Identification of Interspecific Peach and *Prunus* sp. Hybrids Resistant to *Plum Pox Virus* Infection

Jaroslav POLÁK[1] and Ivan OUKROPEC[2]

[1]Department of Virology, Division of Plant Health, Crop Research Institute, Prague-Ruzyně, Czech Republic; [2]Mendel University in Brno, Faculty of Horticulture, Lednice na Moravě, Czech Republic

Abstract

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Interspecific hybrids of *Prunus persica*, Barier, Fire, Cadaman, GF-677, and *Prunus* sp. hybrids and selections, MRS, NBS 540-73, and Pumiselect were evaluated for resistance to *Plum pox virus*. Hybrids were grafted onto trees of a peach cultivar artificially infected with PPV and evaluated for six years for resistance to the virus. The relative concentration of PPV protein was determined by semiquantitative ELISA in June every year. The presence of PPV in peach hybrids was confirmed by IC-RT-PCR in 2007–2008. The presence and intensity of PPV symptoms were evaluated monthly from May to September. The hybrid GF-677 (*P. amygdalus* × *P. persica*) was confirmed as highly resistant to PPV. Hybrids Cadaman (*P. davidiana* × *P. persica*) and Fire (*P. amygdalus* × *P. persica*) were characterized as resistant to PPV. Hybrids GF-677, Cadaman and Fire were selected as candidate sources of resistance to be crossed with peach cultivars susceptible to PPV.

Keywords: Sharka disease; peach; *Prunus* sp.; sources of resistance; determination; intensity of symptoms; semiquantitative ELISA; IC-RT-PCR detection

The investigation of peach (*Prunus persica* L.) resistance to *Plum pox virus* (PPV) started in the nineties of the last century in connection with epidemic damage to peach production in Greece. The first study dealing with resistance of peach cultivars to PPV, based on the evaluation of the intensity of viral symptoms on leaves, was published in Greece (Mainou & Syrgianidis 1992), the next one in Romania (Balan *et al*. 1995). On the other hand, a report on the detection of PPV in asymptomatic peaches, using ELISA, came from the Czechoslovakia (Polák 1989).

An extensive research on the resistance of peach cultivars to PPV was conducted in the Czech Republic, using objective experimental methods. The relative concentration of PPV protein in flowers and leaves of individual peach cultivars, infected both naturally and artificially, was checked (Polák 1995, 1998, 1999; Polák *et al*. 2003) by semiquantitative ELISA. The resistance to PPV was evaluated on 79 peach cultivars in total. None of the evaluated peach cultivars was found to be immune or very resistant.

Recently, *P. davidiana* and *P. amygdalus* were used as PPV resistance donors for improvement of peach resistance to sharka. Kervella *et al*. (1998) used *P. davidiana*, Pascal *et al*. (2003) used both *P. davidiana* and *P. amygdalus*, and Martínez-Gómez *et al*. (2004) employed *P. amygdalus* to improve peach resistance to PPV.

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Seven interspecific hybrids of the genus *Prunus* were tested as candidate sources of resistance to PPV for peach and plum. Preliminary results of three-year evaluation were published previously (Polák & Oukropec 2008). In the present report the original results of six-year evaluation of candidate sources of resistance to PPV for peach and plum are presented.

MATERIALS AND METHODS

Plant material and inoculation of PPV. Interspecific hybrids, most of which involve *Prunus persica*, were used to investigate their resistance to PPV. *Prunus* sp. hybrids and selections MRS, NBS 540-73, and Pumiselect declared by German and Italian breeders as resistant or tolerant to PPV were also included in the investigation. Hybrids Barier (*Prunus davidiana* × *P. persica*), Cadaman (*P. davidiana* × *P. persica*), Fire (*P. amygdalus* × *P. persica*), GF 677 (*P. amygdalus* × *P. persica*), MRS (*P. cerasifera* × *P. spinosa*), NBS 540-73 (*P. cerasifera* × *P. holoserica* × *P. domestica*) and the selection Pumiselect (*P. pumila*) were budded onto 6-years-old peach trees artificially infected with PPV, in spring 2003. The individual hybrids were budded in technical isolation (screenhouse) always onto 3 peach-trees infected with PPV, 6–10 buds per tree. Most buds started to grow in summer 2003. The symptoms of PPV infection were checked on the leaves of peach trees and the occurrence of virus infection was confirmed by ELISA. Trees growing under the permanent virus infection were evaluated six years for resistance to PPV.

