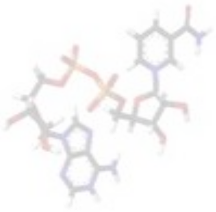
UNIVERSITÀ
CATTOLICA
del Sacro Cuore

Metabolomics in plant-microorganisms interaction

16/09/2024

Biancamaria
Senizza

INTRODUCTION ABOUT THE INTERACTION BETWEEN PLANT SECONDARY METABOLITES AND MICROORGANISMS



WHAT IS METABOLOMICS AND ITS ROLE IN THE INVESTIGATION OF THESE INTERACTIONS

INTRODUCTION ABOUT THE INTERACTION BETWEEN PLANT SECONDARY METABOLITES AND MICROORGANISMS

WHAT IS METABOLOMICS AND ITS ROLE IN THE INVESTIGATION OF THESE INTERACTIONS

Plant metabolites

☐ PRIMARY METABOLITES

☐ Aminoacids

☐ Lipids

☐ Nucleic acids

☐ Carbohydrates..

☐ SECONDARY METABOLITES

☐ Phenolics

☐ Terpenes

☐ N-containing compounds

☐ S-containing compounds

Plant secondary metabolites

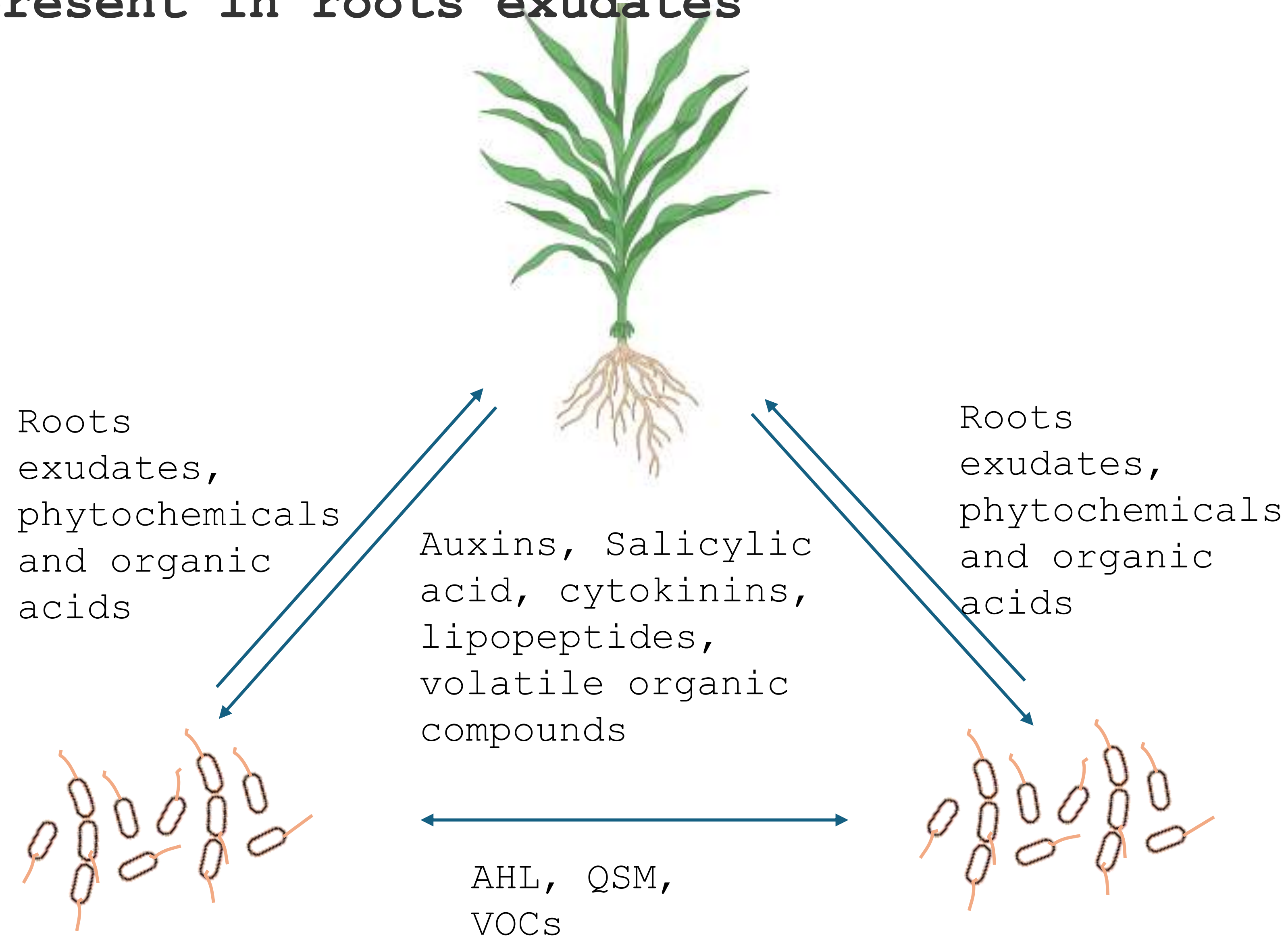
- PRIMARY METABOLITES
- Aminoacids
- Lipids
- Nucleic acids
- Carbohydrates..

- SECONDARY METABOLITES
- Phenolics
- Terpenes
- N-containing compounds
- S-containing compounds

The secondary metabolites are linked to plant microbiomes

This relationship is dynamic and involve several bi-directional exchanges: some microbes adjust the production of secondary metabolites and these bioactive molecules shape the soil microbiome

Compounds secreted by the microbes and metabolite profiles present in roots exudates



Roots exudates, phytochemicals and organic acids

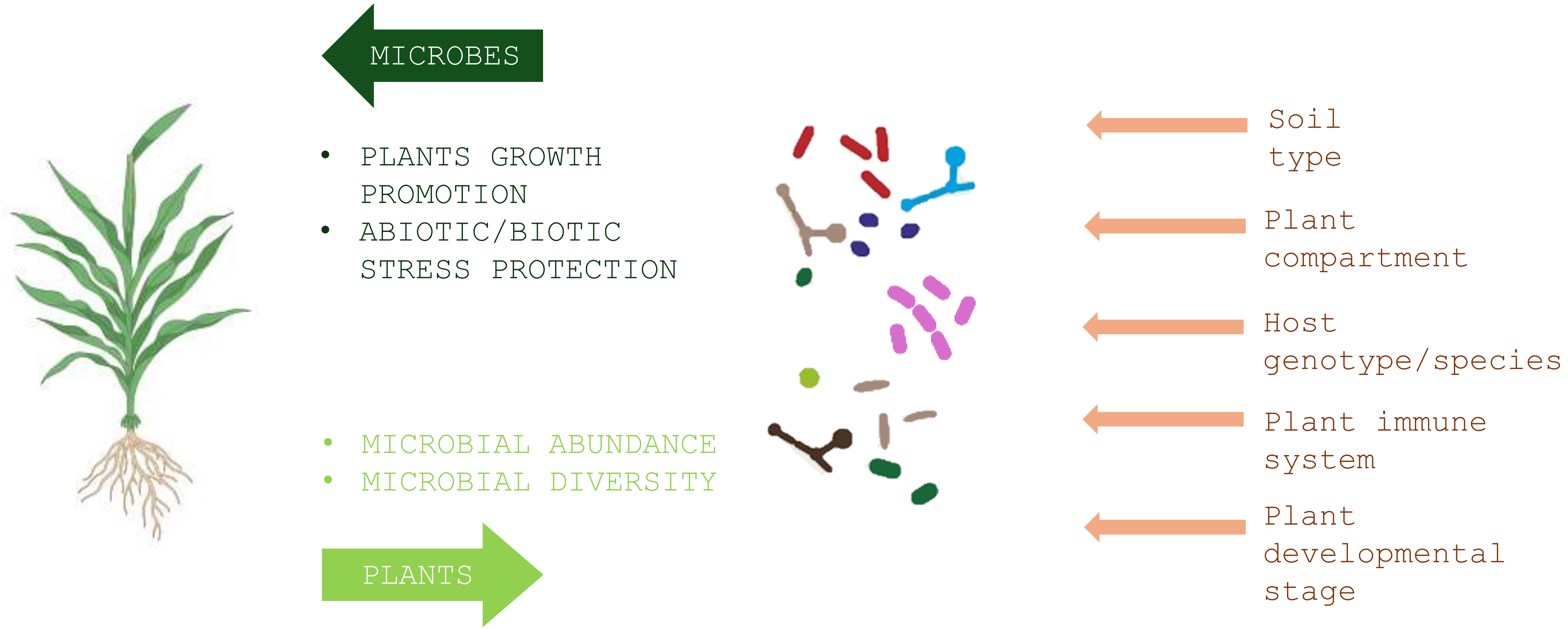
Auxins, Salicylic acid, cytokinins, lipopeptides, volatile organic compounds

