Evaluation of the *Prunus* Interspecific Progenies for Resistance to *Plum Pox Virus*

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Abstract

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Sharka disease caused by the infection with the *Plum pox virus* (PPV) in stone fruit trees is worldwide the most devastating for stone fruit production. Until now, good sources of resistance to PPV within the peach group have not been available. There are no commercial cultivars of peach that are resistant to PPV. Other *Prunus* species are known to show varying levels of resistance. Interspecific hybrids GF 677 (*Prunus amygdalus* × *P. persica*) and Cadaman (*P. davidiana* × *P. persica*) were revealed to be resistant to PPV. The resistance to a Dideron isolate of the descendants of Cresthaven × GF 677 and Cresthaven × Cadaman and their progenitors was evaluated after inoculation by chip-budding in a sealed screenhouse. Results demonstrate a certain level of resistance in both progenies of interspecific hybrids and indicate a potential for PPV resistance transfer to commercial peach cultivars but it will be necessary to perform backcrosses with peach cultivars of agricultural interest in order to return pomological and agronomic traits. For the definitive confirmation of resistance/susceptibility it will be necessary to wait until the adult stage of hybrids.

Keywords: *Prunus amygdalus*; *Prunus davidiana*; *Prunus persica*; PPV; sharka disease; transmissibility

Sharka disease, also known as plum pox, which is caused by *Plum pox virus* (PPV, Potyviruses, Potyviridae, genus *Potyvirus*), is the economically most important virus disease in stone-fruit trees worldwide (Németh 1994; Kölber 2001). It is a very serious problem completely devastating productivity and fruit quality in peach, apricot and plum orchards.

Costly eradication or control efforts exist in many countries. Although successful in a few cases, these efforts have generally slowed the progression of PPV, but not stopped it. Management strategies of plum pox are aimed primarily at preventing introduction by use of virus-tested clean nursery stock (Polák *et al.* 1995). Once detected, strict quarantine, eradication and ongoing surveys are the only useful strategies because a tree, once infected, will never be free of the disease. Insecticide management strategies that keep aphid populations low may help to slow PPV movement in areas where PPV is rare, but may not be a good idea in some situations. Breeding for PPV resistance is the most efficient method to control sharka disease in apricots (Audergon *et al.* 1994; Kegler *et al.* 1998; Egea *et al.* 1999).

In peach (*Prunus persica* /L./ Batsch), all cultivars are susceptible to PPV and the only potential resistance source available *Prunus davidiana* clone P1908 (Anonymous 1974; Decroocq *et al*. 2005) has been identified up to now. Thus,

the isolation and incorporation of genes for resistance to sharka disease into *P. persica* would be of significant economic and environmental benefit. A solution can be found in using PPV resistant wild species closely related to peach such as *P. davidiana* (Anonymous 1974) and *P. amygdalus* (Dicenta *et al.* 2002; Rubio *et al.* 2003a). Interspecific hybrids GF 677 and Cadaman (*Prunus amygdalus* × *Prunus persica* and *P. davidiana* × *P. persica*, respectively) were shown to bear resistance to PPV (Rubio *et al.* 2005; Polák & Oukropec 2010). They represent a good resistance potential to PPV in peach.

The first study of genetic factors involved in resistance to PPV in *Prunus davidiana* was reported in Decroocq *et al.* (2005). It used an F_1 population derived from a cross between *P. persica* cultivar Summergrand and *P. davidiana* clone P1908 and reported a quantitative trait loci (QTLs) 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* clone P1908 was further studied by Marandel *et al.* (2009) in the F_2 population derived from the selfing of the individual (#40) of the F_1 population and demonstrated the conservation of four of the six F_1 QTLs. A total of nine *Prunus davidiana* QTLs involved in PPV resistance were identified in an F_1 population derived from the susceptible peach cultivar Rubira and *P. davidiana* clone P1908 (Rubio *et al.* 2010). All the studies included the mapping of candidate genes or candidate gene-linked simple sequence repeat (SSR) markers.

In the last 20 years strategies for the protection of plants against virus diseases have been expanded to include transgenic plants expressing viral genes, which inhibit virus infection (Sanford & Johnston 1985). The transgenic C5 HoneySweet plum exploiting this technology (Scorza *et al.* 1994) was found to be highly resistant to PPV. However several obstacles need to be overcome in peach. This is especially true for the development of a genotype-independent system for tissue culture and genetic transformation.

The aim of this work was to study the transmission of resistance to PPV from interspecific *Prunus* hybrids GF 677 and Cadaman to the susceptible US peach cultivar Cresthaven by traditional crosses and evaluating the role of these related species as a source of resistance in peach-breeding programs for resistance to PPV.

