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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, insect-borne 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:

- 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)

#### grapevine

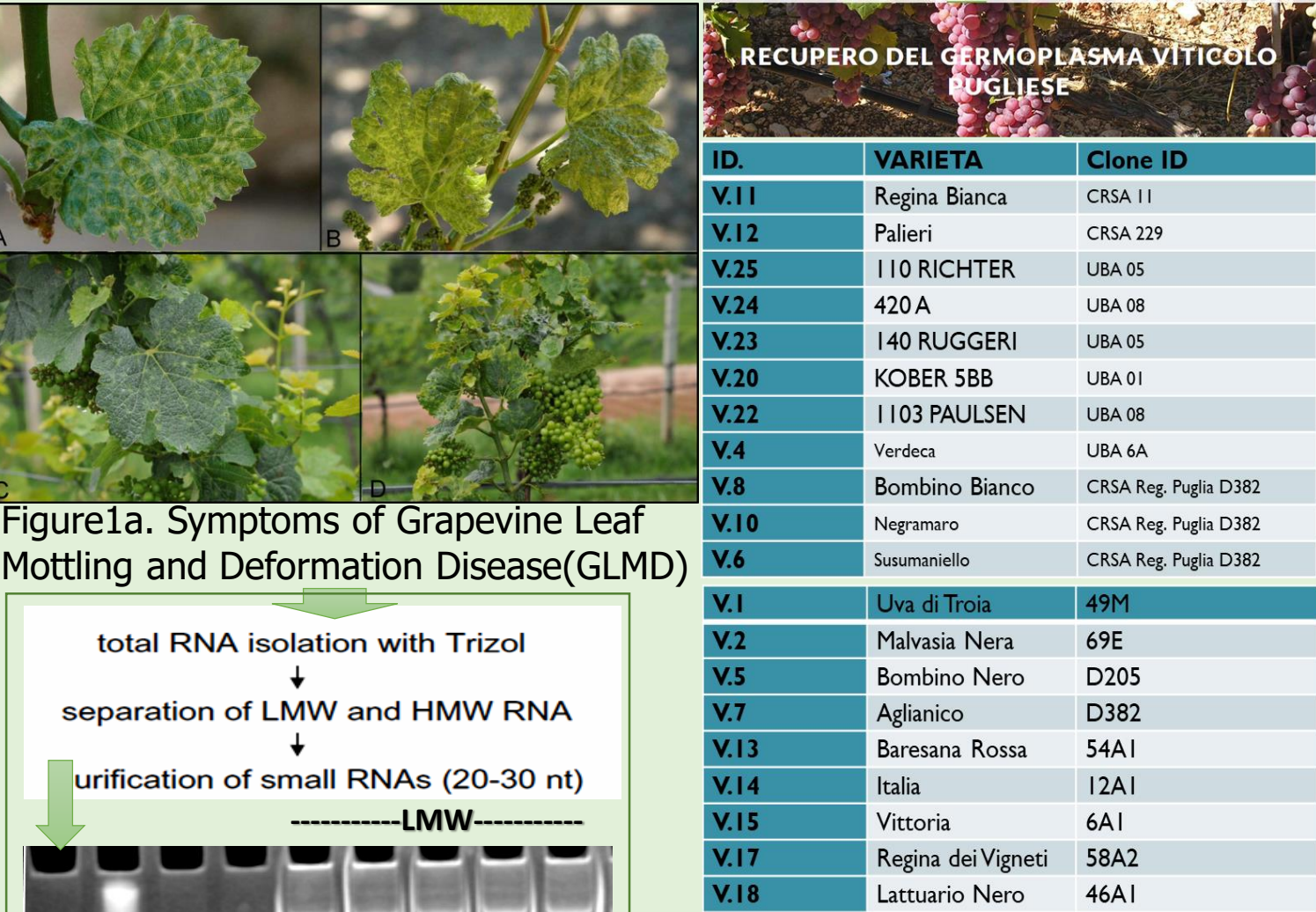


Figure 1a. Symptoms of Grapevine Leaf Mottling and Deformation Disease (GLMD)

