SUSCEPTIBILITY OF PEACH GF 305 SEEDLINGS AND SELECTED HERBACEOUS PLANTS TO PLUM POX VIRUS ISOLATES FROM WESTERN SLOVAKIA

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Summary. – The susceptibility of peach GF 305 seedlings and herbaceous plants to five plum pox virus (PPV) isolates from orchards of western Slovakia was investigated. PPV was isolated from diseased plum, apricot and peach trees, and transmitted by chip-budding to peach GF 305. The herbaceous plants were infected by mechanical inoculation. The transmission was analysed by symptomatology and double sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Infected peaches developed leaf distortion, tissue clearing along the veins and small chlorotic spots (isolate BOR-3). With exception of BOR-3, the PPV isolates transmitted from peach caused local chlorotic spots on *Chenopodium foetidum*. The character of symptoms changed when a sap from PPV-infected *Nicotiana benthamiana* was used as virus inoculum. From *N. benthamiana*, the PPV isolates could be transmitted to *Pisum sativum*, cv. Colmo (light green mosaic), *N. clevelandii* and *N. clevelandii* x *N. glutinosa* hybrid (latent infection or chlorotic spots).

Key words: plum pox virus (PPV); symptomatology; peach GF 305; herbaceous hosts; DAS-ELISA

Introduction

PPV, a member of genus *Potyvirus*, causes economically important sharka disease of stone fruit trees. The disease was recorded for the first time in Bulgaria in 1917 – 1918 on plum cv. Kjustendil, and then in 1933 on apricot (Atanasoff, 1932, 1935; Németh, 1994). On peach, the disease was first reported in 1961 in Hungary (Németh, 1963). Based on biological and and molecular properties, PPV isolates can be divided into 3 groups represented by apricot isolate PPV-D (Dideron) from southeastern France, peach isolate PPV-M (Marcus) from Greece (Kerlan and Dunez, 1979), and the Egyptian isolate PPV-El Amar (Wetzel *et al.*, 1991). PPV was isolated also from sour cherry (Nemchinov *et al.*, 1995) and sweet cherry (Crescenzi *et al.*, 1995). Recently, a new potyvirus, Asian prunus latent virus, has been reported to infect *Prunus* spp. (Hadidi and Levy, 1994).

According to the symptoms on *Chenopodium foetidum*, Sutic *et al.* (1971) classified PPV isolates as yellow, intermediate and necrotic strains. Grüntzig and Fuchs (1986) arranged PPV isolates into two groups. Isolates of the first group were transmissible to *Ch. quinoa*, *Ch. album*, *Ch. ficifolium*, *Ch. amaranticolor* and *Ch. murale*, and had a high immunogenicity. Isolates of the second group did not infect the *Chenopodium* species listed above and had a narrow host range and weak immunogenicity.

On the territory of Slovakia, the sharka disease has appeared first time in the 1950's (Králiková, 1962). A comparative study of several PPV isolates was performed by Paulechová (1981).

In this study, we describe the susceptibility of peach GF 305 seedling and selected herbaceous plants to PPV isolates from orchards of western Slovakia. The results obtained demonstrate differences among isolates with regard to symptoms and infection severity on various indicator plants.

Abbreviations: PPV = plum pox virus; DAS-ELISA = double sandwich ELISA; ELISA = enzyme-linked immunosorbent assay; MoAb = monoclonal antibody; p.i. = post infection

Materials and Methods

Virus isolates. PPV-infected plum, peach and apricot tissues were collected from orchards in western Slovakia (Table 1). All these trees displayed typical symptoms of sharka disease.

Transmission of PPV to peach GF 305. The PPV isolates were transmitted from diseased trees to peach GF 305 by chip-budding during May – June. Then the seedlings were cut off and placed in a greenhouse. Five months after inoculation, they were stored in a dark and cold room. In March the next year, the infected seedlings were placed again in a greenhouse. The evaluation of virus infection was performed visually and by a serological test.

