

Co-funded by the European Union

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### Preparation of the joint agreement within the Consortium on the use of DTHs

**Prof.** Dimitris Tsitsigiannis (Agricultural University of Athens-AUA) and Prof.

Magdalena Cara (Agricultural University of Tirana -AUT)



Harmonization and Innovation in PhD Study Programs for Plant Health in Sustainable Agriculture –HarISA is a Erasmus+ project Cofunded with the suport of the European Union. Project Number: 598444-EPP-1-2018-1-HR-EPPKA2-CBHE-JP (2018-2472 / 001-001)



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# **Description of WP 4**

**Diagnostic and training hubs** (DTH) will be established at PIs in **partner countries** and equipped with additional **equipment** in order to serve as the **future regional** 

centers of excellence in the selected specific fields.















### Plant Disease Clinic

### TEXAS PLANT DISEASE DIAGNOSTIC LABORATORY

Department of Plant Pathology and Microbiology Texas A&M University plantpathology.tamu.edu



BACTERIAL STREA

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# Networking to Improve Diagnostic Efficiency (MODEL)



Communication with first responders: Farm agents, farmers, consultants, etc.

- Organized system of laboratories and personnel communicating with one another and working together
- Hierarchical structure
- Example: U.S. National Plant Diagnostic Network



# **Objectives**



- To develop the selection criteria and identify the excellence of scientific groups within the PIs from partner countries
- To upgrade existing facilities in service of PhD students', staff and professionals' needs
- To establish diagnostic and training hubs with high expertise in particular fields available to serve as regional centers for education and spreading knowledge



# Introduction Healthy Plants = Healthy World

- Plant disease diagnostics has been called art and science.
- Establishment of **Diagnostic and Training Hubs** (DTH) is one of the priority tasks of the international network of universities including researchers and doctoral students in plant health.
- The core of DTHs is the plant protection laboratories, which are already equipped with infrastructure suitable for the identification of plant pathogens/pests by advanced molecular methods, in the framework Erasmus+ CBHE - HarISA project.

# The goal of the DTH:

- to harmonize protocols (Standard Operating Procedures),
- conduct training programs,
- introduce distance diagnostic and
- data management web portal.



# **Material and methods**

- Establishment of a <u>network of diagnostic laboratories</u> of partner universities in ERASMUS PLUS CBHE.
- Identified Capacity Development (Inventory of <u>existing equipment</u> and purchase of <u>new equipment</u>).
- Inter-calibrations between laboratories and proficiency tests that are mandatory in accreditations, especially with PCR, Real Time PCR & LAMP, etc.
- Carrying out joint diagnoses and publication with harmonize protocols.
- Mutual cooperation agreement between laboratories as a HarISA product
- Preparation of training program for Ph.D. Students and young researches.
- **Expanding the network** with partners interested in cooperation.

# Preliminary Results

### Establishment of a network of diagnostic laboratories.

**Diagnosis Training Hubs** 

# This network is led by the Agricultural University of Tirana (AUT) and supported by universities of EU countries.

1a. Agricultural University Tirana (AUT)
1b. University F.S. Noli Korce (UNKO)
2a. University of Sarajevo (UNSA)
2b. University of Mostar (SVEMO)
3a. University of Belgrade (UB)
3b. University of Novi Sad (UNS)

4. Biotechnical University of Montenegro (UOM)



## **Identified Capacity Development**

- Equipment
- Access to biotech materials and supplies
- Updated pest lists
- > Access to library/reference materials

Diagnostics standardization across labs (SOPs)

Purchase of Plant & Equipment

Technology Transfer Methods

## New equipment



# **Standard Operating Procedures**

- Background
- > Sampling
- Symptoms & Signs
- > Media recipes
- Morphological caracterisation
- Serological & Biochemical tests
- > DNA/RNA extraction
- PCR protocols & identification
- Sequencing & Phylogenetic analysis
- Widely tested and validated



#### International Plant Diagnostic Network

Standard Operating Procedure for Plant Diagnostic Laboratories

Banana Fusarium wilt/Panama disease Fusarium axysporum f. sp. cubense

http://www.sun.ac.za/english/faculty/agri/plant-pathology/ac4tr4/Documents/Revised%20SOP\_Panama\_IPDN.pdf

# Sampling preparation for ELISA, DTBIA, PCR and LAMP





+ 5-8 ml buffer (1:10)





Fig. 5 Tissue extracts for ELISA test in DPP laboratory.









