MARS

MARKER ASSISTED RESISTANCE TO SHARKA

Small Collaborative project of the 7th Framework Programme

THEME [KBBE.2013.1.4-07]

[Boosting the translation of FP projects' results into innovative applications in the field of agriculture, forestry, fisheries and aquaculture]

FINAL REPORT

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1. Executive summary

Sharka, caused by the ***Plum Pox Virus* (PPV),** is the most devastating disease affecting stone fruit trees in Europe. In order to respond to this critical situation, the FP7-funded **SharCo** project (2008-2012) devoted a significant part of its efforts on the development of first-generation PPV resistant plant materials, guidelines for new plantings and molecular tools for the implementation of marker assisted selection (MAS) in apricot breeding programs. The first steps of establishment of MAS in SharCo presented some limitations that hampered the EU-wide implementation of a high throughput MAS approach:

1. While most of the PPV resistant individuals displayed the resistant alleles, a significant number of PPV susceptible individuals showed the same resistant haplotype. We thus hypothesize that the presence of other factors or genes involved in the mechanism of resistance to sharka in apricot could explain these unexpected results.
2. Screening of apricot progenies for both PPV resistance and local adaptation to different fruit tree cultivations was limited to few hundreds of individuals due to technical and financial issues.

In this context, the current proposal, **MARS** (for **Marker Assisted Resistance to Sharka**), was designed to boost the production of sharka resistant stone fruit cultivars by improving and transferring efficient and reliable procedures of MAS to SMEs.

First, molecular tools developed in SharCo were upgraded for multiplexing before transfer to SMEs. They then served in the screening of over thirty thousands of apricot progenies in which sharka resistance was combined with locally adapted, high value varieties. About half of the progenies showed resistant alleles over the first locus linked to PPV resistance in SharCo. In the meantime, part of the MARS consortium focused on 1) the identification of the second genetic factor controlling PPV resistance, 2) the development of a new set of molecular markers linked to this second locus and 3) the validation of these new tools before transfer to SMEs. A new set of four molecular markers, complementary to the ones issued from SharCo, were developed. After validation on a subset of apricot progenies and multiplexing, they were also transferred to SMEs. They were then used to screen the promising apricot progenies selected beforehands with the SharCo's tools. They also served to screen progenies in which at least two sources of resistance were combined. In parallel to those molecular approaches, part of the consortium worked on pyramiding several sources of resistance in new breeding programs as well as evaluating the agronomical added-value of the first true resistant material identified both in SharCo and MARS. Moreover, dissemination activities were conducted, targeting fruit producers, nurserymen and extension services, in order to promote the plantation and cultivation of PPV resistant apricot cultivars developed by the MARS partners.

By the end of the MARS project, we can claim on an efficient translation of MAS to SMEs and the implementation of high throughput selection of PPV resistant apricot cultivars adapted for cultivation all over Europe. Such an approach is contributing significantly to building efficient and durable resistance to sharka disease at the European level.

2. Summary description of the project context and objectives

- **MARS context and rationale**

Sharka, caused by the ***Plum Pox Virus*** **(PPV),** is the most devastating disease affecting fruit trees in Europe (cost of 10 billion Euros in the last 30 years for disease management) and has put the world fruit tree industry in a precarious position. In order for Europe to provide breeders with the tools needed to respond to this critical situation, the EC funded the "**SharCo**" project (small Collaborative FP7 project) from 2008-2012. SharCo developed tools such as first-generation PPV resistant plant materials, accurate and reliable methods of PPV detection, guidelines for new plantings, an early warning systems and a decision-support system. Moreover, SharCo devoted part of its effort to the integrated study of genetic resistance to PPV, thus developing molecular tools and knowledge for implementation of marker assisted selection in apricot breeding programs. Molecular marker assisted selection, often simply referred to as marker assisted selection (MAS), involves selection, through molecular markers, of plants carrying genomic regions that are involved in the expression of traits of interest. Whereas at SharCo project end in 2012, part of the genetic tools and plant material were ready for transfer to European breeding programs and private stakeholders such as SMEs, however there were limitations on the translation of these results to realized economic value. This was due to: (i) the restricted number of resistance donors used in SharCo limiting the number of resistance genes available for pyramiding, (ii) the lack of a high throughput method for screening thousands of promising breeding materials which combine both resistance to sharka and locally adapted, high quality value traits. Indeed, although natural sources of resistance have been identified and used in apricots in SharCo and other European breeding programs, they are too limited in number to secure the development of durable sharka resistance, EU-wide. In SharCo, we also demonstrated that some of the parents used for European breeding programmes harbour a restricted number of genetic factors linked to PPV resistance and thus they are only partially resistant to the disease. In the long term this poses a problem of for durable resistance in newly released apricot cultivars.

In this context, the overall aim of MARS was to boost the production of sharka resistant stone fruit cultivars by transferring the efficient and reliable procedures of MAS conceived in SharCo to SMEs to enable targeted, high throughput selection of PPV resistant apricot cultivars adapted for cultivation all over Europe.