Roots exudates, phytochemicals and organic acids

AHL, QSM, VOCs

Rhizobacteria

Rhizobacteria



MICROBES

- PLANTS GROWTH PROMOTION
- ABIOTIC/BIOTIC STRESS PROTECTION

- MICROBIAL ABUNDANCE
- MICROBIAL DIVERSITY

PLANTS

Soil type

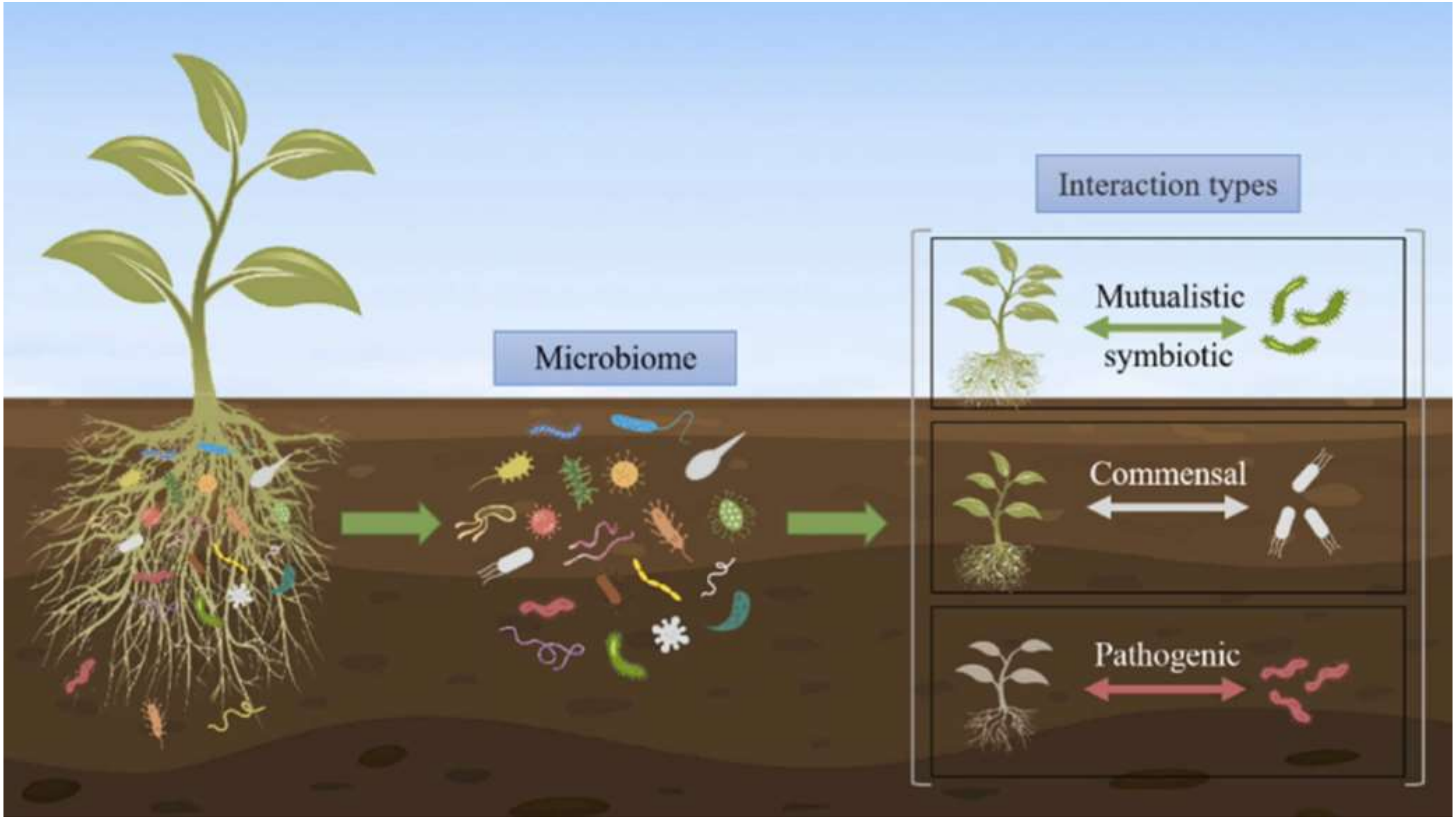
Plant compartment

Host genotype/species

Plant immune system

Plant developmental stage

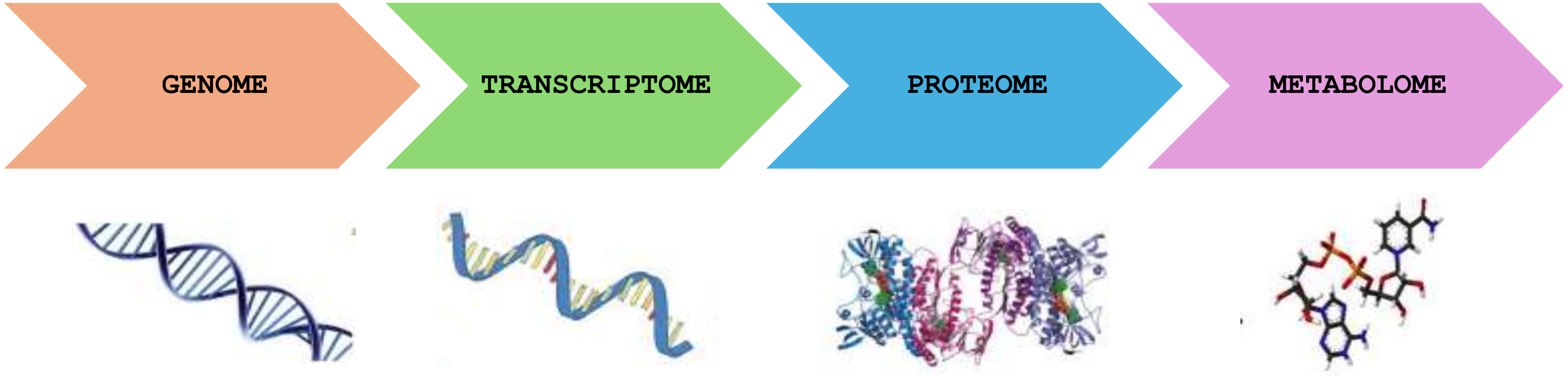
PLANT-MICROBE INTERACTIONS



PLANT METABOLOMICS

-Qualitatively and quantitatively analyze the metabolites of living systems and their dynamic responses to changes in the environment;

-Provides a snapshot of plant metabolism during development or under biotic and abiotic stimuli, including environmental or nutritional stresses.