### 0.5-0.8 gr inde te mostres perfaqesuese





DNA extraction or ELISA/LAMP sap preparation



1 ml crude sap

Liquid-nitrogen

### **ISOLATION PROCEDURES**

### **1. External lesions and/or slight internal rot**



Surface sterilization by NaOCI (2%) for 2 min, rinsed for 1 min by sterile distilled water and airdried in sterile conditions







### 2. Severe and liquid rot



Sterilization by spraying a 70-90% ethanol solution followed by airdrying in sterile conditions





Small tissue portions plated on semi-selective Potato Dextrose Agar (PDA) amended with ampicillin and streptomycin (250 mg/L each) <sup>15</sup>

(Mincuzzi et al., 2020; Garganese et al., 2016)

# Patogenicity test



### **Colletotricum gloesporoides**

Koch's Postulate

# **DNA/RNA extraction, PCR, Sequencings**

















BLAST sequence analysis (accession KY751715) at GenBank revealed 99% similarity with P. capsici AJ854285.



**BLAST sequence analysis (accession KY751715) at** 

GenBank revealed 99% similarity with P. capsici AJ854285.

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## Inter-calibrations between laboratories and proficiency tests

- 1st international external quality assessment studies for laboratory diagnosis of *Xylella fastidiosa*. (35 Laboratories).
- Intercalibrations have been performed between AUT and the University of Bari and CIHEAM for some plant pathogens. (Colletotricum gloesporoides, Rhizopus arizus, Alternaria alternata, etc.
- Intercalibrations with the University of Catania for Colletotricum gloesporoides in citrus.
- Intercalibrations with the Agricultural University of Athens for *Colletotricum acutatum* in olives.

# THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION HORIZON 2020



Xylella Fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy



### EU-XF- PT-2017-02: Proficiency testing for the evaluation of molecular and serological diagnosis of *Xylella fastidiosa*



(organized in accordance with EPPO 7/122 and ISO/IEC 17043 guidelines)

18 EU/non-EU Countries
35 participating laboratories
identified by an anonymous alphanumeric
code to ensure results confidentiality



# Proficiency test (PT) Xylella in Albania test performance study



# **ELISA tests with 2 kits**











Validation data are available on EPPO validation database! <u>http://dc.eppo.int/validationlist.php</u>



### **QIAGEN - Mericon kit extraction**





### Polymerase Chain Reaction Minsavage 1994



### CATEGORIZATION OF THE LABORATORIES BASED ON THEIR PERFORMANCE

Based on the values (%) recovered for the "accuracy" the laboratories were categorized as:

Lab categorization	level of accuracy
highly proficient	100%
proficient	90-100% (1 PD, 1 ND)
non-proficient	<90% (>1 PD, > 1 ND)

The declaration of conformity to the PT assigned to <u>"highly proficient" and "proficient" labs</u>

