

SharCo

Containment of Sharka virus in view of EU-expansion

Small Collaborative project of the 7th Framework Programme

Theme 2

Food, Agriculture, Biotechnologies

DG.1.4

Sources of resistance identified in all countries participating in the project, which will speed the breeding programs for PPV resistance

Due date	28/02/2011
Actual submission date	28/02/2011
Project start date	01/03/2008
Workpackage concerned	WPG.1
Concerned workpackage leader	Maria Luisa Badenes
Dissemination level	PU: Public



Deliverable report structure

1. GENERAL PRESENTATION.....	2
1.1. CONTEXT.....	2
1.2. RATIONAL	2
1. DETAILED DESCRIPTION	3
2. ORIGINAL SPECIFICATIONS AND ACTUAL ACHIEVEMENTS	4
3. USE AND DISSEMINATION OF THE RESULTS.....	7

1. General Presentation

1.1. Context

Availability of *Plum pox virus* (PPV) resistant *Prunus* material is the key for limiting the dissemination of the virus and the impact of the disease, both in nurseries and orchards. Resistance to PPV implies the disability of the virus to infect the plants, thus restricting the spread of the virus. In fact, PPV is able to infect every *Prunus* crop species, from peach, to apricot, plum, almond and cherry, related wild species used in interspecific crosses or as rootstocks included. In comparison, the number of varieties identified as resistant to PPV is very limited to non-existent. In apricot, very few (up to 6) genitors have been identified (Martinez-Gomez et al.,2000). In plum, hypersensitivity to PPV has been described and few genitors are being used to develop cultivars and rootstocks resistant to natural infection(Hartman and Neumuller, 2006) No true resistance to PPV has been identified in *Prunus persica* (peach) and the only solution lies in the interspecific crosses with *P. davidiana* and *P. dulcis* (almond) (Decroocq et al., 2005) Having such limited variability in resistance sources always presents the risk of occurrence and spreading of resistance-breaking PPV isolate(s), thus leaving the fruit industry without alternative solution.

1.2. Rational

In this context, within the workpackage WPG1 of SharCo, the task TG.1.3 entitled 'Molecular survey of local genitors and varieties for PPV resistance' aims at identifying new sources of resistance among the *Prunus* species and germplasm. Those new sources will then be used in breeding programmes for pyramiding with the few genitors already available (see deliverables DG.1.1 to DG.1.3). In the frame of the Sharco project several approaches have been investigated. One strategy was to conduct surveys of native cultivars and screen them for PPV resistance. The institutions and countries involved in this task are USAMV (Romania), ISK (Poland), MKU (Turkey) and FBI (Bulgaria). In apricot, the screening was based on a biological



test both in artificial and natural conditions, completed with a molecular marker assay (issued from deliverable DG1.1, Vera-Ruiz et al., 2011). In plums, only hypersensitivity has been described and is being used as a mean of restriction of PPV infection. When grafted on PPV infected rootstocks, hypersensitive candidates will grow but will eventually die within the next three to five weeks. In fields, under natural conditions of inoculation through aphids, hypersensitive trees will show a high resistance to PPV systemic infection. Based on this behavior, surveys for sources of hypersensitivity and resistance in plums have been slightly adapted.

Nevertheless, in both cases, apricot and plum, the biological test is a lengthy procedure that can take at least 3 vegetative cycles (3 years in the field). The survey starts with the identification of potential PPV resistant material, among germplasm collection, old cultivars, natural mutants and new hybrids. It is then followed by the biological tests, performed both in greenhouses and in the fields, after recovery of the candidate trees as budsticks and grafting on PPV-free and PPV-infected rootstocks. The biological tests have been initiated in the frame of the SharCo project but not completed yet. Moreover, during the procedure, some plants died or some grafts failed, the incidence being higher when the screening was performed in experimental plots and subjected to cold winters, which is the case of every institution involved in this task. We thus had to repeat the whole process in order to secure the survey. In consequence, many of the biological tests will be pursued well after SharCo has ended. However, valuable data have already been obtained and are presented below.

1. Detailed description

Apricot:

The assesment of PPV resistance is performed in natural (e.g. in the field) and artificial conditions (in greenhouse), using a biological indicator (peach 'GF305').

