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# Tackling emerging plant diseases threatening food crops with"omics" technologies



From virus discovery to host-pathogen interaction: past experiences and future challenge

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## BACKGROUND

Climate change and global trade of plant materials are two key drivers responsible for the emergence of plant pathogens ravaging economically important crops. Among different pathogenic microbes, insectborne pathogens (i.e. plant viruses) pose major challenges for their control and are frequently responsible for detrimental diseases. Thus, the accurate and early identification of the causal agent(s) is critical for prevention and disease management. High-throughput sequencing (HTS) allowed for the description of virome profiles of important crops (grapevine, citrus), became widely applied to study the molecular bases of antiviral response and to discover new viruses in emerging diseases of unknown etiology. Moreover HTS providing an high standard for the certification of grapevine material (free of the major listed viral pathogens) was evaluating to be included in the official sanitary certification scheme (1). In 2013 the bacterium Xylella fastidiosa was first identified in Apulia (Italy), causing a severe disease (Olive Quick Decline Syndrome) with loss of the olive trees and productions, and then in other EU countries. HTS and "omics" technologies were exploited in the evaluation of different grade of susceptibility of different cultivars to Xf infection, as well as in the study of the biology, genome structure, genotyping and evolution of the bacterium.

#### Material & Methods:

grapevine

Leaf tissue samples were taken from:

- grapevine showing GLMD symptoms (fig. 1a)
- citrus with unknown etiology (fig. 1c)
- twenty clones of grapevine cultivars and rootstocks previously selected for the assessment of sanitary status by HTS (fig.1b)

**RECUPERO DEL GERMOPL** 

Hosts Virus

# Pathogens



# Material & Methods:

Different strategies and protocols for the enrichment of viral smallRNAs were tested according the different nature of viruses. Bioinformatic pipeline from reads of smallRNAs to assembling of

known/unknown viral genome



	ID.	VARIETA	Clone ID
	<b>V.</b> 11	Regina Bianca	CRSA I I
B	V.12	Palieri	CRSA 229
	V.25	110 RICHTER	UBA 05
	V.24	420 A	UBA 08
	V.23	140 RUGGERI	UBA 05
The season	V.20	KOBER 5BB	UBA 01
	V.22	1103 PAULSEN	UBA 08
A ANTAL AND A ANTAL AND	V.4	Verdeca	UBA 6A
D-	V.8	Bombino Bianco	CRSA Reg. Puglia D382
gure1a. Symptoms of Grapevine Lea	f V.10	Negramaro	CRSA Reg. Puglia D382
lottling and Deformation Disease(GLN	Sector and sector and	Susumaniello	CRSA Reg. Puglia D382
	V.I	Uva di Troia	49M
total RNA isolation with Trizol	V.2	Malvasia Nera	69E
	V.5	Bombino Nero	D205
separation of LMW and HMW RNA	V.7	Aglianico	D382
	V.13	Baresana Rossa	54A1
urification of small RNAs (20-30 nt)	V.14	Italia	12A1
	V.14	Vittoria	6A1
I N/N/		Regina dei Vigneti	58A2
LMW			
	and root	Lattuario Nero b.clones of gr stocks selecte	46AI
Cel 15% denaturing polyacrylamide/urea	V.18 Figure 1 and root assessm	Lattuario Nero b.clones of gra stocks selected ent of sanitary	apevine cultiva

#### Material & Methods:

25 years-old olive trees of the cvs Leccino and Ogliarola salentina. naturally infected, were used in the first transcriptome comparison. Potted olive plants maintained under controlled conditions (25 °C and 70% relative humidity) in the greenhouse and were artificially infected with the Apulian strain De Donno of *X. fastidiosa* subsp. pauca haplotype ST53. Twigs were needle punctured after placing a 10µL drop of bacterial culture Suspension. Xylem tissue (ca. 0.5–1 g) was then recovered, after removing the bark, and processed for DNA or RNA extractions.



RNA interference (RNAi) mechanism as «antiviral plant response»

#### **Results:**

nttp://www.fruttiantichipuglia.it/recuperiamo

in the frame of a regional the virome of a group of commercial clones of grapevine cultivars clones of grapevine cultivars

eomics

Metabolom

Venny comparison

Re.Ge.Vi.P.

HTS technologies were applied Project (Re.Ge.Vi.P) to profile and rootstocks. Genomics

1 sciptomics

RNAs

citrus

ase on lemon trees, reported aries from

Target pathosystems

Citrus, Grape/

viruses

Scheme showing key steps of replication of plant viruses in plant cell

Genomics

**Results:** 

sequence.

