RESEARCH WORKSHOP

Sofia, Bulgaria
September 6 - 7, 2010

Book of Abstracts

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Research Workshop

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SharCO project:
"Containment of sharka virus in view of EU-expansion"

Project part-financed by the European Community's Seven Framework Programme (FP7/2007-2013) under *Grant Agreement* n°204429

The overall aim of the project SharCo is to help the EU face the accession of Member States known as endemic for sharka disease by providing the EU with tools such as marker-assisted selection, *Plum pox virus* (PPV) resistant plant materials, guidelines, early-warning systems, decision-support system.

For that purpose, the project will, **in the field of epidemiology**, identify driving factors of PPV spread and diversification and develop novel and high through-put detection systems for early warning of sharka outbreaks.

In the field of genetics, it will provide molecular markers for the implementation of marker assisted selection of PPV resistant fruit varieties.

In the field of biology, it will assess innovative biotechnological approaches to broaden resistance to PPV in different fruit tree species.

Finally, in order to develop a PPV outbreak management, the project will elaborate i) guidelines for end-users and policy makers concerning cultivation and risk management coupled with a decision support system, ii) an early warning system.

All knowledge and tools developed by the consortium will be widely disseminated all over Europe with special attention made to PPV endemic countries.

SharCo comprises 9 Partners from EU countries (France, Bulgaria, Romania, Spain, Italy, Poland, Czech Republic, Germany and Slovakia) and 2 non EU countries - Serbia and Turkey.

Project duration is 4 years; the project started in March 2008 and will end in March 2012.

Project structure

The project is built on three pillars, each one divided into a number of work packages strongly interacting with each other within and across the pillars.

The ***Epidemiologic Pillar*** encompasses three main aspects, understanding the mechanisms of the disease process on one hand side, developing outbreak detection and risk-assessment systems of the other hand side. The objective is to develop new methods and new tools for monitoring and fighting PPV spread in nursery blocks and orchards. It will also provide data and tools for the evaluation of PPV diversity and factors driving PPV diversification. Methods, tools and knowledge generated in the Epidemiologic Pillar will be used in the Application Pillar for the elaboration of guidelines for integrated sharka management for sharka outbreaks. This research is performed in the following workpackages:

WPE.1: Large scale analysis of PPV worldwide diversity

WPE.2: Improving knowledge of PPV epidemicity and spread dynamics from orchard to regional scales

WPE3: Evaluation of strategies to reduce the incidence of PPV in nurseries blocks

The primary goal of the ***Genetic Pillar*** is to provide stone fruit breeders with genetic knowledge and tools aimed at selecting the resistant varieties. Molecular markers are being developed in apricot, peach and plum, and used for the implementation of marker assisted breeding programmes for resistance to PPV Europe-wide. Unknown mechanisms and sources of resistance are being identified among natural resources or through biotechnological approaches. By the end of SharCo, complementary mechanisms of resistance will be proposed for gene pyramiding enhance PPV resistance in commercial stone fruit varieties. The PPV resistant plant material generated within the SharCo project will be implemented through the cultivation guidelines in the Application Pillar. The works within this Pillar are organised in two workpackages:

WPG.1: Identification of PPV resistance markers and development of marker assisted selection

WPG.2: Characterisation of new and complementary genetic resistance mechanisms

The ***Application Pillar*** is aimed at putting at the disposal of breeders, nursery gardeners and fruit producers, tools and plant material enabling them to prevent sharka outbreak and minimise the impact in case of contamination. It will also support public policy-makers, regulatory bodies, extension services and other stakeholders through the development of an early warning system, the elaboration of risk management guidelines and recommendations for the cultivation of seedlings and grafted material. To facilitate access to knowledge and tools, a decision support system will be initiated and several training workshops will target dissemination and transfer to end-users based in Eastern European regions. This Pillar is organized into two workpackages:

WPA.1: Sustainable sharka containment scheme

WPA.2: Dissemination and transfer

A ***Management Work package*** addressing two principal issues:

Ø Project monitoring

Ø Administrative and financial issues

Workshop Goals

The goal of this workshop is to share existing research efforts and information in the field of biology, epidemiology and genetics concerning sharka disease.

The research workshop is also intended to promote the dissemination of preliminary SharCo outcomes and data to an enlarged scientific community, including non-European countries.

List of Beneficiaries

Number	Beneficiary name	Beneficiary short name	Country
1	Institut National de la Recherche Agronomique	INRA	France
2	AgroBioInstitute	ABI	Bulgaria
3	Universitatea de Stiinte Agronomice si Medicina Veterinara Bucuresti	USAMV	Romania
4	Consejo Superior de Investigaciones Científicas	CSIC	Spain
5	Consiglio Nazionale delle Ricerche-Istituto di Virologia Vegetale	CNR-IVV	Italy
6	Instituto Valenciano de Investigaciones Agrarias	IVIA	Spain
7	Instytut Sadownictwa i Kwiaciarstwa Skierniewice	ISK	Poland
8	Mustafa Kemal University	MKU	Turkey
9	Crop Research Institute	CRI	Czech Republic
10	Technische Universität München	TUM	Germany
11	SavBa, Institute of Virology	SavBa	Slovakia
12	Fruit Research Institute	FRI	Serbia
14	Fruit Growing Institute	FGI	Bulgaria
15	Università degli Studi di Milano	UMIL	Italy
16	Mendel University of Agriculture and Forestry	MUAF	Czech Republic
17	Statiunea de Cercetare-Dezvoltare pentru Pomicultura Bistrita	SCDP	Romania

Scientific Committee

Veronique Decroocq – Project Coordinator (INRA, France)
Miroslav Glasa – WPE.1 Leader (SavBa, Slovakia)
Gerard Labonne – WPE.2 Leader (INRA, France)
Mariano Cambra – WPE.3 Leader (IVIA, Spain)
Maria-Luisa Badenes – WPG.1 Leader (IVIA, Spain)
Juan-Antonio Garcia – WPG.2 Leader (CSIC, Spain)
Donato Boscia – WPA.1 Leader (CNR-IVV, Italy)
Lech Michalczuk – WPA.2 Leader (ISK, Poland)
Ivanka Kamenova – Local organizer (ABI, Bulgaria)

Organizing Committee

Ivanka Kamenova (ABI, Bulgaria)
Rossitza Batchvarova (ABI, Bulgaria)
Marian Minchev (ABI, Bulgaria)
Tanyo Kolev (ABI, Bulgaria)

PROGRAMME

Monday September 6th

14:00 - 14:30	Registration
14:30 – 17:30	**Research workshop: SharCo presentations**
	Chair: Thierry Candresse
14:30 - 15:00	*A. Voloudakis* - Plant virus control employing RNA - based vaccines: A novel non-transgenic strategy
15:00 - 15:15	*Glasa et al.* - A large scale effort to analyze the *Plum pox virus* diversity worldwide
15:15 - 15:30	*Laizet et* al. - The SharCo *Plum pox virus* Database
15:30 - 15:45	*Olmos et al.* - First generation of a mini-oligo array for rapid genome wide analysis of *Plum pox virus*
15.45 - 16:00	*Pleydell et al.* - Estimating risk factors for PPV dissemination from incomplete epidemiological surveillance data
16:00 - 16:15	*Maliogka et al.* - P1 and CP, the two ends of the potyviral polyprotein, are involved in viral pathogenicity and host adaptation
16:15 - 16: 45	Coffee break
	Chair: Gerard Labonne
16:45 - 17:00	*Cambra et al.* - Preliminary results on the efficiency of oil treatments in reducing of natural *Plum pox virus* infection in *Prunus* nurseries
17:00 - 17:15	*Cambra et al.* - *Plum pox virus* containment in nurseries based on epidemiological data
17:15 - 17:30	*Cambra et al.* - Proposal of a guideline for *Plum pox virus* detection in *Prunus* nurseries
19:00	Dinner

