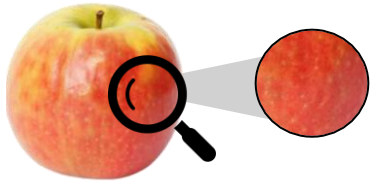
Specialized metabolites analysis of fruits of the DREAM project

Audrey LE CABEC & Massimiliano CORSO

September 29th 2026

Final Meeting - Bologna

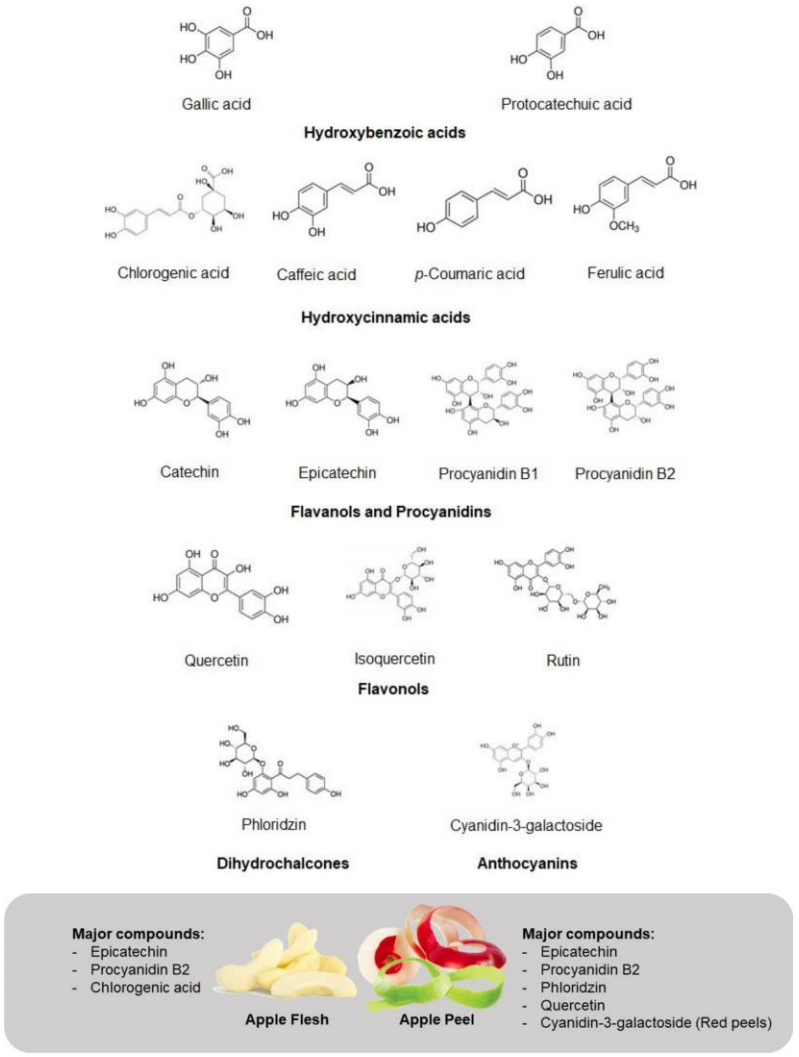
Why analysed specialized metabolites (SMs) ?



→ SMs are sensitive markers of the plant's response to its environment

→ Nutrition, health value and fruits quality depend on specialized metabolites composition

→ SMs profiles can reveal subtle but consistent shifts between agrosystems



Arnold, M., et al. Recent Development on the Chemical Composition and Phenolic Extraction Methods of Apple (*Malus domestica*)—A Review. *Food Bioprocess Technol* **17**, 2519–2560 (2024). https://doi.org/10.1007/s11947-023-03208-9

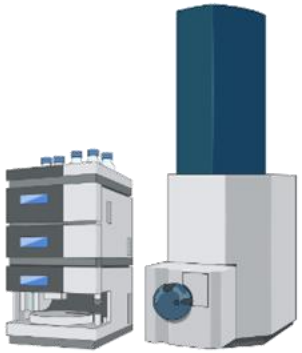
LC-HMRS/MS analysis: principle and methodology