		Pe	rformance	Criteria	Decoding	Results obtained	Accordance/Discordance
Finhpes	Plats A-M	-			11M	Positive	РА
, ,		European RS			5M	Positive	PA
BARI (ITAL V)	Telefor fast films dutine ( and design film) a manifestigation - data and Research for	mus European Union funding for Research & Innovation	Sancihi		4M	Positive	РА
TOMMEN	CFTEMBER 21, 2017				7M	Positive	РА
TO. WHOM IT N	IAY CONCERN				3M	Positive	PA
SUBJECT: EU-) serological diagn	F- PT-2017-02 - Proficiency testing for the en	Valuation of males i			12M	Positive	PA
This	, a la l	and and an and			10M	Positive	PA
Agriculture and E	ertify that the Laboratory of the Department of invironment, Agricultural University	Plant Protection, Faculty of	Sharitin		9M	Positive	PA
Prof. Magdalena ebruary and Ar	Cara, participated to the proficiency test EL	a (Albania), represented by U-XF- PT-2017-02 between			8M	Positive	PA
aboratories involv	ed in the identification of <i>Xylella fastidiosa</i>	performance of diagnostic			1M	Negative	NA
The Labora rological and mo	tory of the University of Tirana, identifie	d as L32, performed both			2M	Negative	NA
ecifically, the lab	oratory L32 performed ELISA test using two	ded by the Organizers. More			6M	Negative	NA
R tests using tw	o different method of DNA extraction.	unterent diagnostic kits, and	ACCUTA		13M	Negative	Not evaluated
Ction method, i. Please feel fr Best regards, Dr. Maria Sap IPSP-CNR, B. On the behalf	e. using the PCR tests. Be to contact us, for any additional clarific onari Merue Set ari of the Organizer	ation.		CEVORESA.			
26		r . 1		APPROVES			
27	Nu						
28		2					
29	N	12				L32	2
31		12				100	%
22	D	erformance criteria:	sensibility specificity accuracy			100	%
22	r.	errormance errena.	sensibility, specificity, accuracy			100	%
33							
34							
35	Labora	tory Code	L01				
36	Ser	nsibility	100%				
3/	Spe		100%	DINA E)	traction.		
30	Ac	curacy	100	A-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1	CARLES CONTRACTOR		
40			The resu	Its obtained in the Laboratory of	the Aminut		
40			theither to the	and Euroratory of	ane Agricultur	al University	of Tirana showed
42			that the Labora	tory performed proficiently thus n	esulted "oont	o man a litter a la se	
43			datastiss	, state in the state of the sta	sounce conf	ormed", with	the most sensitive
4.4			detection metho	od, i.e. using the PCR tests			

# Kit and device icgene

#### ) enbiotech.eu/en/

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#### Company

Enbiotech works in the field of molecular diagnostics, develops and manufactures products and services that are fast and simple to use in the plant pathology, food and environmental safety sectors.

In 2010 a group of researchers with expertise in molecular biology, microbiology, chemistry and biotechnology, create enbiotech with the ambition of transferring new technologies to market by collaborating in an integrated manner with important scientific centers.



# **RT LAMP kit**

**Extraction Strip** 

Primer MIX Strip

Budge Xylella screen glow & FD

LAMP MIX

**Mineral oil** 

**Positive control** 

**Negative control** 





# **Real time LAMP**









### Albanian J. Agric. Sci.

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OCT

### Loop mediated isothermal amplification: an innovative gene amplification technique for plant diseases

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 Abstract:

Nowadays, molecular diagnostic methods of plant pathogens evolved in a fast way, the use of rapid and easy to use detection method is fundamental to prevent pathogens cross border and spread to enhance food quality and security. Real – Time Loop-mediated isothermal amplification (RT-LAMP) is a novel molecular detection method that specifically detects genomic DNA by using a set of six oligonucleotide primers specific to different regions of a target gene and *Bacillus stearothermophilus (Bst)* DNA polymerase protein. This method has been recently modified to be use as a RT-LAMP and then widely applied in many fields for on-site detection and ability to be used in cross border control of plant health, such as quarantine disease diagnosis. The application of rapid and simple DNA extraction method (10 min at 65°C) shortened the detection assay to less than one