Evaluation of PPV symptoms and PPV determination using ELISA. Similarly, the hybrid sprouts growing from buds were evaluated from 2003 to 2008. The presence and intensity of PPV symptoms were evaluated visually at the end of May, June, July, and September. The presence of PPV in the leaves of individual interspecific hybrids was determined using an ELISA kit including PPV polyclonal antibodies (Loewe, Sauerlach, Germany) in 2004-2008.

Determination of relative concentration of PPV protein. The relative concentration of PPV protein in leaves of tested hybrids was checked using semiquantitative ELISA (SQ-ELISA), by determination of the viral protein titre in a homogenate from leaves with PPV symptoms, or in the absence of PPV symptoms from the first three leaves on the sprouts of individual hybrids. The relative concentration of PPV was established by determination of the lowest dilution of extracted sap from leaves of tested trees that showed a positive reaction in ELISA (Albrechtová *et al*. 1986). The titre of PPV in a sample was determined as the dilution of extracted sap with the minimum absorbance value 0.04. The relative concentration of PPV is the reciprocal value of the viral protein titre, e.g. the sap dilution 1:8 = 1.25×10^{-1}. The relative concentration of PPV protein was determined at the each end of May in 2004–2008. The method was described in more details in previously published reports on the investigation of peach resistance to PPV (e.g. Polák *et al*. 2003).

Detection of PPV by IC-RT-PCR. The presence of PPV in leaf extracts from interspecific hybrids and selections was verified by immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) in the last two years of evaluation, in 2007 and 2008. The IC-RT-PCR protocol of Wetzel *et al.* (1992) was used. The Robust II RT-PCR kit (Finnzymes, Espoo, Finland) and the pair of oligonucleotide primers P1/P2 (Candresse *et al*. 1995) were applied in IC-RT-PCR detection. Amplification products were analyzed by electrophoresis of 10 µl aliquots from each reaction mixture on 1.5% agarose gel in Tris-borate-EDTA buffer and visualised by ethidium bromide.

RESULTS

The results of six-year evaluation of seven interspecific hybrids of the genus *Prunus* for resistance to PPV are presented in Table 1. PPV symptoms were evaluated already in the year of grafting on infected peach trees (2003) and in subsequent five years. A semiquantitative ELISA was used in 2004–2008, and the presence of PPV was checked by IC-RT-PCR in the last two years (2007–2008).

The hybrid GF-677 (*P. amygdalus* × *P. persica*) was confirmed to be highly resistant to PPV, and the best hybrid source of resistance. No symptoms (Figure 1A) appeared in the leaves of GF-677 trees in the years 2004–2008. PPV was never detected in leaves or flowers of this hybrid by ELISA and by SQ-ELISA. The results of IC-RT-PCR detection showed a very weak positive reaction in 2007 to 2008, therefore the immunity of GF-677 to PPV was not confirmed.

Table 1. Results of six-year evaluation of seven interspecific hybrids of the genus *Prunus* for resistance to *Plum pox virus*