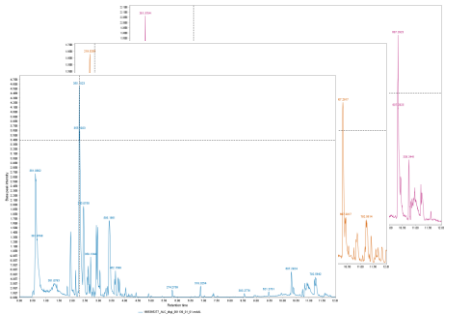
Extraction

Polar and semi-polar compounds





LC-HRMS/MS



Datatreament

mzmine

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MSLEVEL=2
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75.9135 1.2E2
88.6507 1.1E2
91.0579 3.0E2
93.0715 2.1E2
94.0783 1.4E2
95.0877 8.3E2
96.0934 2.5E2
```

Feature list (.mgf)



Feature ID	Ech 1 peak area	Ech 2 peak area
1	0	14000
2	1234	14550
3	10000	0

Quantification table (.csv)



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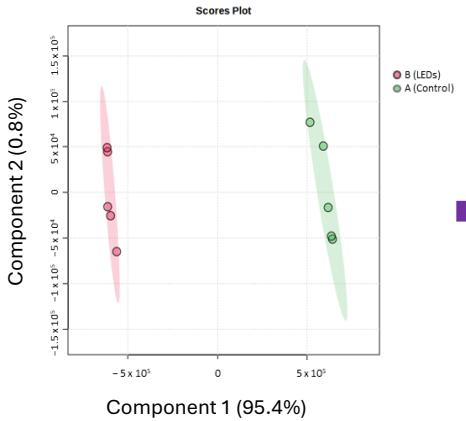
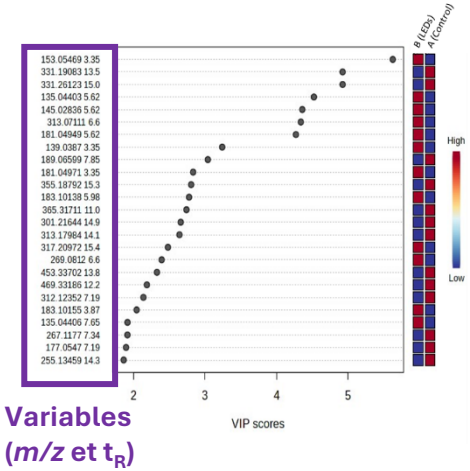
PO-CHEMISTRY
IJPB
METABOLISM

LC-HMRS/MS analysis: principle and methodology



Stastistical analysis





Variables (*m/z* et t_R)

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```

Feature list (.mgf)

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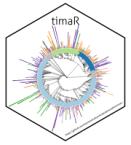
Quantification table (.csv)

Annotation

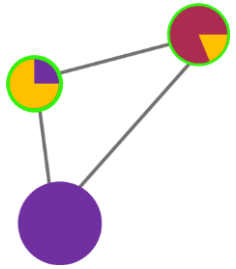


SIRIUS

GNPS

MS2Query

Molecular networking



Apple cultivated in Marocco

