

SharCo

Containment of Sharka virus in view of EU-expansion

Small Collaborative project of the 7th Framework Programme

Theme 2

Food, Agriculture, Biotechnologies

FINAL REPORT

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1. Executive summary

Sharka disease significantly impacts the economics and productivity of stone fruit producing countries in EU and worldwide, affecting a wide range of stakeholders from breeders, plant material gardeners, to fruit producers. The FP7 Small Collaborative SharCo (“Sharka Containment”) project focused on helping the stakeholders ameliorate the different types of risks associated with Sharka disease by providing them with tools such as *Plum pox virus* (PPV) resistant plant materials, accurate and reliable methods of PPV detection, guidelines, warning systems, and a decision-support system. In this regard, in the field of epidemiology, the project surveyed the viral diversity, identified driving factors of PPV spread and diversification and developed novel and highthrough-put detection systems early warning of Sharka outbreaks. In the field of genetics, it provided molecular markers for the implementation of marker-assisted selection of PPV resistant fruit tree varieties. In the field of biology, we assessed innovative biotechnological approaches to broaden resistance to PPV in different fruit tree species. We identified new mechanisms and sources of resistance to PPV that could be combined with the resistant donors currently used in the breeding programs. Finally, to develop a PPV outbreak management scheme, we elaborated: i) guidelines for endusers and policy makers concerning cultivation and risk management, ii) an early warning system coupled with a decision support system. All knowledge and tools developed by the project were widely disseminated in Europe with special attention to PPV-endemic countries.

2. Summary description of the project context and objectives

The concept of SharCo was to combine prophylactic and genetic solutions to prevent or limit the spread of the Sharka *Plum Pox Virus* (PPV). The project scope covered the entire chain from seedling production, grafted material production, to orchard management. It aimed at providing new methods and tools for the containment of Sharka, in orchards and nurseries. For that purpose the project developed specific research activities on a variety of complementary topics: epidemiology, virology, genetic and biotechnological approaches.

The strategic objective of SharCo is to provide the EU with novel methods and tools to face the constant menace of Sharka spreading as well as to reduce the impact of the disease for the various stakeholders, nurserymen and fruit producers. On that purpose, we aimed at

In the field of epidemiology, developing new methods for monitoring and fighting the PPV spread by:

- Identifying the driving factors of the PPV spread and diversification
- Developing novel systems for detecting, assessing and warning Sharka outbreaks
- Defining viral parameters linked to or driving PPV dynamics of dissemination at the field level
- Providing information about the most crucial epidemiological characteristics of PPV in nurseries, in order to design strategies to reduce plum pox incidence in nursery blocks
- Providing information related to the most accurate sampling and testing methods and protocols that would allow analyzing large numbers of nursery plants, thus improving the reliability of PPV detection and consequently the European phytosanitary passport
- Evaluating the effectiveness of mineral oil treatments in reducing PPV infection and spread in nursery blocks.

In the field of biology, develop new genetic tools for selection in view of improving resistance of plants cultivated in orchards by:

- Identifying molecular markers linked to resistance that would help increasing the efficiency of European breeding programs in apricot, peach and plum crop species
- Implementing the marker-assisted selection to speed up the breeding of resistance material

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- Identifying new and complementary genetic resistance mechanisms suitable for pyramiding durable resistance to PPV, thus reinforcing plant resistance.

In the field of agricultural management, help the end-users – notably but not exclusively plant protection officers, breeders, nursery gardeners and fruit producers – to take advantage of the project outcomes by:

- Establishing cultivation guidelines aimed at minimising the virus spread likelihood at the levels of nurseries and orchards
- Proposing tools (decision support system) and recommendations for an early warning system
- Delivering a risk management system designed to minimise the entry, establishment and spreading of the virus and potential new variants in EU.

3. Description of main S & T results/foregrounds

1) **First Epidemiology work package**

- Improving virus typing tools for an early detection of new variant virus outbreaks

The accurate identification of virus isolates that belong to a particular strain is expected to provide insight into the diversity and evolution of the virus, which is especially interesting when the causal agent displays a broad biological and genetic diversity. Such is the case for *Plum pox virus*, the viral agent causing the Sharka disease. Therefore, the objective of the WPE1 task TE1.1 was to develop a mini-oligo array for a rapid genome wide analysis of PPV isolates. In the first step, a first generation PPV oligochip was developed in two phases: the first one consisted in the *in silico* design using the complete sequences of isolates belonging to the main PPV strains using the Array Designer 4 software. The second step consisted in the testing of this first generation array. Subsequently, a second generation PPV oligochip was developed. This second generation contains 10 replicates of 90 probes each constituted of 18 to 22 nucleotides. This second generation oligochip allows the analysis and probing of PPV intra-strain variability within the PPV-D, M and Rec strains. A complete protocol for the use of this PPV mini-oligo array, including spotting of validated probes, post-spotting procedures, hybridization, evaluation of signals using a GenPix 4000B scanner and final signal analysis has been established and assayed in the two distinct laboratories.

In addition, parallel experiments to analyse the variability of PPV isolates were performed using deep sequencing, a more recent and broadest approach, since it analyzes all positions of the viral genome. Deep sequencing was successfully evaluated for its use in the analysis of viral variability and heterogeneity. Several software packages and conditions were checked in order to optimize the *de novo* assembly and to facilitate the reconstruction of viral genomes from small interfering RNAs (siRNAs sequences). In spite of the success of the mini-oligo array technology to characterize PPV isolates, it presents several limitations when compared with deep-sequencing, mainly for its feasibility, reproducibility and capacity to analyse not only intra-strain but also intra-isolate variability.

- Improving our knowledge on Sharka diversity for a better management of the disease outbreaks

The analysis of genetic diversity and the understanding of the relationships between viral strains or isolates are important aspects in the management of viral diseases and in the

analysis of the risk of new emerging viruses. With the aim to provide a precise and broad overview on the current diversity of PPV worldwide, a standardized RT-PCR and sequencing protocol has been established and used by all partners to amplify two highly informative genomic regions (part of the NIb-CP gene and P3-6K1-CI region, respectively). In total, both partial sequences have been generated from about 800 PPV isolates, originating from 29 countries, among which 16 are European Union Member States. This unprecedented effort has furthered our understanding of the genetic complexity and diversity of PPV as more than twice the sequence information has been generated in comparison with what was previously available in the international Genbank database. Moreover, complete epidemiological data (host, location, period of the year, age of the host tree etc...) have been recorded for each isolate (data which generally was not available for isolates in the public database), allowing unprecedented analyses.

Within this PPV diversity survey, we obtained a range of original results on PPV variability and distribution. Higher than previously envisioned intra-strain variability was observed within PPV-D, indicating that the variability of the most widespread PPV strain had previously been underestimated. Within each main strain, divergent isolates were identified, mostly originating from Central- and Eastern Europe. We confirmed the splitting of the PPV-M strain into two major subgroups (Ma and Mb) and obtained for the first time a wide picture of their geographic distribution. A high prevalence of isolates belonging to the newly discovered PPV-T strain in Turkey was also detected.

Two new strains of PPV were identified, one corresponding to the missing ancestor in the evolutionary history that led to the currently known M strain. The second is a novel cherry-adapted strain identified in Russia, further increasing concern about the potential for impact of PPV in cherry crops.

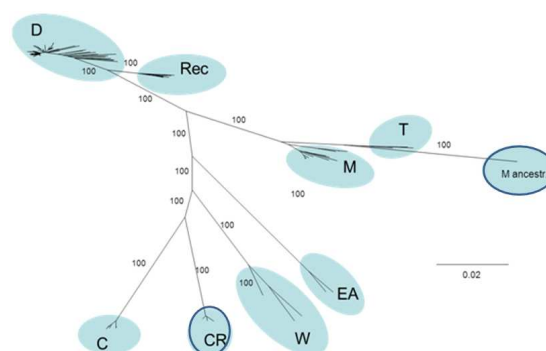
New information on the distribution of PPV strains has been obtained, e.g. the presence of PPV-Rec in Italy, PPV-M in Spain, PPV-W in Latvia and Russia, PPV-T in Albania, PPV-C in Belarus and Russia.

A total of 52 complete genome sequences belonging to 6 of the 7 previously known PPV strains were determined, together with complete sequences for members of the two newly detected strains. The sequences obtained from European PPV-D isolates counterbalanced the scarcity of information in comparison to the North-American or Japanese data, the sequences of PPV-M, Rec and T isolates considerably enriched the previous limited full-length genome data about these strains. Several genome data have allowed to complete the picture of the evolutionary history of PPV. For example, we performed a complete analysis of the divergent

AL-11pl isolate, which originates from Albania. Its complete genome sequence revealed that it represents an “Ancestral” PPV-M isolate from which the current PPV-M strain most probably originated through recombination.

Similarly, we performed a full genome sequence analysis of the PPV-W isolates sampled in Latvia. These isolates appear to be representatives of the “typical” PPV-W strain, which is not affected by recombination events, contrary to the first PPV-W isolate identified in Canada: PPV- W3174. Strikingly, the PPV-W intra-strain variability is substantially higher than for all the other PPV strains. This could impact the ease and reliability of detection of PPV-W isolates, in the near future.

Finally, we also focused our effort on the analysis of PPV diversity on cherry host species. It is indeed acknowledged that PPV only sporadically infects cherry species, with the consequence that at the beginning of the SharCo project, extensive knowledge on the variability and prevalence of the PPV-C strain in Europe, the only strain able to infect (sour and sweet) cherry trees was extremely limited. In the course of the task TE1.1, full genome sequences have been obtained from three PPV isolates found in naturally infected sour cherries in Russia. Those isolates present only ca. 78% of nt identity with other common PPV strains (M, D, Rec), which are not able to infect cherry trees. Complete genome sequencing has confirmed their high divergence from any other known PPV group, PPV-C included. Taking into account their biological properties (ability to infect sour cherry) and genome particularities, the isolates thus represent a new PPV strain, that is able to infect *Prunus cerasus* species. It was later named PPV-CR (Cherry Russian) (see figure 1).



In addition to the classical sequencing approach, the deep sequencing of small RNAs was also assessed. It was successfully applied in the case of the ES-11pe isolate, allowing the determination of its full-length genome sequence from its original host (peach) and confirming the effectiveness of the next-generation sequencing approach for PPV typing and characterization.

➤ Sharing with the public data on PPV diversity analysis and viral collections

All partial and full-length genome sequences have been transferred to the web-queryable SharCo sequence database (<http://w3.pierroton.inra.fr:8060/>), developed in the course of the project. The database contains sequence data and epidemiological information about the isolates, enables view and export of isolate sequences. It lists full data on PPV isolates

including georeferencing, original hosts and location etc... A search procedure using multiple criteria is available, allowing export of results and semi-automatic NCBI import. The current database will be opened to non-SharCo partners by the end of 2012.

The research and practical work in the field of plant virology often requires the availability of a set of reference viruses, strains or isolates. For this purpose, a lyophilized collection of PPV isolates characterized in the frame of the SharCo project has been established. The collection actually contains 654 entries from 22 countries. The isolate naming follows the format used in the SharCo PPV database, allowing easy linkage between sequence and isolate information and the original preserved isolate material. The current number of PPV isolates by country is as follows; Albania (12), Argentina (5), Austria (22), Belarus (15), Bulgaria (73), Chile (1), Cyprus (1), Czech Republic (50), Egypt (1), France (52), Greece (2), Hungary (18), Italy (36), Latvia (3), Moldova Republic (7), Poland (29), Romania (86), Russian Federation (1), Serbia (55), Slovakia (46), Spain (86), Turkey (53). This collection is the most representative of PPV genetic diversity worldwide.

In parallel, we also established a centralized live collection of PPV isolates. This collection is more limited (currently 38 isolates) but it has two main purposes that cannot be achieved easily by the lyophilized collection: i) the maintenance of isolates in their original woody hosts (plum, peach or apricot species) limiting further PPV divergence driven by host adaptation, ii) the access to fully characterized isolates representative of PPV diversity, in a form that can be readily used to inoculate further woody hosts. This material can then be used directly for future analysis of PPV resistance stability and durability, similarly to task TE2.1 in the WPE2 work package. All 38 isolates have been fully sequenced before the establishment of the collection in insect-proof controlled facilities of Partner P5. The collection has been duplicated into an S3 high confinement greenhouse of partner P1, to ensure redundant preservation of the isolates and safeguarding of the collection against potential local incidents.

2) *Second Epidemiology work package*

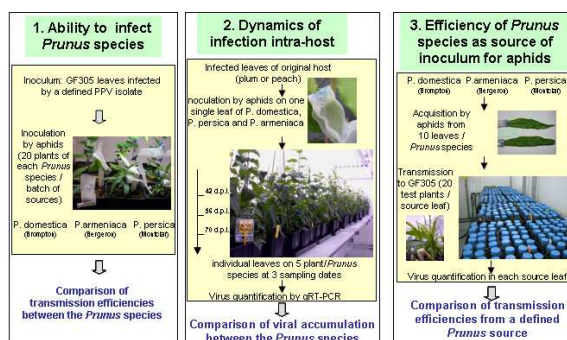
- Searching for pertinent indicators of epidemicity that would help to manage PPV outbreaks in the fields

The increasing knowledge on PPV genetic diversity raises new concerns about the biological properties and related epidemic potential of the identified PPV strains. Indeed, apart from PPV-D and PPV-M, the biological properties of the PPV strains are poorly known.

This situation is partially related to the fact that experimental assays aiming at measuring the biological parameters of a given PPV isolate on a given *Prunus* species are still complicated, long and low throughput. Moreover, the parameters measured in experimental assays are generally not compared with the real epidemiological situation in the field and consequently, the usefulness of the experimental data for risk assessment is rather limited. During the SharCo project, we developed a strategy combining epidemiological surveys in orchards and new experimental assays into a confinement greenhouse to identify and measure pertinent indicators of PPV epidemicity. The experimental assays were designed to gain knowledge about the three successive steps of an infection cycle: (i) the *de novo* infection of a *Prunus* tree after aphid inoculation, (ii) virus multiplication and accumulation in the infected tree after a given incubation time (production of inoculum), and (iii) acquisition of the virus by aphids from this infected tree (virus that will be transmitted to another still-healthy tree: dispersal). Three indicators linked to these three steps were defined:

1. The ability for a given strain / variant to infect certain *Prunus* species after aphid transmission;
2. The dynamics of virus accumulation in *Prunus* hosts;
3. The efficiency of the infected *Prunus* as inoculum sources for the aphids. This last indicator may be correlated to virus accumulation but other factors specific to the *Prunus* species might influence it.

The experimental assays were initially designed to be used in the future as a routine test for every PPV isolate/strain to be characterized. We were thus particularly careful at facilitating the plant material (*Prunus*) production step and at reducing the duration of the overall assays. A specific methodology that enables quantifying and comparing PPV amounts in the three main stone fruit *Prunus* species susceptible to PPV (plum, peach and apricot) was developed. The protocol was designed as indicated on the figure 2 and carried out on one isolate of each



PPV-D, PPV-M and PPV-Rec strains with a duplication of each experiment. While all the results are not yet available, some preliminary conclusions can be inferred:

- The specific strain / *Prunus* interactions evidenced under orchard conditions were not

clearly detected in the experimental assays. More precisely, we detected very few (PPV-D) or exceptional (PPV-Rec) cases of infection in peach orchards whereas in our biological assays,

the PPV-D and PPV-Rec isolates were found able to infect systematically peach seedlings, thus providing a good source of inoculum for further aphid transmission. Such a result may indicate that differences in the ability to infect peach in orchards might not be due to viral factors only but also to environmental and physiological parameters which cannot be easily reproduced in simple experimental assays carried out under high confinement greenhouses.

- The experimental assays evidenced more subtle differences between the strains, and especially in favour of a higher epidemicity of the PPV-M strain. Indeed, the PPV-M isolate used in our biological assays was found to induce faster systemic infections of apricot and was better generalized in peach, generating higher transmission rates by aphids from this *Prunus* species in some conditions. Those differences might be useful for risk assessment and predictions.

For those reasons and because of the numerous difficulties that arose while carrying out the experiments, it is not expected that the same experimental protocol can be used to routinely test any new isolates for risk assessment. However, this does not exclude the possibility that parts of the experimental design targeting specifically the parameters that allowed us to differentiate the isolates can be adapted for further risk assessment.

➤ Challenging *Prunus* sources of resistance with the viral diversity

A set of resistance genitors was challenged with an extended range of PPV isolates that are representative of the viral genetic diversity as defined in the first Epidemiology work package. Among those apricot, peach and almond genitors, only two were truly and stably resistant to infection by eight PPV isolates, apricot cv. 'Harlayne', 'Lito' and in consequence 'SEO', parent of 'Lito'. We are thus recommending them for future breeding programs as described in the Genetic pillar.

