Document downloaded from:

[\[http://redivia.gva.es/handle/20.500.11939/6541\]](http://redivia.gva.es/handle/20.500.11939/6541)

This paper must be cited as:

[Soriano, J. M., Vera-Ruiz, E. M., Vilanova, S., Martínez-Calvo, J., Llácer, G., Badenes, M. L., & Romero, C. (2008). Identification and mapping of a locus conferring plum pox virus resistance in two apricot-improved linkage maps. *Tree Genetics & Genomes*, 4(3), 391-402.]

ivia
Institut Valencià
d'Investigacions Agràries

The final publication is available at

[\[http://dx.doi.org/10.1007/s11295-007-0118-9\]](http://dx.doi.org/10.1007/s11295-007-0118-9)

Copyright [Springer]

Identification and mapping of a locus conferring *Plum Pox Virus* resistance in two apricot improved linkage maps

J.M. Soriano¹, E.M. Vera-Ruiz¹, S. Vilanova^{1,2}, J. Martínez-Calvo¹, G. Llácer¹, M.L. Badenes¹ and C. Romero^{1*}

¹*Instituto Valenciano de Investigaciones Agrarias (IVIA), Apartado Oficial 46113 Moncada, Valencia, Spain.*

²*Present address: Departamento de Genética, IRTA Centre de Cabrils. Ctra. de Cabrils, s/n. 08348 Cabrils (Barcelona) Spain*

* Corresponding author: C. Romero

e-mail: cromero@ivia.es

Tf: 34 96 342 40 00

Fax: 34 96 342 40 01

ABSTRACT

Sharka disease, caused by the *Plum Pox Virus* (PPV), is one of the major limiting factors for stone fruit crops in Europe and America. In particular, apricot is severely affected suffering significant fruit losses. Thus, PPV resistance is a trait of great interest for the apricot breeding programs currently in progress. In this work, two apricot maps, earlier constructed with the F₁ ‘Goldrich x Currot’ (‘GxC’) and the F₂ ‘Lito x Lito’-98 (‘LxL-98’) populations, have been improved including 43 and 37 new simple sequence repeat (SSR) loci, respectively, to facilitate PPV resistance trait mapping. Screening of PPV resistance on the segregating populations classified seedling phenotypes into resistant or susceptible. A non-parametric mapping method, based on the Kruskal-Wallis (KW) rank sum test, was initially used to score marker-trait association and results were confirmed by Interval Mapping. Contrary to the putative digenic model inferred from the phenotypic segregations, all significant markers for the KW statistic

($P < 0.005$) mapped in a unique region of ~21.0 and ~20.3cM located on the upper part of the LG1 linkage group in 'GxC' and 'LxL-98' maps, respectively. According to the data, PPV resistance is suggested to be controlled by at least one major dominant locus. The association between three SSRs, distributed within this region, and PPV resistance was tested in two additional populations ('Goldrich x Canino' and 'Lito x Lito'-00) and breeding program parents. The marker *ssrPaCITA5* showed the highest KW value ($P < 0.005$) in all cases pointing out its usefulness in marker-assisted selection.

Keywords: Apricot linkage map, Marker-Assisted Selection (MAS), PPV resistance

INTRODUCTION

Sharka disease, caused by the *Plum Pox Virus* (PPV), was detected for the first time in Spain in 1984 (Llácer et al. 1985). Since then, it spread throughout the country seriously affecting apricot cultivation as all native cultivars were susceptible to PPV. Attempts to block the spread of the disease through eradication of the infected trees were unsuccessful. In order to solve this problem in the long term, two breeding programs aimed at introducing PPV resistance from apricot sources, were initiated in Spain (Egea et al. 1999; Badenes et al. 2002), as was done earlier in France (Audergon 1995), Italy (Bassi et al. 1995) and Greece (Karayiannis et al. 1999). A few promising resistant selections have already been obtained by conventional breeding (Badenes et al. 2002), however the PPV resistance screening method used is very time consuming and significantly diminishes the selection efficiency being a 'bottle neck' in the selection process. This screening method is based on a biological test that uses susceptible 'GF-305' peach seedlings, as woody indicators of disease, and involves several cold cycles followed by a Double-Antibody Sandwich Indirect Enzyme-Linked Immunosorbent Assay (ELISA-DASI) (Moustafa et al. 2001).

In this context, marker-assisted selection (MAS) for PPV resistance would improve breeding efficiency. Among the different strategies, to search for trait-linked markers genetic maps are one of the most convenient tools. Three *Prunus* linkage maps, based on apricot intraspecific crosses introducing PPV resistance from the North American cultivars ‘Goldrich’ (Hurtado et al. 2002) and ‘Stark Early Orange’ (‘SEO’) (Salava et al. 2002; Vilanova et al. 2003), have been used to analyze PPV resistance. Hurtado et al. (2002) and Vilanova et al. (2003) tentatively mapped the PPV resistance trait in the linkage group 1 (LG1) of the F₁ ‘Goldrich x Currot’ (‘GxC’) and the F₂ ‘Lito x Lito’-98 (‘LxL-98’) population maps, respectively. A similar location was obtained by Salava et al. (2002) in the BC₁ population map ‘LE-3246 x Vestar’ where *PPVres1* was flanked by two AFLP-markers (EAA-MCAG8 and EAG-MCAT14) in a region spanning ~9.3 cM. Additionally, Decroocq et al. (2005) performed a quantitative trait loci (QTL) analysis for PPV resistance on a F₁ population derived from the interspecific cross *Prunus persica* x *Prunus davidiana*, where resistance was introduced from the *P. davidiana* clone P1908. Up to six PPV resistance QTLs were identified on this map and two of them (*PPV-1.1* and *PPV-1.2*) were shown to be located on linkage group 1.

In the three apricot populations analyzed, PPV resistance was mapped as a single-locus controlled trait. However, the segregation ratio obtained in these cases deviated significantly from that expected for a single dominant locus and more closely approximated that for two dominant independent loci (Hurtado et al. 2002; Salava et al. 2002; Vilanova et al. 2003). Thus, these results supported the hypothesis of a digenic inheritance proposed by Dosba et al. (1991) in contrast with the monogenic control proposed by Dicenta et al. (2000) and Karayannis et al. (2006). In addition, as reported above, QTL analysis for PPV resistance on *P. davidiana*, based on an ordinal phenotypic scale to assess PPV infection, also presented evidence supporting the

involvement of several loci in the control of the trait (Decrooq et al. 2005). Nevertheless, the apricot response to PPV infection could only be rated on a binary scale (resistant vs. susceptible) (Salava et al. 2002; Vilanova et al. 2003). The standard QTL mapping approaches assume polygenic inheritance and can behave poorly for binary traits. Several adaptations have been proposed to estimate genetic distance between markers and binary trait locus (BTL) including non-parametric methods (McIntyre et al. 2001).

In this paper, we report the detection and location of a genomic region associated with PPV resistance scored as binary trait. This region was located on very similar positions in two improved apricot maps derived from the F₁ ‘GxC’ and the F₂ ‘LxL-98’ populations. Several markers linked to PPV resistance were identified and tested for MAS in a set of susceptible/resistant apricot cultivars and two additional segregating populations.