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	Eiken GENOME SITE	(http://www.eiken.co.jp/en/)	-merature review	
	LAMP –Loop	o-Mediated Isotherm	nal	
1	- Sensitive, less time-consumi	<b>cation</b> (Notomi <i>et al.</i> , 2000)	quire skilled operators.	1
Bacteria	-Reaction occurs at isothermal c	conditions (65 °C), using one enzyme	Bst DNA polymerase.	
	-Possible on-field application in	a simple heat-block.		
> Viruses <sup>-</sup>	Examples o	of LAMP application in Plant path	nology	
Fungi	Disease	Pathogen	References	S AT
Protista	Tomato and potato late blight	Phytophthora infestans	Hansen et al., 2016	1
	Fusarium wilt of chickpea	Fusarium oxysporum f.sp. ciceris	Ghosh et al., 2015	1 st
> vvorms	Grapevine yellow	Flavescence dorée	Kogovšek et al. ( <u>2015</u> )	a series of the
	Grape powdery Mildew	Erysiphe necator	Thiessen <i>et al.,</i> 2013	100
<b>C</b>	Fire blight	Erwinia amylovora	Temple and Johnson, 2011; Bühlmann <i>et al</i> ., 2012; Moradi <i>et al.</i> , 2012	
	Citrus Bacterial Canker	Xanthomonas spp.	Rigano <i>et al</i> ., 2010	
See 1	Grey Mould	Botrytis cinerea	Tomlinson <i>et al</i> ., 2010	
	Pierce's disease, citrus veinal chlorosis, almond leaf scorch, Olive Quick Decline	Xylella fastidiosa	Harper <i>et al.,</i> 2010 Yaseen <i>et al.</i> , 2015	

## Carrying out joint diagnoses and publication with harmonize protocols



#### **Research article**

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# Isolation of *Rhizopus arrhizus* from Albanian barley

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sustainability

#### Article

Optical Characterization of *Alternaria* spp. Contaminated Wheat Grain and Its Influence in Early Broilers Nutrition on Oxidative Stress

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MDPI



Figure 2. Phylogenetic analysis of *Rhizopus arrhizus* isolated in this study (RO1) and related sequences from GenBank. *Aspergillus fumigatus* was used as the out-group taxon evolutionary history was inferred by using the Maximum Likelihood method. The tree with the highest log likelihood (–1685.5953) is shown. The percentage of trees in which associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutio analyses were conducted in MEGA6.

#### ncbi.nlm.nih.gov/nuccore/MG599472.1 Cara, M., Sanzani, S.M., Mincuzzi, A., Ippolito, A., Cara, O. and AUTHORS Merkuri,J. Isolation of Rhizopus arrhizus from Albanian barley TITLE JOURNAL J. Inst. Brew. (2018) In press Publication Status: Available-Online prior to print REMARK REFERENCE 2 (bases 1 to 593) Cara, M., Sanzani, S.M., Mincuzzi, A., Ippolito, A., Cara, O. and AUTHORS Merkuri,J. TITLE Direct Submission Submitted (01-DEC-2017) Department of Soil, Plant, and Food JOURNAL Sciences, University of Bari Aldo Moro, Via Amendola 165/A, Bari 70126, Italy COMMENT ##Assembly-Data-START## Sequencing Technology :: Sanger dideoxy sequencing ##Assembly-Data-END## FEATURES Location/Qualifiers 1..593 source /organism="Rhizopus oryzae" /mol\_type="genomic DNA" /strain="RO1" /isolation source="barley kernels" /host="Hordeum vulgare' /db xref="taxon:64495" /country="Albania" /note="PCR primers=fwd name: ITS1, rev name: ITS4" <1..>593 misc RNA /note="contains small subunit ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and large subunit ribosomal RNA" ORIGIN 1 gcggaaggat cattaattat gttaaagcgc cttaccttag ggtttcctct ggggtaagtg 61 attgcttcta cactgtgaaa atttggctga gagactcaga ctggtcatgg gtagacctat 121 ctggggtttg atcgatgcca ctcctggttt caggagtacc cttcataata aacctagaaa 181 ttcagtatta taaagtttaa taaaaaacaa cttttaacaa tggatctctt ggttctcgca 241 tcgatgaaga acgtagcaaa gtgcgataac tagtgtgaat tgcatattca gtgaatcatc 301 gagtetttga acgeagettg eactetatgg tttttetata gagtaegeet getteagtat 361 catcacaaac ccacataa catttgttta tgtggtgatg ggtcgcatcg ctgttttatt