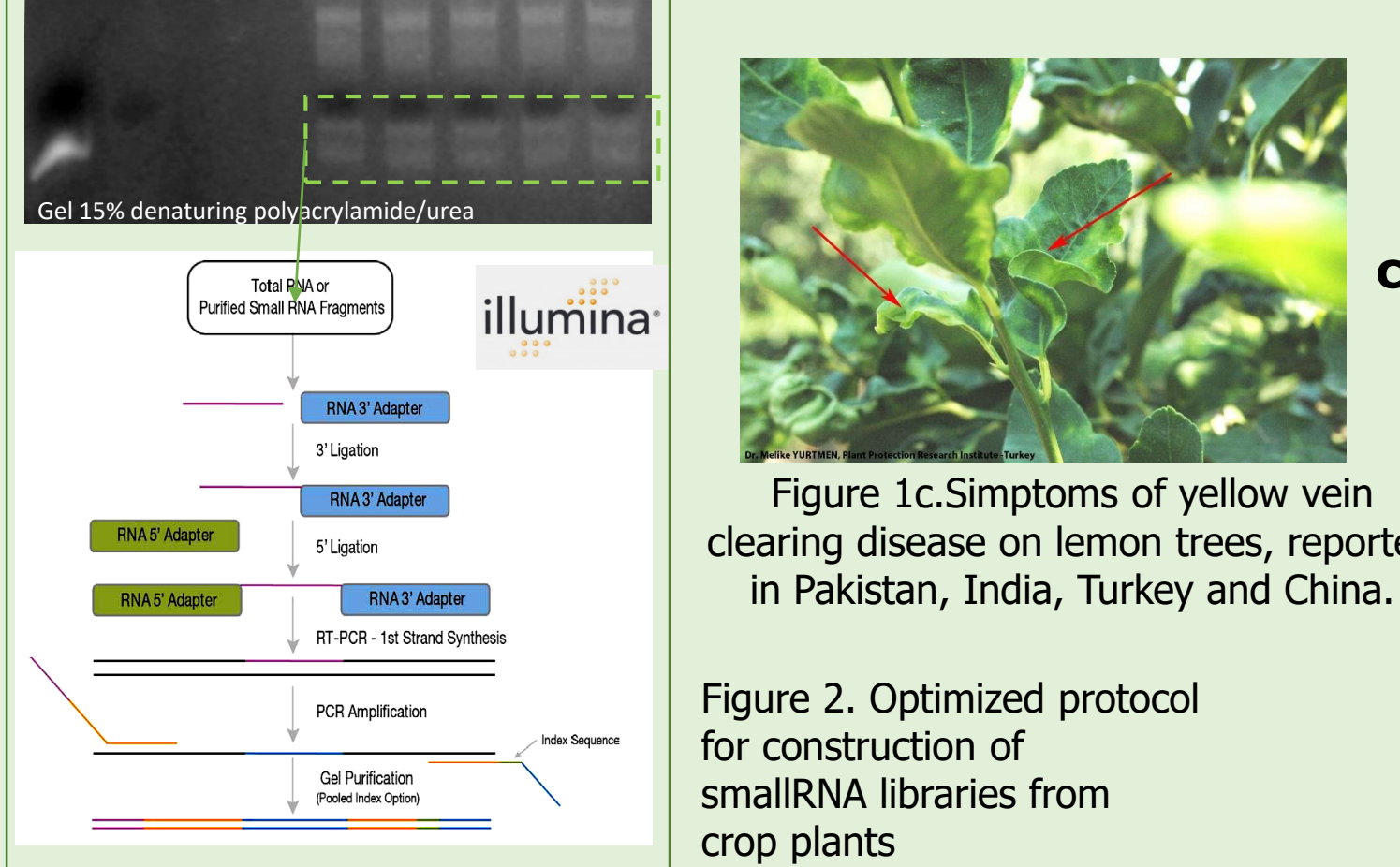


Figure 1b. clones of grapevine cultivars and rootstocks selected for the assessment of sanitary status by HTS