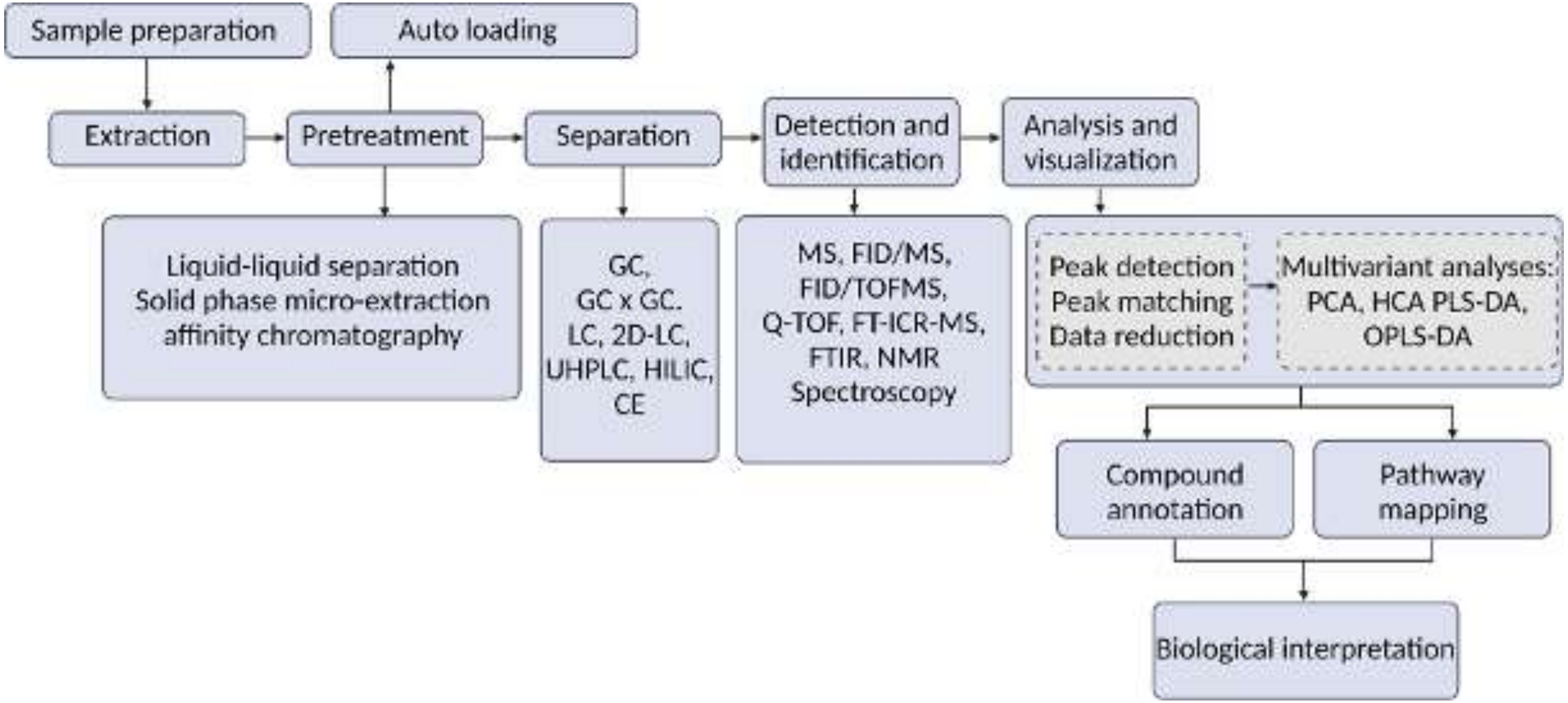
GENOME

TRANSCRIPTOME

PROTEOME

METABOLOME

Metabolomics analysis flow for plant–microbe interactions research



Sample preparation

Auto loading

Extraction

Pretreatment

Separation

Detection and identification

Analysis and visualization

Liquid-liquid separation
Solid phase micro-extraction
affinity chromatography

GC,
GC x GC.
LC, 2D-LC,
UHPLC, HILIC,
CE

MS, FID/MS,
FID/TOFMS,
Q-TOF, FT-ICR-MS,
FTIR, NMR
Spectroscopy

Peak detection
Peak matching
Data reduction

Multivariant analyses:
PCA, HCA PLS-DA,
OPLS-DA

Compound annotation

Pathway mapping

Biological interpretation

Workflow for plant metabolomics

BIOLOGICAL MATRIX

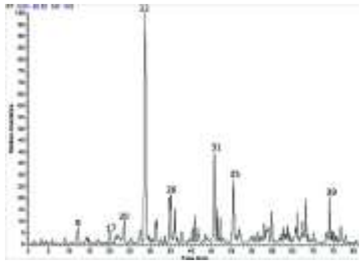
Leaves

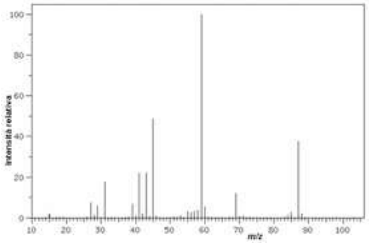
Fruits

Roots

Microorganisms

DATA ACQUISITION AND DATA PROCESSING





Noise filtering
Peak detection
Alignment
Normalization

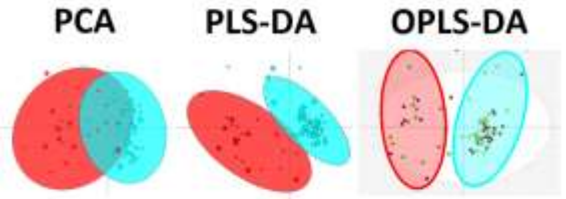
STATISTICAL ANALYSIS

MULTIVARIATE

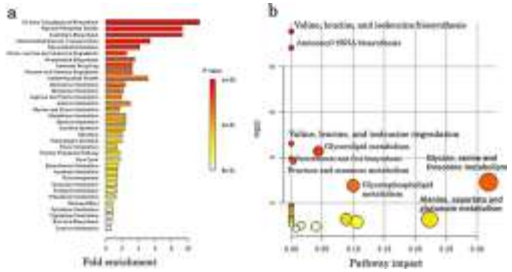
HCA
PCA
PLS-DA
OPLS-DA

UNIVARIATE

ANOVA
T-tests
Linear mode



DATA INTERPRETATION

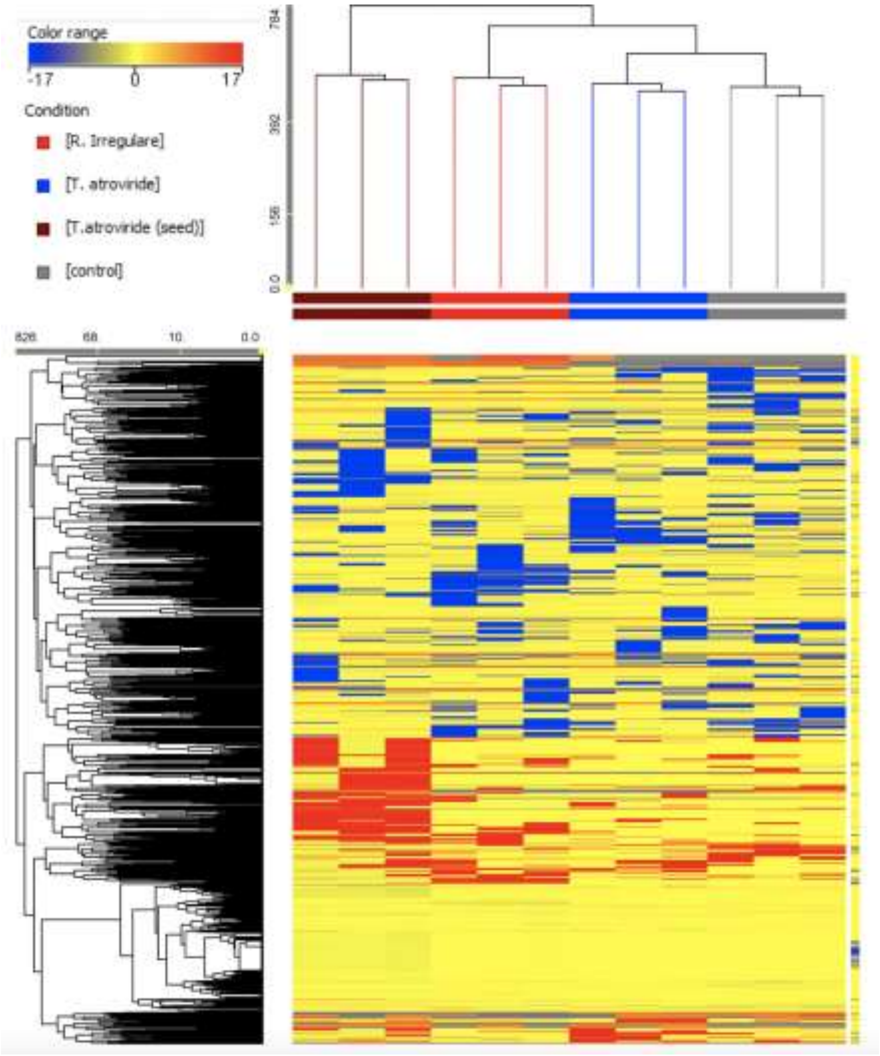


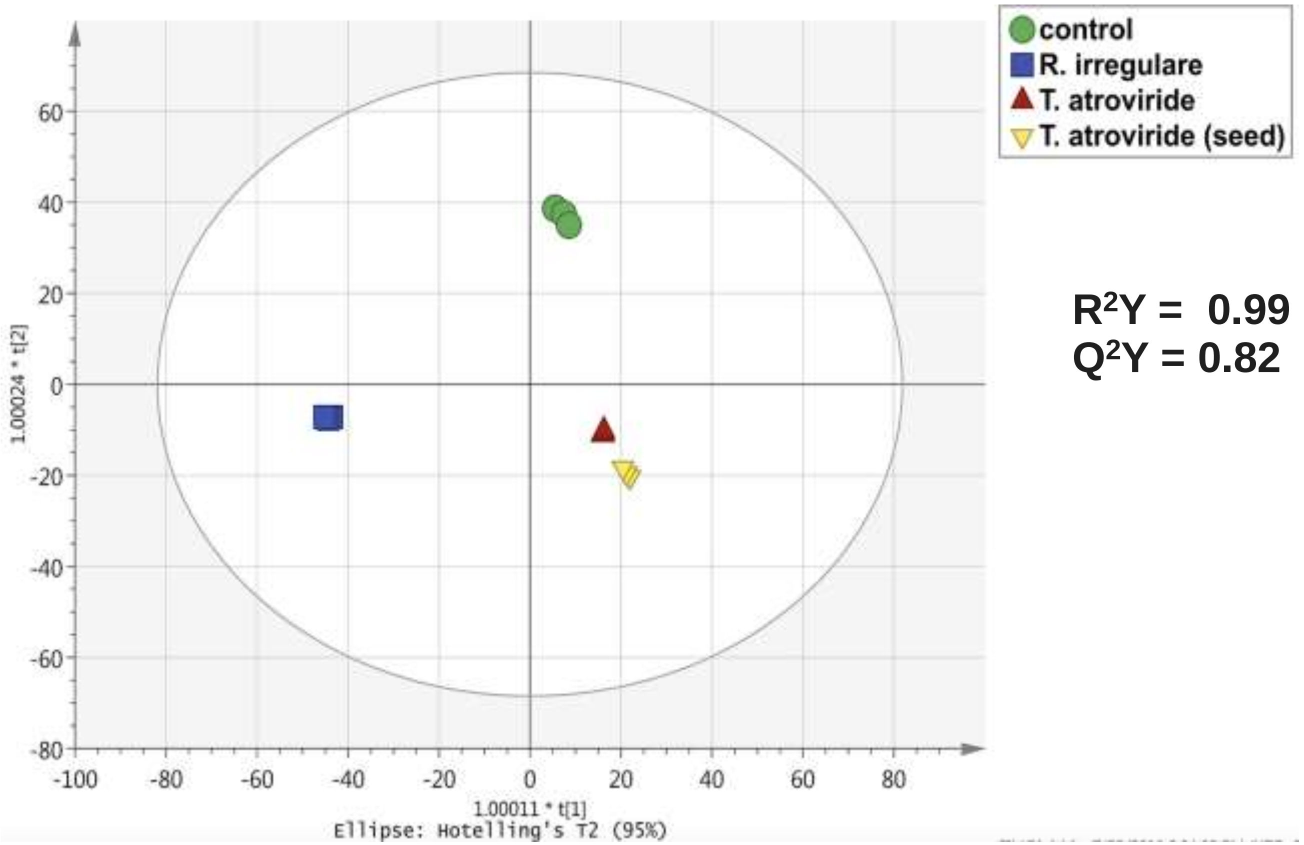
PMN

The Plant Metabolic Network

Is the plant’s secondary metabolism affected by microorganisms?

