

SharCo

Containment of Sharka virus in view of EU-expansion

Small Collaborative project of the 7th Framework Programme

Theme 2

Food, Agriculture, Biotechnologies

Milestone ML.1

PPV resistance molecular markers in apricot

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Workpackage concerned	WPG.1
Concerned workpackage leader	Maria Luisa Badenes



1.-General Presentation

1.1 Objectives of this work package

The SharCo research programme aims at providing new methods and tools for the containment of sharka, in orchards and nurseries. For that purpose the project is developing specific research activities on a variety of topics, epidemiology, genetics, biology and application of EU policy. In the field of genetics, we aim at producing PPV resistant cultivars, adapted to different environmental conditions found all over the European Stone Fruit production areas (South with a Mediterranean climate, East with continental conditions and North with long periods of cold temperature in winter and spring). The general objective of the genetic pillar is indeed to implement breeding programmes for resistance to *Plum pox virus* in European and associated countries where they do not exist (Romania, Bulgaria, Turkey). However, breeding for resistance to PPV encounters the usual problems of breeding perennial plants (extended vegetative periods -3 years in peach, 8 in plum-, 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 the selection of cultivars both agronomically valuable (fruit quality, local adaptation) and PPV resistant, too difficult and expensive. It is therefore of major importance to implement a new strategy of selection of promising individuals assisted by molecular markers. Indeed, 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 seeds) and marker-assisted selection appears like a promising method to select rapidly individuals resistant to sharka disease. The objective of this workpackage (WPG1) is therefore the development of marker assisted selection (MAS) programmes all over Europe, in apricot at first and then in peach and plum.



1.2 Conditioned issue of this milestone:

Two limiting factors are conditioning the successful implementation of MAS:

- 1) The production of a large amount of progenies obtained by crossing PPV resistant genitors with locally adapted cultivars
- 2) **The identification of molecular markers sufficiently linked to resistance in order to be used in future breeding programmes.**

In the first year of the project, SharCo partners initiated or intensified the production of large apricot progenies segregating for resistance to PPV (see Annex 1 of the milestone). The success in the development of molecular markers linked to resistance QTL in apricot is therefore largely conditioning the implementation of MAS in Apricot breeding programmes.

This is why our first milestone is the obtaining of molecular markers linked to PPV in apricot. The objective has been accomplished and a description of the strategies and experiments carried out is presented below.



2.- Detailed Description

The development of molecular markers for use in MAS in apricot breeding programmes is the objective of the TG1.1 task of WPG1 workpackage. It comprises both the development of the markers *sensu stricto* and their validation in selected F1 and F2 apricot populations segregating for resistance to PPV (see annex 1 of the grant agreement, Task TG.1.1 - Development of markers linked to resistance to PPV. Association analysis with selected SSR markers in apricot-). The development of molecular markers in plum will be the subject of another milestone (ML6, month 36) and will therefore not be discussed here.

Resistance to PPV in apricot is controlled by few genetic factors (Soriano et al, 2008; Lambert et al., 2007; Marandel et al., 2009; Pilarova et al., under revision) and most of the trait variance is explained by one major QTL (Quantitative Trait Locus, corresponding to the major genomic region linked to the resistance trait). This major QTL is mapping on the upper arm of linkage group one (LG1) and explains by itself up to 70% of the resistance trait variance. In consequence, the main focus of TG1.1 task was to develop molecular markers located over this major genomic region. When developing markers for MAS in heterozygous plant species, the following criteria are essential: i) high polymorphism of the markers selected, ii) co-dominance of the markers allowing the scoring of both alleles of a diploid genome, iii) good transferability from one breeding population to another. Following those rules, we made the choice to develop SSR (Single Sequence Repeat, also called microsatellite) markers for MAS in apricot breeding programmes.

The development of SSR markers from the LG1 resistance locus was performed in 4 steps:

1) Fine mapping of the LG1 genomic region linked to PPV resistance by saturating the region with 96 SSR markers selected from the Genome Database for Rosaceae (<http://www.bioinfo.wsu.edu/gdr/>). This lowered the exact size of the target region to 13cM.

2) Screening of an Apricot (*cv.* Goldrich) BAC library with probes designed from the above SSR markers, from EST mapping over the region and peach BAC-end sequences of BAC clones mapping in the 13 cM large area. EST and BAC-end sequences were also searched in and extracted from the Genome Database for Rosaceae (see above).

3) A fraction (3/4) of the Apricot BAC clones positive by hybridization was then fingerprinted and 1/3 of those positive clones were sequenced at both ends. BAC-sequencing generated new repeat motifs characteristics of SSR markers, from which we designed new



primers. Following this procedure, we developed 102 primer pairs flanking new SSR markers.

4) Newly-designed SSR markers were then re-mapped in 3 distinct apricot populations (namely F2 'SEO'x'Thyrinthos', F1 'Goldrich'x'Currot' and F1 'Goldrich'x'Canino') in order to confirm their positions within the LG1 major QTL interval, their mono-locus *versus* multi-locus status and evaluate their polymorphism.. All markers together allowed to develop a high resolution map of the upper part of linkage group 1. For example, the genetic map of the LG1 QTL interval comprises 78 co-dominant SSR markers in the 'SEO'x'Thyrinthos' population, and 41 in the 'Goldrich'x'Canino' and 'Goldrich'x'Currot' progenies. The newly-developed markers were finally named 'Gol'.

Beside the transfer to other SharCo partners for validation and use in the first tests of MAS, the above newly-developed SSR markers will allow us to construct a BAC contig and therefore a physical map of the genomic region controlling the largest part of the resistance to PPV in apricot. It is an essential step towards the full sequencing of the region and the identification of candidate genes.