Tuesday September 7th

14:30 - 18.30	SharCo Research Workshop - continuation **Chair: Veronique Decroocq**
14:30 - 14:45	*Lambert et al.* - Quantitative resistance *to Plum pox virus* in *Prunus davidiana* P1908: a possible resource for durable resistance in peach
14:45 - 15:00	*Badenes et al.* - Development of markers for molecular assisted selection of sharka resistance in European breeding programmes
15:00 - 15:15	*Zhao et al.* - Small RNAs in *Plum pox virus* infection and protection
15:15 - 15:30	*Tricon et al.* - Search for natural variants of genes coding eukaryotic initiation factors in *Prunus* species: Identification of new sources of resistance to sharka disease
15:30 – 15:45	*Ravelonandro et al.* - mi-siRNAs and *Plum pox virus*
15:45 - 16:30	Coffee break
16:30 - 18:30	Poster session
19:00 -	Dinner

Invited Speaker

Plant virus control employing RNA-based vaccines: A novel non-transgenic strategy

Andreas Voloudakis (Action Chair): avoloud@aua.gr; **Thomas Hohn** (Action Vice Chair): thomas.hohn@fmi.ch, **Maria Holeva** (Action Secretary): m.holeva@bpi.gr.

COST FA0806 consortium
http://www.aua.gr/COSTFA0806

The current virus control methods are limited in number, efficacy and environmental suitability and current EU decisions restrict crop improvement strategies employing transgenic plants. To protect plants against existing and emerging virus diseases new methods are urgently needed. A very promising approach is the exploitation of RNA silencing, a natural, endogenous mechanism in plants that is a sequence-specific process leading to viral mRNA degradation.

COST Action FA0806* brings together several EU labs in order to develop suitable, efficient and cost-effective methods to induce anti-viral silencing in crops by the transient application of dsRNA, siRNAs and/or artificial small RNAs (collectively designated as "RNA-based vaccines"). These vaccines are produced either *in vitro* or *in vivo* in large quantities and are applied at laboratory or large scale employing specific delivery machinery.

FA0806 is structured in three Working Groups (WGs), WG1: Development of novel non-transgenic strategies for plant virus control, WG2: Application of novel non-transgenic strategies for plant virus control, and WG3: Socio-economic evaluation of the impact of the novel application methods. In the frame of FA0806, Training Schools and Short Term Scientific Missions provide instruments for scientific exchange and training for early-stage and senior researchers alike. Currently, 50 members from 25 COST countries and four non-COST members, from Argentina, New Zealand, South Africa and Mexico, participate in the Action.

WPE1: Large scale analysis of *Plum pox virus* current diversity worldwide

Oral Presentations

A large scale effort to analyze the *Plum pox virus* diversity worldwide

SharCo consortium[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]

[1]Institute of Virology SAS, Bratislava, Slovakia, [2] INRA, Montpellier, France, [3] AgroBio Institute, Sofia, Bulgaria, [4] CSIC, Madrid, Spain, [5] CNR-Istituto di Virologia Vegetale, Bari, Italy, [6] Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain, [7] Instytut Sadownictwa i Kwiaciarstwa, Skierniewice, Poland, [8] Mustafa Kemal University, Antakya, Turkey, [9] Crop Research Institute, Prague, Czech Republic, [10] Fruit Research Institute, Cacak, Serbia, [11] Statiunea de Cercetare, Bistrita, Romania, [12] INRA, Bordeaux, France
Email: **Miroslav.Glasa@savba.sk**

Keywords: PPV, variability, genome, strain, sequencing

The understanding of the geographic distribution and dynamics and of the genetic variability of *Plum pox virus* (PPV) populations on a worldwide is a prerequisite for an efficient management and control of the Sharka disease.
An international effort supported by the European FP7 SharCo project has been initiated with the aim to provide a realistic view on the current diversity of PPV worldwide, by the analysis of a large number of natural field isolates, collected following a standardized sampling protocol. For each isolate, the epidemiologically relevant data (related to locality, host and symptomatology) are systematically collected, isolates are preserved in lyophilized form in a centralized collection and partial sequence data is generated for two highly informative genome portions i.e. P3-6K1 and NIb- CP.
Besides providing information on the presence of particular strains in countries from which they were previously unreported, the results obtained provide a detailed cartography of the prevalence of particular PPV strains in particular countries or host plants. Analysis of the partial sequence data indicate a higher than previously expected genetic diversity within the PPV-D strain and confirm the splitting of the PPV-M strain into two subclusters. Moreover, divergent isolates have been identified in the PPV-D, M, Rec, T and W strains and are currently undergoing full-length genomic sequencing. The detailed synthesis of the results obtained on these various aspects will be presented and discussed.

The SharCo *Plum pox virus* Database

Y. Laizet and T. Candresse

UMR GDPP - IBVM - INRA, BP 81, 33883 Villenave d'Ornon Cedex, France
Email: **ylaizet@bordeaux.inra.fr**

Keywords: PPV, sequence, variability, database, web, strain

The EU FP7-funded SharCo project develops several strategies to study and control the *Prunus* sharka disease. In this frame, SharCo partners have developed an unprecedented collective effort to analyse the variability of *Plum pox virus* (PPV). To ensure efficient data dissemination and usage, standardized origin and sequence information on several hundred PPV gathered as a consequence of this effort have been organised in a web queryable database (http://w3.pierroton.inra.fr:8060/).

The database allows on-line submission of isolates data (origin, typing results, sequence…). Mandatory fields have been specified together with a curation process to allow consistent data searches. Subsequent sequence or chromatogram files submission for an existing isolate is also possible. A semi-automated import procedure of Genbank accessions to centralize all known PPV isolates has also been developed.

Multi-criteria searches of the database or direct access to an isolate and to its sequence information via their identifiers allow for easy data retrieval. Cross-links to available Genbank accessions are displayed for fast navigation and information about the availability of the isolate in the Sharco centralized collection of lyophilized isolates will be added in a next step.

Several tools like blast, geographical mapping, custom fasta sequence and basic statistics have been developed and integrated on the database web site to help researchers to understand the geographic dynamics and the genetic evolution of the PPV population, which is crucial for an efficient management and control of the disease.

The current database release (May 2010) contains 830 sequences from 547 isolates, over 60% of which have been developed by the SharCo partners. At the moment, the database access is restricted to the SharCo partners and collaborators but plans are to rapidly share it with the scientific community.

First generation of a mini-oligo array for rapid genome wide analysis of *Plum pox virus*

A. Olmos[1], G. Ancillo[1], I. López-Fabuel[1], A. R. Corrales[1], M. Cambra[1], T. Candresse[2], M. Glasa[3]

[1] Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada a Náquera km 5. 46113 Moncada, Valencia, Spain
[2] UMR GDPP, INRA et Université Bordeaux 2, IBVM, Centre INRA de Bordeaux, BP 81, 33883 Villenave d'Ornon Cedex, France
[3] Institute of Virology, Department of Plant Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 84505 Bratislava, Slovakia
Email: **aolmos@ivia.es**

Keywords: microarrays, oligoprobes, PPV caractérisation

SharCo project involves multidisciplinary approaches and several work packages to provide strategies and tools for *Plum pox virus* containment in the European Union. In this context, WPE1, entitled "Large scale analysis of *Plum pox virus* current diversity worldwide", includes different tasks and objectives. Task TE 1.1 aims at developing mini-oligo array technologies for the rapid genome wide analysis of PPV isolates. Oligo array technology using short oligos, tends to be lower in density of probes than traditional microarrays based on cDNA. The first generation of oligochips has been produced in the course of the SharCo's first period. The development of the first generation of PPV oligochips was achieved in two phases: the first was *in silico* design and the second one, an *in vitro* assay. These mini-oligo arrays have been produced including 56 selected probes from 18 to 24 nucleotides, covering the diversity of the whole PPV genome present in the public databases. Spotting and pos-spotting procedures have been evaluated using GenPix 4000B and suitability of probes for typing is being performed using well-characterized isolates. Over the second SharCo period, new sequences will complete this first generation of oligochips. By the end of the SharCo project, both the chip and the nucleotide sequences of validated oligoprobes will be released. Rapid characterisation of PPV diversity and PPV typing based on minioligo array are foreseen and this technology will be delivered to interested stakeholders (industry, Plant Protection Services and quarantine services).