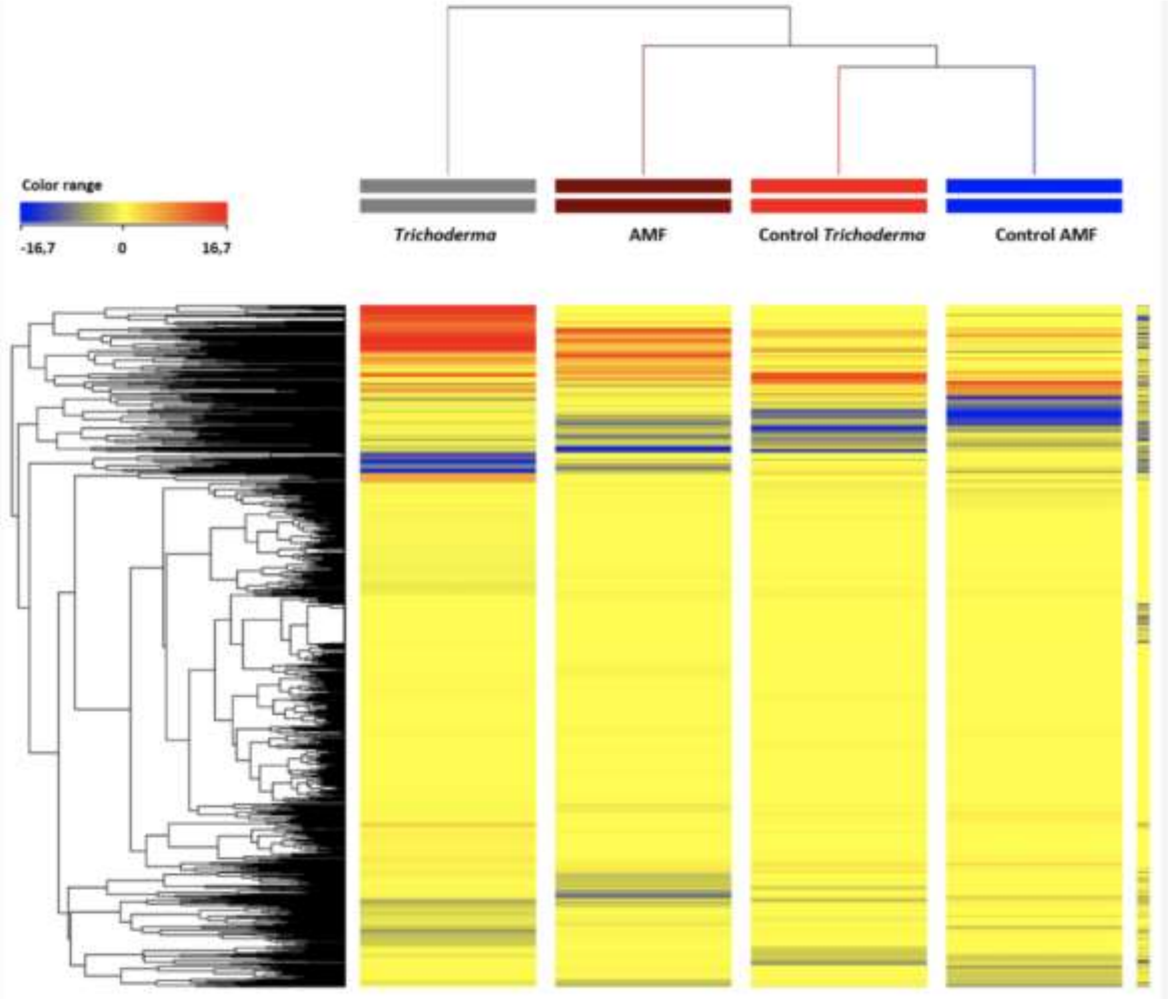
Supervised and unsupervised analysis

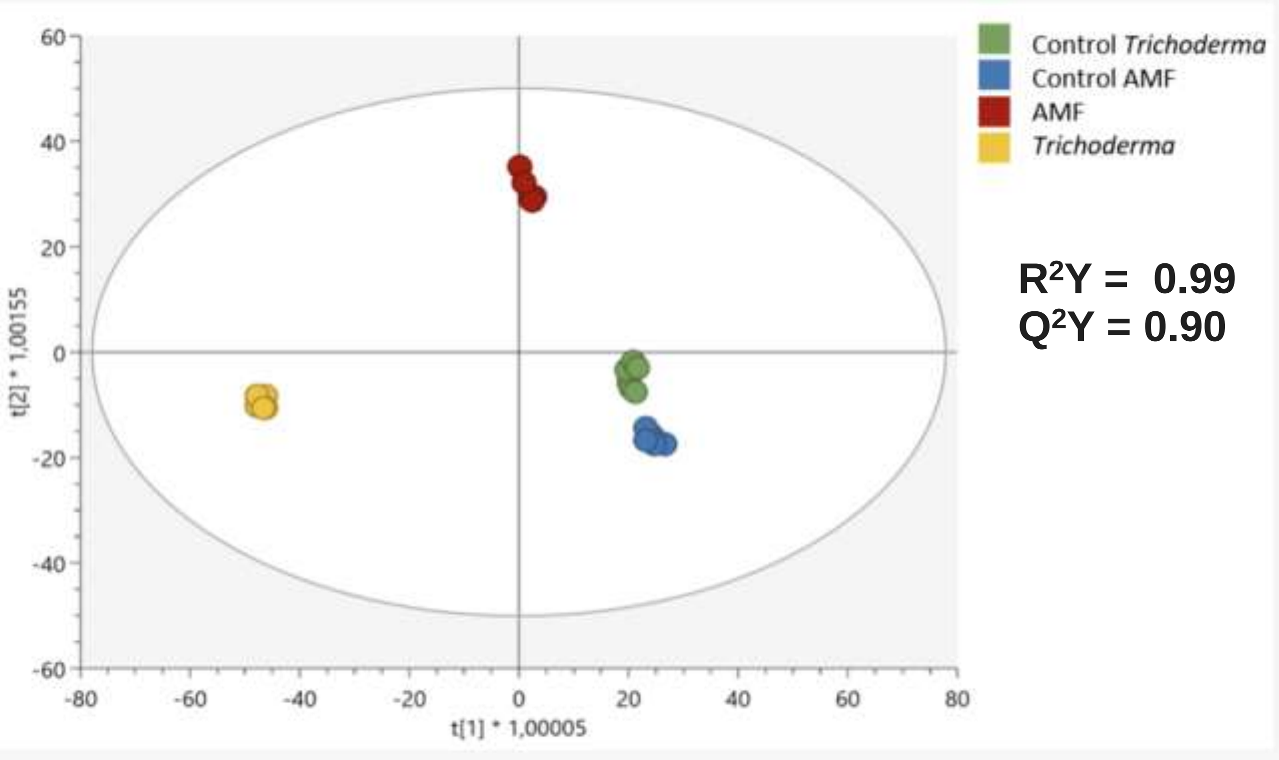




$R^2Y = 0.99$
$Q^2Y = 0.82$

Supervised and unsupervised analysis



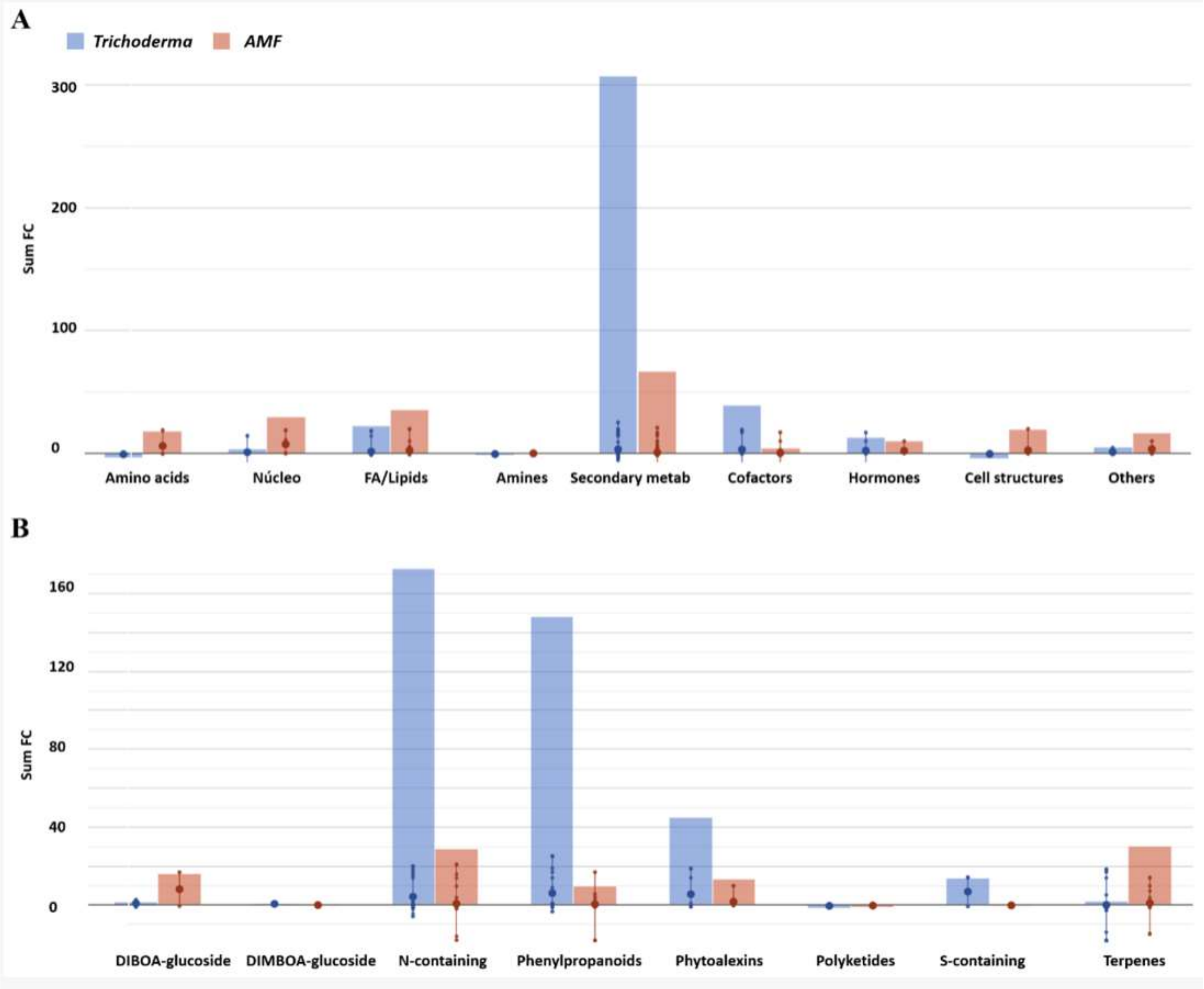


R^2Y = 0.99
Q^2Y = 0.90

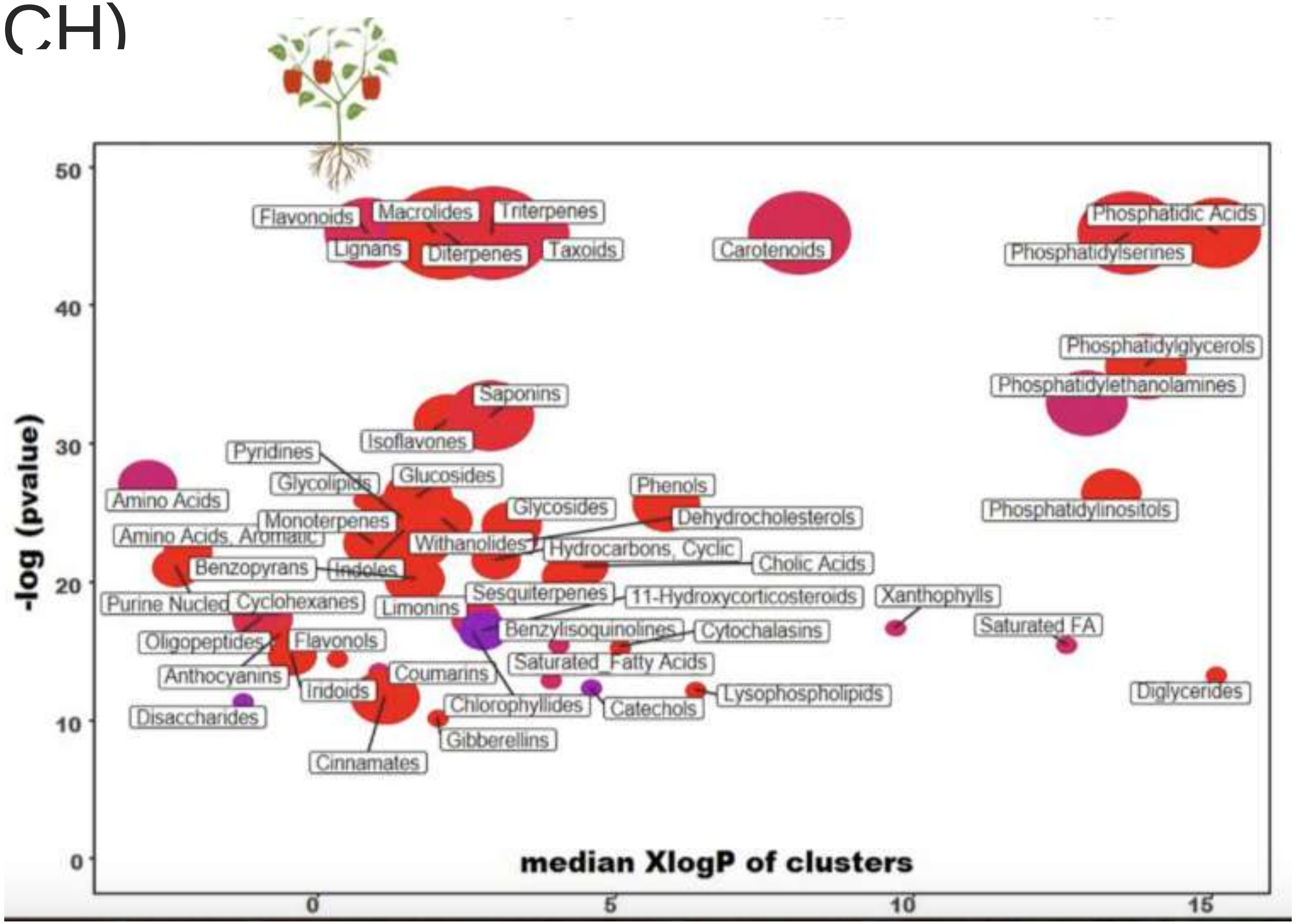
VIP markers

		SCORE	Std error
Phenolics	phaseoluteone	1.44	0.37
	medicarpin-3-*O*-glucoside-6″-malonate	1.43	0.36
	3,6,7,2′,4′-pentamethylquercetagetin	1.41	0.38
	7-hydroxy-4′5′-dimethoxyisoflavone	1.36	0.59
	esculin	1.38	0.69
	robustaquinone H	1.36	0.41
	coniferyl acetate	1.35	0.42