- **MARS scientific and technical objectives**

One of the simplest approaches to controlling the spread and impact of PPV is the development of fruit tree varieties with significant levels of sustainable resistance to the pathogen. For this purpose, the MARS consortium devoted a significant part of its efforts to the integrated study of genetic resistance to PPV, thus developing molecular tools and knowledge for implementation of marker assisted selection (MAS) in apricot breeding programs. However, while developing pathogen resistant new varieties, breeders need also to satisfy consumers taste and meet growers and industry constraints for the production of fruits with optimized flavor, color, texture and resistance to damage during transport. Therefore, it is essential to select PPV resistant material together with these quality criteria in varieties that simultaneously show good productivity and good local adaptation. To maintain their economic position among temperate tree fruits producing countries in the world, stone fruit breeders need to rapidly introduce a high level of resistance to sharka, into high quality value varieties, locally adapted to their environment and cultivation conditions. This process is significantly hampered by the complexities inherent to tree breeding. The first important step is the identification of adequate parents and the development of molecular tools allowing fast and efficient screening of progenies for PPV resistance or for other traits of interest. Plant material issued from controlled crosses between PPV-resistant parents and locally adapted, agronomically valuable varieties is the ultimate of such breeding programmes in order to meet consumers and fruit producers requirements.

Overall, the strategic objective of MARS was to transfer to European SMEs the novel methods and tools originating from SharCo, in order to enable the acceleration of the selection of sharka resistant plant material. For that purpose, MARS aimed at:

- Producing and upgrading reliable molecular tools for MAS in view of improving resistance of plants propagated in nurseries and cultivated in orchards by:
    - Identifying molecular markers linked to a maximum of genetic factors involved in PPV resistance;
    - Reinforcing plant resistance by combining multiple genetic factors in 'elite' germplasm and pre-competitive breeding material;
- establishing a high throughput protocol for MAS transferable to, and applicable by, various end-users such as SMEs/laboratories specialized in biotechnology, diagnosis, legal certification of new cultivars as well as breeding companies of the public and private sectors;
- demonstrating the MAS technology within SMEs established in Europe and Turkey to streamline and accelerate the introgression of resistance into new varieties by:
    - Validating the molecular tools in thousands of progenies set up in various European environmental conditions of cultivation and viral inoculums pressure.

MARS also relied on the SMEs and their respective local network of producers, extension services and growers unions to disseminate the first released apricot varieties and promote the multiplication and planting of sharka-resistant material following the cultivation guidelines established by SharCo.

3. Description of main S & T results/foregrounds

1) *From conventional breeding to marker assisted selection of PPV resistant material*

➢ **Towards the improvement of tools for Marker Assisted Selection**

Resistance to PPV in apricot is controlled by few genetic factors 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) named *PPVres*. 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. A set of three markers (PGS1.21, ZP002 and PGS1.24) targeting the *PPVres* locus was developed in the frame of SharCo (Soriano et al. 2012; Decroocq et al. 2014). In the latest study, we demonstrated that this **first marker set developed SharCo is not broadly applicable for Marker Assisted Selection (MAS)** and that marker assisted breeding based on the sole *PPVres* locus is not sufficient to unambiguously select PPV resistant apricot cultivars. Whereas the major locus is essential for the outcome of viral infection leading to resistance or susceptibility, complete and stable expression of the trait requires at least a second locus (Decroocq et al. 2014). In fact, while most of the PPV resistant individuals displayed the resistant alleles, a significant number of PPV susceptible individuals showed the same resistant haplotype. We thus hypothesize that the presence of other r genes involved in the mechanism of resistance to sharka in apricot could explain these unexpected results. Implementing MAS of PPV resistance in apricot will thus require the identification and targeting with molecular markers of the other locus/loci that potentially modulate the effect of *PPVres*. We thus focused on identifying markers linked to the other genetic factor(s) and to use this data to optimize MAS for resistance to sharka.

For this purpose, we implemented two complementary strategies: **1) a Genome Wide Association Study (GWAS) and 2) a QTL mapping approach in 5 distinct F1 segregating populations**. Mapping of the other genetic factors controlling resistance to sharka is described in details in the **milestone MS1** (available on the MARS collaborative platform).

1. Genome-wide association studies on cultivated and wild accessions

Two distinct loci were identified (Figure 1), which are partly in LD (linkage disequilibrium) and are both mapping on LG1 (linkage group 1 or chromosome 1) (Mariette et al. 2015): One around 10-11 Mbp (could correspond to *MetaPPV1b)* (Marandel et al, 2009) and one at 8 Mbp (possibly corresponding to

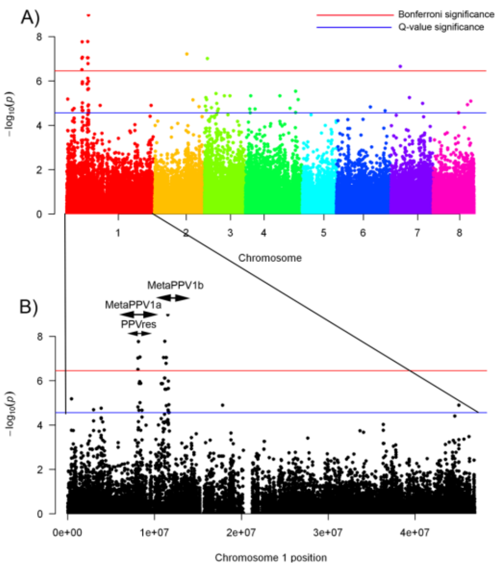


Figure 1: Identification of a second locus controlling the resistance to sharka. Whole-genome scan for association with resistance to PPV across 72 apricot accessions

PPVres) (Soriano et al, 2012), the most significant being the first one and surprisingly, not *PPVres*.