Natural conditions consist in planting seedlings from the genotypes to be tested in fields located in areas with natural inoculum of PPV.

The artificial screening consists in grafting genotypes to be tested on previously infected rootstocks. In this case, the 'GF305' peach rootstock has been selected because it is an indicator of viral infection. After germination, 'GF-305' seedlings were inoculated with a local PPV strain and then grafted with apricot buds from the genotypes to be tested. Following the graft union, the plants are transferred to a cold chamber for a two months cold period. After dormancy, new shoots are developing, they are visually scored for PPV symptoms and analysed by ELISA for presence of the virus. A minimum of three complete cycles are needed for reliable conclusions.

Plums and relatives

In this species resistance has not been described, only hypersensitivity. Similar procedure for screening in greenhouse following the biological test described above was performed for the plum genotypes.

In Romania partner 3 USMVA identified local varieties of plum and apricot in different parts of the country. During 2010, those varieties have been grafted in an experimental plot and are being tested for resistance to PPV using artificial inoculation. The resistance to PPV has been evaluated during this vegetative period



by visual inspection, serological and molecular methods. Results are indicated in Table 1. In apricot DNA has been isolated for further screening of markers linked to PPV resistance.

The experiment on testing plum cultivars for sharka susceptibility was established by Partner 7 (ISK) in an experimental orchard in Skierniewice, Poland in spring 2005, prior to SharCo project commencement. The cultivars: 'Katinka', 'Jubileum', 'Diana', 'Silvia', 'Valjevka', 'Żółta Afaska', 'Common Prune', 'Elena', 'Jojo', 'President' and 'Vision' were planted in 12 replications. In the first year, two trees from each cultivar were inoculated with PPV (PPV-S isolate belonging to D strain) by chip budding whereas the remaining 10 were left to natural infection by aphids. The source of PPV inoculum was provided by two rows of 'Common Prune' trees inoculated with the virus by chip budding. The trees were visually inspected and tested for PPV by ELISA each year.

In Bulgaria no apricot native cultivar was resistant to PPV. The strategy in this case was to introgress the resistance from already described sources (see deliverable DG1.1) into apricot locally well adapted cultivars to Bulgarian conditions. This is part of deliverable DG 1.5. In plum, donor parents of hypersensitivity are 'Stanley', 'Cacanska najbolja', 'Cacanska lepotica', 'Pacific' and 'Ortenauer. They have been used in crosses for introgression of the trait. Subsequently, phenotyping of PPV resistance is being carried out in the family (Althan, s gage x 6-51(Stanley o.p.) and the local plum cultivar Kyustendilska sinja grafted on *P. mariana* GF8.1 inoculated with PPV-M strain (Table 2).

In Turkey according to the references no native resistant cultivars belonging to apricot or plums species were described. However, in the frame of the project, native cultivars are being tested by molecular markers linked to PPV resistance (DG1.1). DNA from the local cultivars 'Hacıhaliloğlu', 'Çataloğlu'; 'Kabaası'; 'Hasanbey' was isolated and the screening is on the way.

2. Original specifications and actual achievements

Apricots

Unfortunately, none of the surveys performed in the different countries, Romania, Bulgaria and Turkey, produced new, still-unknown genotypes resistant to PPV infection, in Apricots.

Plum

Old cultivars located in Romania and Bulgaria are being tested in natural conditions and by artificial inoculation.

More cycles of observation beyond the duration of the project are needed in order to consider these hybrids as an alternative for future breeding, but the preliminary results obtained are a good starting point.

Table 1 summarizes native cultivars screened in Romania, for resistance to PPV. They are a potential new source of resistance, complementary to the ones described in DG1.1 and DG1.3.



Table1. Observation of plum response to PPV-D infection.

PPV was detected in different parts of the graft-inoculated plum genotypes, tested in 4 replicates, under artificial conditions. Highlighted in grey are depicted the most promising genotypes.