Terpenoids	tetraprenyl-beta-curcumene	1.52	0.56
	soyasapogenol -3-*O*-beta-glucuronide	1.39	0.58
	juvenile hormone III	1.37	0.52
	3-hydroxylubimin	1.37	0.79
	kauralexin A2	1.34	0.35
	phytyl monophosphate	1.34	0.44
	Eudesmol/bisabolol	1.33	0.87
	(20S)-ginsenoside	1.53	0.50
Hormones	dihydrozeatin-*O*-glucoside	1.50	0.54
	(22R,23R)-28-homocastasterone	1.40	0.51
	salicyl-6-hydroxy-2-cyclohexene-on-oyl	1.49	0.63
	indole-3-acetyl-beta-6-D-glucose	1.37	0.35

Pathway analysis



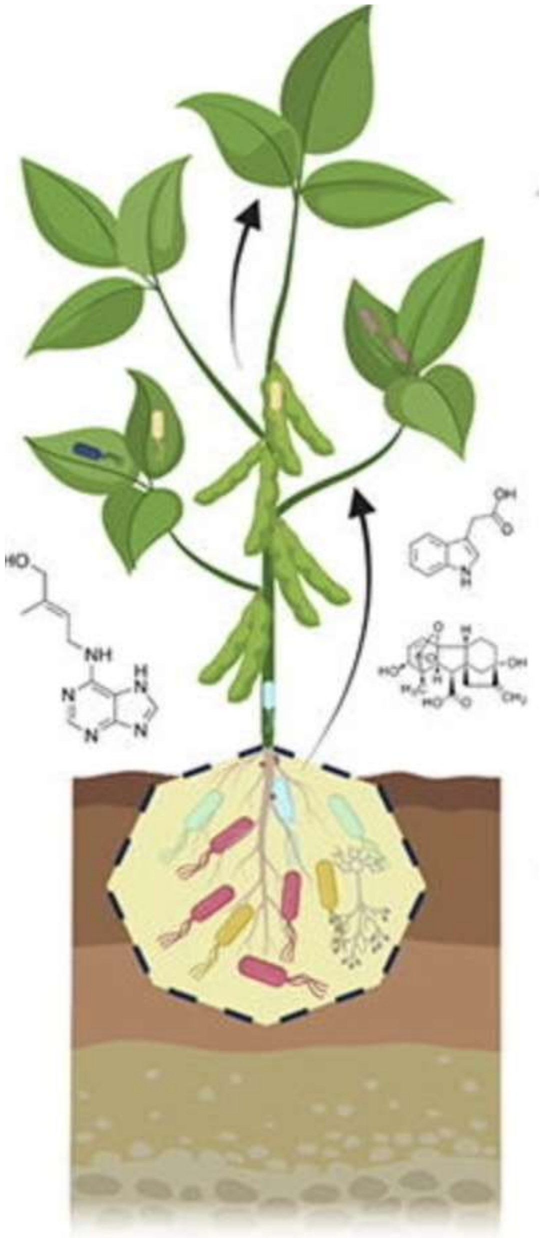
Chemical similarity enrichment analysis (ChemRICH)



