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Identification and characterization of *Apple stem grooving virus* causing leaf distortion on pear (*Pyrus pyrifolia*) in Taiwan

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Abstract A putative virus-induced disease of pear (*Pyrus pyrifolia* var. Hengshen) showing symptoms of reduced size of foliage and leaf distortion was observed in orchards in central Taiwan in 2004. The sap of symptomatic leaf samples reacted positively to an antiserum against *Apple stem grooving virus* (ASGV). Two virus cultures, designated as TS1 and TS2, were isolated from symptomatic pears. Flexuous filamentous virions of ~ 12×600 nm were observed in symptomatic pear leaves and purified virus preparations. Results of back inoculation of pear seedlings with TS1 revealed that ASGV was the causal agent of the disease. Sequence analyses of the cloned coat protein (CP) genes of TS1 and TS2 shared 88–92.4% nucleotide and 90.7–97.1% amino acid identities with those of other ASGV isolates available in GenBank. The polyclonal antibody generated against ASGV TS1 has been routinely used for the detection of the ASGV-infection in the imported pear scions for quarantine purpose via enzyme-linked immunosorbent assays (ELISAs). One of 1,199 samples of pear scions imported from Japan during 2005–2007 was identified as ASGV-positive and the virus was designated as AGJP-22. The CP gene amplified from this AGJP-22 shared 97.9–98.3% amino acid identities to those of the domestic isolates and they were closely related phylogenetically. To date, these data present for the first time conclusive evidence revealing that ASGV is indeed the causal agent of the pear disease displaying symptoms of reduced size of foliage and leaf distortion in Taiwan.

Keywords ASGV · *Capillovirus* · *Rosaceae* · Pome fruit · Pear disease · Phylogenetic analysis

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Introduction

Pear (*Pyrus* spp.) belongs to the plant family *Rosaceae* and is generally divided into two groups, Asian pear and European pear. Asian pears are grown extensively in Mainland China, Japan, Korea and Taiwan (Beutel 1990). The domestic pear cultivar, *P. pyrifolia* var. Hengshen originated from Mainland China and was introduced into Taiwan during the

19th century. The unique pear-topworking cultivation has been adopted to produce high quality Japanese pear fruits in Taiwan by grafting flower bud-containing pear scions imported from Japan onto the local dominant Hengshen pear as rootstock since 1975. Pear has become one of the economically important horticultural crops in Taiwan with approximately 8,400 ha of cultivated area. However, grafting foreign scions onto domestic rootstocks presents the risk of introducing graft-transmissible exotic pathogens into Taiwan (Motoshima et al. 1983).

Rosaceous fruit trees are hosts for at least 25 important RNA viruses (Jones and Aldwinckle 1990; Li et al. 2000; Németh 1986; Russo et al. 2002). Among these viruses, *Apple stem grooving virus* (ASGV) (Lister et al. 1965), *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple mosaic virus* (ApMV), *Tulare apple mosaic virus* (TAMV) (Jones and Aldwinckle 1990), *Apple latent spherical virus* (ALSV) (Li et al. 2000), *Cherry rasp leaf virus* (CRLV) (Jones et al. 1985), *Tomato ringspot virus* (ToRSV) (Stouffer and Uyemoto 1976), *Tomato bushy stunt virus* (TBSV) (Németh 1986) and *Pear latent virus* (PeLV) (Russo et al. 2002) were reportedly able to infect pome fruit trees.

In April 2004, pear plants (*P. pyrifolia* var. Hengshen) showing symptoms of tufted, reduced size of foliage and distorted foliages were observed in orchards in central Taiwan. Symptoms like this on pear have not been characterized previously, and the causal agent of this pear disorder was unclear. We report herein the isolation, serological and molecular characterization, and back-inoculation of the virus that caused size reduction of foliage and leaf distortion on pear plants in Taiwan.

