

EXPERIMENTS ON TRANSMISSION OF *PLUM POX VIRUS* THROUGH *PRUNUS* SEEDS

S. Milusheva¹, P. Gercheva¹, V. Bozhkova¹ and I. Kamenova²

¹ Fruit Growing Institute, Ostromila Street. 12, 4004 Plovdiv, Bulgaria

² AgroBioInstitute, Dragan Tsankov Street. 8, 1164 Sofia, Bulgaria

SUMMARY

Vertical transmission of *Plum pox virus* (PPV) was investigated in seeds collected from three plum (*Prunus domestica* L.) cultivars infected with PPV-M, one apricot (*Prunus armeniaca* L.) cultivar infected with PPV-Rec and *Prunus mahaleb* L. The virus strains were identified before the beginning of the study. The presence of PPV in mature seeds and their parts, as well as in germinating seeds and seedlings was detected by ELISA. The highest percentage of infected whole seeds was registered in cv. Tuleu timpuriu (32%), followed by cvs Stanley (26%), Modesto (24%), and Valjevka (14%). The lowest infection rate was detected in *P. mahaleb* (11%). In the *Prunus* cultivars under study, PPV was detected in 19% to 35% of the seed coats and in 5% to 23% of the cotyledons. None of the embryonic axes was PPV-positive. In germinating seeds, PPV was detected in up to 34% of the seed coats and in up to 8.3% of the cotyledons. The virus was not identified in radicles and plumules. Up to now, all planted seedlings originating from seeds of plum cvs Tuleu timpuriu, Stanley and Valjevka infected by PPV-M, and from seeds of apricot cv. Modesto infected with PPV-Rec, remained symptomless and reacted negatively with an antiserum to PPV.

Key words: stone fruits, Sharka disease, epidemiology, seed transmission.

INTRODUCTION

The problem of seed transmission of *Plum pox virus* (PPV), the causal agent of Sharka disease, is both old and actual. Since PPV belongs in the genus *Potyvirus*, family *Potyviridae*, some members of which are known to be transmitted through seeds (Barnett *et al.*, 1991; Ward *et al.*, 1991), the theoretical possibility exists of its

vertical transmission. In the literature, there are contradictory data on the role of the seeds in the epidemiology of PPV. Szirmai (1961) was first to report PPV transmission through apricot seeds. Afterwards, Savulescu and Macovei (1965) recorded seed transmission in plum, and Coman and Cociu (1976) in peach. Later on, Nemeth and Kolber (1982) detected the virus by ELISA in apricot, peach and plum seedlings originating from PPV-affected trees. Experiments carried out in 1990s using serological and molecular techniques (Eynard *et al.*, 1991; Dulic-Markovic and Rankovic, 1997; Myrta *et al.*, 1998; Pasquini *et al.*, 1998, 2000; Glasa *et al.*, 1999) did not confirm earlier results. However, this problem has a practical importance due to the use of *Prunus* seedlings for nursery production of stone fruit planting material.

Therefore, the aim of the present study was to further address the possible seed transmission of PPV, investigating some pathogen-host (i.e. *Prunus*-PPV strain) combinations. Besides the classical stratification method for obtaining seedlings, an *in vitro* system was also used for a better understanding of the virus localization sites and of the possible changes taking place in the different stages of seed development.

MATERIALS AND METHODS

Investigations were carried out during 2005-2007, using plant species, cultivars and PPV strains reported in Table 1. Virus strains had been identified before the beginning of the study (Kamenova *et al.*, 2002, 2006).

Stones were collected from mature fruits of PPV-infected trees. Seeds from healthy plants of the same cultivars served as controls. Collected seeds were divided into three lots. The first was directly assayed serologically by testing whole seeds as well as seed coats, cotyledons and embryonic axes. The second seed lot was stratified in humid sand at 4°C and allowed to germinate. Then, an aliquot of the seeds was tested for the presence of PPV in the seed coat, cotyledons, radicles and plumule of each individual seed. Seeds of the third lot were cultured *in vitro* (embryoculture). After surface-sterilization, but before placing the seeds in hormone-free artifi-

Table 1. Stone fruit species and cultivars included in this study.

