Experimental Evaluation of Apricot Genotypes for Resistance to Plum Pox Virus*

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Abstract

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The reaction of 19 Slovak apricot cultivars and hybrids (breeding program of Research Breeding Station at Veselé) to infection by M isolates of plum pox virus (PPV-M) was evaluated. The genotypes were inoculated by grafting to naturally infected plum trees in the field and by chip-budding in the glasshouse. Monitoring of PPV infection was done over a 3 year period by visual inspection and DAS-ELISA. In the third year of evaluation the RT-PCR assay was also applied. The tested apricot genotypes differed in their reaction to PPV infection. Most of them developed mild or severe symptoms on leaves in the first year and/or next two consecutive years after artificial inoculation. The four Slovak apricot genotypes Veharda, Vemina, VS 158/1 and VS 157/8 were resistant to PPV-M infection.

Key words: plum pox virus; *Prunus armeniaca*; inoculation; resistance

Plum pox virus (PPV), a member of the *Potyvirus* group, is an important pathogen of stone fruit trees in most European countries, but rarely outside of Europe (SMITH *et al.* 1994; LEVY *et al.* 2000). To date, four subgroups of PPV isolates, namely M, D, EA and C, have been distinguished on the basis of their epidemiological, serological and molecular properties (LÓPEZ-MOYA *et al.* 2000).

PPV-caused sharka disease is widely spread in the fruit growing areas in Slovakia. Although the geographical and climatic conditions are less favorable, the growing of apricots (*Prunus armeniaca* L.) has a long tradition here (BENEDIKOVÁ 1998). Natural infection of apricots was observed in Slovakia around 1960 (KRÁLIKOVÁ 1962), and later GLASA *et al.* (1998) have demonstrated the presence of M and D isolates of PPV in apricot genotypes grown at various localities.

The disease poses a serious danger for apricot growing. Breeding of cultivars resistant to PPV is one of the solutions to sustain stone fruit production in regions affected by sharka disease (HARTMANN 1997). Experimental research on the resistance of *Prunus* to PPV began more than 50 years ago (for a review see KEGLER *et al.* 1998).

An evaluation of PPV resistance of apricot cultivars has been carried out in several European countries. The tests were performed under conditions of natural infection and artificial graft- or aphid inoculation (SYRGIANIDIS 1980; DOSBA *et al.* 1988, 1992; SEDLÁKOVÁ & GALLO 1994; ERDÖS *et al.* 1995; KRŠKA *et al.* 1997; POLÁK *et al.* 1997; KARAYIANNIS 1999). The screening showed the high susceptibility of European cultivars compared to those of North American origin.

The results of an evaluation of Slovak apricot genotypes for resistance to M isolates of PPV under conditions of experimental inoculation are presented in this paper.

MATERIAL AND METHODS

Cultivars and hybrids used: Twenty-one apricot genotypes were evaluated for their reaction to PPV infection. Of these, 19 cultivars and hybrids came from the breeding programme of the Research Breeding Station at Ve-

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selé (Slovakia) (Table 1). For the glasshouse tests two control cultivars were used; Hungarian Best and Stark Early Orange had earlier been described as highly susceptible and resistant, respectively (SEDLÁKOVÁ & GALLO 1994; AUDERGON *et al.* 1995; KARAYIANNIS *et al.* 1999).

Table 1. List of tested Slovak apricot genotypes

Genotype	Parental combination
Barbora	Hungarian Best × (Achrori, Arzami, Zard)
Veharda	Julskij × Hungarian Best
Velita	Hungarian Best × (Achrori, Arzami, Zard)
Vemina	Rakovského × (Achrori, Arzami, Zard)
Vesna	Hungarian Best × (Achrori, Arzami, Zard)
Vesprima	Hungarian Best × (Achrori, Arzami, Zard)
Vestar	Hungarian Best × mixture of Chinese cvs.
VS 046/52	Vesna (free pollination)
VS 1/52	Sunglo × NJA44
VS 155/4	Vesprima × Vestar
VS 156/6	Vesprima × LE 805 + LE 809
VS 157/30	Vesprima × Bergeron
VS 157/8	Vesprima × Bergeron
VS 158/1	Vesprima × Veselka
VS 159/1	Vestar × VS 046/43 (free pollination of Vesna)
VS 2/41	Sunglo × C4R8T22
VS 4/32	Rossošanskij konzervnyj × NJA44
VS 5/135	Sunglo × Borsirozsa Kajszi
VS 86/3	Hungarian Best × Achrori