MATERIALS AND METHODS

Plant material

Two families were used for mapping PPV resistance (Table 1), an F₁ resulting from the cross ‘Goldrich x Currot’ (‘GxC’) and an F₂ derived from the self-fertilization of the PPV resistant cultivar ‘Lito’ (‘LxL-98’), earlier used to construct two apricot linkage maps (Hurtado et al. 2002; Vilanova et al. 2003). ‘GxC’ was previously described by Hurtado et al. (2002) as ‘GxV’ progeny and ‘LxL-98’ as ‘LxL’ by Vilanova et al. (2003). To analyze SSRs selected for MAS, two additional segregating populations were used, ‘Goldrich x Canino’ (‘GxCa’) derived from the cross between the PPV resistant cultivar ‘Goldrich’ and the PPV susceptible cultivar ‘Canino’ and an extension of the ‘LxL-98’ family designated as ‘LxL-00’. A total of 7 PPV resistant (‘SEO’, ‘Lito’, ‘Goldrich’, ‘Harcot’, ‘Sunglo’, ‘Veecot’ and ‘Pandora’) and 11 PPV susceptible

apricot cultivars ('Tyrinthos', 'Currot', 'Ginesta', 'Canino', 'Mitger', 'Palau', 'Bergeron', 'Katy', 'Pepito', 'Moniqui' and 'Colorao') (Martínez-Gómez et al. 2000) were also used to test those SSRs selected for MAS.

DNA isolation

DNA was extracted from 50 mg of young leaves following the method of Doyle and Doyle (1987). DNA quantification was performed by comparison with lambda DNA (Promega, Madison, WI).

Screening for PPV resistance

Evaluation of PPV resistance in the studied families was performed according to the biological test described by Moustafa et al. (2001) using the PPV Dideron strain 3.3 RB (Asensio 1996) and 'GF-305' peach seedlings as woody indicators. The virus presence was analysed by visual scoring of symptoms and confirmed by ELISA-DASI (Lommel et al. 1982) using the 5B-IVIA monoclonal antibody against the PPV coat protein (Cambra et al. 1994). A total of twelve replications of each seedling were evaluated. RT-PCR analyses were performed occasionally to verify uncertain results (Wetzel et al. 1991). Phenotypic scoring based on presence/absence of leaf symptoms classified seedlings into susceptible (0)/resistant (1).

SSR markers

A total of 170 and 150 SSRs from peach and apricot were screened in the 'GxC' and the 'LxL-98' populations, respectively (Table 1 Suppl.). SSR amplifications were performed in a GeneAmp®PCR System 9700 thermal cycler (Perkin-Elmer Corp., Freemont, CA) in a final volume of 10 µl containing 75 mM Tris-HCl pH 8.8, 20 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 0.1 mM of each dNTP, 0.8 µM of each primer, 20 ng of genomic DNA and 1 Unit of Taq polymerase (Invitrogen, Carlsbad, CA) using the following temperature profile: 94°C for 2 min, then 35 cycles of 94°C for 45 s, 50-60°C

for 1 min and 72°C for 1 min and 15 s, finishing with 72°C for 5 min. PCR products were separated by electrophoresis in 3% (w/v) MS-8 agarose (Pronadisa, Madrid, Spain).

Linkage groups

The linkage analysis was carried out using JoinMap 3.0 software (Van Ooijen and Voorrips 2001) with the Kosambi mapping function (Kosambi 1944) used to convert recombination units into genetic distances.

In the ‘GxC’ population two separated genetic linkage maps were constructed for each parent following the “two-way pseudo test-cross” model of analysis (Grattapaglia and Sederoff 1994) and setting a “cross-pollinator” data type. The original maps (Hurtado et al. 2002) were updated by adding 43 new SSR markers. In the ‘LxL-98’ population the linkage analysis was carried out setting F₂ data type and the original map (Vilanova et al. 2003) was updated by adding 37 new SSR markers. Linkage groups were established using as threshold a minimum logarithm of odds (LOD) of 5.0. In general, linkages considered for mapping were those with recombination frequency lower than 0.4 and LOD score larger than 3.0.

Statistical analysis and BTL identification

Binary trait analysis was performed on the two different updated maps (‘GxC’ and ‘LxL-98’) using MapQTL version 4.0 (Van Ooijen et al. 2000). Since PPV resistance scorings were not normally distributed, the Kruskal-Wallis (KW) rank-sum test (Lehmann 1975), with a threshold value of $P < 0.005$, was first applied individually to each segregating locus to test for associations between markers and PPV resistance. Subsequently, Interval Mapping (IM) analysis (Lander and Botstein 1989; Van Ooijen 1992) was performed in order to support the detection of putative BTLs by KW test. The LOD chromosome-wide significance threshold to decide upon the presence or

absence of a BTL for IM (Van Ooijen 1999) was determined with a 5% significance level by using permutation tests (Churchill and Doerge 1994) carried out on LG1 and corresponded to a value of 3.0 in both maps. A confidence interval around the position of the largest LOD was indicated by two-LOD support interval (Van Ooijen 1992).

Putative double recessive epistatic interactions between *ssrPaCITA5* and other co-dominant markers were assessed with the KW test ($P < 0.05$) and multiple comparisons (Dunn 1964) considering differences among the four genotype groups categorized by two SSRs (A-B-/A-Bb/aaB-/aabb).

RESULTS

‘GxC’ and ‘LxL-98’ improved linkage maps

In order to facilitate the identification and location of loci associated with PPV resistance, new SSRs were incorporated into two apricot linkage maps earlier constructed with the intraspecific populations F₁ ‘GxC’ (Hurtado et al. 2002) and F₂ ‘LxL-98’ (Vilanova et al. 2003).

A total of 170 SSRs from different sources were tested in the ‘GxC’ progeny (Table 1 Suppl.) and 148 out of them (87%) could be amplified. Fifty-five out of amplified SSRs were polymorphic (37%), the rest were monomorphic or produced complex patterns. Fourteen out of these polymorphic SSRs were heterozygous in both parents, 26 only in ‘Goldrich’ and 15 only in ‘Currot’.

Thirty-seven SSR loci were incorporated into the ‘Goldrich’ map and distributed throughout the genome, ranging from eight markers in LG1 to one marker in LG3 (Table 2 Suppl.). Twelve out of them correspond to co-dominant loci and 25 to dominant loci. Two additional SSRs tested by Hurtado et al. (2002) but unmapped in the previous map, UDP98-409 and UDP98-412 (Cipriani et al. 1999), were also incorporated into the ‘Goldrich’ map. Four markers (10.5%) deviated significantly from

the expected F_1 segregation ratio at $P < 0.01$. The improved ‘Goldrich’ map is organized in 8 linkage groups covering a distance of 468cM and comprising 139 loci: 63 AFLPs, 48 SSRs, 25 RAPDs and 3 RFLPs (Figure 1). The average distance between adjacent markers was 3.4cM, ranging from 2.4cM in LG6G to 6.3cM in LG8G.

Seventeen SSR loci were incorporated into the 8 linkage groups of the ‘Currot’ map, ranging from one marker in LG2 and LG4 to 5 markers in LG5 (Table 2 Suppl.). Ten resulted in co-dominant loci and 7 in dominant loci. Five markers (29%) showed skewed segregation ratios at $P < 0.01$. The ‘Currot’ map covers a distance of 451cM and comprises 89 loci: 42 AFLPs, 26 SSRs, 21 RAPDs and 1 RFLP (Figure 1). The average distance between adjacent markers was 5.1cM, ranging from 3.0cM in LG8C to 7.0cM in LG4C.