Host species/cultivar	District	Virus strain
<i>P. domestica</i> cv. Stanley	Plovdiv	PPV-M
<i>P. domestica</i> cv. Tuleu timpuriu	Plovdiv	PPV-M
<i>P. domestica</i> cv. Valjevka	Plovdiv	PPV-M
<i>P. armeniaca</i> cv. Modesto	Plovdiv	PPV-Rec
<i>P. mahaleb</i>	Kyustendil	Not tested

cial medium, seed coats were separated and used for serological assays. After chilling at 4°C for 30 days *in vitro*, seeds were placed at 24°C, 2000 lux and 16/8 h photoperiod, and an aliquot of the germinating seeds was tested for PPV presence in the cotyledons, radicles and plumules. *In vitro*-rooted seedlings were acclimated to *ex vitro* conditions. Seedlings obtained by both approaches were planted in individual pots and grown in an insect-proof greenhouse. The plants were observed regularly for leaf symptoms appearance and were periodically tested for PPV.

Virus presence in mature seeds and their parts, as well as in germinating seeds and seedlings was ascertained by ELISA (Clark and Adams, 1977) using a commercial kit (Bioreba, Switzerland). Donor trees were tested serologically also for the presence of *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) using again a commercial kit (Loewe, Germany). Trees infected by PPV only were used in the study.

RESULTS

Detection of PPV in the seeds. As shown in Table 2, the highest percentage of infected whole seeds was registered in cv. Tuleu timpuriu (32%), followed by cvs Stanley (26%), Modesto (24%), and Valjevka (14%). The lowest infection rate was detected in *P. mahaleb* (11%). No significant differences were observed between the results of serological analyses of seed coats tested directly and the coats that had been sterilized before placing in artificial medium (Table 2). Infection levels of directly tested coats were: cv. Stanley, 35.1%; cv. Tuleu timpuriu, 32.8%; cv. Modesto, 22.2%; cv. Valjevka, 19.2%, whereas ELISA-positive sterilized coats were: cv. Tuleu timpuriu, 35%; cv. Stanley, 30.3%, and cv. Modesto, 20.5%. As to cotyledons, PPV was found in approximately 23% of cv. Tuleu timpuriu samples, 9% of cv. Stanley, 7% of cv. Valjevka and 5% of cv. Modesto (Table 2). None of the embryonic axes was PPV-positive.

Detection of PPV in germinating seeds. The analyses performed during germination showed that in cvs. Stanley, Modesto, and Valjevka the percentage of positive coat and cotyledon samples decreased slightly in comparison with the comparable materials tested directly (Table 3 and 4). Only in the embryocultured seeds from cv. Tuleu timpuriu, the percentage of positive cotyledons was reduced to a greater extent (from 23% to 7%). PPV was not identified in any of the samples made up of radicles and plumules (Tables 3 and 4).

Table 2. Detection of PPV in whole seeds and seed parts.

Cultivar	Whole seeds		Seed coats		^(a) Seed coats (vt)		Cotyledons		Embryonic axes	
	n/N	%	n/N	%	n/N	%	n/N	%	n/N	%
Stanley	26/100	26.00	40/114	35.08	33/109	30.27	10/114	8.77	0/114	0
Tuleu timpuriu	40/124	32.25	39/119	32.77	28/80	35.00	27/119	22.65	0/114	0
Valjevka	26/183	14.20	19/99	19.20	-	-	7/99	7.10	0/99	0
Modesto	35/145	24.13	30/135	22.22	24/117	20.5	7/135	5.10	0/135	0
<i>Prunus mahaleb</i>	9/81	11.11	-	-	-	-	-	-	-	-

N = number of tested samples; n = number of positive samples.

^(a)Seed coats (vt) – coats of seeds destined for embryoculture.

Table 3. Identification of PPV in stratified germinating seeds.

Cultivar	Seed coats		Cotyledons		Radicles		Plumules	
	n/N	%	n/N	%	n/N	%	n/N	%
Stanley	14/41	34.14	3/41	7.11	0/41	0.00	0/41	0
Valjevka	5/31	16.13	2/31	6.45	0/31	0.00	0/31	0
Modesto	10/50	20.00	2/50	4.00	0/50	0.00	0/50	0

N = number of tested samples; n = number of positive samples.