WPE1: Large scale analysis of *Plum pox virus* current diversity worldwide

Poster presentations

Assessment of *Plum pox virus* genetic diversity in Slovakia

L. Predajňa, Z. Šubr and M. Glasa

Institute of Virology, Slovak Academy of Sciences, Dúbravska cesta 9, 48505 Bratislava, Slovakia
Email: **Lukas.Predajna@savba.sk**

Keywords: *Plum pox virus*, strain, isolate, variability, Slovakia

Plum pox virus (PPV, genus *Potyvirus*) is the agent responsible for Sharka disease, economically the most important threat of stone fruit production in Slovakia. In order to determine the molecular variability of PPV in Slovakia, two sets of primers targeting distinct parts of the viral genome (i.e. P3-6K1 and NIb-CP) were used for amplification and partial sequencing of more than 50 isolates collected during the 2008-2010 survey in all regions of Slovakia where *Prunus* species are grown. The analysis of sequence data demonstrated that PPV isolates of three major strains PPV-D, -Rec and -M occur in Slovakia, PPV-D being the most prevalent. PPV-M isolates were exclusively found on peach, while PPV-D and Rec isolates were detected predominantly on plum and/or apricot, however without clear-cut regional distribution. The actual PPV diversity and phylogenetic relationship of PPV in Slovakia will be presented and discussed.

Assesment of the diversity of PPV-Rec strain in Bosnia and Herzegovina

D. Jevremović[1], D. Delić[2], B. Lolić[2] and S. Paunović[1]

[1] Fruit Research Institute, Kralja Petra I 9, Čačak, Serbia
[2] University of Banja Luka, Faculty of Agriculture, Bulevar Vojvode Petra Bojovića 1A, Banja Luka, Bosnia and Herzegovina
Email: **darkoj@tfc.kg.ac.rs**

Keywords: *Plum pox virus*, stone fruits, rec strain, ic-rt-pcr, phylogenetic analysis

In order to further investigate the diversity of *Plum pox virus* in Bosnia and Herzegovina 29 samples from *Prunus* trees showing Sharka-like symptoms were collected and analyzed. Twenty five plum, 2 apricot and 3 peach samples from nine localities in the North-western part of the country were analyzed by IC-RT-PCR for the specific detection of PPV-M, -D and –Rec isolates. The PPV-Rec strain was detected in apricot and plum samples. All PPV-Rec isolates originating from plums and apricots were sequenced over two genomic regions, the N-ter CP and P3-6K1 regions. Sequences were compared with previously published sequences of isolates originating from Bosnia and Herzegovina and surrounding countries. Phylogenetic analysis confirmed PCR-typing tests and typical clustering of newly-found isolates with previous PPV-Rec isolates. Clustering of PPV-Rec isolates from Bosnia and Herzegovina and genetic similarity with other isolates will be discussed.

Distribution of *Plum pox virus* strains in Turkey

C.U. Serce, M. Gazel and K. Caglayan

Mustafa Kemal University, Plant Protection Department 31034, Antakya-Hatay/Turkey
Email**: culubas@gmail.com**

Keywords: sharka, strain, Turkey

Plum pox virus (PPV) infection of stone fruit trees in Turkey has been reported since 1968. Nowadays, new PPV outbreaks have been recorded in several regions. Distribution of PPV strains in Turkey tightly linked with the geographical situation. In Marmara and Mediterranean regions, PPV was determined as PPV-M isolates in newly-infected areas. PPV-T was shown to be prevalent in Central Anatolia (Ankara) and the Aegean Regions (Izmir) where PPV is endemic for years.

The most recent outbreak reported in Turkey happened in Hatay, in a nectarine and apricot orchard. This isolate belongs to the PPV-M strain. According to these distributions, new outbreaks appear to happen mainly due to plant importation from foreign countries. They are related to PPV-M introduction in still PPV-free areas whereas PPV-T is spreading in previously infected regions.

Primarily results on the epidemiology of *Plum pox virus* in stone fruit orchards of costal regions of Turkey

K. Caglayan, C.U. Serce, M. Gazel, K. Kaya and F. Can

Mustafa Kemal University, Plant Protection Department 31034, Antakya-Hatay/Tutkey
Email: **kcaglayano@yahoo.com**

Keywords: sharka, vector, Mediterranean, Aegean, Turkey

Although *Plum pox virus* (PPV) was introduced 42 years ago to Turkey, it affects up to now a rather limited number of trees. Our recent results on PPV epidemiology show that PPV was introduced rapidly in new areas (Aegean and Mediterranean costs), which used to be free of the disease. PPV spreading was recorded in these regions in 2009 and 2010. In 2009, the highest aphid population was observed at the end of May in Izmir (Aegean region). Identified aphid species landing on the plants from April to June 2009 in this region were *Myzus (Nectarosiphon) persicae (Sulzer, 1776), Hyalopterus pruni (Geoffroy, 1762), Aphis craccivora (Koch, 1854), Aphis gossypii (Glover, 1877), Acyrthosiphon pisum (Harris, 1776), Anoecia corni (Fabricius, 1775), Hyperomyzus lactucae (Linnaeus, 1758), Macrosiphum euphorbiae (Thomas, 1878), Metopolophium dirhodum (Walker,1849), Capitophorus elaeagni (deI Guercio, 1894)* and *Hyalopterus pruni* (Geoffroy, 1762). The most abundant aphid species were *M. persicae* (27.39 %) and *H. pruni* (23.28 %). Aphids collected from plum and apricot trees were tested individually by ELISA and Real-Time RT-PCR. Among those aphid species only *H. pruni* revealed 40% positive reaction by Real-Time PCR. Epidemiological studies in the Mediterranean cost are still under investigation.

New outbreaks of *Plum pox virus* in Turkey

M. Gazel, C.U. Serce and K. Caglayan

Mustafa Kemal University, Plant Protection Department 31034, Antakya-Hatay/Tutkey
Email: **monagazel@hotmail.com**

Keywords: sharka, new infections, Turkey

Turkey is the leader in apricot production in the world and it ranks 7th for plums. The presence of PPV (Sharka disease) in Turkey has been known since 1968 but the disease was not common, except in apricot and plum trees in home gardens and ornamental parks in restricted areas. After 2006, there have been several reports about new PPV outbreaks in the Turkish Mediterranean regions, which used to be completely free of Sharka disease. First infection in that region of Turkey was recorded in the Western part of the Mediterranean area (Isparta-Egirdir); the PPV isolate was characterized as PPV-Rec. Afterwards, further outbreaks in the Eastern part of the Mediterranean area were detected on apricot, plum, peach and nectarin trees, all being imported cultivars infected with PPV-M. These results show that the new PPV outbreaks in Turkey might be due to the recently imported stone fruit cultivars and failures in quarantine procedures.

WPE2: Improving knowledge of PPV epidemicity and dynamics of spread from orchard to regional scales

Oral Presentations

Estimating risk factors for PPV dissemination from incomplete epidemiological surveillance data

D. R. J. Pleydell[1], G. Th´baud[1], S. Soubeyrand[2], S. Dallot[1], S. Grizard[1], J. Chadoeuf[2], G. Labonne[1]

[1] BGPI, Institut National de la Recherche Agronomique, CIRAD TA 41/K, Campus International de Baillarguet, 34398 Montpellier, France

[2] BioSP, Institut National de la Recherche Agronomique, Site Agroparc, Domaine St Paul, 84914 Avignon, France

Email: **pleydell@supagro.inra.fr**

Keywords: spatio-temporal, epidemiology, compartmental model, Bayesian

Computational simulation is a powerful tool for assessing how epidemics respond to environmental or management changes. However, simulations are often performed with parameters gathered from experiments or literature which are not representative of variation under natural epidemic conditions introducing significant bias. More realistic parameter estimates can be collected from data-sets of epidemic monitoring programs, although doing so requires that mismatches between model requirements and sampling design are addressed correctly.