Fifteen SSRs heterozygous in both parents provided bridges between the two maps. In addition, part of the mapped SSRs allowed us to establish homologies with other *Prunus* maps: twenty-three were held in common with the *Prunus* reference map derived from the almond-peach cross ‘Texas’ x ‘Earlygold’ (Dirlewanger et al. 2004a), 37 with the map of Dirlewanger et al. (2004b) obtained from a myrobolan plum x [almond x peach] progeny and 23 with the peach map of Yamamoto et al. (2005).

A total of 150 SSRs from peach and apricot were tested in the ‘LxL-98’ progeny (Table 1 Suppl.) and segregation was demonstrated for 47 (31%) of them. The rest of SSRs could not be amplified, produced complex patterns or were monomorphic. From the 47 polymorphic SSRs, 37 were mapped in the ‘LxL-98’ map (Table 2 Suppl.), 33 were co-dominant loci and 4 dominant. One SSR tested by Vilanova et al. (2003) but unmapped in the previous map, pchgms2(1) (Sosinski et al. 2000), was incorporated with the new markers. In total, 38 SSR markers were added to the map being distributed throughout the genome and ranging from 15 markers in LG1 to 3 markers in LG2 and

LG3. No markers were mapped on LG4 (Table 2 Suppl.). Nine markers (23%) deviated significantly from the expected F_2 segregation ratio at $P < 0.01$. The map is organized in 8 linkage groups covering a distance of 615cM and comprising 231 loci: 154 AFLPs, 63 SSRs and 14 AFLP-RGAs (Soriano et al. 2005) (Figure 2). The average distance between adjacent markers was 2.6cM, ranging from 1.3cM in LG4 to 8.4cM in LG3. the mapped SSR markers established homologies with other *Prunus* maps: twenty-five were held in common with the *Prunus* reference map (Dirlewanger et al. 2004a), 38 with the map of Dirlewanger et al. (2004b) and 22 with the map of Yamamoto et al. (2005).

Identification of a BTL for PPV resistance

PPV infection in parents and progenies was evaluated after every dormancy cycle by assessing the presence of symptoms and later confirmed by ELISA-DASI. The inoculation efficiency was very high and over 95% of the ‘GF-305’ rootstocks developed symptoms. However, distribution of symptoms was highly irregular among seedling shoots hampering the rating of viral symptoms in intermediate grades. Thus, PPV resistance phenotype was scored as resistant or susceptible to avoid misclassifications. Most susceptible seedlings were detected after the first cycle, but about 15-40% (depending on the population) were scored resistant in cycle 1 and resulted susceptible in cycle 2 (Table 1). Evaluation after additional cycles did not detect significant variations (data not shown).

The resistant/susceptible ratio does not fit with monogenic models neither in the mapping populations nor in the two additional progenies analyses (Table 1). However, all segregations fit better with a digenic model except for ‘GxCa’, where three genes are necessary to explain the segregation obtained (data not shown). Co-segregation analysis between PPV resistance and markers from the ‘GxC’ and ‘LxL-98’ apricot maps

showed that only a few markers located on LG1 showed a recombination frequency (θ) lower than 0.4 and LOD > 3.0 (Table 2). However, the inclusion of the PPV resistance gene in the map modified significantly the original distances among the marker loci, as expected for a trait which segregation fit with a digenic model thus being highly distorted in all populations.

The possible involvement of several loci in the control of the PPV resistance trait was initially studied following the KW non-parametric test. In agreement with the cosegregation analysis, only markers located on the upper part of LG1 in both maps were significant ($P < 0.005$) for the KW test (Table 2 and Figure 3). The $P < 0.005$ significance intervals comprised regions of ~21.0cM in the 'Goldrich' map, between UDAp415 and ssrPaCITA17 (38.1-59.1cM map positions), and ~20.3cM in the 'LxL-98' map, between EAA-MCTA1 and EAC-MCAT13 (25.2-45.5cM map positions) (Figure 3). All the dominant markers in the 'LxL-98' map showing KW significant values come from the resistant parent 'SEO', since those coming from 'Tyrinthos' were linked in repulsion with the resistance, being not included in the analysis.

BTL identification was also performed with the Interval Mapping (IM) procedure in order to support the results obtained with the KW test, and a general agreement was observed between both methods (Figure 3). In spite of the limitation that the binary phenotype distribution supposes for IM, this method confirmed the detection of one BTL on LG1 in both maps. In 'Goldrich' the two-LOD support interval for this BTL is located within a ~11.4cM genomic region (between markers AA-CTT14 and AA-CCC1), and in 'SEO' within a region of ~9.1cM (between EAG-MCTT1 and EAT-MCTC9) (Figure 3). Interestingly, no other BTLs were found by IM. Table 2 shows the statistics summary for the highly significant markers located between two SSRs flanking these intervals (SC6A6 and ssrPaCITA17).

Possible epistatic interactions, that might explain PPV resistance segregations, were tested between the highest KW significant SSR within the BTL associated genomic region identified on LG1 (ssrPaCITA5) and other co-dominant markers residing on a different region of the same chromosome or on different chromosomes. These markers showed relatively high KW values but were non-significant since, as reported above, all the KW significant markers were located in the upper part of LG1. Therefore, as expected from the KW test results, no significant epistatic interactions with a threshold value of $P < 0.05$ were detected in any of the two maps (data not shown).

MAS in apricot breeding

SSRs flanking the BTL associated region identified on LG1 (SC6A6 and ssrPaCITA17) and the SSR showing highest KW and IM LOD score values (ssrPaCITA5) were screened in a set of resistant/susceptible apricot genotypes (Martínez-Gómez et al. 2000) and two additional apricot populations ('GxCa' and 'LxL-00') to validate their association with PPV resistance.

Table 3 shows that ssrPaCITA5 and ssrPaCITA17 alleles linked to PPV resistance were detected in all resistant cultivars but not in susceptible ones. However, SC6A6 PPV resistance linked-allele was also detected in two susceptible cultivars ('Mitger' and 'Palau'). SSRs located on different linkage groups did not show any association with PPV resistance in the set of apricot genotypes analyzed (data not shown).

The association between these markers and PPV resistance in 'GxCa' and 'LxL-00' measured with the KW test (Table 4). Two SSRs, M3b located on the lower region of LG1 and CPPCT-13 located on LG5, were used as negative controls. As previously reported for the 'GxC' population, ssrPaCITA5 showed the highest KW statistic value in the 'GxCa' population. In 'LxL-00' the three markers were highly significant for the KW statistic ($P < 0.0001$) although when merging 'LxL-98' and 'LxL-00' data

ssrPaCITA5 again shows the highest significant value (data not shown). SSR negative controls showed non-significant values in both populations.

To test the potential use of these markers for MAS the percentage of mis-classified seedlings was determined in the four populations studied (Table 5). Seedling classification into resistant or susceptible phenotype based on ssrPaCITA5 is noticeably more accurate than with the other markers (Table 5). Particularly, percentages of seedlings without ssrPaCITA5 classified as PPV resistant (2-10%) are lower than those obtained with SC6A6 or ssrPaCITA17 in all cases (from 6% to 21%).