➤ Identification of genetic determinants of adaptation to *Prunus* species

Some PPV strains or isolates are known for their specificity against *Prunus* or herbaceous host plants. For example, PPV-C is the only strain that infects systemically cherry trees and PPV-Rec has rarely been observed on peach trees. Isolates derived from the PPV-PS isolate (M strain) were shown to have two mutations in the sequence coding for the P1 protein that specifically prevent PPV-PS infection of 'GF305' peach seedlings. Finally, the PPV-D and PPV-R (D strain) isolates are adapted to *Prunus* and *Nicotiana* respectively and have specific determinants at the N-terminal region of the capsid protein (CP) involved in adaptation to these hosts. This material was the basis to study genetic determinants of adaptation to *Prunus* species. For this purpose, we developed PPV-C and PPV-Rec infectious clones, artificial

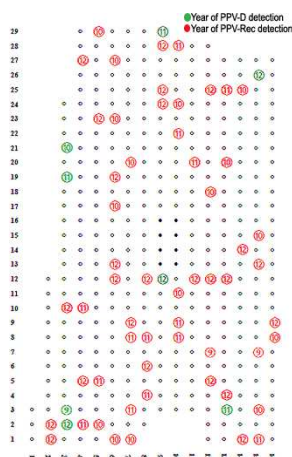
recombinants, site-directed mutagenesis and gene shuffling. Till now, by challenging various hosts with these constructs, we have demonstrated that point mutations in the P1 protein are responsible for symptom expression while other ones in the CP are determining viral systemic infection in peach 'GF305' seedlings and herbaceous plants. A 1.9kb long region was also shown to be essential for PPV-C systemic movement in *P. avium* (cherry trees).

➤ Evaluation of competitiveness and disease dynamics of PPV-Rec in orchards

The prevalence and host distribution of a given PPV strain are driven by its specific biological properties but also by many other factors like the agro-ecological conditions occurring at one location and the historical pathways of PPV strain introductions. A strong geographical effect is thus expected. To disentangle intrinsic properties of the strains from the other factors, surveys using same methodologies and statistical procedures were performed in three distinct countries (Bulgaria, Romania, and Serbia).

As expected, strong geographical effects were recorded both among countries and also among regions of a same country where agronomical conditions differ. PPV-D and PPV-Rec are commonly found in each of the three country, but PPV-M was not found in any region or *Prunus* species in Romania so that it seems still absent in this country.

Data from Bulgaria and Serbia suggested a strong association between PPV-M and peaches, which is consistent with previous data obtained from Europe, but no preference was evident among the three strains and apricot or plum host plants. Indeed, the three strains appear equally fit on these 2 crop species. Multiple infections were shown both at the level of the orchard and within the same trees, leading to mixed infection, especially for plum (10% of plum trees were mixed infected) and at a lower extent for apricot. Every possible type of mixed infections (involving 2 or even 3 strains) was detected in plum and the hypothesis of independence between strains conditionally to the infection by one strain was not rejected. This result suggested that over-infection by any other PPV strain (PPV-M, PPV-D or PPV-Rec) was possible on this *Prunus* species. Extended follow-up of mixed infections might however evidence specific strain competitiveness. The monitoring of over-infections between PPV-D and PPV-Rec isolates in an experimental orchard in Romania is currently under analysis to confirm part of these results. Overall, these data evidenced the potential role of domestic plum in PPV evolution. Indeed, mixed infection constitutes a pre-requisite for the generation of new variants by genome exchanges (recombination).



Three experimental orchards were newly established at the beginning of the project with the aim to monitor the competitive dynamics of PPV-Rec and PPV-D spread (figure 3). A well-characterized primary inoculum of each strain were introduced in the orchards. Infected trees were detected in plum orchards, but, after three vegetative periods, (nearly) all trees that resulted were infected with contaminant inoculums from outside of the experimental orchards. Phylogeny analyses are currently done after partial sequencing to try to identify the exact origins of first cases

and subsequent chains of transmission.

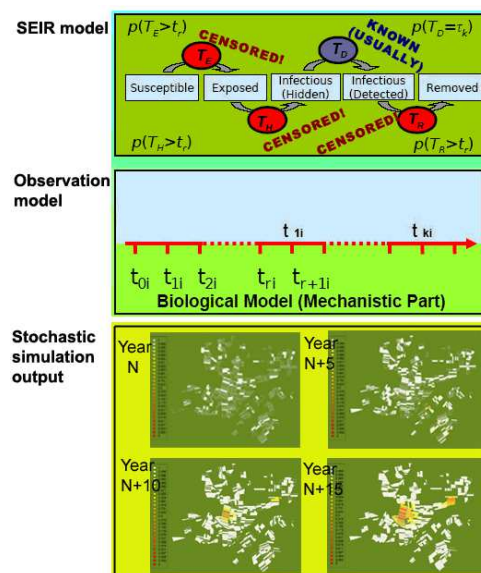
➤ Reconstructing the chains of virus transmission

An experimental and modelling approach was developed to trace back aphid transmission events by combining viral genetic and epidemiological data. The reconstruction of the chains of virus transmission aims at providing information upon the frequency of transmission from one particular source as well as an estimation of the distances of spread. This study was based on a 450 ha area of *Prunus* orchards mixed with other crop, non-host species where every diseased tree (800 in total) was geo-referenced and sampled, giving rise to as many PPV isolates. A previous analysis based on partial viral genome sequencing showed that full-length genome sequencing (10,000 bp) was necessary to get enough resolution at the tree scale. The full-length genomes of 188 PPV isolates corresponding to all diseased trees located in 13 neighbouring orchards were fully sequenced and the genealogical relationships between the sequences were reconstructed. The development of a generic mathematical framework to link genomic and epidemiological information is underway. Expected results should be used in a second step to feed a simulation model of sharka disease spread at a regional scale (see below).

➤ Modelling disease spread at a regional scale

A spatially-explicit model of sharka disease spread at a regional scale has been constructed. It is based on a large regional geo-referenced database supplemented with data of PPV-M epidemics in 1,600 peach orchards located in South-East of France which were surveyed for 14 years. This database was constructed during the first period of SharCo. It includes parameters related to the orchards (location, structural characteristics and conditions of cultivation), to the monitoring (dates of survey, identity of inspectors) and control of the infected trees (dates of removal) as well as to the dynamics of the disease (number of detected

disease cases at each survey date). This hybrid mechanistic-statistical model links real data from the regional database with biological and human processes (dissemination by aphids, latency period, introduction of infected plant material) underlying the observations. The mechanistic part of the model is based on a SEIR model (Susceptible/ Exposed/ Infectious (hidden and detected subclasses)/ Removed) linked to a spatial reference map of the orchards. The statistical component of the model deals with imperfect detection and event-time censoring (figure 4). The core algorithms are the basis of both a simulation model and an estimation model. The estimation model is aimed at assessing the parameter values used in the model (kernel of dissemination, latency period, sources at the origin of the epidemics, ...) following a Bayesian frame. The simulation model has been extracted from this full estimation model and has been adapted to be used under a classical Windows® environment. It will be used to test different strategies of orchard surveillance and disease control.



3) Third Epidemiology work package

The main goal of this work package was to develop strategies to contain PPV in nurseries, thus avoiding PPV introduction and later commercialization of infected nursery plants.

- Towards strategies to limit PPV spreading and establishment in nurseries

At the beginning of the project (spring 2008), six experimental plots were established in six different ecological areas (Bulgaria, Czech Republic, Poland, Romania, Spain and Turkey), close to well-established PPV inoculum sources, following a common statistical experimental design. Using those newly established experimental nursery plots, we have identified *Prunus* rootstock species more resistant to natural PPV infection in field conditions. Twelve *Prunus* rootstock species were evaluated in six different ecological areas to assess their susceptibility to natural PPV infection (Figure 5). In the experimental nursery plots, PPV infection occurred at random in any of the evaluated areas. *Prunus mariana* ‘GF8.1’, ‘Adesoto 101’, ‘Nemaguard’ and myrobalan 29C were the most susceptible rootstock cultivars. Other rootstock genotypes such as ‘Wangenheim’ and myrobalan ‘Alina’ showed a

clear susceptibility. On the other hand, ‘GF677’, ‘Garnem’, ‘Greenpac’ and myrobalan ‘BN4kr’ showed a certain level of resistance to natural PPV infection. ‘Docera 6’, a PPV-hypersensitive rootstock, showed

a relatively good level of resistance to natural PPV infection. When grafting PPV susceptible plum cultivars onto hypersensitive rootstocks, no tree death could be clearly associated up to now with Sharka infection, in the experimental conditions.

The efficiency of horticultural mineral oil (HMO) treatments was also assessed in the same experimental nursery plots. Two PPV susceptible rootstocks were compared: *P.*



Effect of HMO treatments on *P. mariana* ‘GF8-1’ rootstocks

Mariana ‘GF8.1’ and the peach ‘Nemaguard’ cultivar (figure 6). Treatment with 1% emulsion did not avoid PPV natural infection in areas with high PPV-prevalence but significantly reduced the percentage of PPV infected nursery plants, limiting significantly the spread of the disease. Therefore, the use of HMO is highly recommended in nurseries and young plantations in particular during the aphid species peak flight and/or as soon as the first PPV-viruliferous aphids are detected.

- Providing information about the most crucial epidemiological period when nurserymen can interfere with the natural spread of PPV

We monitored and identified the different aphid species that are landing on nursery plants located in different ecological areas, as well as the peak period of aphid populations. The predominance of certain PPV-vector aphid species is dependent on the European region as follows: *Hyalopterus pruni* in Czech Republic, Turkey, Romania, Bulgaria and Poland, *Aphis spiraecola* in Spain and Turkey, *Rhopalosiphum padi* in Poland, *Brachycaudus cardui* *A. craccivora* and *B. helicyrsi* in Turkey, *Phorodon humuli* in Bulgaria and Romania. The maximum peak of aphid flights was scored in springtime in each different ecological area, whereas a more irregular and non-reproducible second peak was observed in autumn, in some areas. PPV-viruliferous aphid individuals were successfully detected by squash real-time RT-PCR. We are thus recommending the use of strategies to limit PPV entry, establishment and

Rootstock	Spain (May 2011)	Poland (June 2011)	Turkey (May 2010)*	Czech Republic (September 2011)	Bulgaria (May 2011)	Romania (May 2011)
<i>P. mariana</i> ‘GF8.1’	131/136 (96.3%)	12/122 (9.84%)	0/17 (0.00%)	76/186 (40.9%)	130/178 (73.0%)	117/187 (63.2%)
‘Nemaguard’	88/122 (72.1%)	26/196 (13.27%)	1/38 (2.63%)	119/170 (70.0%)	141/199 (73.0%)	20/41 (48.8%)
‘Adeco 101’	-	-	-	-	92/169 (54.4%)	114/189 (60.3%)
‘Garnem’	1/157 (0.6%)	-	0/47 (0.00%)	-	73/187 (39.0%)	17/159 (10.7%)
‘GreenPac’	1/161 (0.6%)	-	-	-	49/123 (39.8%)	-
Myrobalan ‘25C’	172/188 (91.5%)	49/198 (24.7%)	7/82 (8.54%)	142/198 (71.7%)	-	116/185 (62.7%)
Myrobalan ‘Alina’	-	7/192 (3.6%)	-	-	-	-
‘Wangenheim’	-	47/196 (24.0%)	-	-	-	-
Myrobalan ‘BN4kr’	-	-	-	0/55 (0.0%)	-	-
‘GF677’	-	-	0/11 (0.00%)	0/155 (0.00%)	-	-
‘St Julien’	-	-	-	30/181 (16.6%)	-	-
Total	393/764 (51.3%)	180/904 (19.9%)	8/195 (4.10%)	367/945 (38.8%)	485/850 (57.0%)	384/759 (50.6%)
PPV inoculum source	D	D	T	D and Rec	M and Rec	D and Rec

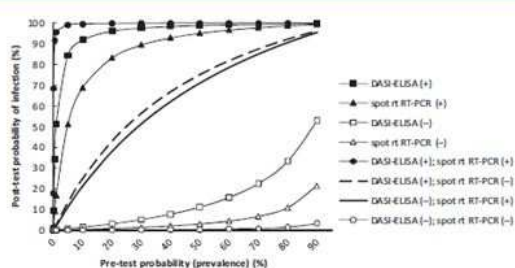
spreading in the nurseries during the aphid flight peak and as soon as the first PPV positive aphid is detected.

The variation of PPV-prevalence during the three-year period, revealed a continuous increase in disease incidence in each agro-ecological condition. The use of the same PPV susceptible rootstock cultivars in all scenarios allows comparing PPV prevalence and incidence in different conditions and shows the ability/efficiency of local aphid species in spreading the different PPV strains present in each location. The most efficient combination of parameters for PPV spread was detected in Spain where PPV-D spreads on *P. mariana* ‘GF8.1’ through the vector *A. spiraecola*, followed by Bulgaria where PPV-M and PPV-Rec were efficiently spread onto *P. mariana* ‘GF8.1’ plantations mainly by *H. pruni* and *P. humuli*. In plots of ‘Nemaguard’ peach seedlings, a similar efficient PPV-spread was estimated in both ecological areas. These results reveal the importance of knowing the vector aphid species prevalent in a specific area. In some situations, the high efficiency of transmission by *A. spiraecola* of PPV-D strain can overcome the potential of PPV-M transmission by *H. pruni* and *P. humuli* (which are less efficient PPV vectors), therefore having significant impact on the theoretical propensity of different PPV isolates to be transmitted in natural conditions.

In addition, the epidemiological role of weed species as PPV reservoirs was evaluated in orchards infected by PPV_M but no PPV infection was detected on weeds.

- Providing information related to the most accurate sampling and testing methods and protocols

The purpose here is to allow the analysis of larger numbers of rootstocks and nursery plants in order to improve the reliability of PPV detection and consequently of the EU phytosanitary passport. Methods for PPV detection in nursery plants were assessed and compared for their diagnostic parameters. Serological tests (ELISA based on 5B-IVIA monoclonal antibodies) and spot real-time RT-PCR were simultaneously evaluated by latent class models using maximum likelihood functions and a Bayesian approach. The sensitivity and specificity of both techniques did not vary according to the latent model applied. Spot real-time RT-PCR was more sensitive while ELISA was more specific for PPV detection



(figure 7). The concordance between both techniques, using the same extract, was almost perfect (Cohen's kappa index of 0.88 ± 0.01) after the analysis of 5,379 plants. The results demonstrate that a coincidental result obtained

by both techniques leads to a practical accuracy of 100% to rule in or rule out the disease in a

specific sample. In addition, spot real-time RT-PCR technique can be successfully applied on composite samples (up to 10 plants, 3-4 leaves or dormant buds/plant) at any vegetative stage or latency period without losing accuracy in detection. By contrast, ELISA showed significant differences in detection accuracy depending on the time of the year and the number of plants pooled together, knowing that spring and summer provided the best sensitivity for detection of PPV. In this case, up to 4 plants (3-4 leaves/plant) could constitute a composite sample. In conclusion, the recommendation is the use of composite samples (4 plants) for accurate PPV detection by ELISA (5B-IVIA based) in spring or summer vegetative periods. However, the use of composite samples (10 plants) is recommended at any vegetative or latency period when analysed by the spot real-time RT-PCR technique using Taqman probe.

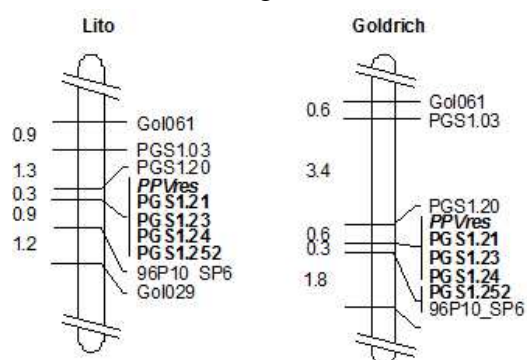
We also compared different methods of sampling, individual analyses *versus* hierarchical methods. The result showed an overestimation of the real PPV incidence in the experimental plot when using the hierarchical method (Table 2). It is worth noting that the hierarchical method is a procedure used widely to detect PPV in an area, starting from a limited number of analyses. Therefore, the cost and time required are reduced when the hierarchical method is used.

4) **First Genetic work package**

The major goal was to implement efficient, rapid and less cost-effective breeding programs for resistance to PPV in European countries. This implies the availability of large progenies segregating for the trait (resistance) and the development of molecular markers.

- To simplify and speed up the procedure of selection of PPV resistant cultivars

In apricot, the development of molecular tools was based on association mapping between PPV resistant phenotypes and genotypes at one specific locus, named *PPVres*. This locus is located at the top of linkage group 1 and was constantly linked to PPV resistance in apricot in the last 10 years. Association mapping was achieved through the construction of apricot linkage maps from previously phenotyped families segregating for the trait.. In those preliminary steps, two resistant donors were used: ‘Goldrich’ and ‘SEO’ (‘Stark Early Orange’). Fine mapping was carried out by increasing the number of markers overlapping *PPVres* and by increasing the number of individuals and families



screened for resistance. Progress in mapping allowed narrowing the region containing the locus linked to Sharka resistance down to 2.1 Mb (figure 8). Forty-three SSR markers designed from the peach genome sequence version 1 were tested in several apricot mapping populations that were already phenotyped for PPV resistance. Three markers, namely PGS1.21, PGS1.23 and PGS1.24, were selected as the best, co-segregating tightly with the resistance. Other extra markers (called MP) were developed from other progenies challenged with the virus in other agro-ecological environments. Those markers were then tested in other progenies already phenotyped by several SharCo partners validating those molecular tools as well as the alleles linked to the resistance in progenies either originating from distinct donors of resistance ('Harlayne', 'Harcot') or tested for resistance to PPV in different agro-environmental conditions.

