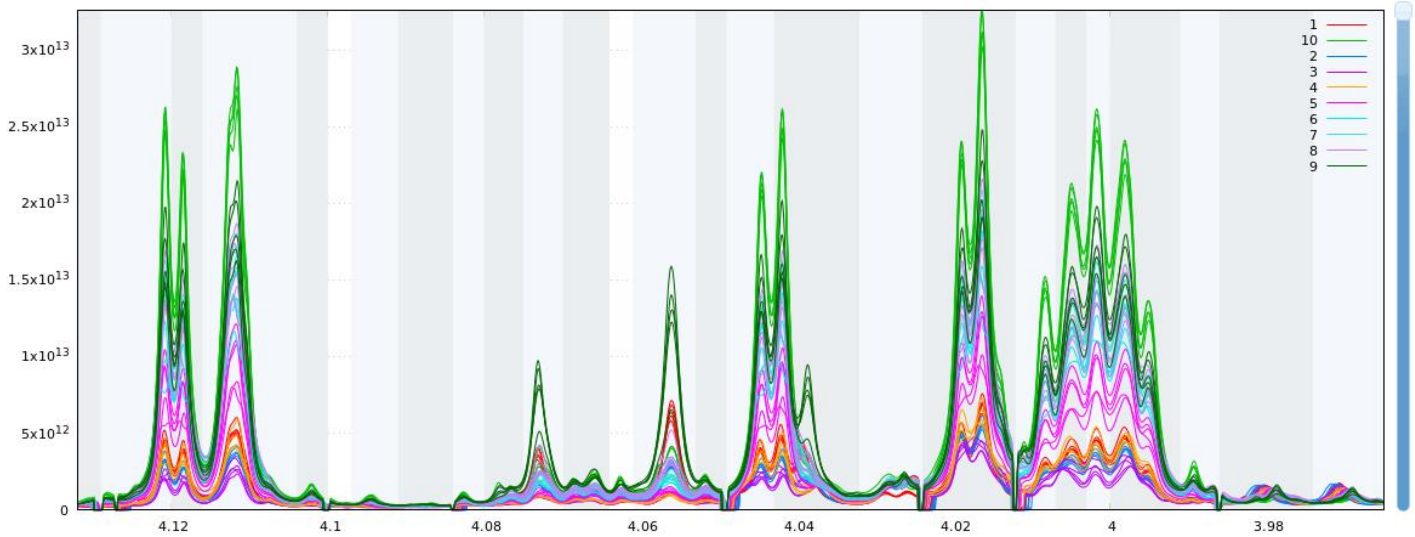
A. Clavé M1 student



1D Spectra Processing tool

51 samples of pepper pericarp extracts
10 developmental stages





Fingerprinting

Spectra preprocessing

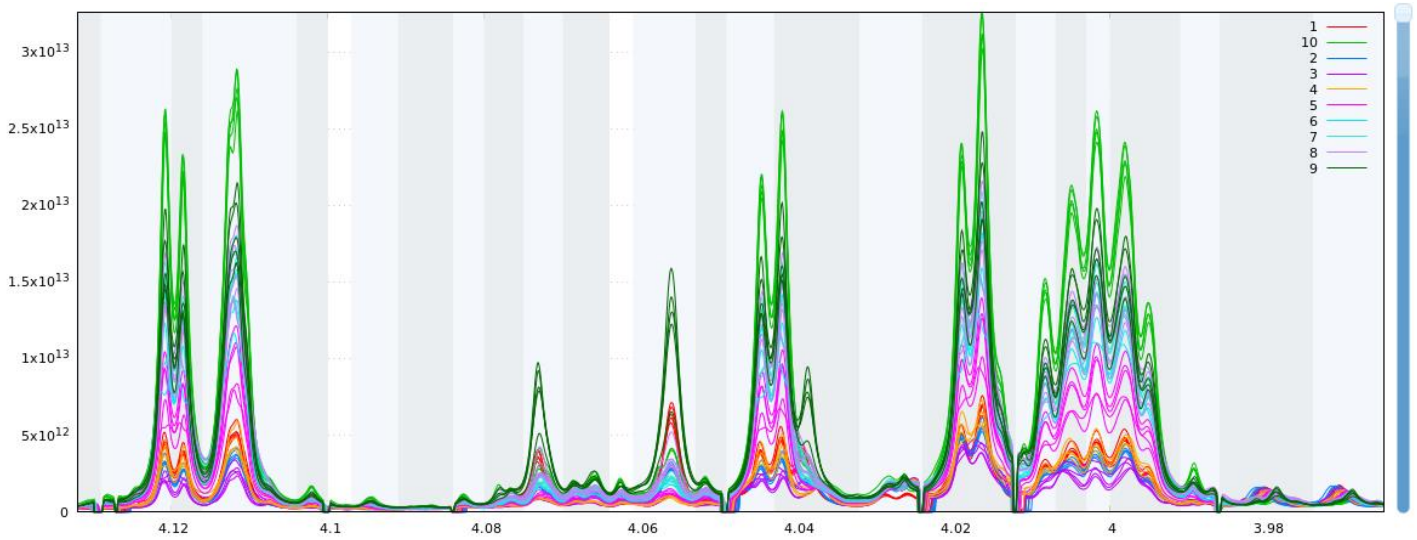
- PPM calibration
- Global and local baseline corrections
- Spectra alignment
- Non-uniform bucketing
- Signal-to-Noise ratio determination
- Total Sum Normalization