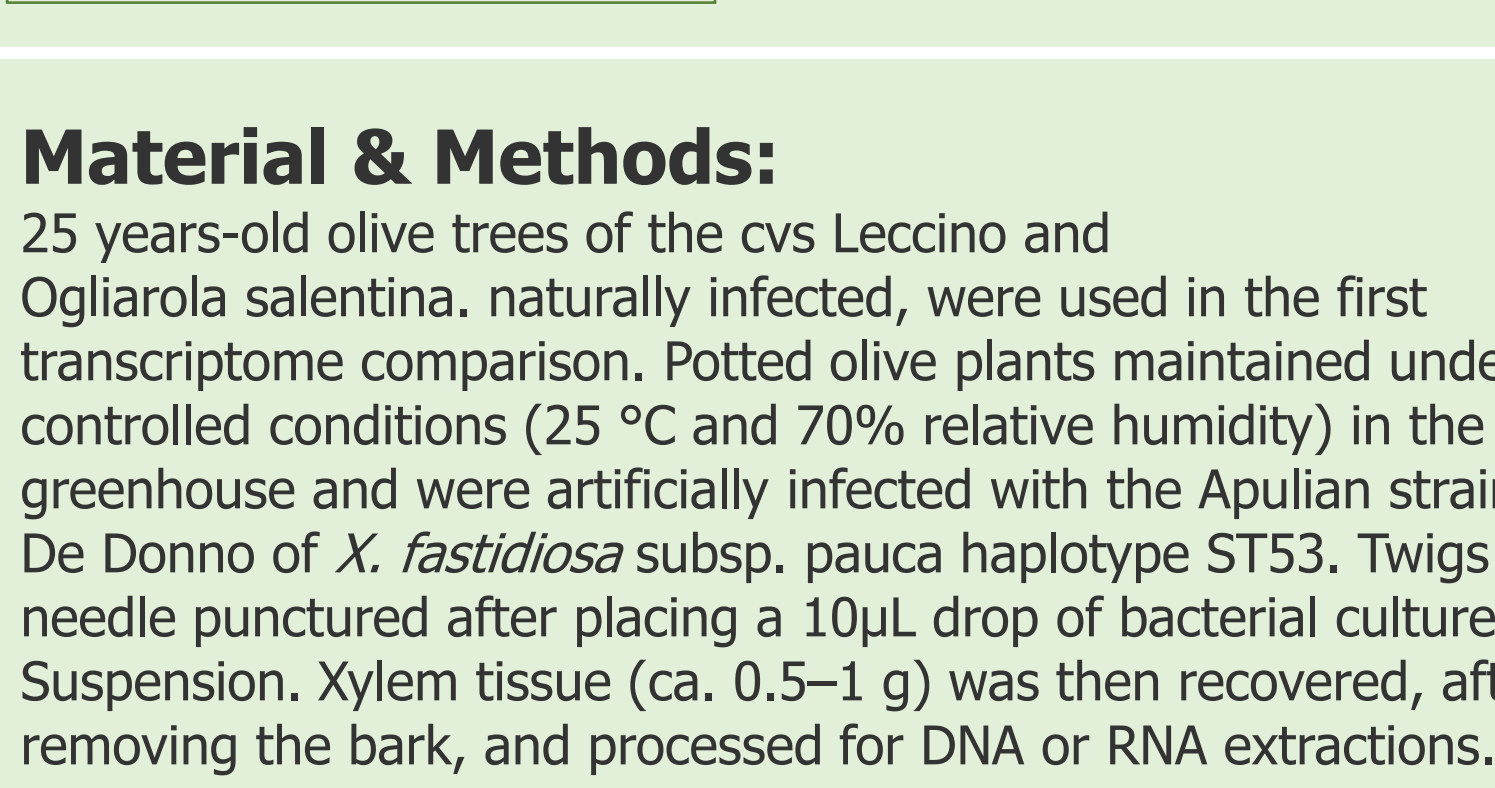
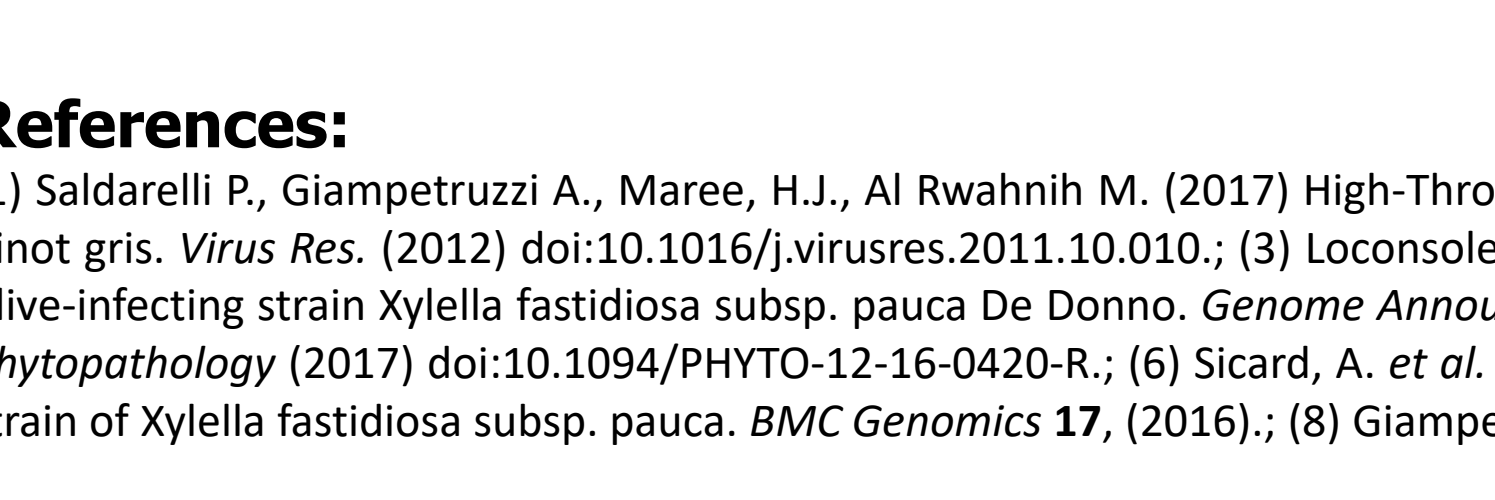
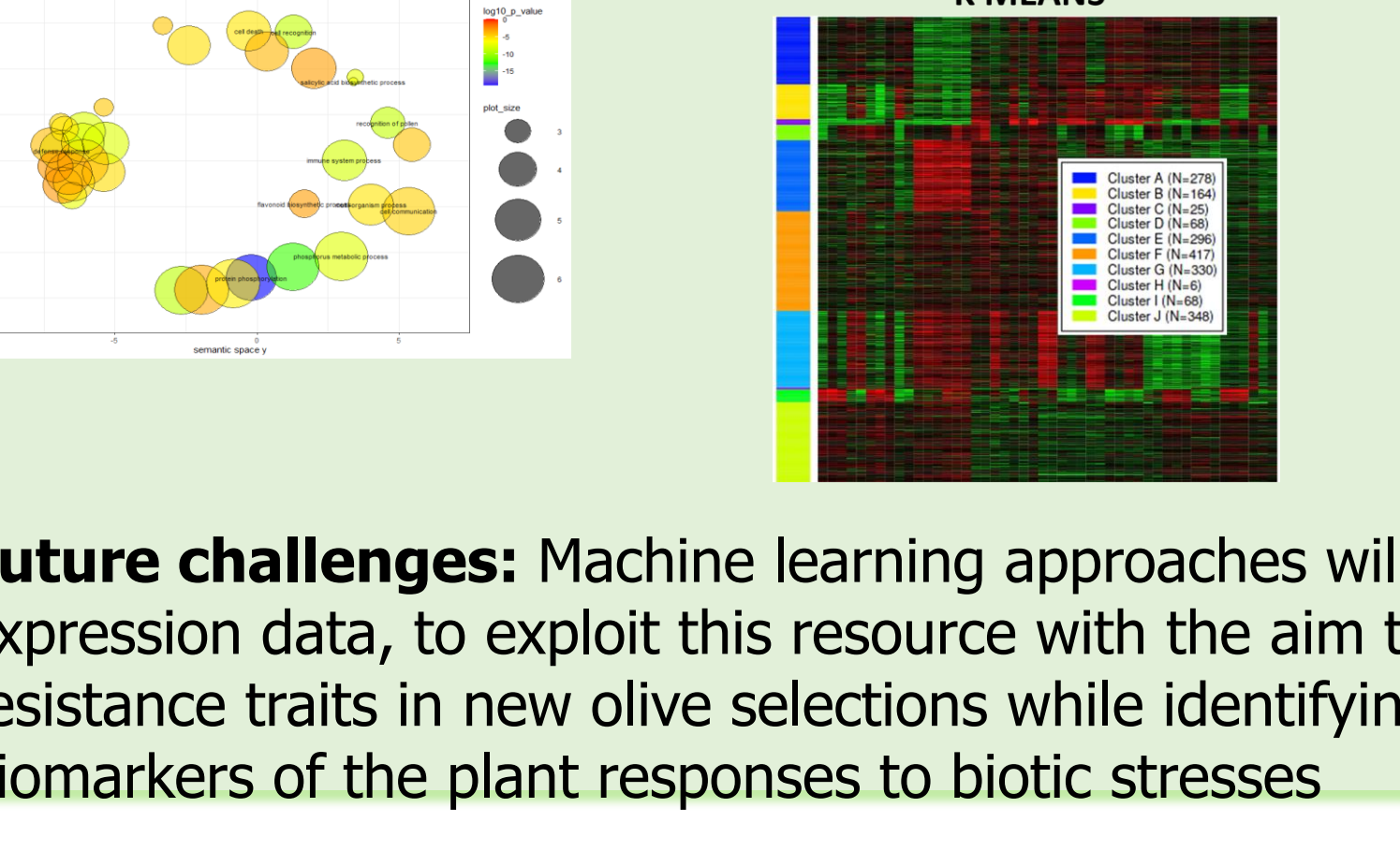