After validation of the markers, transfer of the technology to all other SharCo partners was achieved and implementation of Marker assisted Selection (MAS) was accomplished in the last period of SharCo, both in Western and Eastern European apricot breeding programs. Currently, a total of 8,904 apricot pre-breeding materials are now being screened with the PGS and MP molecular markers. This eliminates the bottleneck of phenotyping thousands of seedlings and speeds up the process of selection of new PPV resistant cultivars, adapted to various European, local environments.

In peach, where no source of resistance was identified, interspecific crosses and progenies using the peach related *P. davidiana* species were used for construction of linkage maps. The quantitative nature of the resistance to PPV lead to the identification of QTL linked to the trait but impeded the development of molecular tools suitable for MAS.

In plum, a resistance trait characterized by hypersensitive reactions that restrict the viral systemic infection was under focus. However, due to the complexity of the European plum genome, which is hexaploid, the development and identification of molecular markers linked to the trait proved tedious and difficult. A transcript profiling approach was thus applied. It provided several cDNA markers that are up-regulated in PPV-infected, hypersensitive individuals. Primer sets were developed from those transcript markers and validation in other plum progenies is still pending.

➤ Looking for new sources of resistance

The introduction of natural resistance to breeding programs, while successful, has limitations: a restricted number of sources for resistance have been found, resistance genes cannot always be easily transferred between species and undesirable traits may be transferred along with the resistance locus. Additionally, we cannot rule out that existing resistances may

be overcome, in the future, by new virus strains. We thus developed methodologies to test new local germplasm for resistance against the main PPV isolates circulating in Europe. Indeed, local germplasm was surveyed by partners in Romania, Poland, Turkey and Bulgaria. In Romania, the local plum cultivars ‘Rival’ and ‘Miroval’ and the apricot varieties ‘Traian’, ‘Auras’, ‘Orizont’, ‘Ovidiu’, ‘Ceres’, ‘Euxin’, ‘Tudor’, ‘Augustin’, ‘Danubiu’, ‘Hristia’ were found resistant to PPV by artificial inoculation. In Bulgaria and Turkey, none of the landraces and hybrids were found resistant to PPV while in Poland, one single plum cultivar (‘Vision’) presented promising behaviour against PPV natural infection.

5) Second Genetic work package

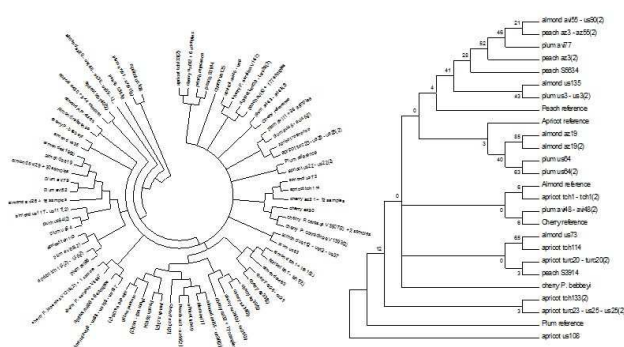
The purpose of this second work package was to enlarge resistance mechanisms available for gene pyramiding with the above sources of resistance. Part of its tasks was the characterization of plant factors involved in PPV replication or propagation.

➤ Identifying alleles of resistance in the *Prunus* germplasm

The EcoTilling approach searched in the natural germplasm of stone fruit trees for rare alleles of translation initiation factors with mutations that interfere with their functions in PPV infection without affecting their function in the host plant. For this purpose, we collected *Prunus* germplasm all over the world, through the scope of another FP7 European People project, “STONE”. One thousand thirty five individuals, including cultivars, accessions and wild representatives, were tested looking for mutations expected to affect the interaction domain or the global protein structure and stability of the eIF4E and eIFiso4E proteins (figure

Mutations at the protein level
in eIFiso4E

Mutations affecting eIFiso4E interaction
domain or stability



9). Fifty-five presented a suspicious mutation (deliverable DG.2.2). Of these, 24 genotypes were introduced in the greenhouse for the purpose of PPV resistance tests. One apricot tree, ‘Kostinskij’, appeared promising and was thus used to complete deliverable DG.1.5 (“Pyramiding different sources

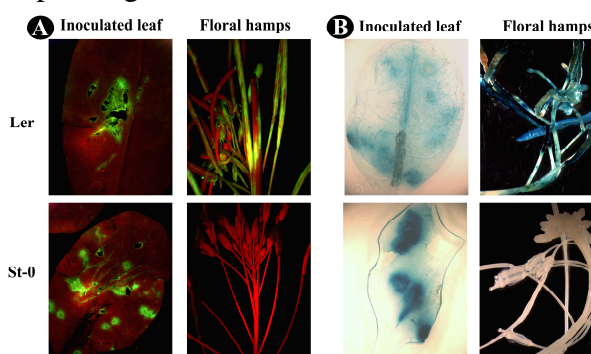
of resistance”). Six other promising peach or interspecific peach cultivars were transferred in the field in Bulgaria to challenge its resistance to PPV infection in natural conditions.

For the second subtask, intron hairpin DNA constructs were developed both for the different isoforms of the eIF4E and eIF4G genes. A total of six constructs were obtained in

the pHANNIBAL or Gateway vectors and then introduced in plums (hexaploid European plum and diploid Japanese plum) by the Chilean partner of a France-CONYCIT program. Ten of the regenerated transformed lines were recently transferred to the high confinement greenhouse in order to challenge them with the virus.

➤ Searching for new mechanisms or genes of resistance to PPV

We also focused on the characterization of plant factors involved in resistance against PPV. A first objective of this task was the cloning of resistance to Plum Pox Virus (*rpv*) genes in *A. thaliana*. Genes coding for host factors contributing to PPV resistance in *A. thaliana* when depleted were identified through different approaches: i) Positional mapping and cloning and ii) Screening of loss-of-function mutants in host factors shown to interact with viral proteins or implicated in the signalosome interacting with viruses. The first approach resulted in the identification and validation of two distinct genes or gene families coding for host factors contributing to PPV resistance, depending on their allelic form as follows: a) *RPV1* gene, which is coding for a cPGK protein that significantly reduces PPV accumulation when silenced and b) *SHA3* gene, which belongs to a TRAF like proteins cluster and its depletion results in complete inhibition of PPV systemic infection (figure 10).



The second approach lead to the demonstration that a functional CSN5 host protein is indispensable for early viral infection of *A. thaliana*. CSN5 is part of the COP9 signalosome complex implicating this pathway in the virus infectious cycle. We thus potentially identified a new mechanism of resistance to PPV infection. A patent was deposited in July 2011, covering its use as strategy to fight against viral infection. All these results are fully described in deliverable DG.2.4.

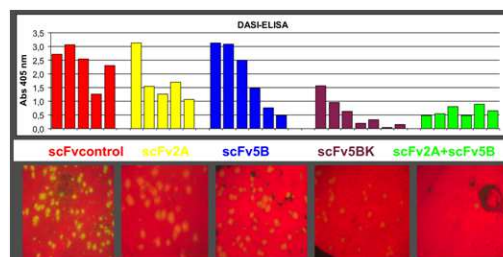
A second objective has been the search for resistance genes activated in herbaceous and woody plants showing a HR to PPV infection. Results related with this objective are also described in the deliverable DG.2.4. We have found four Resistance Gene Analogs (RGA) of the NBS-LRR family in *Chenopodium foetidum* and two in *Nicotiana occidentalis* expressed in plants showing HR after PPV infection. Upregulation after PPV infection has been shown by real time RT-PCR for 5 of these genes, but initial functional analyses with one of them has not revealed antiviral activity. A second approach was taken with the woody host *P. domestica*. A transcriptomic analysis of Jojo variety plants undergoing HR after PPV

infection was performed using RNA deep sequencing. Analysis of the data allowed us to identify 2,794 contigs with expression level variations higher than 2, upon PPV infection and HR induction. One hundred and three of these contigs match genes described in the resistance genes database (pRGDB). In addition, 51 genes with NBS sequences characteristic of the NBS-LRR genes have been identified and at least one of them shows significant variation in expression following infection.

In this work package we also focused on the identification of PPV factors involved in specific resistance against different PPV isolates. For this purpose, we constructed chimeric viruses to identify the determinant for overcoming RTM resistance in *Arabidopsis* at the N-terminal region of the capsid protein (deliverable DG.2.1).

➤ New strategies that interfere with PPV infection

New strategies to interfere with the virus were tested in this work package: the evaluation of the antiviral potential of PPV-specific recombinant antibodies; approaches to interfere with the post-translational modifications of the viral CP; and RNA silencing-related strategies. We generated single chain antibodies (scFV) specific for the CP and the RNA replicase NIb of PPV that have been targeted to three different cell compartments. Transgenic *N. benthamiana* lines with reduced sensitivity to PPV have been obtained by targeting the NIb-specific antibody to any of the three compartments. The efficacy of both NIb-specific and CP-specific recombinant antibodies has been also demonstrated in experiments of transient expression by agroinfiltration (figure 11). The best candidates have been selected to transform woody plants.



Another anti-PPV strategy assessed in this task has been the interference with the *O*-GlcNAcylation and phosphorylation of PPV CP (see deliverable DG.2.4). The *O*-GlcNAcylation is performed in *Arabidopsis* by the glycosyl transferase SEC, and PPV infection is delayed in an *Arabidopsis* mutant deficient in SEC. When downregulated in *N. benthamiana* by RNAi, we observed a drastic reduction in *O*-GlcNAcylation, but the effect in susceptibility to PPV is low. In contrast with animal systems, it appears that the phosphorylation and *O*-GlcNAcylation of PPV CP appears not to be reciprocal. Indeed, phosphorylated residues do not coincide with the *O*-GlcNAcylated ones, and phosphorylation of PPV CP is not enhanced in the *O*-GlcNAcylation-deficient plants.

Another important part of this work package was devoted to assess the performance of different ways of pathogen-derived resistance mediated by RNA silencing (see deliverables

DG.2.3 and DG.2.5). An ihCP-RNA (ihpRNACP) construct, also known as ‘B14’, was engineered into *Nicotiana* and *Prunus* genomes. Regardless to the plant species utilized, the ihpRNACP construct efficiently produced siRNAs and provided stable anti-viral resistance against all the major PPV strains.

We also assessed the efficiency of a novel PDR strategy based on artificial miRNAs

		SEQUENCES	vsIRNAs (-/+)	Accumulation (plus*)		Cleavage on sensors (s/as)		Protection (s/as)
				s	as	plus	*	
NIB	A	5' UGCUCAGUCAGUCAGUAAAG	(1/142)	±/++	+ /+++	-/+	+/-	+ /+++
	B	5' UUCUGUCUCAGUCUCUUCAGU	(8/506)	±/++	+ /+++	+/-	-/+	+++ /+++
	C	5' UACGGGCUUUCUCCAUUUUU	(13/13)	-/+	± /++	-/+	+ /±	+++ /+++
	D	5' UUGGCAUGUAUGCUUUUUCAU	(3/372)	±/++	+ /+++	+/-	+/-	+++ /+++
CP	Dm	5' UUGGCAUGUAUGCUUUUGCGG		±/++	+ /+++	± /±	- /-	± /±
	E	5' UUGGCGUAAUCCAUACCUU	(27/0)	±/++	± /±	+ /±	± /-	+++ /+++
	F	5' AGGUAGAUUUUAUGAUAGUA	(62/17)	±/±	+++ /±	- /±	- /±	+ /+++
	G	5' UAGACUCUACCCAGGUAAG	(9/0)	++ /+++	+++ /+++	- /-	- /-	- /-
3'NCR	H	5' AUGAUUAGACUCUCACCCAGG	(57/8)	++ /+++	+++ /+++	- /-	- /-	- /+++
	Hm	5' UUGAUUAGACUCUCACCCAGG		++ /++	+++ /+++	- /-	- /-	- /+++

(amiRNAs) alone or in combination with the more classical approach based on viral dsRNA expression. We have thus studied the molecular basis of artificial miRNA (amiRNA) expression and activity (figure 12),

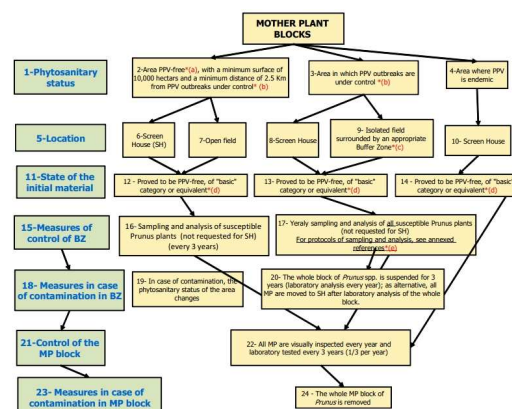
allowing us to design amiRNAs with efficient anti-PPV activity in *N. benthamiana*. Several lines of *N. benthamiana* transgenic plants expressing different single amiRNAs and a double construct have been already obtained, and first experiments suggest that some of these lines have high anti-PPV resistance. *Prunus* transformation with these amiRNA constructs has been started in collaboration with INIA (Chile).

All these results provide a large battery of demonstrated or potential sources of antiviral resistance to be used in future approaches to develop plants with more efficient and durable resistance to Sharka disease.

6) First Application work package

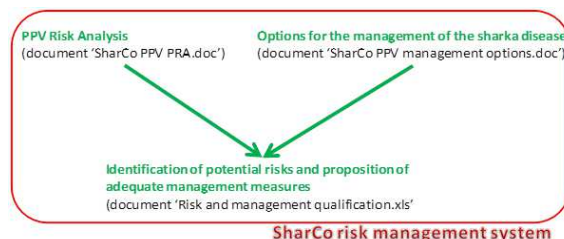
Research activities developed by the SharCo consortium as described above resulted in tools, novel information and expertise. They will be instrumental in containment, and eventually control of the disease. It includes a better view on the diversity of the virus, new knowledge on PPV epidemiology, more sensitive and accurate methods of PPV detection, molecular tools for improvement of the breeding programs, new sources and mechanisms of resistance and new stone fruit cultivars and rootstocks resistant to Sharka. In complement and based on these data, the SharCo consortium prepared the following reports that are expected to support policy-makers, regulatory bodies, extension services and other stakeholders in defining more efficient and realistic measures of Sharka management:

- ✓ Cultivation guidelines that would help to



restrict the virus spread in mother plant blocks, nurseries and orchards (figure 13).

- ✓ Recommendations for the implementation of an early warning system, assessing the risk of occurrence of unknown PPV variants in EU and new PPV outbreaks, mainly in virus-free zones.
- ✓ Guidelines for a risk management system targeted on Sharka outbreaks and spread and applicable everywhere in Europe (figure 14).



To facilitate knowledge and tool access to any stakeholder, three decision support systems (for cultivations of mother plants, for nurseries and for fruit orchards, see figure 15) have been developed and implemented in nine EU member states and two associated countries. They are accessible through institutions websites in eleven National languages.



7) **Second Application work package**

Critical for achieving the goal of efficient transfer of knowledge from researchers to major stakeholders was the dissemination work package. Through various activities it focused on “Dissemination and transfer”, for informing officers of the Plant Protection as well as extension Services, agricultural advisers, and breeders, nurserymen and fruit producers on the know-how, tools and plant material generated by the SharCo project. This was achieved by: i) systematically maintaining the SharCo webpage updated with the project’s public milestones and deliverables and providing on-line access; ii) through training workshops that provided an efficient, inter-active communication platform between the SharCo beneficiaries and all potential endusers; iii) disseminating project’s results to an enlarged scientific community, including non-European countries, through SharCo Research Workshop or communication(s) to more restricted audience and iv) *via* the use of the public media and publications in both local and international, peer-reviewed journals.

8) ***Beyond the SharCo project***

In this project, we have met and exceeded our original project goals as described above. Consequently, we have gained particular insight into certain questions and research areas that should be given priority for continued investigation in the future (see below). This work will be instrumental in translating our results into sustainable management practice for this important disease of fruit trees.

➤ Evaluating new potential risks linked to PPV diversity

Intensive efforts during the four years of SharCo provided original results on PPV variability and distribution, including the discovery of two new PPV strains. The first one is corresponding to the missing ancestor in the evolutionary history that led to the currently known PPV-M strain. The second is a novel cherry-adapted strain identified in Russia, further increasing concerns about the potential for impact of PPV in cherry crops. In addition, our knowledge of the diversity and distribution of PPV-C, the other cherry-infecting strain have been somewhat improved. It seems now possible to try to answer several questions that were outside of the scope of the SharCo project such as: i) what is the extent of the diversity of PPV on cherry host species, ii) what (novel) risk do cherry adapted PPV strains pose to European cherry crops.

➤ Increasing the number of strategies that interfere with PPV infection to promote a more durable resistance to sharka disease

When trying to manage PPV-related risks, the use of genetic resistance is, beyond doubt, the best solution for long term control because it provides effective protection all along the growing season. It allows the new plantations of stone fruit trees in regions where PPV is established. However, too few natural resistance genes have been found in different cultivated and wild species of Prunoideae: one single source in plum and apricot and none in peach. When challenging new genitors with a range of PPV isolates, representatives of the viral diversity, only two or three donors ('Harlayne', 'Lito' and 'SEO') remained durably resistant. Therefore, with a restricted number of sources of resistance, we cannot rule out that the existing mechanisms may be overcome in the future, by new PPV strains or variants. Pyramiding in a commercial cultivar several complementary resistance factors is an alternative that should result in a better durability of resistance to PPV. Such approach has been initiated in the frame of SharCo (DG1.5), in apricot. It is based on the identification of new mechanisms of resistance that interfere with viral infection, most of them being discovered in model plants (*Arabidopsis thaliana*, *Nicotiana benthamiana*) (DG2.4 and DG2.5). It thus requires transfer to *Prunus* crop species before pyramiding with previous

donors of resistance. To be sustainable for the European Fruit production, this should be performed in every major susceptible *Prunus* crop species (peach, apricot and plum). In SharCo, we initiated such approaches (DG1.5) but the unravelling of such new mechanisms of PPV restriction is just beginning and needs to be strongly supported in order to provide enough alternatives that could be easily transferred and combined in all *Prunus* crop species.