Because of the physical proximity of both loci and of the significant LD between them, few recombinants are expected in segregating populations which might not help to distinguish the effect of each locus, their epistatic effect (if any). Thus, ideally, the development of markers should not be in LD, to allow targeting both loci independently, which is not the case in cultivated apricot germplasm. In consequence, a new, complementary approach, based on GWAS in wild, natural populations of apricot segregating for resistance to PPV was initiated. However, due to the time needed for phenotyping, results will be available only by the end of the project.

- ***2.** Screening of populations from controlled crosses between cultivated apricot accessions and **new QTL analyses***

Both at INRA-Avignon (partner P01b) and UNIBO (partner P03), segragating populations which have been already phenotyped were screened for selected markers in the sharka resistance region (upper part of chromosome 1), among which markers developed in the above study. The analysis showed a smaller, although significant peak centred on the position 31 Mbp or 6 Mbp which might be the target for future investigation (Figure 2). After adding the new SNP and SSR markers in the above apricot map frameworks, QTL analysis was performed once again. However, results did not change significantly, compared to the previous ones; no additional region was identified despite the higher marker coverage on linkage group 1 and, at a lower extent, linkage groups 3 and 4. In our opinion, these results are likely due to the genomic structure of the populations (F_1), a relatively low number of individuals that does not allow for breaking linkage and discriminating close QTL regions, if any, and the linkage disequilibrium between the two QTL, *PPVres* and *metaPPV1b*.

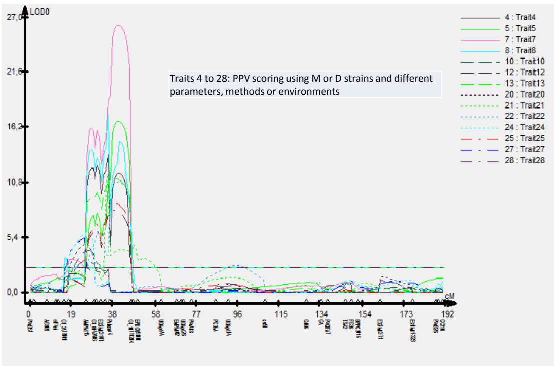
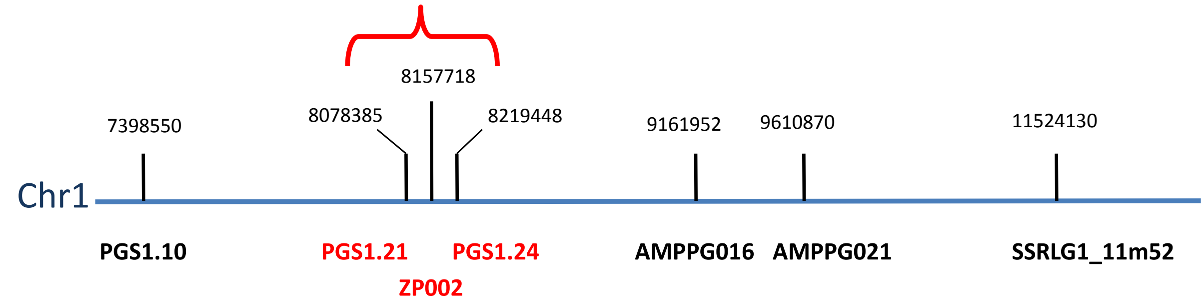


Figure 2: Significant QTLs identified in linkage group 1 of SEO, by Composite Interval Mapping and the new sets of SNPs and SSR markers developed in MARS

At this stage, the next step was then to develop new molecular tools based on the above data and targeting the other factor(s) of resistance, beside *PPVres*. **A set of 68 SNP and 42 SSR markers were developed during the first period of MARS** (see list of markers and details in **Deliverable D1.1**). However, when developing markers for MAB 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, heterozygous 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 as we did in SharCo for the first set of markers. **A set of four SSR markers was selected** (see **Deliverable D1.1** and **milestone MS2**, available on the MARS collaborative platform), based on data obtained on the mapping populations in France and in Italy (see Figure 3 for a representation of the second set of markers, relative to the one developed in SharCo).

Figure 3: Schematic diagram of the upper part of chromosome 1 in apricot showing the positions of the different molecular markers.

In red are represented the markers that belong to the first set of markers developed in SharCo (*PPVres* locus); in black, markers implemented in MARS. Positions of the SSR and SSLP markers are depicted over the figure, in base pairs (bp) from the chromosome start. They correspond to the relative position of the forward primer in the peach annotated genome sequence v1.0 (www.rosacea.org). Chr 1: Chromosome 1.



Conditions of multiplexing were set up in collaboration with the Biotech SMEs, before being used in routine in the second period of the project. While transferring this second set of markers to SMEs, we also tried to validate them in a subset of progenies for which we already knew their resistance or susceptibility status. Indeed, the new set of molecular markers was tested in apricot populations previously phenotyped in France, Italy and Spain (Table 1). However, those studies showed that while the second set of molecular markers helped in some cases to sort out true susceptible progenies that were initially mislabelled as resistant, it did not solve all problems described in Decroocq et al. 2014. Indeed, it appears that additional work will be needed for the development of molecular markers able to screen efficiently seedlings derived from crossing in which alternative sources of resistance such as 'Harcot' are used as parent.

