**EXPERIMENTAL SCREENING OF PLUM GENOTYPES FOR RESISTANCE TO PLUM POX VIRUS (PPV)**

**M. Navrátil, D. Šafářová, R. Karešová, J. Kučerová and F. Paprštein**

**Abstract**

This work reports three-years’ results of artificial inoculation of experimental trees (rootstock-tested segment-sensitive indicator) by PPV isolates classified as member of D or recombinant D/M strains. PPV-D isolate induced more severe reactions in all tested genotypes including sensitive control of ‘Domestic Prune’. No symptoms or in some cases development of vein and even branch necrosis were noticed in infected genotype ‘Izjumnaja’. Evaluated genotypes ‘Jelta Butylkovidna’ and selection ‘XV/4 ŠT’ manifested light sharka symptoms on infected leaves and detection of PPV by ELISA gave low titre or negative results.

**Key words:** sharka, *Plum pox virus*, ELISA, *Prunus* sp., sensitivity

**Introduction**

Sharka, caused by *Plum pox virus* is the most dangerous disease of the *Prunus* species and induces serious yield losses in the commercial production of plums mainly in case of susceptible cultivars. That is why searching for highly tolerant or resistant cultivars in the genetic resources collection is a main topic of the research (Kegler et al. 1998, Minoiu et al. 2002, Hartmann 1998, 2002). A long-term trial aimed at investigation of plum cultivars sensitivity to PPV was established in 1990 (Paprštein and Karešova 1998). In 2001 six perspective genotypes (selection ‘XV/4 ŠT’, ‘Wengerka Pozdnaja 8/30’, ‘Izjumnaja 5/4’, ‘Jelta Butylkovidna’, ‘Bílá Trnečka 6/2’ and ‘Bílá Trnečka 10/83’) and resistant cultivar ‘Jojo’ were selected for more detailed sensitivity study.

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This work reports three-years’ results of artificial inoculation of experimental trees by PPV-D or recombinant PPV-D/M strains.

**Materials and methods**

The perspective cultivars and selection (‘XV/4 ŠT’, ‘Wengerka Pozdnaja 8/30’, ‘Jelta Butylkovidna’, ‘Bílá Trnečka 6/2’ and ‘Bílá Trnečka 10/83’, ‘Jojo’) were chosen on the basis of negative tests for PPV by ELISA from long-term testing of plum germplasm for sensitivity to Plum pox virus (Paprštein et al. 1998). In the case of ‘Izjumnaja 5/4’ a hypersensitive reaction was suspected. The experimental trees, consisting of rootstock (St. Julian) – tested segment – sensitive indicator (‘Domestic Prune’), were grafted in winter 2001/02 and infected in August 2002 by budding of three buds taken from infected tree of ‘Domestic Prune’ on the rootstock. PPV-D and recombinant PPV-D/M (Glasa et al. 2002) strains were used. Five trees for each PPV strain represented each tested genotype minimally. In parallel no inoculated controls were included. Experimental trees were grown under an insect-proof screen-house. The trees were observed for symptoms of infections and presence of PPV in rootstock, tested genotype and sensitive indicator was monitored by DAS ELISA (universal Mab05UP and polyclonal antibodies, produced in our laboratory; Navrátil et al. 1992). PDV, PNRSV and ACLSV were also controlled with ELISA using commercial sets (Bioreba). The leaf samples were used at 1:10 final dilution. Each sample was replicated twice. Titre of PPV in leaves was established with ELISA, in logarithmic dilution series of samples.

**Results**

Results of experimental inoculation of tested genotypes and comparison to long-term field trials are summarized in Table 1. There were noticed differences among PPV strains and analyzed plum genotypes in their virulence and respective sensitivity.

PPV-D strain induced more fast and severe reactions, from light green/yellow mosaic (‘XV/4 ŠT’) to typical severe plum pox symptoms (‘Domestic Prune’). Vein, leaf and tip necrosis followed by branch decline were noticed in genotype ‘Izjumnaja’. None visual symptoms (‘Izjumnaja’, ‘Wengerka Pozdnaja’ and ‘Jelta Butylkovidna’) were induced by PPV-D/M isolate in 2003. In the subsequent year 2004, light symptoms in ‘Jelta Butylkovidna’, typical symptoms at ‘Wengerka Pozdnaja’ and hypersensitive reaction at ‘Izjumnaja’ were observed. Cultivar ‘Domestic Prune’ showed typical severe plum pox symptoms. No symptoms were observed only in ‘Jojo’.

