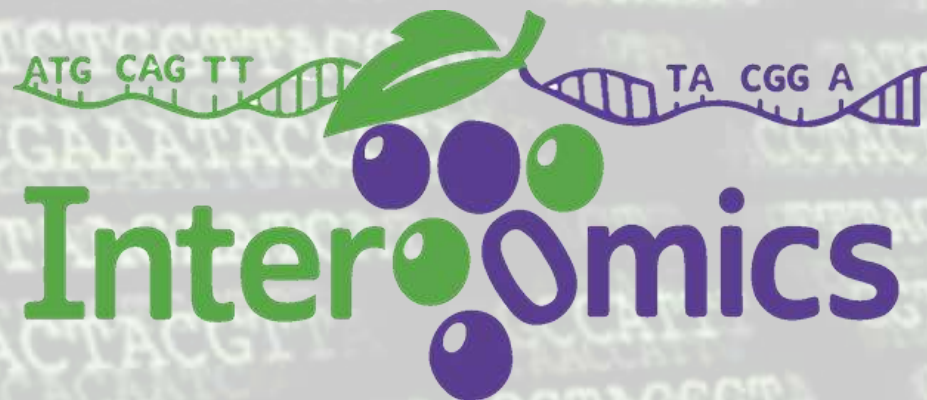


**Kick-off project meeting**  
**13<sup>th</sup> March 2026**

*Decoding interplay between grapevine pathogenic and beneficial microorganisms using multi-omics approach: From transcriptome to metabolome (INTER-OMICS)*

# General information



- Research project (IP-2025-02-8832)
- Financed by Croatian Science Foundation
- Financial value: 198.700,00 EUR
- Period: 31th December 2025 – 30th December 2028

<https://inter-omics.krs.hr/en/>



# Research group



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Tomislav Radić, PhD

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Emanuel Gaši, PhD



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**University of Upper-Alsace, France, Colmar Cedex, France**

Julie Chong, PhD, full prof.

Mary-Lorene Goddard, PhD

Un laboratoire de l'Université de Haute-Alsace





Mate



Emanuel



Katarina



Julie



Mary-Lorene

Tomislav



# INTER-OMICS

Silvija



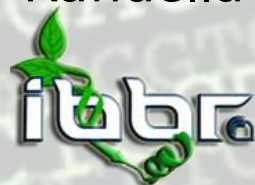
Raffaella



Giorgio



Luca



# About the project idea

**GRAPE-PATH3** - Pathogenic potential of grapevine leafroll-associated virus 3 and its interaction with grapevine hosts (January 2019 – December 2022); PI: K. Hančević

The most important results: GLRaV-3 is dominant pathogenic grapevine virus in Mediterranean Croatia, grapevine physiology and virus replication depend on many factors (virus inocula composition, host, GLRaV-3 genotype (strain) and infection duration), but parameters of oxidative stress are unique to all infected plants and can serve as a cellular biomarker of virus infection ( $\uparrow$  SOD,  $\uparrow$  H<sub>2</sub>O<sub>2</sub>, and disturbed total proteins, trend of  $\uparrow$  MDA, drastic reduction of carotenoids, chlorophyll a and b)

# About the project idea

**MYCO GRAPE** - Arbuscular mycorrhiza potential to modify grapevine defence against viruses (January 2021. – January 2025); PI: T. Radić

The most important results: AMF mitigate pathogenic impact of viruses by restoring the photosynthetic activity (↑ photosynthesis, ↑ growth, ↑ water-use efficiency) in infected grapevine plants, reducing oxidative stress (↓ SOD, ↓ LPO, ↓AsA, ↑ GPOD), modulating the concentration of phytohormones (↑ SA, ↑ ABA) and expression of defense-related genes (↑ EDS1 and ↑ LOX are upregulated, STS1 is higher in non-AMF plants) correlating with higher oxidative burden.

From virological aspects: changes in virus concentration and tissue-distribution, in some cases AMF may increase the accumulation of certain viruses (e.g., GLRaV-3 — requiring further investigation).

