

SharCo

Containment of Sharka virus in view of EU-expansion

Small Collaborative project of the 7th Framework Programme

Theme 2

Food, Agriculture, Biotechnologies

WPG.1

**Identification of PPV resistance markers and
development of marker-assisted selection**

Deliverable DG.1.1

Due date	30/10/2009
Actual submission date	29/10/2009
Project start date	01/03/2008
Workpackage concerned	WPG.1
Concerned workpackage leader	Maria Luisa Badenes
Dissemination level	RE/PU: restricted access during the SharCo timecourse then release in the web queryable database by M48 or through publication.

1.-GENERAL PRESENTATION

The SharCo project is intended to provide tools for *Plum pox virus* containment. The development of those tools is being achieved through a multidisciplinary approach that involves i) a epidemiology pillar for the identification of factors driving PPV spread and the development of highthrough-put detection systems, ii) a genetic pillar for the development of molecular markers and the implementation of marker assisted selection of PPV resistant fruit varieties among many other biotechnological approaches. The general objective of the genetic pillar is indeed to broaden resistance to PPV in various stone fruit species.

In this context, the WPG1 (workpackage genetic 1) objectives are:

- The identification of molecular markers linked to resistance
- The implementation of marker-assisted selection in Western and Eastern European countries.

The rationale of the implementation of MAS in Europe is two folds: i) the necessity to initiate and sustain breeding programmes in Eastern European countries, focusing on the selection of PPV resistant cultivars; ii) the want to speed up the above breeding programmes, through the use of molecular markers.

Indeed, the use of genetic resistance is, beyond doubt, the best solution for the control of virus-induced diseases because it provides effective protection all along the growing season. It allows the new plantations of stone fruit trees in endemic regions, especially in Eastern Europe. However, breeding for resistance to PPV encounters the usual problems of breeding perennial plants (extended vegetative periods, the requirement of time-, labour- and space-consuming experimental nurseries) together with the difficult procedure of screening for PPV resistance that hinder the programmes. Standardisation 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. This slows the breeding process and makes finding new sources of resistance difficult. It is therefore of major importance to develop efficient tools to screen for sharka resistance, particularly where the resistance is only partial. In stone fruit trees, molecular tools have the potential to give us early information on the genetics of *Prunus* progenies (one or two months after sowing seedlings) and marker-assisted selection appears like a promising method to select rapidly individuals resistant to sharka disease.

2.- DETAIL DESCRIPTION

DG1.1 deliverable is focusing on the development of molecular markers for Apricot breeding programmes. Markers for peach and plum will be the object of the two next WPG1 deliverables and will therefore not be discussed in the following report.

Several breeding programmes were initiated years ago in several European countries (Spain, France, Czech republic, Italy) where screening tests of resistance to sharka disease within *Prunus armeniaca* (Apricot) species, cultivars and rootstocks, lead to the selection of few PPV resistant genitors ('Stark Early Orange', 'Goldrich', 'Stella', 'Harcot', 'Harlayne'). Resistance controlled by several genetic factors was identified and characterized. Furthermore, in apricot, using similar but not identical screening procedures, with different strains and genetic back grounds a major QTL was detected and mapped on the upper arm of linkage group one (LG1). Genetic factor(s) located in this major genomic region are controlling up to 70% of the resistance trait. In consequence, the main focus of TG1.1 task was to develop molecular markers located over this major genomic region and linked to PPV resistance. Results and tools obtained in TG1.1 are the object of DG1.1 deliverable. Because most of the *Prunus* species are heterozygous, molecular markers of choice have to be co-dominant (allowing to trace both alleles of the marker) but also highly polymorphic and easily transferable from one Apricot genotype to another. For those reasons, we selected SSR (Single Sequence Repeat) markers, also called microsatellites.

The development of markers in the major genomic region controlling the biggest part of the PPV resistance trait in apricot was achieved in 2 steps:

1) A total of 96 co-dominant SSR markers, which are exclusively mapping on the upper part of LG1 in *Prunus* genetic maps available on the GDR website (Genome Database for Rosaceae: <http://www.bioinfo.wsu.edu/gdr/>), were used in order to saturate the upper part of LG1. After adding 40 more, polymorphic, SSR markers over the region identified by Kosambi mapping function at LOD threshold of 8.0 and Kruskal Walis test, the genetic interval encompassing the major QTL on LG1 reached 13 cM.

2) Overgo probes were designed from ESTs (Expressed Sequence Tags) available from the *Prunus* transcriptomic map, from SSRs gathered within other *Prunus* genetic maps and from peach and apricot BAC-end sequences. Those sequence data are freely available on the GDR (<http://www.bioinfo.wsu.edu/gdr/>) and overgo probes were developed using the following website: <http://www.mouse-genome.bcm.tmc.edu/webovergo>. Radiolabelled

overgo probes were then hybridized against the (PPV resistant) ‘Goldrich’ apricot BAC library. In total, 924 BACs were found positive with the above overgo probes. 733 out of the 924 positive clones were fingerprinted in order to reconstruct the PPV resistance physical region. From the 733 fingerprinted apricot BACs, 370 provided sequence data by BAC–end sequencing. Half of the sequences generated presented homologies with sequences deposited in the GenBank database (<http://www.ncbi.nlm.nih.gov/>) and 31 are homologous to retrotransposons.