Table 4. Identification of PPV in embryocultured germinating plum seeds.

Cultivar	Cotyledons		Radicles		Plumules	
	n/N	%	n/N	%	n/N	%
Stanley	2/24	8.3	0/24	0	0/24	0
Tuleu timpuriu	2/30	6.6	0/30	0	0/30	0

N = number of tested samples; n = number of positive samples.

Data on stratified germinating seeds of cv. Tuleu timpuriu, *P. mahaleb* and embryocultured germinating seeds of cv. Modesto are not presented because of the failure of germination.

Detection of PPV in one-year-old seedlings. Up to now, all planted seedlings originating from seeds of plum cvs. Tuleu timpuriu, Stanley and Valjevka infected by PPV-M and from apricot cv. Modesto infected by PPV-Rec, have remained symptomless and are serologically negative to PPV (Table 5).

Table 5. Results of ELISA testing for one-year-old seedlings.

Cultivar	Seedlings propagated by stratification		Seedlings propagated by embryoculture	
	n/N	%	n/N	%
	Stanley	0/75	0	0/31
Tuleu timpuriu	-	0	0/34	0
Valjevka	0/64	0	-	-
Modesto	0/36	0	-	-

DISCUSSION

The results obtained show that PPV is not transmitted from mother plants to progeny seedlings by seeds, although the virus is present in their tissues. It should be noted that infection levels in whole seeds and in the coats of mature seeds, tested after collection, were lower in comparison with the figures reported by others (Eynard *et al.*, 1991; Myrta *et al.*, 1998), whereas infection levels in the cotyledons were higher. Eynard *et al.* (1991) using ELISA, found that 73% and 90% of mature seeds of apricot cvs. Tonda di Costigliole and Bulida, respectively, were positive, whereas in our tests, the virus was detected up to 32% of the whole seeds. Eynard *et al.* (1991) reported for cvs. Tonda di Costigliole and Bulida, 86% and 95% ELISA-positive coats, respectively. Myrta *et al.* (1998) recorded 70% of infected seed coats of plum and from 89% to 97% infection in seed coats of three apricot cultivars. In our investigation, PPV was detected from 19% to 35% of the seed coats of the studied

cultivars. The above mentioned researchers could not detect PPV in the cotyledons of three apricot and one plum cultivar, whereas in other apricot cultivars, the virus was present to a very low rate, i.e. in approximately 0.5% of the cotyledons of cvs. Cafona and Tyrinthos (Myrta *et al.*, 1998) and 1% of the cotyledons of cv. Bulida (Eynard *et al.*, 1991). In our case, the percentage of infected cotyledons was higher ranging from 5% in cv. Modesto to 23% in cv. Tuleu timpuriu. PPV-infected cotyledons of ripe apricot and peach seeds were also observed by Pasquini *et al.* (1998; 2000).

Tests made during germination, showed that PPV was localized in the seed coats and cotyledons and did not enter the embryo. There were no significant differences between the ELISA responses of seed coats tested directly and the responses of the coats of seeds destined for embryoculture. Similar results were obtained from serological testing of both stratified and embryocultured germinating seeds. Nevertheless, the use of embryoculture yielded more detailed information for each of the *in vitro*-grown genotypes, during their development from seed to seedling.

Our data confirm the results of earlier studies on the lack of PPV transmissibility through seeds (Eynard *et al.*, 1991; Dulic-Markovic and Rankovic, 1997; Myrta *et al.*, 1998; Pasquini *et al.*, 1998, 2000; Glasa *et al.*, 1999). According to Eynard *et al.* (1991), the reason for the conflicting data concerning seed transmission of PPV may be due to the different virus isolates or to the different cultivars used, or both. However, the investigations carried out in the last decade by Pasquini *et al.* (1998, 2000) and Glasa *et al.* (1999), which dealt with several stone fruit species and cultivars infected with PPV-M or PPV-D, showed that there was no PPV passage from seeds to seedlings, notwithstanding the great variety of *Prunus* genotype-PPV strain combinations investigated.

