

No Seed Transmission of Plum Pox Virus in Japanese Apricot

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Abstract

In Japan, a recent outbreak of the *plum pox virus* (PPV)-D strain in Japanese apricot has caused serious plant quarantine concerns. The vertical transmission of the virus from infected Japanese apricot trees to seedlings was investigated in one major cultivar ('Nanko') and two minor cultivars ('Baigo' and 'Komukai'), by immunochromatographic assays using young leaves from 2-month-old seedlings. No PPV-positive seedlings were found among 328 'Nanko', 61 'Baigo', and 8 'Komukai' seedlings. The results indicated that the PPV-D strain in Japan is not vertically transmitted from infected 'Nanko' plants to seedlings. Further experiments are needed to test other cultivars, including 'Baigo' and 'Komukai'.

Discipline: Plant disease

Additional key words: plant quarantine, sharka

Introduction

Plum pox virus (PPV) affects stone fruit worldwide, severely reducing fruit yield and quality. It is efficiently transmitted by aphids (Garcia et al. 2014), and infected trees produce blemished and unmarketable fruit. The disease is also known as "sharka" in some parts of Europe (Garcia et al. 2014). Stone fruit species that are susceptible to PPV include apricots, cherries, nectarines, peaches, and plums (Levy et al. 2000). PPV can also infect wild and ornamental *Prunus* varieties (Levy et al. 2000).

There are at least seven known strains of PPV: D, M, EA, C, W, T (Serçe et al. 2009), and PPV-Rec, which is a recombinant between D and M (Glasa et al. 2004). PPV-D and -M are the most widely distributed strains of PPV worldwide, and M is apparently a severer strain (Levy et al. 2000).

In Japan, PPV was found for the first time in Japanese apricot (*Prunus mume* Sieb. et Zucc) trees grown in the suburbs of Tokyo in around 2006 (Maejima et al. 2010). Later, PPV was reported in several Japanese prefectures. To date, PPV-D is the only strain that has been detected in Japan.

PPV is transmitted by grafting and several aphid

species, and seed transmission is also suspected. The transmissibility of PPV through seeds has already been studied in many countries, but unfortunately with conflicting results (Pasquini & Barba 2006). If PPV is truly transmitted by seeds, then fruits and seeds may play an important role in long-distance dissemination, in which case the control of fruits and seeds through plant quarantines would be fully justified. If not, then there is no reason to block the marketing of infected fruits as long as they are free of aphids, and only the movement of budwood and trees should be controlled to prevent the long-distance spread of disease. For this reason, it is extremely important to determine whether the PPV strain that occurs in Japan is seed-transmissible. In this study, seed transmission tests were conducted on three different cultivars of Japanese apricot using a PPV-D strain from Japan.

Materials and methods

1. Sampling of seeds

In the early summers of 2010 and 2011, green and mature Japanese apricot fruits were harvested from five trees of one major cultivar ('Nanko') and two minor

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cultivars ('Baigo' and 'Komukai'), grown in Ome City, Tokyo, where PPV is prevalent (Table 1). All of the trees were cultivated in commercial orchards in the area where PPV was first found in Japan. The trees were over 20 years old. The fruit flesh was removed and seeds were surface sterilized first with a 10% concentration of commercial bleach (Kao Kitchen bleach, Kao, Tokyo, Japan) and then with 5% 8-hydroxyquinoline sulfate monohydrate. To break dormancy, seeds were wrapped in a paper towel that had been soaked with 5% 8-hydroxyquinoline sulfate monohydrate and kept at 4°C for 5-6 months.

2. Cultivation of seedlings

After dormancy was broken, seeds were sown on sterilized soil in plastic pots, and seedlings were grown in an air-conditioned greenhouse kept at 25°C/20°C (day/night) under natural light for 2-3 months (Fig. 1).

3. Detection of PPV in seedlings

Testing for PPV infections in seedlings was done by immunochromatography using a commercial kit (Plum pox virus Detection Kit, NIPPON GENE CO.,



Fig. 1. Fully expanded 2-month-old seedlings originating from seeds of Japanese apricot (cultivar 'Nanko') trees infected with PPV-D.

