a draft genome sequence of '*Ca.* P. phoenicium' strain SA213, representative of phytoplasma strain populations from different host plants, and determined the genetic diversity among phytoplasma strain populations by phylogenetic analyses of 16S rRNA, *groEL*, *tufB* and *inmp* gene sequences. Sequence-based typing and phylogenetic analysis of the gene *inmp*, coding an integral membrane protein, distinguished AlmWB-associated phytoplasma strains originating from diverse host plants, whereas their 16S rRNA, *tufB* and *groEL* genes shared 100% sequence identity. Moreover, dN/dS analysis indicated positive selection acting on *inmp* gene. Draft genome analyses suggest a parasitism based on the import of glycerol-3-phosphate, a critical mobile inducer of plant systemic immunity. Additionally, integral membrane proteins, effector-like proteins and potential candidates for interaction with hosts were identified. One of the integral membrane proteins was predicted as BI-1, an inhibitor of apoptosis-promoting Bax factor. Bioinformatics analyses revealed the presence of putative BI-1 in draft and complete genomes of other '*Ca.* Phytoplasma' species. The genetic diversity within '*Ca.* P. phoenicium' strain populations in Lebanon suggested that AlmWB disease could be associated with phytoplasma strains derived from the adaptation of an original strain to diverse hosts. Moreover, the identification of BI-1 in '*Ca.* P. phoenicium' draft genome and within genomes of other '*Ca.* Phytoplasma' species suggested its potential role as a phytoplasma fitness-increasing factor by modification of the host-defense response.

THE NEW DEAL IN VIRUS DISCOVERY: A MAJOR CONCERN FOR "MINOR" CROPS. **M. Morelli, M. Chiumenti, P. Saldarelli, A. Giampetruzzi, A. Minafra.** *National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via G. Amendola 122/D, I-70126 Bari, Italy. E-mail: massimiliano.morelli@ipsp.cnr.it*

The use of NGS approach allowed the identification and the complete genome characterization of several plant virus species, previously unknown (*Persimmon cryptic virus*, PeCV; *Mulberry badnavirus-1*, MBV-1), or scarcely characterized and never associated to a specific symptomatology and/or host (*Persimmon rhabdovirus A*, PeVA; *Apple green crinkle associated virus*, AGCaV). Significantly, these findings arose from crops, respectively Japanese persimmon (*Diospyros kaki*) in the case of PeCV and PeVA, quince (*Cydonia oblonga*) for AGCaV and mulberry (*Morus alba*) for MBV-1, disregarded in traditional quest for viral agents and diseases. Routinely used bio- and molecular assays, however modern they are, always hide an *a priori* choice for target, influenced by evident symptomatology, diagnostic purposes prone to certification standard enquiries, availability of literature, etc. NGS analysis, being apart from such constraints, gives the opportunity to widen the target range, thus including also cultivated and wild crops, so far considered not agronomically relevant, or never characterized for their sanitary status. The new approach, accounting on a better feasibility and reliability of detection performance, is leading to noteworthy steps forward for plant virology studies addressed to characterize minor crop infections. A rising number of new species discovered, in once misinvestigated temperate and tropical fruit crops, should not be merely regarded in terms of basic research accomplishments. The meaningful contribution of NGS advent in this field relies in a potential of innovative knowledge to be expended, for instance, in upgrading the extant certification schemes, tracking unforeseen threats in propagative material exchanges and setting new attribution rules for a challenging taxonomical allocation of oncoming species or strains.

SYNTHETIC CLONES OF *GRAPEVINE ALGERIAN LATENT VIRUS* (GALV) DEVELOP A TYPICAL *TOMBUSVIRUS* INFECTION IN *NICOTIANA BENTHAMIANA* INFECTED CELLS. **A. Lovato[1], A. Polverari[1], D. Maffi[2], F. Faoro[2].** *[1]Department of Biotechnology, University of Verona, Strada le Grazie 15, I-37134 Verona, Italy. [2]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy (DISAA), University of Milano, Via Giovanni Celoria 2, I-20133 Milano, Italy. E-mail: franco.faoro@unimi.it*