M. Batsale, M1 student

1D Spectra Processing tool

51 samples of pepper pericarp extracts
10 developmental stages

M. Batsale, M1 student



Variables
(spectral region area or bucket)

$$M = \begin{pmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,n} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,n} \\ \vdots & \vdots & \ddots & \vdots \\ a_{m,1} & a_{m,2} & \cdots & a_{m,n} \end{pmatrix}$$

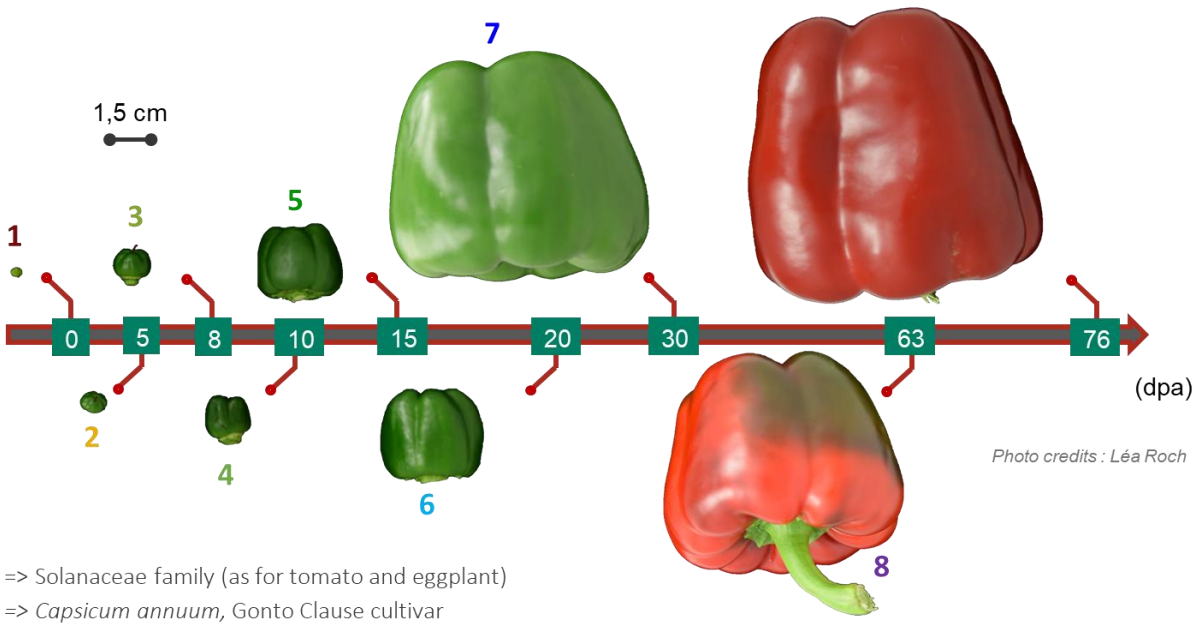
Samples

Data matrix

1D Spectra Processing tool

NMRProcFlow