Table 1: Screening of extensive populations of apricot issued from one single source of resistance, with the two sets of molecular markers implemented in MARS

			Number of samples with resistant alleles			Frequency (%) of seedlings with resistant alleles			Seedlings phenotypically tested		
Partner Nb	**Population tested**	**Seedlings (n)**	**7 loci [a]**	***PPVres* locus [b]**	**ZP002 locus**	**7 loci [a]**	***PPVres* locus [b]**	**ZP002 locus**	**Nb seedlings tested (n)**	**Resistant (%)**	**Susceptible (%)**
P01 (INRA)°											
Total P01°											
P03 (UNIBO)	Lito* x BO81604311	349	181	191	192	51.86	54.72	55.01	342	38.9	61.1
	Harcot* x Real d'Imola	98	34	34	45	34.7	34.7	45.9	96	19.8	80.2
Total P03		447	215	225	237	49.1	50.3	53	438	35.6	64.4
P08 (CEBAS)	Rojo Pasión* × Búlida Precoz	62	0	33	34	0,0	53.22	54.83	62	54.8	45.2
	Goldrich* × Currot	72	29	32	33	40.27	44.44	45.83	72	38.9	61.1
	Orange Red* × Currot	10	1	5	5	10	50	50	10	30	70
	Orange Red* × Palstein	13	3	4	4	23.07	30.76	30.76	13	46.15	53.84
	Orange Red* × Tardif de Bordaneil	77	28	30	31	36.36	38.96	40.26	77	45.5	54.5
Total P08		234	61	104	107	21.94	43.48	44.34	234	43.07	70

[a] 7 marker loci corresponding to the two sets of markers implemented in MARS

[b] PPVres locus corresponds to the first set of markers implemented in MARS and developed in SharCo, initially.

* Resistant parent used in the crosses

° see table 2 for data

- **To build up a durable resistance to PPV**

In the last two years of SharCo, various donors of resistance were challenged with eight PPV isolates, representing five viral strains. Few accessions ('SEO', 'Lito', 'Harlayne' and 'Stella') remained resistant whatever the strain was. This result confirms that in this material, more than one genetic factor participate to the trait. Therefore, MARS also focused on: 1) pyramiding several sources of resistance in breeding populations, thus combining the different factors of resistance in few genotypes, and 2) applying the two sets of molecular markers (one coming from SharCo and the second one developed in the course of MARS see above) on this new material.

Two types of activities were conducted for this purpose in MARS: 1) the characterization of progenies issued from crosses between two sources of resistance and obtained previously to the start of the MARS project (some of them, in the course of SharCo), 2) the crossing between at least two sources of resistance by some MARS partners. The new crosses were performed in the first year of the project (2014). **A total of 12,020 seedlings in which at least two sources of resistance were cumulated** (see complete list in **Deliverable D1.2 and updated in WP01's second periodic report**) were genotyped with the two sets of markers (Table 2). Part of them were also tested for resistance to sharka in greenhouses in order to verify their level of resistance in comparison with seedlings issued from one single source of resistance.

Table 2: Seedlings scored so far for resistance gene pyramiding and results obtained for the two sets of markers implemented in MARS.

A given seedling was scored as genotypically 'resistant' when at least one resistant allele is detected (heterozygous status) at each tested locus.

		Seedlings with resistant alleles (n)			Seedlings with resistant alleles (%)			Seedlings phenotypically tested		
Partner number	**Seedlings***	**7 loci [a]**	***PPVres* locus [b]**	**ZP002 locus**	**7 loci [a]**	***PPVres* locus [b]**	**ZP002 locus**	**Nb seedlings tested (n)**	**Resistant (%)**	**Susceptible (%)**
P01a / P09	173	66	118	171	38.15	68.21	98.84	170*	58.24	41.76
P01b / P11	10193	5760	6861	8240	56.51	67.31	80.83	134	30.60	69.40
P02 / P03	1224	655	806	938	53.51	65.84	76.63	--	--	--
P04	27	12	12	16	44.44	44.44	59.25	27	51.85	48.14
P05	205	80	93	132	39.02	45.36	64.39	--	--	--
P06	33	17	26	28	51.51	78.78	84.84	--	--	--
P07	83	0	76	77	0.0	91.57	92.77	--	--	0.0
P08	82	18	61	64	23.17	74.39	78.04	82	87.80	12.20
Total	**12020**	**6608**	**8053**	**9666**	**54.96**	**66.98**	**75.86**	**413**	**54.72**	**45.28**

-- missing data or ongoing analysis

*Part of the trees from the original population(s) are missing because their genotyping/phenotyping was either delayed or some trees died shortly before sampling.

[a] 7 marker loci corresponding to the two sets of markers implemented in MARS

[b] *PPVres* corresponds to the first set of markers implemented in MARS and developed in SharCo, initially. It includes among the three markers, the ZP002 marker.

All together those preliminary data show that **the frequency of resistant siblings is significantly higher to the ones issued from one single source of resistance** (66.98% for 43% on average for *PPVres*). Full details on this task and results obtained by the MARS consortium are presented in **deliverable D1.2**. Evaluation of the durability and stability of this complex resistance in the obtained breeding material is still ongoing and will require several vegetative cycles.