These corrections often present non-trivial methodological challenges. To this end, hybrid mechanistic-statistical models are becoming increasingly popular since they maintain biological realism whilst accounting for model / data mismatches. Here we present a mechanistic susceptible-exposed-infectious-removed (SEIR) dissemination model which has been adapted to the sampling strategy employed during 14 years of monitoring Sharka disease among peach orchards in southern France. The statistical part consists of a discrete-time survival analysys that models the probability of detecting symptomatic cases on given survey dates. To account for imperfect sensitivity the infectious compartment I is split into detected and non-detected sub-compartments. Parameters relating to the local infection process, disease latency, sensitivity and removal rates of detected cases are estimated. Non-parametric random effects for sensitivity, removal rate and the relative proportions of vector borne / anthropogenically mediated transmission events are included via finite mixture model classifications of peach varieties, farmers, and orchards respectively. Markov chain Monte Carlo strategies are employed for Bayesian inference. We discuss: the spatial range of PPV dissemination by aphid vectors and it's influence on spatio-temporal dynamics of Sharka epidemics; methodological issues concerning estimating Sharka latency parameters from epidemiological data; and, the influence of surveillance and control strategies on control efficiency.

P1 and CP, the two ends of the potyviral polyprotein, are involved in viral pathogenicity and host adaptation

V. I. Maliogka, A. Carbonell, B. Salvador, J. A. García and C. Simón-Mateo
Plant Molecular Genetics, National Centre for Biotechnology-CSIC, Campus de la Universidad Autónoma de Madrid, 28049 Madrid
Email: **vmaliogka@cnb.csic.es**

Keywords: *Plum pox virus*, host adaptation, pathogenicity determinants

Plum pox virus (sharka virus, PPV) is a potyvirus that causes a severe disease of trees of the genus Prunus, which, in addition, is able to infect different herbaceous plants. Even though some pathogenicity determinants of PPV have been identified, the capacity of the virus to establish local and systemic infections in woody and herbaceous hosts has not yet been accurately located.

P1 protein, laying at the 5´terminal region of the potyviral polyprotein, is the most variable protein among the different potyviruses and has been suggested to be involved in host-specific interactions contributing to host-range definition. We have identified two mutations in the P1 gene that are specifically associated with mild pathogenicity of some subisolates of the M-type PPV PS isolate. Site-directed mutagenesis studies demonstrated that both substitutions (W29R, V139E) resulted in decreased PPV infectivity in peach seedlings. In particular, the W29R amino acid change resulted in significantly lower levels of virus accumulation and symptom severity. The presence of both mutations renders the virus completely unable to infect peach. On the other hand, the V139E substitution was found to be responsible for symptom attenuation in herbaceous hosts. The results highlight the relevance of the P1 protein in host adaptation by potyviruses.

On the other hand, the N-terminus of the CP also shows a high variability in length and sequence among potyviruses, prompting the suggestion that it could be involved in specific interactions with host factors. Previous work using chimeric viruses engineered by exchanging fragments of the viral genomes between two infectious cDNA clones of PPV, PPV-R and PPV-D (D strain), indicated that the N-terminus of the CP of PPV includes host specific pathogenicity determinants. In order to further identify these determinants a chimeric virus (PPV-R/D) including the N terminal part of the PPV-D (*P. persicae* adapted) CP was constructed and used for inoculations of *N. clevelandii* and *N. benthamiana* plants**.** The results indicated that the chimera is capable of infecting systemically these plants after the introduction of point mutations in a short region of 30 amino acids in the N terminus of the CP. Six independent amino acid changes (5 substitutions and 1 deletion) were identified in the virus progeny from *N. clevelandii* plants and four in *N. benthamiana*. Three of the selected mutations were common in both hosts. These amino acids map to the region that is removed by the NAT deletion, which has been observed after PPV propagation in herbaceous hosts. In *N. benthamiana* some of the virus progeny did not include any mutations in the under study genomic region. All the selected mutations, but K14R, were stable after one passage in *N. clevelandii* and *N. benthamiana* plants. In addition the virus progeny from *N. benthamiana* that did not show any mutations after one passage in the same plant species accumulated amino acid changes in the same 30 aa region. The results highlight the relevance of the N terminus of the CP in host adaptation of PPV.

WPE3: Evaluation of strategies to reduce PPV incidence in nursery

Oral Presentations

Preliminary results on the efficiency of oil treatments in reducing natural *Plum pox virus* infection in *Prunus* nurseries

M. Cambra[1], V. Bozhkova[2], L. Zagrai[3], E. Vidal[1], S. Milusheva[2], I. Zagrai[3], E. Tasheva- Terzieva[4], I. Kamenova[5], A. Festila[3]

[1] Instituto Valenciano de [1]Investigaciones Agrarias (IVIA), Plant Protection and Biotechnology Centre, 46113 Moncada, Valencia, Spain
[2] Fruit Growing Institute, 4004 Plovdiv, "Ostromila" 12 str., Bulgaria
[3] Fruit Research & Development Station Bistrita, Drumul Dumitrei Nou Street, no. 3, Bistrita, 420127, Romania
[4] Sofia University "St Kliment Ohridski", Faculty of Biology, 1164 Sofia, 8 "Dragan Tsankov" Blvd., Bulgaria
[5] AgroBioInstitute, 1164 Sofia, 8 "Dragan Tsankov"Blvd., Bulgaria

Email: **mcambra@ivia.es**

Keywords: *Plum pox virus*, *Prunus*-rootstocks, nursery, mineral oil, efficiency, aphid vector

The use of mineral oil treatments is one possible strategy to reduce the incidence of *Plum pox virus* (PPV) in nursery plots. The potential of this strategy was evaluated in three countries, *i.e.* Bulgaria, Romania and Spain, therefore in two different environments, Continental and Mediterranean. Tree experimental plots were established with two PPV-susceptible *Prunus*-rootstocks ('Nemaguard' and *Prunus mariana* 'GF 8.1'), in areas with high PPV incidence. Treatments with Sunspray Ultrafine mineral oil 1% were performed all along the vegetative period and PPV incidence was assessed in treated and non-treated blocks. Winged aphid populations visiting the nursery blocks were monitored by using the sticky shoot method in order to determine the maximum peak of flight as well as the different aphid species visiting the blocks. To evaluate the spread of the virus, each plant was serologically tested by DAS-ELISA using 5B-IVIA monoclonal antibody. The dynamic of aphids landing in nursery plots showed a maximum peak of aphid populations at the beginning of June and at the end of October in Bulgaria, at the beginning of June in Romania and at the middle of May in Spain. The most frequent aphid species landing in the experimental block was *Hyalopterus pruni* complex in Bulgaria, *Aphis spp.* in Romania and *Aphis spiraecola* in Spain. The preliminary results on the spread of the virus revealed that mineral oil treatment reduced PPV incidence in all experimental plots but did not stop PPV infection.