#### New virus discoveries:

-Grapevine Pinot gris virus (GPGV) (2) -Citrus yellow vein clearing virus (CYVCV) (3)

> Schematic of the laboratory procedures and the bioinformatic pipeline optimized for the assembly of the genome of a virus by HTS

Analysis of HTS data confirmed the healthy sanitary status of the 20 grapevine clones assessed by traditional diagnostic tools (RT-PCR and ELISA), **Kanscriptomics** which were free from GLRaV-1, -2 and -3, GFLV, ArMV, GVA, GVB and GFkV (Table 1)



_											
		Cultivar/		Redundant reads			Regulated viruses*		GRSPaV		Table 1. Results of NGS and RT-PCR analyses on certified grapevine cultivars and rootstocks. <sup>a</sup> GVA, GVB, GLRaV-1,-2,-3, GFLV, GFKV
ID.	Rootstocks	Clone code	(adpt. trimmed)	Contigs	NGS	PCR	NGS	PCR			
	V.1	Uva di Troia	UBA 49M	16.400.133	2.042	-		+	+	and ArMV, according to	
	V.2	Malvasia Nera	UBA 69E	3.472.517	1.152	-		+	+	Italian (DM 07/07/2006 and	
	V.5	Bombino Nero	CRSA Reg. Puglia D205	22.872.057	1.152	-		+	+	DM 24/06/2008) regulations. Light and dark grey indicate	
	V.7	Aglianico	CRSA Reg. Puglia D382	23.426.017	4.652	-		+	+	extractions from leaf or phloen	
	V.13	Baresana Rossa	CRSA 203	8.276.729	2.274	-		-		tissues, respectively.	
	V.14	Italia	CRSA 121	23.332.063	5.477	-					
	V.15	Vittoria	CRSA 41	22.691.173	3.716	-	-	+	+		
	V.17	Regina dei Vigneti	CRSA 76	21.841.038	6.124	-		+			
	V.18	Lattuario Nero	CRSA 277	3.302.822	302	-		-			
	V.4	Verdeca	UBA 6A	12.128.430	3.173	-	-	+	+		
	V.6	Susumaniello	CRSA Reg. Puglia D382	8.607.208	4.524		-	+			
	V.8	Bombino Bianco	CRSA Reg. Puglia D382	14.014.780	2.351		-	+	+		
	V.10	Negramaro	CRSA Reg. Puglia D382	19.435.282	5.281		-	+	+		
	V.11	Regina Bianca	CRSA 11	6.066.427	1.640			+	+		
	V.12	Michele Palieri	CRSA 229	7.228.556	2.534	-	-	+	+		
	V.20	Kober 5BB	UBA 01	15.016.373	3.415			+	+		
	V.22	1103 Paulsen	UBA 08	21.236.510	9.510			+	+		
	V.23	140 Ruggeri	UBA 05	19.737.418	11.377	-	-	+	+		
	V.24	420 A Mill.de Gr.	UBA 08	14.990.088	9.133	-	-	+	+		
	V.25	110 Richter	UBA 05	13.619.296	8.089	-	-	-	-		

Material & methods:



#### **RNAseq libraries** construction ranscriptome sequencing 1) mRNA isolation

3) generation of sequencing libra

) transcriptome ass

#### Results

**1) Transcriptome profiles** from olives of different cutivars **in natural infection's** conditions (7)







### **Olive/bacteria**



Integration by ity of California (UC **bioinformatics** 

Principal component analysis (PCA)

Effectoromics

and

2) Genomic population study on large scale by whole genome SNP analysis approach revelead the origin (Costa Rica) of Xf introduction in Italy (5)

and PacBio RSII platform







**LUGLIO 2021** 

Bacteria cells of *Xylella fastidiosa(Xf)* were isolated and cultivated in pure culture for DNA extraction and construction of Whole Genome Sequencing libraries. 79 Xf samples were collected from diseased olive trees from Apulian outbreak as well as genomes of the most genetically closely related strains from Central America. Bionformatic pipeline consisting of an hybrid assembly approach is reported below.

Results

**1)** Assembling of the complete genome by Whole Genome Sequencing of the first isolate of Xf associated to Olive Quick Decline Syndrome (4)

infoxylella.i



3 genomes



PONTE RECAS mean length of 8,527 bp

Plant transcriptome analysis after biopesticide treatment, alone or in presence of Xylella to understand the mode of action (MOA), the effects of novel biopesticide application on the plant's defense system 4) Genomic and microevolutionary population study on Apulian outbreak area

> **WCGNA** network analysis

**Future challenges:** Machine learning approaches will be tested on gene expression data, to exploit this resource with the aim to characterize resistance traits in new olive selections while identifying novel diagnostic biomarkers of the plant responses to biotic stresses

approach showed that the outbreak in Apulia is due to a single introduction from Central America that we estimated to have occurred in 2008 (6)

by whole genome SNP analysis

Amnibacteriu Pantoea Kineosporia Wolbachia

Geographic structure and genetic distributior

of 70 Apulian isolates

aimed at characterizing the microbiome for the search for antagonistic endophytic microorganisms (8)

trom Costarica



92 / COF0324

5) Assembling genomes of Xf isolated from different subsequent outbreaks in **Europe** (Tuscany, Spain, XYL1966\_18 XYL1981\_18 XF\_3960\_18 XYL1968\_18 Balearic island , Lazio) = ST87

For more informations: H2020 european projects focused on X. fastidiosa https://www.ponteproject.eu/ https://www.xfactorsproject.eu/, https://biovexo.eu/

PONTE PESOTO ACTORS CONSTRUCTION ACTORS

#### **References:**

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