3.- Means of verification and actual achievements

The accuracy, the polymorphism and the position of the newly-developed Gol SSR markers were verified in three apricot segregating populations. Resistance to PPV was previously scored and mapped in those populations (Soriano et al, 2008) and a genetic map was built. The five first Gol markers (see annex 2) are being transferred to partners P1, P9 and P15 for validation in other apricot related (the same 'SEO' or 'Goldrich' PPV resistant genotypes were used as genitors) or un-related (other PPV resistant parents, 'Harlayne' or 'Harcot', were used in the initial crosses) progenies. Genotyping and phenotyping of the PPV resistance were also realised previously in those populations (Lambert et al., 2007; Marandel et al., 2009; Pilarova et al., in press; Rubio et al., in press; Dondini et al., submitted). The five first Gol markers were selected for their polymorphism and for their even distribution over the resistance locus (see annex 2). Co-segregation of the Gol markers with resistance to PPV will be verified in the next 6 months, in a total of 8 apricot segregating populations (see Annex 3).

Following this verification, those molecular markers will be transferred to partners P3, P8, P14 and P16 for implementation of the marker assisted selection in the breeding programmes they initiated during the first period (see below, annex 1). Those apricot progenies were obtained by crossing PPV resistant genitors ('SEO', 'Goldrich', 'Lito', 'Harlayne', 'Stella'



and 'NJA2') with cultivars well adapted to local conditions. Association study of the Gol markers with resistance to PPV in those progenies is expected to be completed by the end of the second period.

3.- Contingency plans

No contingency plan is proposed since this milestone was successfully achieved. If some of the five SSR markers transferred to other SharCo partners are not usable in their segregating populations (due to a lack of polymorphism or amplification), they will be replaced by others selected from the 97 molecular markers left. In such situation, we propose to screen the remaining 97 SSR markers for polymorphism in the corresponding 'defaulting' progeny; successful markers will later be used for MAS in the partner's breeding programmes.



References

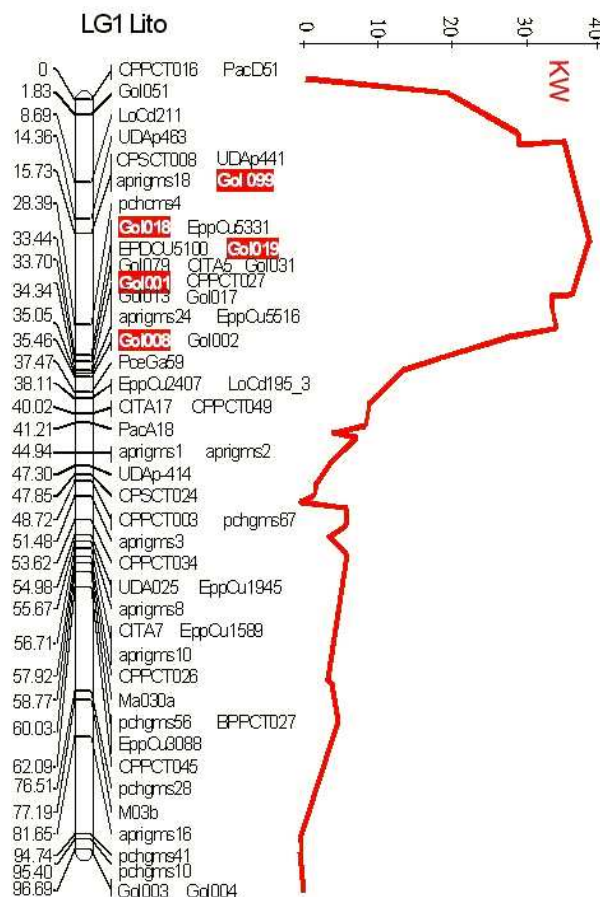
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Annex 1: Pre-breeding material selected or prepared in the first period for implementation of marker assisted selection

Partner	species	Number of new crosses performed in the first period	Size of the population (number of individuals)
P1	Apricot and peach	Pre-breeding material	3,600
P3	apricot	16	252
P6	apricot	2 back-crosses	1,400
P8	apricot	50	2,549
P14	Apricot	7	342
P15	apricot	34	5,944
P16	apricot	4	139
Total material available for MAS in the second period			= 14,226

Annex 2: Mapping of the Gol SSR markers (in red in the figure) that will be validated in the second period and later used in MAS in apricot.



The curve is depicting the position of the genomic interval detected by Kruskal-Wallis test (KW) as linked to resistance to PPV in apricot. The peak of the curve corresponds to the region presenting the most significant linkage to PPV resistance.

Annex 3: Mapping populations used to validate the newly-developped SSR molecular markers

Population name	cross	Type of population	SharCo partner	Size of the population (number of individuals)
PS	Apricot 'Polonais' x 'SEO'*	F1	P1	142
GoMo	Apricot 'Goldrich'* x 'Moniqui'	F1	P1	183
GoCa	Apricot 'Goldrich'* x 'Canino'	F1	P6	196
GC	Apricot 'Goldrich'* x 'Currot'	F1	P6	81
LxL	'Lito'* selfpollination	F2	P6	400
HM	Apricot 'Harlayne'* x 'Marlen'	F1	P9 and P1	147
639	Apricot 'Harcot'* x 'Reale di Mola'	F1	P15	116
616	'Lito'* selfpollination	F2	P15	119
Total individuals used for validation of newly-developped SSR markers				= 1,384

* PPV resistant genitor. 'Lito' is an F1 individual issued from the PPV resistant genitor 'SEO' ('Stark Early Orange').