Plum pox virus containment in nurseries based on epidemiological data

M. Cambra[1], Boscia[2], E. Vidal[1], T. Malinowski[3], W. Warabieda[3], K. Caglayan[4], C. Ulubas[4], M. Gazel[4], J. Polak[5], J. Patockova-Olbrechtova[5], M. Neumüller[6], L. Mühlberger[6], V. Bozhkova[7], S. Milusheva[7], I. Kamenova[8], E. Tasheva-Terzieva[9], L. Zagrai[10], I. Zagrai[10]

[1] Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada, Valencia, Spain
[2] Consiglio Nazionale delle Ricerche-Istituto di Virologia Vegetale (CNR-IVV). Bari, Italy
[3] Instytut Sadownictwa i Kwiaciarstwa (ISK). Skierniewice, Poland
[4] Mustafa Kemal University, Antakya-Hatay, Turkey
[5] Crop Research Institute (CRI). Prague, Czech Republic
[6] Technische Universität München (TUM). Munich, Germany
[7] Fruit Growing Institute (FGI) Plovdiv, Bulgaria
[8] AgroBio Institute (ABI) and University of Sofia. Sofia, Bulgaria
[9] Sofia University "St Kliment Ohridski", Faculty of Biology, 1164 Sofia, 8 "Dragan Tsankov" Blvd., Bulgaria
[10] Statiunea Cercetare-Dezvoltare pentru Pomicultura (SCDP). Bistrita, Romania

Email: **mcambra@ivia.es**

Keywords: PPV-susceptibility, rootstocks, aphids, nursery, ELISA 5B, real-time RT-PCR

Plum pox virus (PPV) is currently spreading in new areas in spite of the available knowledge on PPV management, of the quarantines rules and of the requirement of a proper EU phytosanitary passport. PPV is "legally" distributed over large distances by the transport of latently infected plant material that have escaped quality controls. The main goal of the SharCo third Epidemiology workpackage (WPE3) is to develop strategies to contain PPV. The susceptibility of the most commonly planted Prunus rootstocks to natural PPV-infection is being established in experimental nurseries in Bulgaria, Czech Republic, Poland, Romania, Spain and Turkey. Rootstocks originating from plum species are currently the most susceptible, whereas almond x peach hybrids and PPV-hypersensitive plum varieties are the less susceptible to natural PPV-infection. The maximum peak of aphid populations is being determined in the different areas. *Hyalopterus pruni* complex and *Brachycaudus* spp. in continental central European areas and *Aphis spiraecola* in Mediterranean areas are the prevalent aphid PPV-vector species. The use of less sensitive rootstocks to PPV-infection or the combination in the same nursery block of susceptible and PPV-resistant rootstocks, together with mineral oil treatments during the peak of aphids flights, can significantly reduce PPV spread. In addition, the use of physical barriers and/or the isolation of nurseries in areas free of natural PPV susceptible hosts, are key strategies to avoid natural infection and unexpected outbreaks. These strategies must be combined with accurate PPV-detection methods and with systems able to detect as early as possible the first PPV-viruliferous aphids landing on the nursery plants.

Tentative guidelines for accurate, sensitive and reproducible *Plum pox virus* detection in nurseries

M. Cambra[1], E.Vidal[1], E. Bertolini[1], M. T. Gorris[1], M. C. Martínez[1], A. Olmos[1], D. Boscia[2]

[1] Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada a Náquera km 5. 46113 Moncada, Valencia, Spain
[2] Consiglio Nazionale delle Ricerche-Istituto di Virologia Vegetale (CNR-IVV). Via Giovanni Amendola, 122/O - 70126 Bari, Italy
Email: **mcambra@ivia.es**

Keywords: sampling, hierarchical method, composite samples, dormant and vegetative periods, ELISA 5B, real-time RT-PCR, diagnostic parameters

The only way PPV can spread over long distances is through the exchange of latently infected plant materials that have escaped quality controls and/or the quarantine procedures. Consequently, protocols and validated laboratory analyses for accurate PPV detection are needed. Nowadays, there is a wide range of detection and sampling procedures, available apparatus for sample processing, specific and validated reagents and new methods for universal PPV detection and that leads us to propose changes in the traditional ways and procedures related to PPV detection. The EU-SharCo project aims to improve different steps of sharka early warning system and thus to prepare, as a first step, a specification sheet for accurate, sensitive and reproducible PPV detection. Conventional sampling and extract preparation methods for ELISA must be modified to reduce risk of contamination when highly-sensitive molecular amplification methods are used. The modifications include: hand collection of samples, hierarchical sampling, sample management, extract preparation into plastic bags, and grouping of samples. Different systems are proposed for large scale sampling starting from dormant buds or spurs, from expanded leaves collected in winter or from leaves collected all along the vegetative period. Serological ELISA 5B-IVIA/AMR Lab based, the most specific ELISA in our hands, and molecular Spot real-time RT-PCR, the most sensitive method, have been validated for PPV detection in single and multiple, bulked samples. With these optimised specifications, we were able to detect PPV all along the year, spring and summer being the most appropriate periods followed by fall and winter. ELISA-5B supplies the most accurate technology for sharka disease in positive *versus* negative samples and Spot real-time RT-PCR, the most sensitive in dubious, negative samples. The combination of both techniques is providing a practically perfect PPV detection methodology, especially in symptomless nursery plants.

WPG1: Identification of molecular markers linked to resistance to PPV and marker-assisted selection

Oral presentations

Quantitative resistance *to Plum pox virus* in *Prunus davidiana* P1908: a possible resource for durable resistance in peach

P. Lambert[1], M. Rubio[2], C. Tuero[1], A. Bachellez[1] and T. Pascal[1]

[1] INRA, Unité de Génétique et d'Amélioration des Fruits et Légumes, Domaine Saint Maurice, BP 94, 84143 Montfavet Cedex, France
[2] Department of Plant Breeding, CEBAS-CSIC, PO. Box 164, Murcia, Spain
Email: **patrick.lambert@avignon.inra.fr**

Keywords: quantitative trait loci, sharka, *Prunus davidiana*, durable resistance, genetic map

The clone P1908 of *Prunus davidiana,* a peach-related species*,* is currently the only source of resistance to *Plum pox virus* (PPV)) that has been so far studied for resistance, both at the phenotypic and molecular levels, and which might be useful in peach (*Prunus persica*) breeding programs. Two previous studies using F_1 and F_2 populations derived from the nectarine cv. Summergrand and *P. davidiana* P1908 identified a total of six *P. davidiana* quantitative trait loci (QTLs) involved in PPV resistance (Marcus strain). The current study evaluated the incidence of PPV infection in a F_1 population derived from the susceptible peach cv. Rubira and *P. davidiana* P1908 and identified nine regions involved in differential symptom expression, among which, six were common with the previous studies. However several discrepancies were observed, suggesting interactions between the genetic background of the susceptible parent and that of *P. davidiana* P1908. Based on these findings, sequence analysis of previously published candidate genes was undertaken in order to detect SNPs useful for Marker Assisted Breeding (MAB). Part of these results will be presented and discussed but as a preliminary outcome, they suggest that i) *P. davidiana* P1908 would be a limited resource in breeding programs aimed at PPV resistance if used alone, ii) it might still remain an interesting quantitative resistance source if combined with medium to strong resistance sources such as those provided by some almond cultivars (*Prunus dulcis*).

Development of markers for molecular assisted selection of sharka resistance in European breeding Programs

Sharco Consortium*

* SharCo Consortium members involved in this work are: INRA-Bordeaux, INRA Avignon, USAMV-Bucarest, IVIA Valencia, TUM Munich, MKU Antakya, ISK Skierniewice, FGI Bulgaria, UMI Milan, Mendel University, Lednice
Email: **badenes_mlu@gva.es**

Keywords: MAS, breeding efficiency, PPV resistance,

In the framework of the SharCo project aimed at containing the spread of sharka in stone fruit trees in Europe, we have developed molecular markers as a way to increase the efficiency of the breeding programs aiming at introgressing resistance to sharka in apricot, peach and plum cultivars.
The strategies used were different according to the biology of the species, source and type of the natural resistance. In apricot, few sources of resistance were identified, they were used for the construction of linkage maps and the fine-mapping of regions involved in resistance. A major QTL identified in linkage group one was saturated with markers and those markers are being tested in different apricot progenies in order to select the best markers for MAS (marker or molecular assisted selection). In peach, there is no source of resistance known up to now. Only one single clone of *Prunus davidiana*, a wild peach related species, (P1908) displayed resistance to PPV; it has been used for introgression of PPV resistance in peach. Linkage maps were dobtained and several QTL, identified. However, since in *P. davidiana* P1908 the resistance is quantitiative, this approach is not sufficient to produce accurate markers tightly linked to every PPV resistance locus and a new strategy based on candidate genes is being carried out. In Plum (*Prunus domestica* L), an hexaploid *Prunus* species, the strategy used has been the identification of genes linked to the hypersensitivity response, a trait that restricts the spread of the virus into the plant.
It is expected that data resulting from the above approaches will provide markers useful for the rapid and accurate selection of genotypes resistant to sharka, at an early stage of the breeding programmes, increasing their efficiency and shortening the period between the production of agronomically valuable, PPV resistant cultivars and their delivery to nurseries and fruit producers.