These results suggest that metabolomics could discriminate the different microbial/fungi inoculations and how these can modulate the roots/exudates profile→ contribute to the elucidation of such complex processes;

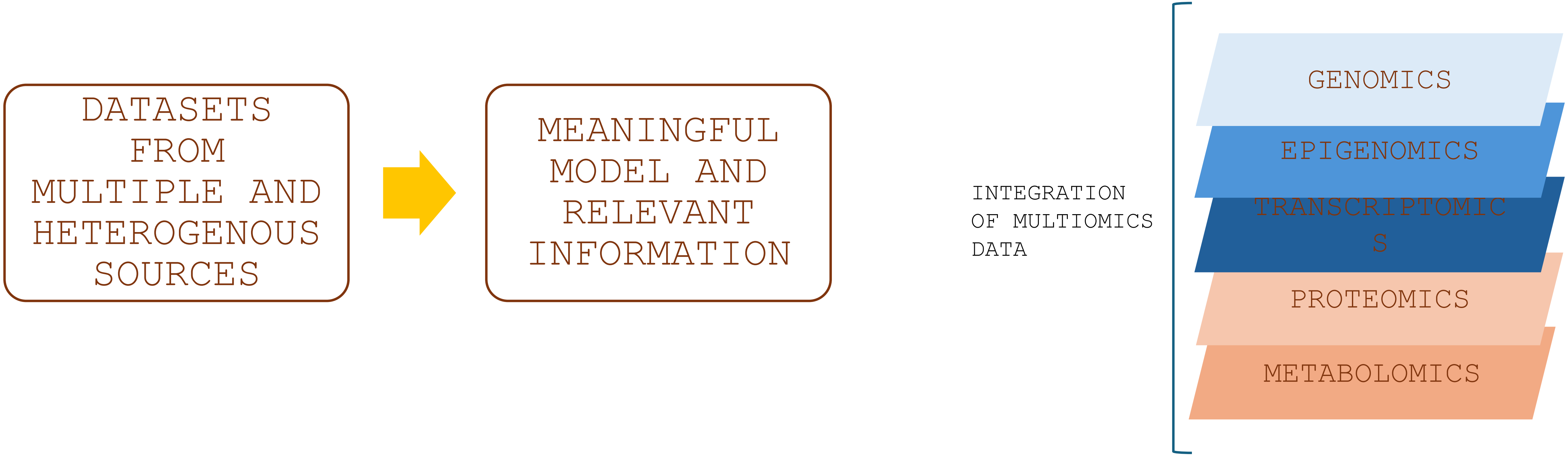
Know more about the modulation of the phytochemical composition of the roots/ exudates could contributes to better understand the beneficial/harmful microbial-induced effect on plant growth.

How to assess simultaneously in one experimental design the changes in both the plant host metabolism and its microbiota?



Multi-omics approach

The multi-omics approach could be used as an integrated analytical strategy to resolve the coordinated and complex dynamic interactions between the plant and its rhizosphere bacteria



