Prune Cv. Jojo Resistance to Different Strains of *Plum Pox Virus*

Jaroslav POLÁK, Jitka PÍVALOVÁ and Jiří SVOBODA

Department of Virology, Division of Plant Medicine, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Abstract

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Trees of prune (*Prunus domestica* L.), cv. Jojo, were inoculated by chip budding with three different strains of PPV isolated from European plum in the Czech Republic. These isolates included *Plum pox virus* M strain (PPV-M), *Plum pox virus* D strain (PPV-D) and a PPV-recombinant both strains (PPV-Rec). The results of the evaluation of the inoculated trees over 2 years are presented. Trees of plum cv. Jojo behaven differently to infection with the three PPV strains. A strong hypersensitive reaction appeared a year after inoculation with PPV-M and PPV-Rec strains, although not all inoculated tree died. PPV must have been present in the tissue of cv. Jojo because the virus was transferred to the rootstock St. Julien. Plants of the rootstock became systemically infected with the PPV-M and PPV-Rec strains, showing severe PPV symptoms. The presence of PPV was proved by ELISA in leaves of rootstock St. Julien, but not in leaves of cv. Jojo. Inoculation with strain PPV-D resulted in partial hypersensitive reaction of plants of cv. Jojo, but after initial stunting and partial death of shoots recovering of plants was observed.

Keywords: prune; resistance; hypersensitivity; *Plum pox virus*; virus strains

Most of prune (*Prunus domestica* L.) cultivars are very susceptible to sharka (*Plum pox virus,* PPV). Breeders, together with plant virologists, have tried for over 50 years to release prune cultivars with high quality of fruits and resistant, or immune, to PPV. In 1947 were reported the prune cultivars Anna Späth, Bühler Frühzwetsche, Montfort (Christoff 1947) and the Czar to be resistant to PPV (Kegler *et al*. 1998). The most extensive programme of breeding for resistance in prune is Cacak, Jugoslavia. In this programme several cultivars including Cacanska Rodna, Cacanska Lepotica, Cacanska Najbolja, or Valevka (Ranković 1986) were studied. In *P. domestica,* the quality of all plum cultivars resistant to PPV is lower than the PPV susceptible ones, e.g. Požegača, Deutsche Hauszwetsche, Švestka domácí. Fruit production in highly infected regions is only possible with tolerant or resistant cultivars (Hartmann 1997). In 1993 a prune breeding programme was started at Hohenheim (Germany), to release new sharka-resistant cultivars with better fruit quality (Hartmann 1998). The first breeding cycle resulted in the resistant or tolerant cultivars Hanita, Katinka, Elena, Tegera and Presenta. It was supposed that absolute resistance of prunes to PPV may be the result of qualitative resistance based on hypersensitivity. A hybrid hypersensi-

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tive to PPV, derived from Ortenauer × Stanley 13, was obtained at Hohenheim, registered as prune cultivar Jojo, and declared as absolutely resistant to PPV (Hartmann 1998). The importance of hypersensitivity for breeding prunes resistant to PPV was presented by Hartmann (2002).

Trees of cv. Jojo are also grown in the Czech Republic. Prune cultivar Jojo with good quality of fruits and a qualitative resistance to PPV based on hypersensitivity could decrease problems with sharka in regions of the Czech Republic with endemic presence of PPV. On the other hand absolute resistance is immunity, and the immunity is very different from the hypersensitivity. The hypersensitivity can be also disrupted by new strain of virus. Therefore we have tested the reaction of cv. Jojo to infection with three different strains of *Plum pox virus* present in the territory of the Czech Republic.

MATERIALS AND METHODS

Plant material, PPV inoculation and visual evaluation. Two year old trees of prune cv. Jojo grafted onto rootstock St. Julien were planted in the field in October 2002. The plantation was isolated by surrounding with grass and cereals for a diameter of 1500 m. In April 2003 the trees were inoculated by chip budding with grafts from plum trees infected with the three different PPV strains, PPV-M, PPV-D and PPV-Rec (PPV-M × PPV-D) isolated from prune in the Czech Republic. Three different PPV strains are maintained in depository of the Department of Virology, Research Institute of Crop Production, Prague-Ruzyně. Four trees of cv. Jojo were used for each combination (PPV-Rec strain – samples No. 5–8; PPV-M strain – samples No. 13–16; PPV-D strain – samples No. 9–12), with two infected buds per tree. Four non-inoculated trees were used as control (samples No. 1–4). Growth of buds was checked in 2003. At least one bud was growing on every inoculated tree. The reaction of the plants was evaluated during 2003 and 2004.