LTD., Tokyo, Japan (<http://www.nippongene.com/kensa/products/immuno/ppv/ppv.html>)), essentially following the instructions provided by the producer. Briefly, a few young tender leaves were taken from the seedlings and ground in a buffer provided in the kit. Then, an immunostrip in the kit was immediately soaked with the sap. For a positive control, typical symptomatic leaves (Fig. 2) collected from PPV-infected trees in Ome City, Tokyo, and then kept in a deep-freezer at -80°C, were used. Visual ratings were made noting the absence or presence of the PPV-specific band on the immunostrip after 15 min.

Results

Seeds germinated well and most seedlings grew vigorously in the greenhouse. A total of 397 seedlings from three cultivars were grown in two separate experiments in the winters of 2010 and 2011. The seedlings were cultivated for about 2 months until most seedlings had more than 10 leaves in 2010. In 2011, the seedlings were grown for about 3 months. In both experiments, no symptoms appeared on any of the seedlings (Fig. 3). All of these healthy-looking seedlings tested negative when the immunochromatographic assay was conducted using young leaves from near the tops of the plants (Table 1).

Discussion

Some researchers have reported the seed transmission of PPV, whereas others have claimed that PPV is not transmissible through seeds. Such contradicting results arise from confusion regarding the term "seed



Fig. 2. Typical symptomatic leaves on a PPV-D-infected Japanese apricot (cultivar 'Nanko') tree in Ome City, Tokyo (photo taken in spring 2009).

transmission.” Németh and Kölber (1982) wrongly claimed the seed transmission of PPV after detecting PPV in some seed parts. Using this experimental design, they only observed the transmission of PPV to seeds. The term “seed transmission” should entail the transmission of a virus to progenies through seeds, not the transmission from parental reproductive tissues to seed tissues. Some positive results in older reports including one by Szirmai (1961) should be questioned due to the lack of a vector control in the seedlings tested (Pasquini & Barba 2006). Pasquini and Barba (2006) cited two early works by Coman and Cociu (1976) and Săvulescu and Macovei (1965) that did find seed transmission in plums and peaches, respectively. However, these manuscripts were not available to the authors, making it difficult to evaluate the technical plausibility of these works.

From the 1990s, negative reports on seed transmission



Fig. 3. Close-up of the tested seedlings.

No symptoms appeared on any leaves of the seedlings obtained from seeds of Japanese apricot (cultivar ‘Nanko’) trees that were infected with PPV-D.

have been accumulating (Eynard et al. 1991, Dulić-Marković & Ranković 1997, Myrta et al. 1998, Pasquini et al. 1998, 2000, Glasa et al. 1999). Recently, Milusheva et al. (2008) reported that all of the 1-year-old seedlings originating from the seeds of three plum cultivars infected with PPV-M, and from the seeds of an apricot cultivar (‘Modesto’) infected with PPV-Rec, remained symptomless and tested negative by ELISA using PPV-antiserum. All of these authors claim that PPV was indeed detected in seeds (seed coats), and occasionally in cotyledons, but never in grown seedlings (Eynard et al. 1991, Dulić-Marković & Ranković 1997, Pasquini et al. 1998, 2000, Milusheva et al. 2008). One of the few exceptions is the study conducted by Slovakova et al. (2002) in which PPV was detected in a low number of plums and apricots from a few seedlings. However, they stated that further work was needed to confirm seed transmission. In short, there has yet to be any reliable evidence presented to support the seed transmission of PPV.

In our study, no symptoms appeared and no PPV was detected in the 397 seedlings obtained from PPV-infected Japanese apricot trees. The results indicated that PPV is not vertically transmitted from infected trees to their fully expanded seedlings. Seed transmissibility may vary among PPV strains or host cultivars (Levy et al. 2000), and depends on the PPV strain and host cultivar combinations. It is clear that the PPV-D strain in Japan is not transmitted to seedlings from infected trees of ‘Nanko’—the major Japanese apricot cultivar. Further experiments may be needed to test other cultivars, including ‘Baigo’ and ‘Komukai’.

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Table 1. Feed composition of ear corn parts and machine harvested ECS ingredients.

Cultivars	Tree ID number	Fruit maturity	Sampling Year	Positive ¹	Negative ²
Nanko	Tree-1	immature	2010	0	20
	Tree-2	mature	2010	0	226
	Tree-3	mature	2011	0	82
Baigo	Tree-1	mature	2011	0	61
Komukai	Tree-1	immature	2010	0	8
Total				0	397

¹ Number of seedlings that tested positive for PPV.

² Number of seedlings that tested negative for PPV.

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