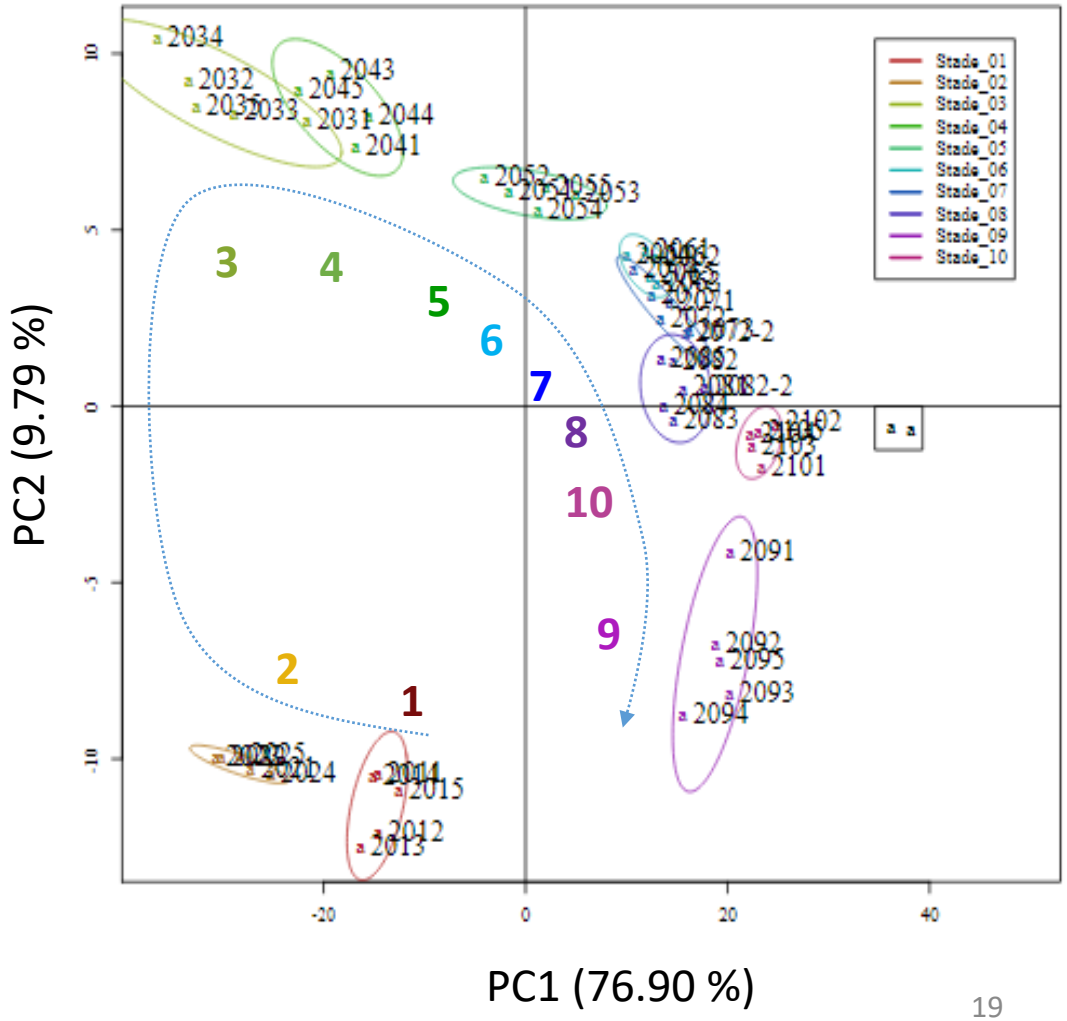
51 samples of pepper pericarp extracts
10 developmental stages



=> Solanaceae family (as for tomato and eggplant)
=> *Capsicum annuum*, Gonto Clause cultivar
=> 10 development stages, at least 5 replicates for each stage 51 samples of pool of pericarp

⇒ Trajectory
⇒ Earliest stages isolated

PCA analysis (CSN, UV scaling)
with 496 variables (spectral regions or buckets)



M. Batsale, M1 student

Selection of several developments and applications in NMR-based metabolite profiling

