

# Quantitative trait loci analysis of *Plum pox virus* resistance in *Prunus davidiana* P1908: new insights on the organization of genomic resistance regions

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**Abstract** No valuable source of resistance to *Plum pox virus* (PPV), the causative agent of sharka disease, has been found in peach (*Prunus persica*), but polygenic resistance to PPV was described in *Prunus davidiana* clone P1908. Two previous studies using F<sub>1</sub> and F<sub>2</sub> populations derived from the nectarine cv. Summergrand and *P. davidiana* P1908 identified a total of six *P. davidiana* quantitative trait loci (QTLs) involved in PPV resistance (Marcus strain). In an effort to verify the QTL stability in a large progeny and to search for possible interactions of the genetic backgrounds, the current study evaluated the incidence of PPV infection in an F<sub>1</sub> population derived from the susceptible peach cv. Rubira and *P. davidiana* P1908 over three growth periods using an improved method of PPV phenotyping referred to as “heavy test.” The phenotypic dataset was analyzed using similar methods as the previous studies and a newly developed simple-sequence-repeat-based *P. davidiana* map. Nine regions involved in differential symptom expression were identified among which six were common between studies. However, the level of resistance observed in the population was very low compared to the other studies, and the main QTL previously identified in linkage group 6 was not conserved,

suggesting strong interaction of the genetic background of the susceptible parent with that of *P. davidiana* 1908. Consequently, this could be a limiting factor for developing resistant cultivars derived from *P. davidiana* P1908.

**Keywords** Quantitative trait loci · Sharka · *Prunus davidiana*

## Introduction

Sharka disease, whose causative agent is the *Plum pox virus* (PPV), is one of the most limiting factors for stone-fruit tree production all over the world, the economic losses being estimated at over 10,000 million euros over the last 30 years (Cambra et al. 2006). Peach [*Prunus persica* (L.) Batch] is one among the species most affected by this disease which causes severe symptoms on leaves and flowers and leads to malformation of the fruits, making them often not marketable. This viral disease is characterized by its difficult control due to fast transmission by aphids in a nonpersistent way (Labonne et al. 1994) and is disseminated at long distance by infected *Prunus* material. Short-term control methods in the field include removal of diseased plants and the planting of virus-free-certified material (Ravelonandro et al. 2000), but the cultivation of resistant cultivars seems to be the only long-term solution. As a consequence, the development of resistant peach genotypes is one of the most important objectives in *Prunus* breeding programs (Martínez-Gómez et al. 2000).

To date, no natural PPV resistance source has been identified in peach germplasm (Gabova 1994; Escalettes et al. 1998; Pascal et al. 2002). Most of the cultivars which have been assayed were reported as susceptible, particularly to PPV M (Marcus) isolates (Mainou and Syrgiannidis

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1992; Dosba et al. 1994). However, inside the widespread susceptibility existing in peach trees, it is possible to find an important variability, some cultivars being highly susceptible while others generally present no symptoms in leaves (“Moon Grand” and “Red Bird Cling”) or in fruits (“Dupniska” and “Superior Pacific Star”) in field conditions (Gabova 1994). Escalettes et al. (1998) reported two resistant ornamental peaches, the “S2844” clone of “Russel Red” originating from the UK and the “S2873” clone of “Early Double Red” originating from the USA, but these were later confirmed as susceptible to a PPV M isolate by Pascal et al. (2002). Hopefully, beside the lack of PPV resistance sources in peach, wild-peach-related species are an interesting option for finding potential resistance sources. *Prunus davidiana* is one of these; it is close to cultivated peach (Grasselly 1974; Hesse 1975), and the clone 1908 has been used at INRA Avignon in a peach breeding program for resistance to several pests and diseases including PPV (Kervella et al. 1998; Pascal et al. 1998), during which several interspecific crosses have been generated. Among them, three connected progenies (F<sub>1</sub>, F<sub>2</sub>, and BC<sub>2</sub>) derived from crosses between *P. persica* cv. Summergrand and *P. davidiana* P1908 were used for constructing genetic maps, and Foulongne et al. (2003) demonstrated suitability of *P. davidiana* to introgress resistance characters into peach.

The first study of the genetic factors involved in the resistance to PPV in *P. davidiana* was reported in Decroocq et al. (2005). It used the same F<sub>1</sub> population (referred to as SD) and genetic map as Foulongne et al. (2003) and reported a quantitative trait loci (QTL) analysis which identified six genomic regions related to PPV resistance in *P. davidiana* P1908 and thus demonstrated the polygenic character of the resistance carried by this clone. The heritability of the PPV resistance originating from *P. davidiana* P1908 was further studied by Marandel et al. (2009) in the F<sub>2</sub> population derived from the selfing of the individual (#40) of the F<sub>1</sub> population and demonstrated the conservation of four of the six F<sub>1</sub> QTLs. Both studies included the mapping of candidate genes or candidate-gene-linked simple sequence repeat (SSR) markers and suggested the possible involvement of components of the eukaryotic translation initiation complex in PPV resistance. However, these studies evaluated a low number of individuals for PPV resistance, and the phenotyping method used had to be improved. As a consequence, it was important to confirm these findings in a larger progeny using a reliable method of symptom evaluation. Moreover, as the resistance to potyviruses was known to involve components of eukaryotic translation initiation complex and appeared mainly recessive (Ruffel et al. 2002; Kang et al. 2005), it was important to search for possible interactions of the genetic backgrounds, in the case that recessive factors would be involved in part of the resistance carried by *P. davidiana*.

For this purpose, we used an interspecific F<sub>1</sub> population referred to as RD derived from a cross between “Rubira,” a peach cultivar used as a rootstock showing a high susceptibility to PPV, and *P. davidiana* P1908. The aim of the present study was to build an SSR-based map of *P. davidiana* P1908 anchored to the general map for *Prunus*, to develop a reliable method of symptom evaluation, and to compare the behavior of the progeny and the positions of the QTLs with those of the previous studies, the final aim being to analyze the potential of *P. davidiana* for introgressing PPV resistance factors into peach cultivars and to develop reliable markers for marker-assisted breeding of new cultivars.

## Materials and methods

### Plant material

The F<sub>1</sub> progeny ( $n=171$ ) used for PPV phenotyping and mapping was derived from a controlled interspecific cross between *P. persica* cv. Rubira (female parent) and *P. davidiana* P1908. “Rubira” is a peach rootstock resistant to green peach aphid (*Myzus persicae*) and highly susceptible to PPV. *P. davidiana* P1908 is resistant to PPV, peach powdery mildew (*Sphaerotheca pannosa*), green peach aphid, and peach leaf curl (*Taphrina deformans*).

### Phenotyping procedure

Virus-free “GF305” peach seedlings used for grafting were supplied by a commercial nursery (Lafond, France). They were inoculated with a Marcus-type isolate called “Vallier” by grafting an infected chip-bark from other “GF305” seedlings showing strong sharka symptoms. Three replicates of each of the 171 individuals and parents were grafted onto these inoculated rootstocks. Four weeks after grafting, they were taken to the cool chamber, at 7°C in darkness, for 2 months of dormancy. Plants were then transferred to the greenhouse, and rootstocks were pruned above the grafting point to allow the sprouting of genotype buds. Three cycles of evaluation were performed over one and a half year (i.e., 2 months in a cold chamber and 4 months in a greenhouse) in 2007 and 2008. During each growth period, seedlings were observed for leaf symptoms. Intensity of leaf symptoms (I) and distribution of symptoms on whole plant (D) were scored independently on each scion using the phenotypic scoring system and the ordinal scale from 0 (absence of symptoms) to 4 (numerous discolorations affecting the whole leaf and associated with leaf distortions for I; symptoms on more than 30% of leaves for D) described in Decroocq et al. (2005) and Marandel et al. (2009). In addition, a third parameter (G) was defined:

the global behavior of each seedling; it was used by Decroocq et al. (2005) and Marandel et al. (2009) and corresponds to the mean of the sum of the intensity and distribution scores observed on each scion. During the three cycles of evaluation, an enzyme-linked immunosorbent assay (ELISA)–double-antibody sandwich assay was applied to the leaves using a monoclonal antibody against the coat protein of PPV (BIOREBA®) to ascertain the presence or absence of PPV in the genotypes (one leaf with symptoms for symptomatic individuals and five leaves taken at random for the other ones). Optical densities (OD) were recorded at 405 nm, after 60 min. In accordance with Sutula et al. (1986), samples with OD double that of the healthy controls were considered ELISA positive.

