

# **SharCo**

## **Containment of Sharka virus in view of EU-expansion**

Small Collaborative project of the 7<sup>th</sup> Framework Programme

Theme 2

Food, Agriculture, Biotechnologies

### **DE.1.5**

**Centralized collection in a lyophilized form of all (700-1000) isolates analyzed**

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**Deliverable report structure**

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# 1. General Presentation

## 1.1. Context

Plum pox virus (PPV), a member of the genus Potyvirus, is the causal agent of Sharka, considered as the most detrimental viral disease of *Prunus* spp. worldwide, including economically important stone fruit crops (peach, apricot, plum, Japanese plum). Viral particles contain a single stranded genomic RNA of positive polarity (9741-9795 nucleotides in length) encapsidated by a single type of capsid protein subunit.

On the basis of serological and molecular differences, seven PPV strains are now recognized. From those, PPV-M, D and Rec are the most widespread while the four additional minor strains (EA, C, T, W) have a much more limited prevalence and/or geographical distribution.

Genetic variability is an essential feature of RNA viruses, which are characterized by high mutation rates resulting from the lack of proofreading activity of their RNA-dependent RNA polymerases. In addition, RNA recombination has been shown to represent another evolutionary mechanism shaping the populations of RNA viruses and contributing to their diversity. Both processes can lead to the emergence of new viral strains and variants better adapted to the ever-changing environmental conditions or with an increased fitness in their natural hosts.

The understanding of the genetic variability of PPV populations is an important prerequisite for an efficient diagnosis, management and long term control of the Sharka disease. The use of rapid and robust diagnostic tools is essential to control or prevent the Plum pox virus spread. Generally, the development of accurate, specific and sensitive diagnostic methods requires knowledge of the diversity of target pathogen and availability of viral isolates for optimization/validation of detection tools.

One of the main aspects of the Work package E1 of the SharCo project was to establish a database containing sequences of a large number of PPV isolates representative of European and non-European countries (TE1.2, TE1.3). To optimize the use of those genomic data, the database has been linked to a long-term centralized reference collection of the above-mentioned PPV isolates, either lyophilized or in live (TE1.4).

## 1.2. Rationale

The research and practical work in the field of plant virology often require the availability of a set of reference viruses, strains or isolates. A virus collection is commonly preserved to capture the variability of a species, in terms of pathogenicity, virulence and strain affiliation. Despite this fact, long-term preservation of the diversity of phytopathogenic viruses is an area generally overlooked. In the case of PPV, collections have often been generated and maintained more or less efficiently on the long term by individual researchers. The problem of such collection is their limited range of survey (mainly locally or regionally oriented), their generally poor accessibility over time (conservation method, removal of the collection when the researcher changes of field of interest or retires, insufficient quantities stored preventing distribution...), the unavailability of standardized epidemiological or sequence data related to the preserved isolates.

The greater the number of isolates maintained in a collection, that are known to be different, the greater the chance of developing and validating a diagnostic tool that can identify as many as possible variants of the target species. As a consequence, the availability of a well-characterized PPV collection would be a major asset for monitoring changes in the pathogen distribution over time and for supporting quarantine efforts with diagnostic tools able to discriminate among particularly aggressive strains and/or to identify all known strains.

Infected lyophilised plant material can be stored at +4°C for years without loss of physical structure, infectivity or degradation of viral RNA or viral capsid protein. This strategy was therefore selected in SharCo.

## 2. Detailed description

The isolates were either lyophilized locally by the partners or sent fresh to the centralized collection in Spain and lyophilized on site.

The fresh material (aprox. 40 g) was weighted and cut in small pieces, frozen at –80C ° and then liquid nitrogen was added before lyophilization. The lyophilisation was performed in a freeze dryer (Christ Alpha1-2) during 72 h. The lyophilised material was disrupted in a coffee grinder (Moulinex) and weighted. The powder was introduced in 6 Eppendorf tubes and in two vials of 5 ml (if enough material). The containers were introduced in plastic bags and sealed under vacuum conditions (Tecnotrip). The collection is maintained into special hermetic plastic boxes containing silica gel and kept at room temperature in a conditioned room of controlled access. The collection is managed with a stock programme. A database including country code, sample's number, host, geographical localization-GIS, and authority sender was generated. A computerized barcode system for sample identification was implemented and directly linked to the PPV database referencing system (deliverable DE1.7). A connexion with SharCo web page will be introduced in the Partner 6 web page in order to facilitate the access to the list of the available PPV isolates.

## 3. Original specifications and actual achievements

The initial proposition as described in the DOW was to set up the centralized collection in a lyophilized form of all (700-1000) isolates analyzed.

A total of 653 isolates have been currently lyophilized. Most of lyophilized isolates (except isolates from France and Turkey) are already kept in the collection of P6. P1 and P8 will deliver the lyophilized samples (processed in their own laboratories) to P6 in July 2011.

### Overview of lyophilized PPV isolates until June 2011

COUNTRY		TOTAL SAMPLES
ALBANIA	AL	12
ARGENTINA	AR	6
AUSTRIA	AT	22
BELARUS	BY	5
BOSNIA	BiH	3
BULGARIA	BG	78
CHILE	CL	1
CYPRUS	CY	1
CZECH REPUBLIC	CZ	24
EGYPT	EG	1
FRANCE*	FR	76

GREECE	GR	2
HUNGARY	HU	18
ITALY	IT	36
LATVIA	LT	1
MOLDOVA	MO	7
POLAND	PL	29
ROMANIA	RO	86
SLOVAKIA	SK	54
SPAIN	ES	85
SERBIA	RS	54
TURKEY*	TK	52
	TOTAL	<b>653</b>

\* Lyophilisation done in the partner laboratory

Part of the samples couldn't be delivered to the reference collections due to unforeseen problems (technical problems, unavailability of a sufficient amount of material especially in the case of isolates provided by third parties etc.). In this case, partners stored the infected material in their own deep-freezers.

## 4. Use and dissemination of the results

The deliverable DE1.5 is represented by a lyophilized collection of precisely identified (Database) and characterized (by partial sequencing TE1.2) isolates representative of the PPV genetic diversity. Services provided by this comprehensive and centralized Plum pox virus lyophilized collection (including reference isolates, but also variable or highly divergent isolates, thus encompassing the presently known PPV variability) can be summarized as follows:

- 1) 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).
- 2) provide well characterized or reference PPV isolates for development and validation of future robust, reliable and efficient detection tools, or typing tools specifically targeting particular strain or PPV group.
- 3) 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.
- 4) Preservation of the biodiversity of an important plant pathogen.

A system has been developed, so that interested researchers may order specific PPV isolates from the centralized collection, irrespective of their participation to the SharCo consortium. The collection will thus be freely available to the whole scientific community, as a

contribution of the SharCo project. However, access to a complete set of isolates (duplication of the collection will require negotiations with the SharCo consortium.

The collection and its open access policy will be publicized in the forthcoming SharCo publications and on the SharCo Web site.



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