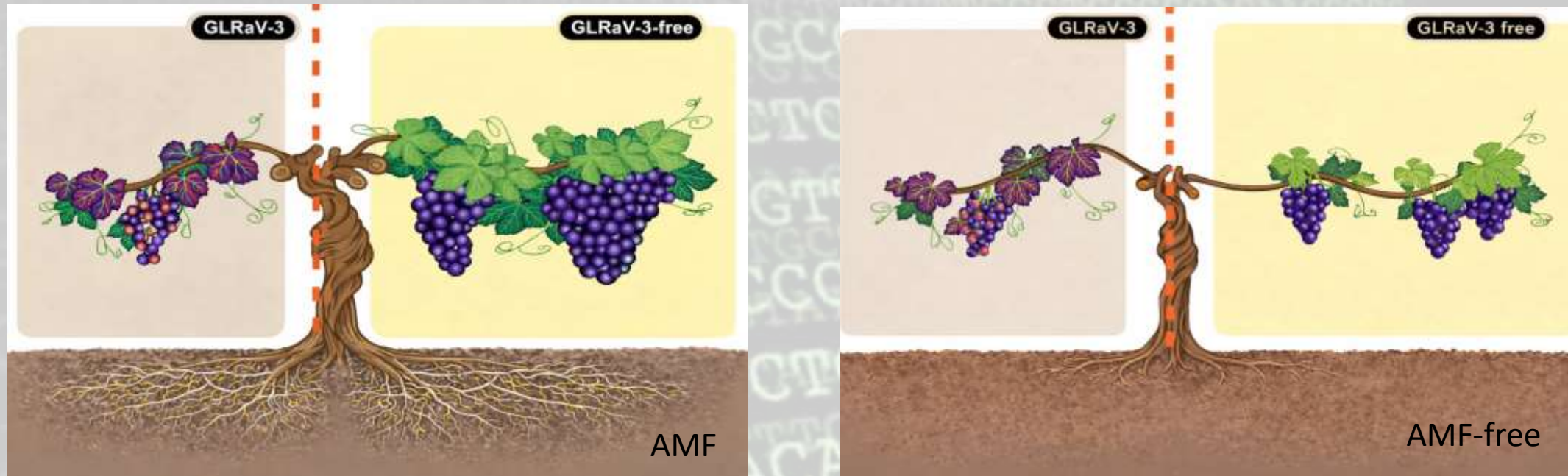


## INTER-OMICS

Integrative grapevine and AMF response to plant-microbial interaction (Tribidrag, GLRaV-3 and *Rhizophagus irregularis*) studied by HTS and untargeted metabolome screening-new methodological approach

In-depth study on processes in grapevine triggered by virus infection, by AMF symbiosis and by virus-AMF interaction to know more how these microorganisms alone and in combination, affect processes in grapevine and how symbiosis may mitigate virus negative impact (apart from already familiar facts)

# What we know so far



Initial illustrations by Marco Martini

- AMF: Improves grapevine nutrition and resistance to stresses (water stress, resistance to pathogens) through changes in primary and secondary metabolism
- GLRaV-3: Alters oxidative balance and stress response, reduces photosynthesis, affects sugar transport, changes secondary metabolism
- AMF and viruses in grapevine: Induce beneficial impact on photosynthesis, modulate phytohormonal pathways and reduce oxidative stress



## INTER-OMICS - Tasks

1. To analyse differentially expressed genes (DEGs) in grapevine leaves and to detect the most significant grapevine responses at the transcriptional level
2. To explore AMF and grapevine DEGs in roots and to detect the most significant grapevine and AMF responses at the transcriptional level
3. To determine biochemical changes upon metabolomic profiling of grapevine leaves and roots, as well as grapevine agronomic performance
4. To ascertain major genetic and biological processes or pathways triggered by these interactions

The most differentially expressed functional genes will be defined and the most discriminant metabolome profiles among treatments will be identified.

Expected new skills learned: knowledge in utilizing bioinformatic tools for functional detection of DEGs upon RNA-Seq and their biological interpretation.