GALV is a 30 nm icosahedral virus (*Tombusvirus* genus), with a positive ssRNA genome of 4731 nucleotides including at least five Open Reading Frames (ORFs) encoding for replicase proteins (p33 and p92), the coat protein (p40), the movement protein (p24) and the multifunctional p19 protein, which also functions as a silencing suppressor. We have previously produced a synthetic GALV construct complying with the available genome sequence of a GALV isolate from nipplefruit (GALV-nf). This clone systemically infected both grapevine and *Nicotiana benthamiana* plants causing severe symptoms. In order to use GALV-nf as a VIGS (Virus Induced Gene Silencing) vector, we modified it by a single nucleotide substitution on the p19 ORF, to reduce the silencing suppressor activity of the virus, hence increasing the plant silencing efficiency against a target gene. The cytopathology of this mutant (GALV-nf-Δ) and the unmodified clone have been compared to verify if a less functional p19 allowed a correct, though reduced, virus synthesis. Ultrastructural analysis revealed the presence of isometric virus particles and multivesicular bodies (MVBs), typical of tombusviruses, in the apical leaves infected with either GALV clones but more consistent in GALV-nf infection. MVBs mainly originated from peroxisomes, though it cannot be exclude that also mitochondria could be involved in their formation. Immunogold labelling using an anti p33 serum showed that viral replicase is mainly located in the peripheral membrane of MVBs in both synthetic virus constructs, demonstrating that p19 modification does not affect GALV localization and replication but strongly interferes in terms of virus virulence.

Opificio delle idee

DIPLODIA SAPINEA AND CLIMATE CHANGE: SPECIES DISTRIBUTION MODELS OF THE MOST IMPORTANT PINE SHOOT PATHOGEN IN ITALY. **L. Bosso[1], H. Rebelo[2,3], N. Luchi[4], G. Maresi[5], D. Russo[1,3], G. Cristinzio[1].** *[1]Department of Agricultural Sciences, University of Napoli "Federico II", Via Università 100, I-80055 Portici (NA), Italy. [2]CIBIO-Centro de Investigacao em Biodiversidade e Recursos Genéticos da Universidade do Porto, Campus Agrário de Vairão, R. Padre Armando QuintasVairão, Portugal. [3]School of Biological Sciences, University of Bristol 24, Tyndall Avenue, BS8 1TQ, Bristol, United Kingdom. [4]National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Via Madonna del Piano 10, I-50019 Sesto Fiorentino (FI), Italy. [5]Technology Transfer Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. E-mail: luciano.bosso@unina.it*

Species Distribution Models (SDMs) provide realistic scenarios to explain the influence of bioclimatic variables on plant pathogens distribution. In this study, we develop a maximum entropy model for *Diplodia sapinea* in Italy to reach the following goals: i) to carry out the first geographical distribution analysis in Italy and determine which Eco-Geographical Variables (EGVs) may affect its distribution; ii) to detect the effect of climate change on the species' geographic range by 2050 and 2070. To develop SDMs for *D. sapinea* we used Maxent vers. 3.3.3k, the most popular approach to model species distributions with scarce presence-only data. Future climate projections for *D. sapinea* were derived from six Global Climate Models (GCMs) (BCC-CSM1-1, CCSM4, GISS-E2-R, MIROC5, HadGEM2-ES, MPI-ESM-LR) for two representative concentration pathways (RCP 4.5 and RCP 8.5) and two time projections: 2050 and 2070. The most important EGVs for the current distribution were found to be land cover, altitude and mean temperature of wettest quarter. The distribution of *D. pinea* essentially increased in Central and Southern Italy and shifted upwards by 90 m. Moreover, this fungus expanded its range in response to an increase in mean temperature of wettest and driest quarter in all GCMs of 1.9 and 5°C, respectively. Validation statistics (AUC, AUC_{diff} TSS) showed that our models achieved high performances (> 0.8). Our study shows that under different climate change scenarios *D. sapinea* damages will likely affect larger areas of pine forests in the country probably causing heavy effects on dynamics and evolution of these stands or perhaps playing as constrains factor to their survival.

RAPID SPREAD AND GENETIC DIVERSITY OF *PEPINO MOSAIC VIRUS* IN TOMATO CROP IN SICILY. **S. Panno[1], S. Davino[1,2,3], G. Iacono[4], M. Davino[4].** *[1]Euro-Mediterranean Institute of Science and Technology (IEMEST), Via E. Amari 123, I-90139 Palermo, Italy. [2]Department of Agricultural and Forest Sciences (SAF), University of Palermo, Viale delle Scienze Ed. 5, I-90123 Palermo, Italy. [3]National Research Council of Italy, Institute for Sustainable Plant Protection (IPSP), Strada delle Cacce 73, I-10135 Torino, Italy. [4]Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 100, I-95123 Catania, Italy. E-mail: pannostefano@virgilio.it*

Pepino mosaic virus (PepMV), belong to the genus *Potexvirus* of the family *Alphaflexiviridae* cause one of the most destructive diseases of tomato (*Solanum lycopersicum*) crops worldwide. In Sicily, the first outbreak of PepMV has been detected in a single greenhouse in the year 2005 and has been quickly eradicated. After this first report, PepMV has not been detected in Sicily until the end of 2008, when, in this case the disease was impossible to eradicate. The purpose of this study was to assessed the dispersion and the genetic diversity of PepMV in Sicily and to compare it to other PepMV isolates in order to know what factors are determinant for the evolution and epidemiology of this virus. A total of 1,800 samples from symptomatic and asymptomatic plants were randomly collected in Sicily during the period 2001-2013. The incidence of the virus increased rapidly from 13% in 2011 to 63% in 2013. Based on the molecular analysis and host range we can highlight two subgroups of PepMV isolates belonged to the clade CH2: one composed exclusively from sicilian isolates that was extremely virulent and cause symptoms on tomato fruits and another composed from foreign isolates that cause mild symtoms on tomato plants. From an epidemiological point of view more restrictive controls are required to avoid PepMV spreading to other Italian Regions.