Sample preparation and transmission of PVP to herbaceous plants. Leaves of infected peaches were ground in 0.1 mol/l phosphate buffer pH 8.0 (1:10, w/v). The sap was used for mechanical inoculation of *N. benthamiana* Domin. and *Ch. foetidum* Schrad. The re-inoculation tests included *N. benthamiana*, *N. clevelandii* Gray, *N. clevelandii x N. glutinosa* hybrid, *Ch. foetidum* and *P. sativum* L., cv. Colmo.

Table 1. Origin of PPV isolates

Isolate	Locality	Notes (age)
VAR	Bratislava-Vajnory	Peach, cv. Harland (10 years)
CAH-2	Čachtice	Apricot, cv. Maďarská (15 years)
BOJ-3	Bojnice	Plum, cv. BO-4-75 (10 years)
BIII/2	Nitra	Plum, cv. Bystrická (8 years)
BOR-3	Borovce	Apricot, cv. VS 123/9 (5 years)

DAS-ELISA. The experiments were evaluated by DAS-ELISA according to Clark and Adams (1979) using monoclonal antibodies (MoAbs) provided by Dr. M. Navrátil, Palacký University, Olomouc (Hilgert *et al.*, 1993). The assay was performed on infected peaches after budbreak and immediately before inoculum preparation. In infected herbaceous plants, the viral antigen was tested in the basal, medium and apical parts. Uninfected and mock-infected plants were used as controls.

Results

The next year after chip-budding inoculation, the peaches showed characteristic symptoms on leaves (Table 2). There were three types of symptoms on the leaves: distortion, tissue clearing along the veins, and small chlorotic spots. Besides, the PPV isolates differed in severity of infection.

In further step, the PPV isolates were characterised by symptomatology on *N. benthamiana* indicator plant. As a source of inoculum, infected leaves of peach seedlings were used. About 20 days after mechanical inoculation, the PPV isolates produced on tobacco leaves chlorotic spots

Table 2. Symptoms of PPV infection on peach GF 305 leaves

Isolate	Symptoms on leaves		A_{405} (DAS-ELISA)
VAR	D, TC	++	0.657
CAH-2	D, TC	++	0.745
BOJ-3	D, TC	+	0.634
BIII/2	TC	+	0.282
BOR-3	SC	+	0.108
None[a]	–		0.045

D = distortions, TC = tissue clearing along the veins, SC = small chlorotic spots, (++) = severe infection, (+) = mild infection. [a]Uninfected control.

Table 3. Symptoms of PPV infection on *Chenopodium foetidum*

Isolate	Symptoms on leaves	
VAR	C[a]	CN[b]
CAH-2	C[a]	N[b]
BOJ-3	C[a]	0[b]
BIII/2	C[a]	C[b]
BOR-3	0[a]	C[b]

C = chlorotic spots, CN = chlorotic spots with necrotic centres, N = necrotic lesions, 0 = unsuccessful transmission. [a]Transmission from infected peach seedlings. [b]Transmission from infected *N. benthamiana* plants.

and dark green flecks. Later, approximately 28 days p.i., also pucker leaves and dark green flecks along the veins were visible. The infection was systemic.

To test the character of PPV infection on widely used *Ch. foetidum* indicator plant, young leaves of this plant were inoculated with a sap from infected peaches and *N. benthamiana* plants. With exception of BOR-3 where a repeated transmission was unsuccessful, all the isolates transmitted from peach caused chlorotic spots on *Ch. foetidum* leaves. When a sap from *N. benthamiana* was used as source of inoculum, the character of symptoms was different (Table 3). The symptoms appeared 7 – 12 days p. i. The infection of *Ch. foetidum* was local.

The results of PPV transmission from *N. benthamiana* to other herbaceous plants are presented in Table 4. As it was demonstrated by DAS-ELISA, the PPV isolates caused in plants listed in this table a systemic infection, though the infection with isolates BOJ-3, BIII/2 and BOR-3 produced no symptoms on tobacco leaves. A growth reduction of infected *N. clevelandii* and *N. clevelandii x N. glutinosa* plants was observed too. The symptoms on tobacco plants appeared 7 – 21 days p.i. and on pea cv. Colmo 14 – 21 days p.i.