# Experimental design

## Four treatments:

1. Tribidrag without GLRaV-3 and with AMF
2. Tribidrag with GLRaV-3 and with AMF
3. Tribidrag without GLRaV-3 and without AMF
4. Tribidrag with GLRaV-3 and without AMF

## Two tissue types:

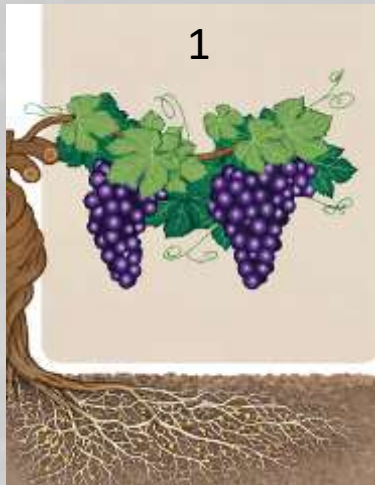
1. Leaves
2. Roots

For transcriptome analysis: Three biological replicates

**In total: 24 samples**

For metabolome analysis: At least four biological replicates

**At least: 32 samples but prefer 48**



# Schematic overview of the experiment workflow

Omega Grafting  
for Virus Transfer

Stratification & Rooting

Potting & AMF Inoculation

AMF Colonization Check  
(2 months PI)

Virus Presence Check  
(4 months PI)

Sampling for  
Transcriptome  
& Metabolome Analysis  
(5/6 months PI)



**First year of the project:** Virus testings, experimental set up, HTS and metabolome analysis, growth parameters described, website of the project activated, kick-off meeting, attendance of two workshops on HTS bioinformatic analysis, equipment and consumables purchased

**Second year of the project:** Analysis of HTS and metabolome results, biochemical changes upon metabolomic profiling described, RT-qPCR validation of HTS results, one scientific conference attendance, one training completed

**Third year of the project:** RT-qPCR validation, results assembly (DEGs in grapevine leaf and roots and major processes or pathways triggered by these interactions identified), dissemination and education

In total:

4 conference attendance

2 scientific papers published

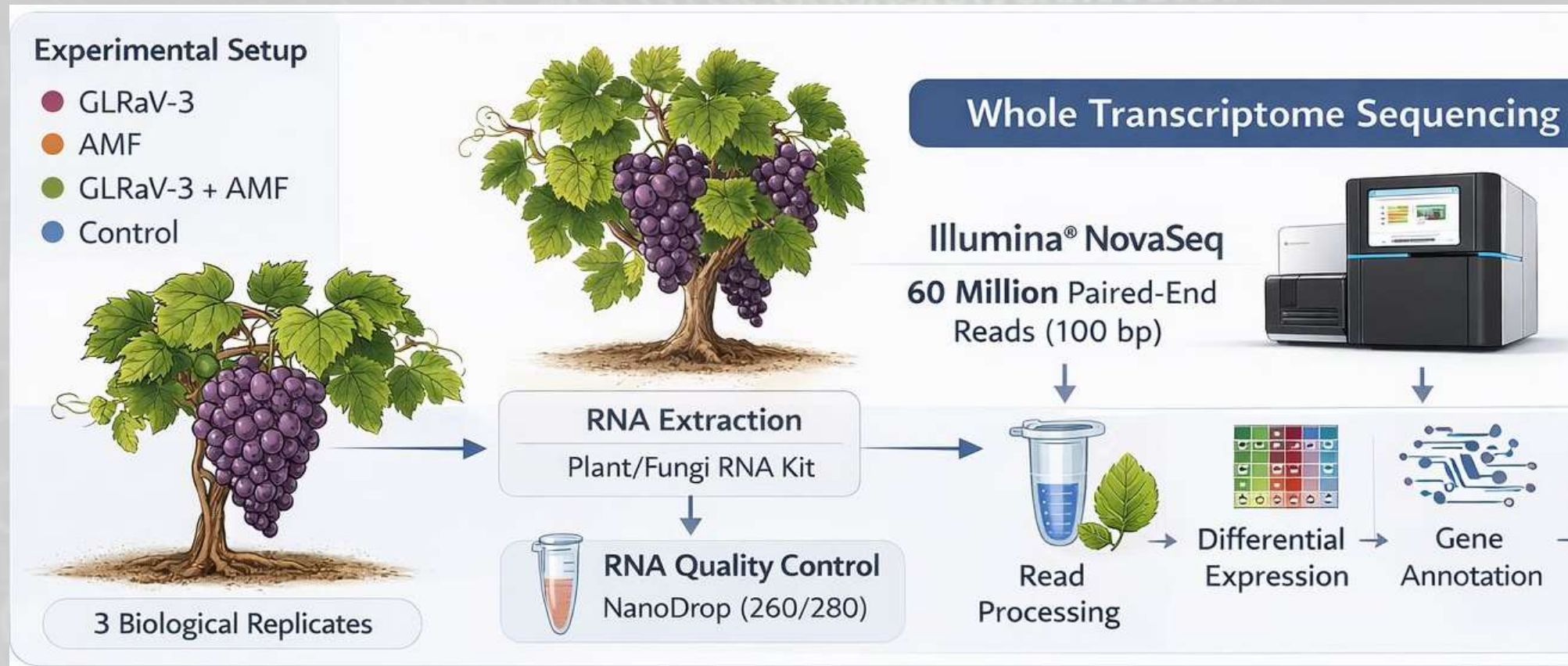
3 workshops on HTS analysis ( 2 on-site, one on-line)