Interspecific hybrid	Year of evaluation													Resistance to PPV
	2003	2004		2005		2006		2007			2008			
	symptoms	symptoms	ELISA	symptoms	ELISA	symptoms	ELISA	symptoms	ELISA	IC-RT-PCR	symptoms	ELISA	IC-RT-PCR	
NBS 540-73 *P. cerasifera* × *P. holoserica* × *P. domestica*	M	MM	4.7×10^{-4}	SM	3.3×10^{-3}	SM	1.56×10^{-2}	SM	3.74×10^{-2}	+	SM	3.9×10^{-2}	+	susceptible
Barier *P. davidiana* × *P. persica*	DS	DS, VC	4.5×10^{-2}	VC	3.7×10^{-2}	VC	1.9×10^{-2}	DS	1.25×10^{-1}	+	DS	1.25×10^{-1}	+	medium susceptible
MRS *P. cerasifera* × *P. spinosa*	DS	DS	5.0×10^{-1}	DS	4.3×10^{-2}	DS	3.7×10^{-2}	DS	2.57×10^{-1}	+	DM	1.56×10^{-2}	+	medium susceptible
Pumiselect *P. pumila*	VC	NS	1 (1:1)	NS	1.7×10^{-1}	VC	4.6×10^{-2}	NS	1.7×10^{-1}	+	VMM	1.25×10^{-1}	+	medium resistant
Cadaman *P. davidiana* × *P. persica*	NS	VMM	1	NS	1	VMM	1	VMM	1	+	VMM	1	+	resistant
Fire *P. amygdalus* × *P. persica*	NS	NS	2.3×10^{-2}	NS	0	NS	0	NS (VMM)	1	+	NS	1	+	resistant
GF-677 *P. amygdalus* × *P. persica*	NS	NS	4.7×10^{4}	NS	0	NS	0	NS	0	(±+)	NS	0	±	higly resistant

ELISA – relative concentration of PPV by quantitative ELISA; M – mosaic; MM – mild mosaic; SM – severe mosaic, VMM – very mild mosaic; VC – vein clearing; DS – diffuse spots; NS – no symptoms; + – positive; ± – suspicious, very weak reaction