Materials and methods

Virus source, host range and back-inoculation

In April 2004, two pear plants (*Pyrus pyrifolia* var. Hengshen) showing disease symptoms of reduction in size of foliage and leaf distortion (Fig. 1a) were observed in orchards in Taichung County, central Taiwan. Possible presence of common fruit viruses in the diseased pear samples was initially analyzed by enzyme-linked immunosorbent assay (ELISA) using commercial antisera against ASGV, ACLSV, ASPV (Bioreba, Nyon, Switzerland), ToRSV, TBSV, *Peach rosette mosaic virus* (PRMV) (ADI, LLC., Fayetteville, AR, USA), ApMV, *Plum pox virus* (PPV) (ADGEN Ltd., Scotland, UK) and the POTY monoclonal antibody for detecting potyviruses (ADI, LLC., Fayetteville, AR, USA), according to the manufacturers' instructions. Moreover, rabbit antisera against tospoviruses (Zheng et al. 2008) and *Lisianthus necrosis virus* (LNV) (Chen et al. 2001) generated in our laboratory were also used for ELISA. Two virus cultures, designated as TS1 and TS2, were isolated from disease pear leaves by triple single-lesion isolations in *Chenopodium quinoa*, in which the viruses were propagated for the subsequent analyses. The TS1 isolate was biologically characterized on 23 herbaceous plant species representing 6 families by mechanical inoculation using 30-fold dilution (w/v) of the inoculum prepared by grinding TS1-infected *C. quinoa* leaf tissues in 10 mM potassium phosphate buffer (pH 7.0) (Jan et al. 2000). Pear seedlings (var. Hengshen) germinated from seeds were also mechanically inoculated with the isolate TS1 for the pathogenicity tests. Four-week old pear seedlings after germination were used for the back-inoculation assay. The inoculum was prepared as described above. To increase the efficiency in back-inoculation tests of isolate TS1, the pear seedlings were mechanically inoculated twice. The second inoculation was performed two weeks after the first inoculation. Two pear seedlings were also mechanically mock-inoculated with potassium phosphate buffer as the negative control. All the tested plants were maintained in a greenhouse for symptom observation for at least 2 months. Both symptomatic and asymptomatic inoculated-plants were analyzed by RT-PCR and ELISA using an antiserum produced against the isolate TS1 to confirm virus infection.

Virus purification, electron microscopy and polyclonal antibody production

Viral particles were purified from TS1-infected *C. quinoa* plants as described by de Sequeira and Lister (1969) with minor modifications. The extract of the infected *C. quinoa* leaves in 10 mM potassium phosphate buffer (pH 7.0) was clarified with 0.2% bentonite (vol./vol.), and the virus was partially purified from the clarified extract by 8% polyethylene glycol (PEG, MW 6,000)-0.02 M sodium chloride