DISCUSSION

Improved apricot linkage maps

Saturation of the ‘GxC’ and ‘LxL-98’ apricot linkage maps (Hurtado et al. 2002; Vilanova et al. 2003) was moderately high, although clearly insufficient in ‘GxC’ where not all the groups corresponding to the basic chromosome number of *Prunus* (n=8) could be defined. Moreover, the number of co-dominant markers contained in both maps was fairly small. Thus, to facilitate the use of these maps for MAS or synteny studies up to 43 and 37 new SSR markers, respectively, were incorporated in this work.

The new ‘Goldrich’ map covers 468cM with an average distance between markers of 3.4cM, significantly lower than the 3.9cM determined in the original map (Hurtado et al. 2002). As cited above, initially only 5 of the 8 expected linkage groups were identified (Hurtado et al. 2002), but in the improved map 8 groups were obtained noticing that two of the 5 previous groups were formed by joining together 2 different groups (LG1G-LG8G and LG2G-LG5G). The new ‘Currot’ map covers 451cM with an average distance of 5.1cM in contrast with the 5.8cM of the previous map (Hurtado et al. 2002). Sixteen SSRs established bridges between the 8 linkage groups of both maps maintaining the co-linearity in the majority of them. The apricot genome length

estimated using the method of Meagher et al. (1988) was between 800 and 1200 cM, therefore the 'Goldrich' map may cover from 39% to 58% of the nuclear genome and the 'Currot' map from 37% to 56%.

The new 'LxL-98' map covers 615cM with an average distance of 2.6cM lower than the 3.3cM of the previous map (Vilanova et al. 2003) and lower than those obtained in most of the *Prunus* maps (Bliss et al. 2002; Dirlewanger et al. 2004b; Yamamoto et al. 2005) except Dirlewanger et al. (2004a). According to Meagher et al. (1988) this linkage map may cover from the 52% to 77% of the nuclear genome. In these apricot maps LG1 and LG6 were the largest linkage groups in agreement with results observed in most *Prunus* maps (Dirlewanger et al. 2004a; Dirlewanger et al. 2004b; Yamamoto et al. 2005) and with cytogenetic data suggesting the existence of a chromosome larger than the rest in *Prunus* (Jelenkovic and Harrington 1972).

Segregation distortion at $P < 0.01$ was observed for 17% of markers in 'Goldrich' and 22% in 'Currot'. These markers were distributed throughout the genome but on LG5 appears a cluster with 9 skewed markers in 'Goldrich' and 5 in 'Currot' suggesting that this region may be subjected to selection. In the 'LxL-98' map 54% of the distorted loci were located on the LG1 upper part. Vilanova et al. (2003) suggested a selection at pre- or post-zygotic level against lethal or sub-lethal genes located in this region. In addition, 27% of the distorted loci were placed on LG6 due to the semi-compatible self-fertilization of 'Lito' controlled by the *S*-locus located on LG6 (Vilanova et al. 2003).

New SSRs on the 'GxC' and 'LxL-98' maps established homologies with other *Prunus* maps (Dirlewanger et al. 2004b; Yamamoto et al. 2005) and increased the number of anchor markers with the *Prunus* reference map (Dirlewanger et al. 2004a) from 14 to 23 in 'GxC' and from 22 to 25 in 'LxL-98'. These markers were essentially collinear with other *Prunus* maps supporting the high degree of synteny observed within

this genus (Dirlewanger et al. 2004a). Comparative mapping studies developed in the last years will facilitate the future use of MAS in fruit breeding.

Genetic control and mapping of PPV resistance trait

To date genetic control system of PPV resistance remains unknown and this is being an important handicap for the breeding programs. In addition, attempts to locate this trait in the available maps have not been completely successful hindering the MAS development. Several reasons may explain this situation: the strong environmental dependence of PPV resistance scoring (Decroocq et al. 2005), the difficulty into evaluating this trait on large-scale experiments and the differences in the methods of evaluation used by research groups (Llácer et al. 2006).

In this work we report the analysis of PPV resistance segregation data in four populations: two F_1 , 'GxC' (Hurtado et al. 2002) and 'GxCa', and two F_2 , 'LxL-98' (Vilanova et al. 2003) and 'LxL-00'. All observed segregations deviated significantly from the expected ratio for a single dominant locus (1:1 in F_1 and 3:1 in F_2) but fit with a model of two dominant independent loci controlling PPV resistance (1:3 in F_1 and 9:7 in F_2) except 'GxCa' consistent with a trigenic model. These results suggest that several loci may be involved in the resistance control. The digenic model would correspond to a double recessive epistasy in which the dominant alleles of both genes are necessary to provide resistance. However, a digenic control for plant pathogen resistance does not seem to be very frequent. In one of the few examples available from the literature, Suwabe et al. (2003) initially found two SSRs linked to two possible independent genes involved in the resistance to clubroot in *Brassica rapa* L., but final results supported an oligogenic control.

If monogenic control is not assumed it is not possible to map the PPV resistance trait as a single marker and QTL approaches become necessary. Since attempts to determine

intermediate phenotypes with certain guarantees were not completely successful, PPV resistance was scored as a binary trait (resistant vs. susceptible). These kind of traits, non-normally distributed, are not infrequent but the use of standard QTL approaches for such traits may lead to low power or unacceptably high false positive rates (Kruglyak and Lander 1995). The non-parametric mapping method based on the KW rank sum test enables mapping QTL when a spike in the phenotype distribution occurs and therefore the usual normality assumption can not be made (Broman 2002). However, the use of parametric statistical methods is recommended to confirm non-parametric QTL mapping results when there is non-normality (Kruglyak and Lander 1995; Caranta et al. 1997).

Binary traits analysis for PPV resistance performed with the KW test and confirmed by IM revealed the presence of a putative single BTL in the upper region of LG1 in two different apricot improved maps. The KW test defined BTL confidence intervals, comprising markers significant at $P < 0.005$, very similar in size (~21.0 cM) and location in the two maps. The consistency of these results is reinforced if we consider that these two maps are based on two different populations F_1 and F_2 derived from two different resistance donors ('Goldrich' and 'SEO'). Moreover, although not totally coincident, map localizations proposed for PPV resistance trait are consistent with those determined by Salava et al. (2002) in the apricot 'LE-3246 x Vestar' cross and Decroocq et al. (2005) in the *P. persica* x *P. davidiana* cross.

The identification of only one genomic region involved in PPV resistance by KW and IM analyses disagrees with the digenic (or even trigenic) model suggested by the segregations observed. Two main hypotheses may explain these contradictory results. First, a possible bias in the PPV resistance evaluation due to the mis-classification of resistant seedlings as susceptible. In fact, the latent resistance of seedlings and

selections after showing symptoms of PPV infection has been already documented in apricot (Karayiannis 2006). This possibility would approximate segregations to those expected for one single locus. An alternative explanation might be that KW and IM analyses only detected a major gene located on LG1 but not some modifier genes with minor effects located throughout the genome. A similar model has been proposed previously for the resistance against *Cucumber Mosaic Virus* in pepper (Caranta et al. 1997), for the mildew resistance in sunflower (Gentzbittel et al. 1998) and for the resistance to *Plasmodiophora brassicae* in Chinese cabbage (Kuniguki et al. 1997).