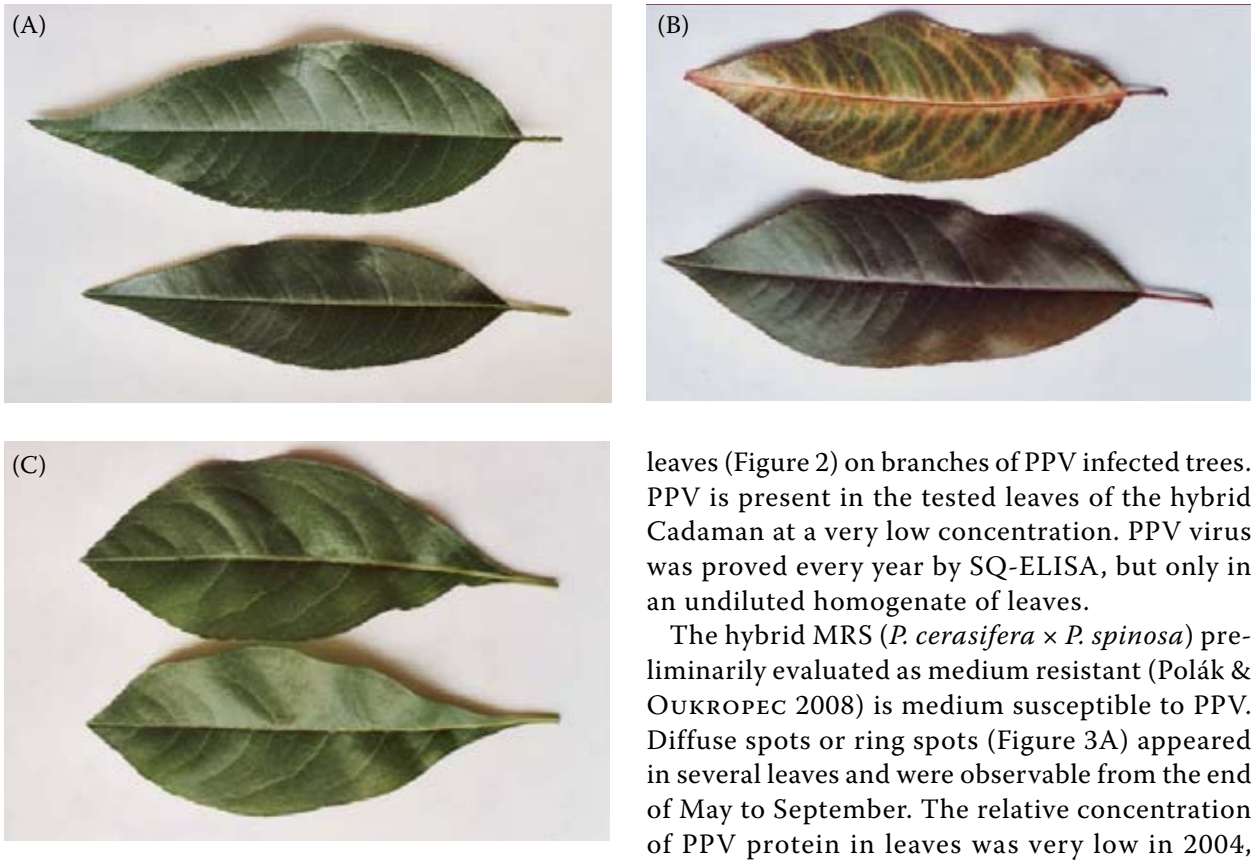

Figure 1. PPV infected hybrid GF-677 (A), Fire (B) and Pumiselect (C), no symptoms in leaves

The hybrid Fire (*P. amygdalus* × *P. persica*) is resistant to PPV. No symptoms appeared in leaves (Figure 1B) during the period of evaluation. PPV was detected by SQ-ELISA in the first year after grafting onto PPV infected peach trees and the relative concentration of PPV protein was 2.3×10^{-2}. PPV was not detected by ELISA in 2005–2006, but it was detected again in 2007 and 2008 in an undiluted homogenate of leaves only.

The hybrid Pumiselect (*P. pumila*) is characterized as medium resistant to PPV. Vein clearing was observable in some leaves of trees in the year of grafting onto PPV infected peach trees. No symptoms (Figure 1C) or occasionally vein clearing of the first growing leaves at the beginning of vegetation period were found in 2004–2008. PPV is present in asymptomatic leaves of infected plants at a low concentration. Plants of *P. pumila* can be latently infected with PPV. The relative concentration of PPV protein in leaves is low, but the virus was detected by SQ-ELISA in all years of evaluation (2004–2008).

The hybrid Cadaman (*P. davidiana* × *P. persica*) is resistant to PPV. No symptoms appeared in leaves (Figure 2) on branches of PPV infected trees. PPV is present in the tested leaves of the hybrid Cadaman at a very low concentration. PPV virus was proved every year by SQ-ELISA, but only in an undiluted homogenate of leaves.

The hybrid MRS (*P. cerasifera* × *P. spinosa*) preliminarily evaluated as medium resistant (Polák & Oukropec 2008) is medium susceptible to PPV. Diffuse spots or ring spots (Figure 3A) appeared in several leaves and were observable from the end of May to September. The relative concentration of PPV protein in leaves was very low in 2004, but it slightly increased in subsequent years. The reaction of the hybrids Barier and MRS to PPV infection is very similar.

The hybrid Barier (*P. davidiana* × *P. persica*) is medium susceptible to PPV. Diffuse spots or mild mosaic symptoms appeared in older leaves already in the year of grafting. Diffuse spots (Figure 3B) or vein clearing appeared in the first two or three leaves of some branches every year, and were observable from the end of May to September. The intensity of symptoms was low, but stable in 2003–2008. The relative concentration of PPV

Figure 2. PPV infected hybrid Cadaman, occasionally very mild diffuse spots in leaves