ELISA evaluation. PPV polyclonal antibodies were used in DAS-ELISA (Clark & Adams 1977) to detect PPV in leaf tissues of the prune cv. Jojo, and in leaves of the rootstocks St. Julien. Samples for ELISA were prepared by grinding 0.2 g of leaf tissues in phosphate buffered saline, pH 7.4 with 2% polyvinylpyrrolidone and 0.2% of egg albumin in the ratio 1:20. Microplates were rated by using a MR 5000 Dynatech reader at 405 nm. Samples with $OD_{405} > 0.10$ were considered as positive, and samples with $OD_{405} < 0.03$ were rated as negative.

RESULTS AND DISCUSSION

The vegetation period in 2003 was very dry and warm, and the trees grew very slowly. For this reason, no symptoms of PPV appeared on the trees (Figure 1). Thus, in the year of inoculation no difference was observed between the groups of trees inoculated with three different strains of PPV, neither between inoculated and non-inoculated prunes. However, substantial differences between non-inoculated control trees and PPV-inoculated trees of cv. Jojo were observed in 2004. Shoots of

Figure 1. Control healthy (noninoculated with PPV) trees of *Prunus domestica* L., cv. Jojo (sample No. 1–4 from the left to the right) by the end of June 2004

Figure 2. Dying trees of *P. domestica*, cv. Jojo (sample No. 5–8 from the left to the right) inoculated with PPV-recombinant strain

PPV-inoculated trees started to grow in April, but they grew slowly and part of the shoots began to wilt in June. In addition different reactions appeared between trees and groups of trees inoculated with individual PPV strains.

Figure 3. Growing rootstock St. Julien with severe PPV symptoms PPV-recombinant strain in leaves. Rootstock is growing under the dying tree of plum cv. Jojo inoculated with PPV-recombinant strain

The PPV-Rec strain and PPV-M strain were the most pathogenic. Trees infected with PPV-Rec (samples No. 5–7) were dead at the beginning of July (Figure 2). The rootstocks St. Julien of dead trees started to grow and severe systemic infection by the PPV-Rec strain was apparent (Figure 3). Sample No. 8 remained stunted, but no shoots of rootstock St. Julien appeared. Trees inoculated with PPV-M (samples No. 13, 14 and 16) died at the beginning of July (Figure 4). Shoots from their rootstocks St. Julien appeared to be systemically infected with PPV-M. Sample No. 15 was stunted, and new shoots started to grow from the bottom of the stem. Trees inoculated with PPV-D (samples No. 9–12) were much less damaged (Figure 5). Samples No. 9, 10 and 12 were stunted, but new shoots of samples No. 10 and 12 from the bottom of the stem grew fast. The crown of sample No. 11 died, but new shoots of cv. Jojo from the bottom of the stem developed quickly. No shoots of rootstocks St. Julien appeared.

PPV was not proved by ELISA in the leaves of the following dying trees: samples No. 5–8 inoculated with PPV-Rec, samples No. 9–12 inoculated with PPV-D, and samples No. 13–16 inoculated with PPV-M. PPV was not proved by ELISA in control trees (samples No. 1–4). On the other hand, PPV was proved by ELISA (absorbance values over 1.0) in leaves of rootstocks St. Julien of trees samples No. 5–7, and of trees samples No. 13, 14 and 16. The evaluation of PPV symptoms and results of ELISA detection were summarised in Table 1.

The results proved that trees of plum cv. Jojo reacted differently to the inoculation with three PPV strains. A strong hypersensitive reaction appeared

Figure 4. Dying trees of *P. domestic*a, cv. Jojo (samples No. 13–16) inoculated with PPV-M strain

Figure 5. Recovering trees of *P. domestica*, cv. Jojo (samples No. 9–12) inoculated with PPV-D strain. Some of shoots died, only in tree 11 died the whole crown, but recovering of tree is going on from the bottom of stem

in the second year after inoculation of trees with PPV-M and PPV-Rec strains. Not all inoculated trees died. Trees of cv. Jojo are hypersensitive to PPV-M and PPV-Rec strains, but not "absolutely resistant" as published by Hartmann (2004). PPV must have been present in the tissues of cv. Jojo, because the virus was transferred via cv. Jojo to the rootstock. Plants of rootstock St. Julien became systemically infected with PPV-M and PPV-Rec strains, causing severe PPV symptoms. The presence of PPV was proved by ELISA in leaves of rootstock St. Julien, but not in leaves of cv. Jojo (Table 1). A partial hypersensitive reaction of plants of cv. Jojo appeared after inoculation of trees with strain PPV-D.