Testing of genotypes by grafting on infected trees: The cultivars and hybrids were grafted in April onto the branches of 10-year old plum trees (cv. Bystrická) showing severe symptoms of sharka disease (chlorotic spots and rings on the leaves, fruit pox marks and dropping). The trees were grown in an experimental orchard, and all were naturally infected by a local plum pox virus isolate from the M subgroup as previously determined by using *RsaI* polymorphism (GLASA, unpublished). Three to four grafts per genotype were used. The grafts come from an experimental space-isolated plantation of the Research Breeding Station at Veselé. The reaction of the genotypes was evaluated for 3 years (1997–1999) on leaves of developed shoots by a combination of visual inspection, double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR). Fruits that appeared randomly in some genotypes were not evaluated.

Glasshouse tests: Twelve apricot genotypes (Table 3) selected from the test-collection (Table 1) and including the two control cultivars were simultaneously tested by the glasshouse method (DOSBA *et al.* 1988; FAGGIOLI 1997). Two buds of a genotype were T-budded in August onto a healthy seedling of peach GF 305 in two replications. Next year the apricot shoots at a stage of 20 cm long were inoculated by chip-budding. Peach GF 305, experimentally infected with isolate PPV-VAR belonging to the PPV-M subgroup (GLASA *et al.* 1998), was used as a source of inoculum. Inoculated plants were monitored for 3 years (1998–2000) and periodically checked for presence of PPV. During evaluation the plants were kept in a glasshouse from February to October, and then placed in a cold room for 3.5 months.

DAS-ELISA: Serological detection of PPV in leaf samples was performed by standard DAS-ELISA (CLARK & ADAMS 1977) using universal anti-PPV monoclonal antibodies (MAbs) from the Palacky University Olomouc, Czech Republic (HILGERT *et al.* 1993). PPV antigen presence was checked three times during the vegetation period (from May to July in the field experiment, and from March to June in the glasshouse, respectively).

RT-PCR: The primers amplifying the 243 bp fragments of 3 termini of CP gene (WETZEL *et al.* 1991) were used in RT-PCR (KÚDELA *et al.* 1998). The leaf samples were prepared as described previously (GLASA *et al.* 1998).

RESULTS

Testing of genotypes by grafting on infected plum trees: No symptoms were developed on the leaves of grafted apricot shoots in the year of grafting, and repeated DAS-ELISA was also negative. In contrast, characteristic symptoms and a high concentration of PPV antigen were detected in the leaves of plum trees. On 12 apricot genotypes (Barbora, Vesna, Vesprima, Vestar, VS 046/52, VS 1/52, VS 155/4, VS 156/6, VS 159/1, VS 2/41, VS 4/32, VS 5/135) symptoms appeared in the second year of evaluation. Their intensity varied according to the genotype (Table 2). The PPV origin of symptoms was confirmed by DAS-ELISA. The genotypes Veharda, Velita, Vemina, VS 157/30, VS 157/8, VS 158/1 and VS 86/3 were symptomless throughout the period of evaluation. No latent infection was identified in 1997 and 1998. However, in the third year of evaluation latent infection was detected by DAS-ELISA in three of these genotypes (Velita, VS 157/30 and VS 86/3).

The genotypes having no leaf symptoms of PPV infection and/or giving negative or suspicious results in serological tests were subjected at the end of the third year (July 1999) to RT-PCR assay (Table 2).

The grafting experiments, including visual, serological and RT-PCR analyses, have demonstrated that four genotypes (Veharda, Vemina, VS 158/1 and VS 157/8) are resistant to infection by PPV-M.