- **Diversification of the sources of resistance to sharka**

Most of the sources of resistance used up to now as parents (donors of the resistance) in European conventional breeding programs share the same genetic origin, a Central-Asian material introduced in North-American breeding programs over 50 years ago. Therefore, regardless to the implementation of high throughput MAB, we cannot rule out that identified resistances may be overcome, in the future, by new virus strains. Therefore, it was important to diversify those sources of resistance and identify new, still-unknown germplasm for future breeding programmes. The above two sets of molecular markers were used to survey the apricot germplasm as well as local, old/ancient cultivars, to try to identify additional resistant parents or varieties.

Two types of plant material were gathered for this task (see complete list into the **Deliverable D1.3**):

1) Cultivated, current or ancient landraces kept at the French National genetic resource collection, located at INRA, Avignon (174 accessions) and at UMIL (P02) (132 accessions);

2) Apricot accessions from wild, natural populations together with local ancient cultivars and landraces originating from Central Asia and the Irano-Caucasian region (552 accessions currently maintained by partner P01, INRA-Bdx, UMR BFP). The sampling of the second type of plant material was performed in the last 5 years in the frame of the STONE FP7 IRSES project (coordinated by partner P01a).

A summary of the results is presented in the Tables 3 and 4 below as well as in the **deliverable D1.3**, together with a full list of individuals screened. It showed a higher proportion of *PPVres* resistant alleles in the apricot natural populations and in landraces originating from the area of origin and domestication of this species.

Table 3: Marker Assisted screening of the irano-caucasian and central Asian apricot germplasm

			Frequency (%) of resistant alleles		
Eco-geographic area	**Country**	**Nb tested**	**7 loci**	**3 *PPVres* loci**	**ZP002 locus**
Irano-Caucasia	**Turkey**	20	0	5	5
	Azerbaijan	37	0	2.70	2.70
Central Asia	**Kazakhstan**	316	5.69	25.94	40.82
	Uzbekistan	14	0	7.14	28.57
	Kyrgyzstan	111	3.60	13.51	27.92
	China	28	3.57	14.28	32.14
	Pakistan	26	0	0	0

Table 4: Marker Assisted screening of modern and old/ancient apricot cultivated germplasm

	Nb accessions genotyped	accessions with resistant alleles (n)			accessions with resistant alleles (%)			accessions phenotyped		
Partner		7 loci	*PPVres* loci	ZP002 locus	7 loci	*PPVres* loci	ZP002 locus	Nb assessed (n)	Nb resistant (%)	Nb susceptible (%)
P01b INRA.Avi	174	--	14	32	--	8.04	18.39	45	11.11	88.89
P02 UMIL	118*	17	31	44	14.41	26.27	37.29	25	52	48

-- missing data or ongoing analysis

* Accessions used as control and for which genotype and phenotype were already known were removed from the original dataset (N= 132)

A subset of this original material was tested for resistance to sharka by infecting them with PPV-Marcus strain (Figure 3 for the wild germplasm).

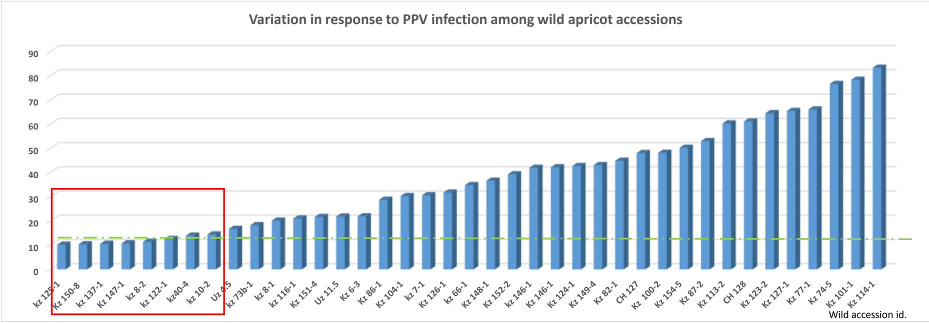


Figure 3: Distribution of the first 39 wild apricot accessions according to their mean viral accumulation value as a percentage of optical density value, over two vegetative cycles. Individuals are ordered from the lowest to the highest. Accessions are considered as resistant when their mean OD value is inferior or equal to the mean OD value of the negative control (green dotted line). The red box displays the most probable, highly resistant wild accessions.

In summary, in the apricot collections of MARS partners, there are, up to now, 858 apricot accessions available. **153 out of the 858 are bearing alleles associated to PPV resistance according to the three *PPVres* markers**. However, after phenotypic validation, in total, **we confirmed 25 new sources of resistance** (8 in the wild germplasm and 16 in the National collections of apricot cultivars). The new putative parents will be made available to European breeders in order to improve durability of PPV resistance in the future cultivars by combining different sources of resistance.

2) *Establishment and transfer to SMEs of a high throughput marker assisted technology of selection of PPV resistant apricot trees*

Europe is characterized by its large variability in agronomical and environmental conditions of stone fruit cultivation, from continental climates characterized by cold winter periods to Mediterranean, arid areas with mild winter periods. This means that, when setting up the production of PPV resistant material for re-planting, it is essential to cover the diversity of agro-ecological conditions of apricot cultivation in Europe. Because of this diversity in environmental conditions, we need to optimize the selection of sharka resistant material which has to be adapted to various European agronomical conditions. In consequence, in MARS, we set up the screening of thousands of apricot progenies, in order to be in adequation with various local cultivation conditions and market requirements, which obviously vary from Mediterranean regions to Eastern European countries. This explains the establishment in MARS of a high-throughput protocol of MAS, for the screening of several thousands of apricot breeding material and the selection of new cultivars adapted to the diversity of apricot production in Europe.