421 acagtgagca cctaaaatgt gtgtgatttt ctgtctggct tgctaggcag gaatattacg

481 ctggtctcag gatctttttt tttggttcgc ccaggaagta aagtacaaga gtataatcca

541 gtaactttca aactatgatc tgaagtcagg tgggattacc cgctgaactt aag

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Short communication

*Colletotrichum gloeosporioides sensu stricto* as causal agent of anthracnose on pomegranate fruit in Albania

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Fig. 3. Bayesian inference phylogenetic tree of *Collectorichum glocoperioides* species complex demonstrating the strain from this study (in bold) belongs to C. glocoportioides sensu arites. The tree has been reconstructed from a combined multi-locus sequence alignment. Bayesian posterior probability (BPP) values (above 0.50) are represented by thickness of the node. i lant Discuse



#### First Report of *Colletotrichum acutatum* Causing Anthracnose on Olives in Albania

Journal:Plant DiseaseManuscript IDDraftManuscript Type:Plant Disease NoteDate Submitted by the Author:n/aComplete List of Authors:Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Illaid, Maria; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science ScienceKeywords:Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas		
Manuscript IDDraftManuscript Type:Plant Disease NoteDate Submitted by the Authorin/aComplete List of Authors:Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Iliadi, Maria; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop ScienceKeywords:Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas	Journal:	Plant Disease
Manuscript Type;Plant Disease NoteDate Submitted by the Author;n/aComplete List of Authors;Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Iliadi, Maria; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science Merkuri, Jordan; Didactic and Scientific Research Center rsitsiglannis, Dimitrios; Agricultural University of Athens, Crop ScienceKeywords:Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas	Manuscript ID	Draft
Date Submitted by the Author:n/aComplete List of Authors:Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Iliadi, Maria; Agricultural University of Athens, Crop Science Lagogianni, Christina; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science Merkuri, Jordan; Didactic and Scientific Research Center Tsitsigiannis, Dimitrios; Agricultural University of Athens, Crop ScienceKeywords:Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas	Manuscript Type:	Plant Disease Note
Complete List of Authors:Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Iliadi, Maria; Agricultural University of Athens, Crop Science Lagogianni, Christina; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science Merkuri, Jordan; Didactic and Scientific Research Center Tsitsigiannis, Dimitrios; Agricultural University of Athens, Crop Science Merkuri Science Science Merkuri Science Merkuri Scien	Date Submitted by the Author:	n/a
Keywords: Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas	Complete List of Authors:	Cara , Magdalena; Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment Iliadi, Maria; Agricultural University of Athens, Crop Science Lagogianni, Christina; Agricultural University of Athens, Crop Science Paplomatas, Epapeimondas; Agricultural University of Athens, Crop Science Merkuri, Jordan; Didactic and Scientific Research Center Tsitsigiannis, Dimitrios; Agricultural University of Athens, Crop Science
	Keywords:	Fungi < Causal Agent, tree fruits < Fruit < Crop Type, Pathogen detection < Subject Areas, Etiology < Subject Areas



Figure S1. Symptoms of anthracnose on olive fruits and morphology of the Colletotrichum acuto

Symptoms of mummified olive fruits (A), acervuli of C. acutatum on olive fruits after artificial in

(B), colony growth on PDA (C) and conidia (D) (5 days on PDA, 400X magnification).



### **Twig and Shoot Dieback of Citrus, a New Disease Caused by** *Colletotrichum* **Species**

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Editor: Nihal Buzkan, Kahramanmaraş Sütçü Imam University, Turkey.