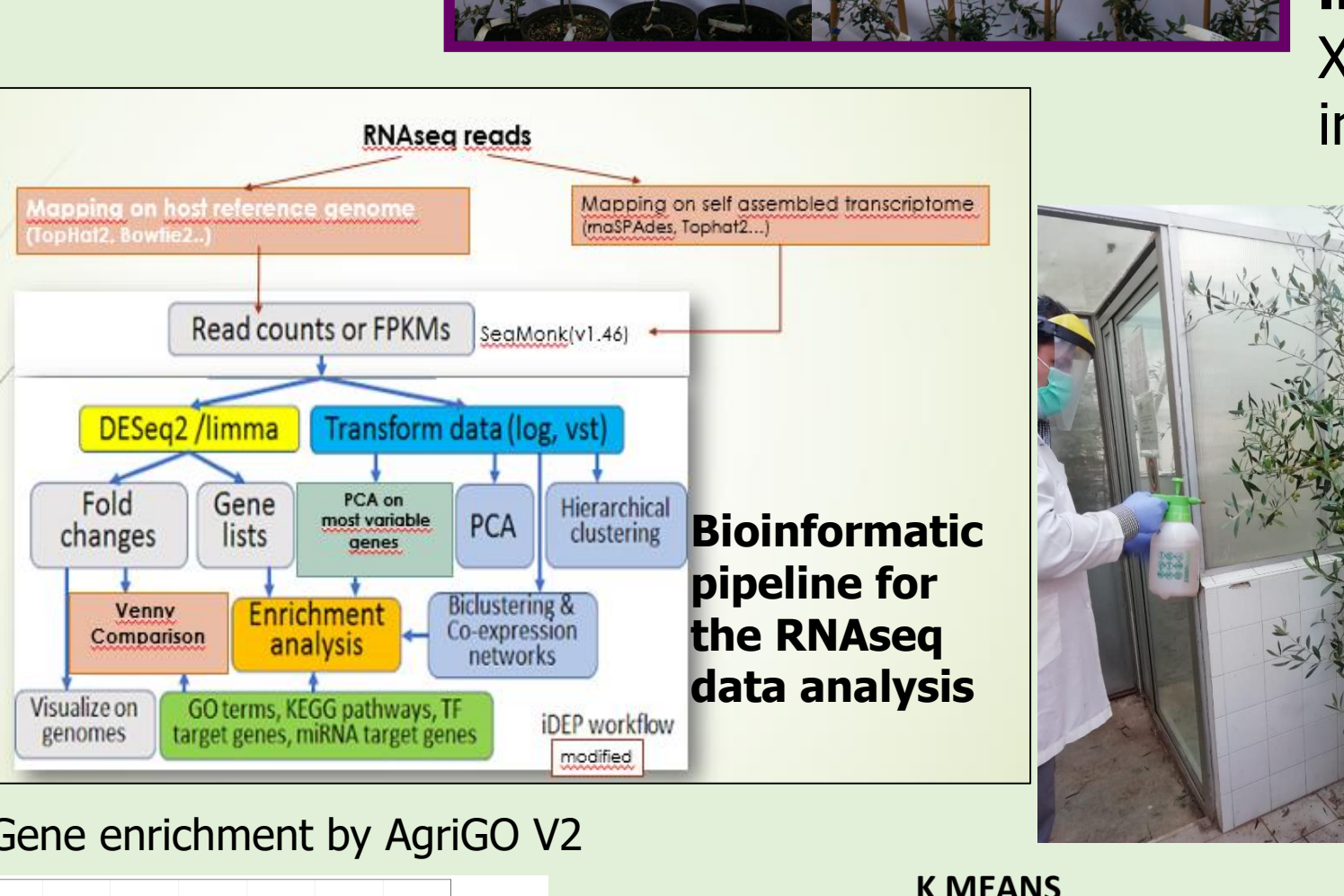
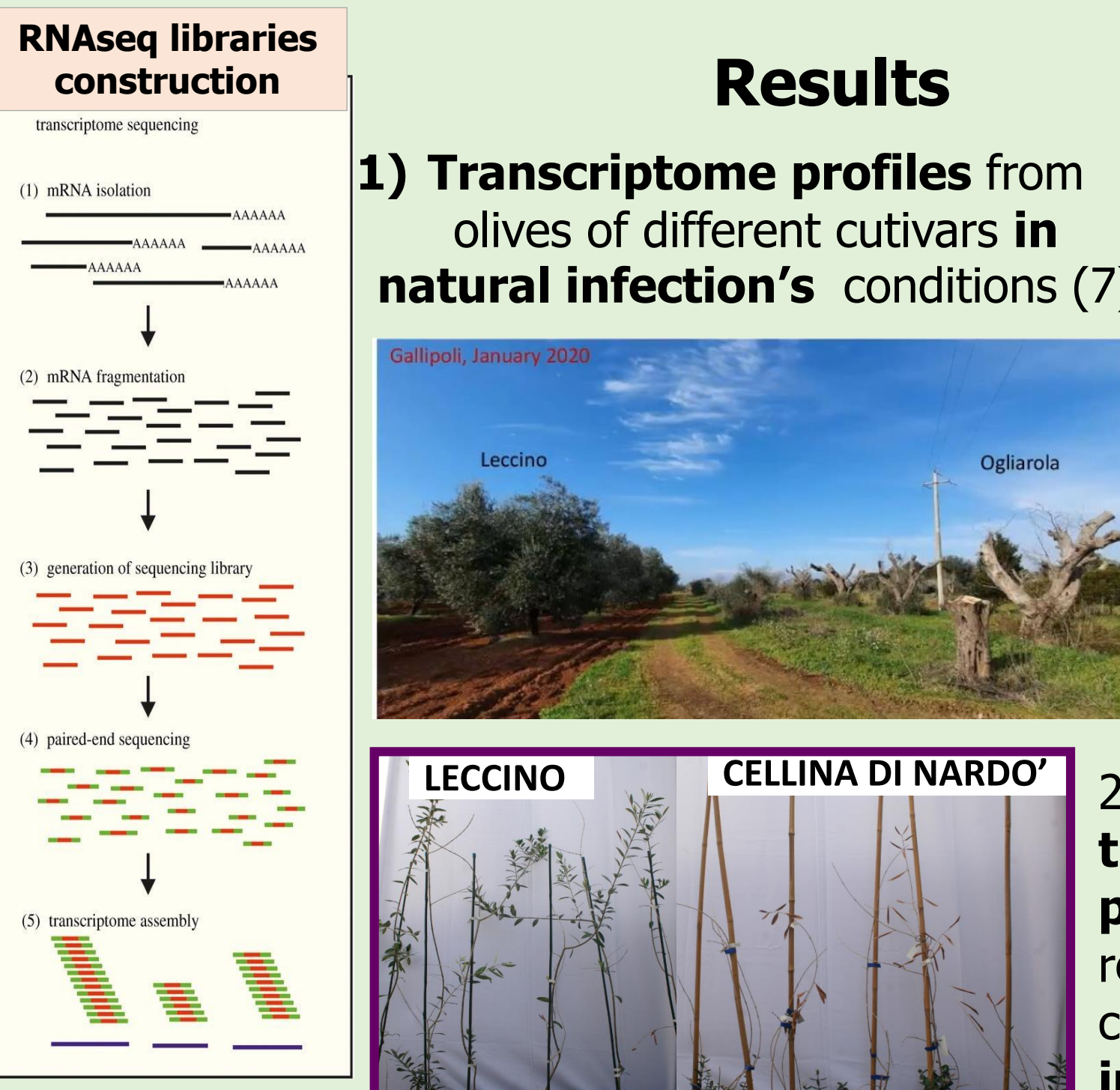
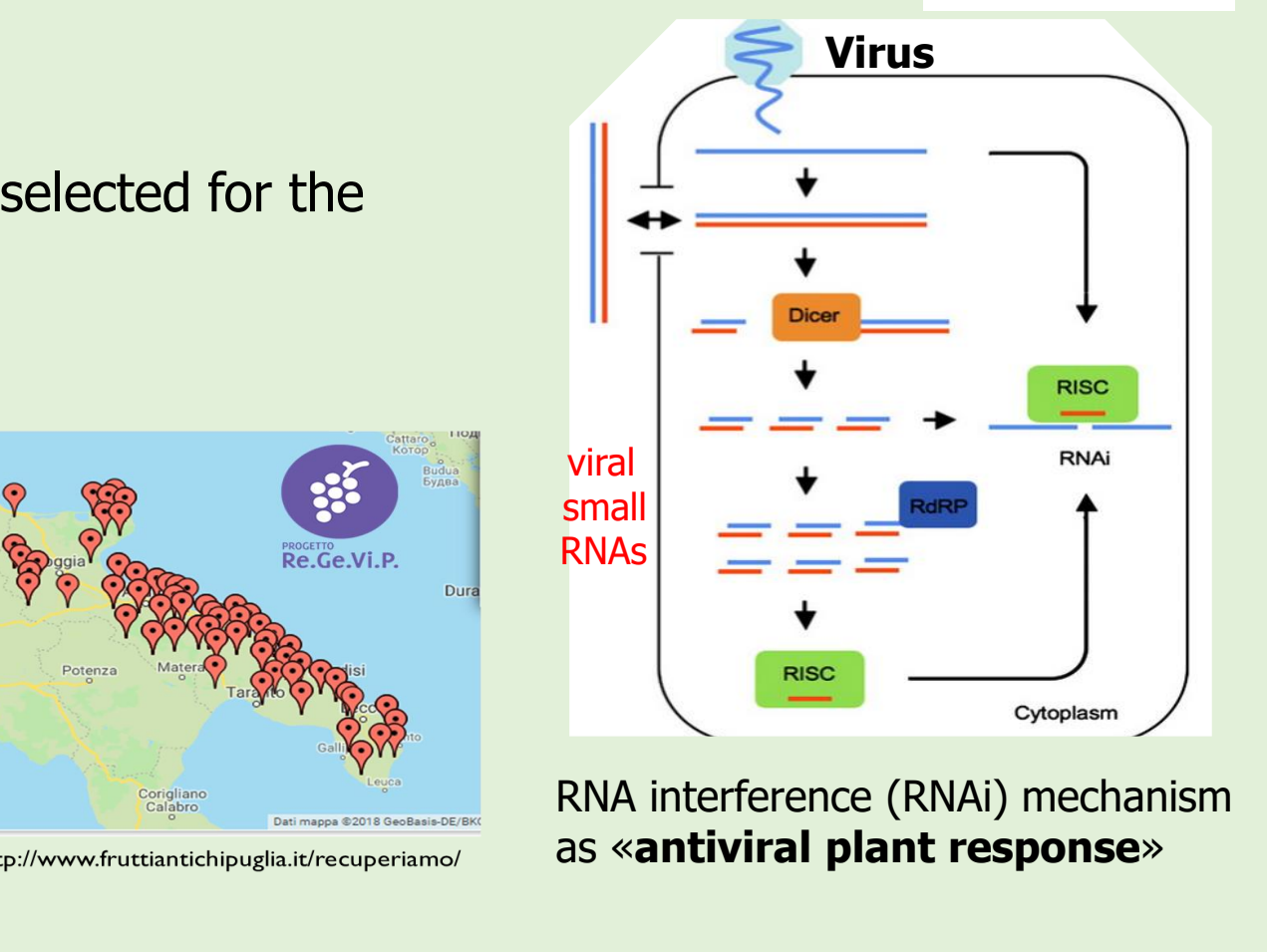