Glasshouse tests: Three apricot genotypes (Barbora, VS 156/6 and Hungarian Best) developed typical PPV symptoms on leaves about 3–4 months after chip-inoculation. A latent infection was serologically detected in genotypes VS 046/52, VS 157/30 and VS 159/1 (Table 3); in the second year (1999) these genotypes developed the

Table 2. Evaluation of apricot resistance to PPV by grafting on sharka-infected plums

Cultivar hybrid	Year of evaluation						
	1997		1998		1999		
	symptoms	ELISA	symptoms	ELISA	symptoms	ELISA	RT–PCR
Barbora	0	–	ss	++	ss	++	n.t.
Veharda	0	–	0	–	0	–	–
Velita	0	–	0	–	0	+	+
Vemina	0	–	0	–	0	–	–
Vesna	0	–	ss	++	ss	++	n.t.
Vesprima	0	–	(s)	+	0	–	+
Vestar	0	–	s	+	s	(+)	+
VS 046/52	0	–	s	+	ss	+	n.t.
VS 1/52	0	–	s	+	s	+	n.t.
VS 155/4	0	–	s	+	0	+	+
VS 156/6	0	–	ss	++	ss	++	n.t.
VS 157/30	0	–	0	(+)	0	+	n.t.
VS 157/8	0	–	0	–	0	–	–
VS 158/1	0	–	0	–	0	–	–
VS 159/1	0	–	s	+	ss	+	n.t.
VS 2/41	0	–	s	+	0	(+)	+
VS 4/32	0	–	ss	++	ss	++	n.t.
VS 5/135	0	–	s	+	ss	++	n.t.
VS 86/3	0	–	0	–	0	+	+
Bystrická (plums)	ss	++	ss	++	ss	++	+

Symptoms: 0 = no symptoms, (s) = uncertain or very mild symptoms, s = mild symptoms on a few leaves, ss = severe symptoms on majority of leaves

ELISA: – negative, (+) suspicious, + positive reaction exceeding the healthy control by 3–10 times, ++ more than 10 times

RT-PCR: + positive; – negative; n.t. not tested

symptoms of PPV infection on leaves. The symptomatology corresponded to the results of DAS-ELISA.

The plants 'GF 305 rootstock/apricot genotypes' Veharda, Vemina and VS 157/8 showed no symptoms either on apricot or on GF 305 leaves. However, after reinoculation of these plants by chip-budding into apricot and GF 305 shoots, symptoms were observed on GF 305 in the next vegetation period, while the apricot leaves were symptomless and no virus was detected by DAS-ELISA (Table 3).

At the conclusion of this evaluation (May 2000), randomly selected leaves from the basal and apical parts of apricot shoots negatively reacting in DAS-ELISA were tested by RT-PCR. The tests confirmed that apricot genotypes Veharda, Vemina, VS 157/8, VS 158/1 and Stark Early Orange were resistant to infection by PPV-VAR (M subgroup).

DISCUSSION

The sharka disease is a serious threat for apricot production because of reduced quality and premature fruit dropping of affected trees of susceptible genotypes. Due to the wide host range of PPV and its non-persistant mode of transmission by numerous aphid vectors, the control of the disease is very difficult. Therefore, planting of genotypes resistant to sharka seems to be the most effective means of control.

Several problems can complicate the evaluation of resistance of genotypes to PPV (delayed response of *Prunus* to inoculation, various physiological state of plants, differences in the virulence of PPV isolates, environmental conditions). For these reasons, two methods of artificial inoculation (grafting on infected trees in the field, and chip-budding in the glasshouse) were applied in our experiments.

A comparison at the end of 3 years evaluation showed that the two methods of inoculation had no significant influence on the reaction of genotypes to PPV-M infection. However, a delay of viral translocation was observed when apricots were grafted on infected plum trees. In this case, susceptible apricot genotypes showed no symptoms in the year of grafting (first year of evaluation), and DAS-ELISA assays were also negative.