Besides building an efficient and durable resistance to sharka disease at the European level, the MARS project also aimed at boosting the production of sharka resistant stone fruit cultivars by transferring the above procedures of MAS to SMEs to enable targeted, high throughput selection of PPV resistant apricot cultivars adapted for cultivation all over Europe. First of all, a standardised protocol was established to ease the running of molecular analyzes in different biotech laboratories located in different environments, on several thousands of apricot seedlings (**Deliverable D2.1**). This protocol was implemented in laboratories in France, Greece and Turkey, initially with the first set of markers developed in SharCo and then for the second set, developed in MARS. In total, 31,584 apricot seedlings were screened initially with the first set of markers (**Deliverable D2.2**). Material that was scored as resistant in deliverable D2.2 was then tested with the second set of markers, in order to validate and refine the previous data (**Deliverable D2.3**). Results of this high throughput screening for PPV resistant apricot seedlings are depicted in Table 5, below. Overall, **only 9.35% of the seedlings displayed resistant alleles for the two sets of markers, at the seven marker loci, for 54.96% when cumulating at least two sources of resistance** (see Table 2).

Table 5: PPV resistant apricot progenies scored with the first and second set of markers

	Nb seedlings processed with 1rst set	Nb seedlings scored as resistant or recombinant with 1rst set*	Nb seedlings processed with 2nd set	Nb seedlings scored as resistant with 2nd set**	Freq resistant genotype (%)
P01/P11	18,432	10,080	16,128	1,872	10.16
P02/P03	8,736	4,896	4,896	839	9.60
P04	480	384	384	25	5.21
P05	384	288	288	16	4.17
P06	2496	1344	1344	95	3.81
P07	288	192	192	24	8.33
P08	576	384	384	35	6.08
P09	192	192	192	48	25$^{\Omega}$
Total number of progenies screened	31,584	17,760	23,808	2,954	9.35

*scored as resistant when all three loci of the first set of markers displayed allele(s) associated to resistance following Decroocq et al. (2014)

**scored as resistant when apricot samples showed resistance-associated alleles at the 4 marker loci of the second set of markers

Ω This strikingly higher % of resistant genotypes corresponds to one single cross which combines two sources of resistance

This promising material was or is currently being transferred to nurseries for agronomical evaluation.

3) *Selection of new PPV resistant cultivars adapted to local conditions of apricot cultivation and with a high quality and commercial value*

In order to fit the fruit market requirement as well as various cultivation conditions, in parallel to the translation of the tools and methodology to SMEs, a portion of the MARS effort was directed at understanding how to develop a durable resistance to PPV in any European and extra-European orchard. All along the procedure, we kept on feeding the MAS programs established by the SMEs in order to optimize the selection of sharka resistant material adapted to various European agronomical conditions and consumers' taste. For this purpose, we established procedures of agronomical evaluation of putatively resistant apricot pre-breeding material. Agronomical trait evaluation covered from the floral biology, to the fructification, the fruit quality, the yield as well as the tree features (vigour, growth habit, thining and tree management…). Many of those tasks related to the agronomical evaluation of promising, PPV resistant apricot seedlings are still ongoing but orchards were established in each partner country (**Deliverable D2.4**). **A total of 15 varieties resistant to sharka were released** in the course of MARS as described below in Table 6.

Table 6: PPV resistant apricot accessions currently under development in the MARS nurseries

Breeding partner	SME/nursery	Number seedlings	Number of pre-selection	Number of selection	New varieties resistant to sharka	Name of commercial varieties
P01	P11	11,192	300	20	4	Meligat, Elgat, Congat, Anegat
P02/P03	P12	6,000	190	47	--	
P04	P14	244	55	84	--	
P05	P14	348	105	--	--	
P06	nd	1,755	881	--	--	
P07	P16	400	--	--	6	Chariessa, Neraida, Laias, Danais, Tyrvi, Niiris
P08	P15	360	--	101	5	Rojo Pasion, Mirlo blanco, Mirlo anaranjado, Mirlo rojo, Murciana, Valorange
P09	nd	200	117	67	--	
nd: not determined						
-- ongoing task						

In summary, MARS delivered the following key results within the 2 years of the project work-plan (in brackets, corresponding deliverable):

1. Validated tools for SMEs:
   - Set of molecular markers developed in SharCo and validated in MARS (D1.1). This tool was produced by targeting the main locus controlling the resistance trait in apricot donors;
   - Optimized set of molecular markers targeting the other genetic factor linked to the resistance (D1.1). This new set will complement the one developed in SharCo in order to implement a more reliable and broad-spectrum technology of MAS;
2. apricot parents and hybrids which display a high level of resistance to PPV (D1.2 and D1.3);
3. recommendations for breeders:
   - for the establishment of MAS for obtaining PPV resistant varieties (D2.1);
   - for the crosses of PPV resistant parents with locally adapted cultivars (D2.2);
4. Standards for the planting of PPV resistant apricot varieties EU-wide, other than in Turkey (D2.3).