#### DNA isolation

Samples of young expanded terminal leaves from both parents and the 171 hybrids were collected in May 2006 and kept at  $-80^{\circ}\text{C}$  until DNA isolation. Genomic DNA isolation was then performed following the protocol of Bernatzky and Tanksley (1986). DNA concentrations were measured using a spectrophotometer Thermo Scientific

NanoDrop™. DNA was further diluted to a final concentration of  $250\text{ ng }\mu\text{l}^{-1}$ .

#### SSR analysis

A set of 329 SSR primer pairs developed from several *Prunus* species was tested for the study (Table 1). SSRs were first screened on both parents and then on a set of eight individuals of the mapping population when it was necessary for establishing segregation patterns. They were polymerase chain reaction (PCR)-amplified in a volume of  $15\text{ }\mu\text{l}$  containing  $10\text{ mM Tris-HCl pH }9$ ,  $50\text{ mM KCl}$ ,  $0.1\%$  of Triton X100,  $1.5\text{ mM MgCl}_2$ ,  $0.2\text{ mM}$  each dNTP,  $0.25\text{ U Taq DNA polymerase}$  (Promega, Madison, WI, USA),  $20\text{--}30\text{ ng}$  of genomic DNA,  $0.2\text{ }\mu\text{M}$  of the first primer, and  $34\text{ pM}$  of the second primer end-labeled with  $\gamma\text{-}[33\text{P}]\text{ATP}$  using T4 polynucleotide kinase. PCRs were performed in an MJR PTC-100 thermal cycler (MJ Research, Waltham, MA, USA) under the following conditions: an initial denaturation for  $3\text{ min }30\text{ s}$  at  $95^{\circ}\text{C}$ , followed by 30 cycles of  $1\text{ min}$  at  $94^{\circ}\text{C}$ ,  $1\text{ min}$  at  $49\text{--}57^{\circ}\text{C}$ ,  $30\text{ s}$  at  $72^{\circ}\text{C}$ , and a final extension of  $5\text{ min}$  at  $72^{\circ}\text{C}$ . The PCR products were further separated by electrophoresis on a  $6\%$  polyacrylamide and  $7.5\text{ M}$  urea sequencing gel (Sequagel-National

**Table 1** Origin of microsatellites tested and mapped in *Prunus davidiana*

Terminology	Species	Origin	Tested	Amplified	Heterozygous	Mapped	Reference SSR source
AMPA	Apricot	Genomic	17	15	9	4	Hagen et al. (2004)
AMPA	Apricot	cDNA	10	9	4	2	Hagen et al. (2004)
BPPCT	Peach	Genomic	39	38	20	16	Dirlewanger et al. (2002)
CPDCT	Almond	Genomic	30	28	15	2	Mnejja et al. (2005)
CPPCT	Peach	Genomic	27	26	15	8	Aranzana et al. (2002)
CPSCT	Plum	Genomic	25	23	12	3	Mnejja et al. (2004)
EMPA	Sweet cherry	Genomic	3	3	1	0	Clarke and Tobutt (2003)
EPPB	Peach	cDNA	3	3	1	1	Dirlewanger (personal communication)
EPPCU	Peach	cDNA	6	5	4	2	Howad et al. (2005)
M	Peach	cDNA	6	4	2	2	Yamamoto et al. (2002)
MA	Peach	Genomic	21	19	12	8	Yamamoto et al. (2002)
Pac	Apricot	cDNA	8	7	1	1	Decroocq et al. (2003)
PaCITA	Apricot	Genomic	21	18	11	5	Lopes et al. (2002)
PceGA	Sour cherry	Genomic	1	1	1	1	Downey and Iezzoni (2000)
Pchcms	Peach	cDNA	5	5	3	2	Sosinski et al. (2000)
Pchgms	Peach	Genomic	6	6	3	2	Sosinski et al. (2000)
PdavW	<i>P. davidiana</i>	Genomic	1	1	0	0	Lambert et al. (2004)
PMS	Sweet cherry	Genomic	5	4	0	0	Cantini et al. (2001)
PS	Sour cherry	Genomic	5	2	0	0	Sosinski et al. (2000)
UDA	Almond	Genomic	2	2	2	0	Testolin et al. (2004)
UDAp	Apricot	Genomic	63	56	24	14	Messina et al. (2004)
UDP	Peach	Genomic	16	15	8	7	Cipriani et al. (1999)
UDP	Peach	Genomic	9	7	4	4	Testolin et al. (2000)
Total			329	297	152	84	

diagnostics) for 2.5 h at 70 W with 1× TBE. Following electrophoresis, the gel was dried and exposed to an X-ray film (Kodak X-Omat) for 1–4 days. Segregating SSRs with easily readable profiles were further selected from their position in the “Texas” × “Earlygold” general map for *Prunus* (hereafter referred to as T×E) or in the other *Prunus* maps and mapped in the whole population using the multiplex protocol.

#### Multiplex protocol and genotyping

The whole population was PCR-amplified by using the QIAGEN® Multiplex PCR Kit (Qiagen Inc., Valencia, CA, USA) with three to six primer pairs simultaneously (0.2 and 0.4 μM concentration for each IRD700- and IRD800-labeled primers, respectively) with 2× QIAGEN multiplex PCR master mix (final concentration 1×) and 5× of Q-Solution (final concentration 0.5×). The same concentration was used for the complementary primers. Twenty nanograms of template DNA were used in a final reaction volume of 10 μl. DNA amplifications were carried out in a Mastercycler® ep gradient thermal cycler (Eppendorf GmbH, Germany) using the universal multiplex cycling protocol (QIAGEN Multiplex PCR kit; QIAGEN): 15 min at 95°C (initial activation step) followed by 35 cycles consisting of 94°C for 30 s, 57°C for 90 s, and 72°C for 60 s, with a final extension of 10 min at 72°C. The Multiplex PCR products were then diluted in formamide blue in a 1:40 ratio and denatured at 95°C for 3 min. Then, 0.5–0.8 μl of each sample was loaded on a 6% polyacrylamide sequencing gel and run at constant power (1,500 W) for 1–2 h using a LI-COR (IR2) sequencer (Model 4200, LI-COR, NE, USA).

#### Marker scoring and linkage analysis

Due to the poor level of heterozygosity in “Rubira,” only the genetic map of *P. davidiana* was constructed, following the “double pseudo-testcross” model of analysis (Grattapaglia and Sederoff 1994). Markers heterozygous in *P. davidiana* were scored for the presence or absence of the band considered. Departures from the 1:1 Mendelian ratio were tested by using chi-squared goodness of fit on segregation data. Linkage analysis was performed using Mapmaker/exp 3.0 software (Lincoln et al. 1992). Linkage groups were established using a critical logarithm of the odds (LOD) threshold of >8.0 and a recombination fraction of 0.30. Marker distances were calculated using the Kosambi (1944) mapping function.

#### Statistical analysis

Mean and standard deviation were calculated, and PPV resistance scores were tested for normality. Broad-sense

heritability ( $h$ ) of the genotypic mean values was estimated using the formula  $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/n)$ , where  $\sigma_g^2$  is the genotypic variance,  $\sigma_e^2$  the environmental variance (error effects), and  $n$  the number of replicates.