Seeds from different *Prunus* genotypes infected by isolates of PPV-M and PPV-Rec (Kamenova *et al.*, 2002, 2006) were also used in the present study. All progeny seedlings, originating from seeds of plum cvs. Tuleu timpuriu, Stanley, and Valjevka infected with M strain, were virus-free and, in this respect, the obtained data confirm the results of previous investigations, indicating that PPV-M is not transmitted through *Prunus* seeds (Myrta *et al.* 1998; Glasa *et al.* 1999; Pasquini *et al.* 2000). We have also shown that isolate PPV-Rec used in this study is not transmitted vertically through the seeds of cv. Modesto.

Among the hypotheses about the impossibility of PPV to be vertically transmitted, one possible explanation is that the virus is inactivated due to particle-breakdown in the mature seeds (Eynard *et al.*, 1991). Dulic-Markovic and Rankovic (1997) consider that, in the seeds, PPV localizes in the integuments, the unabsorbed parts of the endosperm and nucellus, i. e. the tissues which burst and fall during stratification and ger-

mination. According to Pasquini and Barba (2006) “hypothetically, the only possibility of seed transmission would arise from a mutation in the helper component of the virus, associated with high susceptibility of the infected *Prunus* cultivar”.

ACKNOWLEDGEMENTS

This investigation was supported by the Bulgarian Ministry of Education and Science-Contract R11/2005. We are also grateful to Mrs. Yuliana Sotirova, Mrs. Daniela Mazneva and Mrs. Jaroslava Mincheva for their technical help in realization of the study.

REFERENCES

- Barnett O.W., 1991. *Potyviridae*, a proposed family of plant viruses. *Archives of Virology* **118**: 131-141.
- Clark M.F., Adams A.N., 1977. Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**: 475-483.
- Coman T., Cociu V., 1976. Transmission de la Sharka par le pollen et par les graines. *Bulletin d'Information Sharka* **2**: 15-21.
- Dulic-Markovic I., Rankovic M., 1997. An experiment with plum pox virus transmission by apricot and peach seeds. *Proceedings of Middle European Meeting '96 on Plum Pox, Budapest 1996*: 117-119.
- Eynard A., Roggero P., Lenzi R., Conti M., Milne R.G., 1991. Test for pollen and seed transmission on *Plum pox virus* (Sharka) in two apricot cultivars. *Advances in Horticultural Science* **5**: 104-106.
- Glasa M., Hrinovsky I., Kudela O., 1999. Evidence for non-transmission of *Plum pox virus* by seeds in infected plum and myrobalan. *Biologia* **54**: 481-484.
- Kamenova I., Dallot S., Dragoiski K., Milusheva S., 2006. Strain status of *Plum pox virus* in Bulgaria. *Plant Science* **43**: 403-407.
- Kamenova I., Milusheva S., Borisova A., Stoev A., Myrta A., 2002. Typing of *Plum pox virus* strains in Bulgaria: Preliminary results. *Biotechnology and Biotechnological Equipment* **16**: 10-13.
- Myrta A., Di Terlizzi B., Savino V., 1998. Study on the transmission of Plum pox potyvirus through seeds. *Phytopathologia Mediterranea* **37**: 41-44.
- Nemeth M., Kolber M., 1982. Additional evidence on seed transmission of *Plum pox virus* in apricot, peach and plum, proved by ELISA. *Acta Horticulturae* **130**: 293-300.
- Pasquini G., Simeone A.M., Conte L., Barba M., 1998. Detection of *Plum pox virus* in apricot seeds. *Acta Virologica* **42**: 260-263.
- Pasquini G., Simeone A.M., Conte L., Barba M., 2000. RT-PCR evidence of non-transmission through seeds of *Plum pox virus* strains D and M. *Journal of Plant Pathology* **82**: 221-226.
- Pasquini G. and Barba M., 2006. The question of seed transmissibility of *Plum pox virus*. *Bulletin OEPP/EPPO Bulletin* **36**: 287-292.
- Savulescu A., Makovei A., 1965. Studies on the sharka (Plum pox) virus and related line pattern virus. *Zastita bilja* **16**: 357-366.
- Szirmai J., 1961. Report on fruit tree virus diseases in Hungary. *Tijdschrift Planteavl* **65**: 220-229.
- Ward C.W., Shukla D.D., 1991. Taxonomy of *Potyvirus*: current problems and some solutions. *Intervirology* **32**: 269-296.