COMPARATIVE GENOMICS BETWEEN THE INVASIVE FOREST PATHOGEN *HETEROBASIDION IRREGULARE* AND THE NATIVE SIBLING SPECIES *H. ANNOSUM* PROVIDE A GLIMPSE INTO THEIR DIVERGENT ADAPTIVE EVOLUTION. **F. Sillo[1], M. Garbelotto[2], P. Gonthier[1].** *[1]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Paolo Braccini 2, I-10095 Grugliasco (TO), Italy. [2]Department of Environmental Science, Policy and Management, Forest Pathology and Mycology Laboratory, University of California Berkeley, 54 Mulford Hall, 94720 Berkeley, California, USA. E-mail: paolo.gonthier@unito.it*

The fungal plant pathogens *Heterobasidion irregulare* and *H. annosum* have been evolving allopatrically for 34-41 million of years. *Heterobasidion irregulare* was recently introduced from North America to Italy, within the natural range of *H. annosum*, generating hybrid swarms. Divergent adaptive evolution affecting the genomes of these pathogens is still poorly studied. Here, a comparative genomic approach was used to determine which gene groups were affected by divergent positive selection during the allopatric phase. In particular, it was tested the hypothesis that genes involved in pathogenicity are not as divergent between the two species compared to genes involved in sabrobic ability and sporulation, as previously demonstrated in phenotypic observations. Results based on the whole-genome sequencing of three genotypes per species confirmed their status as sister taxa, despite a large macrosynteny was observed. Genes involved in pathogenicity appeared to be more conserved between the two species compared to genes involved in saprobic growth and sporulation. This finding provided genomic evidence that differences in fitness are more likely to be determined by these two last functions, as previously documented by *in vitro* experiments. A large fraction of genes under positive selection was described as involved in transcriptional functions and mitochondrial factors. Genes in interspecific structural variations were also found to be related to these two categories and to transposable element activity. The study has shown at the genomic level that factors related to transmission rather than those related to pathogenicity might explain the invasiveness of exotic pathogens.

LASER MICRODISSECTION OF GRAPEVINE LEAVES HIGHLIGHTS SITE-SPECIFIC TRANSCRIPTIONAL CHANGES AT THE EARLY STAGES OF DOWNY MILDEW INFECTION. **L. Lenzi[1,2], C. Caruso[2], P.L. Bianchedi[3], I. Pertot[1], M. Perazzolli[1].** *[1]Research and Innovation Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Italy. [2]Department of Agrobiologia and Agrochimica (DABAC), University of Tuscia, Via San Camillo de Lellis s.n.c., I-01100 Viterbo, Italy. [3]Technology Transfer Centre, Edmund Mach Foundation, Via E. Mach 1, I-38010 San Michele all'Adige (TN), Trento, Italy. E-mail: luisa.lenzi@fmach.it*

Grapevine (*Vitis vinifera*) is one of the world's major fruit crops, but most of the commercial cultivars are susceptible to downy mildew, caused by *Plasmopara viticola*. Transcript profiling has largely been used to investigate gene expression changes of the interaction between grapevine and *P. viticola*, but these studies have generally involved the use of RNA from whole grapevine leaves. *Plasmopara viticola* infects grapevine leaves and young berries by stomata and develops intercellular mycelium in the mesophyll. Only a small fraction of leaf cells is in contact with the pathogen at the early stages of infection and the large portion of not-infected cells could mask the transcriptional changes related to defence activation. Laser microdissection (LMD) technique allows the isolation of specific cell types from heterogeneous tissue. LMD was used to specifically collect cells at the site of *P. viticola* infection or at the adjacent layers from inoculated leaves of *in vitro*-grown grapevines. Protocols for sample fixation, laser microdissection and RNA isolation from group of cells were optimized and the expression of ten genes involved in the grapevine defence response was analysed by Real-time RT-PCR. The expression level of the selected genes was generally greater at the site of infection compared to the whole infected leaf, and expression profiles in infected and adjacent cells differed according to the tested genes. These results get new insights on the activation of specific processes at the sites of *P. viticola* infection, which were masked in the whole-leaf analysis, and the optimized protocols will be further used for site-specific transcriptomic studies.

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Difesa delle piante per l'alimentazione e l'energia

BOOK OF ABSTRACTS

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