On the other hand, more efficient and durable protection will also be achieved by deploying resistant cultivars together with agro technical ‘packages’ associating diverse phytosanitary measures and cultural practices aimed at preventing, delaying or reducing virus spread in orchards and nurseries (see DA1.2). These ‘packages’ will not only enhance resistance efficiency at the crop level but may also contribute to reducing the risk of occurrence of virulent variants by decreasing the virus inoculum pressure on resistant plants. We thus recommend the future deployment of those PPV resistant commercial varieties only together with the application of the SharCo cultivation guidelines.

- Understanding the biological parameters of the three main PPV strains and linking them to differential spreading risks

Despite a large effort devoted in SharCo on this matter, our knowledge of the epidemic properties of the most common PPV strains (PPV-D, -M and -Rec) remains very partial. Aphid transmissibility and dynamics of multiplication of one isolate for each of the three PPV strains was evaluated in the most important *Prunus* crop species, using experimental conditions and by analysis of their respective prevalence in endemic regions. Of significant concern, we identified rather frequent cases of mixed infection, in particular in plums in some countries. This poses a clear risk of evolution of the pathogen diversity and thus properties through recombination. However, our preliminary analysis of data collected experimentally did not show clear-cut differences in PPV/host interactions between the three strains and the three *Prunus* species (peach, apricot and plum). No pertinent indicators directly linked to the biological cycle of the virus for a given strain in a given host was identified in our conditions, despite the fact that the behavior of the various strains may be markedly different under field conditions. In the prospect of sharka management, it remains crucial to characterize the biological parameters determining the epidemic properties of PPV strains, including transmissibility by aphids and pathogenicity on *Prunus* hosts. Indeed, once identified and validated, these indicators are expected to be used to predict the epidemicity of any PPV variant in a given agro-system, thus modulating the response of the National Plant Protection officers depending on the occurrence or not, of new strains or isolates with novel properties with the potential to further degrade the situation in any given area.

4. Potential impact and main dissemination activities and exploitation results

- From the characterisation of PPV diversity to the detection of unknown PPV variants and development of early warning tools

The objective of establishing a current view on the diversity of PPV has been largely achieved. The sequence data generated during the project has widely contributed to update the views of the international scientific community on PPV diversity. This sequence data and the associated biological and geographical data are made available to all parties interested through the web queriable SharCo database. Among other uses, this sequence information can further serve to improve the robustness and specificity of detection tests (designing new primers, etc.). Both PPV collections (centralized lyophilized collection and in vivo grafted collection) include reference isolates, but also variable or highly divergent isolates, thus encompassing the presently known PPV variability. Therefore, their services can be summarized as follows: i) provide well-characterized inoculum maintained in the *Prunus* hosts, which can be used in the evaluation of resistant germplasm and help to standardize the resistance test procedures, ii) provide well characterized or reference PPV isolates for development and validation of future robust, reliable and efficient detection tools, or specific typing tools targeting particular isolate(s) or PPV strain(s), iii) provide a reference baseline for future monitoring of changes in the pathogen distribution or for further studies of PPV population genetics, variability and evolution (e.g. using next generation sequencing tools), iv) provide well characterized or reference PPV isolates for validation efforts of protocols or detection techniques, including procedures of optimization and demonstration of performance characteristics and evaluation of sensitivity and specificity, v) preservation of the biodiversity of an important plant pathogen.

- From experimental characterisation of PPV variants epidemic properties to the understanding of agro-system-wide spreading factors

Simple and highly significant indicators of epidemicity of PPV strains on specific *Prunus* crop species were unfortunately not obtained from the experiments realized under confined conditions. Instead, some subtle differences were set out that constitute the starting point(s) to design better adapted tests. While disappointing, this result implies important consequences.

We clearly demonstrated that the three tested PPV strains are able to infect the three tested *Prunus* species, while real differences were observed in field conditions. These differences are thus not due to an intrinsic property of the virus to infect or not a given *Prunus* species. Indeed, it points out that field differences can be due to the impact of the physiological state of the trees in field conditions that give subtle but efficient advantages to a given strain and not to another one. The results stress also that none of the present tests are really appropriate at identifying strain specific resistances in *Prunus*; the classical test used in genetics is probably the best one as it covers several physiological conditions during three vegetation cycles.

The identification of mutations in the P1 and CP proteins of PPV that cause host-specific pathogenicity properties could facilitate the unravelling of host factors interacting with these proteins, which would be excellent targets for antiviral actions. The results point to the existence in *Nicotiana* species of an anti-PPV resistance mechanism targeting the N-terminal region of the CP protein, which potentially could be transferred to *Prunus* hosts once identified. The construction of libraries of PPV clones with random sequences will facilitate the design of experimental approaches for forced evolution allowing the identification of virus factors involved in PPV adaptation to particular hosts. As stressed in the previous paragraph, these approaches are meaningful when host preferences are clear-cut, that seems not to be the case except maybe for PPV-C. But they are very useful to find new ways of hampering host-virus interactions.

The survey of strain prevalence under different agronomical situations points to the risk of emergence of new recombinants through the role of *P. domestica* that seems to be a host suitable for all the strains and which is found with a significant percentage of mixed infection when the situation is favourable. This point is relevant when considering the preparation of cultivation guidelines and early warning systems: the monitoring of plum orchards to quickly detect abnormal situations is a priority.

The simulation model is close to a functional version and will quickly be used at exploring different control scenarios or at comparing different agrosystem situations. As a tool for PPV control strategies, it can be transferred, when validated, to official plant protection agencies. It can be used to test different strategies through the sensitivity to a range of possible human control actions (i.e., detection time, monitoring system, impact of quick removing, orchard structure, inter-orchard distances...), at time and spatial scales that are not accessible to practical experimentation. While useful to test different strategies, the outputs of the model are impacted by the precision of the main biological parameters it includes (i.e., range of

dissemination by aphids, latency period, rate of increase). These parameters are still imprecise. Several approaches were employed to improve the precision on these parameters: inference through regional data bases via the estimation from the regional estimation model; reconstruction of the chains of dissemination events on a defined 400 ha area; experimental orchards with a defined PPV strain as primary inoculum. All these approaches will continue to bring practical results in the next two years, particularly on distances of spread and thus possibility to recommend improved regulatory rules.

Many data are still currently under analysis. The first outcomes provided valuable information on PPV epidemiology in nurseries and orchards that were thus included in the cultivation guidelines (deliverable DA1.2) as well as in the risk management system (deliverable DA1.5). Other ones will be added if necessary as the analyses will progress.

- For the definition of guidelines in support of stone fruit tree propagation in nurseries

Following SharCo outcomes, significant reduction of the risks of entry and establishment of PPV in nursery blocks can be achieved by the use of proper and accurate protocols for sampling and testing nursery *Prunus* plants as described in the deliverables DE3.2 and 3.3. By following those procedures, identification as early as possible of contaminated plant material and/or latent PPV infection that is usually overseen during visual inspections is expected. Also it was demonstrated that natural infection of propagated plant material is significantly limited by the use of mineral oils at the peak period of aphid flights. It is thus also recommended to monitor aphid flights, in order to apply adequate means of management of PPV natural vectors.

To reduce the establishment and further spread of the virus in the nursery, we advise using rootstocks less susceptible to PPV infection as the ones described above and in the deliverable DE3.2. At least, nursery blocks of PPV susceptible stone fruit trees should alternate with non-host or less susceptible propagating material.

When applied all together, these measures of PPV management are expected to significantly reduce the occurrence and the incidence of PPV in nursery blocks as well as secure the trade of stone fruit, PPV-free, propagation material. In consequence, all these data served to elaborate recommendations for PPV management in nurseries; they are detailed in the DE3.3 deliverable.

- From conventional breeding to marker-assisted selection of PPV resistant material

The use of genetic resistance is, beyond doubt, the best solution for the control of virus-induced diseases because it provides effective protection throughout the growing season. It allows the new plantings of stone fruit trees in areas where PPV is present. However, breeding for resistance to PPV encounters the usual problems of breeding in perennial plants that include extended vegetative periods, high labor cost, large space needed for phenotyping and difficult and lengthy procedures of screening for PPV resistance. Standardization of the resistance tests has proved difficult because of delayed response to inoculation, variability of the virus, physiological state of the host plant and inoculation method. To test an interesting cultivar, one needs 3 years of monitoring after infection to assess the level of resistance or susceptibility. The breeding programs that are focused on the selection of cultivars with agronomic value, fruit quality, local adaptation and PPV resistance, become a long, difficult and expensive procedure. It is therefore of major importance to implement the selection of promising individuals by molecular assisted selection (MAS). Molecular markers linked to the trait have the potential to select the genes of interest (for instance PPV resistance genes) in vegetative tissues, giving information early in the plant development, at an early stage such as young seedlings. The screening of PPV resistant plant material through molecular markers, once they are identified, is a simple, fast and reliable procedure. In the Genetic pillar, markers linked to resistance to PPV in apricot were developed and new breeding programs have been implemented and supplemented with molecular markers. In consequence, selection of pre-breeding plant material takes now a few months instead of 3 to 4 years. This accelerates the introgression of PPV resistance into locally adapted apricot cultivars, in many agro-ecological environments.

All together these results are increasing the efficiency of the breeding programs, as initially planned, but also impact the deciphering of the inheritance and genetics of Sharka resistance, opening new opportunities for genetic strategies in Sharka containment.

➤ Towards the development of a durable resistance to PPV

Another of our goals, to increase the collection of potential sources of resistance to PPV, has been largely achieved. These new sources of resistance cover a broad range of mechanisms, largely differing in strength of protection and proximity to practical application.

The stimulation of antiviral RNA silencing by expression of ih (intron hairpin) viral constructs has been proven to provide efficient and durable protection to plum trees against the major PPV strains, and joins to the already approved in the USA Honey Sweet cultivar as an answer to the Sharka problem very close to practical application. Expression of amiRNAs

is another way to exploit RNA silencing to engineer antiviral resistance, which lacks the environmental risks associated with transgenic expression of long viral sequences, and is expected to have reduced off-target effects. Our results on the molecular analysis of amiRNA expression and activity could help us to design more efficient anti-PPV amiRNAs. Moreover, we have designed amiRNAs with efficient anti-PPV activity in *N. benthamiana*. If these results are reproduced in *Prunus*, we would have available another interesting source of resistance to confront Sharka disease.

An intense EcoTilling approach applied to a gene essential for PPV infection, the eIFiso4E protein, identified *Prunus* individuals in natural germplasm with promising PPV resistance that are already being tested in natural conditions, and could be introduced in breeding programs in the very near future. Ongoing approaches to silence by RNAi the eIFiso4E and different isoforms of the eIF4G factor, one of which is also essential for PPV infection, in *Prunus* cultivars are expected to yield PPV-resistant plants to be used when European concerns in transgenic plants decline.

Work in *A. thaliana* has allowed the identification of three additional sources of PPV resistance based on host proteins required for PPV infection. These proteins appear to be involved in different steps of the virus cycle, enhancing their utility in pyramiding strategies. If the role of these proteins is conserved in the *Prunus* hosts of PPV, they can be also the targets of EcoTilling approaches to look for functional alleles unable to support PPV infection, and of genetic engineering approaches to silence them in case they are not essential for the host, or to selectively disturb their virus-related activities. Another host target for anti-PPV action is the *Prunus* homolog of the *A. thaliana* SEC protein, which *O*-GlcNAcyates PPV CP. The results of this work package show that *O*-GlcNAcylation of PPV CP is not essential but has a fine tuning effect in PPV infection. It was recently demonstrated that quantitative (partial) resistance traits enhance the durability of protection provided by strong resistance genes. Thus, combining down regulation of SEC-like genes with other antiviral strategies could have a positive effect in protecting plants against Sharka disease. Similarly, the expression of PPV-specific recombinant antibodies, which in the laboratory only partially protects against PPV infection, could be very useful to protect plants against Sharka disease in natural conditions.

Our transcriptomics analysis of herbaceous and woody plants displaying an HR in response to PPV infection did not allow the definitive recognition of host factors involved in this defensive reaction. However, the identification of a set of genes whose expression was

altered by the HR reaction should help us to discover genes whose disturbance could potentiate HR-related anti-PPV defense.

- For an upgraded and harmonized management of PPV outbreak

The application of the recommendations and tools we proposed for Sharka containment is expected to significantly reduce the spread and impact of the disease at the European, national and regional level. They should help implement sustainable plant, fruit quality and yield with obvious positive effects on rural socio-economical issues, including job opportunities, maintenance of agricultural activities in many low to mid-income Southern European rural regions, in particular in marginalized lands.

Based on the results obtained in the scope of SharCo, we proposed more acceptable cultivation guidelines (DA1.2), an early warning system (DA1.4) and a risk management system (DA1.5) to European policy makers, national plant protection services and governments. We also formulated recommendations for nurseries gardeners and fruit producers of best-practices all along the fruit tree multiplication and production process. Therefore, stakeholders range from EU policy makers, plant protection services, extension personnel, nursery gardeners, fruit growers, and fruit industry representatives. Likewise, certification standards at the European level are needed to guarantee PPV-free production, transport, and sale of nursery stock and budwood. To facilitate knowledge and tool access to any stakeholder, a decision support system has been developed and several training workshops were organized, targeting information dissemination and transfer to European and PPV endemic regions. They took place successively in Poland, Bulgaria, Turkey, Romania and Czech Republic. A major Research workshop focusing on Sharka was organized also in Sofia (Bulgaria) and gathered over 80 international scientists and officers of the Plant Protection Services from most of the European member states.

5. Use and dissemination of foreground

Section A: Dissemination of foregrounds

For extra details, see deliverable DA2.4.

Original research papers published in peer-reviewed journals

1. Candresse T., Saenz P., Garcia J.A., Boscia D., Navratil M., Gorris M.T., Cambra M., 2011. Analysis of the epitope structure of *Plum pox virus* coat protein. *Phytopathology* 101: 611-619. (IF 2.799)

Abstract

Typing of the particular Plum pox virus (PPV) strain responsible in an outbreak has important practical implications and is frequently performed using strain-specific monoclonal antibodies (Mabs). Analysis in Western blots of the reactivity of 24 Mabs to a 112-amino-acid N-terminal fragment of the PPV coat protein (CP) expressed in *Escherichia coli* showed that 21 of the 24 Mabs recognized linear or denaturation-insensitive epitopes. A series of eight C-truncated CP fragments allowed the mapping of the epitopes recognized by the Mabs. In all, 14 of them reacted to the N-terminal hypervariable region, defining a minimum of six epitopes, while 7 reacted to the beginning of the core region, defining a minimum of three epitopes. Sequence comparisons allowed the more precise positioning of regions recognized by several Mabs, including those recognized by the 5B-IVIA universal MAb (amino acids 94 to 100) and by the 4DG5 and 4DG11 D serogroup-specific Mabs (amino acids 43 to 64). A similar approach coupled with infectious cDNA clone mutagenesis showed that a V74T mutation in the N-terminus of the CP abolished the binding of the M serogroup-specific AL MAb. Taken together, these results provide a detailed positioning of the epitopes recognized by the most widely used PPV detection and typing Mabs.

2. Calvo M., Dujovny G., Lucini C., Ortuño J., Alamillo J.M., Simón-Mateo C., López-Moya J.J., García J.A., 2010. Constraints to virus infection in *Nicotiana benthamiana* plants transformed with a potyvirus amplicon. *BMC Plant Biology* 10, art. no. 139. (IF 3.447)

Abstract

Background: Plant genomes have been transformed with full-length cDNA copies of viral genomes, giving rise to what has been called 'amplicon' systems, trying to combine the genetic stability of transgenic plants with the elevated replication rate of plant viruses. However, amplicons' performance has been very variable regardless of the virus on which they are based. This has boosted further interest in understanding the underlying mechanisms that cause this behavior differences, and in developing strategies to control amplicon expression. Results: *Nicotiana benthamiana* plants were transformed with an amplicon consisting of a full-length cDNA of the potyvirus Plum pox virus (PPV) genome modified to include a GFP reporter gene. Amplicon expression exhibited a great variability among different transgenic lines and even among different plants of the same line. Plants of the line 10.6 initially developed without signs of amplicon expression, but at different times some of them started to display sporadic infection foci in leaves approaching maturity. The infection progressed systemically, but at later times the infected plants recovered and returned to an amplicon-inactive state. The failure to detect virus-specific siRNAs in 10.6 plants before amplicon induction and after recovery suggested that a strong amplicon-specific RNA silencing is not established in these plants. However, the coexpression of extra viral silencing suppressors caused some amplicon activation, suggesting that a low level of RNA silencing could be contributing to maintain amplicon repression in the 10.6 plants. The resistance mechanisms that prevent amplicon-derived virus infection were also active against exogenous PPV introduced by mechanical inoculation or grafting, but did not affect other viruses. Amplicon-derived PPV was able to spread into wild type scions grafted in 10.6 rootstocks that did not display signs of amplicon expression, suggesting that resistance has little effect on virus movement. Conclusions: Our results suggest that amplicon-derived virus infection is limited in this particular transgenic line by a combination of factors, including the presumed low efficiency of the conversion from the transgene transcript to replicable viral RNA, and also by the activation of RNA silencing and other defensive responses of the plant, which are not completely neutralized by viral suppressors.