#### Quantitative trait loci analysis

Datasets obtained with the three phenotypic parameters listed in the phenotyping procedure, symptom intensity (I), symptom distribution (D), and global behavior (G), were used independently for further analysis. Two groups of data were established for each parameter. The first one was composed of the mean of the replicate scores computed for each genotype; the second one was composed of the maximal score observed among replicates for each genotype; this “maximal score” was hypothesized to represent the potential of symptom development which could be attained by each of the hybrids and it was interesting to compare the effects of both scores on QTL detection in order to choose the most relevant for further studies. In total, six datasets were used for QTL detection and for each evaluation cycle: IAv (average symptom intensity), IMax (maximal symptom intensity), DAv (average symptom distribution), DMax (maximal symptom distribution), GAv (average global behavior), and GMax (maximal global behavior). They are further referred to as “traits.”

QTL detection was performed by composite interval mapping (CIM; Zeng 1994) and stepwise regression analysis (MultiRegress model or MR) with Windows-QTL-Cartographer V2.5 software (Wang et al. 2007; <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>). In addition, since PPV accumulation departed from normality for some traits, a nonparametric test based on the Kruskal–Wallis (KW) methodology (Kruglyak and Lander 1995) was performed using the MapQTL 4.0 program (Van Ooijen et al. 2002). For stepwise regression analysis and Kruskal–Wallis tests, a significance threshold of 0.5% was used as recommended by Van Ooijen et al. (2002) to declare putative QTLs, but a less stringent threshold of 5% was considered for comparison with the previous studies which chose this threshold to declare putative QTLs. For CIM, a 1,000-permutation test was performed for each cycle and each trait in order to estimate the most appropriate LOD threshold to declare a QTL putative (type I error  $\alpha=0.05$ ), and cofactors were selected by forward–backward stepwise regression. The likelihood value of the presence of a QTL was estimated by the point where the LOD score was found maximum, and 95% confidence interval was assessed from this point decreased by one LOD unit. The percentage of phenotypic variation explained ( $R^2$ ) was estimated for each QTL. The one explained by all the QTLs ( $R^2_t$ ) was computed by multiple-regression analysis.

## Results

### Map construction

Among the 329 SSR primer pairs tested, 14 were heterozygous in “Rubira” (4.2%), among which four markers were included in the mapping set. This was insufficient to construct a map for “Rubira.” The genetic linkage map of *P. davidiana* consisted of the expected eight linkage groups; they were composed of 88 markers covering 454.2 cM (Fig. 2). Four among the 84 SSR primer pairs used for mapping amplified two different loci. Fifty-two (62%) were developed from *P. persica*, confirming a good level of transferability between peach and *P. davidiana*. Among all the markers mapped, 13 in total (14.8%) deviated significantly from their chi-squared expectations: five (5.7%) at the 5% threshold ( $\alpha$ ) and eight (9.1%) at the 1% threshold. Except one in LG2, they were clustered in LG7 (8) and LG8 (4). Five markers mapped in the central region of LG7 were heavily skewed ( $p < 0.001$ ). Forty SSRs (48%) mapped in *P. davidiana* were common with the general T×E *Prunus* map. The map coverage was estimated to 82% of the T×E map by comparison with the published *Prunus* maps, with some disparities (from 95%

for LG6 to 55% for LG8). The number of SSR loci mapped in each linkage group ranged from eight (LG5 and LG8) to 14 (LG1 and LG6) with an average of 11. The length of each linkage group was comprised between 89.4 cM (LG1) and 39.0 cM (LG2), and the average distance between loci ranged from 3.5 cM (LG2) to 7.7 cM (LG7) with an average of 5.4 cM in total. Four gaps longer than 15 cM were observed in three of the eight linkage groups (LG1, LG2, and LG5).

### Phenotypic evaluation of resistance to PPV

Due to the dominant gene (*Gr*) responsible for red color in “Rubira,” all the individuals were red in early stages, but the red color was later hidden by chlorophyll. A line pattern similar to typical PPV was observed in the plants, not yellow or bleached as usual but green–blue and usually darker than the rest of the leaf. However, this feature was without incidence on the scoring process. Table 2 summarizes the data obtained with the six traits studied over three vegetative cycles, and Fig. 1 shows symptom distributions over the 18 trait/cycle combinations analyzed. The distribution of the F<sub>1</sub> population was continuous in the disease score classes, indicating that several genetic factors were

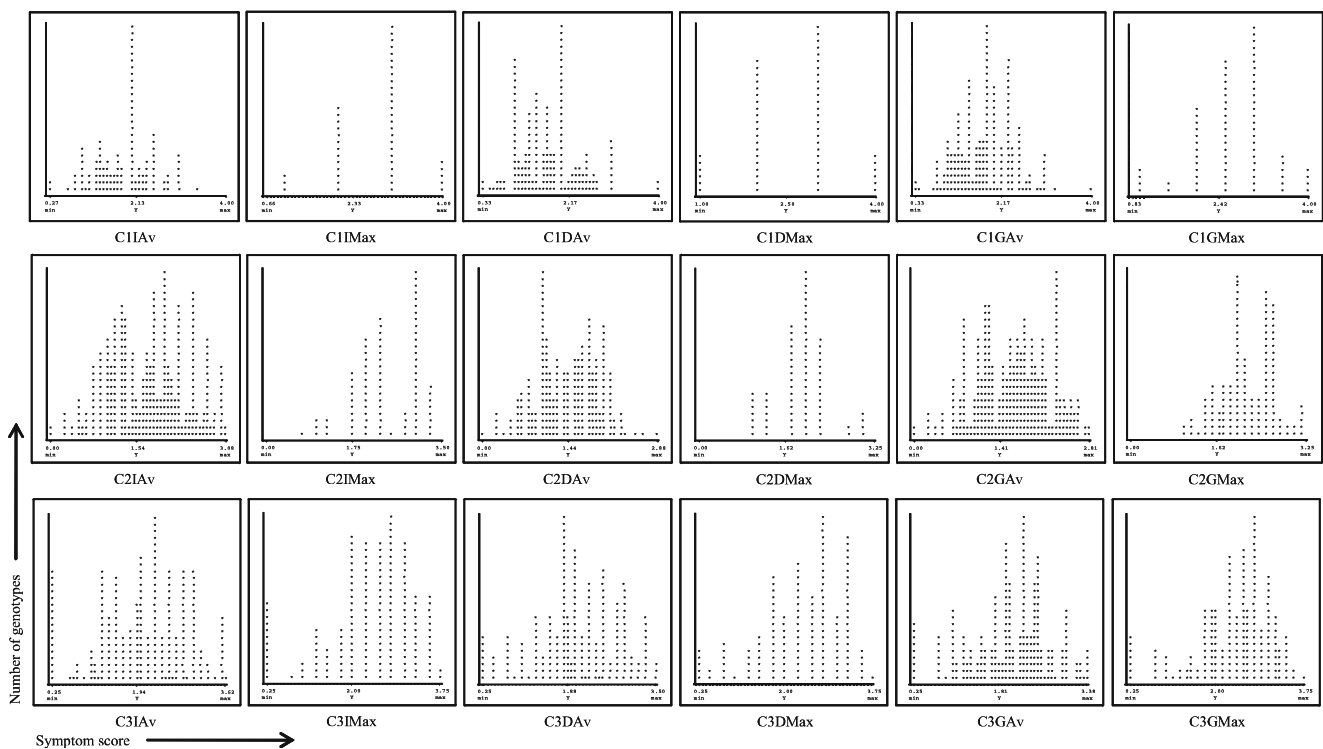
**Table 2** Summary of the statistics computed with the phenotypic data obtained with the “Rubira” × *P. davidiana* (RD) progeny