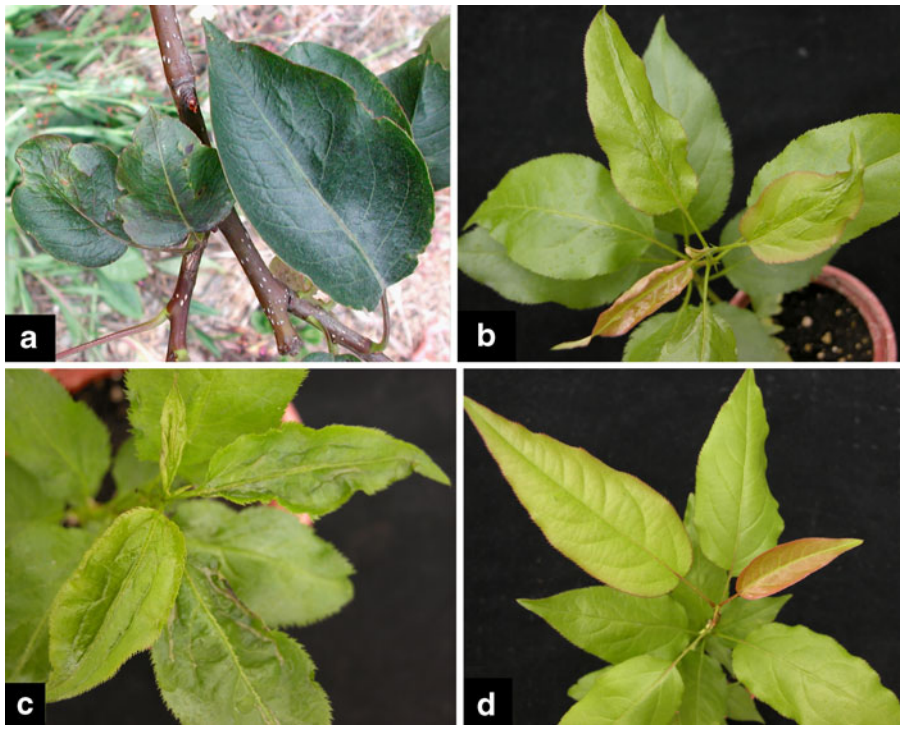

Fig. 1 (**a**) The symptoms of reduced size of foliage, deformed and/or distorted leaves observed in diseased pear (*Pyrus pyrifolia* var. Hengshen) plants from orchards in central Taiwan. (**b**) Pear seedling inoculated with isolated ASGV TS1 displaying reduced size of foliage and distorted symptoms on new emerged leaves. (**c**) The symptoms of severely deformed leaves and drastic reduction in foliage size were observed on diseased pear plant 2 month after inoculation. (**d**) No systemic symptoms were observed in mock-inoculated pear plant as a negative control

precipitation. The virus was further purified by 10–40% sucrose density gradient centrifugation. The purified virions were dialyzed and preserved in 10 mM potassium phosphate buffer (pH 7.0). The samples of purified virus preparations and crude saps of the infected leaf tissue were separately placed on formvar-coated carbon grids which were then stained with 2% uranyl acetate (pH 4.2) before being examined with a JEOL 200 CX transmission electron microscope (JEOL Ltd., Tokyo, Japan) (Jan and Yeh 1995). Purified virus was also used to immunize a New Zealand white rabbit for the production of a polyclonal antibody (PAb) as described previously (Jan and Yeh 1995).

Molecular cloning and sequence analysis

Total RNAs were extracted from healthy and virus-infected leaves of *C. quinoa*, pear plants or flower buds of pear scions imported from Japan during 2005–2007, as described by Napoli et al. (1990). Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described by Jan et al. (2000). For RT-PCR amplification of the ASGV genomic segment corresponding to the coat protein (CP) gene, the upstream primer FJJ01-104 (5′-AGAAGGTCACGCTCAAT-3′) and downstream primer FJJ01-24 (5′-TCACACGACTCCTAAC-3′) were designed based on the reported ASGV genomic sequences available in the National Centre for Biotechnology Information (NCBI) GenBank database. The first strand cDNA was synthesized from the total RNAs by using the *Moloney murine leukemia virus* reverse transcriptase (M-MLV RT) kit (Epicentre, Madison, WI, USA) following the manufacturer's instructions. Subsequently, PCR was performed using *Taq* DNA polymerase to amplify the expected product of 0.8 kb. The RT-PCR was performed in a 25 μl volume using 3 μl total RNAs and denatured by heating at 95°C with 2 μl downstream primer (200 ng/μl) for 5 min followed by chilling on ice for 5 min. First-strand cDNA was synthesized using 2.5 μl 10×reverse transcriptase reaction buffer, 2 μl dNTP (2.5 mM), 3 μl dithiothreitol (DTT) (0.1 M) and 10 units of M-MLV RTase at 37°C and 42°C for each 30 min. PCR were performed in a 100 μl volume containing 5.5 μl cDNA, 10 μl of 10× *Taq* reaction buffer [500 mM KCl, 100 mM Tris-HCl (pH 9.0), 1% Triton X-100], 8 μl $MgCl_2$ (25 mM), 2 μl dNTP (2.5 mM), 2 μl of primer pairs (200 ng/μl) and 0.5 μl *Taq* DNA polymerase (5.0 U/μl). PCR was performed for 30

cycles of 1 min for denaturation at 94°C, 1 min for annealing at 50°C, and 1 min for synthesis at 72°C and the final cycle at 72°C for 5 min. The amplified DNA product was cloned into pCRII-TOPO vector (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The cloned fragment was sequenced on an automatic DNA sequencer ABI PRISM 3730 (Applied Biosystems, Hammonton, NJ, USA) at the Biotechnology Center, National Chung Hsing University, Taiwan.