3. Capote N., Bertolini E., Olmos A., Vidal E., Martínez M.C., Cambra M., 2009. Direct sample preparation methods for the detection of *Plum pox virus* by real-time RT-PCR. *International*

Microbiology 12: 1-6.

Abstract

Direct systems to process plant materials allowed high-throughput testing of Plum pox virus (PPV) by real-time reverse transcription (RT)-PCR without nucleic acids purification. Crude plant extracts were diluted in buffer or spotted on membranes to be used as templates. Alternatively, immobilized PPV targets were amplified from fresh sections of plant tissues printed or squashed onto the same supports, without extract preparation. Spot real-time RT-PCR was validated as a PPV diagnostic method in samples collected during the dormancy period and showed high sensitivity (93.6%), specificity (98.0%), and post-test probability (97.9%) towards sharka disease. In an analysis of 2919 *Prunus* samples by spot real-time RT-PCR and DASi-ELISA 90.8% of the results coincided, demonstrating high agreement ($k = 0.77 \pm 0.01$) between the two techniques. These results validate the use of immobilized PPV targets and spot real-time RT-PCR as screening method for large-scale analyses.

4. Carbonell A., Dujovn G., Garcia J.A., Vall A., 2012. The *Cucumber vein yellowing virus* silencing suppressor P1b can functionally replace HCPro in *Plum pox virus* infection in a host-specific manner. *Molecular Plant-Microbe Interactions* 25: 151-164. (IF 4.431)

Abstract

Plant viruses of the genera *Potyvirus* and *Ipomovirus* (*Potyviridae* family) use unrelated RNA silencing suppressors (RSSs) to counteract antiviral RNA silencing responses. HCPro is the RSS of potyviruses, and its activity is enhanced by the upstream P1 protein. Distinctively, the ipomovirus *Cucumber vein yellowing virus* (CVYV) lacks HCPro, but contains two P1 copies in tandem (P1aP1b), the second of which functions as RSS. Using chimeras based on the potyvirus *Plum pox virus* (PPV) we found that P1b can functionally replace HCPro in potyviral infections of *Nicotiana* plants. Interestingly, P1a, the CVYV protein homologous to potyviral P1, disrupted the silencing suppression activity of P1b and reduced the infection efficiency of PPV in *N. benthamiana*. Testing the influence of RSSs in host specificity, we found that a P1b-expressing chimera infected poorly PPV's natural host *Prunus persica*. Conversely, P1b conferred PPV chimeras the ability to replicate locally in cucumber, CVYV's natural host. The deleterious effect of P1a on PPV infection is host-dependent, since the P1aP1b-expressing PPV chimera accumulated in cucumber to higher levels than PPV expressing P1b alone. These results demonstrate that a potyvirus can use different RSSs, and that particular RSSs and upstream P1-like proteins contribute to defining the virus host range.

5. Dallot S., Glasa M., Jevremovic D., Kamenova I., Paunovic S., Labonne G., 2011. Mediterranean and central-eastern European countries host viruses of two different clades of *Plum pox virus* strain M. *Archives of Virology*. 156 (3): 539-542. (IF 2.111)

Abstract

The genetic diversity of *Plum pox virus* strain M (PPV-M) was assessed by analyzing 28 isolates collected in 8 European countries. Two genomic fragments spanning the (Cter)P3-6K1-(Nter)CI coding region as well as the full coat protein coding region were sequenced directly from PCR products. Phylogenetic analysis showed that the geographical origin of the collected isolates was clearly associated with two different PPV-M clades. Moreover, the pattern of substitutions in the CP gene shed light on the evolutionary relationships between PPV-M and the recombinant strains PPV-Rec and PPV-T.

6. Dallot S., Decroocq V., Thébaud G., Candresse T., Borron S., Labonne G., 2012. Sharka, mieux comprendre pour mieux gérer en verger. *Phytoma* 654 (in press).

Abstract

Sharka disease is a detrimental viral disease of stone fruits (*Prunus*) present in most *Prunus*-growing nations. The disease causes yield losses and reduced fruit quality. The causal agent is a RNA virus (Plum pox virus, PPV) transmitted by vegetative propagation of infected plant material and by many aphid species. Insecticide treatments are not useful to limit the spread of the disease due to the non-persistent virus transmission mechanism (rapid acquisition-transmission process). This article presents the progress on sharka research (viral genetic diversity, diagnosis and strain typing tools, epidemiology, disease management and control) developed under the framework of the European research program SharCo. Advances have been made in many aspects and will help in the next future to optimize the strategies of survey and disease control.

7. Decroocq V., Salvador B., Sicard O., Glasa M., Cosson P., Svanella-Duma L., Revers F., Garcia J.A., Candresse T., 2009. The determinant of potyvirus ability to overcome the RTM resistance of *Arabidopsis thaliana* maps to the N-terminal region of the coat protein. *Molecular Plant-Microbe Interactions* 22 (10): 1302-1311. (IF 4.431)

Abstract

In *Arabidopsis thaliana* Columbia (Col-0) plants, the restriction of *Tobacco etch virus* (TEV) long-distance movement involves at least three dominant RTM (restricted TEV movement) genes named RTM1, RTM2, and RTM3. Previous work has established that, while the RTM-mediated resistance is also effective against other potyviruses, such as *Plum pox virus* (PPV) and *Lettuce mosaic virus* (LMV), some isolates of these viruses are able to overcome the RTM mechanism. In order to identify the viral determinant of this RTM-resistance breaking, the biological properties of recombinants between PPV-R, which systemically infects Col-0, and PPV-PSes, restricted by the RTM resistance, were evaluated. Recombinants that contain the PPVR coat protein (CP) sequence in an RTM-restricted background are able to systemically infect Col-0. The use of recombinants carrying chimeric CP genes indicated that one or more PPV resistance-breaking determinants map to the 5' half of the CP gene. In the case of LMV, sequencing of independent RTM-breaking variants recovered after serial passages of the LMV AF199 isolate on Col-0 plants revealed, in each case, amino acid changes in the CP N-terminal region, close to the DAG motif. Taken together, these findings demonstrate that the potyvirus CP N-terminal region determines the outcome of the interaction with the RTM-mediated resistance.

8. Gil M., Esteban O., García J.A., Peña L., Cambra M., 2011. Resistance to *Plum pox virus* in plants expressing cytosolic and nuclear single-chain antibodies against the viral RNA Nib replicase. *Plant Pathology* 60: 967-976. (IF 2.125)

Abstract

The expression of engineered single-chain variable fragments specific to the Nib RNA replicase of *Plum pox virus* (PPV) (scFv2A) in transgenic plants was successfully used as a strategy to interfere with viral infection. Different scFv2A fusion proteins were constructed to target those subcellular compartments, such as the cytosol, endoplasmic reticulum (ER) membrane structures and the nucleus, where Nib protein presumably accumulates. Several transgenic lines of *Nicotiana benthamiana* plants expressing the scFv2A targeted to the cytosol (2A lines), ER (6K2 lines) and nucleus (NLS lines) were obtained. The protective effect of scFv expression was determined by mechanical virus inoculation in five 2A, three 6K2 and four NLS transgenic lines. The strongest resistance was afforded with the 2A-3 (six non-infected plants out of 10), 6K2-1 (17 out of 33) and NLS-11 (16 out of 19) transgenic lines. The success of this interference with PPV infection opens new possibilities for the control of this RNA virus and could be exploited not only to confer resistance in transgenic plants, but also to elucidate the role of the non-structural Nib protein in different cell compartments during viral infection.

9. Glasa M., Malinowski T., Predajňa L., Pupola N., Dekena D., Michalczyk L., Candresse T., 2011. Sequence variability, recombinations analysis and specific detection of the W strain of *Plum pox virus*. *Phytopathology* 101: 980-985. (IF 2.799)

Abstract

Plum pox virus (PPV), a member of the genus *Potyvirus*, is the causal agent of Sharka, the most detrimental disease of stone-fruit trees worldwide. PPV isolates are grouped into seven distinct strains. The minor PPV-W strain was established recently for the divergent W3174 isolate found in Canada. Here, the partial or complete genomic sequences of four PPV-W isolates from Latvia have been determined. The completely sequenced isolates LV-141pl and LV-145bt share 93.1 and 92.1% nucleotide identity, respectively, with isolate W3174, with two regions of higher (>20%) divergence in the P1/HC-Pro and NIa (VPg) regions. Further analyses demonstrated that these two regions correspond to two independent recombination events in the W3174 genome, one involving PPV-M (approximate genome positions 692 to 1424) and the other PPV-D (nucleotides 5672 to 5789). The LV-141pl and LV-145bt isolates appear to be representatives of the “ancestral” PPV-W strain, not affected by recombination. The PPV-W intrastrain variability is substantially higher than that of all other PPV strains, with potential implications for the serological detection of PPV-W isolates. A PPV-W-specific primer pair has been developed, allowing the specific reverse-transcription polymerase chain reaction detection of all five presently available W isolates. The characterization of these new PPV-W isolates sheds light on PPV-W evolutionary history, further supports the hypothesis of its East-European origin, and opens the way for the biological and epidemiological characterization of this poorly known PPV strain.

10. Ion L., Hoza D., Moale C., Petrica A.M., Neagu T., Zagrai I., Isac M., Preda S., 2011. Determination of resistance to sharka (*Plum pox virus*) in Romanian apricot and rootstocks. *Acta Hort.* 899:117 -122.

Abstract

The “sharka” virus (*Plum pox virus* or PPV) is the most devastating viral disease affecting stone fruit crops in Europe. It causes important loss of fruit, mainly in apricot and European plum. The spreading of the PPV may be limited: by using the resistant cultivars to sharka and also by using the resistant rootstocks. In spring 2008, at USAMV Bucharest, a breeding program to develop cultivars and rootstocks resistances to PPV was initiated and a efficient procedure for the determination of sharka resistance in the progenies was needed. Like methodology used to germination and growing GF 305 is a IVIA Valencia protocol (Tarek et al. 2001). The rootstocks “GF

305” peach was used as indicator (susceptible) to PPV in comparison with the Mirobolan BN 4 Kr considered to be resistant to sharka (Zagrai et al. 2009). The subsequent grafting protocol was optimized, and a better source of inoculum was identified. The both species to rootstocks was grafted with apricot progenies in the same conditions and by chip-budding before artificial inoculation with PPV.

- 11. Ion L., Moale C., 2011. A new breeding program for resistance to PPV (*Plum Pox Virus*) in several Romanian apricot progenies. Scientific papers, Journal of RIFG, Pitesti, vol. XXVII, pp. 70-75.**

Abstract

Breeding for fruit resistant to pests and diseases has become a major objective for many research laboratories. Excessive use of pesticides is increasingly denounced by consumers and the rules controlling their use (particularly with respect to toxic residues) are increasingly restricting. The use of resistant cultivars reduces production costs and increases workers safety.

Prospecting through Romanian apricot collections has lead us to the discovery of several sources of resistance to sharka. The different Romanian hybrids or local apricot varieties was chosen among the different sources of resistance as it could also be used to develop a weeping variety in the some breeding program. To start with, the resistance mode of her ability was studied by creating F1 generations from the resistant parent crossed with a sensitive one, Mari de Cenad The resistance character is dominant and monogenic (symbols Rm1/m1). Breeding was the continued by creating the F2 generations using by resistance parent “ Stark Early Orange” and “ NJA 2” and after that to improve fruit quality as parent to introduce the organoleptic characters. The paper describes the breeding work involved in this program.

The resistance/susceptibility level of 213 descendants from three different crosses between the Romanian apricot cultivar ‘Mari de Cenad’ (susceptible to Plum pox virus, sharka), and the North American cultivar ‘Stark Early Orange’, and “NJA 2” (resistant) was evaluated during four cycles of study under controlled greenhouse conditions. Resistant: susceptible ratios were 91:16 in the case of the ‘Stark Early Orange’ open-pollination descendants, 52:26 in ‘Mari de Cenad’ – ‘Stark Early Orange’ descendants.

- 12. Ion L., Hoza D., Zagrai I., Moale C., Preda S., Stoian V., 2011. The behaviour of the Romanian plum genotypes to artificial infection with PPV (*Plum pox virus*). Scientific papers, Journal of R.I.F.G., Pitesti, Vol. XXVII, pp. 82-86.**

Abstract

Plum pox virus (PPV) is a potyvirus that causes sharka disease in infested stone fruit trees (*Prunus* species, peach, apricot, plum). It causes severe losses in productivity and fruit quality in European stone fruit orchards. As PPV is transmitted by aphids in a non-persistent manner, the use of pesticides to reduce PPV dissemination is ineffective (Atanassov, 1932). Therefore, breeding new plum cultivars resistant or tolerant to sharka disease is necessary for effective control of PPV in orchards and nurseries.

The majority of existed plum cultivars show different level of susceptibility to PPV. The Romanian cultivar ‘Andreea’ belongs to highly tolerant plum cultivars: only few symptoms are observed on leaves and virus particles are present in plant tissues in low concentration (Zagrai I. et al. 2005,). Also the study of local plum genotypes concerning the resistance to PPV, is an important precondition for improvement a new breeding program in plum.

- 13. Kamenova I., Milusheva S., Dragoyski K., Borissova A., Dallot, S., Mavrodieva V., Levy L., 2011. An overview of sharka research in Bulgaria. Acta Hort. 899: 19-27.**

Abstract

Since first being recorded in Bulgaria in 1917-1918 and described as a viral disease in 1932 (Atanasoff, 1932/1933) sharka (Plum pox) disease has progressively spread via infected plant material to be present nowadays all over the country. This overview is an attempt to synthesize almost 80 years of sharka disease investigations in Bulgaria in several aspects as: economical importance, hosts, diagnosis, identification and strain characterization of the pathogen, aphid vectors and the control measures applied. Overall, the history of PPV control in Bulgaria is one of unsuccessful eradication but successful spreading and contamination.

- 14. Kamenova, I., Dallot S., Bozkova V., Mikusheva S., 2011. First report of *Plum pox virus* recombinant strain on peach in Bulgaria. Plant Disease 95(10): 1320. (IF 2.449)**

Abstract

Plum pox virus (PPV) causes sharka, the most damaging viral disease of stone fruit species. Seven distinct PPV strains are known; PPV-M, PPV-D, and PPV-Rec are the most common (3). PPV-Rec is a unique recombinant (3) between PPV-M and PPV-D and has been reported from plum, apricot, Japanese plum, myrobalan, and blackthorn in eastern and central Europe, but has never been found in peach as a single natural infection (2). A

survey was conducted during spring 2009 in eight peach orchards located in the southwest, southeast, and south central regions of Bulgaria to assess the incidence of PPV infection. A total of 98 leaf samples from individual trees showing PPV-like symptoms were collected and analyzed by triple-antibody sandwich (TAS)-ELISA with the universal monoclonal antibody (MAb) 5B (AgriTest, Valenzano, Italy). Sixty one samples reacted positive for PPV (optical density 0.161 to 1.267) and these samples were further analyzed with PPV-M (AL) and PPV-D (4DG5) specific MAbs (1). All 61 samples reacted positively with PPV-M specific MAbs. To distinguish PPV-M and PPV-Rec strains, which are serologically identical, immunocapture (IC)-reverse transcription (RT)-PCR was carried out with PPV-M (CIP-M: 5'-GTC GCA GCA TTT GTA GCC CTT GTT-3', CIP-MR: 5'-CCA ACA CGT TAA CGC CAT GCT TCA-3') and PPV-D (CIP-D: 5'-ATG ATG CTG TTT GAC TCG GAG CGA-3', CIP-DR: 5'-TCG CAA CTG CTT GCA CAC ATT CTC-3') specific primers targeting the 6K1-CI genomic region. A PCR fragment of ~880 bp amplified with PPV-M specific primers obtained from 59 samples confirmed that these were PPV-M isolates. However, the remaining two samples (both coming from infected trees located in two different orchards in the southwest region) yielded a 468-bp PCR fragment with PPV-D specific primers, suggesting that these two samples belonged to PPV-Rec strain. These samples together with controls of PPV-M, PPV-D, and PPV-Rec strains were further analyzed by RTPCR using mD5/mM3 primers spanning the recombination breakpoint (4). Both peach samples and the PPV-Rec strain control produced a single 605-bp PCR product. The two peach amplicons were purified and sequenced directly with the same primers. The nucleotide (nt) sequences obtained were 100% identical to each other. BLAST analysis of the two samples with PPV-Rec (No. AF421118.1) showed maximum nt identity of 98%. Percent maximum nt identity with PPV-M (No. AY324837.1) and PPV-D (No. AB576062.1) were 93 and 87%, respectively. The deduced amino acid sequences of the two isolates were 98% identical to PPV-Rec (No. AF421118.1), 93% identical to PPV-M (No. M92280.1), and 84% identical to PPV-D (No. AB576062.1). Analyzed samples were further transmitted from the diseased trees to peach seedlings (GF 305) by chip-budding in a greenhouse during the fall of 2009. Six months later, faint vein clearing on the leaves of inoculated seedlings was observed. The presence of PPV was confirmed by TAS-ELISA and PPV-Rec presence was shown by IC-RT-PCR (mD5/mM3 primers). One of the generated 605-bp products was sequenced and showed 100% nt identity with the isolate used for inoculation. To our knowledge, this is the first identification of PPV-Rec strain in naturally infected peach trees, a finding that calls for further large-scale investigations of PPV-Rec incidence in peach in Bulgaria.