Figure 1c. Symptoms of yellow vein clearing disease on lemon trees, reported in Pakistan, India, Turkey and China.



### Hosts



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

### Pathogens



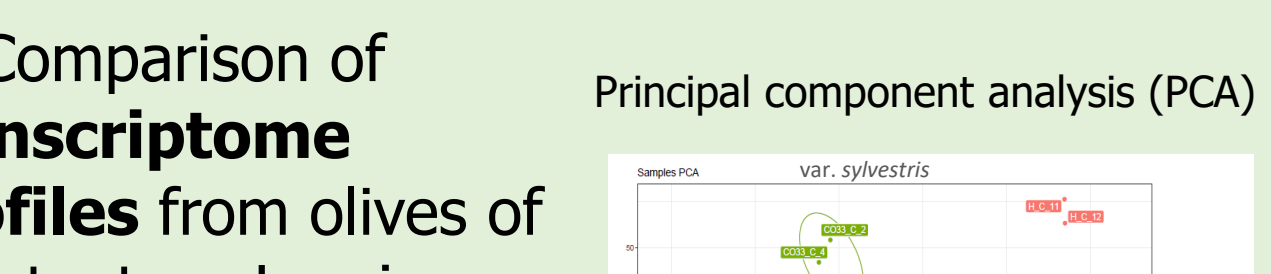
Scheme showing key steps of replication of plant viruses in plant cell

### 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 sequence.

### Results:

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



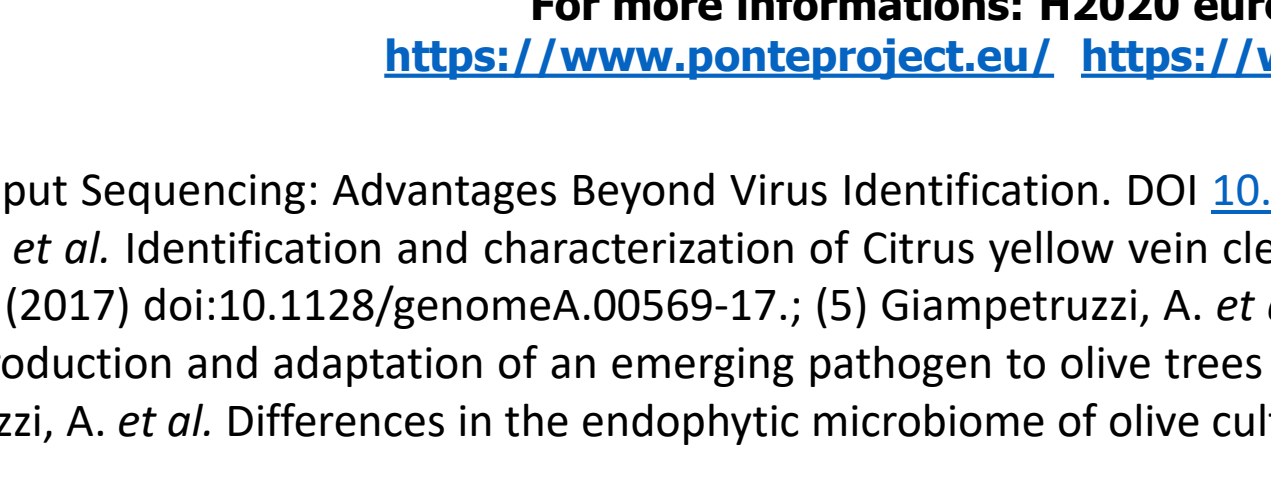
Schematic of the laboratory procedures and the bioinformatic pipeline optimized for the assembly of the genome of a virus by HTS