The CP sequences of the virus isolates collected from diseased pear plants and pear scions were compared with those of the following 15 ASGV isolates available in the NCBI GenBank (GenBank accession numbers and geographical origins in parenthesis): lily isolate (AB004063 and D16681 from Japan), apple isolates (D14995 and NC_001749 from Japan; AF438409 from Brazilian; FM204881 from India; EU236258 from Mainland China), pear isolates (AF465354 and AY596172 from Korea; AY886760 from Mainland China), kiwifruit isolate (AF522459 from New Zealand), and citrus isolates (CTLV) (AY646511 from Taiwan; D14455 and D16368 from Japan; and DQ273564 from Mainland China). Nucleotide and amino acid sequence comparisons were preformed in the DNASTAR Lasergene software (DNASTAR, Inc., Madison, WI, USA). The multiple sequence alignments were preformed by CLUSTAL W program (Thompson et al. 1994). Phylogenetic analyses using neighbor-joining method (Saitou and Nei 1987) was performed with MEGA (Molecular Evolutionary Genetics Analysis) software version 4.0 (Tamura et al. 2007) and bootstrap was tested in 1,000 replications. *Cherry virus A* (Genus *Capillovirus*) was used as an outgroup.

Indirect ELISA

Indirect ELISA was performed as described by Clark and Adams (1977) with required modifications. Two hundred microlitres of the crude saps extracted from young emerged leaves or flower buds of pear scions with coating buffer (0.05 M sodium carbonate, pH 9.6, 0.02% sodium azide) at 50-fold dilution were coated in polystyrene microtitration plates (Greiner Bio-One, Frickenhausen, Germany) and incubated at 37°C for 1 hr. The plates were then washed with PBST (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20) three times (3 min each). The ASGV-TS1 polyclonal antibody at 4,000-fold dilution in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.2% ovalbumin) was added 200 μl per well for immunoreaction. The plates were incubated at 37°C for 1 hr and then washed three times with PBST. Then 200 μl of goat anti-rabbit IgG-alkaline phosphatase (AP) conjugate (Jackson Immuno Research Lab. Inc., West Grove, PA, USA) in 5,000-fold dilution in conjugate buffer was added to each well and incubated at 37°C for 1 hr. After further washing, 200 μl per well of the alkaline phosphatase substrate solution was added. The substrate solution was prepared by dissolving the alkaline phosphateas tablets (Sigma-Aldrich, St. Louis, MO, USA) in the substrate buffer (9.7% diethanolamine, and 0.02% sodium azide, pH 9.8) to a final concentration of 1 mg/ml. The plates were incubated at room temperature and the level of reaction were recorded at 405 nm using a Labsystems Multiskan EX microplate reader (Labsystems, Vantaa, Finland) from 5 min to 60 min after the addition of the substrate solution.

Western blotting analysis

Immunoblotting was performed with the protocol described by Jan and Yeh (1995). Leaf extracts of healthy and TS1-infected pear plants were separated by 10% SDS-PAGE and then transferred to PVDF membrane (PerkinElmer, Waltham, MA, USA). The membrane was incubated with antiserum against ASGV TS1 at 4,000-fold dilution, followed by incubating in the AP conjugated goat anti-rabbit IgG at 5,000-fold dilution and the color was developed by treatment of chromogenic substrates (NBT/BCIP) (Amresco, Solon, OH, USA).

Results

Virus isolation

Two diseased pear leaves bearing symptoms of small foliage and leaf distortion (Fig. 1a) reacted positively only to a commercial antiserum against ASGV. Two virus cultures designated as TS1 and TS2 were isolated from the ASGV-ELISA positive diseased pear samples and established in *C. quinoa* plants via

triple single-lesion isolations. The *C. quinoa* plants inoculated with the isolate TS1 or TS2 reacted positively only to the ASGV antiserum in ELISA (data not shown) and they displayed symptoms of chlorotic local lesions on the inoculated leaves 10–12 days after infection and foliar distortion symptoms on systemic leaves thereafter.