DATASETS FROM MULTIPLE AND HETEROGENOUS SOURCES

MEANINGFUL MODEL AND RELEVANT INFORMATION

INTEGRATION OF MULTIOMICS DATA

GENOMICS

EPIGENOMICS

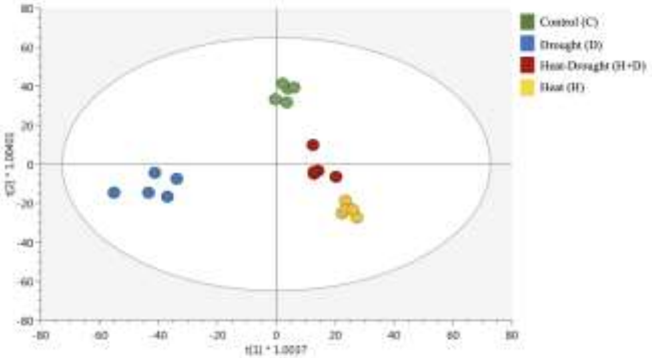
TRANSCRIPTOMICS

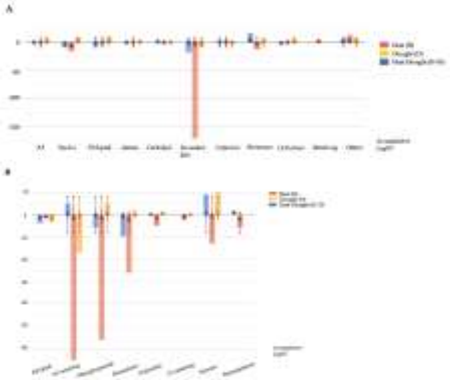
PROTEOMICS

METABOLOMICS

Metabolomics

ROOTS/EXUDATES

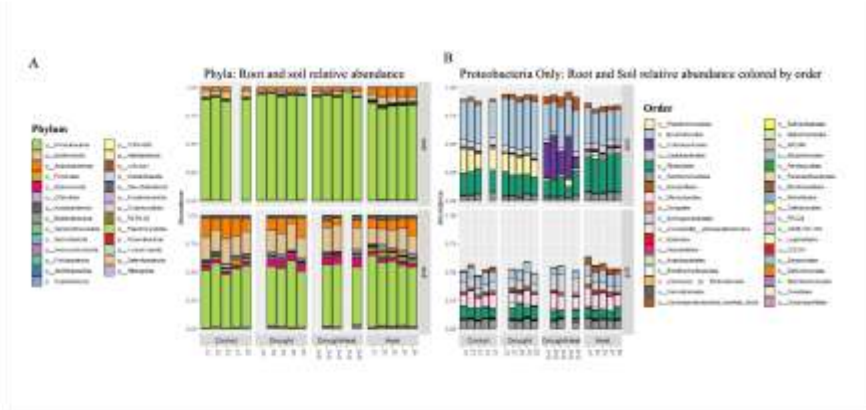


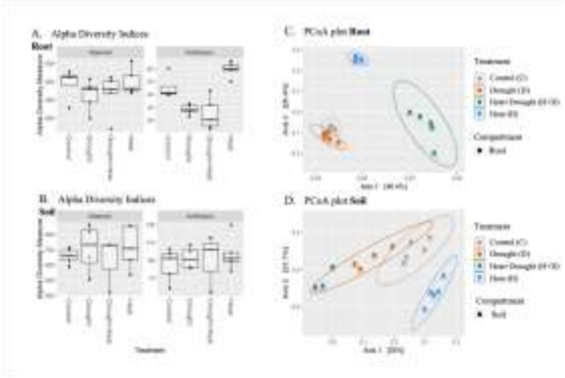


MultiOmics integration

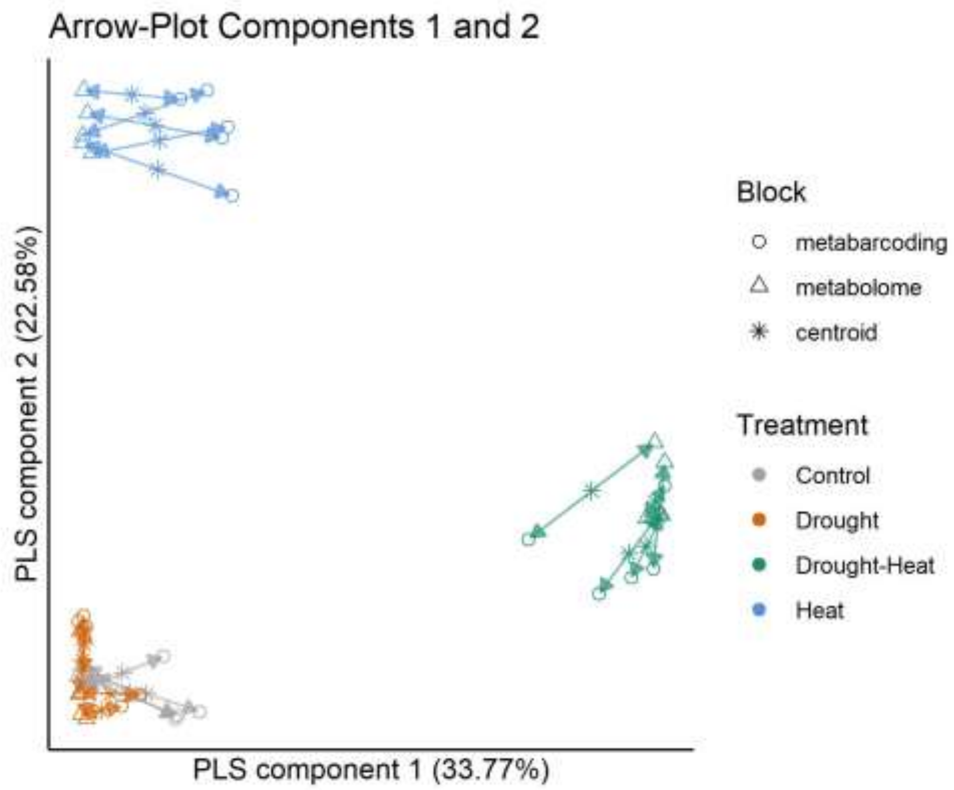
ROOTS-RHIZOSPHERE

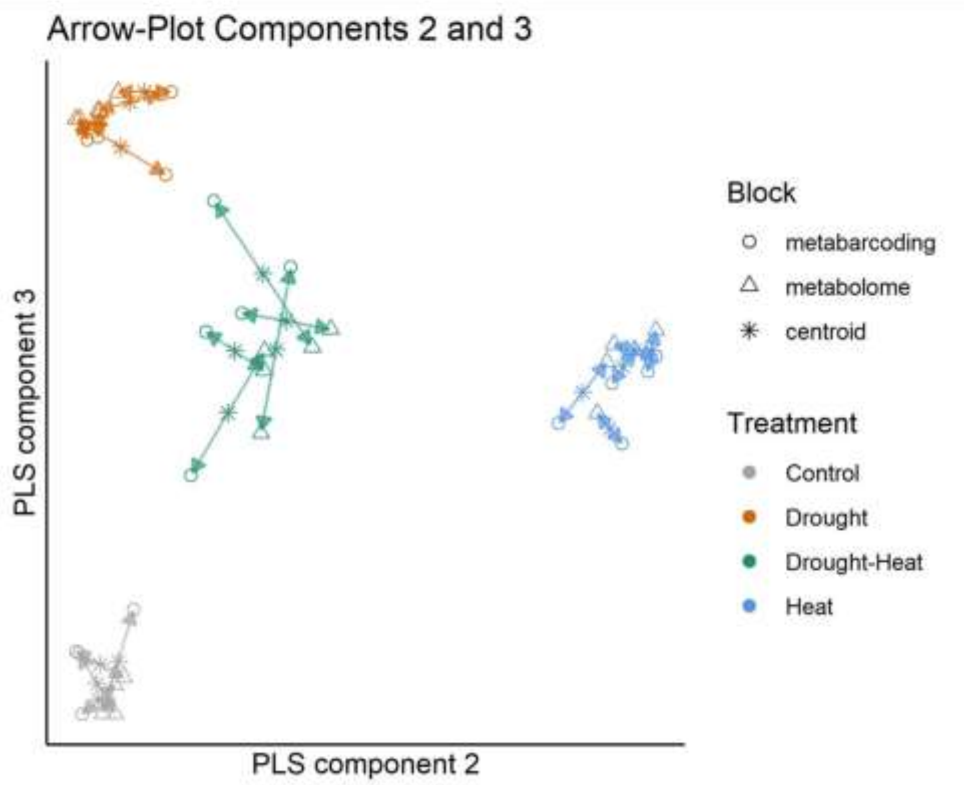
Metagenomics





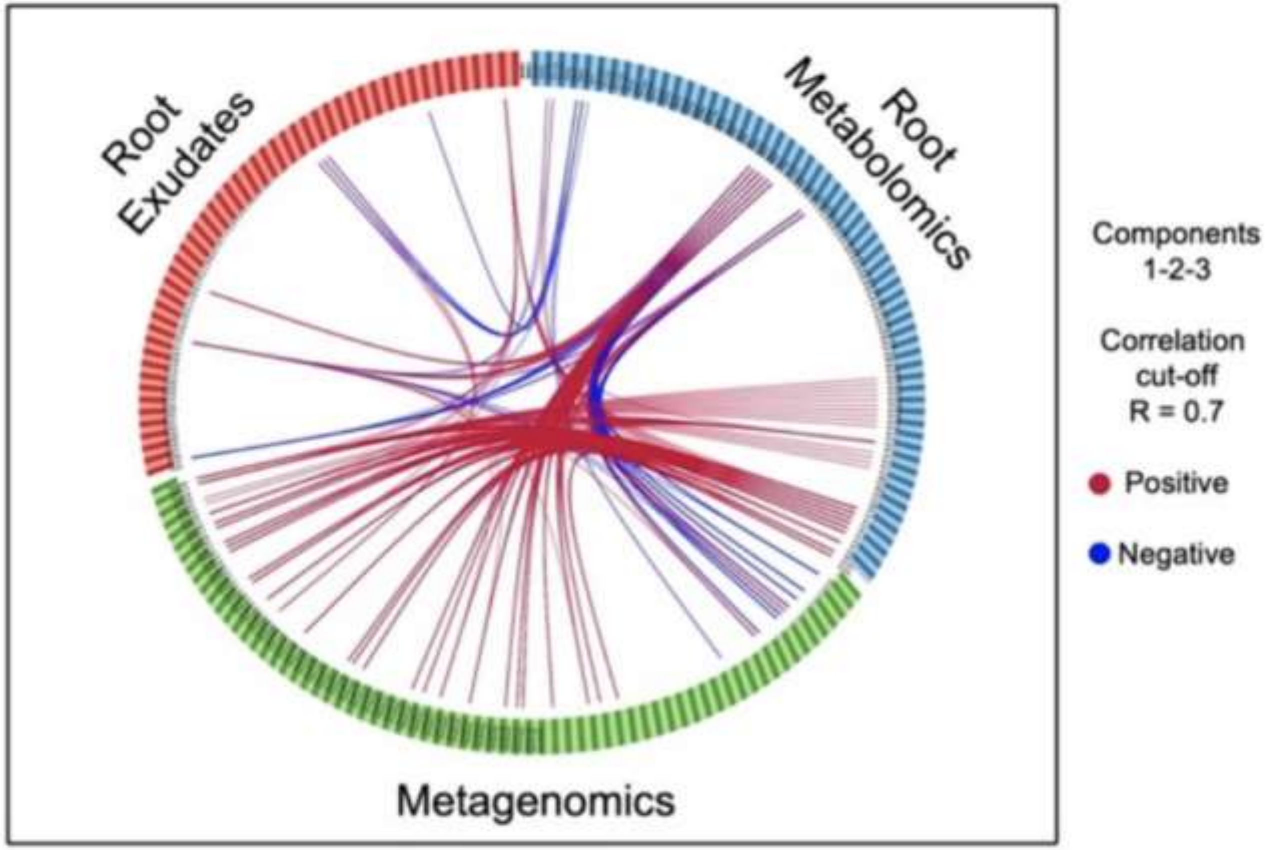
DIABLO framework with multiblock sparse PLS-DA





The datasets are highly correlated for all three components.
The agreement between metabarcoding and metabolomics is high for all samples and treatments.

Circos plot from multiblock sPLS-DA



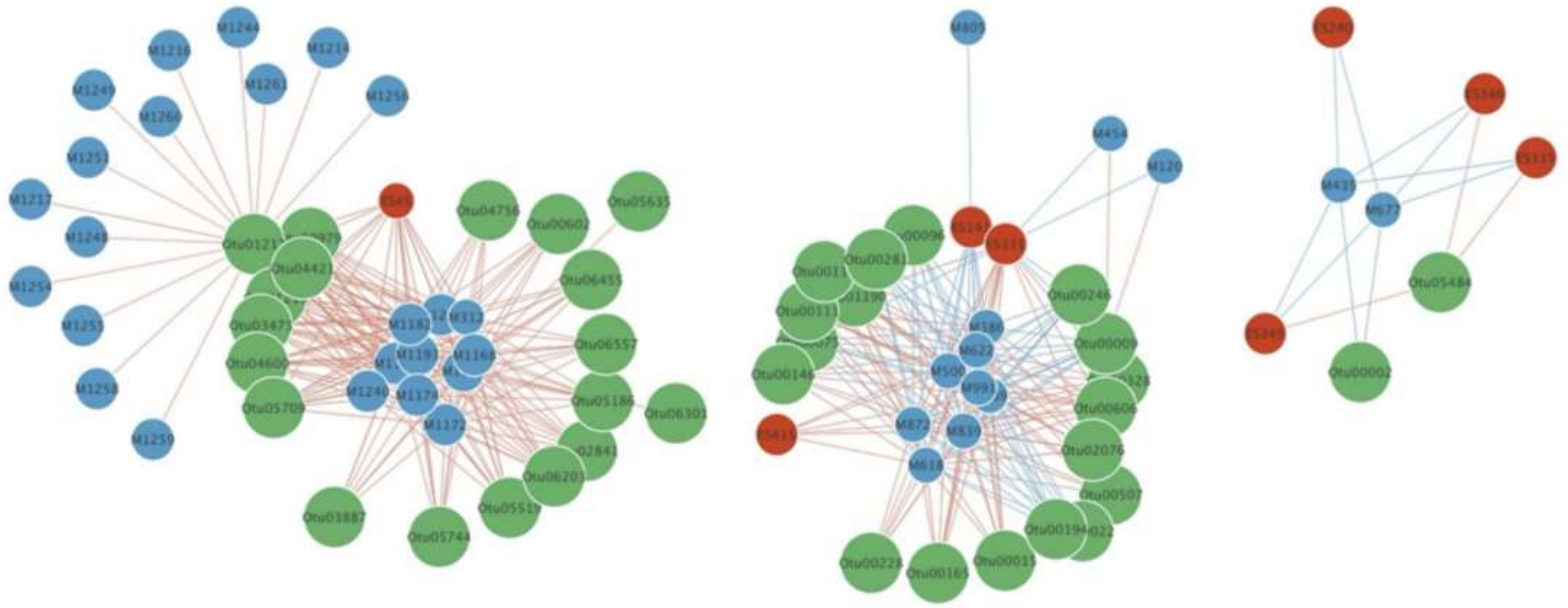
Correlations > 0.7 between variables of different types

NETWORK ANALYSIS

to provide information on variables belonging to the same biological pathway.