Traits	Population size in the corresponding cycle	Mean <sup>a</sup> (SD)	Range <sup>b</sup>	Skewness	Kurtosis	<i>S</i> test value	Heritability ( $h^2$ )
C1-IAv	170	1.85 (0.65)	0.27–4	0.07	0.60	2.301	0.32
C1-IMax		2.70 (0.78)	0.66–4	−0.18	1.11	4.066	0.39
C1-DAv		1.71 (0.64)	0.33–4	0.17	0.67	16.903	0.53
C1-DMax		2.54 (0.82)	1–4	−0.08	1.17	1.999	0.32
<i>C1-GAv</i>		<i>1.78 (0.60)</i>	<i>0.33–4</i>	<i>0.07</i>	<i>0.46</i>	<i>4.579</i>	<i>0.37</i>
C1-GMax		2.62 (0.71)	0.83–4	−0.12	0.81	3.986	0.34
C2-IAv	169	1.73 (0.67)	0–3.08	−0.02	0.50	2.723	0.24
C2-IMax		2.34 (0.69)	0–3.5	−0.21	0.74	12.098	0.29
C2-DAv		1.41 (0.51)	0–2.88	−0.01	0.19	0.681	0.32
C2-DMax		1.89 (0.54)	0–3.25	−0.04	0.35	7.826	0.35
<i>C2-GAv</i>		<i>1.57 (0.58)</i>	<i>0–2.81</i>	<i>−0.03</i>	<i>0.28</i>	<i>2.267</i>	<i>0.34</i>
C2-GMax		2.12 (0.56)	0–3.25	−0.12	0.40	17.937	0.28
C3-IAv	148	2.01 (0.71)	0.25–3.62	−0.18	1.32	3.127	0.37
C3-IMax		2.32 (0.85)	0.25–3.75	−0.53	1.85	17.721	0.39
C3-DAv		2.00 (0.75)	0.25–3.5	−0.14	0.91	2.838	0.69
C3-DMax		2.30 (0.82)	0.25–3.75	−0.40	1.40	11.987	0.76
<i>C3-GAv</i>		<i>2.01 (0.72)</i>	<i>0.25–3.38</i>	<i>−0.15</i>	<i>0.76</i>	<i>4.283</i>	<i>0.47</i>
C3-GMax		2.32 (0.74)	0.25–3.75	−0.36	1.14	22.101	0.67

Six traits were scored for each cycle (C1, C2, and C3): IAv (average symptom intensity), IMax (maximal symptom intensity), DAv (average symptom distribution), DMax (maximal symptom distribution), GAv (average global behavior), and GMax (maximal global behavior). The critical values for the rejection of normality are 5.99 and 9.21 at the 5% and 1% levels, respectively, for the test statistics *S*. Italicized rows represent the traits used for QTL analysis in the previous studies

<sup>a</sup> Mean of symptom scores; in brackets are standard deviations

<sup>b</sup> Range: minimum and maximum observed values for the corresponding trait



**Fig. 1** Seedling distributions in the different symptom classes according to the trait analyzed. Scores are figured on the abscissa ( $x$ ) and number of genotypes on the ordinate ( $y$ ). The figure shows the three cycles of evaluation (C1, C2, C3) with the six traits used for QTL detection: IAv (average symptom intensity), IMax (maximal

symptom intensity), DAv (average symptom distribution), DMax (maximal symptom distribution), GAv (average global behavior), and GMax (maximal global behavior). Graphs are issued from QTL Cartographer outputs

involved in the expression of susceptibility. Compared with the previous studies, the response was close to a normal distribution for most of the 18 trait/cycle combinations since only seven were rejected at the 5% level (Table 2), among which six involved maximal scores (Table 2 and Fig. 1). Seven, eight, and three plants in total showed no symptom and were ELISA negative during the first, second, and third cycles, respectively; among these, only one genotype was scored “0” for the three replicates (cycle 2). In addition, 19, 27, and 16 genotypes (11.1%, 15.9%, and 10.8% for each of the three cycles, respectively) showed a low incidence of PPV with mean scores comprised between 0 and 1. *P. davidiana* P1908 was confirmed as resistant as neither symptoms nor positive ELISA were observed during the three cycles of evaluation. The mean score for “Rubira” was 2.45 (GAv).

The mean values were generally lower during the second cycle, with the lowest for C2-DAv (1.41). As expected, the highest mean values were scored in those traits representing the maximal symptoms expression, with the highest one in the first cycle for C1-IMax (2.70). The differences between mean and maximal intensities were higher in the first cycle and lesser in the third one. The range of symptom scores was wide

with the narrower in cycle 2 (0–2.81) and the widest in cycle 1 (0.27–4). Heritability was heterogeneous, ranging from 0.24 (C2-IAv) to 0.76 (C3-DMax), and demonstrated important environmental effects.

#### QTL analysis

QTL analysis was performed for each of the trait/cycle combinations. A total of nine regions were identified at least once with one method (KW, MR, or CIM) and the less stringent threshold (5%) for KW or MR. These QTLs were named *PPV.RD-1.1*, *PPV.RD-2.1*, *PPV.RD-2.2*, *PPV.RD-4.1*, *PPV.RD-5.1*, *PPV.RD-6.1*, *PPV.RD-6.2*, *PPV.RD-7.1*, and *PPV.RD-7.2* according to the name of the population (RD), the linkage group on which they were identified, and a number; their locations and effects are summarized in Table 3 and their positions in the linkage groups in Fig. 2. The variation explained by the models fitting all the QTLs was low and ranged from 9.7% (C1-GAv) to 30.1% (C3-DMax). The most significant QTLs were detected for several trait/cycle combinations with KW ( $p$  value equal or lower than  $5 \times 10^{-3}$ ) and one or both additional methods (MR or CIM); their positions are shown in Fig. 3. Four main genomic regions

**Table 3** Summary of the QTLs detected for each scoring dataset by Kruskal–Wallis test (KW), multiple regression (MR), and composite interval mapping (CIM)