In conclusion, based on the available evidence, we suggest that PPV resistance in apricot is controlled by at least one major dominant gene located in the upper region of LG1 although the involvement of other minor genes can not be discarded at this time.

MAS in apricot breeding

The evaluation of PPV resistance is one of the main limitations for the apricot breeding programs due to the high costs of time and space that are required. At the IVIA since 1993 near 30 progenies comprising more than 4000 individuals have been generated. Every year, if the conditions are favourable, 500 seedlings can be analyzed requiring total of 13 months for each. Four months are necessary to grow the woody indicators and evaluation itself takes 9 months, including the bud grafting, two cold treatments and two periods of symptoms observation. In this context, the use of molecular markers linked to PPV resistance would significantly increase the breeding program efficiency.

Among the selected SSR tested for MAS *ssrPaCITA5* was the most effective. Depending on the population type the proportion of susceptible seedlings misclassification with this marker varies from 41% to 69%. However, if we use this marker to discard those seedlings without the marker ~50% of the F₁ seedlings and ~25% of

the F₂ seedlings would be removed, the majority of them susceptible, while preserving most resistant seedlings (>90% in F₁ and >95% in F₂ populations). These results, although modest in comparison with MAS studies in other species like rice (Hittalmani et al. 1995) or apple (Kellerhals 2000; Tartarini et al. 2000) might still be useful in apricot breeding programs considering the huge limitations reported above particularly relevant in fruit tree crops. Saturation of LG1 should be pursued in the future to facilitate MAS in breeding programs and to tackle map based cloning of the major PPV resistance gene.

Moreover, the presence of the *ssrPaCITA5* and *ssrPaCITA17* PPV resistance linked-alleles in all resistant cultivars studied is specially interesting since four different sources of PPV resistance ('SEO', 'Sunglo', 'Reliable' and *Prunus mandchurica* sp.) are represented in the 7 resistant cultivars analysed (Karayiannis 2006). These results suggest a possible common origin for the PPV resistance however, this remains to be investigated.

Acknowledgements We want to thank Dr. A.G. Abbott and Dr. M.J. Aranzana for their helpful revision of the manuscript. This research was supported by a grant from the Ministerio de Ciencia y Tecnología (AGL2004-04126-C02-02/AGR). The authors also thank D. Archelós and L. Ramirez, for their technical contributions. J.M.S. and E.M.V.R. were funded by a fellowship from the Ministerio de Educación y Ciencia of Spain. All the experiments described in this paper comply with the current laws of Spain.

REFERENCES

Abernathy D, Zhebentyayeva T, Abbott AG, Vilanova S, Badenes ML, Salava J, Polák J, Krska B, Damsteegt VD (2004) Molecular genetic mapping of the *plum pox virus* resistance genes in apricot. *Acta Hort* 657:283-288

425 Aranzana MJ, García-Mas J, Carbó J, Arús P (2002) Development and variability
 426 analysis of microsatellite markers in peach. *Plant Breed* 121:87-92
 427 Asensio M (1996) El virus de la sharka (plum pox virus): caracterización, diagnóstico y
 428 detección mediante anticuerpos monoclonales específicos. Tesis doctoral. Universidad
 429 Politécnica de Valencia. 193 pp
 430 Audergon JM (1995) Variety and Breeding. *Acta Hort* 384:35-45
 431 Badenes ML, Martínez-Calvo J, Llácer G (2002) Estado actual del programa de mejora
 432 genética del albaricoquero en la Comunidad Valenciana. *Actas de Horticultura* 29:637-
 433 643
 434 Bassi D, Bellini D, Guerriero R, Monastra F, Pennone F (1995) Apricot breeding in
 435 Italy. *Acta Hort* 384:47-54
 436 Bliss FA, Arulsekhar S, Foolad MR, Becerra V, Gillen AM, Warburton ML, Dandekar
 437 AM, Kocsisne GM, Mydin KK (2002) An expanded genetic linkage map of *Prunus*
 438 based on an interspecific cross between almond and peach. *Genome* 45:520-529
 439 Broman KW (2002) Mapping quantitative trait loci in the case of a spike in the
 440 phenotype distribution. *Genetics*. 163:1169-1175
 441 Brooks RM, Olmo HP (1997) The Brooks and Olmo register of fruit and nut varieties.
 442 3rd ed. ASHS Press, Alexandria, Va.
 443 Cambra M, Asensio M, Gorris MT, Camarasa E, García JA, Moya JJ, López-Abella D,
 444 Vela C, Sanz A (1994) Detection of Plum pox potyvirus using monoclonal antibodies to
 445 structural and non-structural proteins. *EPPO Bull* 24:569-578
 446 Caranta C, Palloix A, Lefebvre V, Daubèze AM (1997) QTLs for a component of
 447 partial resistance to cucumber mosaic virus in pepper: restriction of virus installation in
 448 host cells. *Theor Appl Genet* 94:431-438

449 Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait
 450 mapping. *Genetics* 138:963-971

451 Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT
 452 and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation,
 453 characterisation and cross-species amplification in *Prunus*. *Theor Appl Genet* 99:65-72

454 Decroocq V, Foulongne M, Lambert P, Le Gall O, Martin C, Pascal T, Schurdi-Levraud
 455 V, Kervella J (2005) Analogues of virus resistance genes map to QTLs for resistance to
 456 sharka disease in *Prunus davidiana*. *Mol Genet Genomics* 272:680-689

457 Della Strada G, Pennone F, Fideghelli C, Monastra F, Cobianchi D (1989) Monografia
 458 di cultivar di albicocco. Ministero dell'Agricoltura e delle Foreste. Direzione Generale
 459 della Produzione Agricola. Istituto Sperimentale per la Frutticoltura, Roma, Italia

460 Dicenta F, Martínez-Gómez P, Burgos L, Egea P (2000) Inheritance of resistance to
 461 plum pox potyvirus (PPV) in apricot (*Prunus armeniaca* L.) *Plant Breed* 119:161-164

462 Dirlewanger E, Cosson P, Howad W, Capdeville G, Bosselut N, Claverie M, Voisin R,
 463 Poizat C, Lafargue B, Baron O, Laigret F, Kleinhentz M, Arús P, Esmenjaud D (2004b)
 464 Microsatellite genetic linkage maps of myrobalan plum and an almond-peach hybrid –
 465 location of root-knot nematode resistance genes. *Theor Appl Genet* 109:827-838

466 Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P,
 467 Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.)
 468 Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus*
 469 *avium* L.). *Theor Appl Genet* 105:127-138

470 Dirlewanger E, Graziano E, Joobeur T, Garriga-Calderé F, Cosson P, Howad W, Arús P
 471 (2004a) Comparative mapping and marker-assisted selection in *Rosaceae* fruit crops.
 472 *Proc Natl Acad Sci USA* 101:9891-9896

