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DISEASE NOTE

First report of plum pox virus infecting Japanese apricot (*Prunus mume* Sieb. et Zucc.) in Japan

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Abstract For the first time, plum pox virus (PPV) has been detected in commercial Japanese apricot (*Prunus mume*) trees in Tokyo, Japan. These trees had ringspot or mottle on leaves, color breaking of petals and, occasionally, mild ringspots and malformation on fruits. The virus was identified based on the morphology of virus particles, serology, and RT-PCR. The amplified nucleotide fragment shared 100% identity with a partial coat protein gene of PPV-D isolates.

Keywords Plum pox virus (PPV) · Japanese apricot · *Prunus mume* Sieb. et Zucc. · Stone fruit

Japanese apricot (*Prunus mume* Sieb. et Zucc.), which was introduced to Japan from China ca. 2000 years ago (Hayashi et al. 2008), is one of the most popular fruit trees in Japan. Approximately 121 thousand tons of fruit were produced in 2008. Japanese apricot is also favored as an ornamental flowering tree and is grown widely throughout the country. Only two viruses, cucumber mosaic virus and prunus necrotic ringspot virus, have been reported on Japanese apricot in Japan (Kurihara et al. 1995).

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Plum pox virus (PPV), a member of the genus *Potyvirus* in the family *Potyviridae*, is an RNA virus with a flexuous filament particle of approximately 660–770 × 12.5–20 nm containing a positive-sense single-stranded RNA genome of about 9.8 kb (Kegler and Sutic 1996). Seven strains of PPV are recognized (D, M, Rec, EA, C, W, and T) (Serçe et al. 2009). In Europe, PPV is the most destructive viral pathogen of stone fruit species (*Prunus* spp.) such as plum, apricot, and peach (Németh 1986). PPV causes severe fruit symptoms such as premature drop, malformation, and ringspots, resulting in reduced fruit yield and quality. The economic impact of PPV on the stone fruit industry worldwide is estimated to be about 50 billion yen per year (Cambra et al. 2006). PPV is transmitted by a number of aphid species in a non-persistent manner, as well as by grafting, but it is not transmitted vertically through seeds (Pasquini and Barba 2006). The PPV epidemic originated in Eastern Europe and has spread progressively to a large portion of Europe, the Middle East (Turkey, Iran and Syria), Africa (Egypt and Tunisia), Asia (Russia, India, China, Kazakhstan, and Pakistan), and the Americas (Chile, Argentina, USA, and Canada) (EPPO Bulletin 2006). In Japan, the importation of stone fruit trees is under strict phytosanitary control, and the occurrence of PPV has never been reported.

Since the 1990s, ringspot and mottle symptoms on leaves, not yet reported, have been observed in Japanese apricot orchard trees by growers in the city of Ome, Tokyo. In July 2008, the Plant Clinic of the University of Tokyo was asked by the Tokyo Metropolitan Agriculture and Forestry Research Center to diagnose this case. In field surveys from July 2008 to May 2009, we found that some Japanese apricot cultivars such as Nanko, Baigo, Komukai, Gyokuei, and Shirokaga, and non-grafted seedlings had chlorotic to yellowish ringspot and mottle patterns on

leaves (Fig. 1a). Furthermore, color break of petals and, occasionally, mild ringspots and malformation of fruits were observed (Fig. 1b, c). These symptoms closely resembled those of PPV-infected plum, apricot, and peach (Llácer and Cambra 2006) as well as those on leaves of Japanese apricot experimentally graft-inoculated with PPV (Hamdorf 1975). Suspecting that this was an unreported disease of Japanese apricot infected with PPV, we performed a series of experiments, as described next to diagnose the disease.