● Metabolomics
● Metagenomics
● Root exudates

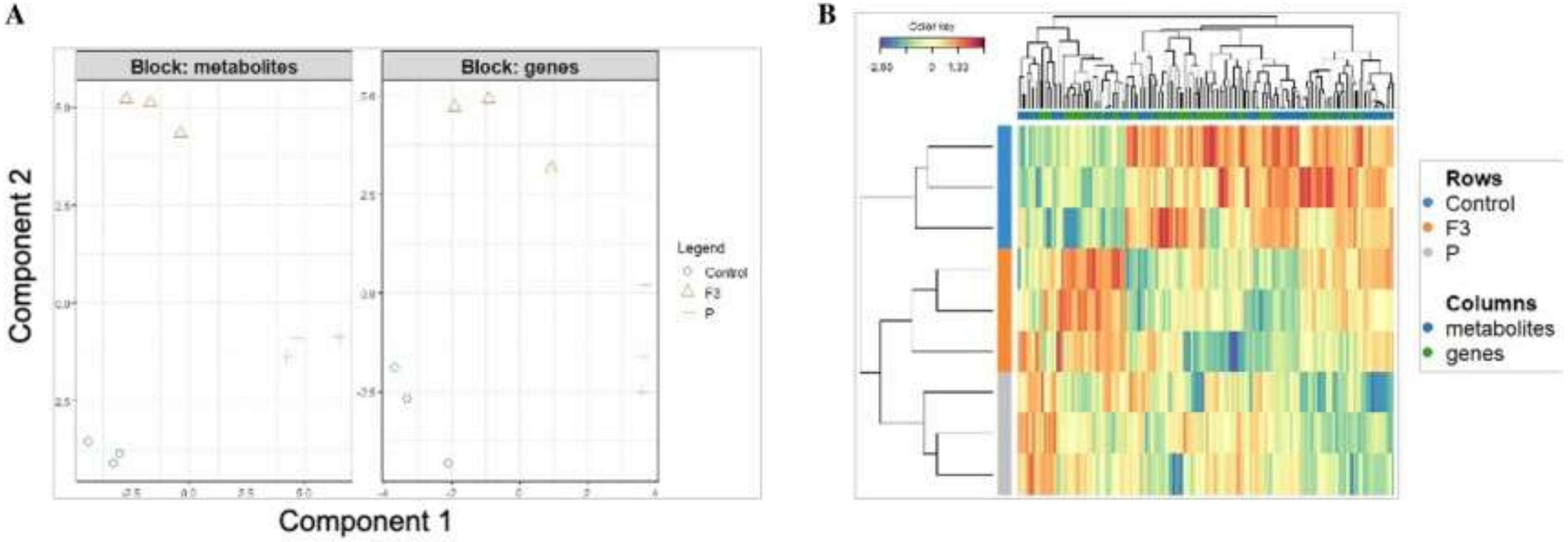
Pearson's correlation analysis ($r > 0.7$)



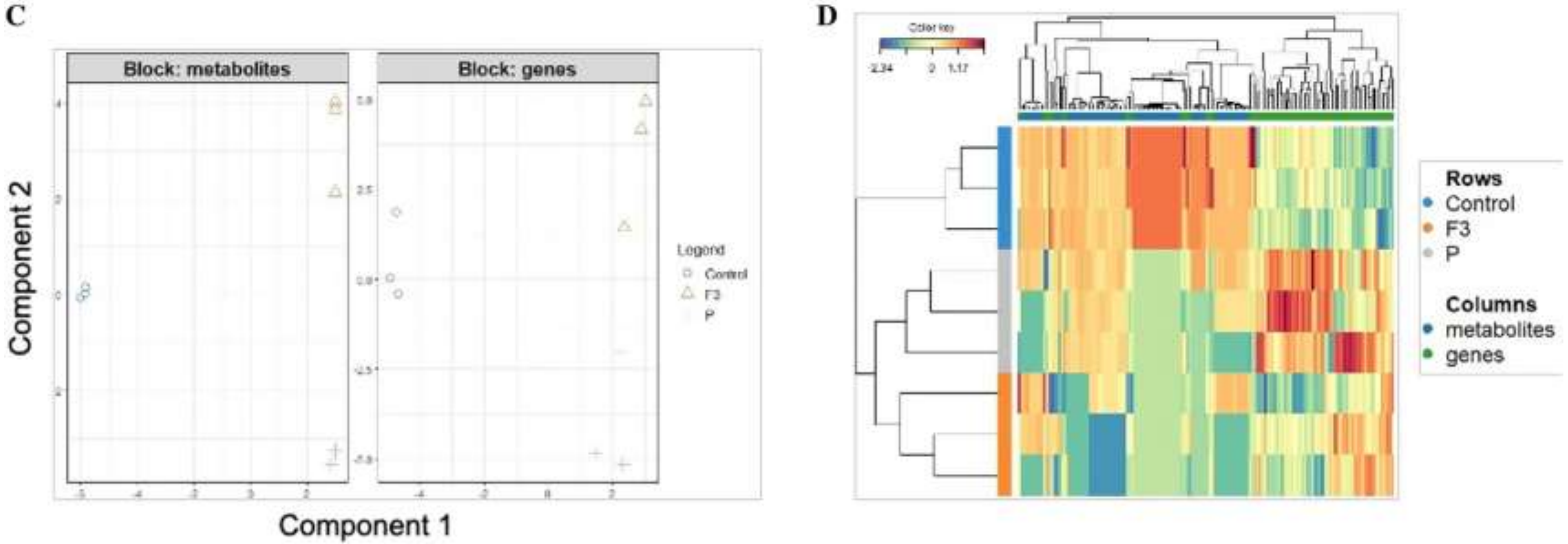
Network made with the variables highly correlated among the three datasets. The line color indicates the positive (red) or negative (blue) correlations. The node size is correlated to the degree of their interactions.

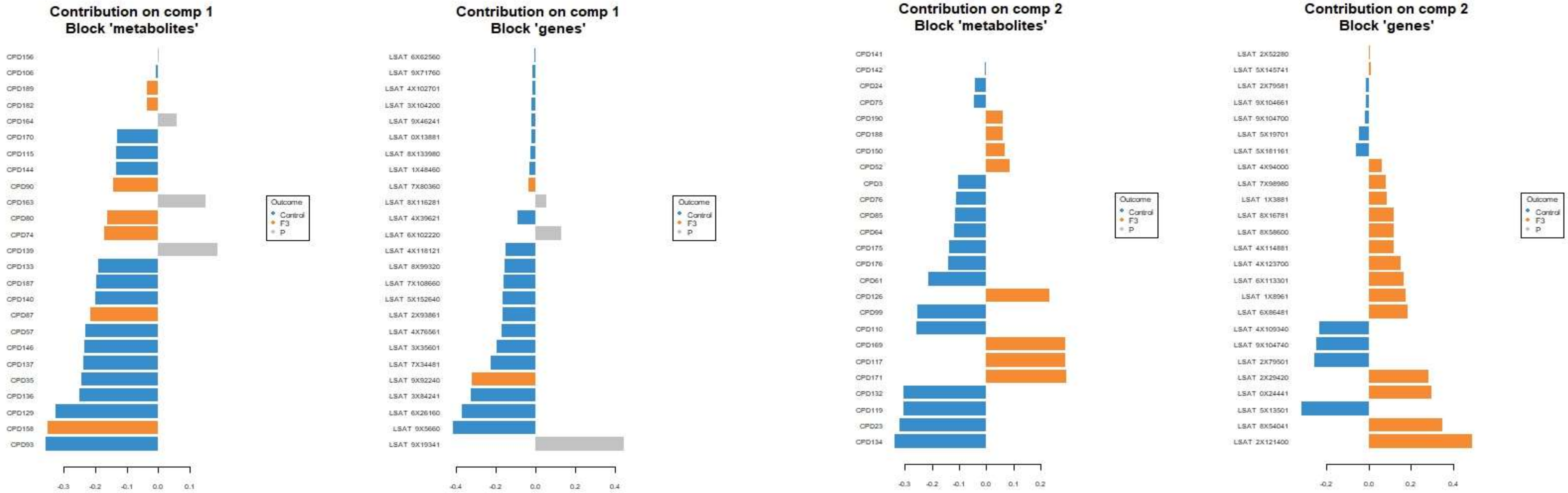
Block contribution and cluster analysis

Non-stress condition



Stress condition





Significant features (metabolites and genes) mainly responsible for the discrimination between the treatments

Conclusions

The omics sciences coupled with tools that filter and analyze huge amount of data, permit to understand the complex interactions between plants and microbes at molecular level;

Data integration may provide a comprehensive view of the mechanisms of biological process and pinpoint the molecular markers by disclosing the regulatory units across diverse omics layers

THANK YOU FOR YOUR ATTENTION