473 Dosba F, Denise F, Maison P, Massonié G, Audergon JM (1991) Plum Pox Virus
 474 resistance of apricot. Acta Hort 293:569-579
 475 Doyle JJ, Doyle JL (1987) A rapid isolation procedure for small quantities of fresh leaf
 476 tissue. Phytochem Bull 19:11-15
 477 Dunn OJ (1964) Multiple comparisons using rank sums. Technometrics 6:241-252
 478 Egea J, Burgos L, Martínez-Gómez P, Dicenta F (1999) Apricot breeding for sharka
 479 resistance at the CEBAS-CSIC, Murcia (Spain). Acta Hort 488:153-157
 480 Gentzbittel L, Mouzeyar S, Badaoui S, Mestries E, Vear F, De Labrouhe DT, Nicolas P
 481 (1998) Cloning of molecular markers for disease resistance in sunflower, *Helianthus*
 482 *annus* L. Theor Appl Genet 96:519-525
 483 Grattapaglia D, Sederoff RR (1994) Genetic linkage maps of *Eucalyptus grandis* and *E.*
 484 *urophylla* using a pseudotest-cross strategy and RAPD markers. Genetics 137:1121-
 485 1137
 486 Hittalmani S, Foolad MR, Mew T, Rodríguez RL, Hang N (1995) Development of a
 487 PCR-base marker to identify rice blast resistance gene, *Pi-2(t)*, in segregating
 488 population. Theor Appl Genet 91:9-14
 489 Hurtado MA, Romero C, Vilanova S, Abbott AG, Llácer G, Badenes ML (2002)
 490 Genetic linkage maps of two apricot cultivars (*Prunus armeniaca* L.) and mapping of
 491 PPV (sharka) resistance. Theor Appl Genet 105:182-191
 492 Jelenkovic G, Harrington E (1972) Morphology of the pachytene chromosomes in
 493 *Prunus persica*. Can J Genet Cytol 14:317-324
 494 Karayiannis I (2006) Conventional breeding for *Plum Pox Virus* resistant apricots
 495 (*Prunus armeniaca* L.) in Greece. EPPO Bull 36 (In press)
 496 Karayiannis I, Mainou A, Tsaftaris A (1999) Apricot breeding in Greece for fruit
 497 quality and resistance to plum pox virus. Acta Hort 488:111-117

498 Kellerhals M (2000) Marker-Assisted Selection in apple breeding. Acta Hort 521:255-
499 265

500 Kosambi DD (1944) The estimation of map distance from recombination values. Ann
501 Eugenics 12:172-175

502 Kruglyak L, Lander ES (1995) A nonparametric approach for mapping quantitative trait
503 loci. Genetics 139:1421-1428

504 Kuginuki Y, Ajisaka H, Yui M, Yoshikawa H, Hida K, Hirai M (1997) RAPD markers
505 linked to a club-root resistance locus in *Brassica rapa* L. Euphytica 98:149-154

506 Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits
507 using RFLP linkage maps. Genetics 121:185-199

508 Lehmann EL (1975) Nonparametrics: Statistical Methods Based on Ranks. Holden and
509 Day, San Francisco

510 Llácer G, Badenes ML, Romero C (2006) Problems in the determination of inheritance
511 of plum pox virus resistance in apricot. Acta Hort (In press)

512 Llácer G, Cambra M, Laviña A (1985) Detection of plum pox virus in Spain. EPPO
513 Bull 15:325-329

514 Lommel SA, McCain AH, Morris TJ (1982) Evaluation of Indirect-Linked
515 Immunosorbent Assay for the detection of plant viruses. Phytopathology 72:1018-1022

516 Lopes MS, Sefc KM, Laimer M, da Câmara Machado A (2002) Identification of
517 microsatellite loci in apricot. Mol Ecol Notes 2:24-26

518 Martínez-Gómez P, Dicenta F, Audergon JM (2000) Behaviour of apricot (*Prunus*
519 *armeniaca* L.) cultivars in the presence of sharka (plum pox potyvirus): a review.
520 Agronomie 20:407-422

521 McIntyre LM, Coffman CJ, Doerge RW (2001) Detection and localization of a single
522 binary trait locus in experimental populations. Gen Res 78:79-92

523 Meagher RB, McLean M, Arnold J (1988) Recombination within a subclass of
 524 restriction fragment length polymorphisms may help link classical and molecular
 525 genetics. *Genetics* 120:809-818

526 Messina R, Lain O, Marrazzo MT, Cipriani G, Testolin R (2004) New set of
 527 microsatellite loci isolated in apricot. *Mol Ecol Notes* 4:432-434

528 Moustafa TA, Badenes ML, Martínez-Calvo J, Llácer G (2001) Determination of
 529 resistance to sharka (plum pox) virus in apricot. *Sci Hort* 91:57-70

530 Russell D (1998) The stonefruit cultivar system (a database of worldwide stonefruit
 531 cultivars and rootstocks). Department of Primary Industries, Queensland, Australia

532 Salava J, Wang Y, Krska B, Polak J, Kominek P, Miller W, Dowler W, Reighard GL,
 533 Abbott AG (2002) Identification of molecular markers linked to resistance of apricot
 534 (*Prunus armeniaca* L.) to plum pox virus. *J Plant Dis Prot* 109:64-67

535 Soriano JM, Vilanova S, Romero C, Llácer G, Badenes ML (2005) Characterization and
 536 mapping of NBS-LRR resistance gene analogs in apricot (*Prunus armeniaca* L.). *Theor*
 537 *Appl Genet* 110:980-989

538 Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S,
 539 Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite markers in
 540 peach [*Prunus persica* (L.) Batsch]. *Theor Appl Genet* 101:421-428

541 Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Fujimura M, Nunome T, Fukuoka
 542 H, Matsumoto S, Hirai M (2003) Identification of two loci for resistance to clubroot
 543 (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. *Theor Appl Genet* 107:997-
 544 1002

545 Syrgianidis GD, Mainou AC (1993) Deux nouvelles variétés d'abricotier résistantes à
 546 la maladie à virus de la sharka (Plum Pox) issues de croisements. In *Deuxièmes*

547 recontres sur l'abricotier, Avignon, France, 27-31 May 1991. Edited by JM Audergon.
 548 Commission des Communautés Européennes, Luxembourg. Pp. 135-136
 549 Tartarini S, Sansavini S, Vinatzer B, Gennari F, Domizi C (2000) Efficiency of marker
 550 assisted selection (MAS) for the *vf* scab resistance gene. Acta Hort 538:549-552
 551 Testolin R, Marrazzo T, Cipriani G, Quarta R, Verde I, Dettori MT, Pancaldi M,
 552 Sansavini S (2000) Microsatellite DNA in peach (*Prunus persica* L. Batsch) and its use
 553 in fingerprinting and testing the genetic origin of cultivars. Genome 43:512-520
 554 Van Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous
 555 species. Theor Appl Genet 84:803-811
 556 Van Ooijen JW (1999) LOD significance thresholds for QTL analysis in experimental
 557 populations of diploid species. Heredity 83:613-624
 558 Van Ooijen JW, Voorrips RE (2001) JoinMap®3.0, Software for the calculation of
 559 genetic linkage maps. Plant Research International, Wageningen, The Netherlands
 560 Van Ooijen JW, Boer MP, Jansen RC, Maliepaard C (2000) MapQTL (tm) 4.0:
 561 Software for the calculation of QTL positions on genetic maps. Plant Research
 562 International, Wageningen, The Netherlands
 563 Vilanova S, Romero C, Abbott AG, Llácer G, Badenes ML (2003) An apricot (*Prunus*
 564 *armeniaca* L.) F₂ progeny linkage map based on SSR and AFLP markers, mapping
 565 plum pox virus resistance and self-incompatibility traits. Theor Appl Genet 107:239-
 566 247
 567 Vilanova S, Soriano JM, Lalli DA, Romero C, Abbott AG, Llácer G, Badenes ML
 568 (2006) Development of SSR markers located in the G1 linkage group of apricot (*Prunus*
 569 *armeniaca*) using a bacterial artificial chromosome library. Mol Ecol Notes 6:789-791
 570 Wetzel T, Candresse T, Ravelonandro M, Dunez J (1991) A polymerase chain reaction
 571 assay adapted to plum pox potyvirus detection. J Virol Methods 33:355-365