3- Case study in plant

Projects from the last 5 years in metabolomics – a selection

Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine *Vitis vinifera*  	Shoot Root Leaf Berry (skin, pulp)	Grafting Drought and Grafting Pathogen infection Heat Stress	X X X X	 X X	International National Regional National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
Buxus sempervirens 	Leaf Root	Insect Herbivory	X	X	National
Citrus	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

Projects from the last 5 years in metabolomics – a selection

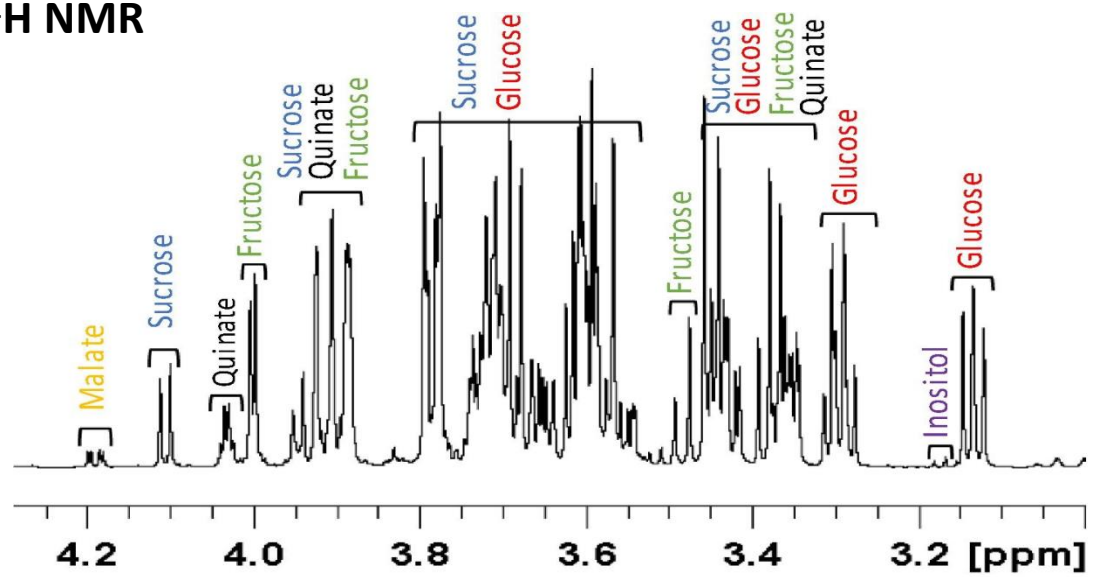
Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine *Vitis vinifera*  	Shoot Root Leaf Berry (skin, pulp)	Grafting Drought and Grafting Pathogen infection Heat Stress	X X X X	 X X	International National Regional National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
Buxus sempervirens 	Leaf Root	Insect Herbivory	X	X	National
Citrus	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

NMR-based metabolite identification: major metabolites of kiwi (*Actinidia deliciosa*) fruit pericarp