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4. Potential impact and main dissemination activities and exploitation results

- **Expected impact of the implementation of a highthrough-put Marker Assisted Selection for resistance to sharka in apricot**

Attracting end-users such as breeding companies for the selection of PPV resistant fruit trees

The development of Marker Assisted Selection is expected to attract end-users such as private or public breeding companies, European- and world-wide. Up to now, to test an interesting cultivar, one needs 4 years of monitoring after infection to assess the level of resistance. This slows the breeding process, as it is a labour-intensive, time consuming and expensive process the selection of material resistant to PPV . In woody plants, molecular tools have the potential to give us early information on the genetics of *Prunus* progenies and marker-assisted selection is the only short-term solution to rapidly select individuals resistant to sharka disease.

The production and maintenance of a minimum of 6,000 hybrids in an apricot breeding programme costs about 30,000 Euros per year (data provided by nursery SME CEP Innovation). Knowing that the evaluation of PPV resistant material requires a minimum of 4 years, switching from traditional breeding to MAS would allow saving at least 120,000 Euros, per breeding programme. For 8 countries involved in apricot breeding programmes in Europe, MAS would allow saving about 1 million Euro for each year all over Europe.

By the end of the MARS project, a new set of molecular markers and guidelines for the implementation of MAS for resistance to sharka will be proposed to public and private breeders and certification services (legal offices of registration of new cultivars from the CPVO – European Community Plant Variety Office), it will promote the use and plantation of PPV resistant varieties.

Sustainibility of the stone fruit industry

Plantation of PPV resistant cultivars EU-wide is expected to help not only in the management of the sharka disease but also in the sustainable production of apricot fruits and industries (mostly dried fruits in Mediterannean and Eastern rural areas. Transfer of the resistance mechanism(s) available in apricot to other stone fruit species such as diploid plum could also be facilitated, in the long term, with the help of molecular markers linked to the apricot genetic determinants. Indeed, MAS is useful for backcross breeding for the introgression of resistance genes from wild or distantly related species while selecting against the undesirable characteristics of the wild or distinct species. Backcross of the resistance to diploid plum and then to peach is thus 'feasible', at least if molecular markers are available and through the implementation of high throughput MAS. However, it will still require years and several generations to achieve it.

In order to accelerate the selection of sharka resistant and agronomically valuable fruit trees for cultivar deployment, all this plant material and molecular tools was combined to switch from conventional breeding to marker assisted breeding. MARS thus brought methodology, innovative molecular tools and breeding expertise together for the deployment of resistance in apricot cultivars circulating in Europe or under selection.

➢ **Promotion of PPV resistant breeding and pre-breeding material for plantation**

One whole workpackage (Dissemination, or WP3) aimed at putting at the disposal of breeders, nurserymen and fruit producers, resistant apricot plant material limiting the impact of the Sharka disease. For this purpose, part of the MARS activities were devoted to the promotion of sharka resistant breeding material to a range of key stakeholders, including decision makers from industry, early-stage & experienced researchers, farmers, policy makers and consumers. Know-how and plant material will be used to draw standards for the deployment of PPV resistant material. MARS' results was also disseminated through the MARS dedicated website (www.inra.fr/mars) as well as the establishment of a PPV resistance genotyping database (http://users.unimi.it/apricotmap).

We thus aimed at bridging this gap by organising training and research workshops on PPV with special attention paid to end-users such as fruit producers and nurserymen, in Western and European countries. Leaflets, in nationale language, were produced and distributed to gardening shops and nurseries as well as leaflets describing agronomical, morphological and sensorial quality features of newly produced or/and ready-to-be released, sharka resistant cultivars (see Deliverable . They were transferred to farmers, nurseries and extension services by MARS partners; and uploaded on the MARS website, for further dissemination and printing.

Four types of dissemination activities were performed in the course of MARS, as follows:

- **Training workshops on the multiplication and cultivation of PPV resistant apricot material** were organised during the first period of MARS, in Spain and Romania, in national languages. Other workshops were organised over the second period, in Spain, Bulgaria, Turkey, Greece, Italy and France. They addressed to fruit producers, managers of major nurseries and advisers from Agricultural Extension Services all over Europe (see all details, copies of the work programme and minutes of the day in the WP3's second periodic report, available on the MARS collaborative platform). For a complete list of training workshops that took place in the second period, see Table 7 or **Deliverable D3.2**.

Table 7: MARS training workshops organised by the MARS partners

Partner(s) organising the workshop	Place of the training workshop	Date	Nb of attendees
First period			
P04	Bucharest-Romania	20/11/2014	107
P15	Barranda,Murcia-Spain	30/04/2014	200
Second period			
P02	Metaponto-Italy	25/06/2015	200
	Cesena,Martorano-Italy	10/07/2015	90
P05	Plovdiv-Bulgaria	26/06/2015	60
P06	Adana-Turkey	14/05/2015	72
	Malatya-Turkey	05/10/2014	156
P07	Platanoreum,Servia-Greece	07/03/2015	80
P11	Lyon-France	11/07/2015	165
P12	Metaponto-Italy	25/06/2015	200
	Cesena,Martorano-Italy	10/07/2015	90
P15	Murcia-Spain	22/05/2015	60
P16	Platanoreum,Servia-Greece	07/03/2015	80

The MARS leaflet produced in the first period by partners P12 and P06 was translated in national languages and disseminated during their respective training workshops or within their stakeholders' networks.