### 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.

### Results

- 1) Transcriptome profiles from olives of different cultivars in natural infection's conditions (7)
- 2) Comparison of transcriptome profiles from olives of resistant cv. Leccino vs cv. Cellina artificially infected by different Xylella's strain (CO33) in controlled condition
- 3) 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 (BIOVEXO ongoing project)



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

### Material & Methods:

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

ID	Cultivar/Rootstocks	Clone code	Redundant reads	Regulated genes	GRSPV
V1	Uva di Troia	USA 49H	16,400,133	2,942	-
V2	Malvasia Nera	USA 69E	3,472,517	1,152	-
V5	Bombino Nero	CRSA Reg. Puglia D205	22,872,927	1,152	-
V7	Aglianico	CRSA Reg. Puglia D382	25,428,917	4,452	-
V13	Baresana Rossa	CRSA 203	8,279,229	2,274	-
V14	Italia	CRSA 121	23,332,263	5,477	-
V15	Victoria	CRSA 41	22,681,173	3,718	-
V17	Regina dei Vigneti	CRSA 79	21,841,928	6,124	-
V18	Lattuario Nero	CRSA 277	3,382,422	362	-
V4	Verdeca	USA 6A	12,128,430	3,173	-
V8	Susumaniello	CRSA Reg. Puglia D382	8,887,236	4,254	-
V10	Negamaro	CRSA Reg. Puglia D382	14,914,780	3,521	-
V11	Regina Bianca	CRSA 11	6,916,427	1,840	-
V12	Paleri	CRSA 229	7,228,558	2,534	-
V20	KCSER SBB	UBA 01	15,918,373	3,415	-
V22	I103 Paulsen	UBA 06	21,236,510	5,910	-
V23	I40 Ruggeri	UBA 05	18,737,410	11,377	-
V24	420 A Villa de Gr.	UBA 08	14,980,980	8,133	-
V25	I10 Richter	UBA 05	13,919,296	8,089	-

Table 1. Results of NGS and RT-PCR analyses on certified grapevine cultivars and rootstocks: GVA, GVB, GLRaV1-2-3, GFLV, GPGV and ArMV, according to Italian DM 07/07/2006 and CM 24/05/2006 regulations. Light and dark grey indicate extractions from leaf or phloem tissues, respectively.

### Material & Methods:

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. Bioinformatic 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)
- 2) Genomic population study on large scale by whole genome SNP analysis approach revealed the origin (Costa Rica) of Xf introduction in Italy (5)
- 3) Metagenomic analysis of plants infected with *X. fastidiosa* aimed at characterizing the microbiome for the search for antagonistic endophytic microorganisms (8)
- 4) Genomic and microevolutionary population study on Apulian outbreak area by whole genome SNP analysis 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)
- 5) Assembling genomes of Xf isolated from different subsequent outbreaks in Europe (Tuscany, Spain, Balearic island, Lazio)