Sap from symptomatic Japanese apricot leaves was negatively stained with 2% phosphotungstic acid (pH 7.0) and examined with a JEM-1010 electron microscope (JEOL, Tokyo, Japan). We observed filamentous potyvirus-like particles with an average size of 744 × 12.5 nm ($n = 19$) (Fig. 1d). We did not detect any other virus-like particles. Moreover, in an immunochromatographic assay using PPV AgriStrip (Bioreba, Reinach, Switzerland), we obtained a positive reaction with extracts from the symptomatic leaves, but not with extracts from healthy control leaves (data not shown). To confirm these results, reverse transcription-polymerase chain reaction (RT-PCR) amplification and sequence analysis of a partial PPV coat protein gene was carried out using the forward primer P2 (5′-CAG ACT ACA GCC TCG CCA GA-3′) and the reverse primer P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′) (Wetzel et al. 1991). A 243-bp DNA fragment was amplified from the total RNA fraction extracted separately from the symptomatic leaves of four Japanese apricot cultivars (cv. Nanko, Baigo, Shirokaga, and Komukai) and of a non-grafted seedling. The amplified products were treated with ExoSAP-IT (GE Healthcare, Buckinghamshire, UK) to remove the unincorporated dNTPs and PCR primers, followed by direct sequencing using an ABI 3130*xl* genetic analyzer (Applied Biosystems, Carlsbad, CA, USA) with the same primers. The obtained forward and reverse sequences were assembled using ATGC ver. 4.3.3 software (Genetyx, Tokyo, Japan). As a result, the 243-bp fragments obtained from symptomatic Japanese apricot leaves had 100% identity with each other. These sequences had 100% identity with the corresponding sequences of PPV-D isolates reported from different parts of the world, but had relatively low identities with that of PPV-M, the most epidemic strain, and of PPV-C, the cherry strain (Candresse and Cambra 2006) (Fig. 2). PPV-D is the most widely distributed strain of plum pox virus worldwide (EPPO Bulletin 2006) and has a wide experimental host range among *Prunus* spp. (Damsteegt et al. 2007).

This is the first report of PPV in Japan. Notably, in Ome city, PPV was detected from all symptomatic Japanese apricot trees examined, which implies that PPV was the causal agent of the disease. A graft transmission test is now underway to confirm the pathogenicity of PPV toward Japanese apricot. Moreover, PPV was detected in several different Japanese apricot cultivars and non-grafted seedlings, indicating aphid transmission of the virus. Our results also show, for the first time, that PPV can naturally infect and spread among Japanese apricot in which only experimental infection has been reported (Damsteegt et al. 2007; Hamdorf 1975). Therefore, further investigation of the extent of yield losses of Japanese apricot fruit due to PPV infection will be required. This outbreak of PPV in Japanese apricot raises concerns about a pandemic spread among other stone fruit species in Japan. We need to