MATERIAL AND METHODS

One hundred and fifty-six individuals from 2 different crosses were studied: Cresthaven × GF 677 (110 descendants) and Cresthaven × Cadaman (46 descendants). Cresthaven is a US peach cultivar susceptible to PPV, while GF 677 and Cadaman are French interspecific hybrids (*Prunus amygdalus* × *Prunus persica* and *P. davidiana* × *P. persica*, respectively) rootstocks resistant to PPV (Rubio *et al.* 2005, Polák & Oukropec 2010).

Cresthaven is a peach cultivar developed at South Haven, USA, by crossing cultivars Kalhaven and South Haven 309 (SH 50 × Redhaven). It is a late self-pollinating cultivar, with great taste and very juicy fruits. The peach cultivar Cresthaven sus-

Table 1. Scheme of five-year evaluation cycle for resistance to *Plum pox virus* (PPV)

Procedure	Date
Crosses	April 2005
Artificial stratification	January 2006–March 2006
Transplanting of seedlings	April 2006
Inoculation	August 2006
Chilling treatment	December 2006–March 2007
Symptom observation 1	May 2007
Symptom observation 2	June 2007
ELISA	June 2007
Re-inoculation	July 2007
Chilling treatment	December 2007–March 2008
Symptom observation 1	June 2008
Symptom observation 2	July 2008
ELISA	May 2008
Chilling treatment	December 2008–March 2009
Symptom observation 1	May 2008
Symptom observation 2	July 2009
Chilling treatment	December 2009–March 2010
Symptom observation 1	June 2010
Symptom observation 2	July 2010
Chilling treatment	December 2010–March 2011
Symptom observation 1	June 2011
Symptom observation 2	July 2011
Chilling treatment	December 2011–March 2012
Symptom observation 1	May 2012
Symptom observation 2	June 2012
RT-PCR	June 2012

ceptible to PPV was crossed as a female parent to PPV resistant interspecific hybrids Cadaman (*P. persica* × *P. davidiana*) and GF 677 (*P. persica* × *P. amygdalus*) in 2005. Seeds resulting from the crosses were stratified and after that sown in a greenhouse.

Plants were grown in 3.81-l pots in an insect-proof screenhouse. The plants were transferred to a cold chamber (7°C, darkness) periodically, and successive evaluations were conducted. The general timetable of the experimentation is presented in Table 1. Each progeny was grown on its own roots (1 tree).

The isolate assayed was a PPV-Dideron type strain originally isolated from an apricot in south-eastern France. Each plant was inoculated by chip-budding with one infected bud. The buds came from an apricot infected with the isolate and showing sharka symptoms on leaves. Symptoms were also observed on leaves of shoots growing from the inoculum bud (effective inoculation). Plants without sharka symptoms on leaves of these shoots growing from the inoculum bud and with negative enzyme-linked immunosorbent assay (ELISA) reaction were re-inoculated. At the beginning of each growth period, pruning was performed to induce vigorous new shoots for symptom scoring.

The resistance of the individuals was evaluated following the method of Salava *et al.* (2005). Symptoms of sharka were visually scored on leaves from 0 (no symptoms) to 4 (maximum intensity of symptoms) (Table 2) after two months in the screenhouse. Plants were studied for five vegetative cycles. Individuals were considered susceptible when they developed chlorotic discoloration and distortion of leaves characteristic of PPV and assayed positive by ELISA or RT-PCR during at least one of the last three growth periods assayed (Table 3).

At the same time, in the first and second cycle of study, an ELISA (Polák *et al.* 1997) was applied to confirm the presence or absence of the virus in the plant. Finally, in the fifth cycle of the study, RT-PCR using the specific primers P1 and P2 (Wetzel *et al.* 1991) was applied in some samples to verify the absence of PPV.

RESULTS AND DISCUSSION

The reaction of the 156 *Prunus* interspecific hybrids to PPV inoculation is summarized in Table 4.

It appears from results that the progenies studied are heterogeneous for resistance to PPV. The level of resistance/susceptibility to PPV was different in particular hybrids.

The range of maximum and minimum observed values for the trait intensity of symptoms was from 0 to 4 (both populations).

The range of maximum and minimum mean values for the trait was from 0.6 to 3.6 (Cresthaven × GF 677) and 0.6–3.1 (Cresthaven × Cadaman).

Susceptible plants developed chlorotic discoloration and distortion of leaves characteristic of PPV and were assayed positive by ELISA or RT-PCR during at least one of the last three growth cycles assayed.

Plants would be considered as resistant if they did not show any symptoms and positive ELISA or RT-PCR reaction in the last 3 growth periods evaluated.