Traits	QTL	LG	Closest marker	KW <i>P</i> value	MR Posit. <sup>a</sup>	<i>P</i> value	CIM Posit. <sup>a</sup>	LOD <sup>b</sup>	LOD <sub>t</sub> <sup>c</sup>	Add. <sup>d</sup>	<i>R</i> <sup>2</sup> <sup>e</sup>	<i>R</i> <sup>2</sup> <sub>t</sub> <sup>f</sup>
C1-IAv	<i>PPV.RD-2.1</i>	2	UDP98-025	$1 \times 10^{-3}$	–	–	6.47	2.41	2.37	0.322	7.1	18.3
	<i>PPV.RD-2.2</i>	2	CPPCT017	$5 \times 10^{-3}$	–	–	–	–	–	0.259	4.4	
	<i>PPV.RD-5.1</i>	5	Pchgms4	$5 \times 10^{-3}$	36.31	$8 \times 10^{-3}$	–	–	–	0.217	2.5	
C1-IMax	<i>PPV.RD-2.1</i>	2	UDP98-025	$< 10^{-4}$	6.67	$3.4 \times 10^{-3}$	5.18	3.33	2.38	0.469	8.3	22.7
	<i>PPV.RD-2.2</i>	2	CPPCT017	$5 \times 10^{-4}$	–	–	–	–	–	0.235	4.2	
	<i>PPV.RD-7.2</i>	7	CPPCT033	–	–	–	42.84	2.54	–	0.597	7.1	
C1-DAv	<i>PPV.RD-1.1</i>	1	PaCITA5	–	–	–	24.64	2.41	2.40	0.512	6.8	15.6
	<i>PPV.RD-2.1</i>	2	UDP98-025	$1 \times 10^{-2}$	–	–	–	–	–	0.165	2.5	
	<i>PPV.RD-2.2</i>	2	M1a	$1 \times 10^{-3}$	23.7	$1.4 \times 10^{-2}$	–	–	–	0.221	2.9	
C1-DMax	<i>PPV.RD-2.1</i>	2	MA024a	$2 \times 10^{-2}$	–	–	–	–	–	0.293	3.1	14.3
	<i>PPV.RD-2.2</i>	2	BPPCT013	$2 \times 10^{-2}$	–	–	–	–	–	0.307	3.4	
	<i>PPV.RD-5.1</i>	5	Pchgms4	$3 \times 10^{-2}$	–	–	–	–	–	0.316	3.6	
	<i>PPV.RD-7.2</i>	7	UDAp-407	–	23.83	$3 \times 10^{-3}$	–	–	–	0.391	4.5	
<b>C1-GAv</b>	<b><i>PPV.RD-2.1</i></b>	<b>2</b>	<b>UDP98-025</b>	<b><math>2 \times 10^{-3}</math></b>	–	–	–	–	–	<b>0.257</b>	<b>4.4</b>	<b>9.7</b>
	<b><i>PPV.RD-2.2</i></b>	<b>2</b>	<b>M1a</b>	<b><math>6 \times 10^{-3}</math></b>	–	–	–	–	–	<b>0.140</b>	<b>1.1</b>	
	<b><i>PPV.RD-5.1</i></b>	<b>5</b>	<b>Pchgms4</b>	<b><math>6 \times 10^{-3}</math></b>	<b>36.31</b>	<b><math>6.5 \times 10^{-3}</math></b>	–	–	–	<b>0.191</b>	<b>2.5</b>	
C1-GMax	<i>PPV.RD-2.1</i>	2	UDP98-025	$5 \times 10^{-4}$	4.56	$1.5 \times 10^{-2}$	5.18	3.67	2.49	0.435	8.9	22.5
	<i>PPV.RD-2.2</i>	2	M1a	$1 \times 10^{-3}$	–	–	–	–	–	0.219	2.1	
	<i>PPV.RD-6.1</i>	6	BPPCT025	–	–	–	36.35	3.51	–	0.757	8.2	
C2-IAv	<i>PPV.RD-2.2</i>	2	CPPCT017	$8 \times 10^{-4}$	24.67	$3.5 \times 10^{-3}$	24.78	3.31	2.41	0.399	8.5	12.0
	<i>PPV.RD-4.1</i>	4	AMPA103	–	44.42	$2 \times 10^{-2}$	–	–	–	0.202	2.2	
C2-IMax	<i>PPV.RD-2.2</i>	2	CPPCT017	$5 \times 10^{-3}$	28.56	$1.4 \times 10^{-2}$	–	–	–	0.493	5.8	12.1
	<i>PPV.RD-6.2</i>	6	AMPA121	$2 \times 10^{-2}$	–	–	–	–	–	0.224	2.5	
C2-DAv	<i>PPV.RD-2.1</i>	2	UDP98-025	$4 \times 10^{-3}$	6.67	$1 \times 10^{-2}$	–	–	–	0.228	4.6	15.4
	<i>PPV.RD-2.2</i>	2	CPPCT017	$5 \times 10^{-3}$	–	–	–	–	–	0.209	2.1	
	<i>PPV.RD-7.1</i>	7	AMPA107	$2 \times 10^{-3}$	3.83	$9 \times 10^{-4}$	2.94	2.33	2.35	0.252	5.7	
C2-DMax	<i>PPV.RD-2.2</i>	2	CPPCT017	$1 \times 10^{-2}$	–	–	–	–	–	0.144	1.6	13.7
	<i>PPV.RD-5.1</i>	5	Pchgms4	$1 \times 10^{-3}$	–	–	–	–	–	0.262	4.6	
	<i>PPV.RD-7.1</i>	7	AMPA107	$1 \times 10^{-3}$	3.83	$1 \times 10^{-4}$	2.35	2.40	2.39	0.273	5.9	11.9
<b>C2-GAv</b>	<b><i>PPV.RD-2.1</i></b>	<b>2</b>	<b>UDP98-025</b>	<b><math>4 \times 10^{-3}</math></b>	–	–	<b>6.47</b>	<b>2.39</b>	<b>2.40</b>	<b>0.291</b>	<b>6.0</b>	<b>14.1</b>
	<b><i>PPV.RD-2.2</i></b>	<b>2</b>	<b>CPPCT017</b>	<b><math>1 \times 10^{-3}</math></b>	<b>24.67</b>	<b><math>4.5 \times 10^{-3}</math></b>	<b>24.78</b>	<b>2.99</b>	<b>2.40</b>	<b>0.320</b>	<b>7.4</b>	
	<b><i>PPV.RD-4.1</i></b>	<b>4</b>	<b>AMPA103</b>	–	<b>44.42</b>	<b><math>2 \times 10^{-2}</math></b>	–	–	–	<b>0.169</b>	<b>2.1</b>	
C2-GMax	<i>PPV.RD-2.2</i>	2	CPPCT017	$2 \times 10^{-3}$	24.67	$9.1 \times 10^{-3}$	23.84	3.17	2.36	0.481	8.2	17.5
	<i>PPV.RD-5.1</i>	5	Pchgms4	$2 \times 10^{-2}$	–	–	–	–	–	0.120	1.1	
	<i>PPV.RD-6.2</i>	6	AMPA121	$1 \times 10^{-2}$	–	–	–	–	–	0.220	3.8	
C3-IAv	–	–	–	–	–	–	–	–	–	–	–	
C3-IMax	<i>PPV.RD-7.2</i>	7	UDAp-460	–	37.83	$3.5 \times 10^{-2}$	42.84	3.52	2.38	0.908	14.3	25.1
C3-DAv	<i>PPV.RD-7.2</i>	7	UDAp-407	$1 \times 10^{-4}$	29.83	$< 10^{-4}$	30.43	4.21	2.43	0.579	13.1	20.4
C3-DMax	<i>PPV.RD-2.2</i>	2	M1a	$1 \times 10^{-2}$	–	–	–	–	–	0.258	2.4	30.1
	<i>PPV.RD-4.1</i>	4	BPPCT040	$9 \times 10^{-3}$	–	–	–	–	–	0.279	2.7	
	<i>PPV.RD-7.1</i>	7	AMPA107	$2 \times 10^{-3}$	–	–	–	–	–	0.195	1.5	
	<i>PPV.RD-7.2</i>	7	UDAp-407	$< 10^{-4}$	29.83	$< 10^{-5}$	30.43	5.40	2.41	0.701	15.6	
<b>C3-GAv</b>	<b><i>PPV.RD-7.2</i></b>	<b>7</b>	<b>UDAp-407</b>	<b><math>1 \times 10^{-2}</math></b>	<b>33.83</b>	<b><math>1.3 \times 10^{-2}</math></b>	<b>30.43</b>	<b>2.71</b>	<b>2.45</b>	<b>0.420</b>	<b>7.6</b>	<b>24.5</b>
C3-GMax	<i>PPV.RD-7.2</i>	7	UDAp-407	$8 \times 10^{-4}$	33.83	$1.6 \times 10^{-3}$	42.84	5.60	2.47	0.928	20.1	26.1

C1, C2, and C3 are the evaluation cycles, followed by the trait name: IAv (average symptoms intensity), IMax (maximal symptom intensity), DAv (average symptom distribution), DMax (maximal symptom distribution), GAv (average global behavior), and GMax (maximal global behavior). Closest marker is given by the Kruskal–Wallis test. *P* value is the significance of the association between the marker and the QTL. *P* value comprised between  $5 \times 10^{-2}$  and  $5 \times 10^{-3}$  are in italics for KW. Only QTLs above or close (in italics) to the empirical threshold are listed for CIM. The reader is advised to read across the table to assign QTLs to the MR, CIM, and KW analysis, respectively. Rows in bold represent the traits used for QTL analysis in the previous studies

LG linkage group

<sup>a</sup> Position of the QTL peak from the upper part of the linkage group in centimolar