-

Analysis performed in April 2025

المعهد الوطني للبحث الزراعي
ⴰⵙⵉⵏⴰⴳ ⴰⵏⴰⵎⵓⵔ ⵏ ⵓⵔⵣⵣⵓ ⵜⵙⵏⵏⵍⴰⵃⵜ
Institut National de la Recherche Agronomique

Apple cultivated in Marocco : sampling

المعهد الوطني للبحث الزراعي
Institut National de la Recherche Agronomique

DREAM system



Golden delicious



Starking delicious



Story



King roat



Anna



Dorset

6 species

Conventional system



Golden delicious



Starking delicious

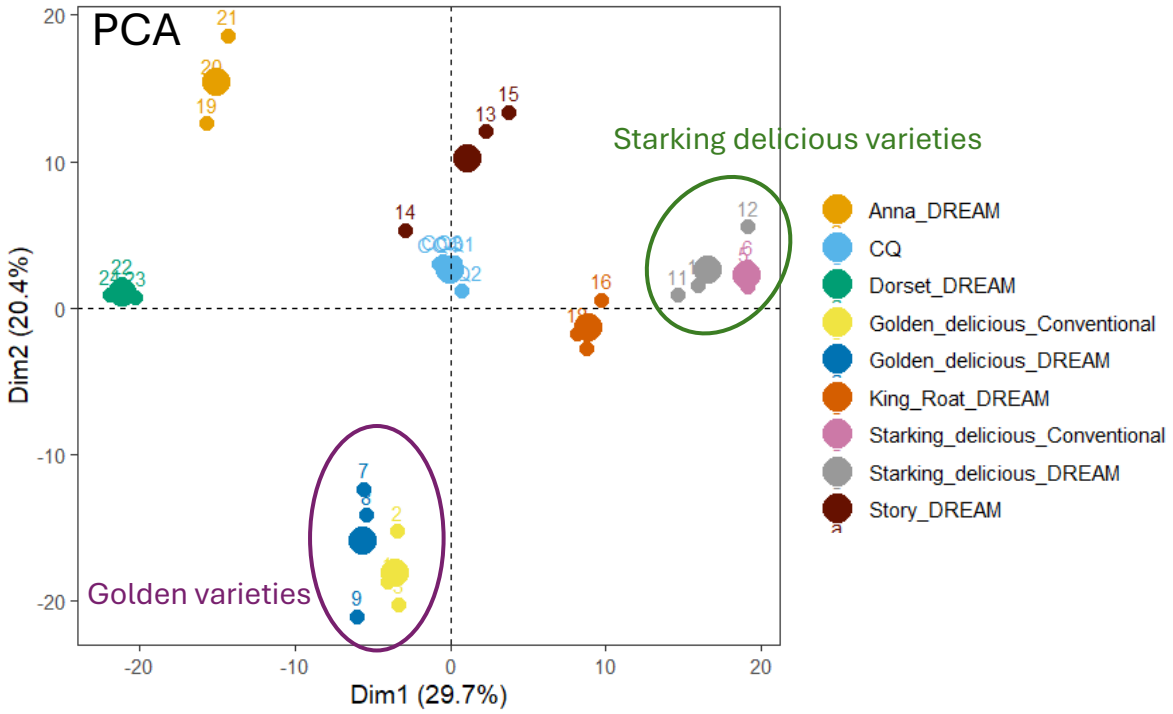
2 for comparison
Conventional vs DREAM

Does the growing system (conventional vs DREAM) affect the metabolome of apple varieties/cultivars ?

Do the metabolomes of the 6 apple varieties/cultivar differ under DREAM growing conditions ?

Apple cultivated in Morroco : statistical analysis

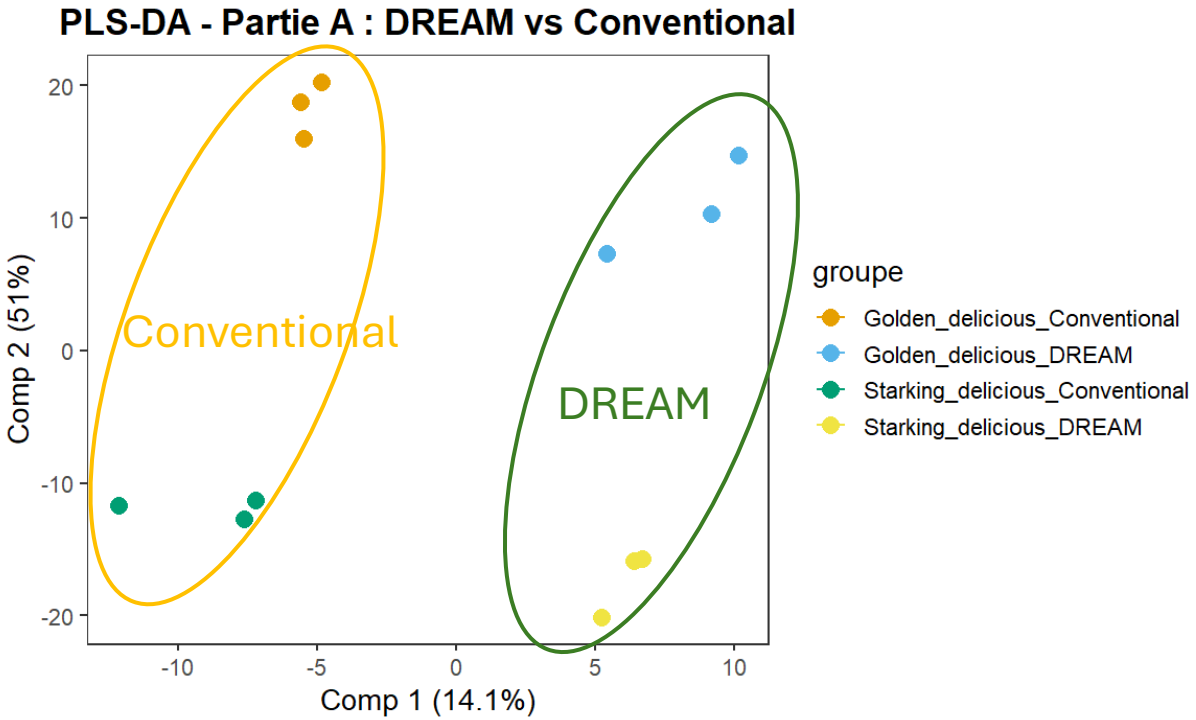
Does the growing system (conventional vs DREAM) affect the metabolome of apple varieties/cultivars ?

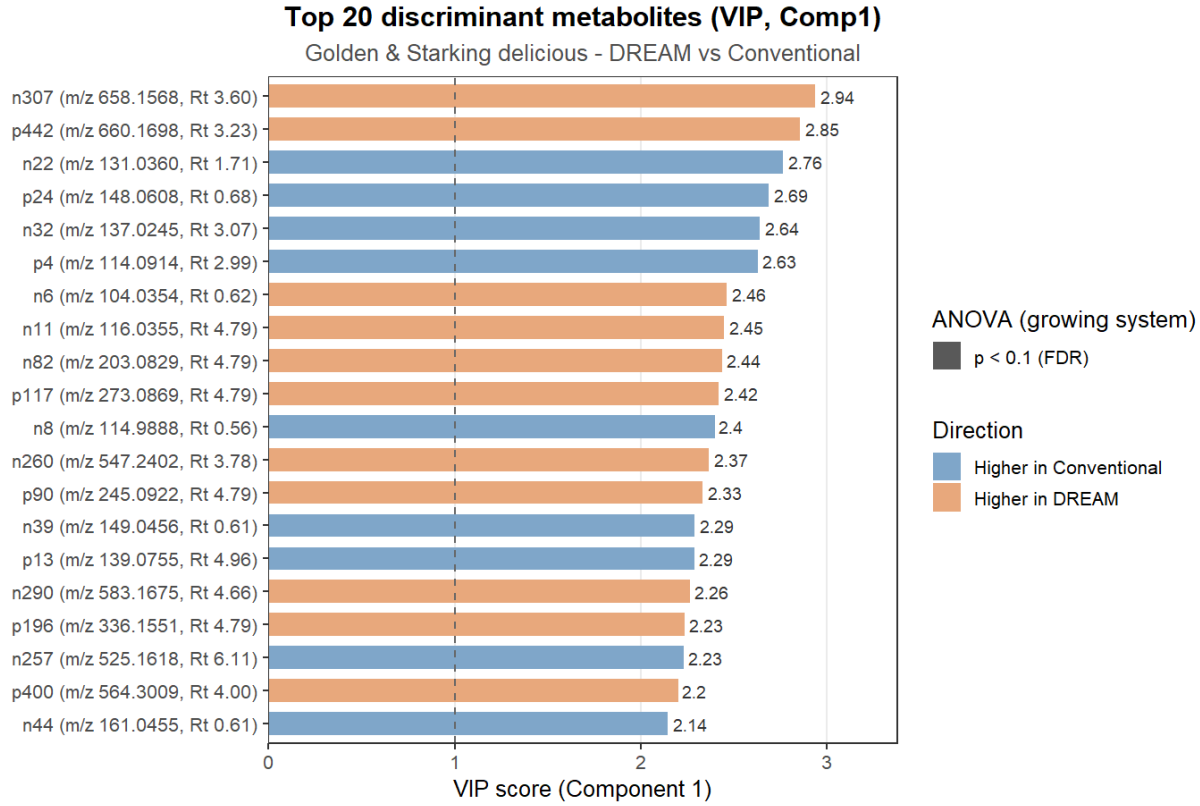


Growing system affects the apple metabolome, but cultivar/variety identity remains the dominant source of variation.