#### Short Notes

#### Detection and phylogeny of viruses in native Albanian olive varieties

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Summary. Forty samples representing 14 native Albanian and two foreign olive varieties were collected from an olive varietal collection plot in the Valias region (Tirana, Albania). The samples were assayed by RT-PCR for presence of olive-infecting viruses, including arabis mosaic virus (ArMV), cherry leaf roll virus (CLRV), cucumber mosaic virus (CMV), olive latent ringspot virus (OLRSV), olive latent virus 1 (OLV-1), olive leaf vellowing-associated virus (OLYaV), strawberry latent ringspot virus (SLRSV) and by PCR for the bacterium Xylella fastidiosa (Xf). Ninety-eight percent of the samples were infected with at least one virus. OLYaV was the most prevalent (85% of samples), followed by OLV-1 (50%), OLRSV (48%), CMV (28%), SLRSV (3%) and CLRV (5%), whereas ArMV and Xf were absent. Fifty-five percent of the samples were infected with one virus, 13% with two viruses, 20% with three, and 5% with four. Analyses of the nucleotide sequences of the Albanian virus isolates generally showed low genetic variability, and that most were phylogenetically related to Mediterranean isolates, in particular to those from Greece and Italy. Five olive trees, representing three native cultivars ('Managiel', 'Kalinjot' and 'Kushan-Preze') and one foreign ('Leccino'), were found to be plants of the Conformitas Agraria Communitatis ("CAC") category i.e. free of ArMV, CLRV, SLRSV and OLYaV. Only one tree of the native cultivar 'Ulliri i kuq' was free of all tested viruses, so this is plant material of the "Virus-tested" category. Olives derived from both categories could be used for propagation of standard quality plant materiel in a future certification programme for olive in Albania. This is the first report of CLRV, OLRSV, CMV and OLV-1 in Albania. The study also reveals the precarious health status of native olive varieties in the Valias varietal collection plot. However, the discovery of six plants representing two certifiable categories is a first step in a future olive tree certification program in the country.

**Table 2.** Olive samples collected from the varietal collection plot of Valias region (Tirana, A<sup>\*\*</sup> - \* assayed by RT-PCR for the presence of seven olive-infecting viruses. \* *CAC* category plants; \*\* *tested* category plant.

N°. cv	Cultivar	N°. sample	ArMV	SLRSV	CLRV	OLYaV	OLRSV	CMV	OLV-1
		1	-	-	-	+	+	-	-
1	Boç	2	-	-	-	+	+	-	-
		3	-	-	-	+	+	-	-
2	Managial	1*	-	-	-	-	+	-	+
2	Managjei	2	-	-	-	+	+	-	+
		1	-	-	-	+	-	-	+
3	Frangu	2	-	-	-	+	-	-	+
		3	-	-	-	+	-	-	+
		1	-	-	-	+	-	-	+
4	Kushan	2	-	-	-	+	+	-	+
		3	-	-	-	+	-	-	+
	I bardhi i	1	-	-	-	+	+	-	+
5	Tiranes	2	-	-	-	+	+	-	+
		3	-	+	-	+	+	-	+
		1*	-	-	-	-	+	+	-
6	Kalinjot	2*	-	-	-	-	-	+	-
		3	-	-	-	+	-	+	-
_	Krypsi i	1	-	-	-	+	+	+	-
7	Krujes	2	-	-	+	+	+	+	-
		3	-	-	-	+	+	-	-
0	Kokermadhi i	1	-	-	+	+	-	+	-
8	Beratit	2	-	-	-	+	-	+	+
		3	-	-	-	+	-	+	+
0	I holli i	1	-	-	-	+	+	-	-
9	Himares	2	-	-	-	+	+	-	-
			-	-	-	+	+	-	-
10	Miyan	2	-	-	-	+	-	Ŧ	-
10	WIIXall	2		-	-	+		+	-
		1					-	-	
11	Lecino Valias	2	-	-	_	+	-	_	_
11	Leemo vanas	3*	-	-	_	-	-	_	+
	Frantoio- Valias	1		-	-	+	+	-	-
12		2	-	-	-	+	+	+	+
	Kushan- Preze	1*	-	-	-	-	-	_	+
13		2	-	-	-	+	-	-	+
	I bardhi i	1	-	-	-	+	-	-	-
14	Tiranes-	_							
	Preze	2	-	-	-	+	-	-	+
15	Mixan- Preze	1	-	-	-	+	-	-	+
16	Ulliri i kuq- Preze	1**	-	-	-	-	-	-	-
	No infected trees		0	1	2	34	19	11	20
	% infected trees		0	2.5	5	85	47.5	27.5	50