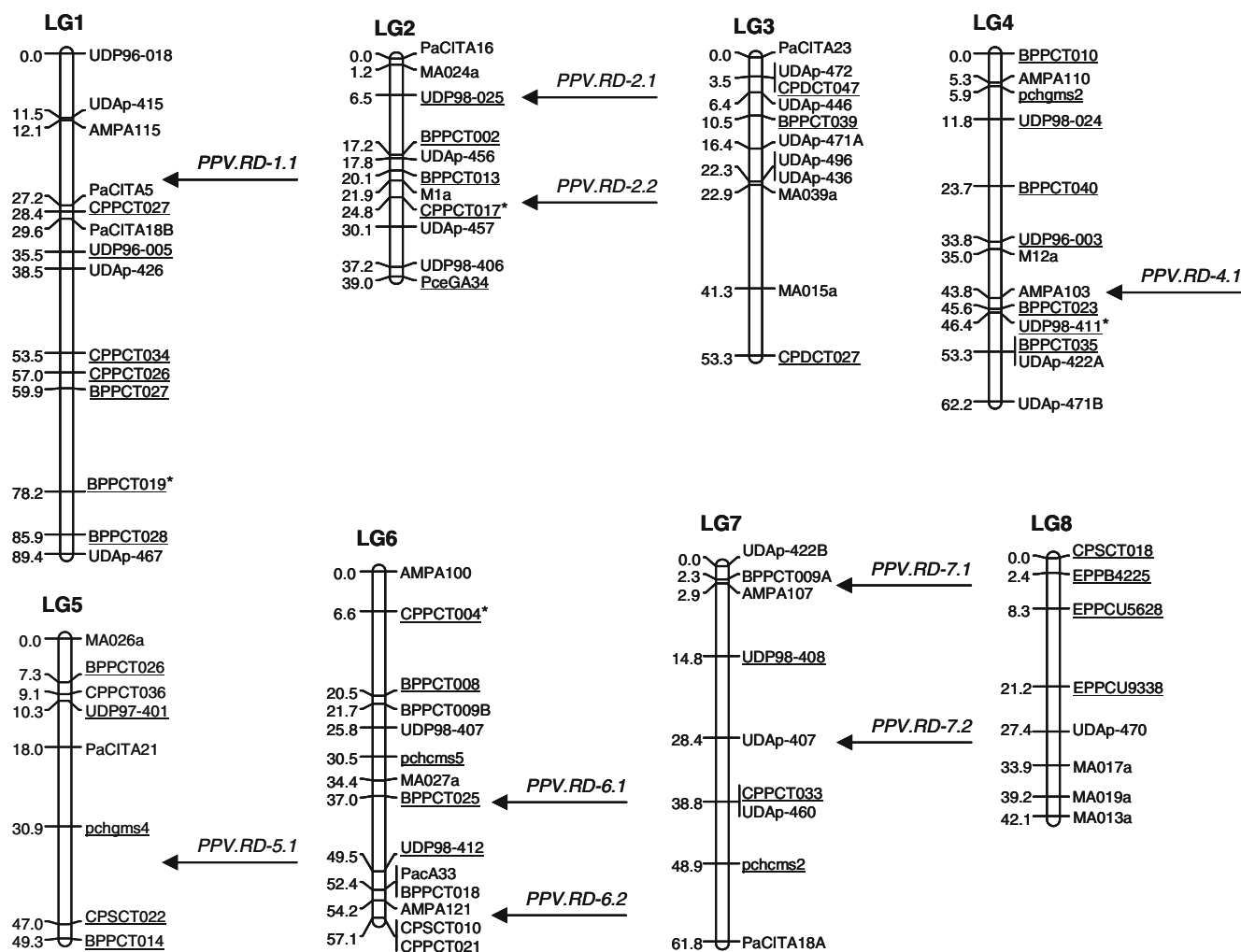
<sup>b</sup> Logarithm of odds score under composite interval mapping

<sup>c</sup> LOD threshold under composite interval mapping

<sup>d</sup> Additive effect

<sup>e</sup> Individual contribution to the variance accounted for by the QTL (%)

<sup>f</sup> Total variance explained by the model (%)



**Fig. 2** Linkage map of *P. davidiana* P1908 derived from the RD population. Markers mapping several loci are figured with a capital letter (A, B) following the locus name. Markers placed in the general

map for *Prunus* (T×E) are underlined; loci followed by *asterisk* were mapped in different linkage groups in T×E. QTLs are figured with an *arrow* on the *right* of the linkage groups

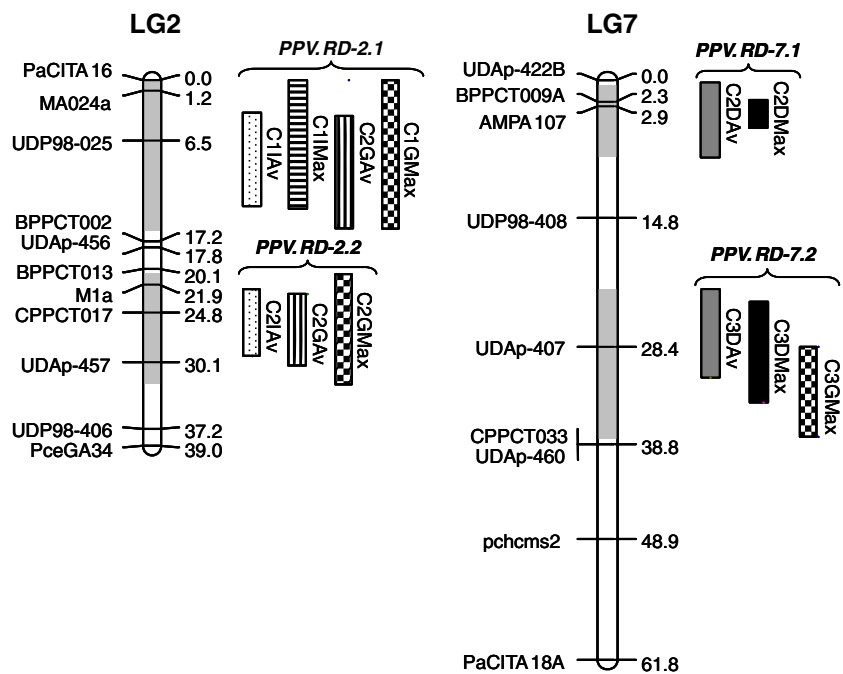
involved in PPV resistance were thus identified. Two of these were detected in LG2 and the other ones in LG7. The phenotypic variation explained individually ranged from 2.5% to 8.9% for *PPV.RD-2.1*, 1.1% to 8.5% for *PPV.RD-2.2*, 1.5% to 5.9% for *PPV.RD-7.1*, and 4.5% to 20.1% for *PPV.RD-7.2* (Table 3). The QTL with the strongest effect (*PPV.RD-7.2*) was detected for the third cycle of evaluation but was not detected with KW for the other cycles. The QTLs in LG2 (*PPV.RD-2.1* and *PPV.RD-2.2*) were only detected for the first two cycles. Five additional regions (*PPV.RD-1.1*, *PPV.RD-4.1*, *PPV.RD-5.1*, *PPV.RD-6.1*, and *PPV.RD-6.2*) spread over four linkage groups were identified. Among these, *PPV.RD-5.1* was identified for two vegetative cycles and five trait/cycle combinations among which two with KW at the 0.05% threshold (Table 3). The other ones were detected only once or with a significance level close to the threshold.

## Discussion

### Marker polymorphism and map comparison

The SSRs tested in the RD population were developed from several *Prunus* species. Their ability to amplify DNA from the population and consequently the level of transportability in *P. davidiana* was very high, except for those from sour cherry (50%), but with a limited set of SSRs (6): 93.7% for almond, 92.7% for peach, 92% for plum, 88.2% for apricot, 87.5% for sweet cherry, with an average of 90.3% in total. These species belong to the three *Prunus* subgenus, and there is no obvious correlation between the SSR origin and the amplification in *P. davidiana*. These results are in agreement with those reported in Dirlewanger et al. (2006) for peach. Among the 329 SSRs tested, 152 were heterozygous in *P. davidiana* (46.2%). This level of heterozygosity is intermediate between that of peach (5% to

**Fig. 3** Linkage groups 2 and 7 of *P. davidiana* P1908 showing the location of the QTLs which were detected by at least two statistical methods including KW at  $P < 5 \times 10^{-3}$ . Vertical bars on the right indicate the position and LOD-1 confidence interval for each QTL. The shaded areas in the linkage groups represent the QTL position and interval



28%) and that of almond (44% to 88%), close to that of apricot (24% to 65%) as reported by Sánchez-Pérez et al. (2006). In comparison, only 14 SSRs were heterozygous in “Rubira” (4.2%), showing that *P. davidiana* has apparent genetic distance to this cultivar.