2 trainings in partners institutions

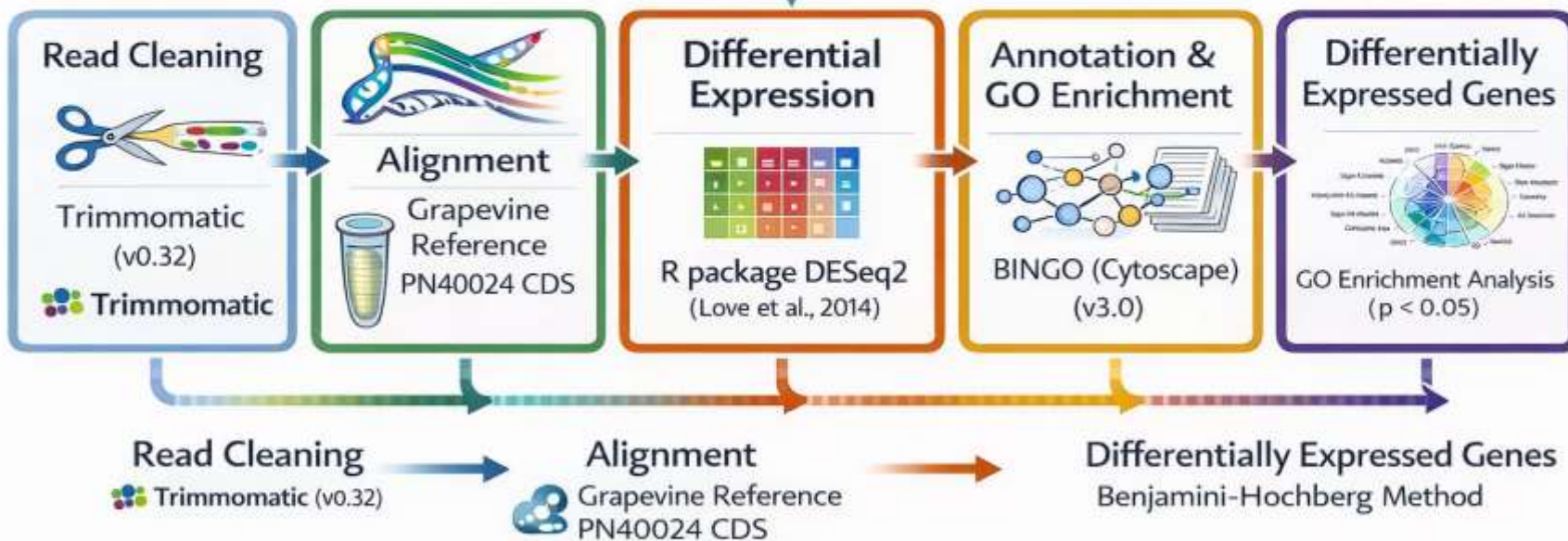
# Methods used/to be used

- RT-qPCR, RT-mPCR and PCR for RNA and DNA viruses detection and quantification in Tribidrag and GLRaV-3 donor-plant prior grafting and after grafting
- Microscope detection and quantification of AMF colonization in grapevine roots
- Illumina Novaseq-HTS in external service (we will perform sample preparation)
- Analysis of HTS results and identification of DEGs
- Validation and quantification of DEGs by RT-qPCR
- Untargeted metabolome analysis (GC-EI-Q-MS, LC-ESI-QTOF-MS)
- Growth parameters and symptoms observation