#### **POMEGRANATE STRAINS**

		AP19-A	lternaria alternata morphotype alternata strain 19						
		KU933229.1-Alternaria alternata morphotype alternata strain A65							
		AP18-Alternaria alternata morphotype alternata strain 18							
		AP17-A	lternaria alternata morphotype alternata strain 17						
		AP15-A	lternaria alternata morphotype alternata strain 15						
	00	AP14-A	lternaria alternata morphotype alternata strain 14						
	33	AP3- Al	ternaria alternata morphotype alternata strain 3						
		AP2-Alt	ernaria alternata morphotype alternata strain 2						
		- MG063	726.1-Alternaria alternata morphotype alternata f.sp. strain CBS 10727						
		AP9-Al	ternaria alternata morphotype alternata strain 9						
	63	AP10-4	Alternaria alternata morphotype alternata strain 10						
	05	AP16-4	AP16-Alternaria alternata morphotype alternata strain 16						
		MG063725.1-Alternaria alternata morphotype alternata strain CBS 112249							
		AP6-Alternaria alternata morphotype tenuissima strain 6							
	9	AP8-Alternaria alternata morphotype tenuissima strain 8							
		AP5-Alternaria alternata morphotype tenuissima strain 5							
		JQ800561.1-Alternaria alternata morphotype tenuissima isolate 37FrB							
			MG063729.1-Alternaria alternata morphotype limoniasperae CBS 102.595						
65	1		AP1-Alternaria alternata morphotype limoniasperae strain 1						
			AP4-Alternaria alternata morphotype limoniasperae strain 4						
			AP7-Alternaria alternata morphotype limoniasperae strain 7						
		99	AP11-Alternaria alternata morphotype limoniasperae strain 11						
			AP12-Alternaria alternata morphotype limoniasperae strain 12						
			AP20-Alternaria alternata morphotype limoniasperae strain 20						
			KU933223.1-Alternaria alternata morphotype limoniasperae strain A42						
			<ul> <li>KY561993.1-Alternaria solani</li> </ul>						



#### alternaria tenuissima limoniasperae

#### **CITRUS STRAINS**



Eur J Plant Pathol https://doi.org/10.1007/s10658-021-02240-9

Mycotoxigenic fungi contaminating greenhouse-grown tomato fruit and their alternative control

Simona Marianna Sanzani - Ferielle Djenane -Ornella Incerti - Naouel Admane -Annamaria Mincuzzi - Antonio Ippolito



Detection of key genes of biosynthesis of secondary metabolites

Mycotoxins AOH/AME Phytotoxins ACT & pksl ACR actt1-2 & acrt1-2



ACT-toxin R = 0H = 0 OH = 0

CIHEAM

BARI





RESEARCH ARTICLE

Characterization of Citrus-Associated Alternaria Species in Mediterranean Areas

Francesca Garganese<sup>1</sup>, Leonardo Schena<sup>2</sup>, Ilenia Siciliano<sup>3</sup>, Maria Isabella Prigigallo<sup>2</sup>, Davide Spadaro<sup>3,4</sup>, Anna De Grassi<sup>5</sup>, Antonio Ippolito<sup>1</sup>, Simona Marianna Sanzani<sup>1</sup>\*

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### PRESENCE OF PKSI GENE







# **Preliminary results of DTHs**

Sequencing and registration in repositories of economically relevant pathogens, some of them:

- Colletotricum gloeosporioides in citrus and pomegranate,
- Phytophthora nicotianae and P. citrophthora in citrus,
- Colletotricum acutatum in olive,
- Phytophthora capsici in cucumber, etc.
- More than 70 sequences were registered in GenBank.
- 32 strains of Alternaria alternata are already submitted in ncbi
- These pathogens are stored in standard cultural collections at the AUT and in partner universities.