The previous map of *P. davidiana* derived from the SD F<sub>1</sub> population was mainly composed of RFLPs and dominant markers (Foulongne et al. 2003; Decroocq et al. 2005) not useful for map comparison. In addition, two groups (LG3 and LG7) were insufficiently covered. The new map of *P. davidiana* was constructed with 84 *Prunus* SSRs which covers most of the T×E general map for *Prunus* (Joobeur et al. 1998; Aranzana et al. 2003; Genome Database for Rosaceae <http://www.bioinfo.wsu.edu/gdr/>), allowing map comparison with the T×E map and numerous published *Prunus* maps (Dirlewanger et al. 2004, 2006; Lambert et al. 2004; Yamamoto et al. 2005; Verde et al. 2005; Dondini et al. 2007; Soriano et al. 2008). Its length is similar to that of the T×E map which covers 506 cM (Genome Database for Rosaceae <http://www.bioinfo.wsu.edu/gdr/>). No significant difference in locus order was observed between the map of *P. davidiana* and these *Prunus* maps. Nine SSRs mapped at new loci were compared to their position in the other maps: four derived from apricot (PacA33, PaCITA18B, UDAp-422B, and UDAp471A and B) and five derived from peach (CPPCT017, UDP98-411, BPPCT009, BPPCT019, and CPPCT004). In LG7, UDAp-422B and AMPA107 mapped at inverted positions in the “Lito” and “BO81604311” maps (Dondini et al. 2007). In LG1, it is also the case for CPPCT034 and CPPCT026 markers compared to the T×E

map, but these markers mapped to similar positions in the “Garfi” × “Nemared” map (Jáuregui et al. 2001). Due to the low number of markers in common, the comparison of the two maps of *P. davidiana* (SD and RD populations) was only possible between six groups with the intermediate use of the T×E map. The mean lengths of linkage groups were similar but with some discrepancies depending on the group considered. Linkage groups were longer for the map derived from the SD progeny for LG1, LG2, and LG6 and shorter for the others. This could be explained by different recombination rates in relation with the female parent or errors in the scoring of dominant markers. The region flanking the self-incompatibility locus in LG6 did not contain skewed markers in the new map unlike the homologous region of the F<sub>2</sub> map derived from *P. davidiana* (SD40<sub>2</sub>) and numerous *Prunus* maps (Joobeur et al. 1998; Foulongne et al. 2003; Lambert et al. 2004; Soriano et al. 2008). Most of the skewed loci were concentrated in LG7. This fact was not reported in Foulongne et al. (2003) for the previous map of *P. davidiana*, but this group was partially covered and split into two parts. This configuration suggests the presence of a small number of loci under strong selection pressure influencing flanking marker transmission by linkage.

#### Phenotyping

Assessing PPV resistance in peach and more generally in *Prunus* is an uneasy task since PPV shows an irregular behavior even in herbaceous host like the model plant

*Arabidopsis thaliana* (Decroocq et al. 2006; Sicard et al. 2008). It is essentially based on visual observation of symptoms in the plants followed by confirmation of the virus accumulation with ELISA or PCR techniques. In most studies, PPV resistance was considered as a qualitative trait (Martínez-Gómez et al. 2000; Rubio et al. 2007; Soriano et al. 2008; Lalli et al. 2008). Trees were scored as susceptible or resistant, and the ratio obtained was statistically tested against theoretical ratios for one or several genes. In our study, we assume the hypothesis of polygenic control of PPV resistance in *P. davidiana* as demonstrated in Decroocq et al. (2005) and Marandel et al. (2009) and scored symptom expression as a quantitative trait. Consequently, the phenotyping method used was a key point for obtaining reliable results useful for QTL mapping, and a strong attention was put on this point in order to determine which parameters would be most appropriate. Phenotypic evaluations were carried out following the model of rootstock inoculation called “heavy test,” used for PPV evaluation in Martínez-Gómez and Dicenta (2000b), Moustapha et al. (2001), and Rubio et al. (2003). Direct inoculation of cultivar or “standard test” has been more widely used (Dosba et al. 1991; Audergon et al. 1995; Pascal et al. 2002; Decroocq et al. 2005; Marandel et al. 2009) as the latter is thought to be very similar to natural transmission by aphids (Labonne et al. 1994) and allows the study of the virus movement towards the rootstock (Dicenta et al. 2003; Ferri et al. 2002; Decroocq et al. 2005). Recently, Rubio et al. (2008b) suggested that rootstock inoculation would be more suited to sharka evaluation in the framework of a breeding program. These authors demonstrated that this method is faster and more reliable because it is simpler to realize; prior inoculation of the rootstock prevents the cultivar from escaping inoculation; it allows for first-symptom scoring directly after the cultivar grafting without prior chilling, and it is easier to score symptoms on the plants thanks to a generally homogeneous reduced foliar mass.

In this study, 170 individuals were evaluated (90% with three replicate), as opposed to about 70 in the F<sub>1</sub> SD and F<sub>2</sub> SD40<sub>2</sub> populations evaluated in Decroocq et al. (2005) and Marandel et al. (2009), respectively; this high number of individuals makes the work carried out consistent. However, our results strikingly contrasted with those of Decroocq et al. (2005) and Marandel et al. (2009). The incidence and severity of observed symptoms were far higher than had been observed in both previous populations. The mean symptom scores (GA<sub>v</sub>) ranged from 4.2- to 12.6-fold (6.3-fold on average) and from 1.2-fold to fivefold (2.2-fold on average) higher than those reported in the SD and the SD40<sub>2</sub> populations (Decroocq et al. 2005; Marandel et al. 2009). The genotype distributions in the disease score classes were close to normality in the RD

population for most of the trait/cycle combinations, and only one genotype was considered totally resistant after three growth periods; this is consistent with a polygenic model in which the factors involved in PPV resistance have cumulative low individual effects. To the contrary, the response was not normally distributed in both other studies due to a large proportion of asymptomatic individuals in the populations; moreover, *P. davidiana* and the whole SD population showed no symptoms but “Summergrand” in the first vegetative cycle (year 1997) and very mild symptoms in 1999 (Decroocq et al. 2005). This could suggest that a high level of PPV resistance in the SD population or that inoculum pressure and, consequently, virus accumulation were limited in the plants and would not allow proper development of symptoms in the leaves. In fact, vegetative development was much higher in the standard test compared to the heavy test in which foliar mass was strongly reduced. This probably induced a lower virus accumulation in the leaves of the SD population and consequently weaker, scattered symptoms that were more difficult to identify. In spite of the fact that two different female parents were used for constructing the F<sub>1</sub> populations (SD and RD), these important dissimilarities suggest that the “heavy test” would be more appropriate for PPV evaluation than the “standard test,” as already reported in Moustapha et al. (2001) and Rubio et al. (2008b).

We also made an attempt to separate factors involved in virus accumulation and virus long-distance movement in the plants as they are potentially different (Ion-Nagy et al. 2006); plants were thus independently scored for symptom intensity and distribution as reported in Decroocq et al. (2005), in order to establish possible association of these traits with different regions of the genome. A good correlation was found between intensity and distribution which validates, a priori, the use of a global score that integrates both scores for determining the level of susceptibility of a given cultivar for breeding purposes. On the other hand, the QTL analysis results show that symptom intensity seems more likely to be linked to LG2 and symptom distribution to LG7, suggesting that scoring these parameters independently would be more appropriate to decipher plant/virus interactions at the genome level. These findings are in agreement with those reported in Decroocq et al. (2005) who identified two loci in LG7 involved in the restricted downward movement (RDM) of PPV.