Yamamoto T, Mochida K, Imai T, Shi YZ, Ogiwara I, Hayashi T (2002) Microsatellite markers in peach [*Prunus persica* (L.) Batsch] derived from an enriched genomic and cDNA libraries. Mol Ecol Notes 2:298-301

Yamamoto T, Yamaguchi M, Hayashi T (2005) An integrated linkage map of peach by SSR, STS, AFLP and RAPD. J Jap Soc Hort Sci 74:204-213

Figure legends and tables

Table 1.- PPV resistance segregating populations used in this study

Population	Resistance donor	Type	Cross date	Evaluation date	N ^b	Res./Suscep. ^c		χ^2 (P-value) ^d	χ^2 (P-value) ^e
						1 st cycle	2 nd cycle	mono. ratio	di. ratio
'GxC'	'Goldrich'	F ₁	1995	1998-99 (2) ^a	81	41/40	21/60	18.78 (<0.0001)	0.04 (0.847)
'LxL-98'	'SEO'	F ₂	1998	2000-01 (3)	81	54/27	50/31	7.61 (0.006)	0.99 (0.321)
'LxL-00'	'SEO'	F ₂	2000	2002-03 (3)	249	195/54	152/97	25.87 (<0.0001)	2.35 (0.125)
'GxCa'	'Goldrich'	F ₁	2001	2004-05 (2)	171	51/120	28/143	77.34 (<0.0001)	6.79 (0.009)

^a Number of vegetative cycles analysed

^b Number of seedlings evaluated

^c Number of Resistant/Susceptible seedlings observed after one or two dormancy cycles

^d χ^2 and P-value for the expected Resistant/Susceptible ratio in a monogenic model (1:1 in F₁ and 3:1 in F₂)

^e χ^2 and P-value for the expected Resistant/Susceptible ratio in a digenic model (1:3 in F₁ and 9:7 in F₂)

Table 1 Supplementary.- Origin and sources of SSR markers tested in the ‘GxC’ and ‘LxL-98’ populations

Loci acronym	Source	SSR origin	<u>‘GxC’ population</u>		<u>‘LxL-98’ population</u>	
			Tested	Mapped	Tested	Mapped
UDP	Peach	Cipriani et al. 1999	4	2	---	---
pch/ps	Peach	Sosinski et al. 2000	9	1	---	---
pchgms	Peach	Testolin et al. 2000	---	---	17	1
BBPCT	Peach	Dirlewanger et al. 2002	37	11	37	10
CPPCT	Peach	Aranzana et al. 2002	22	3	---	---
MA/M	Peach	Yamamoto et al. 2002	36	9	36	10
ssrPaCITA	Apricot	Lopes et al. 2002	22	8	22	7
aprigms	Apricot	Vilanova et al. 2006	16	1	16	6
UDAp	Apricot	Messina et al. 2004	20	6	20	1
SC	Apricot	Abernathy et al. 2004	4	2	2	2
Total			170	43	150	37

605 **Table 2 Supplementary.-** Summary of SSRs mapped in ‘Goldrich’, ‘Currot’ and ‘LxL-
606 98’ maps.

SSR marker	Linkage	Goldrich	Currot	LxL	SSR marker	Linkage	Goldrich	Currot	LxL
	Group	(χ^2) ^a	(χ^2)	(χ^2)		Group	(χ^2) ^a	(χ^2)	(χ^2)
aprigms1	LG1	---	---	11.4*	UDAp404	LG4	0.4 ^D	---	---
aprigms2	LG1	1.4 ^D	---	9.1	UDAp416	LG4	2.1	---	---
aprigms3	LG1	---	---	3.1	UDAp418	LG4	0.9 ^D	---	---
aprigms8	LG1	---	---	2.5	UDAp419	LG4	2.6	---	---
aprigms10	LG1	---	---	9.9*	UDP98-024	LG4	0.9 ^D	---	---
aprigms16	LG1	---	---	2.0	BPPCT017	LG5	11.4*	11.4*	0.4
BPPCT011	LG1	---	---	6.3	BPPCT020	LG5	0.1 ^D	---	---
CPPCT26	LG1	2.5 ^D	---	---	BPPCT026	LG5	---	0.9 ^D	---
CPPCT34	LG1	1.8 ^D	---	---	BPPCT037	LG5	39.2*	39.2*	1.2
MA030a	LG1	---	---	7.0	BPPCT038	LG5	22.3*	22.3*	31.2* ^D
M3b	LG1	0.5 ^D	---	3.3	ssrPaCITA21	LG5	---	2.8 ^D	2.2
pchgms10	LG1	---	---	1.8	BPPCT009	LG6	1.4 ^D	---	2.7
SC6A6	LG1	3.3	3.3	3.9	BPPCT018	LG6	2.0	2.0	---
SC19A6	LG1	6.3	6.3	11.0*	BPPCT042	LG6	---	---	78.0*
ssrPaCITA5	LG1	0.5 ^D	---	8.4	MA027a	LG6	5.8	5.8	4.0
ssrPaCITA7	LG1	2.5 ^D	---	4.8	MA040a	LG6	0.3 ^D	---	18.8*
ssrPaCITA17	LG1	1.7 ^D	---	10.3*	M5a	LG6	3.9 ^D	---	---
UDAp415	LG1	1.6 ^D	---	---	UDP98-412	LG6	0.0 ^D	---	---
BPPCT002	LG2	2.0 ^D	---	0.0	CPPCT22	LG7	42.3*	42.3*	---
BPPCT030	LG2	0.0 ^D	---	---	MA010a	LG7	0.0 ^D	---	1.6
MA007a	LG2	0.1 ^D	---	---	MA020a	LG7	---	---	17.1*
ssrPaCITA14	LG2	---	---	1.2	pchgms2(1)	LG7	---	---	9.4*
ssrPaCITA16	LG2	0.0 ^D	---	1.7	UDAp407	LG7	---	---	0.1
ssrPaCITA19	LG2	0.5	0.5	---	UDP98-405	LG7	---	32.0* ^D	---
UDAp413	LG2	0.0 ^D	---	---	BPPCT012	LG8	---	---	0.3 ^D

BPPCT007	LG3	---	---	2.5	MA006b	LG8	---	---	3.0
BPPCT024	LG3	---	---	0.5 ^D	MA013a	LG8	0.9 ^D	---	0.6
BPPCT039	LG3	---	0.2 ^D	---	MA019a	LG8	---	0.2 ^D	7.2
MA034a	LG3	---	---	0.6 ^D	M6a	LG8	---	0.1 ^D	---
ssrPaCITA4	LG3	1.4	1.4	---	Ps1h3	LG8	0.4 ^D	---	---
BPPCT040	LG4	0.1 ^D	---	---	ssrPaCITA15	LG8	---	---	3.7
ssrPaCITA6	LG4	---	0.2 ^D	---	UDP98-409	LG8	1.1	---	---

* Markers showing distorted segregation at $P < 0.01$.