## Mutual cooperation agreement between laboratories as a HarISA product













#### Co-Funded by the European Union

#### University of Zagreb Faculty of Agriculture Svetosimunska street 25, 10000 Zagreb, Croatia Contact: <u>harissa@agr.hr</u> <u>www.agr.hr</u>





LOGO here

UNIVERSITY OF SARAJEVO FACULTY OF AGRICULTURE AND FOOD SCIENCES Zmaja od Bosne 8, 71000 Sarajevo Bosnia and Herzegovina

And

UNIVERSITY OF ... FACULTY OF... Address Country

Conclude an

#### AGREEMENT OF SCIENTIFIC COOPERATION

#### I General

University of Sarajevo, Faculty of Agriculture and Food Sciences and University of ... Faculty of ... (hereinafter referred to as "Parties") wishing to promote scientific, academic and educational cooperation between two Institutions, in the terms stated henceforth, have concluded the present Agreement of Scientific Cooperation.

This Agreement aims to provide a general framework for facilitating institutional collaboration and cooperation between the Parties in areas of common interest.

#### II Subject of the Agreement

Cooperation will be implemented through, but may not be limited to, the following activities:

- Networking and mutul exchange of academic staff, research personnel, students, and
  other associates for lectures, visits and transfer of knowledge,
- Joint educational, training and research activities,
- Participation in the implementation of expert activities,
- Realization of scientific-educational programs of doctoral and postdoctoral studies and projects,
- Joint participation and applying for funds designated for research and education,
- Joint organization of conferences, symposia, congresses, semianrs, courses, summer schools, workshops, and other meetings,
- Exchange of proessional literature, textbooks, and other university publications.
- Exchange of information in fields of interest to both Parties.

In the implementation of specific cooperative programs, a writen agreement covering all relevant aspects including funding and other obligations to be undertaken by each party will be negotiated, mutually agreed and formalized in writing, prior to the commencement of the program.

#### III Duration of the Agreement

This Agreement will become effective upon the date of signature of both institutions. It will be in force for five (5) year and may be renewed by the Parties for further period.

Amendments and changes will be possible only in case of written consent by both Parties and will be added to this Agreement.

This Agreement may be terminated before the expirary of the five (5) year period only upon written consent of both Parties. Either Party wishing to make changes or terminate the Agreement shall notify the other institution in writing os such intention no later than six (6) months before the suggested changes or termination should come into effect. The Agreement cannot be terminated if this action will jeopardize the implementation of any of the agreed activities, or before the deadline set for completion of such activities.

#### IV Implementation of the Agreement

Each institution will appoint a contact person to coordinate the implementation of this Agreement. Both Parties have committed not to execute activities related to this Agreement without previous consultations.

Both Parties agree that all financial agreements necessary to implement this Agreement must be negotiated and will depend upon availability of funds. Neither institution shall have any financial obligation to the other institution based on this Agreement.

In witness whereof, the parties hereto affix their signatures below this

For University of Sarajevo Faculty of Agriculture and Food Sciences For University of ... Faculty of ....

Prof. dr.

Prof. dr. Muhamed Brka, Dean Date: Date: Nr.: Nr.:



Harmonization and Innovation in PhD Study Programs for Plant Health in Sustainable Agriculture –HarISA is a Erasmus+ project Co-Funded with the suport of the European Union. Project Number: 598444-EPP-1-2018-1-HR-EPPKA2-CBHE-JP (2018-2472 / 001-001)

## **Preparation of training program for Ph.D. Students and young researches.**



Plant Disease

Insect Clinic

# Manual on PLANT HEALTH CLINIC





2020

# **Expanding the network** with

## partners interested in cooperation.

- Dept. Agriculture, Food and Environment, University of Catania (ERASMUS ICM)
- CIHEAM, Bari (Master thesis supervisor)
- Plant Pathology Lab, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki (ERASMUS ICM applic.)
- University of the West of Scotland (ERASMUS ICM)
- CNR Bari Italy (Bilateral Project)

# **Projects that support DTH**

- Horizon Europe focus for all researchers
- ERASMUS PLUS CBHE & ICM
- Jean Monnet (Module, chair, network, excellence center)
- COST Action (excellent networks, COST Inclusiveness Target Countries (ITCs)
- Maria Sklodowska Curie Ph.D. students
- National and Bilateral projects Ph.D. students



### Co-funded by the European Union







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