- 15. Krška B., Vachun Z., Nečas T., Ondrášek I., Salava J., Polák, J., 2010. Šlechtění meruněk na resistenci k PPV v ČR a v Evropě, Nové směry-molekulární markery (Breeding apricots for resistance to PPV in the Czech Republic and Europe, new trend-molecular markers). Proceedings of Conference: Šarka peckovin-současný stav problematiky v České republice a v Evropě, 28-29.06.2010 VÚRV, Praha. ISBN:978-80-7427-039-0.**

Abstract

The example of research apricot resistance to Plum pox virus (Plum pox virus - PPV), we will demonstrate the problems of stone fruit resistance to Plum pox virus. The most important growing areas of apricot production in the European countries around the Mediterranean Sea, Spain, France, Italy, Greece and Turkey, most of which belong to the Asian continent. Apricots are also important for the Czech Republic, fruit species, although the area is growing in comparison with those countries is much smaller. In the CR year 3000 až harvested 20,000 tons of apricots. Apricots are, in areas of intensive plantations, the third most important species. In 2007, CR 1526 in the thousands. ha apricots. In the present market value is expressed primarily apricot fruit quality and their specific habitat requirements, respectively. their adaptability to the environment, including the requirement for the main resistance to harmful agents. In the article were given the possibility of reducing of the PPV-resistant primarily through MAS breeding and selection.

- 16. Krška B., Vachun Z., Necas T., Ondrasek I., 2011. Resistance breeding of apricots at the Horticultural Faculty in Lednice. Acta Hort. 899: p.123-130.**

Abstract

During the breeding process of apricots in the Horticultural Faculty in Lednice we searched for donors against sharka disease (Plum pox virus -PPV). As donors of resistance to PPV for crossing" as the choice of cultivars as 'Stark Early', 'Orange' 'Orangered, Harlayne'. 'Henderson', 'Goldrich', 'Orangered'. 'Harlayne' 'Mari de Cenad' and 'Alfred'. From crossing of this combinations we obtained results indicating to a polygenic character, when in case of the SEO resistance donor the segregation ratio 3:1 was susceptible/resistant. Back crossing the progenies with this donor we obtained either the same ratio or 5:3. The progeny with immune cultivar 'Harlayne' had the ratio of 7: 1.

- 17. Maliogka V., Calvo M., Carbonell A.T., García J.A., Valli A., 2012. Heterologous RNA silencing suppressors from both plant- and animal-infecting viruses support *Plum pox virus* infection. Journal of General Virology (in press). (IF 3.363)**

Abstract

HCPPro, the RNA silencing suppressor (RSS) of viruses belonging to the Potyvirus genus in the Potyviridae family, is a multifunctional protein presumably involved in all essential steps of the viral infection cycle. Recent studies have shown that *Plum pox potyvirus* (PPV) HCPPro can be successfully replaced by *Cucumber vein yellowing ipomovirus* P1b, a sequence unrelated RSS from a virus of the same family. In order to gain insight into the requirement of a particular RSS to establish a successful potyviral infection, we tested the ability of different heterologous RSSs from both plant- and animal-infecting viruses to substitute HCPPro. Making use of engineered PPV chimeras, we show that PPV HCPPro can be functionally replaced by some, but not all, unrelated RSSs, including the NS1 protein of the mammalian-infecting Influenza A virus. Interestingly, the capacity of a particular RSS to replace HCPPro does not strictly correlate with its RNA silencing suppression strength. Altogether, our results suggest that not all suppression strategies are equally suitable for an efficient escape of PPV from the RNA silencing machinery. The approach followed here based on using PPV chimeras in which an under-consideration RSS substitutes for HCPPro could further help to study the function of diverse RSSs in a "highly-sensitive" RNA silencing context, such as that taking place in plant cells during the process of a viral infection.

- 18. Maliogka V.I., Salvador B., Carbonell A., Sáenz P., San León D., Oliveros J.C., Delgadillo M.O., García J.A., Simón-Mateo C., 2012. Virus variants with differences in the P1 protein coexist in a *Plum pox virus* population and display particular host-dependent pathogenicity features. Molecular Plant Pathology (in press) (IF 3.899)**

Abstract

Subisolates segregated from an M-type *Plum pox virus* (PPV) isolate, PPV-PS, differ widely in pathogenicity despite their high degree of sequence similarity. A single amino acid substitution, K109E, in the helper component proteinase (HCPPro) protein of PPV caused a significant enhancement of symptom severity in herbaceous hosts, and notably modified virus infectivity in peach seedlings. The presence of this substitution in certain subisolates that induced mild symptoms in herbaceous hosts and did not infect peach seedlings suggested the existence of uncharacterized attenuating factors in these subisolates. In this study, we show that two amino acid changes in the P1 protein are specifically associated with the mild pathogenicity exhibited by some PS subisolates. Site-directed mutagenesis studies demonstrated that both substitutions, W29R and V139E, but especially W29R, resulted in lower levels of virus accumulation and symptom severity in a woody host, *Prunus persica*. Furthermore, when W29R and V139E mutations were expressed concomitantly, PPV infectivity was completely abolished in this host. In contrast, the V139E substitution, but not W29R, was found to be responsible for symptom attenuation in herbaceous hosts. Deep sequencing analysis demonstrated that the W29R and V139E heterogeneities already existed in the original PPV-PS isolate before its segregation in different subisolates by local lesion cloning. These results highlight the potential complexity of potyviral populations and the relevance of the P1 protein of potyviruses in pathogenesis and viral adaptation to the host.

- 19. Marandel G., Pascal T., Candresse T., Decroocq V., 2009. Quantitative resistance to *Plum pox virus* in *Prunus davidiana* P1908 linked to components of the eukaryotic translation initiation complex. Plant Pathology 58: 425-435. (IF 2.125)**

Abstract

A complex, polygenic resistance to Plum pox virus (PPV) was previously described in a wild peach-related species, *Prunus davidiana* clone P1908. In the current study, an analysis of quantitative trait loci (QTL) was performed on an F2 population comprising 99 individuals obtained by selfing the F1 individual #40 of an interspecific cross between susceptible nectarine cv. Summergrand and the resistant *P. davidiana* clone P1908. Six QTL were identified using both parametric and non-parametric methods of detection, individually explaining 5–28% of the phenotypic variance. The total phenotypic variation explained ranged from 29 to 58%. Alignment of the genetic map of the F2 cross with the *P. davidiana* parent map showed consistency of QTL over generations, with three of the six QTL co-localizing at the 1- LOD interval and another one at the 2-LOD interval. Two of the QTL were mapped onto linkage group one, where resistance to PPV was previously mapped in apricot. Development and mapping of new microsatellite markers linked to candidate genes revealed a striking co-localization of three of the detected QTL with gene copies coding for eukaryotic translation initiation factors eIF4E and eIF(iso)4G. As co-localization of one QTL with candidate gene eIF(iso)4E was previously reported in the F1 population, the results reported here strongly reinforce the idea that components of the eukaryotic translation initiation complex are correlated with resistance to PPV in *P. davidiana* P1908.

20. Marandel G., Salava J., Abbott A., Candresse T., Decroocq V., 2009. Quantitative trait loci meta-analysis of *Plum pox virus* resistance in apricot (*Prunus armeniaca* L.): new insights on the organization and the identification of genomic resistance factors. *Molecular Plant Pathology* 10: 347-360. (IF 3.899)

Abstract

Plum pox virus (PPV) is responsible for sharka disease, one of the most detrimental stone fruit diseases affecting *Prunus* trees worldwide. Only a few apricot cultivars have been described as resistant, most originating from North American breeding programmes. Several PPV resistance quantitative trait loci (QTLs) have been mapped in various progenies, consistently highlighting the contribution to the resistance of the upper part of linkage group 1 (LG1). However, to date, no consensus has been reached on the precise number of QTLs linked to the resistance to PPV in apricot and *P. davidiana* or on their accurate position on the genetic linkage map. In the present study, the quantitative resistance of cultivar 'Harlayne' was analysed over five growth periods in a large F1 population. Four QTLs were identified, three mapping on LG1, explaining between 5% and 39% of the observed phenotypic variance. In an effort to further this analysis of PPV resistance in apricot, these results were merged in a single QTL meta-analysis with those of five other PPV resistance analyses available in the literature. Three consensus QTL regions were identified on LG1 and a putative fourth region on LG3. QTL meta-analysis also revealed the contribution of each resistant cultivar to meta QTLs, providing interesting comparative data on the resistance factors shared between the resistance sources used in the various studies. Finally, it was shown that one of the metaQTLs co-localizes with the eukaryotic translation initiation factor eIF4E, thus providing new hypotheses on the mechanisms of PPV resistance in apricot.

21. Martinez J., Ll cer G., Badenes M.L., 2010. Rafel and Xelva, two apricot varieties resistant to sharka. *HortScience* 45: 1904-1905. (IF 0.778)

Abstract

'Rafel' and 'Belgida' are mid- to early ripening apricot cultivars (*Prunus armeniaca* L.) with good yield, excellent fruit quality, self-compatibility, and resistance to Sharka, a disease caused by the Plum pox virus, a serious limiting factor for apricot production in Europe. Their fruits have excellent organoleptic characteristics and are larger than the traditional Valencian cultivars. 'Rafel' and 'Belgida' are very well adapted to the climatic conditions of the Valencia and Murcia areas.

22. Martinez J., Ll cer G., Badenes M.L., 2011. Moixent apricot cultivar resistant to sharka. *HortScience* 46: 655-656. (IF 0.778)

Abstract

'Moixent' is an early ripening apricot cultivar (*Prunus armeniaca* L.) with excellent fruit quality, self-compatible and resistant to sharka (plum pox virus), a serious limiting factor for apricot fruit production in affected areas. 'Moixent' fruits have excellent organoleptic characteristics, improving notable the early varieties available in the market. 'Moixent' has good adaptability to areas with warm winter and Mediterranean climate, showing a good fruit set and yield.

23. Nagyov  A., Kamencayov  M., Glasa M.,  ubr Z., 2012. The 3'-proximal part of the *Plum pox virus* P1 gene determinates the symptom expression in two herbaceous host plants. *Virus Genes* 44: 505-512. (IF 1.845)

Abstract

Three major strains of the *Plum pox virus* (PPV) are the most important in Europe: PPV-D, PPV-M, and PPV-Rec. By combining the genomes of two different strains of PPV (PPV-D with PPV-Rec; PPV-D with PPV-M), 20 inter-strain chimeric infectious clones (CICPPV) were constructed. Biological properties of CICPPV were tested by inoculating them on different herbaceous host species susceptible to PPV. Four of the seven species tested, exhibited visible symptoms. In *Nicotiana benthamiana* all CICPPV induced systemic mosaic and leaf malformation. *Pisum sativum* showed a broad range of symptom severity (systemic chlorotic and necrotic lesions) but neither qualitative nor quantitative aspects of symptomatology were related to a single PPV genome locus. *Nicotiana occidentalis* and *Nicandra physaloides* proved to be suitable for symptom-based differentiation. Depending on the virus strain/chimera, *N. occidentalis* showed two types of symptoms: mild systemic chlorotic spots or local necrotic lesions/systemic vein necroses. *N. physaloides* reacted to the PPV infection either symptomless or by local necrotic lesions. Our results demonstrated that the P1/HC-pro region of the PPV genome appears to be the determinant of the symptom manifestation in these host plants. In silico analysis mapped it to the 30-proximal part of the P1 gene.

24. Pagny G., Paulstephenraj P.S., Poque S., Sicard O., Cosson P., Eyquard J-P., Caballero M., Chague A., Gourdon G., Negrel L., Candresse T., Mariette S., Decroocq V., 2012. Family based linkage and association mapping reveals novel genes affecting *Plum Pox Virus* infection in *Arabidopsis thaliana*. *New Phytologist*, DOI 10.1111/j.1469-8137.2012.04289.x. (IF 6.645)

Abstract

Sharka is a devastating viral disease caused by the *Plum Pox Virus* (PPV) in stone fruit trees and few sources of resistance are known in its natural hosts. Since knowledge gained in *Arabidopsis* on plant virus susceptibility factors is likely to be transferable to crop species, *Arabidopsis* natural variation was searched for host factors essential for PPV infection. To locate regions of the genome associated with susceptibility to PPV, linkage analysis was performed on six biparental populations as well as on multiparental lines. To refine QTL mapping, a genome-wide association analysis was carried using 147 *Arabidopsis* accessions. Evidence was found for linkage on chromosome 1, 3 and 5 with restriction of PPV long distance movement. The most relevant signals occurred within a region at the bottom of chromosome 3, which comprises seven RTM3-like TRAF domain containing genes. Since the resistance mechanism analyzed here is recessive and the *rtm3* knock-out mutant is susceptible to PPV infection, it suggests that other gene(s) present in the small identified region encompassing RTM3 is necessary for PPV long distance movement. In consequence, we report here, for the first time to our knowledge, the occurrence of host factor(s) indispensable for virus long distance movement.

25. Pallas V., Garcia J.A., 2011. How do plant viruses induce disease? Interactions and interference with host components. *Journal of General Virology* 92: 2691-2705. (IF 3.363)

Abstract

Plant viruses are biotrophic pathogens that need living tissue for their multiplication and thus, in the infection-defence equilibrium, they do not normally cause plant death. In some instances virus infection may have no apparent pathological effect or may even provide a selective advantage to the host, but in many cases it causes the symptomatic phenotypes of disease. These pathological phenotypes are the result of interference and/or competition for a substantial amount of host resources, which can disrupt host physiology to cause disease. This interference/competition affects a number of genes, which seems to be greater the more severe the symptoms that they cause. Induced or repressed genes belong to a broad range of cellular processes, such as hormonal regulation, cell cycle control and endogenous transport of macromolecules, among others. In addition, recent evidence indicates the existence of interplay between plant development and antiviral defence processes, and that interference among the common points of their signaling pathways can trigger pathological manifestations. This review provides an update on the latest advances in understanding how viruses affect substantial cellular processes, and how plant antiviral defences contribute to pathological phenotypes.

26. Pilařová P., Marandel G., Decroocq V., Salava J., Krška B., Abbott A.G., 2010. Quantitative trait analysis of resistance to *Plum pox virus* in the apricot F1 progeny 'Harlayne' × 'Vestar'. *Tree Genetics & Genomes* 6: 467–475. (IF 2.335)

Abstract

Plum pox virus (PPV), the agent of the sharka disease, is the most devastating viral disease of fruit tree species in the subfamily Prunoideae. Thus, natural resistance to *Plum pox virus* is one of the most important traits of interest to stone fruit breeders. Few PPV resistant cultivars have been previously identified in apricots and of those which have, most of them have originated from North American breeding programs. In earlier studies, one single, major PPV resistance locus was mapped in different progenies derived mainly from the 'Goldrich' and 'Stark Early Orange' genitors. In the present study, we started with an F1 progeny issued from a cross between 'Harlayne', as a PPV resistant parent, and 'Vestar' as a susceptible parent. The hybrids were grafted simultaneously and subsequently inoculated with the PPV-M and D strains. The symptom scoring on leaves was performed nine times over two vegetative cycles. Marker – trait associations were analyzed using the Kruskal-Wallis (KW) non-parametric test and the PPV resistance loci were mapped using composite interval mapping (CIM). In the current paper, we show that both analyses (KW and CIM) highlighted the upper part of linkage group 1 of the apricot 'Harlayne' genitor.

27. Pilařová P., Krška B., 2009. Inheritance of resistance to *Plum Pox Virus* in the progeny of the apricot cv. 'Harlayne'. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* LVII (5): 243-249. ISSN 1211-8516.

Abstract

Natural resistance to *Plum pox virus* (PPV), the agent of sharka disease, is one of the most important traits of interest to stone fruit breeders, although few sources of resistance have been identified. One of the few apricot cultivars which does show resistance, 'Harlayne', was chosen for a study of the genetics of PPV resistance. It

was crossed with three different cultivars, two susceptible ('Vestar' and 'Strepel') and one immune ('Orangered'). Four different lines (since there was one reciprocal combination) were established and the F1 crosses were subsequently inoculated with the PPV-M and PPV-D strains by grafting infected buds. A woody indicator *Prunus persica* 'GF 305' was then also top-grafted onto the plants of three of these F1 populations. The observations of leaf symptoms and accompanying ELISA tests were performed over three, or in some cases five, growing seasons and then hybrids were classified accordingly, as either resistant or susceptible. The resistant : susceptible ratios were calculated and compared with expected theoretical ratios using the χ^2 -test. The ratios of resistant to susceptible plants in the progeny derived from the four apricot crosses are compatible with the hypothesis of three dominant genes being responsible for PPV resistance, with 'Harlayne' being heterozygous for all three genes. However, the possibility that resistance is controlled by just two dominant genes can not be ruled out just yet.