^a Chi-square of the goodness of fit for the segregation at $P < 0.01$.

^D SSR dominant markers

Figure 1.- Genetic linkage maps of ‘Goldrich’ and ‘Currot’. Linkage groups were numbered according to the nomenclature of Dirlewanger et al (2004a). New SSR markers are *black boxed*. *Solid circles* indicate anchor markers with other *Prunus* maps: Dirlewanger et al. (2004a), Dirlewanger et al. (2004b), Yamamoto et al. (2005) an the ‘LxL-98’ map. *Asterisks (**)* indicate markers with distorted segregations at $P < 0.01$. Distances in cM are shown on the left in ‘Goldrich’ an on the right in ‘Currot’.

Figure 2.- Genetic linkage map of ‘LxL-98’. Linkage groups were numbered according to the nomenclature of Dirlewanger et al. (2004a). New SSR markers are *black boxed*. *Solid circles* indicate anchor markers with other *Prunus* maps: Dirlewanger et al. (2004a), Dirlewanger et al. (2004b), Yamamoto et al. (2005) an the ‘GxC’ map. *Asterisks (**)* indicate markers with distorted segregations at $P < 0.01$. Distances in cM are shown on the left.

Figure 3.- Kruskal-Wallis (KW) statistic values (solid line) and Interval Mapping (IM) LOD score (pointed line) at markers on LG1. Horizontal dashed line corresponds to the $P < 0.005$ KW significance level. **a** ‘Goldrich’ map. **b** ‘LxL-98’ map. Only SSR and highly significant AFLP markers are shown. Bars at the bottom indicate the KW

interval for $P < 0.005$ significant markers (solid bar) and the IM two-LOD support interval (pointed bar).

Table 2.- Results of co-segregation, KW and IM analysis for PPV resistance on ‘SEO’ and ‘Goldrich’ maps. SSRs flanking significance intervals (SC6A6 and *ssrPaCITA17*) and highly significant markers are shown.

Map	Marker	Co-segregation			KW test		Interval Mapping		
		Pos ^a	r^b	LOD ^c	KW ^d	$P(KW)^c$	IM ^f	$R^2(\%)^g$	a^h
SEO	SC6A6	21.0	0.39	1.26	8.61	<0.05	2.74	14.4	0.263
	EAA-MCTA(1)	25.2	0.31	2.56	12.04	<0.001	2.77	14.6	0.206
	EAG-MCTT(1)	31.5	0.27	5.29	20.57	<0.0001	5.14	25.4	0.356
	<i>ssrPaCITA5</i>	33.4	0.38	2.36	13.54	<0.005	3.59	18.5	0.288
	EAT-MCTC(9)	40.5	0.33	2.95	12.71	<0.0005	3.09	16.1	0.325
	<i>ssrPaCITA17</i>	42.9	0.43	0.83	6.59	<0.05	1.43	7.8	0.198
Goldrich	SC6A6	28.7	0.30	0.16	2.78	---	2.03	14.2	
	UDAp415	38.1	0.29	2.21	9.81	<0.005	5.31	34.8	
	AG-CTT6	43.3	0.26	4.17	17.13	<0.0005	6.05	36.8	
	<i>ssrPaCITA5</i>	49.2	0.32	3.40	13.68	<0.001	3.57	21.6	
	AA-CTT7	52.0	0.29	3.72	15.16	<0.0005	3.71	21.8	
	<i>ssrPaCITA17</i>	59.1	0.39	1.92	7.94	<0.005	2.40	18.0	

^a Position in cM on LG1

^b Recombination frequency between markers and PPV resistance trait.

^c LOD score for co-segregations.

^d Kruskal-Wallis test statistic values.

^e Probability associated with the KW value.

^f LOD score under Interval Mapping.

^g Percentage of the contribution to the total variance.

^h Additive effect.

641 **Table 3.-** PCR-amplification of selected SSR markers in 7 Resistant (R) and 11
642 Susceptible cultivars (S). The presence (amplification) of the PPV resistance linked
643 allele is indicated by (+) and the absence by (-).

Cultivar	Pedigree	R/S	ssrPaCITA5	ssrPaCITA17	SC6A6
SEO	Unknown ^a	R	+	+	+
Lito	SEO x Thyrinthos ^b	R	+	+	+
Pandora	SEO x Thyrinthos ^b	R	+	+	+
Sunglo	Unknown ^a	R	+	+	+
Goldrich	Sunglo x Perfection ^a	R	+	+	+
Veecot	Reliable (open pollination) ^a	R	+	+	+
Harcot	[(Geneva x Naramata) x Morden 604] x NJA1 (Phelps x Perfection) ^a	R	+	+	+
Tyrinthos	Unknown ^c	S	-	-	-
Bergeron	Unknown ^c	S	-	-	-
Moniquí	Unknown ^c	S	-	-	-
Colorao	Unknown ^c	S	-	-	-
Canino	Unknown ^c	S	-	-	-
Currot	Unknown ^d	S	-	-	-
Ginesta	Unknown ^d	S	-	-	-
Mitger	Unknown ^d	S	-	-	+
Palau	Unknown ^d	S	-	-	+
Pepito	Unknown ^d	S	-	-	-
Katy	Zaiger's Genetics (USA) ^e	S	-	-	-

^a Brooks and Olmo (1997)

^b Syrgianidis and Mainou (1993)

^c Della Strada et al. (1989)

^d IVIA

^e Russell (1998)

Table 4.- Association between selected SSRs and PPV resistance in ‘GxCa’ and ‘LxL-00’ progenies based on the Kruskal-Wallis test. KW: Kruskal-Wallis test statistic values; DF: Degrees of freedom; P: probability associated with the KW value.

Marker	Linkage	‘GxCa’			‘LxL-00’		
	Group	KW	DF	P(KW)	KW	DF	P(KW)
SC6A6	LG1	3.201	2		60.494	2	<0.0001
ssrPaCITA5	LG1	20.169	3	<0.0001	56.274	2	<0.0001
ssrPaCITA17	LG1	12.475	1	<0.0001	51.562	2	<0.0001
M3b	LG1	2.909	1		2.175	2	
CPPCT-13	LG5	0.166	1		2.885	2	

Table 5.- MAS for PPV resistance. Percentages of mis-classified seedlings in the four populations analysed: R- indicates percentage of seedlings without the marker classified as PPV resistant, S+ indicates percentage of seedlings carrying the marker classified as PPV susceptible and Total indicates percentage of total seedlings mis-classified.

Marker	‘GxC’			‘GxCa’			‘LxL-98’			‘LxL-00’		
	R-	S+	Total	R-	S+	Total	R-	S+	Total	R-	S+	Total
SC6A6	21	62	49	11	76	65	6	69	29	8	47	23
ssrPaCITA5	10	41	32	7	47	41	2	69	27	8	49	24
ssrPaCITA17	15	48	39	14	49	44	8	70	31	7	55	25

659

660

Figure 2

Figure 3