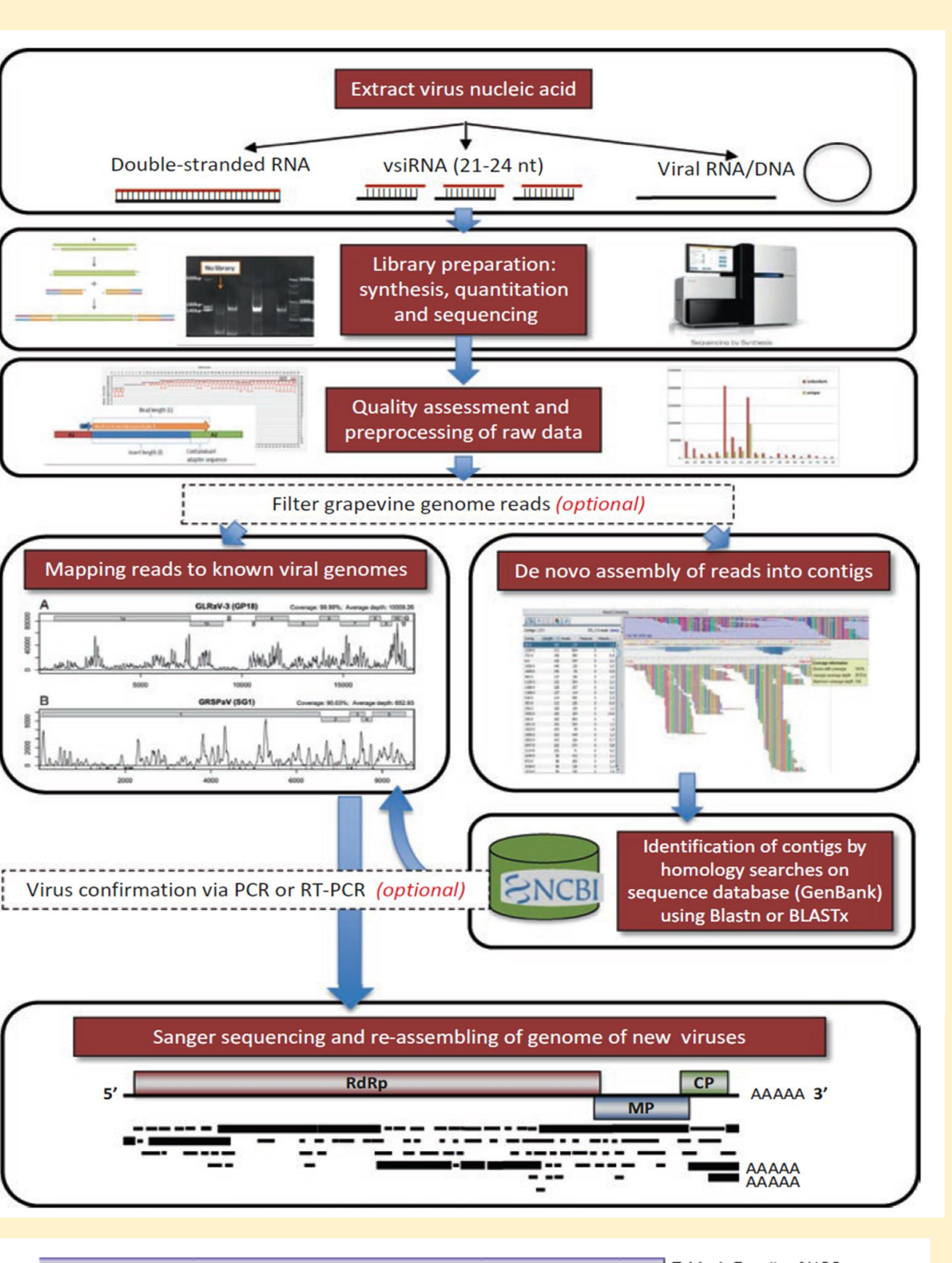
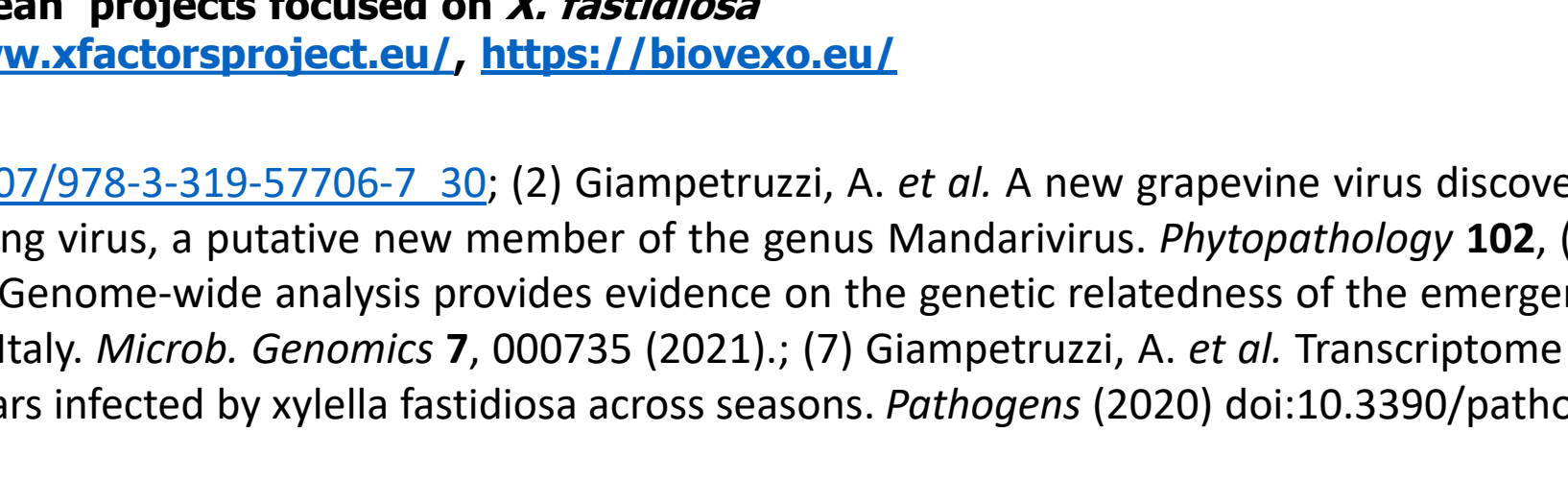
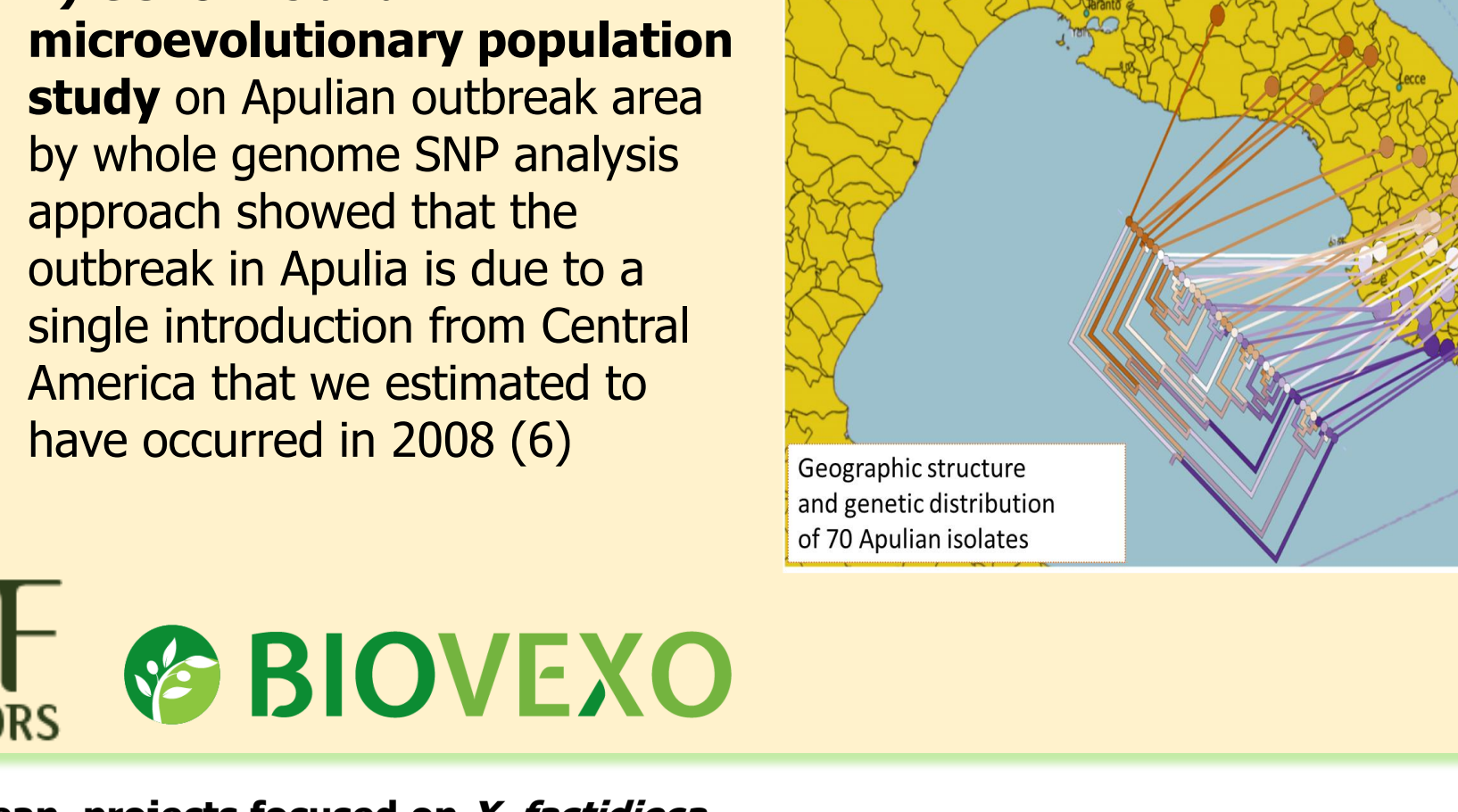


