

Annual Herbs – Possible Reservoirs of Sharka Disease?

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Abstract

Nineteen annuals – herbaceous indicators – were used for elucidation of *Plum pox potyvirus* seed transmission possibility after artificial inoculation with 9 different naturally infected PPV M and D strain sources. The seeds of positive enzyme-linked immunosorbent assay (ELISA) tested herbaceous indicators were collected and planted for germination. Successful seed transmission was detected after ELISA in 3 weeks old plants as follows: *Nicotiana benthamiana* 3.75% (source of PPV M *Prunus domestica* L. cv. unknown); *N. clevelandii* 3.5% (source of PPV M *P. armeniaca* (L.) Batsch cv. V 66052); *N. benthamiana* 8.42% and *N. acuminata* 1.97% (source of PPV D *P. domestica* L. cv. Althane); *N. benthamiana* 12.73% (source of PPV M *P. domestica* L. cv. Bystrická); *N. acuminata* 1.84% and *N. occidentalis* 15.1% (source of PPV D *Rubus fruticosus* Agg.); *N. occidentalis* 19.23% (source of PPV M *Juglans regia* L. isolate O 15); *N. occidentalis* 12.0% (source of PPV M *J. regia* L. isolate H1). These preliminary results suggest that PPV seed transmission by annual species may serve as a potential source of a virus spreading to the new plantations of the stone fruit trees by aphids transmission.

Keywords: *Plum pox potyvirus*; herbaceous indicators; annuals; seed transmission; ELISA

INTRODUCTION

A rapid spreading of sharka disease, caused by the *Plum pox potyvirus* (PPV) – not only in the Europe but also in other continents – establish the need to study new possible sources of its spreading. PPV belongs to the family *Potyviridae* with definitive 118 species from which only 14 has been reported to be transmitted by seed or pollen (BRUNT *et al.* 1996). Except of well-known ways little is known about PPV spreading by pollen and seeds either of the stone fruit trees or weeds. There are contradictory reports about PPV seed transmissibility by stone fruits (NÉMETH & KÖLBER 1982; DULIC-MARKOVIC & RANKOVIC 1992; PASQUINI *et al.* 1998; GLASA *et al.* 1999). KRÖLL (1973) and SPYCHER (1975) have, for example, reported that sharka could be isolated from 10 plant species growing on the fields. Many authors have successfully transmitted the PPV to the great number of the herbaceous species in an artificial

way (VAN OOSTEN 1974; KRÖLL 1978; ZAWADZKA & SMOLARZ 1978; MORVAN & CHASTELLIÈRE 1980; ŠUTIC 1977). From this point of view, it is interesting to know, whether seeds of herbaceous plants could also transmit the PPV. These plants may serve as a potential source of a virus for the new plantations of the stone fruit trees, which is commonly transmitted by aphids.

MATERIALS AND METHODS

The annual herbaceous indicators (*Chenopodium amaranticolor*; *C. foetidum*; *C. murale*; *C. quinoa*; *Cucumis sativus* cvs. Laura, Delikates and Znoj-mia; *Momordica balsamina*; *Nicotiana acuminata*; *N. benthamiana*; *N. clevelandii*; *N. clevelandii* × *glutinosa*; *N. glutinosa*; *N. megalosiphon*; *N. occidentalis*; *N. tabacum* vars. Samsun, White Burley and Xanthi; *Nicandra physaloides*) were inoculated with different isolates of the PPV (SLOVÁKOVÁ *et al.* 2002) by