Table 1. Results of observation of PPV symptoms and ELISA in plants cv. Jojo and rootstock St. Julien

Cultivar	PPV-M		PPV-Rec		PPV-D	
	symptoms	ELISA	symptoms	ELISA	symptoms	ELISA
Jojo	dying of 3 trees stunting of 1 tree	–	dying of 3 trees stunting of 1 tree	–	partly dying and stunting, later recovering	–
St. Julien	oak mosaic, rings	+	oak mosaic, rings	+	no growing rootstock	not tested

Recovery of plants from PPV-D infection was observed. Thus, isolation of the virus by necrosis is incomplete and there is partial dying of shoots.

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References

Clark M.F., Adams A.N. (1977): Characteristic of the microplate method of enzyme-linked immunosorbent assay for the detection of plant virus. Journal of General Virology, **34**: 51–857.

Christoff A. (1947): Sharka disease of plum. Bulletin de la Chambre de culture Nationale de Sofia, Série Biologique, **1**: 261–296 (in Bulgarian).

Hartmann W. (1997): Cultivation of plums in sharka-infected regions. In: Proceedings of the Middle European Meeting '96 Plum Pox. Budapest, 1996: 49–52.

Hartmann W. (1998): Breeding of plums and prunes resistant to *Plum pox virus*. Acta Virologica, **42**: 230–232.

Hartmann W. (2002): The importance of hypersensitivity for breeding plums and prunes resistant to *Plum pox virus* (Sharka). Acta Horticulture, **577**: 33–37.

Hartmann W. (2004): "Jojo" – the first absolutely sharka resistant plum variety. In: 8th International Symposium on Plum and Prune. Genetics, Breeding and Pomology. Book of Abstracts. Lofthus, Norway: 84.

Kegler H., Fuchs E., Grüntzig M., Schwarz S. (1998): Some results of 50 years of research on the resistance to *Plum pox virus*. Acta Virologica, **42**: 200–215.

Ranković M. (1986): Ispitivanje otpornosti nehih novijih sorti šljiva prema virusu šarke. Jugoslovensko Vocarstvo, **20**: 601–606.

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Abstrakt

Polák J., Pívalová J., Svoboda J. (2005): **Rezistence švestky domácí cv. Jojo k různým kmenům *Plum pox virus*.** Plant Protect. Sci., **41**: 47–51.

Stromy švestky domácí cv. Jojo byly inokulovány metodou „chip budding" třemi různými kmeny *Plum pox virus* izolovanými z evropského typu švestky v České republice. Tyto izoláty zahrnovaly *Plum pox virus* kmen M (PPV-M), *Plum pox virus* kmen D (PPV-D) a PPV-rekombinant obou kmenů (PPV-Rec). Během dvouletého hodnocení se stromy švestky cv. Jojo chovaly k infekci třemi kmeny různě. Silná hypersenzitivní reakce se objevila rok po inokulaci kmeny PPV-M a PPV-Rec, i když ne všechny inokulované stromy odumřely. PPV musel být v pletivu cv. Jojo přítomen, protože virus byl přenesen do podnože St. Julien. Rostliny podnože byly systémově infikovány kmeny PPV-M a PPV-Rec a objevily se na nich silné příznaky PPV. Přítomnost PPV byla prokázána pomocí ELISA v listech podnože St. Julien, nikoli však v listech cv. Jojo. Výsledkem inokulace kmenem PPV-D byla částečná hypersenzitivní reakce rostlin cv. Jojo, avšak po počátečním zakrsávání a částečném odumírání výhonů bylo pozorováno ozdravení rostlin.

Klíčová slova: slivoň; rezistence; hypersenzitivita; *Plum pox virus*; kmeny viru

Corresponding author:

Doc. Ing. Jaroslav Polák, DrSc., Výzkumný ústav rostlinné výroby, odbor rostlinolékařství, oddělení virologie, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 402, fax: + 420 233 311 592, e-mail: polak@vurv.cz