Host reactions and back-inoculation

The host range of TS1 was studied with 23 herbaceous plant species from 6 families by mechanical inoculation, of which 15 species were found susceptible to the virus. All of the infections by isolate TS1 were determined by symptom expression and indirect ELISA using the generated polyclonal antibody. Among these susceptible species, *Gomphrena globosa* (*Amaranthaceae*), *Cucurbita pepo* var. zucchini (*Cucurbitaceae*), *Datura stramonium*, *Nicotiana benthamiana*, *N. glutinosa, N. tabacum* cv. Xanthi, *N. tabacum* cv. Samsun and *Solaum esculentum* (*Solanaceae*) developed systemic symptoms with mostly leaf mottling, mosaic, foliar distortion or chlorotic spots. The virus isolate TS1 induced symptoms of necrotic local lesions and mild leaf mottling, respectively, on the inoculated and systemic leaves of *D. stramonium*, while it induced chlorotic spots on inoculated leaves of *C. quinoa* (*Chenopodiaceae*) and caused leaf malformation with chlorotic spots on systemic leaves. Systemic infection of TS1 was also found in *Amaranthus gangeticus* (*Amaranthaceae*) without apparent symptom. The other five susceptible species including *C. amaranticolor*, *C. murale* (*Chenopodiaceae*), *Phaseolus vulgaris*, *Vigna unguiculata* and *V. radiate* (*Leguminosae*) were locally infected with chlorotic spots on the inoculated leaves but not systemically infected by TS1. *Brassica chinensis* (*Brassicaceae*), *Cucumis melo*, *C. sativus* (*Cucurbitaceae*), *Vicia mungo* (*Leguminosae), N. occidentalis, N. edwardsonii, N. tobacum* cv. Vam-Hicks and *Capsicum frutescens* (*Solanaceae*) were not infected by TS1 as determined by symptom expression and ELISA.

To determine whether ASGV was the causal agent of the pear disease observed in orchards, TS1 was back-inoculated onto 25 Hengshen pear seedlings by mechanical inoculation. Twenty-four pear seedlings inoculated with TS1 displayed symptoms of leaf distortion on newly emerged leaves (Fig. 1b) similar to those observed on the naturally infected plants (Fig. 1a) about 45 days after inoculation. The initial symptoms occurred at both sides of the vein. The symptoms of sunken streaks from the back of the leaves to front part were displayed on the newly emerged leaves at the early stage. Then the typical symptoms of leaf distortion developed on the infected pear plants. The distorted leaves displayed symptoms of severe deformation and drastic reduction in foliage size after two months (Fig. 1c). No systemic symptoms were observed on new leaves of the mock-inoculated Hengshen pear plants (Fig. 1d). Infections were also confirmed by positive reactions of ELISA tests using antiserum against isolate TS1. All ASGV-ELISA positive pears were also confirmed with ASGV infection by RT-PCR but not with ACLSV and ASPV (Fig. 2).

Electron microscopy and serological characterization

Electron microscopic examination of the negatively stained purified virus preparations and leaf crude saps of TS1-infected pear revealed the presence of flexuous filamentous particles measuring about 12× 600 nm (Fig. 3). One structural protein of 27–28 kDa was found in purified virus preparations in SDS-PAGE. The similar band of ~28 kDa was observed in western blotting of leaf extracts from TS1-infected pear using 4,000-fold diluted rabbit PAb (data not shown).

Molecular cloning and sequence analysis

To determine the taxonomic relationships of the ASGV isolate from diseased pear, cDNA fragments of around 800 base pairs (bp) were amplified from the total RNAs isolated from TS1- and TS2-infected *C. quinoa* by RT-PCR with the primers FJJ01-104 and FJJ01-24 designed for amplifying the CP gene. The RT-PCR product was cloned and sequenced. The TS1 CP gene is 711 bp in length and encodes a predicted protein of 237 amino acids with a calculated molecular mass of 27.2 kDa. The CP gene of TS1 shared 99.7% nucleotide and 99.6% amino acid identities to that of TS2. Comparisons of the CP genes of the TS1 and TS2 with those of fifteen ASGV isolates available in the GenBank revealed 88–92.4% nucleotide and 90.7–97.1% amino acid identities. These data indicated that TS1 and TS2 were two isolates of ASGV. The CP gene phylogenetic analysis