During the scoring process, the score attributed to each replicate measured the level of symptoms expression and not the level of resistance/susceptibility; this level varies according to different parameters including the vegetative state of the plant and its interaction with the environment. For this reason, we considered useful using the score attributed to the replicate showing the strongest symptoms

(maximal score) for each genotype in addition with the mean value generally used for QTL analysis since it could contribute to reduce the variability due to environmental effects. The results obtained are contrasting; the maximal score is a reliable parameter as it was generally able to detect the same QTLs as those detected with the mean scores and, occasionally, with higher significance levels or LOD scores. However, it was not the case for all the trait/cycle combinations, and some of the QTLs with low effects were identified with either the maximal or the mean scores but not with both. Consequently, the maximal score adds significant and complementary value to the information but has to be combined with the mean scores in order to have a complete view on the genetic control of PPV resistance underlying the behavior of the plants. Nevertheless, for breeding purposes, it seems more appropriate for showing the genetic potential of a given progeny to express susceptibility; it could thus minimize some of the difficulties encountered in sharka evaluation assays, like erratic virus distribution, time of the evaluation, inoculum source, or culture conditions (Ferri et al. 2002; Ll acer et al. 2008; Rubio et al. 2008a).

#### Consistency of the QTLs across genetic backgrounds

To date, only two studies (Decroocq et al. 2005; Marandel et al. 2009) described the complex genetic basis of quantitative resistance to PPV in *P. davidiana* P1908. These authors used F<sub>1</sub> and F<sub>2</sub> progenies derived from another peach cultivar (“Summergrand”) as female genitor and identified seven genomic regions involved in PPV resistance in *P. davidiana*. Taking our results as a whole, our study identified nine genomic regions which were rather involved in modulating disease incidence or virus distribution in the plants than resistance since only one genotype was asymptomatic. Based on comparative mapping through the general *Prunus* map and common anchor loci, six among these ones were homologous to regions identified in Decroocq et al. (2005) and/or Marandel et al. (2009) in LG1, LG2, LG4, LG5, LG6, and LG7. Apart from the fact that if we consider those identified with the same parameter as these authors, that is mean global behavior (GA<sub>v</sub> in our study), only five remain (*PPV.RD-2.1*, *PPV.RD-2.2*, *PPV.RD-4.1*, *PPV.RD-5.1*, and *PPV.RD-7.2*); these convergent results confirm that the polygenic resistance carried by *P. davidiana* is based on QTLs with cumulative minor individual effects and, despite their dissimilar effects, suggest consistency of most of them over successive generations and genetic backgrounds. However, behind these findings, some significant discrepancies exist which induce questioning and the first of which is the high level of symptom severity observed in the RD population compared to both other populations. Two main

hypotheses could explain this result: the influences of the test (standard vs heavy) and that of the genetic background as the female parents were different. We showed above that the standard test could have been at issue due to the large foliar mass making more difficult symptom identification; however, most of the QTL regions previously identified were detected at least with one trait/cycle combination, suggesting that the genetic background of the susceptible parent is most likely responsible for the low QTL effects and thus to the higher susceptibility observed. Genetic background effects on quantitative traits have been well documented in numerous annual species (Liao et al. 2001), and it is likely that “background” (modifying) or “complementary” loci could have interacted with resistance factors of *P. davidiana* and thus modified their effects. Nevertheless, the hypothesis of recessive resistance factors has to be taken into account as well, even if Marandel et al. (2009) demonstrated that the presence of a least one *P. davidiana* allele was enough to induce resistance. Recessive resistance against *potyviruses* has been described in different species, particularly in *Solanaceae* where half of the 16 well-characterized resistance genes are recessive (Parella et al. 2002). The implication of components of the eukaryotic translation initiation complex has been reported in several virus/plant pathosystems including *A. thaliana* (Maule et al. 2007; Nicaise et al. 2007), and among these ones the eukaryotic initiation factor 4E (*eIF4E*) has been identified as a recessive inherited resistance locus in pepper, lettuce, and pea against several *potyvirus* (Kang et al. 2005). Decroocq et al. (2005) and Sicard et al. (2008) mapped several candidate genes involved in the eukaryotic translation initiation complex in peach and apricot and suggested the involvement of some of them in resistance to PPV. In addition, Marandel et al. (2009) mapped SSRs linked to a component of the eukaryotic translation initiation complex (*eIF(iso)4G*) to the region of the major QTL in LG6, even if these authors did not demonstrate a link between the resistance and a specific allele. In Decroocq et al. (2005) and Marandel et al. (2009), the main QTLs were identified in LG6 and LG7 whereas our study did not confirm the prominence of LG6; two QTLs were identified in this group, but their contributions to the phenotypic variance were low. In the contrary, two QTLs were identified in LG7 among which one QTL accounts for the strongest contribution (*PPV.RD-7.2*). Most of the markers were skewed, but Xu (2008) demonstrated that, whether segregation distortion is detrimental to the power of detecting QTL with dominant effect, for 44% of the time, segregation distortion loci (SDL) are beneficial to QTL mapping with additive effect, which is the case in our study. The LOD-2 support of *PPV-7.a* in Marandel et al. (2009) was found overlapping both QTLs in our study; in addition, two candidate genes for PPV resistance (Cd201 and Cd89)

colocalized with *PPV-7a* (Marandel et al. 2009). In Decroocq et al. (2005), two regions were identified in this group, among which one encompasses a main QTL (*PPV-7.1*); both regions were involved in restricted downward movement of the virus (RDM1 and RDM3) whereas, in our study, both regions in LG7 were found more likely associated to virus distribution. These findings suggest that this common region probably contributes to a major part of the PPV resistance in *P. davidiana* P1908 but seems to be more associated to virus accumulation and distribution in the plant than to symptom severity. This suggests that symptom severity and virus accumulation are associated with different genomic regions as reported in Sicard et al. (2008) who detected QTL associated to symptom severity (SYMP) and virus accumulation (ACC) for PPV infection in *A. thaliana*. Besides this, these findings suggest that the major QTL in LG6 is probably the one which contributes the most to the reduction of symptom severity since a correlation seems to exist between the high level of symptom severity and the low effect of this QTL in the RD population.

Two among the remaining QTLs (*PPV.RD-1.1*, *PPV.RD-5.1*) have a peculiar interest as they were previously identified in homologous regions in apricot. *PPV.RD-1.1* was homologous to the main dominant QTL mapped in LG1 in apricot in the neighborhood of PaCITA5 SSR marker (Lambert et al. 2007; Sicard et al. 2007; Soriano et al. 2008; Lalli et al. 2008). The position of this QTL is well documented now in apricot, and our study confirms synteny of this factor between both species thanks to the SSR anchor loci. The dissimilar QTL effects between *P. davidiana* and apricot are probably due to allelic variants of the gene responsible for PPV resistance which could lead to contrasting responses to virus infection. For *PPV.RD-5.1*, a putative recessive QTL was identified in the same region close to pchgms4 SSR marker in the apricot maps of “Stark Early Orange” and “Polonais” (Lambert et al. 2007), demonstrating the possible involvement of recessive factors in PPV resistance. In our study, it is one among the most conserved QTL among trait/cycle combinations. These results underline the difficulties encountered in translating at a genomic level the interactions between the plant and the virus in a polygenic model for PPV resistance such as *P. davidiana* P1908 in which resistance is probably the result of the effects of dominant and recessive factors. It demonstrates that the expression of symptoms is strongly dependent upon the erratic movement of the virus in the plants and some other environmental conditions but, mainly, to components of the genetic background which modulate the QTL effects; these components probably include allelic variants of recessive factors. The hypothesis of genetic recessive factors has to be confirmed by further

studies and cross-evaluations, but the genetic background of the susceptible parent has therefore to be taken into account as it could be a limiting factor for developing resistant cultivars derived from *P. davidiana*. It leads up to the reconsideration of the breeding methods used and favoring those using molecular markers derived from validated candidate genes in order to combine favorable alleles of the cultivars used as female genitors with those of *P. davidiana*. This work is currently in progress.

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