Apple cultivated in Morroco : statistical analysis

Is it possible to separate the sample according to the cultivation mode ?





Apple cultivated in Italy (Bologna)

–

Analysis performed in March 2026

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Apple cultivated in Italy : sampling

DREAM system : 8 varieties


Pink Lady


Abbondanza


Campanino


Renetta Gris de Torriana (RGT)


Gala


Red Pulp

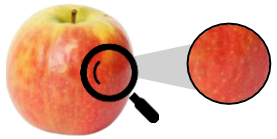

RedUNIBO

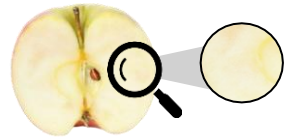

Smeralda

Conventional system : 1 variety


Pink Lady

2 tissues


skin


flesh

Irrigation : 2 conditions

Full irrigation (FI) :

Irrigation of the plants at 100% of crop evapo-transpiration (ETc).

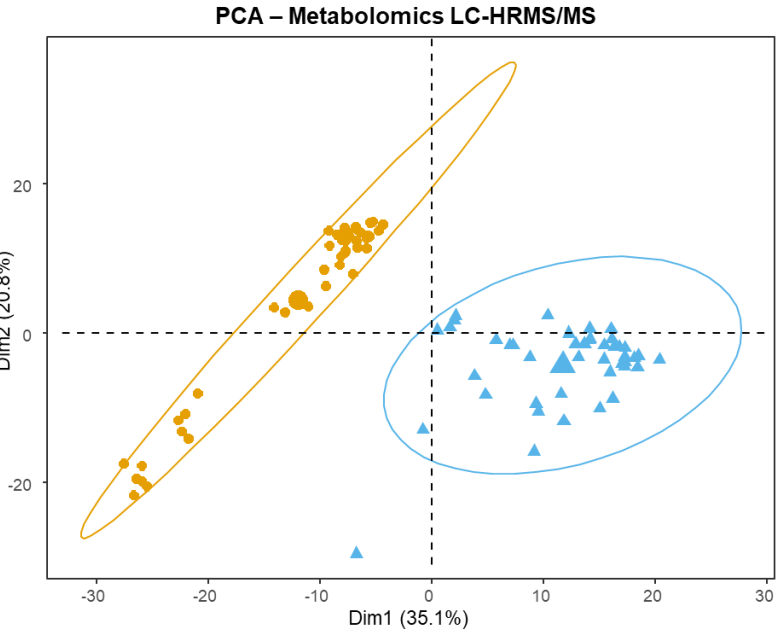
Deficit irrigation (DI) :

From June - 50% of the full irrigation

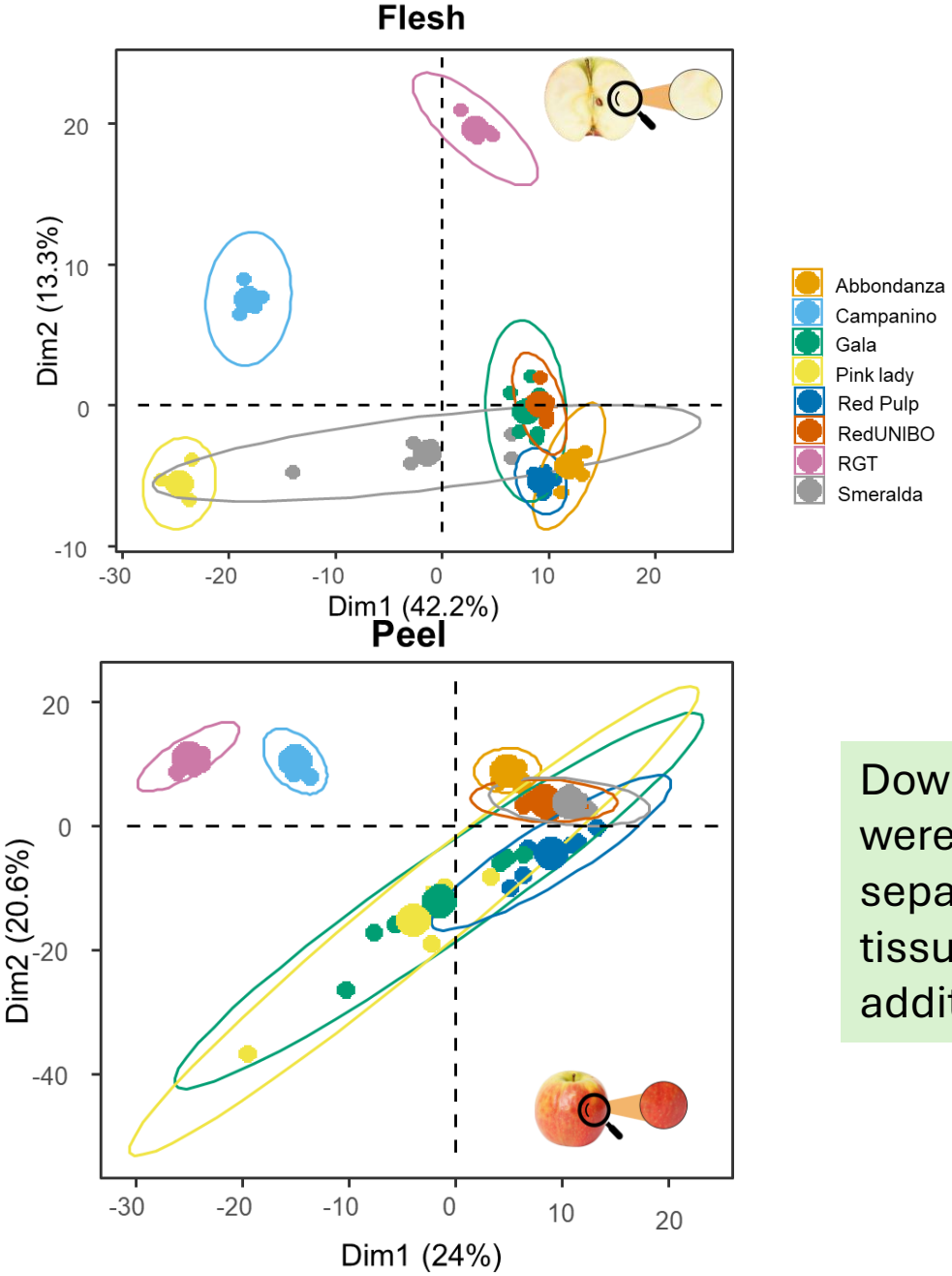
Does the growing system (conventional vs DREAM) affect the metabolome of apple varieties/cultivars (Pink Lady)?
Do the metabolomes of the 8 apple varieties/cultivar differ under DREAM growing conditions ?