WPG1: Identification of molecular markers linked to resistance to PPV and marker-assisted selection

Poster Presentations

Towards sharka containment, the 'SharCo' project: genetic approach, development of molecular markers for MAS in apricot breeding

J. M. Soriano, E. Zuriaga, E. M. Vera-Ruiz[1], C. Romero[1], T. Zhebentyayeva[2], A. Abbott[2], G.Llácer[1] and M. L. Badenes[1]

[1] Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera, km 4.5, 46113 Moncada, Valencia, Spain
[2] Department of Genetics and Biochemistry, Clemson University, 100 Jordan Hall, Clemson, SC 29634, USA
Email: **badenes_mlu@gva.es**

Keywords: breeding, *Plum pox virus*, MAS (Marker Assisted Selection), *Prunus*

Sharka disease, caused by the *Plum pox virus* (PPV), produces important economic losses in apricot (*P. armeniaca L.*). Natural resistance to PPV has been found only in some North American apricot cultivars and efforts are being made to introduce this trait into Mediterranean germplasm and breeding material.
Studies on the segregation of the resistance trait in different intraspecific apricot crosses suggest that PPV resistance is controlled by at least one major dominant locus located on the upper part of linkage group 1 (LG1). Using microsatellites already mapped in different *Prunus spp.,* we have built a high density genetic map on LG1 comprising the region linked to PPV resistance in apricot. In addition, overgo probes designed from SSR clone sequences have been used to screen an apricot Bacterial Artificial Chromosome (BAC) library. Positive clones are being used to construct a BAC contig, spanning the apricot genomic region linked to PPV resistance. In this study, a total of 50 and 36 SSRs were incorporated onto the LG1 maps corresponding to 'Lito' and 'Goldrich' PPV resistant cultivars, producing high resolution maps with a density of 0.70 and 0.68 markers/cM, respectively. This saturation is specially high over the PPV resistance locus where marker density doubled that of the whole LG1 map, reaching 1.5 to 1.3 markers/cM in 'Lito' and 'Goldrich', respectively. Results allow to obtain SSR markers linked to PPV resistance. Validation for MAS is currently in progress in 5 different apricot breeding programs all across Europe.

Genomic organization of the resistance to *Plum pox virus* in distinct apricot progenies through a quantitative meta-analysis

V. Decroocq[1], G. Marandel[1], J. Salava[2], A. Abbott[3], J-M Audergon[4], P. Lambert[4], T. Candresse[1] and M. L. Badenes[5]

[1] UMR GDPP INRA-Université Bordeaux II, 71 Avenue Edouard Bourlaux, 33140 Villenave d'Ornon (France)

[2] Division of Plant Medicine, Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic

[3] Department of Genetics, Biochemistry, and Life Science Studies, Clemson University, 100 Jordan Hall, Clemson, SC 29634, USA

[4] UGAFL, INRA, Domaine St Maurice, BP 94, 84143 Montfavet Cedex, France

[5] IVIA Instituto Valenciano de Investigaciones Agrarias, Apartado Oficial, 46113 Moncada, Valencia, Spain

Email: **decroocq@bordeaux.inra.fr**

Keywords: quantitative trait locus, meta-analysis, quantitative resistance

The *Plum pox virus* (PPV), the causal agent of the sharka disease, is the most detrimental virus on stone-fruit trees, worldwide. Infected fruits are not marketable. To date, few sources of resistance have been identified and mapped or are being mapped in several apricot progenies issued from the genitors 'Stark Early Orange' (SEO), 'Goldrich', 'Harcot', and 'Harlayne'. However, whether those sources of resistance are distinct or not is still questionned.

Within the SharCo consortium, a meta-analysis of the apricot resistance to PPV was performed. The purpose of this study was i) to integrate all PPV resistance QTL information available in the literature in a QTL meta-analysis in order to detect consensual, most probable genomic regions linked to resistance to sharka disease, ii) to compare the genomic organisation of those resistance loci in different genitors. The first step was to include another apricot resistance source (cv. 'Harlayne') to the previous ones ('SEO', 'Goldrich', 'Lito'), giving access to a larger mapping population. The second step consisted in merging the above data with previously published PPV resistance loci in an extensive QTL meta-analysis. Starting from a consensus genetic linkage map, this statistical algorithm based analysis enabled to pinpoint the best model fitting the observed QTL. Resistance QTLs from the Apricot 'Harlayne' cultivar were added to the analysis. Data were projected on the Prunus consensus map as well as on the physical map. The QTL meta-analysis was performed on populations originating from apricot cv. 'Golrich', 'SEO' and 'Harlayne'. It provided evidence on the occurrence of three Meta-QTL in the upper part of LG1 in Apricot and one on linkage group 3 (Marandel et al., Molecular Plant Pathology, 2009). While the Apricot cultivar 'Goldrich' bears one single QTL, 'Harlayne' and 'SEO' share two QTLs, explaining the higher resistance level of those two cultivars. In conclusion, this analysis enabled to refine the boundaries of the genomic region controlling PPV resistance in the current genitors of resistance to PPV in P. *armeniaca.*

Determination of resistance to sharka (*Plum pox*) in some Romanian apricot progenies and rootstocks

L. Ion[1], D. Hoza[1], C. Moale[2], A. M. Petrica[1], T. Neagu[1], I. Zagrai[3], M. Isac[4] and S. Preda[4]

[1]University of Agronomy Sciences and Veterinary Medicine Bucharest, Marasti Street, no. 59

[2]Fruit Research & Development Station Constanta, Romania

[3]Fruit Research & Development Station Bistrita, Romania

[4] Fruit Research & Development Station Valcea, Romania

Email: **ioannagyligia@yahoo.fr**

Keywords: *Plum pox virus*, apricot, cultivars, rootstocks, resistance.

Sharka, caused by *Plum pox virus* (PPV), is the most devastating viral disease affecting stone fruit crops in Europe. It causes important economic losses in the fruit production, mainly in apricot and European plum. The spreading of PPV might be limited by planting PPV resistant or at least less-susceptible rootstocks on which PPV resistant scions have been grafted. In spring 2008, at USAMV Bucharest, Romania, a breeding program aiming to develop cultivars and rootstocks resistant to PPV was initiated and an efficient procedure for the determination of sharka resistance within the progenies was established. It is based on the methodology developed at IVIA Valencia, Spain (Tarek el al., 2001).

The peach 'GF 305' rootstocks were used as indicators for susceptibility to PPV in comparison with the Mirobolan BN 4 Kr considered to be resistant to sharka (Minoiu et al., 2002, Zagrai et al. 2009). The subsequent grafting protocol was optimized, and a Romanian PPV-D isolate was identified and used as inoculum source. The two rootstocks were grafted with apricot individuals originating from crossing between a PPV resistant genitor (e.g. 'SEO', 'Stella') and Romanian preferred varieties. Grafted plants were inoculated by chip-budding and monitored by visual inspection and ELISA, completed by IC/RT-PCR for the PPV negative plants. Preliminary data of the Romanian PPV resistance breeding programmes will be presented.