Plants were classified as medium resistant if they showed very light yellow (chlorotic) discolorations on one or two leaves of shoots in some years and were ELISA mostly negative and RT-PCR positive in the last 3 growth periods evaluated.

The results showed no resistant hybrids in either population (Table 4). Only very few hybrids (10.9% and 4.5%, respectively) from both populations showed resistance to the PPV-D isolate assayed after five years of study. This resistance was lower than that of the parents.

Table 2. Phenotypic scoring system used to evaluate *Plum pox virus* (PPV) infection

Class	Criteria used for evaluating resistance and susceptibility to PPV	
	intensity of leaf symptoms	extent of symptoms on whole plant
0	no symptoms	no leaves with symptoms
1	mild vein clearing	on 1–2 leaves of shoots
2	vein clearing, mild interveinal mosaic	on first 3–4 leaves of shoots, present till the end of June
3	vein yellowing, medium interveinal or oak mosaic	on first 5–6 leaves of shoots, present even in July
4	severe vein yellowing, severe yellow and oak mosaic, and/or twisting of leaves and thickening of leaf blades	on first 7 or more leaves of shoots, present the whole growth period

Table 3. Classification of genotypes based on leaf symptoms, ELISA and RT-PCR tests

Class of resistance	Symptoms*	ELISA	RT-PCR
Resistant	≤ 0.5	negative	negative
Medium resistant	> 0.5 and ≤ 1	mostly negative	positive
Medium susceptible	> 1 and ≤ 2	positive	positive
Susceptible	> 2 and ≤ 3	positive	positive
Highly susceptible	> 3	positive	positive

*Mean intensity of *Plum pox virus* (PPV) symptoms over the whole evaluation

Table 4. Number and percentage (in brackets) of individuals of both populations included in different classes of resistance

Resistance class	Cresthaven × Cadaman	Cresthaven × GF 677
Resistant	0 (0)	0 (0)
Medium resistant	5 (10.9)	5 (4.5)
Medium susceptible	10 (21.7)	4 (3.6)
Susceptible	25 (54.3)	92 (83.6)
Highly susceptible	6 (13.1)	9 (8.3)
Total	46 (100)	110 (100)

Not all plants showing symptoms also gave positive ELISA readings. Some samples that were positive by PCR were not positive by ELISA. A higher sensitivity has been reported for RT-PCR in comparison with ELISA (Wetzel *et al.* 1991; Martínez-Gómez *et al.* 2003).

Some hybrids showed important changes in symptom severity and virus accumulation over time. This may be due to a delay in PPV accumulation, or to plant recovery and elimination of the virus.

A decrease in the symptom intensity of seedlings in the fourth cycle was observed probably because of the age of the plants grown in pots in controlled conditions in the screenhouse (Rubio *et al.* 2003b). However, the symptom intensity returned to the previous level in the fifth growth period. It indicates that the decrease in symptom intensity could be due to the environmental conditions.

Some contradictory results concerning the level of resistance of almond hybrid rootstock GF 677 and *P. davidiana* hybrid rootstock Cadaman have been reported. Pascal *et al.* (2002) reported susceptibility in Cadaman and GF-677 to a PPV-M isolate which was different from the PPV-D isolates used by Rubio *et al.* (2005), Polák and Oukropec (2010) and in this study. Thus these differences can be explained by the use of different isolate types.

Concerning the genetic aspects, the determination of resistance to PPV observed in Cadaman and GF 677 is not known. But the low number of resistant hybrids selected from crosses Cresthaven × GF 677 and Cresthaven × Cadaman makes us think that it is probably polygenic. So, PPV resistance breeding involves the selection of numerous progenies and the use of early selection tests.

To go ahead, a genetic map will be constructed using an F_1 population between interspecific hybrid GF 677 and peach (Cresthaven). Important genomic regions controlling resistance to PPV will be identified. The map and loci linked to resistance to PPV will provide a useful tool for marker-assisted selection as well as introgression of almond genes into peach cultivated cultivars.

In conclusion we can state that Cadaman and GF 677 show approximately the same ability in transmitting resistance to their descendants. They could be directly exploited in rootstock breeding programs. For the selection of peach cultivars, breeding programs will have to take in account fruit characteristics. In this context, *P. davidiana* presents the disadvantages of transmission of poor fruit quality to peach (Moing *et al.* 2003), which was not a problem in advanced almond-derived peach selections (Gradziel 2003).

We have to realize that in order to obtain the final results, it will be necessary to wait until the adult stage to evaluate symptoms on fruits, productivity and fruit quality.

Crosses between a recurrent cultivar and the selected hybrids should allow accumulating agronomic traits, while preserving the resistance level.

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