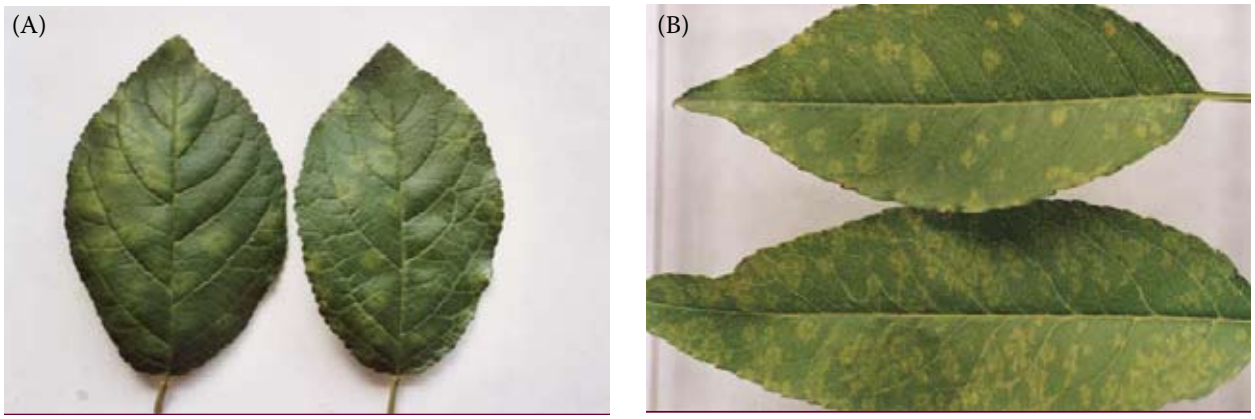

Figure 3. PPV infected hybrid MRS (A) and Barier (B), leaves with diffuse spots and rings

protein in leaves was ten to hundred times lower in comparison with the hybrid NBS 540-73.

The hybrid NBS 540-73 (*Prunus cerasifera* × *P. holoserica* × *P. domestica*) was proved to be susceptible to PPV. Mosaic symptoms in leaves of NBS 540-73 trees already appeared in the year of grafting onto peach trees artificially infected with PPV. Severe mosaic symptoms (Figure 4) were observable from the end of May to September in 2005–2008. The relative concentration of PPV protein in leaves was high in 2004 (4.7×10^{-4}) and decreased in each subsequent year to 1.25×10^{-1} in 2008.

DISCUSSION

The hybrid GF-677 was identified as the best source of resistance to PPV for crosses with peach cultivars of high quality. These experiments brought about two relevant observations. Firstly, the hybrids Fire and Cadaman were identified as appropriate sources of resistance to PPV for peach. Secondly, in turn, the hybrid Barier as medium susceptible to PPV is not relevant to be crossed with peach cultivars, and the hybrids and selections of *Prunus* sp. declared by plum breeders as resistant to PPV were proved to be susceptible (NBS 540-73) or medium resistant to PPV (MRS, Pumiselect). We state that these latter hybrids could not be recommended to growers as resistant fruit-trees to PPV infection. The six-year evaluation of interspecific hybrids of peach revealed that *Prunus amygdalus* and *Prunus davidiana* are suitable donors of resistance to PPV for peach. PPV resistance of interspecific hybrids of *Prunus persica* used in the present experiment could enable to obtain PPV resistant peach cultivars with high agronomic value faster than to employ the species *P. davidiana* or *P. amygdalus*.

Figure 4. PPV infected hybrid NBS 540-73. Younger leaves with diffuse spots and rings

Recently, the use of wild species *Prunus davidiana* and almond (*Prunus amygdalus* Batsch) as sources of PPV resistance in peach breeding was problematic. Kervella *et al.* (1998) investigated an interspecific cross between *P. davidiana* and the peach cultivar Summergrand. The problem of this source of resistance to PPV was a very low agronomic value of progenies in the first generations. Pascal *et al.* (2003) used both *P. davidiana* and *P. amygdalus* as PPV resistance donors for the improvement of peach resistance to sharka. Martínez-Gómez *et al.* (2004) studied different almond cultivars as sources of PPV resistance for peach. The resistance of almond cultivars has been successfully transmitted to descendants. Six out of eight genotypes from interspecific almond × peach crosses were resistant to PPV.

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Corresponding author:

Doc. Ing. Jaroslav Polák, DrSc., Výzkumný ústav rostlinné výroby v.v.i., odbor rostlinolékařství, oddělení virologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 315, e-mail: polak@vurv.cz