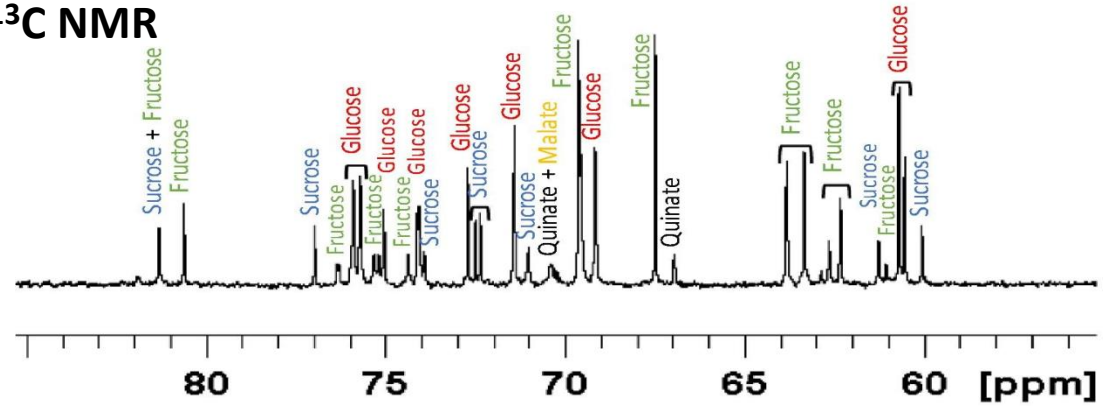
Léa Roch
Thesis 2018

NMR spectra of kiwi polar extracts

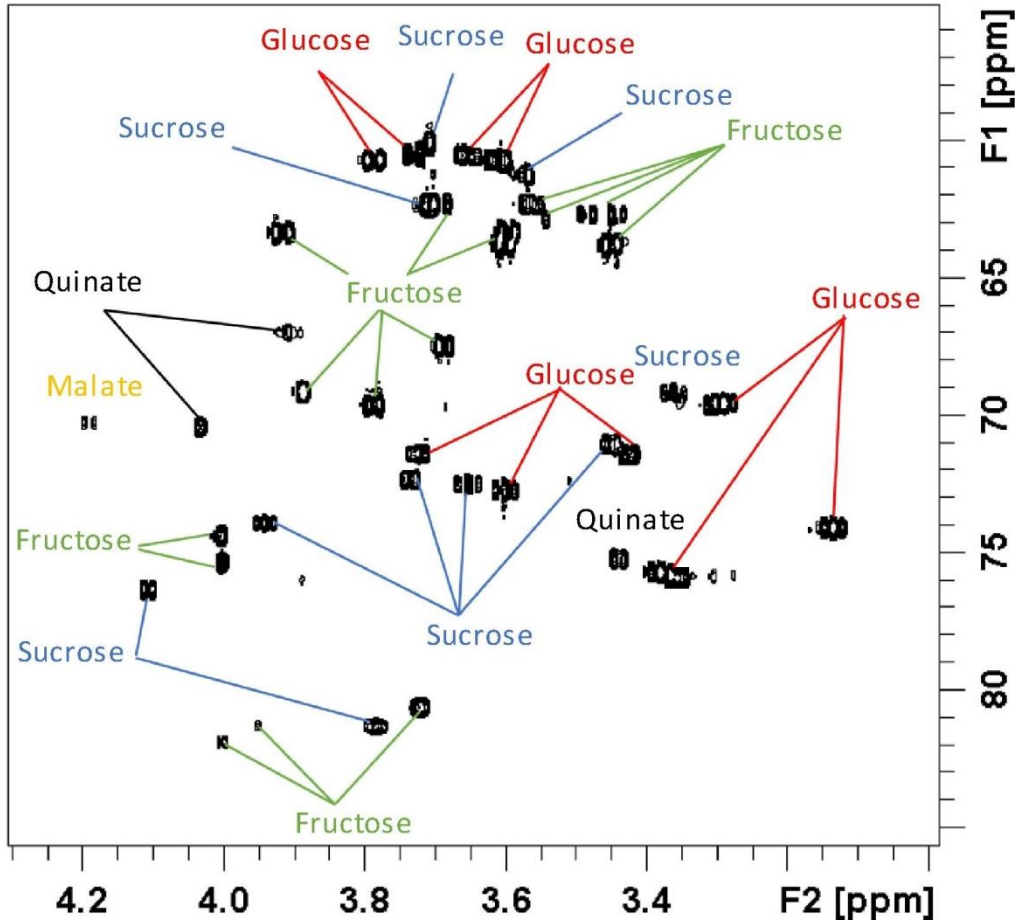
^{1}H NMR



^{13}C NMR



2D ^{1}H-^{13}C **HSQC** @ 700 MHz

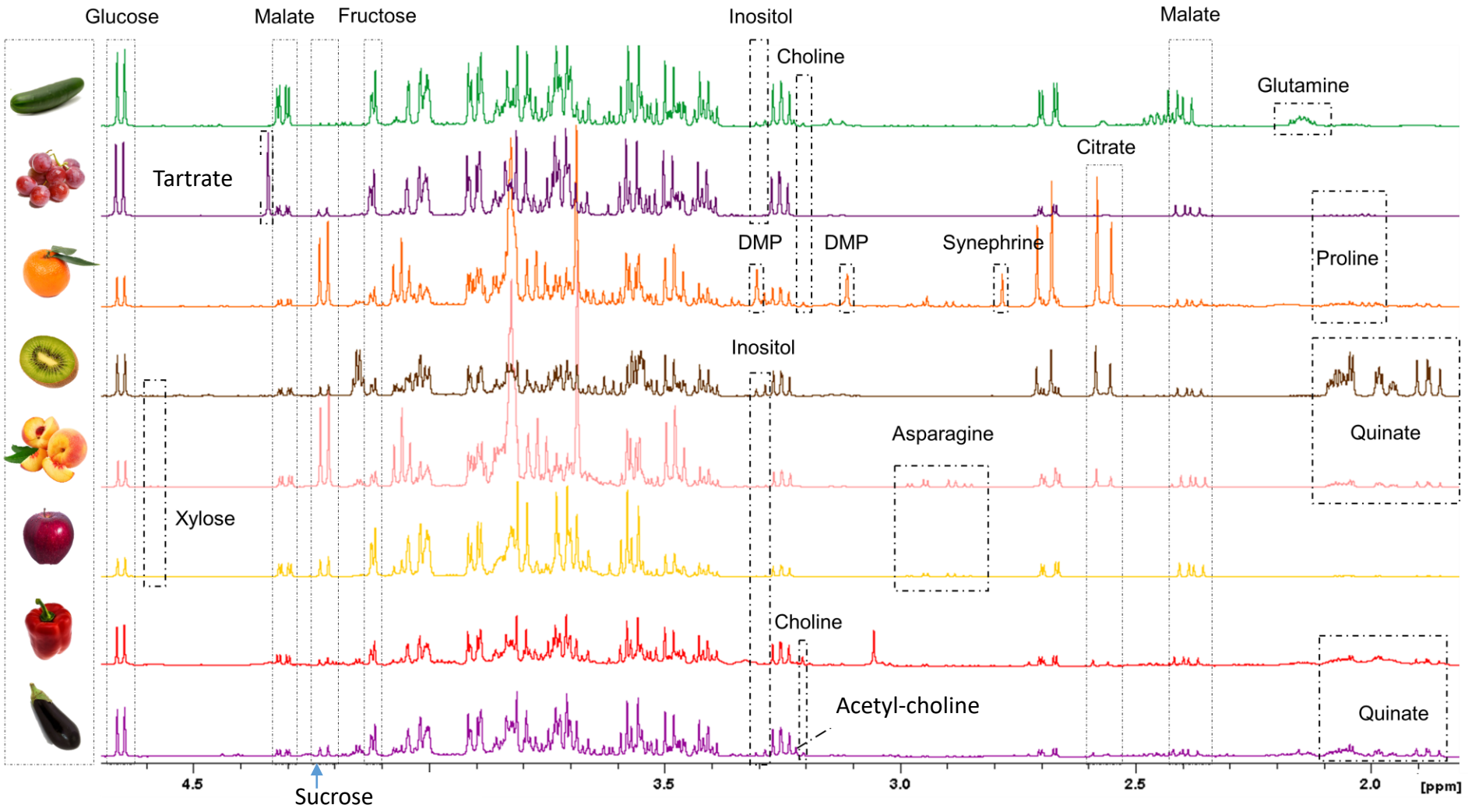


Deborde *et al.*, **Plant metabolism as studied by NMR spectroscopy.** *Prog Nucl Mag Res Spec 2017 102-103:61-97*

Coll CEISAM, Univ Nantes

^{1}H NMR-based metabolic profiling of fruit: major metabolites common or specific to 8 species

^{1}H-NMR spectra of polar extracts + dedicated NMR experiments for spectra annotation…



<u>Sugars and cyclitol</u>
glucose, fructose, sucrose & inositol

Kiwi : raffinose

<u>Organic acids</u>
citrate or malate

Kiwi : isocitrate
grapevine : tartarate

<u>Amino acids and N-containing compounds</u>
alanine, valine & aspartate

Eggplant : histamine
Cucumber : citrulline
Clementine : synephrine & dimethyl-proline