Analysis of the resistance to PPV in a F1 progeny derived from the apricot cultivars 'Goldrich' and 'Moniqui' - Identification of the QTLs involved

P. Lambert, G. Roch, A. Graillat, J. Leonetti, E. Martin, A.M. Ferreol, A. Blanc and J. M. Audergon
INRA– UR1052 UGAFL – Domaine St Maurice – BP94 – F84143 Montfavet cedex (France)
Email: **patrick.lambert@avignon.inra.fr**

Keywords: *Prunus armeniaca*, sharka, *Plum pox virus,* genetic resistance, QTL, genetic map

Within the framework of the SharCo project, the genetic factors controlling resistance to PPV in the apricot 'Goldrich' cultivar were investigated using a F1 progeny. The aim was to complete our knowledge on the genetic determinism of the resistance carried by 'Goldrich' and to compare it with other apricot resistance sources.
A progeny of 180 hybrids derived from a cross between 'Goldrich' (resistant) and 'Moniqui' (susceptible) has been maintained and two genetic maps composed of 120 and 94 SSR markers widely distributed throughout the genome were then constructed for 'Goldrich' and 'Moniqui', respectively.
Concurrently, the susceptibility *vs* resistance to sharka disease, after incoulation with PPV-M, was scored in an insect-proof greenhouse, on all the F1 seedlings, and the parents, using 5 replicates for each. The observations were performed during each vegetative cycle onto both the rootstock and the scion. They were completed by a systematic ELISA test. A minimum of 5 vegetative cycles were conducted for each genotype.
The data obtained are consistent with those reported in previous studies on 'Goldrich' or other resistant cultivars. However, while 'Goldrich' carries a major resistant factor located on LG1 which is associated with symptom intensity and localization, an additional QTL was detected on LG7 and found associated with the so-called latency period, defined as the period (expressed in number of vegetative cycles) before the inoculation and the appearance of the first symptoms. 'Moniqui' carries two susceptible factors located on LG2 associated with the latency period before symptom appearance and the persistence of the observed symptoms.

WPG2: Characterization of new and complementary genetic resistance mechanisms

Oral Presentations:

Small RNAs in *Plum pox virus* infection and protection

M. Zhao, D. San León, J. C. Oliveros, J. A. García and C. Simón-Mateo

Centro Nacional de Biotecnología-CSIC, Campus Universidad Autónoma de Madrid, 28049 Madrid
Email: **jagarcia@cnb.csic.es**

Keywords: virus resistance, viral small RNAs, deep sequencing, artificial miRNAs

MicroRNAs (miRNAs) and other small noncoding RNAs have been recognized as key components in several regulatory processes in animals and plants. Recently, endogenous miRNAs have been shown to target engineered plant viruses containing miRNA target sequences, and specific virus resistance has been achieved by transgenic expression of virus-specific artificial miRNAs (amiRNA). These amiRNAs consist in engineered precursors of abundant plant miRNAs containing complementary sequences to particular plant viruses. Compared with homology-dependent gene silencing, amiRNAs lack the environmental risks associated with transgenic expression of long viral sequences, and is expected to have reduced off-target effects. We are interested in a rational application of the amiRNA technology to generate resistance against *Plum pox virus* (PPV). In order to select sequences to be included in the anti-PPV amiRNAs, we have searched for targets that could be well accessible to the silencing machinery by *in silico* analyses and by mapping small RNAs and 5' ends of viral RNA fragments accumulating in PPV infections. Ten amiRNAs have been constructed, and the accumulation of the two strands of these amiRNAs and their anti-PPV efficacy are being analysed in agroinfiltration assays, as a first step in the assessment of the broadness and durability of amiRNA-derived anti-PPV resistance in *N. benthamiana* and in the natural *Prunus* hosts of PPV.

Search for natural variants of genes coding eukaryotic translation initiation factors in *Prunus* species: Identification of new sources of resistance to sharka disease

D. Tricon, S. Mariette and V. Decroocq

UMR GDPP INRA-Université Bordeaux II, 71 Avenue Edouard Bourlaux, 33140 Villenave d'Ornon (France)

Email: **dtricon@bordeaux.inra.fr**

Keywords: targeted natural mutation, eukaryotic translation initiation factors, susceptibility allele

Viruses are obligatory parasites. The virus infectious cycle is a complex process that includes expression of the viral genome, suppression of post-transcriptional gene silencing, virus replication, cell-to-cell movement via plasmodesmata and long distance movement through the vascular system. This multi-step process requires interactions between host factors and viral proteins. Host factors indispensable to the virus are thus envisionned as susceptibility factors that can be surveyed for mutations leading to recessive resistance to viruses. Indeed, in the last few years, recessive genes were identified as natural resistance genes especially against several important potyviruses in vegetable crops. They all encode one of the translation initiation factors (or isoforms) of the eIF4F complex. In the case of *Plum pox virus* (PPV), we showed that a knock-out mutant for *eiF(iso)4E* or for *eiF(iso)4G* in *Arabidopsis thaliana* is resistant to PPV infection (Decroocq et al., 2006, Nicaise et al., 2007). It suggests that PPV uses those translation initiation factors to complete its life cycle in *Arabidopsis* and thus possibly in stone fruit trees. This prompted us to search for PPV resistant alleles for the translation initiation factors in *Prunus* species. Due to their susceptibility to PPV, we postulate that most of the *Prunus* cultivated species bear a susceptibility allele for *eIF4E* and *eIF4G* and that natural variants for these genes are present naturally in wild *Prunus* populations and/or germplasm collections. As a proof-of-concept, we surveyed 700 individuals from peach, apricot, almond, cherry and plum trees for *eiF4E* and *eIF(iso)4E* variants diverging from the PPV susceptible 'GF305' rootstock. Individuals presenting mutations in the plant-virus interaction domains have been identified and are currently tested for resistance to *Plum pox virus*.

We thank the CRG (Centre de Ressources Génétiques) (INRA, Bordeaux and Avignon, France), MUAF (Lednice, Czech republic), ARS-USDA (Davis, USA), Inonu University (Turkey), AGRI (Bakou, Azerbaijan), JAAS (Nanjing, China) and the apricot repository in Xiongyue (Lianoning, China) for access to valuable *Prunus* germplasm material.

Immunomodulation of *Plum pox virus* infection by transient expression of different scFv fragments specific to structural and non-structural viral proteins

M. Gil[1], O. Esteban[1*], J. A. García[2], L. Peña[1] and M. Cambra[1]

[1] Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada a Náquera km 5. 46113 Moncada, Valencia, Spain
[2] Dpto. Genética Molecular de Plantas, Centro Nacional de Biotecnología (CNB), CSIC. Campus de la Universidad Autónoma, 28049 Cantoblanco (Madrid), Spain
* Pressent address: Laboratorio de Bionanofotónica. Instituto de Bioingeniería de Cataluña. Universidad de Barcelona. Baldiri Reixac 21-25, 08028 Barcelona, Spain
Email: **mcambra@ivia.es**

Keywords: recombinant antibodies, resistance, agroinfiltration, PPV-GFP

The constitutive expression of recombinant antibodies in transgenic plants could be an alternative to conventional breeding to generate PPV resistance. The single chain variable antibody fragments (scFv) specific to different viral proteins has been used to interfere with viral infections. Immunomodulation of factors playing a role in the viral infection process can provide a powerful tool to produce resistant plants. The effectiveness of the scFv2A fragment (specific for PPV-NIb replicase protein), and of the scFv5B and scFv5BKDEL fragments (specific for PPV-CP), to interfere with PPV infection, were evaluated. *Nicotiana benthamiana* leaves were co-agroinfiltrated with *Agrobacterium tumefaciens* cultures carrying an infective PPV-GFP clone mixed with *A. tumefaciens* cultures carrying scFv constructs specific for PPV (scFv2A, scFv5B, scFv5BKDEL, alone or combined) or scFv construct specific for *Citrus tristeza virus* as control. After three days agroinfiltrated leaves were excised, observed under a stereomicroscope with UV light and photographed. A significant reduction on the number and size of the fluorescent foci and of the viral accumulation (estimated by DASI-ELISA), was observed with all scFv fragments, compared with the control. The scFv5BKDEL was the most efficient single version for immunomodulation. The combined expression of both 2A and 5B fragments was more efficient than the single expression. The use of PPV-GFP allowed infection monitoring at early stages. Transient expression is an interesting methodology to the stable transformation to evaluate the ability of scFvs to interfere with PPV.