# Transcriptomic workflow



**AIR RNA-seq**  
Artificial Intelligence RNA-seq  
(<https://transcriptomics.cloud>)



# Metabolomic workflow

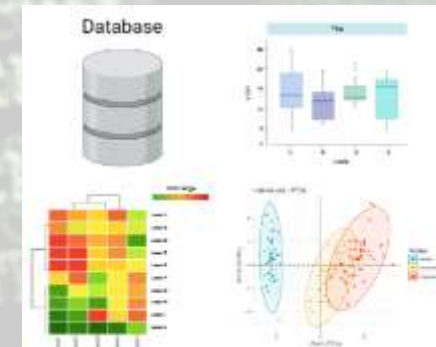
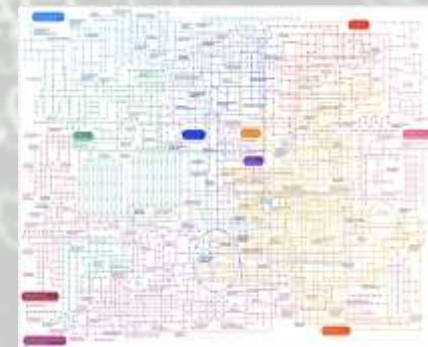
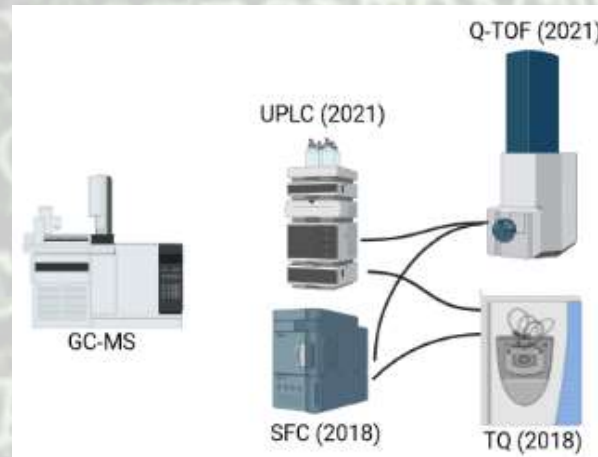
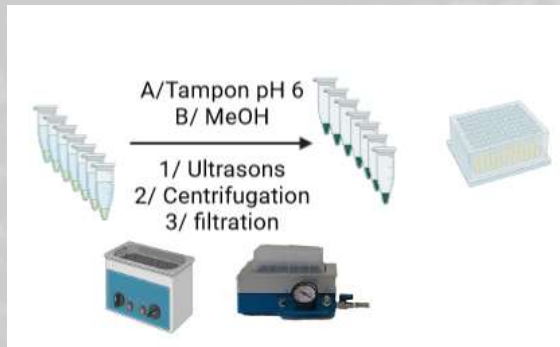
Biological experiment

Metabolite extraction

Chemical analyses

Annotation

Data analysis



Chemical library ~ 300 natural products (aa, sugars, flavonoids, stilbenes, ...)