From the above sequences, 102 primers flanking microsatellite repeat motifs have been designed and the corresponding newly-developped SSR markers were re-mapped in 3 distinct apricot genetic maps (issued from F2 ‘SEO’x’Thyrinthos’ and F1 ‘Goldrich’x’Currot’ and ‘Goldrich’x’Canino’ progenies). ‘SEO’ (‘Stark Early Orange’) and ‘Goldrich’ are the PPV resistant genitors used in those crosses. All newly-developped SSR markers together gave rise to a highly saturated resolution map of the upper part of linkage group 1. By including those new markers, the ‘SEO’ x ‘Thyrinthos’ genetic map includes now 78 codominant markers in the target area, while the consensus map of ‘Goldrich’ includes 41 codominant markers in the target region.

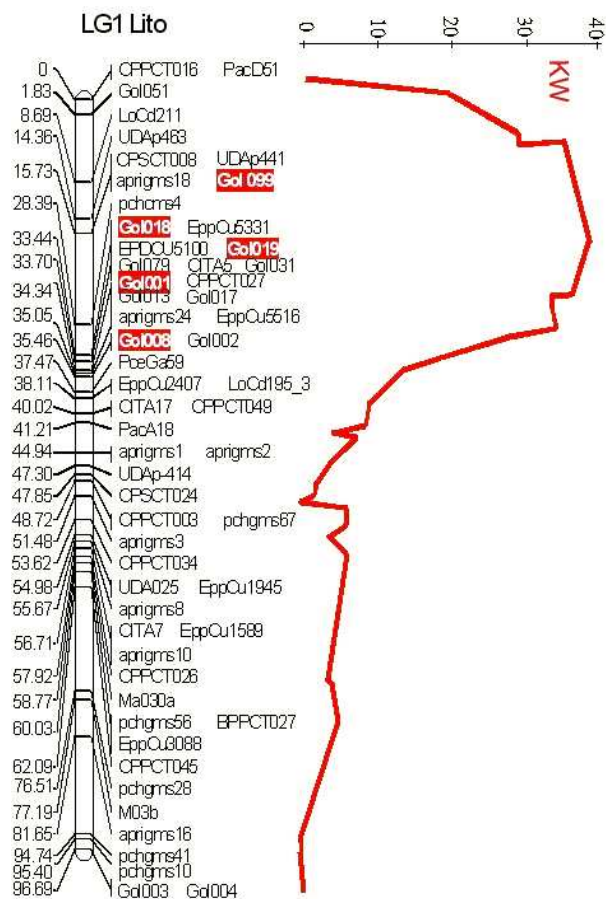
Finally, a contig of the major genomic region linked to PPV resistance is under construction. The above 924 BAC clones identified by hybridization with the overgo probes together with fingerprinting and BAC-end sequences are being processed through the FPC software (Soderland et al, 2000) to generate a physical map of the target PPV resistant region.

3. ACTUAL ACHIEVEMENTS

102 new SSR markers have been produced, a fraction of them are presenting criteria interesting for Marker Assisted Selection:

- They are highly polymorphic and can be used in many apricot progenies
- They are mapping over the genomic region linked to resistance to PPV and are distributed evenly over the target area.

They were named Gol and their position relative to the resistance interval depicted by the highest KW curve peak is presented in the figure below:



Newly-developed SSR markers selected for further validation in eleven other apricot progenies genotyped and phenotyped by the WPG1 SharCo partners (partner P1, P6, P9 and P15) are highlighted in red in the figure above. Other promising SSR markers were identified previously (Marandel et al., 2009; Sicard et al., 2008). All those molecular markers will be transferred after validation to SharCo partners' developing new apricot populations segregating for resistance to sharka disease (partners P3, P8, P14 and P16).

Moreover, the progress made in fine mapping of the PPV resistant apricot genomic region, along with its physical map is allowing to construct a contig of apricot BAC clones covering the target area. The meta QTL analysis developed by partner P1 allows to link alleles to QTL of PPV resistance and to different sources of resistance.

4.- USE AND DISSEMINATION OF THE RESULTS

Molecular markers (SSR) developed in the course of the SharCo first period or identified previously as linked to the resistance trait are being transferred to members of the SharCo consortium for validation and for implementation of Marker Assisted Selection. Over the next SharCo periods, molecular markers can be provided to non-SharCo members under the

signature of a MTA (Material Transfer Agreement) and a confidentiality statement. By the end of the SharCo project, they will be released freely.

Publication(s) on the characterisation of PPV resistance in apricot, on the molecular markers linked to the resistance and the implementation of marker assisted selection are foreseen and if so, will be delivered on M48

Up to date, preliminary results impacting on WPG1 tasks have been or are being published in scientific journals and international meetings as follows:

Marandel G. et al. (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 resistance genomic factors. Molecular Plant Pathology, 10, 3: 347-360.

Rubio M., P. Lambert, A. Bachellez, T. Pascal. Quantitative trait loci analysis of Plum pox virus resistance in Prunus davidiana P1908: new insights on the organization of genomic resistance regions. Tree Genetics and Genomes, in press.

References cited in the text:

Sicard O., Marandel G., Soriano J.M, Lalli D.A. , Lambert P., Salava J., Badenes M.L., Abbott A. and Decroocq V. (2008b). Flanking the major Plum pox virus resistance locus in apricot with co-dominant markers (SSRs) derived from candidate resistance genes. *Tree Genetics & Genomes*, 4:359–365.

Marandel G., Salava J., Abbott A., Candresse T. and 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 resistance genomic factors. *Molecular Plant Pathology*, 10, 3: 347-360.