Table 3. Evaluation of apricot cultivars in the glasshouse after chip-inoculation

Cultivar hybrid	Year of evaluation						
	1998		1999		2000		
	symptoms	ELISA	symptoms	ELISA	symptoms	ELISA	RT–PCR[1]
Barbora	ss[2]/ss[3]	++/++	ss/ss	++/++	n.t.	n.t.	n.t.
Veharda	0/0	–/–	0/0*	–/–	ss/0	++/–	–
Vemina	0/0	–/–	0/0*	–/–	ss/0	++/–	–
VS 046/52	0/0	+/+	ss/ss	++/++	n.t.	n.t.	n.t.
VS 155/4	s/0	++/–	ss/0	++/+	ss/0	++/+	n.t.
VS 156/6	ss/ss	++/++	ss/ss	++/++	n.t.	n.t.	n.t.
VS 157/30	s/0	++/++	ss/ss	++/++	n.t	n.t.	n.t.
VS 157/8	0/0	–/–	0/0*	+/–	ss/0	++/–	–
VS 158/1	ss/0	++/–	ss/0	++/–	ss/0	++/–	–
VS 159/1	s/0	++/+	ss/ss	++/++	n.t.	n.t.	n.t.
VS 2/41	0/0	–/–	s/0	+/+	ss/0	++/(+)	+
VS 5/135	0/0	–/–	0/s	+/++	ss/ss	++/++	n.t.
Stark Early Orange	ss/0	++/(+)	ss/0	++/–	ss/0	++/–	–
Hungarian Best	ss/ss	++/++	ss/ss	++/++	n.t.	n.t.	n.t.

[1] apricot leaves tested; [2] GF 305 rootstock/[3] apricot cultivar

Symptoms: 0 = no symptoms, s = mild symptoms on a few leaves, ss = severe symptoms on majority of leaves, * = chip–reinoculation;
ELISA: – negative, (+) suspicious, + positive reaction exceeding the healthy control by 3–10 times, ++ more than 10 times
RT–PCR: + positive; – negative; n.t. not tested

Resistance to PPV was first detected in cultivars Stark Early Orange and Stella by SYRGIANIDIS (1979). Later, the resistant reaction of these cultivars was confirmed by many authors (DOSBA *et al.* 1992; KARAYIANNIS & MAINOU 1994; AUDERGON *et al.* 1995; KRŠKA *et al.* 1997). In our glasshouse experiments Stark Early Orange also displayed resistance to the applied Slovak PPV-M isolates.

In Slovakia, a collection of several cultivars and hybrids had previously been evaluated by SEDLÁKOVÁ and GALLO (1994). They did not find viral antigen in the Slovak genotypes Vegama, Veharda and VS 9/83 after artificial inoculation.

From the set of Slovak cultivars and hybrids examined in our investigation, the resistance to infection by PPV-M could be confirmed in genotypes Veharda, Vemina, VS 158/1 and VS 157/8. PPV could not be identified in these genotypes during 3 years by symptomatology, DAS-ELISA and RT-PCR both in field and glasshouse tests. However, further investigation to identify the level of resistance of these genotypes to different PPV isolates (PPV-D subgroup) is needed.

In the case of some genotypes, detection of PPV by ELISA gave negative or ambiguous results, but the RT-PCR assays were apparently positive. We conclude that tests based on the more sensitive RT-PCR should be performed to evaluate new "resistant" apricot genotypes.

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Súhrn

GLASA M., BENEDIKOVÁ D., GLASOVÁ Ž., HRIČOVSKÝ I, KÚDELA O. (2000): **Experimentálne hodnotenie genotypov marhúľ na rezistenciu k vírusu šarky slivky**. Plant Protect. Sci., **36**: 123–127.

Zhodnotila sa reakcia 19 slovenských odrôd a hybridov marhúľ (šľachtiteľský program Výskumno-šľachtitelskej stanice Veselé) voči infekcii M izolátmi vírusu šarky slivky (PPV-M). Genotypy boli inokulované v poľných podmienkach navrúbľovaním na prirodzene infikované stromy sliviek a v skleníku štítkami kôry. Sledovanie PPV infekcie sa uskutočnilo počas 3 rokov vizuálnym pozorovaním a metódou DAS-ELISA. V 3. roku hodnotenia sa aplikovala aj RT-PCR. Testované marhule sa odlišovali v ich reakcii na PPV infekciu. Väčšina z genotypov prezentovala mierne alebo vážne príznaky na listoch v prvom a/alebo v nasledujúcich 2 rokoch po inokulácii. Štyri genotypy – Veharda, Vemina, VS 158/1 a VS 157/8 – vykázali rezistenciu k PPV-M infekcii.

Kľúčové slová: vírus šarky slivky; *P. armeniaca*; inokulácia; rezistencia

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