- Research Institutions and SMEs participated at **meetings and orchard visits** under Plant Protection Service and their own organizations, respectively. They were mainly dedicated to PPV control strategies, their effectiveness, their cost-effectiveness, and the end-users perception, including the development of resistant cultivars.

- **MARS last dissemination event** was organised concomitant to the Fruit Attraction fair, at IFEMA, Madrid, on the 29th of October 2015. Three communications were delivered:
  - Welcome and Introduction of the MARS project by Véronique Decroocq (INRA, partner P01)
  - The Marker Assisted Selection technology: Legacy of the SharCo FP7 project by Daniele Bassi and Filippo Geuna (UMIL, partner P02)
  - The MARS results by Fabienne Moreau (ADNid, partner P10) and Manuel Rubio (CEBAS-CSIC, partner P08)

MARS partners also presented the outcomes of the MARS project in their own breeding or research program, through the display of posters, on site. All communications and posters are available online at www.inra.fr/mars/.

- **Publications in peer-reviewed international journals as well as local, vulgarisation newspapers and participation to international symposium and conferences**, especially to the World Expo in Milano, where the outcomes of the FP7 SharCo and MARS projects were presented at the EU pavilion, on the 15th of July 2015 (see From SharCo to MARS: A translational research path to solve a major phytosanitary problem http://europa.eu/expo2015/node/731). See the complete list of dissemination tools in the section *Use and Dissemination of the Foreground*.

- **A portal and a database for Marker Assisted Selection of PPV resistant apricot trees**

A relational database, based on the 4D (4th Dimension) software has been designed in order to gather and store MARS genotyping data. This database was set up for running under the Windows 2012 Server operating system on an HP Proliant server hosted at the University of Milano (partner P02, UMIL) (see **Deliverable D3.3**). The database has been fed with genotyping data from the first set of markers developed in SharCo. The process of feeding up the database with data from the second set of markers is still ongoing. Those data are coming from around **25,090 individuals which after screening with the two sets of molecular markers provided 71,608 genotypes**. The database is accessible and queryable by the partners and, ultimately (once initial results are suitably published), will be opened to the international community, once the last batch of data is uploaded. An hyperlink will be placed on the international Genome Database for Rosaceae (GDR) (www.rosaceae.org).

5. Use and dissemination of foreground

Section A: Dissemination of foregrounds

Original research papers issued from MARS, published in peer-reviewed journals and cited in the text

1. Decroocq, S., Chague, A., Lambert, P., Roch, G., Audergon, J. M., Geuna, F., Chiozzotto, R., Bassi, D., Dondini, L., Tartarini, S., Salava, J., Krska, B., Palmisano, F., Karayiannis, I., Decroocq, V. (2014). Selecting with markers linked to the *PPVres* major QTL is not sufficient to predict resistance to *Plum Pox Virus* (PPV) in apricot. *Tree Genetics and Genomes*, 10 (5), 1161-1170. DOI : 10.1007/s11295-014-0750-0.

2. Mariette S., Wong Jun Tai F., Roch G., Barre A., Chague A., Decroocq S., Groppi A., Laizet Y., Lambert P., Tricon D., Nikolski M., Audergon J-M, Abbott AG, Decroocq V (2016) Genome wide association links specific genes to resistance to *Plum Pox Virus* in apricot (*Prunus armeniaca*). *The New Phytologist* 209: 773–784. doi:10.1111/nph.13627.

Section B: Use of Foregrounds

PATENTS and TRADEMARKS, REGISTERED DESIGNS

Not applicable here

FOREGROUNDS

For more details concerning the MARS exploitation plan, please see **deliverable D2.5**

Exploitable foreground	Exploitable products or measures	Sectors of application	Timetable / commercial use	Patents or other IPR exploitation	Owners and other beneficiaries
Natural resistance to sharka	PPV resistant apricot material (pre-selection)	breeding	>2 years for PPV resistance confirmation	MTA	P08 and MARS partners involved in characterization
Natural resistance to sharka	PPV resistant apricot material (pre-selection)	breeding	Not defined yet	MTA	P01 and P11
Natural resistance to sharka	PPV resistant apricot material (breeding stock)	breeding	timetable for use in breeding (> 2 years)	MTA	P01 and Pomological institute in Almaty and Botanic garden in Bishkek
Natural resistance to sharka	PPV resistant apricot material (breeding stock)	breeding	timetable for use in breeding : 2 years	MTA	all except P10, P13 and P17
Natural resistance to sharka	PPV resistant apricot material (cultivars)	nurseries, growers, marketers	within 5 to 8 years	NPA or ML	P1, P2, P4, P5, P7, P8, P9, P11, P12
Marker Assisted Breeding for resistance to sharka	Molecular diagnosis tool using markers implemented by the MARS partners	breeding, biotech companies	now	none	P10, P13, P17, and P1, P2, P3
Marker Assisted Breeding for resistance to sharka	Molecular diagnosis tool based on markers under development in MARS	breeding, biotech companies	within the next 5 years	exclusivity on the knowledge for future development of new products	P10, P13, P17
Genotyping data in apricot progenies and accessions	Genotyping database	breeding, extension services, Plant Genetic Resources	now	none	P2 and data from the whole MARS consortium