The both genotypes of ‘Bílá Trnečka’ and ‘Wengerka Pozdnaja’ were evaluated as highly sensitive comparable to control ‘Domestic Prune’. In the case of analyzed genotypes ‘Jelta Butylkovidna’ and selection ‘XV/4 ŠT’ sharka symptoms on in-
Results of testing genotypes to PPV sensitivity (all the tested plants were free from PDV, PNRSV, and ACLSV)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PPV strain</th>
<th>Field inoculation</th>
<th>Experimental inoculationa</th>
<th>leaf symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA positive 2003/%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ELISA positive 2004/%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>titre of positive segments</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>leaf symptoms</td>
<td></td>
</tr>
<tr>
<td>'Izjumnaja'</td>
<td>D</td>
<td>leaf and tip necrosis</td>
<td>10 6/60 7/70 (3)b</td>
<td>10b</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>10 1/17 1/17 (6)b</td>
<td>10b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rarely light mosaic</td>
<td>10 7/70 9/90 (1)b</td>
<td>10b</td>
</tr>
<tr>
<td>'Wengerka Pozdnaja'</td>
<td>D</td>
<td>leaf and tip necrosis</td>
<td>7 0/0 5/71 (1)b</td>
<td>10b</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>7 1/17 1/17 (2)b</td>
<td>10b</td>
</tr>
<tr>
<td>'Bílá Trnečka 6/2'</td>
<td>D</td>
<td>rarely light mosaic</td>
<td>6 5/83 6/100 (1)c</td>
<td>none 2003, typical 2004</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>5 1/20 3/60 (1)</td>
<td>none 2003, typical 2004</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>9 1/17 1/17 (2)d</td>
<td>light 2003, 2004</td>
</tr>
<tr>
<td>'XV/4 ŠT'</td>
<td>D</td>
<td>rarely light mosaic</td>
<td>6 1/17 2/33 (4)c</td>
<td>none 2003, light 2004</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>6 1/17 2/33 (4)c</td>
<td>none 2003, light 2004</td>
</tr>
<tr>
<td>'Domestic Prune'</td>
<td>D</td>
<td>severe symptoms</td>
<td>9 9/100 9/100 (10)</td>
<td>none 2004</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>9 9/100 9/100 (10)</td>
<td>none 2004</td>
</tr>
<tr>
<td>'Jojo'</td>
<td>D</td>
<td>nt</td>
<td>2 nt</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D/M</td>
<td>nt</td>
<td>2 nt</td>
<td>0</td>
</tr>
</tbody>
</table>

a Only evidentially inoculated trees included in.
b Number of no proliferating, i.e. no tested, segments.
c Only one analyzed sample.
2004 Symptoms analyzed in 2004.
"+" positive, "−" negative, nt – not tested.
fected leaves were light and/or none. Since the second year after inoculation the buds usually have not proliferated and low titre of PPV or negative results in ELISA were obtained. No symptoms or in some cases development of vein and even branch necrosis were noticed in infected genotype ‘Izjumnaja’. Only samples from early vein necrosis gave in ELISA positive results.

Virus concentration was the highest in control high sensitive ‘Domestic Prune’ (Table 1). The titre of PPV-D was always higher than the titre of PPV-D/M in all evaluated genotypes. In generally, the virus titre corresponded with severity of leaf symptoms except genotypes ‘Jelta Butylkovida’ and ‘XV/4 ŠT’ where relatively high virus titre was found out only in sporadic occurred symptomatic leaves.

Discussion

Two different types of resistance of plums to sharka disease have been described (Hartmann 1998, Kegler et al. 1998). Quantitative resistance we could find in tolerant and resistant prune cultivars. Qualitative resistance is mostly based on hypersensitive reaction of the plant. Genotypes with hypersensitive reaction are mostly field resistant that means an infection by aphids is not possible. At present, only a small amount of information on genetic bases of plum resistance is known.

Despite the high inoculation pressure applied to tested plants used in evaluation test, the variable levels of sensitivity as well as hypersensitive reaction in the set of analysed genotypes were reported. It seems that reaction depends not only on the genetics of the plant and PPV isolate, but also on an inoculation method. Dosba et al. (1994) consider this kind of massive inoculation suitable for fast screening but variable compared to less severe classical tests as chip budding or to an aphid transmission. Some contradictory results concerning the level of resistance ‘Bílá Trnečka’ genotypes have been reported. Genotypes had shown a “good behaviour” against the PPV in field conditions after artificial budding directly in the crown of tested cultivar (Paprštein and Karešová 1998). In our very severe conditions these genotypes have quickly expressed strong symptoms on leaves and since the second year they have stopped proliferating and they could not be tested.

The hypersensitive reaction of ‘Izjumnaja’ inoculated with PPV-D and PPV-D/M was confirmed. Hartmann (2002) pointed out the importance of virus dose for hypersensitivity screening. Our tested segments were permanently under infection pressure from inoculated rootstock resulting in veinal necrosis and branch dieback. It is necessary to give attention to natural aphid transmission of PPV to ‘Izjumnaja’ in field conditions to confirm qualitative type of resistance.

These experimental results correspond with long-term field observations except high sensitivity of both genotypes ‘Bílá Trnečka’. There were noticed partial differences in reaction of all tested genotypes on PPV infection as a lower sensitivity, sporadic occurrence of symptomatic leaves, and weaker symptoms on inoculated field trees. Different methods of inoculation as well as different infection doses can lead to different reactions of the host plants and, consequently, to differ-
ent kinds of evaluation (Dosba et al. 1992). Inoculation of sensitive rootstock of tested genotypes used in our experiments is more intensive procedure than chip budding used in the field trials (Paprštein et al. 1998). Evaluation could be affected by the others factors like virus concentration, virus invasion, virus strains, susceptibility and age of host genotypes and environmental influences (Karešová and Pluhař 1988, Polák 1989).

**Literature**


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