Nr.crt	Genotype	Analyzed part of plant	2010 (1 st scoring)			2010 (2 nd scoring)	
			DASI-ELISA (DO= 405nm)	IC-RT-PCR	PPV symptoms intensity	DASI-ELISA	PPV symptoms intensity
1	Myrobolan 4Kr	bottom half	++	-	-	++	-
		top half	-	-	-	-	-
2	'Andreea'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
3	'Local de Dragasani'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
4	'Carpatin'	bottom half	+++	+	++	+	+
		top half	+	+	+	+	+
5	'Centenar'	bottom half	+	+	++	+	+
		top half	+	+	-+	+	+
6	BN 3/2-98	bottom half	+	+	+	+	-
		top half	-	+	-	+	-
7	'Pixy'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
8	'Otesani 11'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
9	'Scoldus'	bottom half	+	-	-	+	-
		top half	-	-	-	-	-
10	'Calugaresti'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
C-	Negative control	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
C+	'Tuleu dulce' (positive control)	bottom half	++++	+	+++++	+++++	+++++
		top half	++++	+	+++	++++	+++

In Poland, the results obtained by 2010 show that among the cultivars tested, 'Jojo' and 'Vision' are resistant to PPV infection by chip budding. Trees of 'Jojo' and 'Vision' planted in the experimental field were also not infected by aphids (see Table 2). However, sharka was detected on one 'Vision' tree planted nearby, but not included in the experiment. Of the remaining 9 cultivars, all were infected by chip budding, but showed differentiated rate of infection by aphids, ranging from 12% of infected trees ('Elena') to 80% ('Katinka').

During 6 years of experimentation, some trees were damaged by frost. Especially susceptible was 'Silvia' (40% tress damaged), followed by 'Elena' (20% trees damaged). The table bellow shows the summarized results of the 6 year testing.

Table 2: Susceptible and resistant responses of plum genotypes to PPV-S (PPV-D) infection in Skierniewice

In grey, promising, PPV resistant cultivars.

Cultivar	No. of trees left in 2010	No. of trees infected during 6 years of experimentation	% of infected trees
'Katinka'	10	8	80
'Jubileum'	9	4	44
'Diana'	10	2	20
'Silvia'	6	1	17
'Valjevka'	9	2	22
'Żółta Afaska'	10	4	40
'Common Prune'	10	7	70
'Elena'	8	1	12
'Jojo'	9	0	0
'President'	9	5	55
'Vision'	9	0	0

The above data demonstrates that 'Jojo' is fully resistant to PPV infection, both by artificial inoculation and by aphids. Thus, it can be recommended for planting in areas where sharka is endemic. Another promising cultivar is 'Vision'. Although not fully resistant, it showed very low susceptibility to the disease. Besides, we cannot also rule out a mistake in the true-to-type identity in the close-by tree. Nevertheless, its susceptibility shall be further tested to confirm its behavior in various agroclimatic conditions.

Table 3 summarizes data obtained in Bulgaria.

In those countries where local cultivars were surveyed before this project started and where no resistance has been found, PPV resistance has been introgressed from sources described elsewhere (DG1.1 and DG1.3) in the most popular native cultivars.

Table 3: Phenotyping of old plum cultivars in Bulgaria

Genotype	Artificial inoculation PPV M strain	Visual inspection
'Stanley'	+	Performed
'Cacanska najbolja'	+	Performed
'Cacanska lepotica'	+	Performed
'Pacific'	+	Performed
'Ortenauer'	+	Performed
Kyustendilska sinya	+	Performed

3. Use and dissemination of the results

The cultivars identified as resistant to sharka will be transferred to all partners for introgression of resistance to PPV in future breeding programmes and pyramiding with already known, PPV resistant genitors.

References

V. Decroocq Æ M. Foulongne Æ P. Lambert Æ O. Le. GallC. Mantin Æ T. Pascal Æ V. Schurdi-Levraud Æ J. Kervella .2005. Analogues of virus resistance genes map to QTLs for resistance to sharka disease in *Prunus davidiana*. *Mol Gen Genomics* **272**: 680-689

Martínez-Gómez P, Dicenta F, Audergon J-M (2000) Behavior of apricot (*Prunus armeniaca* L.) cultivars in the presence of sharka (*plum pox potyvirus*): A review. *Agronomie-Paris* 20: 407-422

Hartmann, W. and M. Neumuller (2006). Breeding for resistance: breeding for Plum pox virus resistant plums (*Prunus domestica* L.) in Germany. *Bulletin OEPP/EPPO Bull.* 36(2):332-336.

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