# Chemical analyses

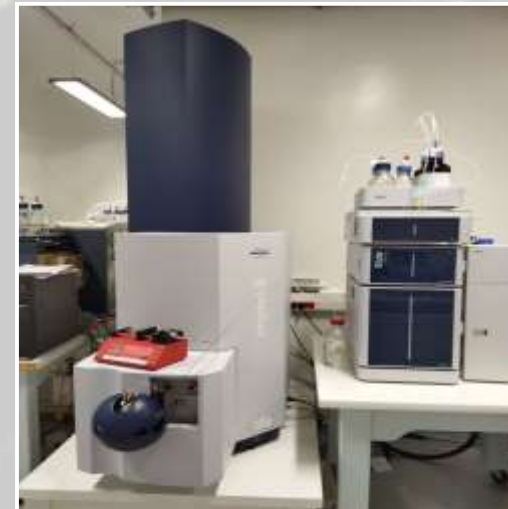


## GC-MS + derivatization

- Amino acids
- Sugars and derivatives
- Small organic acids

## RP-LC-MS/MS

- Flavonoids
- Stilbenes
- Other aromatic compounds





## Activities and participants' roles

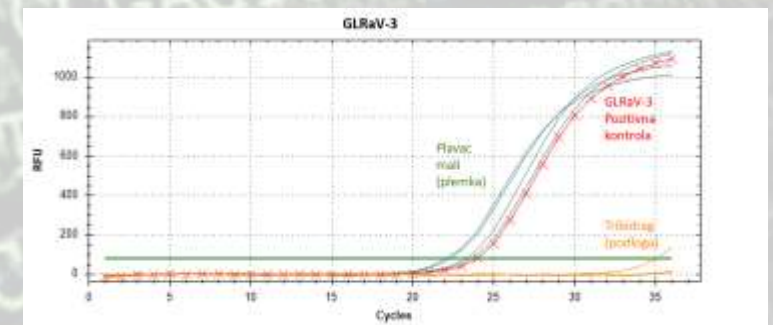
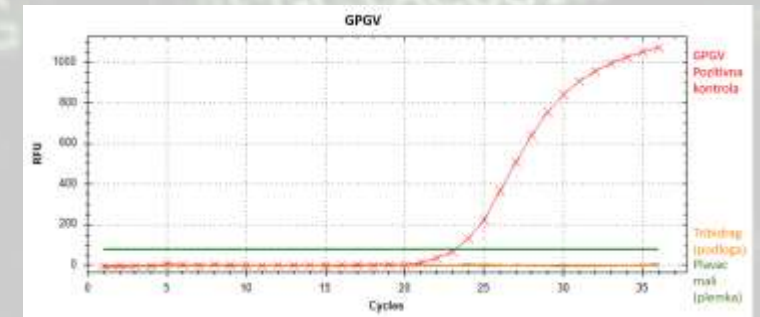
- Experiment set-up (Hančević, Čarija, Gaši, Radić)
- Detection and monitoring of viruses (Hančević, Čarija, Gaši, Černi)
- Monitoring success of AMF colonization and quantification (Gaši, Radić)
- Optimization of protocols for RNA extraction and preparation for RNA sequencing (Hančević, Čarija, Černi, Gambino, Balestrini)
- Sampling (Čarija, Černi, Gaši, Radić, Hančević)
- Training in bioinformatics (Čarija, Gaši, Nerva)
- Construction of RNA-seq data libraries (Nerva, Gambino, Balestrini, Čarija, Gaši)
- DEGs identification (Balestrini, Gambino, Nerva, Hančević, Čarija, Gaši)
- Selected genes' expression validation (Hančević, Čarija, Gaši, Radić)
- Metabolome analysis and interpretation (Chong, Goddard)
- Publications preparation and dissemination (all members)

# What we have done so far:

One clone of Tribidrag and one virus-donor plant were pruned

Ten viruses checked and confirmed virus-free sanitary status of Tribidrag and the presence of only GLRaV-3 in grapevine virus-donor plant:

1. Arabis mosaic virus (ArMV)
2. Grapevine fanleaf virus (GFLV)
3. Grapevine leafroll-associated virus 1 (GLRaV-1)
4. Grapevine leafroll-associated virus 2 (GLRaV-2)
5. Grapevine leafroll-associated virus 3 (GLRaV-3)
6. Grapevine fleck virus (GFkV)
7. Grapevine virus A (GVA)
8. Grapevine virus B (GVB)
9. Grapevine Pinot gris virus (GPGV)
10. Grapevine Roditis leaf discoloration-associated virus (GRLDaV)



# What we have done so far:

Tribidrag plants were Omega grafted for virus transfer and put to rooting in sterile substrate in controlled screenhouse





Thank you for your attention!