mi-siRNAs and *Plum pox virus*

M. Ravelonandro[1], R. Scorza[2], P. Briard[1] and J. M Hily[1,2]

[1]UMR 1090-GDPP, INRA-Bordeaux, France
[2]Appalachian Fruit Station, ARS-USDA, Kearneysville, W-V, USA
Email: **ravelona@bordeaux.inra.fr**

Keywords: RNA silencing, virus resistance, RNAi, amiRNA, siRNA

Plant biotechnology is facing a future where efficiency is among the challenging demand from growers. During these last decades, only a few examples reached the marketable step. Fight against *Plum pox virus* (PPV) is requiring an applicable technology whatever technological advances through genetic engineering should provide tools and science-based knowledge to block PPV spread in the environment. Over the last 18 years, the experience gained through the characterization of transgenic Honeysweet resistant plum represents a substantial support to challenge PPV infection within the silencing model based onto small interfering RNAs. For this purpose, the two kinds of RNAi technology (mi and si-RNAs) have been developed. To follow up the successful characterization of siRNAs from HoneySweet plum, we chose to focus our investigation through the better studies of the capsid protein cistron. The bioinformatic approach, we performed, has permitted to identify some folded RNA segments potentially exploitable. Downstream analyses *in planta* permitted to confirm that siRNA technologies confer a stable resistance to plum. Whether the transient transcription studies with herbaceous plant models showed that either mi- or/and siRNAs can be detected, there is not doubt that the efficiency of these RNAi technologies can only be verified in genetically engineered plants.

WPG2: Characterization of new and complementary genetic resistance mechanisms

Poster Presentations:

Engineering the *Prunus* translation initiation factors in stone fruit trees for resistance to *Plum pox virus*

V. Decroocq[1], J. R. Astudillo[2], M. Acuña Soto[2] and H. Prieto[2]

[1] UMR GDPP INRA-Université Bordeaux II, 71 Avenue Edouard Bourlaux,

33140 Villenave d'Ornon (France)
[2] INIA, La Platina Station, Santa Rosa 11610, La Pintana, SANTIAGO de Chile
Email: **decroocq@bordeaux.inra.fr**

Keywords: eukaryotic translation initiation factor, hairpin construct, endogenous susceptibility gene silencing

Viruses are obligatory parasites with a very small genome. They rely on the host cellular machinery to complete the different steps of their cycle. Recent results suggest that recessive resistances against viruses are more likely to correspond to a passive mechanism due to the absence or to the inappropriate nature of a host factor specifically required by the virus to complete its cycle. The dominant allele can then conceptually be envisioned as encoding a susceptibility factor needed by the virus to be able to infect the host plant. Indeed, one of the major findings in the last few years was the identification of natural recessive resistance genes against several important potyviruses in vegetable crops, all of them encoding the translation initiation factor eIF4E or one of its isoforms (Ruffel et al., 2002; Nicaise et al., 2003; Gao et al., 2004; Stein et al., 2005; Ruffel et al., 2005). We also showed in the model plant Arabidopsis thaliana that a knock-out mutant for *eIF(iso)4E* or for *eIF(iso)4G* is resistant to *Plum pox virus* (Decroocq et al., 2006; Nicaise et al., 2007). These results suggest that many if not all Potyviruses probably use identical or closely related host factors in widely different plants to complete their life cycle. As a consequence (and as validated by the repeated discovery of the role of eIF4E and eIF4G in Potyvirus-plants interactions), the identification of a plant susceptibility factor in any given Potyvirus-plant pathosystem is very likely to be transferable to a number of other pathosystems and, in particular, to stone fruit trees. In addition, given the dominant nature of the susceptibility allele, inactivation of the relevant gene is likely to interfere with the virus cycle and to result in resistance. The objective of this project is to test the hypothesis that in *Prunus* species, translation initiation factors are also playing a central role in PPV infection and that the inactivation of one or the other form of the *eIF4E* or *eIF4G* genes leads to resistance to sharka disease. For this purpose, eIF4E and eIF4G orthogues in *Prunus* species have been searched for and consequently mapped in the peach genome in order to identify independent copies of each gene. Consequently, RNAi-hairpin based constructs specific to each copy were obtained and are currently transferred to peach and Japanese plum by *Agrobacterium* transformation.

Novel mechanisms of resistance to *Plum pox virus* are being unravelled in the model plant, *Arabidopsis thaliana*

G. Pagny, P. Paulstephenraj, S. Poque, G. Gourdon, P. Cosson, T. Candresse and V. Decroocq

UMR GDPP INRA-Université Bordeaux II, 71 Avenue Edouard Bourlaux, 33140 Villenave d'Ornon (France)
Email: **decroocq@bordeaux.inra.fr**

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In plant-virus interactions, recessive resistance has been associated, up to now, to a passive mechanism in which virus infection is impaired due to the lack of a specific host factor. The identification of a recessive resistance gene and, symmetrically, of the corresponding susceptibility allele is thus expected to provide valuable information on the minimal requirements for successful virus infection of the host plant. Gaining access to such information is thus expected to result in new candidate genes for manipulation across species and genera and in the deployment of new resistance sources in agronomically important crop species, such as stone fruit trees. We showed few years ago that *Plum pox virus* (PPV) is able to infect the model plant *Arabidopsis thaliana* and that a large diversity of phenotypes was observed, from susceptibility with symptoms to complete resistance (Decroocq et al., 2006). Consecutively, up to 44 *Arabidopsis* accessions have been screened for differential susceptibility to PPV isolates, as a way to identify potential resistant phenotypes. Such studies have contributed to the identification of natural resistance to PPV, and among them, of several recessive resistance mechanisms. Distinct host factors that determine either PPV systemic infection, long-distance movement or virus titer have been identified. We are presently fine-mapping some of those host determinants in order to clone them. The cloning and characterisation of *Arabidopsis* recessive resistance genes against PPV is an exciting project that has the potential to provide both important information on host-potyvirus interactions and new resistance genes that can be harnessed in stone fruit species.

Differential gene expression in susceptible and hypersensitive plum genotypes infected with *Plum pox virus*

L. Michalczuk[1], K. Kowalczys[1], M. Klekowicka[1], M. Neumüller[2] and L. Mühlberger[2]

[1]Research Institute of Pomology and Floriculture, Skierniewice, Poland
[2]Technische Universität München, Freising, Germany
Email: **lmichal@insad.pl**

Keywords: *Plum pox virus*, *Prunus domestica*, hypersensitivity, differential gene expression

Hypersensitive and susceptible to PPV plum rootstocks were infected with the virus and RNA samples were isolated from both the infected and healthy (control) plants. Gene expression was analyzed by cDNA-AFLP on bulked RNA samples using 256 primer/restriction enzyme combinations. Amplification products showing differential expression pattern (either up - or down-regulated) in control and infected plants were re-amplified from polyacrylamide gel, cloned, sequenced and subjected to BLAST analysis. Of 25 differentially expressed transcript fragments detected, one showed homology to gene encoding protein-binding protein involved in activation of transcription, one to DnaJ protein involved in posttranslational protein modification, two to GTP-binding proteins involved in signal transduction and 3 to genes encoding enzymes involved in secondary metabolism. For the rest of the transcripts no homology to genes encoding functional proteins was found. Expression pattern of 15 transcripts suggests that they may be putative markers of hypersensitivity.

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