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means of a homogenate of symptomatic leaves using karborundum as an abrasive. The strain M was originated from *Prunus domestica* L. cvs. Bystrická and unknown, *P. persica* L. cv. Redhaven, *P. armeniaca* cv. V 66052 and *Juglans regia* L. ser. O15 and H1. The strain D was originated from *P. domestica* L. cvs. Althane and Stanley and *Rubus fruticosus* Agg. The amount of the virus antigen in the examined objects was determined by the DAS-ELISA method (CLARK & ADAMS 1977) using polyclonal antibody against *Plum pox virus* (PPV), *Apple chlorotic leaf spot virus* (ACLV), *Cherry leafroll virus* (CLRV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) from Loewe Phytodiagnostica and Biochemica GmbH. The determination of the PPV strains by the DAS-ELISA (CAMBRA *et al.* 1994) was carried out using monoclonal antibodies against the strains M (Marcus), EA (El Amar), C (Cherry) prepared by Dr. Myrta and co. (MYRTA *et al.* 1998) and Mab 05 prepared by Dr. Navrátil and co. (NAVRÁTIL *et al.* 1992). Seeds of the PPV positive herbaceous indicators were collected and

planted for germination. The three weeks young plants were tested again by the DAS-ELISA method and kept for seeds production.

RESULTS

The ELISA examination revealed also that the mother trees were infected not only with PPV, but with other viruses as well (plums with CLRV, PNRSV; peach with CLRV; apricot with ACLV, CLRV; walnuts with CLRV; blackberry with CLRV). Transmission of mentioned viruses to herbaceous plants was sometimes manifested by different symptoms, sometimes was symptom-less. Not all of the PPV positively reacting plant indicators were able to produce seeds. Some of them dried out during cultivation. In two-years observations seeds were produced only by some plants of *Nicotiana* species. These seeds were collected and planted (3–4 seedlings per pot) for the purposes of germination. The results of DAS ELISA examination are presented in Table 1.

Table 1. Transmission of *Plum pox potyvirus* (PPV) strains by seed of some artificially infected herbaceous plants

Source of virus strains	PPV positive herbaceous plants (ELISA)	Seeds planted (No.)	Seeds emerged (No.)	Seedlings infected from seed (No.)	Seed transmission (%)
Results of experiments in the year 2001					
<i>Prunus domestica</i> L. cv. unknown (M)	<i>Nicotiana benthamiana</i>	100	84	3	3.75
<i>Prunus armeniaca</i> (L.) Batsch cv. V 66052 (M)	<i>Nicotiana clevelandii</i>	250	200	7	3.50
Results of experiments in the year 2002					
<i>Prunus domestica</i> L. cv. Althane (D)	<i>Nicotiana benthamiana</i>	250	190	16	8.42
	<i>Nicotiana acuminata</i>	200	152	3	1.97
<i>Prunus domestica</i> L. cv. Bystrická (M)	<i>Nicotiana benthamiana</i>	250	212	27	12.73
<i>Rubus fruticosus</i> Agg. (D)	<i>Nicotiana acuminata</i>	200	163	3	1.84
	<i>Nicotiana occidentalis</i>	350	331	50	15.10
<i>Juglans regia</i> L. isol. O15 (M)	<i>Nicotiana occidentalis</i>	100	52	10	19.23
<i>Juglans regia</i> L. isol. H1 (M)	<i>Nicotiana occidentalis</i>	200	150	18	12.00

DISCUSSION

The large PPV host range can be important for the disease epidemiology. This concerns not only perennial species, where by controlling the sharka disease in nurseries, a lasting success can be reached by elimination diseased tree combined with weed control (as reported by KRÖLL (1973)). As seen from our results, annual species of sharka hosts can to some extent represent a dangerous reservoirs of PPV spreading. By using of Mabs to strains M, C, EA and 05 B in the indirect ELISA method it has been found out that the M strain of PPV was successfully transmitted by seeds of *N. occidentalis* and *N. benthamiana* whiles the strain D by seeds of *N. benthamiana* and in low degree by *N. acuminata*. These findings contribute to our previous results (DANADOVÁ *et al.* 2002), which reported transmission only by the strain M not only in the first generation but also in the second generation of tested plants. The further experiments which can bring more knowledge about this problem are still in preparation.

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