28. Predajňa L., Šubr Z., Candresse T., Glasa M., 2012. Evaluation of the genetic diversity of *Plum pox virus* in a single plum tree. *Virus Research* (in press). (IF 2.941)

Abstract

Genetic diversity of *Plum pox virus* (PPV) and its distribution within a single perennial woody host (plum, *Prunus domestica*) has been evaluated. A plum tree was triply infected by chip-budding with PPV-M, PPV-D and PPV-Rec isolates in 2003 and left to develop untreated under open field conditions. In September 2010 leaf and fruit samples were collected from different parts of the tree canopy. A 745-bp N1b-CP fragment of PPV genome, containing the hypervariable region encoding the CP N-terminal end was amplified by RT-PCR from each sample and directly sequenced to determine the dominant sequence. In parallel, the PCR products were cloned and a total of 105 individual clones were sequenced. Sequence analysis revealed that after 7 years of infection, only PPV-M was still detectable in the tree and that the two other isolates (PPV-Rec and PPV-D) had been displaced. Despite the fact that the analysis targeted a relatively short portion of the genome, a substantial amount of intra-isolate variability was observed for PPV-M. A total of 51 different haplotypes could be identified from the 105 individual sequences, two of which were largely dominant. However, no clear-cut structuration of the viral population by the tree architecture could be highlighted although the results obtained suggest the possibility of intra-leaf/fruit differentiation of the viral population. Comparison of the consensus sequence with the original source isolate showed no difference, suggesting within-plant stability of this original isolate under open field conditions.

29. Predajňa L., Nagyová A., Glasa M., Šubr Z., 2012. Cloning of the complete infectious cDNA of the *Plum pox virus* strain PPV-Rec. *Acta Virologica* 56 (2) (in press) (IF 0.682)

Abstract

Plum pox virus (PPV) is the causal agent of Sharka, considered to be the most detrimental viral disease of *Prunus* spp. worldwide. So far, several PPV strains have been recognized, three of them (PPV-D, PPV-M and PPV-Rec) having shown serious economic impact in the European area. Infectious cDNA clones of plant RNA viruses are excellent tools for functional studies of viral genomes. Preparation and use of PPV-D and PPV-M infectious clones have been previously reported. Here we describe the construction of an infectious cDNA clone of the strain PPV-Rec (isolate BOR-3) by the strategy involving the subsequent exchanges of homologous BOR-3 genome parts in the backbone of the previously prepared PPV-D infectious construct. The infectivity of each intermediate chimeric cDNA as well as that of the final construct (pIC-PPV-Rec) was confirmed by biolistic transfection of *Nicotiana benthamiana* plants. Complete sequence of the cloned viral BOR-3 cDNA revealed 0.14% of difference at the nucleotide level compared to original BOR-3 sequence, resulting in four amino acid changes. This slight inequality was related to the population heterogeneity of the initial BOR-3 isolate; no difference in the amino acid sequence resulted from the cloning steps performed.

30. Rubio M., Pascal T., Bachellez A., Lambert P., 2010. Quantitative trait loci analysis of *Plum pox virus* resistance in *Prunus davidiana* P1908: new insights on the organization of genomic resistance regions. *Tree Genetics & Genomes* 6: 291-304. (IF 2.335)

Abstract

No valuable source of resistance to Plum pox virus (PPV), the causative agent of sharka disease, has been found in peach (*Prunus persica*), but polygenic resistance to PPV was described in *Prunus davidiana* clone P1908. Two previous studies using F1 and F2 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). In an effort to verify the QTL stability in a large progeny and to search for possible interactions of the genetic backgrounds, the current study evaluated the incidence of PPV infection in an F1 population derived from the susceptible peach cv. Rubira and *P. davidiana* P1908 over three growth periods using an improved method of PPV phenotyping referred to as "heavy test." The phenotypic dataset was analyzed using

similar methods as the previous studies and a newly developed simple-sequence-repeat based *P. davidiana* map. Nine regions involved in differential symptom expression were identified among which six were common between studies. However, the level of resistance observed in the population was very low compared to the other studies, and the main QTL previously identified in linkage group 6 was not conserved, suggesting strong interaction of the genetic background of the susceptible parent with that of *P. davidiana* 1908. Consequently, this could be a limiting factor for developing resistant cultivars derived from *P. davidiana* P1908.

- 31. Scholthof K.B.G., Adkins S., Czosnek H., Palukaitis P., Jacquot E., Hohn T., Hohn B., Saunders K., Candresse T., Ahlquist P., Hemenway C., Foster G.D. 2011. Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology* 12: 938-954. (IF 3.899)**

Abstract

Many scientists, if not all, feel that their particular plant virus should appear in any list of the most important plant viruses. However, to our knowledge, no such list exists. The aim of this review was to survey all plant virologists with an association with *Molecular Plant Pathology* and ask them to nominate which plant viruses they would place in a 'Top 10' based on scientific/economic importance. The survey generated more than 250 votes from the international community, and allowed the generation of a Top 10 plant virus list for *Molecular Plant Pathology*. The Top 10 list includes, in rank order, (1) *Tobacco mosaic virus*, (2) *Tomato spotted wilt virus*, (3) *Tomato yellow leaf curl virus*, (4) *Cucumber mosaic virus*, (5) *Potato virus Y*, (6) *Cauliflower mosaic virus*, (7) *African cassava mosaic virus*, (8) *Plum pox virus*, (9) *Brome mosaic virus* and (10) *Potato virus X*, with honourable mentions for viruses just missing out on the Top 10, including *Citrus tristeza virus*, *Barley yellow dwarf virus*, *Potato leafroll virus* and *Tomato bushy stunt virus*. This review article presents a short review on each virus of the Top 10 list and its importance, with the intent of initiating discussion and debate amongst the plant virology community, as well as laying down a benchmark, as it will be interesting to see in future years how perceptions change and which viruses enter and leave the Top 10.

- 32. Simon-Mateo C., Garcia J.A., 2011. Antiviral strategies in plants based on RNA silencing. *Biochimica et Biophysica Acta* 1809: 722-731.**

Abstract

One of the challenges being faced in the twenty-first century is the biological control of plant viral infections. Among the different strategies to combat virus infections, those based on pathogen-derived resistance (PDR) are probably the most powerful approaches to confer virus resistance in plants. The application of the PDR concept not only revealed the existence of a previously unknown sequence-specific RNA-degradation mechanism in plants, but has also helped to design antiviral strategies to engineer viral resistant plants in the last 25 years. In this article, we review the different platforms related to RNA silencing that have been developed during this time to obtain plants resistant to viruses and illustrate examples of current applications of RNA silencing to protect crop plants against viral diseases of agronomic relevance. This article is part of a Special Issue entitled: MicroRNAs in viral gene regulation.

- 33. Soriano J.M., Domingo M., Zuriaga E., Romero C., Zebentayeva T., Abbott A., and Badenes M.L., 2011. Identification of SSR markers tightly associated to PPV resistance in apricot. *Molecular Breeding* (in press) DOI 10.1007/s11032-011-9685 (IF 2.852)**

Abstract

Sharka disease, caused by the *Plum Pox Virus* (PPV), is one of the major limiting factors for stone fruit production in Europe and America. Attempts to stop the disease through the eradication of the infected trees were unsuccessful. Thus, introgression of PPV resistance for crop improvement is the most important goal in the *Prunus* breeding programs. Due to time and labour consuming protocols, phenotyping for sharka still is the major bottleneck in the breeding pipeline. In this context, screening seedlings at early stages of development and marker assisted selection (MAS) provide the best solution for enhancing a breeding efficiency. In this study, we have generated 42 SSR markers from the Peach genome assembly v1.0 and an apricot BAC clone identified in the physical map of the PPV resistance locus previously defined in apricot. Using a linkage mapping approach, we have found SSR markers tightly linked to PPV resistance trait in all our progenies. Three SSR markers, PGS1.21 PGS1.23 and PGS1.24, showed allelic variants associated with the PPV resistance with no recombinants in the analyzed crosses. These markers unambiguously discriminated resistant from susceptible accessions in different genetic backgrounds. The results presented here are the first successful application for their use in MAS for resistance breeding in *Prunus* species.

- 34. Šubr Z., Kamencayová M., Nováková S., Nagyová A., Nosek J., Glasa M., 2010. A single amino acid mutation alters the capsid protein electrophoretic double-band phenotype of the *Plum pox virus* strain PPV-Rec. *Archives of Virology* 155: 1151-1155. (IF 2.111)**

Abstract

Plum pox virus (PPV) isolates differ by their capsid protein (CP) mobility in SDS-PAGE. These electrophoretic phenotypes are likely to result from posttranslational modifications of the CP. We demonstrated that the CP mobility was solely determined by the CP N-terminal region. Sequence comparison pinpointed a possible role of mutations at position 66 in determining the CP phenotype of PPV-Rec isolates. Site-directed mutagenesis of a chimeric clone demonstrated that Gly(66) in the CP resulted in the double-band phenotype, while Arg(66) led to a single-band CP pattern, possibly by preventing the phosphorylation of a nearby Ser residue by steric hindrance.

- 35. Ulubas Serce C., Candresse T., Svanella-Dumas L., Krisbai L., Gazel M., Caglayan K., 2009. Further characterization of a new recombinant group of *Plum pox virus* isolates, PPV-T, found in orchards in the Ankara province of Turkey. *Virus Research*: 142: 121-126. (IF 2.941)**

Abstract

Sixteen Plum pox virus (PPV) isolates collected in the Ankara region of Turkey were analyzed using available serological and molecular typing assays. Surprisingly, despite the fact that all isolates except one, which was a mix infection, were typed as belonging to the PPV-M strain in four independent molecular assays, nine of them (60%) reacted with both PPV-M specific and PPV-D specific monoclonal antibodies. Partial 5' and 3' genomic sequence analysis on four isolates demonstrated that irrespective of their reactivity towards the PPV-D specific monoclonal antibody, they were all closely related to a recombinant PPV isolate from Turkey, Ab-Tk. All three isolates for which the relevant genomic sequence was obtained showed the same recombination event as Ab-Tk in the HC-Pro gene, around position 1566 of the genome. Complete genomic sequencing of Ab-Tk did not provide evidence for additional recombination events in its evolutionary history. Taken together, these results indicate that a group of closely related PPV isolates characterized by a unique recombination in the HC-Pro gene is prevalent under field conditions in the Ankara region of Turkey. Similar to the situation with the PPV-Rec strain, we propose that these isolates represent a novel strain of PPV, for which the name PPV-T (Turkey) is proposed. Given that PPV-T isolates cannot be identified by currently available typing techniques, it is possible that their presence has been overlooked in other situations. Further efforts should allow a precise description of their prevalence and of their geographical distribution in Turkey and, possibly, in other countries.

- 36. Valli A., Oliveros J.C., Molnar A., Baulcombe D., García J.A., 2011. The specific binding to 21-nt double-stranded RNAs is crucial for the anti-silencing activity of *Cucumber vein yellowing virus* P1b and perturbs endogenous small RNA populations. *RNA* 17: 1148-1158. (IF 5.095)**

Abstract

RNA silencing mediated by siRNAs plays an important role as an anti-viral defense mechanism in plants and other eukaryotic organisms, which is usually counteracted by viral RNA silencing suppressors (RSSs). The ipomovirus *Cucumber vein yellowing virus* (CVYV) lacks the typical RSS of members of the family Potyviridae, HCPro, which is replaced by an unrelated RSS, P1b. CVYV P1b resembles potyviral HCPro in forming complexes with synthetic siRNAs in vitro. Electrophoretic mobility shift assays showed that P1b, like potyviral HCPro, interacts with double-stranded siRNAs, but is not able to bind single-stranded small RNAs or small DNAs. These assays also showed a preference of CVYV P1b for binding to 21-nt siRNAs, a feature also reported for HCPro. However, these two potyvirus RSSs differ in their requirements of 2-nucleotide (nt) 3' overhangs and 5' terminal phosphoryl groups for siRNA binding. Co-purification assays confirmed in vivo P1b-siRNA interactions. We have demonstrated by deep sequencing of small RNA populations interacting in vivo with CVYV P1b that the size preference of P1b for small RNAs of 21 nt also takes place in the plant, and that expression of this RSS causes drastic changes in the endogenous small RNA populations. In addition, a site-directed mutagenesis analysis strongly supported the assumption that P1b-siRNA binding is decisive for the anti-silencing activity of P1b and localized a basic domain involved in the siRNA-binding activity of this protein.

- 37. Vera E.M., Soriano J.M., Romero C., Zuriaga E., Terol J., Zebentayeva T., Llácer G., Abbott A., Badenes M.L., 2011. Narrowing down the apricot *Plum pox virus* resistance locus and comparative analysis with the peach genome syntenic region. *Molecular Plant Pathology* DOI: 10.1111/J.1364-3703.2010.00691. (IF 3.899)**

Abstract

Sharka disease, caused by the *Plum pox virus* (PPV), is one of the main limiting factors for stone fruit crops worldwide. Only a few resistance sources have been found in apricot (*Prunus armeniaca* L.), and most studies have located a major PPV resistance locus (*PPVres*) on linkage group 1 (LG1). However, the mapping accuracy was not sufficiently reliable and *PPVres* was predicted within a low confidence interval. In this study, we have constructed two high-density simple sequence repeat (SSR) improved maps with 0.70 and 0.68 markers/cm, corresponding to LG1 of 'Lito' and 'Goldrich' PPV-resistant cultivars, respectively. Using these maps, and excluding genotype-phenotype incongruent individuals, a new binary trait locus (BTL) analysis for PPV

resistance was performed, narrowing down the *PPVres* support intervals to 7.3 and 5.9 cm in 'Lito' and 'Goldrich', respectively. Subsequently, 71 overlapping oligonucleotides (overgo) probes were hybridized against an apricot bacterial artificial chromosome (BAC) library, identifying 870 single BACs from which 340 were anchored onto a map region of approximately 30–40 cm encompassing *PPVres*. Partial BAC contigs assigned to the two allelic haplotypes (resistant/susceptible) of the *PPVres* locus were built by high information content fingerprinting (HICF). In addition, a total of 300 BAC-derived sequences were obtained, and 257 showed significant homology with the peach genome scaffold_1 corresponding to LG1. According to the peach syntenic genome sequence, *PPVres* was predicted within a region of 2.16 Mb in which a few candidate resistance genes were identified.

- 38. Vidal E., Moreno A., Bertolini E., Cambra M., 2012. Estimation of the accuracy of two diagnostic methods for the detection of Plum pox virus in nursery blocks by latent class models. Plant Pathology 61: 413–422. (IF 2.125)**

Abstract:

The control of Plum pox virus (PPV), the most important viral disease that affects stone fruit trees, requires the use of reliable detection methods. The effectiveness of spot real-time reverse transcriptase polymerase chain reaction (RT-PCR) for the detection of PPV in samples collected from nursery blocks was compared with a validated PPV detection technique, the double antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA) using the PPV-specific monoclonal antibody 5B-IVIA/AMR. In total, 5047 nursery plants were analysed by both techniques. The agreement between the techniques was almost perfect (Cohen's kappa index of 0.88 ± 0.01). The diagnostic parameters (sensitivity, specificity and likelihood ratios) of both techniques were simultaneously evaluated in 2473 nursery plants by latent class models using maximum likelihood functions and a Bayesian approach. The sensitivity and specificity of both techniques did not vary according to the latent model applied. Spot real-time RT-PCR was more sensitive while DASI-ELISA was more specific for PPV detection. In addition, the findings demonstrate that latent class models are a flexible and potent statistical method to estimate the accuracy of diagnostic tests for plant pathology.

- 39. Vidal E., Zagrai L., Milusheva S., Bozhkova V., Tasheva-Terzieva E., Kamenova I., Zagrai I., Cambra M. 2012. Control of Plum pox virus in nursery blocks using horticultural mineral oils treatments in different ecological scenarios. Submitted to Annals of Applied Biology. (IF 2.179)**

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Abstract

Plum pox or Sharka, caused by *Plum pox virus* (PPV) is considered as the most destructive disease of plum. Although PPV is widespread in all plum growing areas from Romania and causes serious yield losses, little is known about the variability of its isolates at country level. For this reason, a large-scale study was performed with the aim to get a picture of the prevalence and distribution of PPV strains in plum. During three years surveys, 200 PPV isolates collected from 23 different plum orchards from Transylvania, Moldavia and Muntenia areas were investigated. DAS-ELISA and IC/-RT-PCR were used for PPV detection. PPV strains were serologically determined by TAS-ELISA using PPV-D and PPV-M specific monoclonal antibodies. Molecular strain typing was done by RT-PCR targeting three genomic regions corresponding to (Cter)CP, (Cter)NIb/(Nter)CP and CI. RFLP analysis was used to distinguish D and M strains, based on *RsaI* polymorphism located in (Cter)CP. All PCR products targeting (Cter)CP and 13 PCR products spanning the (Cter)NIb/(Nter)CP were sequenced. The typing of PPV isolates revealed that PPV-D is the prevalent strain in all the three areas. The higher incidence of PPV-D was noticed in Moldavia (84%) and the higher rate of PPV-Rec was recorded in Transylvania (18%). The mixed infections (D+Rec) was more frequent in Muntenia (24 %). Overall results provided that in Romania the predominant strain is PPV-D (73%), follow with a much lower frequency by PPV-Rec (14%). Mixed infections (PPV-D+PPV-Rec), which might generate additional variation by recombination, are also frequent (13%).