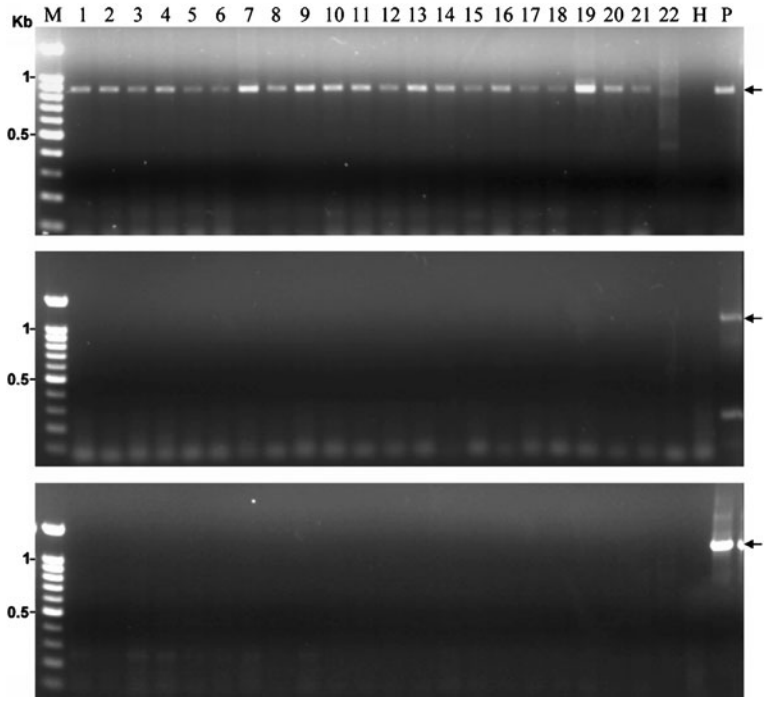

Fig. 2 Agarose gel electrophoretic analysis of DNA products derived from amplification of total RNAs of the 22 ASGV TS1-infected pear plants (lane 1-22) with ASGV- (top), ACLSV- (middle) and ASPV- (bottom) CP gene primers by RT-PCR. The arrows were indicated the expected sizes of RT-PCR products amplified with each virus-specific primer. Total RNAs isolated from mock-inoculated pear plant (lane H), and ASGV-, ACLSV- and ASPV-positive pear plants (lane P) were used as negative and positive controls for RT-PCR. Lane M is the standard DNA molecular weight markers

revealed that ASGV isolates from Taiwan (TS1 and TS2) were closely related to pear isolates from Japan (AGJP-22) and Korea. The ASGV isolates from pear formed a major group close to, but distinct from, the branches containing the isolates from apple and citrus. The citrus and lily isolates from Japan were closely related to apple isolates from Japan. The apple isolate from Brazil were closely related to an apple isolate from India (Fig. 4).

Detection of ASGV from imported pear scion from Japan

The production of Japanese pear fruits in Taiwan have been achieved by unique topworking cultivation by grafting the flower bud-containing pear scions imported from Japan onto domestic Hengshen pear. For quarantine purpose, the developed ELISA and RT-PCR are being routinely applied for the detection

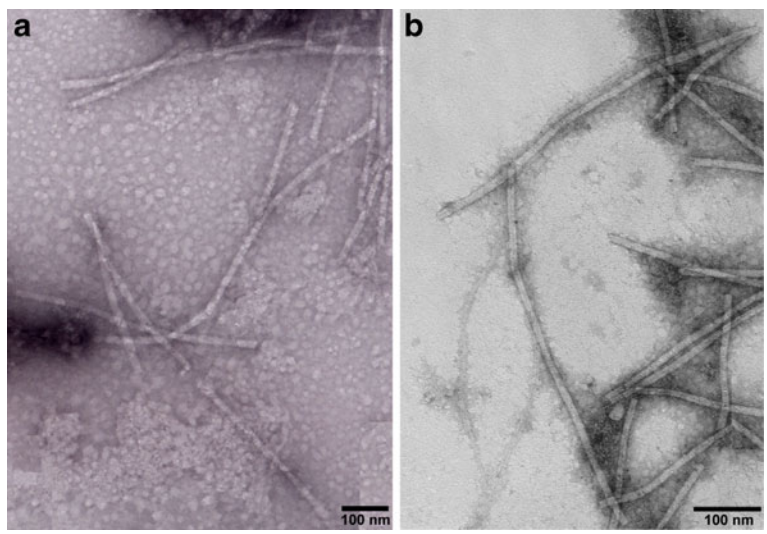

Fig. 3 Electron micrographs of virus particles of the TS1 isolate of *Apple stem grooving virus* (ASGV). (**a**) The flexuous filamentous virus particles in leaf dips of ASGV TS1-infected *Pyrus pyrifolia* var. Hengshen plants and (**b**) the purified ASGV TS1 virions were observed. Bar represents 100 nm