Discussion

The peach GF 305 seedling is generaly used as indicator plant for PPV detection. The PPV infection of this plant is

Table 4. Symptoms on herbaceous plants after mechanical transmission of PPV isolates from *Nicotiana benthamiana*

Isolate	Symptoms on leaves		
	N. clevelandii	*N. clevelandii x N. glutinosa*	*Pisum sativum* cv. Colmo
VAR	C	C	LGM
CAH-2	C	C	LGM
BOJ-3	0	0	LGM
BIII/2	0	0	LGM
BOR-3	0	0	LGM

C = chlorotic spots, LGM = light green mosaic, 0 = no visible symptoms, latent infection.

displayed mainly by tissue clearing along the veins and distortion of young leaves (Németh, 1986). Three types of symptoms could be observed after peach infection with PPV isolates in the present study. The PPV isolates VAR (peach), CAH-2 (apricot) and BOJ-3 (plum) caused a leaf distortion and tissue clearing along the veins. BIII/2 (plum) isolate developed only tissue clearing along the veins. Atypical small chlorotic spots were observed on peach leaves infected with BOR-3 (apricot) isolate. According to the intensity of symptoms, the examined PPV isolates can be divided in severe (VAR, CAH-2) and mild (BOJ-3, BIII/2, BOR-3) ones.

Tobacco *N. benthamiana* is often used for detection, maintenance and symptom analysis of PPV (Németh, 1986). By susceptibity and symptomatology, this plant turned out as a suitable herbaceous host also for the detection and propagation of our PPV isolates. The symptoms (chlorotic spots, dark green flecks and pucker leaves) were easily identifiable and the virus could be detected by ELISA for a long period.

Of other herbaceous plants suitable for PPV isolation, differentiation of PPV isolates, and antiserum preparation, *Ch. foetidum* (Németh, 1963) and *N. clevelandii* (Kassanis and Sutic, 1965) should be mentioned. According to the reaction of *Ch. foetidum*, Sutic *et al.* (1971) identified three types of PPV strains, designated yellow, intermediate and necrotic. When we used a sap from infected peach as inoculum, the isolates VAR, CAH-2, BOJ-3 and BIII/2 behaved as yellow strains. The isolate BOR-3 could not be transmitted to *Ch. foetidum*. Different results were obtained with a sap from infected *N. benthamiana* plants used as inoculum. By symptomatology on infected *Chenopodium* leaves, we could identify yellow strains (BIII/2, BOR-3), intermediate strain (VAR) and necrotic strain (CAH-2). Isolate BOJ-3 produced no symptoms, and DAS-ELISA was also negative after inoculation. There have been described also other isolates of PPV which could not be transmitted to *Ch. foetidum* after mechanical inoculation (Németh, 1986).

We used the sap of infected *N. benthamiana* plants as inoculum for transmission of the PPV isolates also to *N. clevelandii*, *N. clevelandii x N. glutinosa* and *P. sativum*, cv. Colmo. The infection of *N. clevelandii* and *N. clevelandii x N. glutinosa* with isolates BOJ-3, BIII/2 and BOR-3 was latent, without visible leaf symptoms (DAS-ELISA was positive). VAR and CAH-2 isolates produced on these plants chlorotic spots. A *N. clevelandii x N. glutinosa* hybrid has been used by Albrechtová *et al.* (1986) for propagation and maintenance of PPV-W isolate. The use of *P. sativum* as host for PPV investigation has been proposed by Kerlan and Dunez (1976). E.g., cvs. Serpette d' Auvergne, Express généreux (Kerlan *et al.*, 1981), Zeiners Grüne Bastard (Paulechová, 1981), Glória (Németh, 1986) and Colmo (Adamolle, 1993; Candresse *et al.*, 1995) have been employed. PPV infection of these cultivars was manifested mostly by tiny chlorotic spots (light green mosaic) on leaves (Paulechová, 1981; Németh, 1986; Adamolle, 1993). Such type of symptoms was produced also by our PPV isolates under study. We detected the presence of virus in leaves, stalks, flowers and young pods of pea plants. It is the first succesful transmission of Slovak PPV isolates to *P. sativum* cv. Colmo ever recorded.

The experiments on the susceptibility of peach GF 305 seedlings and some herbaceous plants to PPV isolates from western Slovakia have demonstrated that these isolates differ in severity of infection, host range and symptomatology.

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