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Original specifications and actual achievements of the dissemination plan

The results of the research conducted within SharCo project were published in peer-reviewed research journals and presented at scientific conferences and symposia. Publication started as early as in the second year of the project and remained very active throughout the subsequent years with the peak of conference reports in 2010 and of original research papers in 2011 (Figure 16).

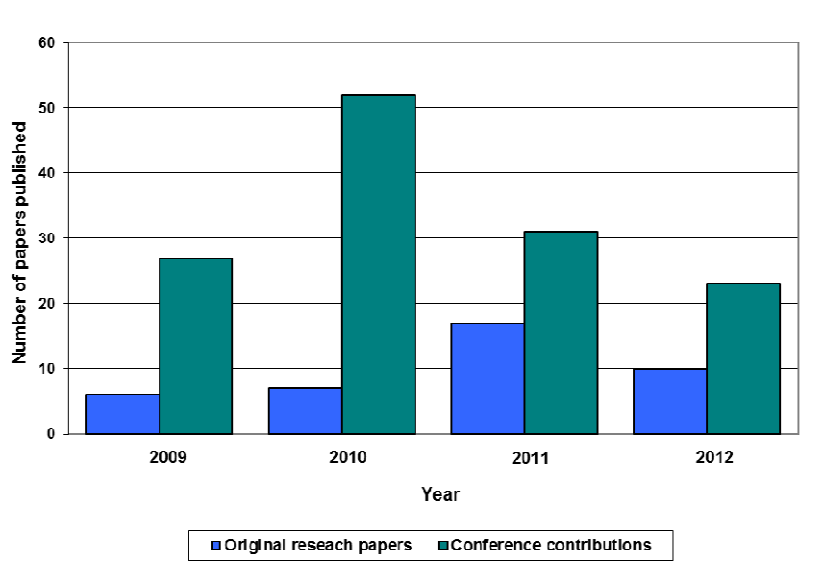


Figure 16. Original research papers and conference reports published during 4 years of the project duration.

Of 40 original research papers, 29 (72.5%) were published in journals listed in Journal Citation Report database of the Thompson Reuters. The most frequently the results of the SharCo research were published in prestigious journals with Impact Factor ranging between 2 and 3, but some were also published in journals with IF exceeding 5 (Figure 17 below).

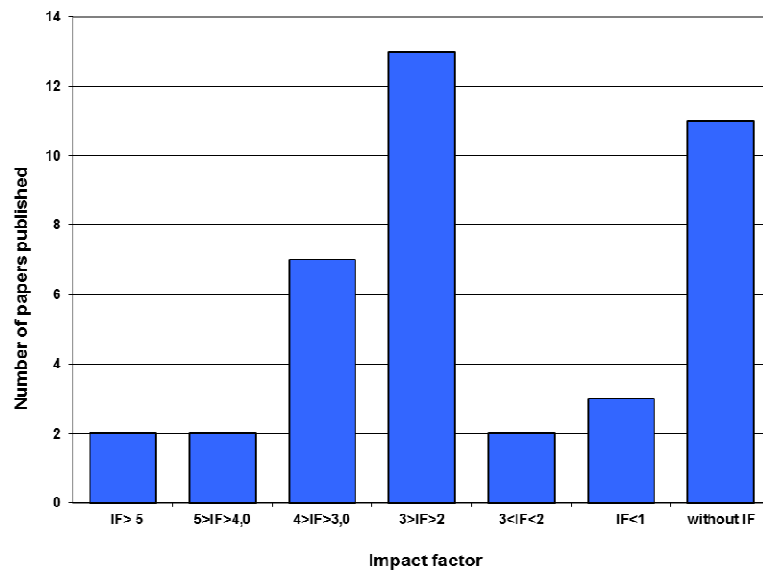


Figure 17. Original research papers published in peer-reviewed journals as related to impact factor.

Section B: Use of Foregrounds

PATENTS and TRADEMARKS, REGISTERED DESIGNS

Table 1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter. The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified.

TABLE B1 : LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.			
Type of IP Rights: Patents, Trademarks, Registered designs, Utility models, etc.	Application reference(s) (e.g.EP123456)	Subject or title of application	Applicant (s) (as on the application)
Patent	FR 11 56258 (submitted 11/07/2011) Extension to EU under consideration	Procédé d'amélioration de la résistance des plantes aux virus	CHEVALIER Christian, DECROOCQ Véronique, DELMAS Frédéric, HERNOULD Michel
IPPC-FAO Registered standard protocols for PPV detection	ISPM 27 Annex 02 * DP 2:2012	Diagnostic protocols for regulated pests: Adopted diagnostic protocol for PPV detection	Cambra M., Levy L., Candresse T., Palkovics L., Glasa M., Boscia D., James D., Gentit P.

*See links: https://www.ippc.int/file_uploaded/1346166095_ISPM_27_2006_En_2012-08-28.pdf

Annex 2: https://www.ippc.int/file_uploaded/1336641118_DP_02_2012_En_2012-05-08.pdf

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FOREGROUNDS

		OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND					
Workpackage	ID	Exploitable Foreground (description)	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable, commercial use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
Epidemiology WPE1	1	PPV web-queryable database	Database of partial and complete genome sequences of PPV isolates	Scientific community	Within the next year, following public release of the publication	none	P1 (INRA) and SharCo consortium
Epidemiology WPE1	2	Oligo chips	Mini oligo arrays for rapid PPV typing	Scientific community National plant protection services	Within the next year, following public release of the publication	none	P6 (IVIA) P1 (INRA)
Epidemiology WPE1	3	Sequence of primer pairs for PPV-W isolates	Primer pair W8328F/W8711R for specific RT-PCR detection of PPV-W strain	Scientific community National plant protection services	immediate	none	P11 (SAVBA) P1 (INRA)
Epidemiology WPE1	4	Sequence of primer pairs for routine amplification of PPV isolates	Primer pair NCuniFor/NCuniRev for routine and broad RT-PCR detection of PPV (all strains, including CR)	Scientific community National plant protection services	immediate	none	P11 (SAVBA)
Epidemiology WPE1	5	Lyophilised collection of characterized PPV isolates	Partially sequenced and strain-characterised PPV isolates	Scientific community	Within the next year, following public release of the publication	none	P6 (IVIA) and SharCo consortium
Epidemiology WPE1	6	In vivo collection of characterized PPV isolates	In vivo maintained full-genome characterized PPV isolates	Scientific community Breeding programs	Within the next year, following public release of the publication	none	P5 (CNR IVV), P1 (INRA) and SharCo consortium
Epidemiology WPE2	7	Protocol for simultaneous extraction of RNA and DNA of Prunus species	Method of PPV quantification in Prunus	Scientific community	Within the next year, following public release of the publication	none	P1 (INRA)
Epidemiology WPE2	8	Model of PPV spread	Simulation model at a regional level	Policy makers National plant protection services	Within the next two years, following public release of the model	none	P1 (INRA)
Epidemiology WPE2	9	Infectious cDNA clones of a PPV isolate of the C strain	Study of PPV pathogenicity determinants, and forward and reverse genetics in cherry using virus-	Scientific community Biotech companies	From immediate to 2 years	none	Partner 4 (CSIC)

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			induced gene silencing				
Epidemiology WPE2	10	Infectious cDNA clone of a PPV isolate of the Rec strain	Study of PPV pathogenicity determinants	Scientific community, Biotech companies	From immediate to 2 years	none	Partner 11 (SavBa)
Epidemiology WPE3	11	Ranking of Prunus rootstocks for their resistance to natural PPV infection	List of rootstocks for decision makers.	Prunus industry in general and nurserimen.	Within the next year, following public release of the publication	None	Partners: 02 (ABI), 06 ((IVIA), 07 (ISK), 08 (MKU), 09 (CRI), 10 (TUM), 14 (FGI), and 17 (SCPD).
Epidemiology WPE3	12	Protocols for accurate sampling and analysis of nursery and adult mother plants	Specific protocols , methodologies and recommendations.	Plant Protection Services, Plant Health Organizations, (OEPP/EPPO, IPPC-FAO), policy makers, Prunus industry in general and nurseryment.	Immediate	None	Partner 06 (IVIA)
Epidemiology WPE3	13	Horticultural mineral oils (HMO) treatments in nursery blockand young plantations	Methodology for HMO treatments, optimal period and PPV-viruliferous aphid species monitoring	Prunus industry in general and nurserimen.	From immediate, following public release of the data.	None	Partners: P2 (ABI), 06 ((IVIA), 08 (MKU), 14 (FGI), and 17 (SCPD)
Genetic WPG1	14	Molecular markers linked to resistance to PPV in apricot	Markers indispensable for the implementation of efficient Marker Assisted Selection	Breeding programs	Within the next two years, following public release of the data	none	P1 (INRA), P6 (IVIA)
Genetic WPG1	15	Molecular markers linked to resistance to PPV in peach	Markers indispensable for the implementation of efficient Marker Assisted Selection	Breeding programs	Within the next two years, following public release of the data	none	P1 (INRA)
Genetic WPG1	16	Apricot full genome sequences	Development of new markers more closely linked to the resistance to PPV	Breeding programs, Scientific community	Within the next two years, or upon public release of the data	none	P1 (INRA and ANR) UMR BFP and UGAFL Clemson University and USDA for part of the sequences (background) P15 (UMIL) P09 (CRI)
Genetic WPG1	17	Apricot progenies segregating for PPV resistance for a total of 8,904 individuals under screening	Apricot cultivars resistant to PPV	Breeders, Fruit growers, Nurseries,	From five to ten years (depending on the selection strategy), given the timeframe required for the propagation and field evaluation of the interesting selections in several apricot-growing locations	PVR (Protected Varietie Registration) application could be issued at the time the interesting selections will be introduced as new cultivars	Partners P1 (INRA),3 (USAMV),6 (IVIA),8 (MKU),14 (FGI),15 (UMIL),16 (MUAF) in cooperation with other parties interested in the commercial development of new cultivars resistant to PPV

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Genetic WPG1	18	One to two hybrids from crossing Harlayne x Marlen that show high level of resistance.	New PPV resistant apricot varieties	Fruit growers	5-7 years after pomological evaluation	PVR application for new cultivar release	Partner P16 (MUAF)
Genetic WPG1	19	Implementation of MAS selection in National breeding programs targeting PPV resistance	Apricot breeding programs	Breeders	Immediate	no	Partners P1 (INRA),3 (USAMV),6 (IVIA),8 (MKU),14 (FGI),15 (UMIL),16 (MUAF)
Genetic WPG1	20	Partial characterization of the apricot germplasm for its susceptibility to PPV	Characterization of genitors to be used in breeding program	Breeding programs	Immediate	none	Partners P1 (INRA), 3 (USAMV), 6 (IVIA)
Genetic WPG1	21	Validation of a robust methodology for assessing Prunus susceptibility to PPV	Robust testing procedure	Breeding programs	Immediate	none	P1 (INRA) for testing in confined conditions, P14 (FGI) for testing under natural infection
Genetic WPG1	22	New apricot varieties resistant to PPV	Registered varieties	Fruit production (nurseries and growers)	The time established for registration of varieties (5 years registration number and immediately in the temporary registration number)	Registration on the CPPVO	Partner P6 (IVIA), P1 (INRA), P15 (UMIL), P16 (MUAF)
Genetic WPG1	23	Sequence and procedure for tracking the genes involved in natural resistance to PPV in apricot	Identification of gene(s) involved in natural resistance to PPV in apricot	Breeding institutes involved in apricot breeding, Biotech companies	2 years ahead of a patent application	Under discussion for application of a patent	Partner P6 (IVIA)
Genetic WPG1	24	Locally-adapted, PPV resistant varieties in apricot	Traian,Auras,Ceres,Euxin, Tudor,Augustin	Nurseries, Producers	Already registered. If not, 5 to 10 years	none	Partner P3 (USAMV)
Genetic WPG1	25	Plum hybrids issued from a cross between the hypersensitive cultivar 'Jojo' and the fruit varieties 'Prune d'Ente' or 'Reine Claude dorée'	PPV resistant plum varieties for Prune or fruit (Quetsche, Greengage) production	Breeding programmes, Fruit producers	From 5 to 20 years	Eventually, application for PVR (cultivar registration and protection)	P1 (INRA), P10 (TUM), P14 (FGI)
Genetic WPG1	26	Locally-adapted, PPV resistant genotypes in plum	Mirobolan 4Kr, Otesani 11, Pixy, Scoldus, Calugaresti, Miroval, Rival and the varieties Andreea and Loacal de Dragasani	Nurseries, Producers	Immediate if registered	none	Partner P3 (USAMV)
Genetic WPG1	27	Molecular markers linked to hypersensitive reaction to PPV in European	Markers will be used for early selection in plum breeding programs	Breeding programs	The markers will be used within 2 years, following public release	none	P7 (IO) P10 (TUM)

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		Plums					
Genetic WPG1	28	Plum (<i>Prunus domestica</i>) cultivar 'Vision' evaluated for very low susceptibility to PPV	Recommendation for planting in commercial orchards and as a source of resistance in plum breeding programmes	Fruit production, breeding programmes	Within 4 years, upon evaluation of agricultural and quality properties, this cultivar will be included on the list of recommended cultivars	none	Partner P7 (IO)
Genetic WPG1	29	use of <i>Prunus</i> rootstocks with hypersensitivity resistance to PPV	rootstock cultivars	nurseries, fruit growers	vegetative propagation of 'Docera 6' rootstock in vitro (in 2012 and the following years), grafting of rootstocks in commercial nurseries in 2013	'Docera 6' rootstock : application for PVR in 2013 (or 2014 latest, depending on when first trees can be sold to fruit growers)	Partner P10 (TUM)
Genetic WPG2	30	List of <i>Prunus</i> trees bearing mutations in genes essential for viral infection	Pyramiding with existing sources of resistance New sources of resistance to be used as donors of resistance in breeding programmes	Breeding programs, Biotech companies	From 2 to 10 years or upon public release of the data	none	Partner P1 (INRA)
Genetic WPG2	31	Gene constructs targeting silencing of host factors indispensable for viral infection in model and crop species	Gene constructs to be introduced into PPV host plants	Biotech companies for plant transformation Breeder for assessment of transgenic lines	Immediate to 10 years, depending on the availability of the plant transformation technology	none	P1 (INRA) INIA Santiago de Chile (Chile) USDA Kearneysville (USA)
Genetic WPG2	32	Host factors involved in susceptibility and resistance to PPV	Development of resistant new cultivars	Breeding programs, Biotech companies	From 2 to 10 years or upon public release of the data.	Yes, in the case of the CSN5 gene	P1 (INRA) and University of Bordeaux
Genetic WPG2	33	Genes whose expression was altered by HR to PPV infection	Markers associated to hypersensitive resistance to PPV; study of HR-mediated resistance to PPV	Scientific community, Biotech companies	From immediate to 10 years, conditioned by release of the data through publication(s)	none	Partner 4 (CSIC) Partner 10 (TUM)
Genetic WPG2	34	Infectious cDNA clones of a PPV mutant with non- <i>O</i> -GlcNAcylated CP	Study of post-translational modifications of PPV CP	Scientific community	Immediate	none	Partner 4 (CSIC)
Genetic WPG2	35	Transgenic <i>Nicotiana benthamiana</i> lines with the SEC gene downregulated by RNAi	Studies on the functional relevance of <i>O</i> -GlcNAcylation of virus and plant proteins	Scientific community	Immediate	none	Partner 4 (CSIC)
Genetic WPG2	36	Gene constructs expressing PPV-specific recombinant antibodies	Development of resistant new cultivars	Breeding programs, Biotech companies	From 2 to 10 years	none	Partner 6 (IVIA), Partner 4 (CSIC)

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Genetic WPG2	37	amiRNA constructs able to provide anti-PPV resistance	Development of resistant new cultivars for fruit production (plum, peach)	Breeding programs, Biotech companies	From two to ten years, depending of the availability and feasibility of gene transfert in fruit trees	none	Partner 4 (CSIC), INIA, Santiago de Chile
Genetic WPG2	38	New virus gene constructs aiming at the control of PPV	New resistant Plum cultivars (<i>Prunus domestica</i>)	Breeding programs, Stone-fruit industry	From 4 years and according to the public perception	Yes, following to the expansion of HoneySweet plum.	P1 (INRA) USDA Kearneysville, USA
Application WPA1	39	Cultivation guidelines for mother plant blocks, nurseries and orchards	Cultivation guidelines aimed at restricting the virus spread in Europe	Policy makers, National plant protection services, Nurseries, Growers	Immediate (30.04.2011)	none	SharCo consortium for public use
Application WPA1	40	Decision Support System for Integrated Sharka Management	Online Decision Support System for the containment of Sharka in mother plant blocks, nurseries and orchards	Nurseries, growers, Plant Protection Services	Immediate (31.08.2012)	none	Partner P7 (IO) and whole SharCo consortium for public use
Application WPA1	41	SharCo risk management system	Guidelines for the establishment of a PPV risk management system	Policy makers, National plant protection services	Immediate (31.08.2012)	none	SharCo consortium for public use
Application WPA1	42	Early Warning System	Early Warning System aimed at harmonizing and optimizing the PPV surveys	Policy makers, National Plant Protection Services, Nurseries and growers	Immediate (31.08.2012)	none	SharCo consortium for public use