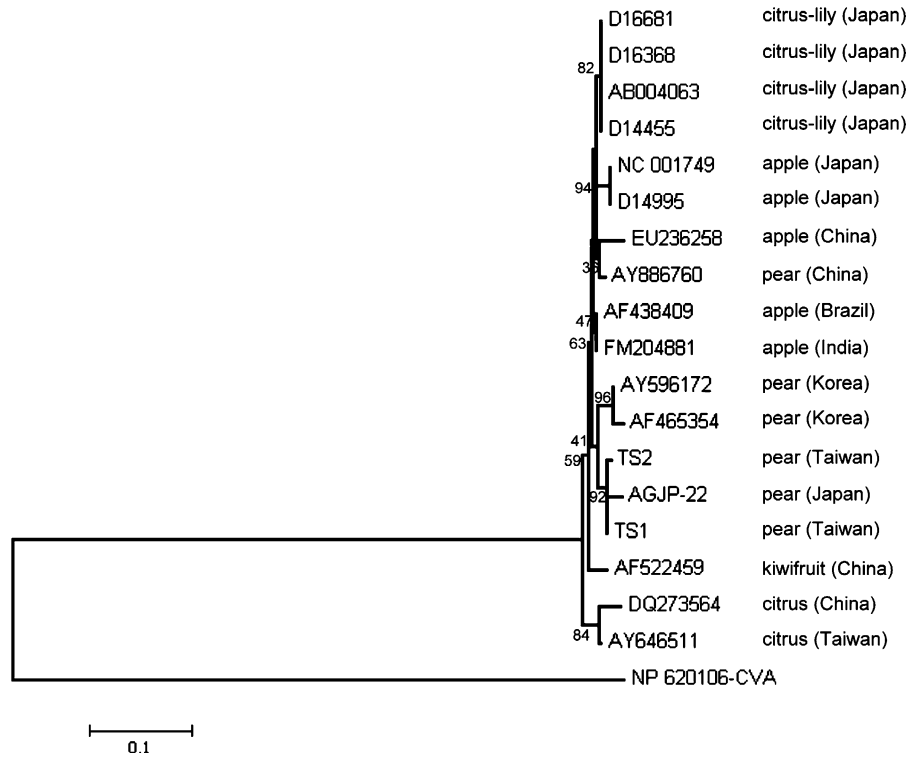

Fig. 4 Phylogenetic relationships of the ASGV coat protein gene of Taiwanese isolates TS1 and TS2, Japanese isolate AGJP-22 from imported pear scion and fifteen other isolates. Phylogenetic trees were constructed by CLUSTAL W and the Neighbor-Joining method with 1,000 bootstraps using the MEGA version 4.0 program. The scale bar representing the genetic distance is indicated at the left bottom

of ASGV-infection in imported pear scions. One of the 1,199 tested pear scions imported from Japan during 2005–2007 was confirmed ASGV-positive by indirect-ELISA using the TS1 antiserum. The cloned CP gene amplified from this ASGV-positive sample, designated as AGJP-22, shared 97.9–98.3% amino acid identities with those of the domestic isolates (TS1 and TS2). The CP genes of the TS1, TS2 and the Japanese AGJP-22 isolates grouped together in a sub-cluster which diverged from other isolates (Fig. 4).

Discussion

Our results have demonstrated that the virus TS1 causing the symptoms of reduced size of foliage and leaf distortion on one of the important pear cultivar in Taiwan, *P. pyrifolia* var. Hengshen, is an isolate of *Apple stem grooving virus* (ASGV). The phylogenetic analyses of the CP gene revealed that the Taiwanese ASGV isolates TS1 and TS2 were closely related to Japanese pear isolate.