Figure 9. Material & Methods for virus discovery

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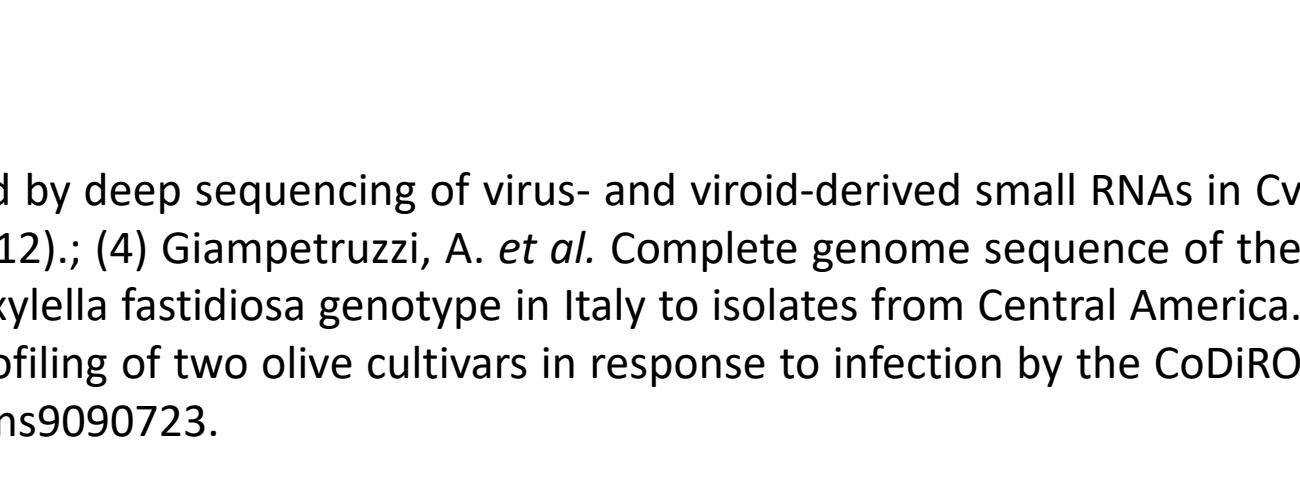
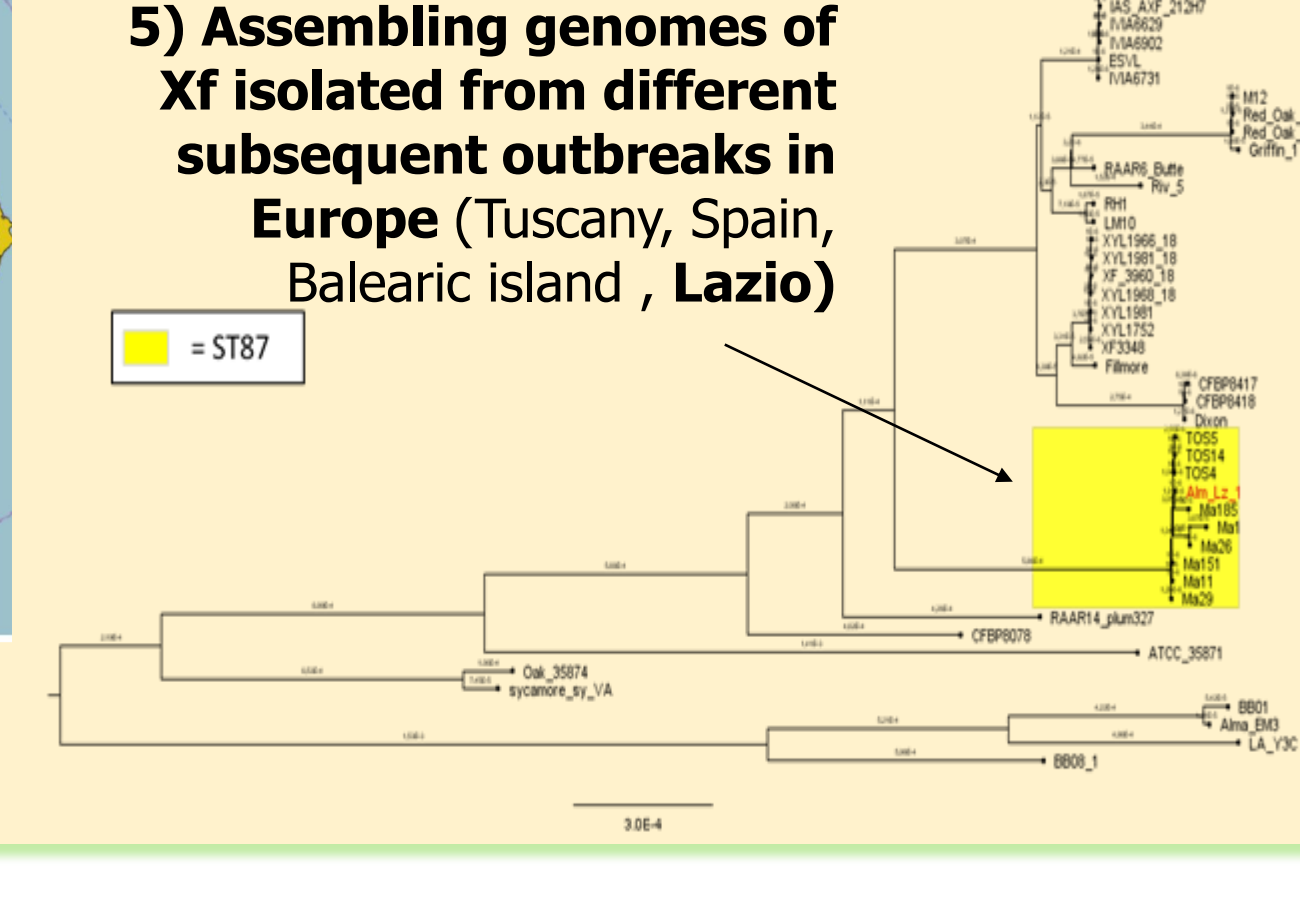
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**Future challenges:** Machine learning approaches will be tested on gene expression data, to exploit this resource with the aim to characterize resistance traits in new olive selections while identifying novel diagnostic biomarkers of the plant responses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