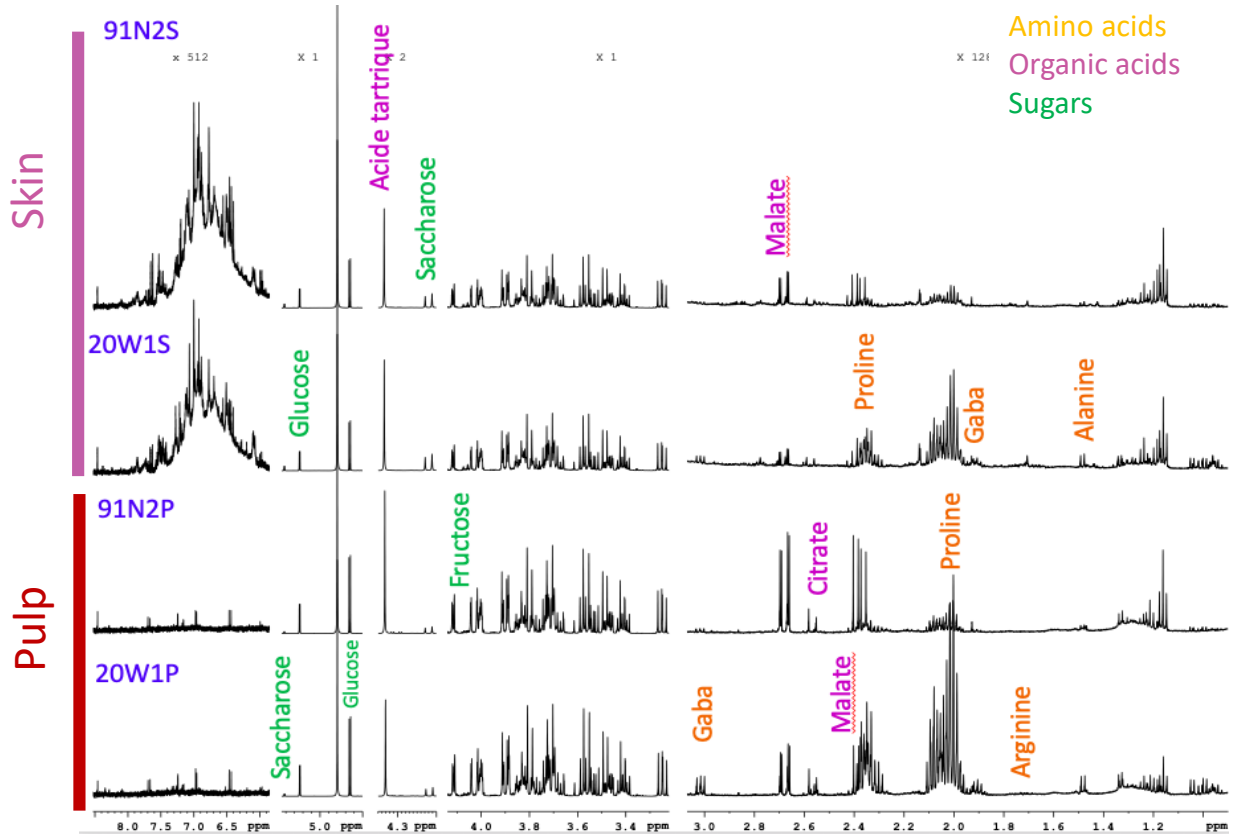
Roch et al., **Biomass composition explains fruit relative growth rate and discriminates climacteric from non-climacteric species.** *J. Exp. Bot.* 2020.

Projects from the last 5 years in metabolomics – a selection

Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine *Vitis vinifera*  	Shoot Root Leaf Berry (skin, pulp)	Grafting Drought and Grafting Pathogen infection Heat Stress	X X X X	X X	International National Regional National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
Buxus sempervirens 	Leaf Root	Insect Herbivory	X	X	National
Citrus	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

^{1}H NMR-based metabolic profiling of grape berry under mild heat stress in vineyard

Two examples of hot plots in the Saint-Emilion vineyard



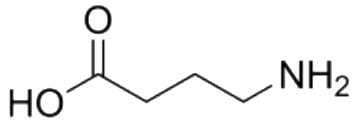
Fingerprinting with annotation of polar extract

-> Multivariate and univariate analyses

Hot plots :

+ Amino acids (Proline ; GABA)