The herbaceous host range of ASGV by mechanical inoculation have been reported for apple (Yoshikawa 2000), kiwifruit (Clover et al. 2003), citrus (Lovisolo et al. 2003) and lily (Inouye et al. 1979) isolates, but not for pear isolate. Though ASGV TS1 and previously described isolates differ among themselves in host-preference, they all could infect *C. quinoa*, *N. glutinosa* and *P. vulgaris*. ASGV isolate TS1 but not citrus isolates could infect *A. gangeticus* and *D. stramonium* (Lovisolo et al. 2003). *D. stramonium* showed necrotic local lesions on the inoculated and mild leaf mottling on the systemic leaves when infected by pear TS1 isolate but was symptomless when infected by lily and apple isolates (Inouye et al. 1979; Lister et al. 1965). Tobacco *N. tabacum* cv. Samsun could be infected by TS1, but not by lily isolate (Inouye et al. 1979). *N. occidentalis* and *C. sativus* were not hosts for TS1 and citrus isolates, but were hosts for kiwifruit isolate (Clover et al. 2003). *C. pepo* could be infected by apple (Uyemoto and Gilmer 1971), citrus (Lovisolo et al. 2003), lily (Inouye et al. 1979) and TS1 isolates.

ASGV is a graft-transmissible viral pathogen that frequently occurs in *Rosaceae* fruit trees such as apple (Jones and Aldwinckle 1990; Magome et al. 1997; Nickel et al. 2001), pear (Jones and Aldwinckle 1990; Shim et al. 2004; Yoshikawa et al. 1996), apricot

(Németh 1986), or cherry (Yoshikawa 2000). In addition, ASGV has been reported to infect citrus (Lovisolo et al. 2003; Magome et al. 1997), lily (Inouye et al. 1979), and kiwifruit (Clover et al. 2003). Although most of ASGV isolates cause symptomless latent infection in the commercial apples and pear cultivars (Yoshikawa 2000), several ASGV isolates induce stem grooving, necrosis and deformation on top-grafting unions in susceptible cultivars (Motoshima et al. 1983; Németh 1986; Yoshikawa 2000). The uncharacterized symptom of reduced size of foliage and leaf distortion on Hengshen pear in Taiwan is different from the symptoms described previously (Yoshikawa 2000). These symptoms also differ from those on pear (*Pyrus pyrifolia*) infected with the Korean isolate which induced black necrotic leaf spot (Shim et al. 2004) and caused serious yield loss in pear cultivation in Korea. The pear disease characterized in this study is a new disease caused by ASGV. Based on the differences in symptom developments induced by ASGV TS1 when compared to those of other reported ASGV isolates in pear plants, the newly characterized pear disease in Taiwan could be proposed as the pear leaf distortion disease (PLD).

ASGV has been reported to be seed-transmissible in apple (*Malus platycarpa*) (6%) (Šutić et al. 1999; van der Meer 1976), lily (*Lilium longiflorum*) (2%) and *C. quinoa* (2.5–60%) (Inouye et al. 1979). However, seed transmission of ASGV in pear has not been found. In addition, Shim et al. (2006) indicated that ASGV could be transmitted by a fungus *Talaromyces flavus* to pear (20% infectivity) and *P. vulgaris* (35–90% infectivity) plants by direct infiltration into leaves with ASGV-infected *T. flavus*. The pear and *P. vulgaris* plants inoculated with ASGV-infected *T. flavus* developed similar symptoms of black necrotic leaf spot and chlorotic spots, respectively, as those observed in plants which were mechanically inoculated with the crude saps from ASGV-infected *C. quinoa*.

The pear fruit production, in terms of yield and quality, in Taiwan relies upon top-grafting cultivation using imported pear scions. The CP gene (AGJP-22) amplified from the ASGV-infected scion imported from Japan shared 97.9–98.3% amino acid identities to those of the domestic isolates and was closely related phylogenetically. It not only showed the possibility that ASGV might be introduced into Taiwan with imported pear scions, but also showed the flower-bud of the pear could be a carrier of a transmissible ASGV.

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