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Plum pox virus (PPV) was first detected in Canada in 2000. Since then an intensive survey program has be undertaken to determine the distribution of the virus with the goal of eradicating the virus from Canada. A number of Canadian isolates have been characterized and only isolates of PPV strain D have been found in commercial orchards. This research was conducted in part to: a) determine the relationship of PPV D isolates found in commercial orchards with PPV D isolates found in homeowner or residential properties; and b) analyze unusual isolates to confirm strain and/or determine identity. A total of 5 homeowner isolates were obtained for analysis. Four isolates were confirmed as strain D isolates, and formed 3 distinct clades. One of these isolates (H- 0170) grouped with the Canadian Subgroup II, 2 isolates (H-4688, H-4880) grouped with the Canadian Subgroup I, and the fourth isolate (H-4782) formed a separate and distinct clade. The fifth homeowner isolate was strain typed as a member of the strain PPV Rec.

Key words: *Pepper veinal mottle virus*, Incidence, Severity, Pepper, Agroecological Zones.

Biolistic transfection of plants by infectious cDNA clones of Plum pox virus

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Plant biolistic transfection by two *Plum pox virus* (PPV) infectious cDNA clones (strains PPV-M and PPV-D) using the gene gun apparatus PDS 1000-He (Biorad) was optimized. *Nicotiana benthamiana* plants were germinated by five on Petri dishes (diameter 6 cm) with MS growth medium. In the age of four weeks (5 – 6 leaf stage, total leaf surface about 1.5 cm2 per plant) the plants were subjected to biolistic transfection and three days later they were transplanted into common soil substrate. The plant survival after transplantation was about 70 %, the transfection efficiency was over 80 % (compared to 6 % efficiency reached by mechanical plant inoculation). The plants showed typical PPV symptoms two weeks post transfection (leaf distortions and mosaic). The virus presence was confirmed by immunoblotting, RT-PCR, as well as by successful transmission by sap to healthy plants and subsequent virus purification. The cotransfection of *N. benthamiana* plants by PPV-M and PPV-D led to mixed infection with prevalent PPV-D.

In vivo thermotherapy and in vitro chemotherapy of plums, apricots and peaches artificially infected with PPV-D and PPV-M strains.

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Plum cultivars Čačanská lepotica and Švestka domácí, apricot cvs. Leskora and Velkopavlovická, peach cvs. Redhaven and Earliglo artificially infected with PPV-D and PPV-M were treated by in vivo thermotherapy at 37°C. A successful treatment was recorded in cases of plum cv. Čačanská lepotica and apricot cvs. Leskora and Velkopavlovická. Plum cv. Čačanská lepotica and apricot cv. Velkopavlovická were PPV-D free, apricot cv. Leskora was PPV-M free seven and nine months after finishing the in vivo thermotherapy. However, both of the peach cultivars remained PPV infected after the treatment. Furthermore, five peach trees died during the treatment. In vitro cultures of plum cv. Bluefree and apricot cv. Hanita infected with *Plum pox virus* (PPV) were used for the virus elimination by chemotherapy. Low ribavirin concentrations of 5 and 10 mg.l-1 in MS medium were applied in the treatment. PPV was completely eliminated by ribavirin in concentration of 5 mg.l- 1 in plum cv. Bluefree within twenty weeks, and in apricot cv. Hanita in twelve weeks of the application. The presence of PPV was not proved by RT-PCR. Clones of plum cv. Bluefree and apricot cv. Hanita were re-tested by RT-PCR one year after the termination of the ribavirin treatment and negative results confirmed the elimination of PPV. PPV free clones rooted in modified MS medium by Paunovic (2007) during six weeks.

Distribution of Plum pox virus strain in natural sources in the Czech Republic.

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In the Czech Republic, the distribution of *Plum pox virus* (PPV) has been monitored for the last 15 years. Also, individual strains of PPV have been monitored since the end of the 20th century. PPV-M was typed in natural sources of plum, myrobalan and blackthorn from 1999 to 2004. PPV-M was detected in 5, 88% of investigated plum trees; 7, 41% of myrobalan trees and 4, 0% of blackthorn shrubs, respectively. Distribution of PPV-D, PPV-M and PPV-Rec was investigated in 2005-2008. 52-94 samples of plum, myrobalan and blackthorn were tested in individual years. PPV was detected by DAS-ELISA with specific polyclonal antibodies; PPV-M by DASI-ELISA with specific monoclonal antibodies; PPV-D, PPV-M and PPV-Rec were detected by RT-PCR. The presence of PPV-D varied from 94, 7% to 100%, the presence of PPV-M from 0, 0% to 3, 2% and the presence of PPV-REC from 0, 0% to 2, 1% during 2005-2008. More than 95% of natural sources of PPV were infected with PPV-D and less than 2, 5% of natural sources of PPV were infected with PPV-M or PPV-Rec. The presence of PPV-C and PPV-ElAmar was not proved in plum, myrobalan and blackthorn trees infected with PPV.

Typing and distribution of Plum pox virus isolates in Romania

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Plum pox or Sharka, caused by *Plum pox virus* (PPV) is considered as the most destructive disease of plum. Although PPV is widespread in all plum growing areas from Romania and causes serious yield losses, little is known about the variability of its isolates at country level. For this reason, a large-scale study was performed with the aim to get a picture of the prevalence and distribution of PPV strains in plum. During three years surveys, 200 PPV isolates collected from 23 different plum orchards from Transylvania, Moldavia and Muntenia areas were investigated. DAS-ELISA and IC/-RT-PCR were used for PPV detection. PPV strains were serologically determined by TAS-ELISA using PPVD and PPV-M specific monoclonal antibodies. Molecular strain typing was done by RTPCR targeting three genomic regions corresponding to (Cter)CP, (Cter)NIb/(Nter)CP and CI. RFLP analysis was used to distinguish D and M strains, based on RsaI polymorphism located in (Cter)CP. All PCR products targeting (Cter)CP and 13 PCR products spanning the (Cter)NIb/(Nter)CP were sequenced. The typing of PPV isolates revealed that PPV-D is the prevalent strain in all the three areas. The higher incidence of PPV-D was noticed in Moldova (84%) and the higher rate of PPV-Rec was recorded in Transylvania (18%). The mixed infections (D+Rec) was more frequent in Muntenia (24 %).Overall results provided that in Romania the predominant strain is PPV-D (73%), follow with a much lower frequency by PPV-Rec (14%). Mixed infections (PPV-D+PPV-Rec), which might generate additional variation by recombination, are also frequent (13%).

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Cloning and sequencing of a mild naturally induced PPV isolate

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A mild PPV isolate (PPV-B2) was naturally induced after low temperature treatment of *Nicotiana benthamiana* plants infected with the local isolate PPV-DGR. Compared to the mother isolate, PPV-B2 is not aphid transmissible, replicates less efficiently in *N. benthamiana* and *N. clevelandii* and causes no symptoms on the last mentioned experimental host. Both isolates were cloned, sequenced and 14 amino acid substitutions were determined between them as follows: two in P1, two in HC-Pro, two in P3, one in