Apple cultivated in Italy : tissue composition differ between flesh and peel

Metabolomic data were processing using a workflow including filtering, weight normalization, log-transformation and Pareto scaling prior to statistical analysis.





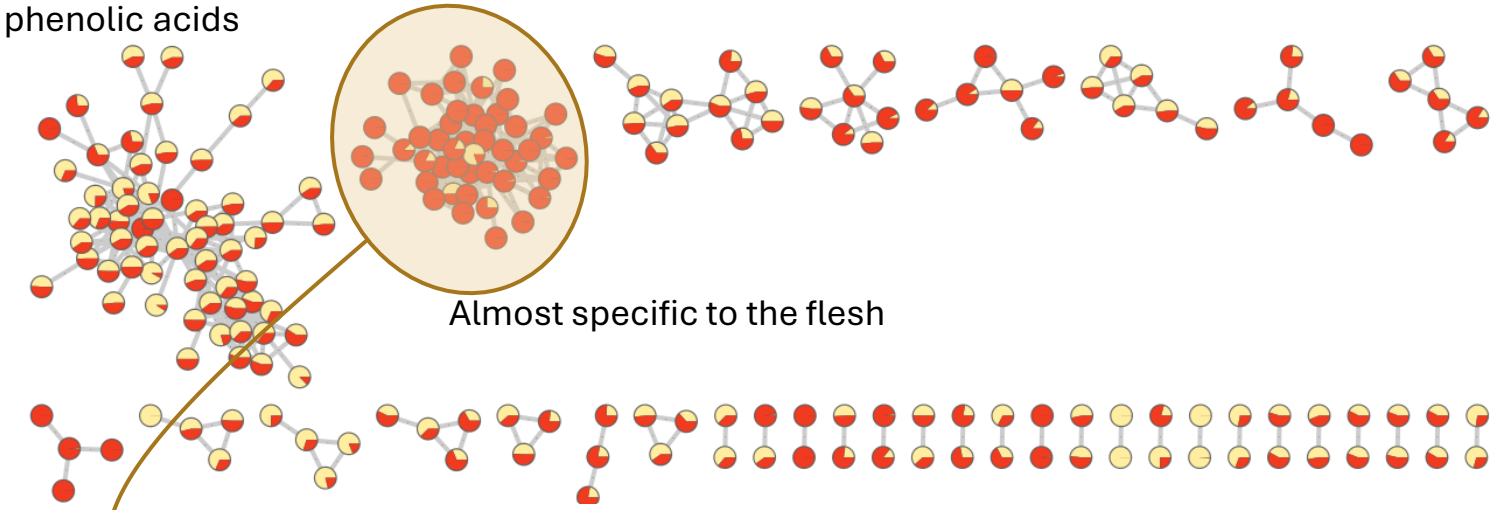


Downstream analysis were conducted separatly for each tissue to better resolve additional effect.

Apple cultivated in Italy : tissue composition differ between flesh and peel

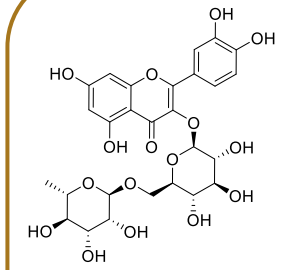


saccharide and small phenolic acids

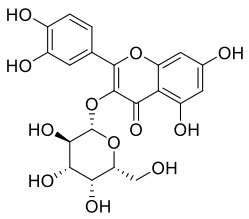


Almost specific to the flesh

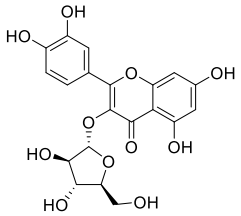
Metabolites annotated in the flesh



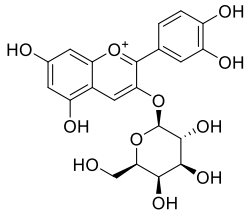
rutin



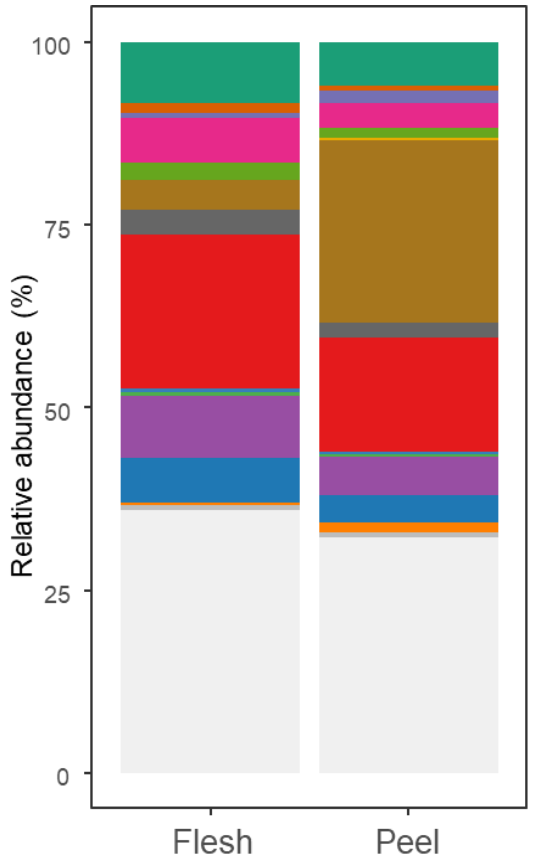
hyperoside



quercetin derivative



cyanidin



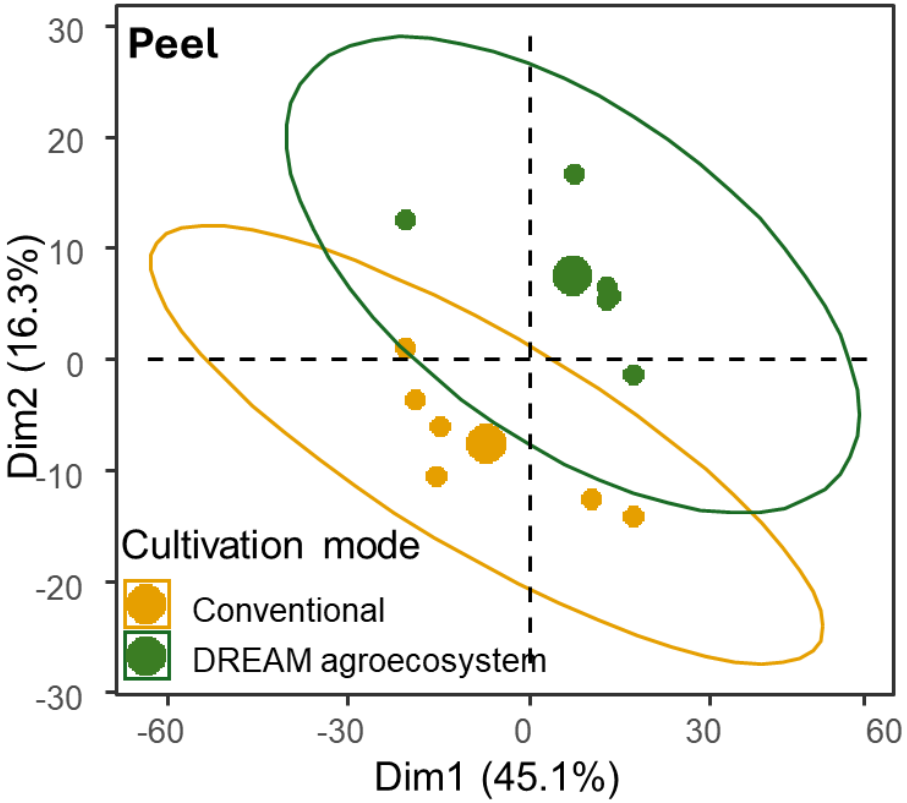
Apple cultivated in Italy : tissue composition differ between flesh and peel



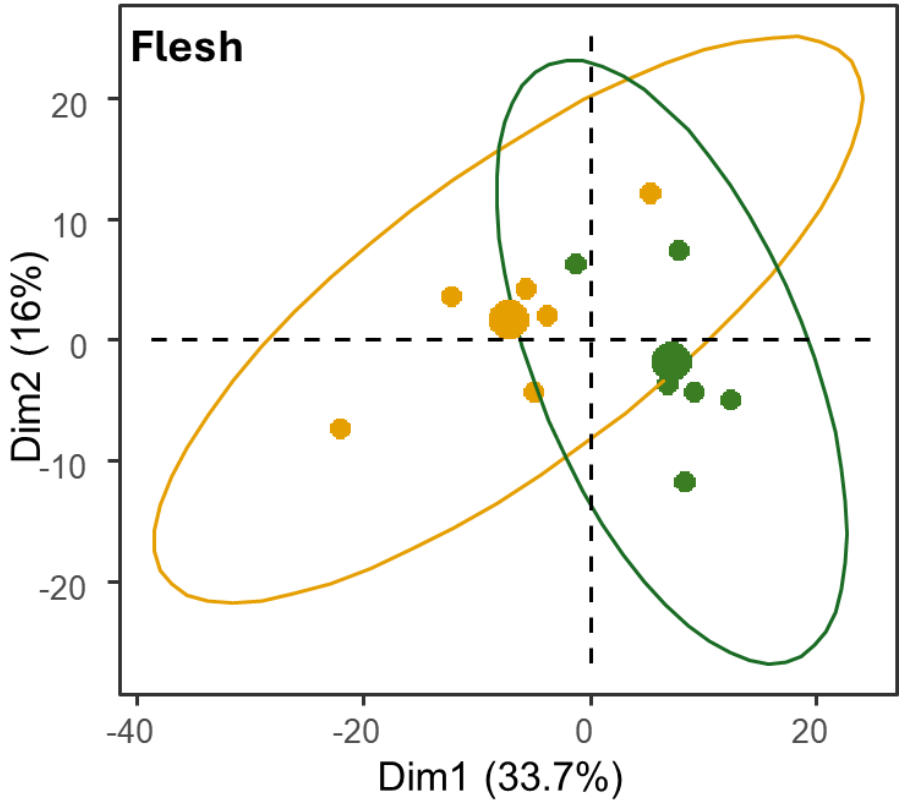
Pink Lady

Is the DREAM system impact the metabolisme of the apple ?

PCA : Peel - Conventional vs DREAM