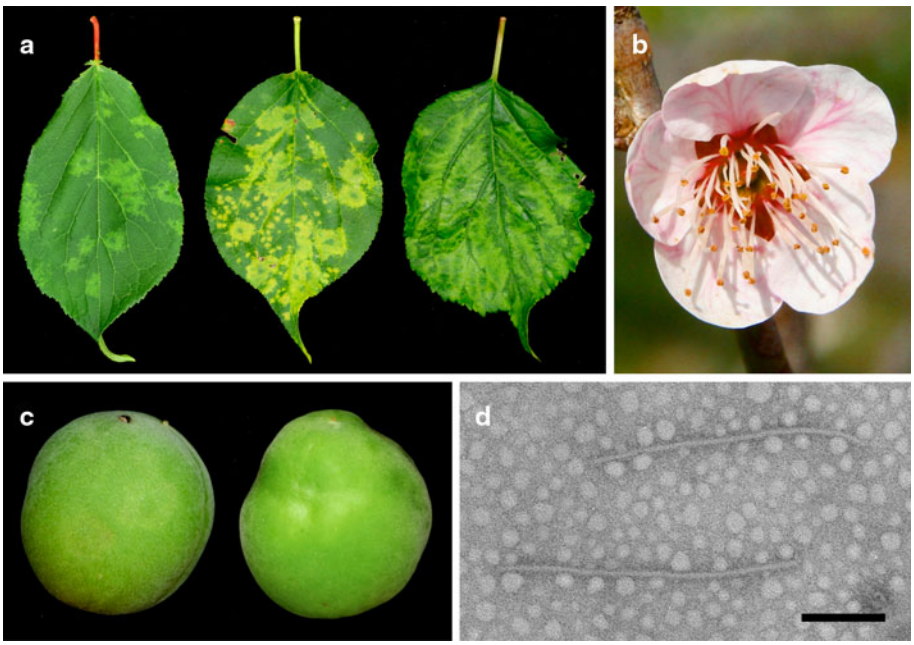

Fig. 1 **a** Chlorotic ringspots (*left* cv. Komukai), yellowish ringspots (*middle* a pollinizer) and mottling (*right* cv. Komukai) on leaves, **b** color break (cv. Nanko) on petals, and **c** mild ringspots (*left* cv. Gyokuei) and malformation (*right* unknown cultivar) on fruits of Japanese apricot. **d** Transmission electron micrograph of virus particles in symptomatic leaf sap of Japanese apricot. *Scale bar* 200 nm

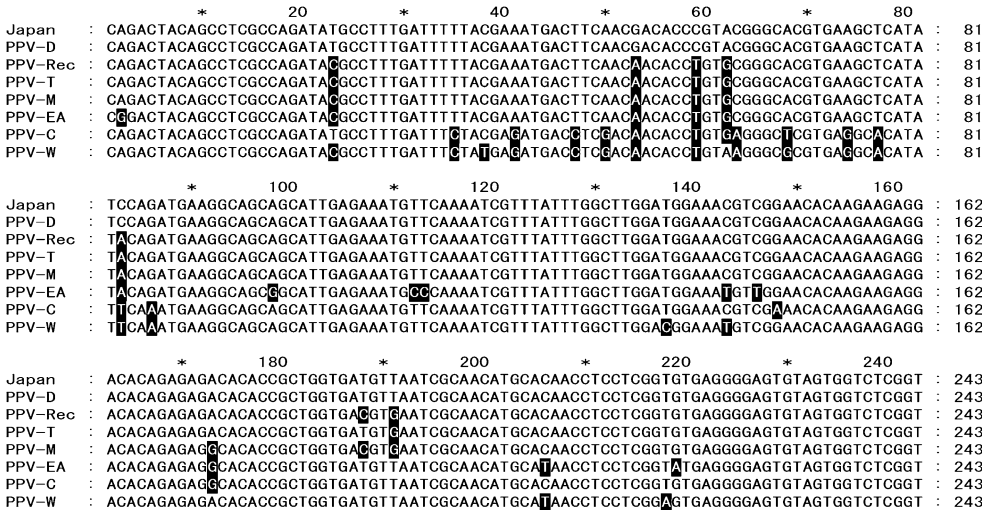

Fig. 2 Multiple sequence alignment of Japanese plum pox virus (PPV) isolates and seven strains of PPV (partial coat-protein-coding region). Accession numbers for the viral sequences used in this study are *Japan*: AB539016 (amplified from cv. Nanko), AB539017 (amplified from cv. Baigo), AB539018 (amplified from cv. Shirokaga), AB539019 (amplified from a nongrafted seedling) and AB539020 (amplified from cv. Komukai); *PPV-D*: DQ299538 (Argentina), AM260934 (Bulgaria), AY912056 (Canada), AF440741 (Chile), AY750961 (China), X16415 (France), X81077 (Germany), FN179152 (Hungary), AY591253 (Kazakhstan), and AF401295 (USA); *PPV-Rec*: AY028309; *PPV-T*: EU734794; *PPV-M*: M92280; *PPV-EA*: DQ431465; *PPV-C*: AY184478; *PPV-W*: AY912055. Nucleotides 1–20 and 224–243 correspond to the P2 and P1 primer sequences, respectively. The alignment was constructed using GeneDoc software (http://www.psc.edu/biomed/genedoc/). Nucleotides that differ from PPV-Japanese isolates are shaded

investigate the distribution of PPV in other plant hosts including fruit trees other than Japanese apricot and weeds throughout Japan carefully and in detail.

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