- Organic acids (Malate ; Citrate)



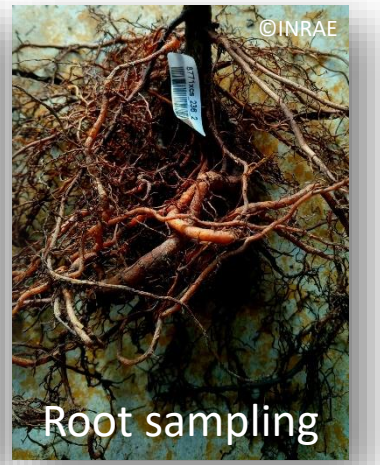
GABA

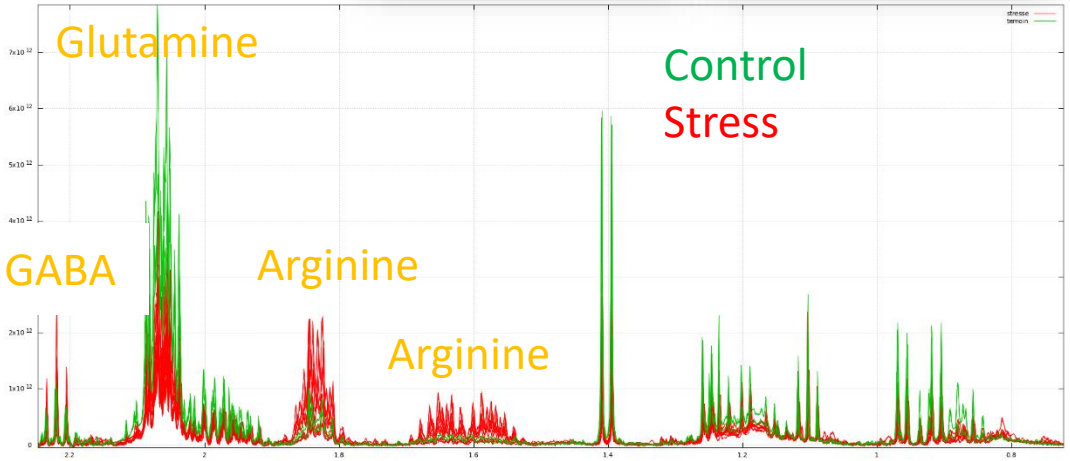
In collaboration with G Hilbert, UMR EGFV, ISVV, Bordeaux

Projects from the last 5 years in metabolomics – a selection

Species	Tissue or Organ	Biological question	Fingerprinting	Profiling	Collaboration
Grapevine	Shoot	Grafting	X		International
Vitis vinifera	Root	Drought and Grafting	X	X	National
 	Leaf Berry (skin, pulp)	Pathogen infection Heat Stress	X X	 X	Regional National
	Fruit Pericarp Leaf	Fruit metabolism Drought		X	National
Buxus sempervirens 	Leaf Root	Insect Herbivory	X	X	National
Citrus	Leaf Root	Pathogen infection Ploidy and grafting	X		International
Crops Wheat, Maize, Sunflower 	Spikelet Leaf Plantlets	Pathogen infection Stress & genotype selection	X	X	National

^{1}H NMR-based metabolic profiling of vitis root under water stress in greenhouse





Profiling of root tip polar extract

-> Multivariate and univariate analyses

Water stress on root composition :
Amino acids :
+ Arginine, GABA
- Glutamine

In collaboration with N Ollat, UMR EGFV, ISVV, Bordeaux

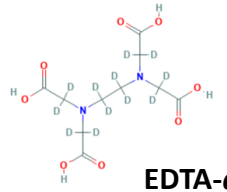
Conclusion – Take home message

- NMR remains a useful tool for plant metabolomics approach

Characterization of Organ, Tissue or whole organism composition & Metabolism,

Biomarkers of plant performance, of biotic or abiotic effect

- real need to take into account sample chemical diversity for plant tissues specificity: pH and paramagnetic ions concentrations



EDTA-*d12*

- NMRProcFlow fulfils the need for 1D NMR profiling processing of small to large sample series

- Don’t do Metabolomics alone

Thank you for your attention

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P Pétriacq (Facility leader since Nov 2018)

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