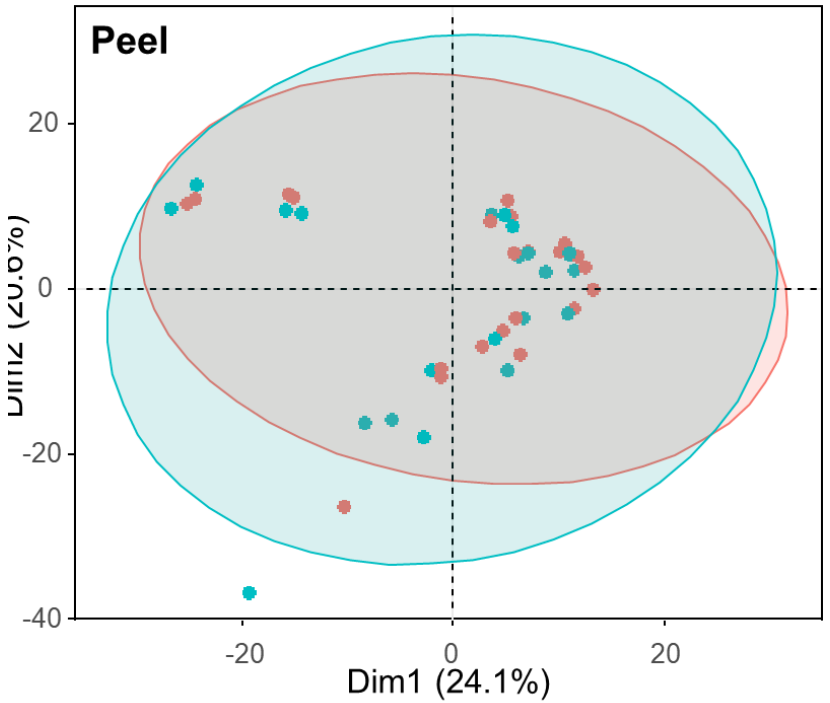
PCA : Flesh - Conventional vs DREAM



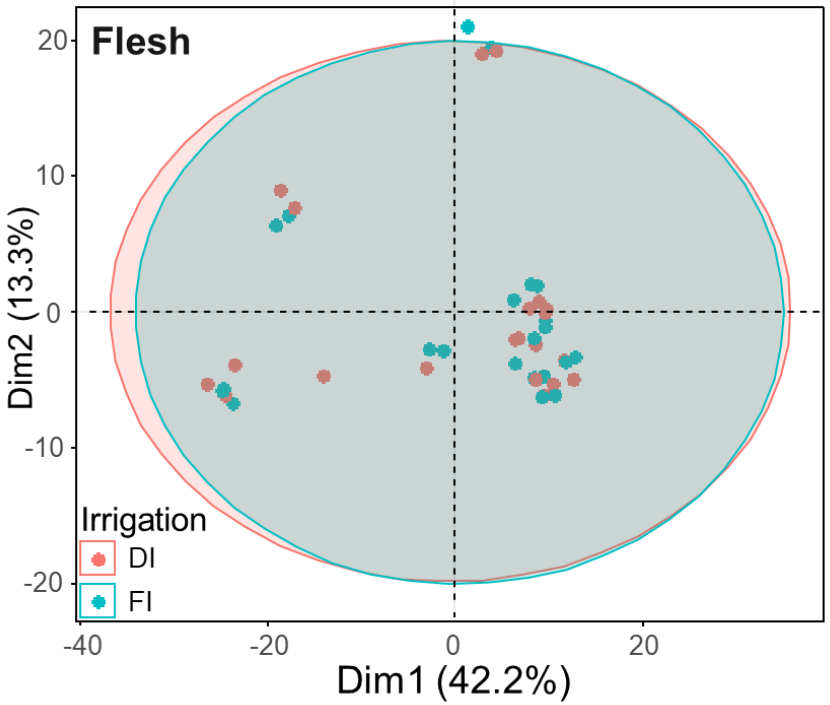
Comparison of Pink Lady apples with and without the DREAM systems showed no clear metabolic effect in the flesh, whereas the peel appeared more affected suggesting a tissue-dependent response

Apple cultivated in Italy : Impact of the irrigation of the plants on the fruit metabolites

PCA : Peel - Conventional vs DREAM



PCA : Flesh - Conventional vs DREAM



No clear metabolic differences were observed between irrigation conditions (FI: full irrigation and DI: deficit irrigation) in either peel or flesh, as also indicated by poor classification performance in PLS-DA between irrigation groups and high cross-validation error rates.

Under the tested conditions, reducing water input does not significatyl alter the overall metabolite composition of the fruit. These results indicate a potential for water-saving irrigation strategies without major impact on fruit metabolic profile

Pear cultivated in Spain

-

Ongoing analysis

Instituto Murciano de Investigación
y Desarrollo Agrario y Medioambiental

Pear cultivated in Spain : sampling

Tissue : 2 compartiments



skin flesh

Other conditions (only for Ercolini varieties) :

- **Cover :** cover vs no cover
- **Treatment :** monovarietal vs multivarietal

Varieties cultivated : 7 varieties



Ercolini



Magallona



Tosca



Etrusca

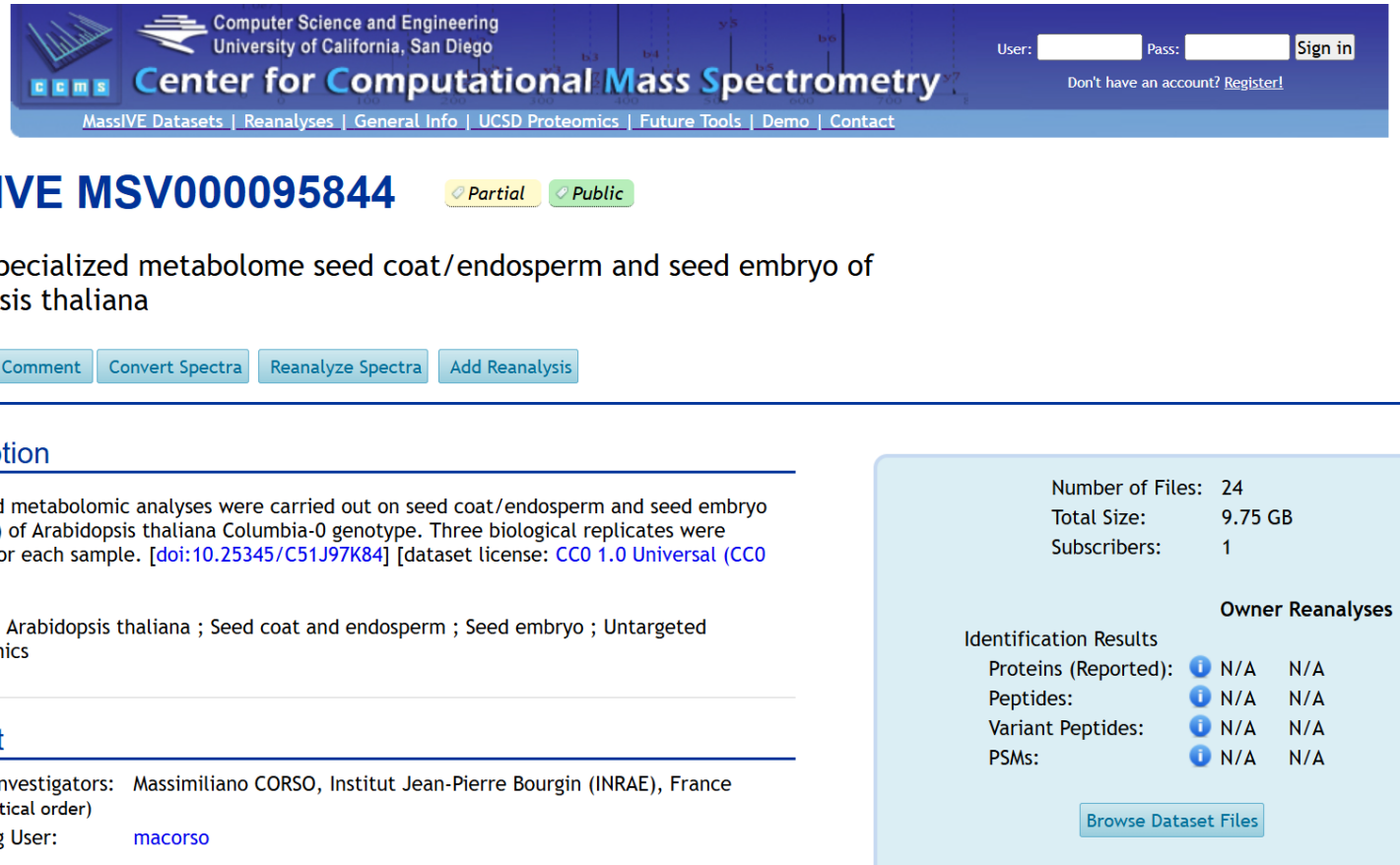


Blanquilla

Moratin

Creation of a dataset from the metabolomic analysis

Example of dataset submitted on Massive



Possibility to add metadata or annotation files in order to use / re-use the data

Conclusion

Apple from Marocco :

- harvesting 2024 : analysis of the sample in negative and positive mode performed

Apple from Italy :

- harvesting 2025 : analysis of the sample in positive mode performed – which comparison need to be performed ?
- harvesting 2026 : ?

Pear from spain

- harvesting 2025 : no pear
- harvesting 2026 : analysis plan on October 2026

Database / dataset

MAMBO

Project ANR – 25 – GDBB – 0004

Component of the distributed infrastructure of the « Grand Défi » for biostimulation and biocontrol for agroecology

Molecul**A**r **M**odelling to Optimize and Develop Tools for Evaluating **B**io-solutions for S**O**il and Plant Health

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   Study of the molecular mechanism of action using multi-omic analyses
5. **Data processing**
   Data integration, biostatistics, prediction and modeling

Coordinators :
Massimiliano Corso, INRAE
Jean-Luc Cacas, AgroParisTech
Manager: Ophélie Merzeaud

The MAMBO team



Massimiliano CORSO
Coordinator of MAMBO



Jean-Luc CACAS
Coordinator of MAMBO



Ophélie MERZEAUD
Manager of MAMBO

SEED-DREAM team

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Nadia BESSOLTANE (Bio-informatician)
Céline BROSSE (IE)
Baptiste GUILLON (PhD student)
Ophélie MERZEAUD (Project manager)
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Damaris GRAIN



Loïc LEPINIEC
DR



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OV-Chimie, Metabolomic Platform IJPB



Stéphanie BOUTET



François PERREAU

Jean-Chrisologue
TOTOZAFY