MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF AN ISOLATE OF *APPLE STEM PITTING VIRUS* CAUSING PEAR VEIN YELLOWS DISEASE IN TAIWAN

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SUMMARY

Symptoms of vein yellows were observed on the leaves of the domestic pear cultivar, *Pyrus pyrifolia* var. Hengshen, in central Taiwan. A virus with filamentous particles *ca* 12×800 nm in size (isolate VY1) was recovered from one symptomatic leaf and established in *Chenopodium quinoa*. VY1 was confirmed to cause pear vein yellows by back inoculation. Sequence analysis of the cloned coat protein (CP) gene of VY1 revealed 80.5-86.7% amino acid identity with comparable genes of 19 reported *Apple stem pitting virus* (ASPV) isolates. A conserved region of 220 amino acids was identified at the C terminal of the CP genes of these 20 viral isolates (3'-CP region). Three German isolates, two Polish pear and apple isolates as well as 13 other ASPV isolates were divided into four groups, A, B, C and D, respectively. Isolates included in each of the groups A, B, or C shared 97.3-100% amino acid identity in the CP gene. Sequence comparisons of CP genes at the inter-group level, showed 74.8-91.2% and 79.4-93.7% nucleotide and amino acid identity, respectively. However, when the nucleotide and amino acid sequences of the 660 nt 3'-CP conserved regions were compared, sequence identity values rose to 81.1-95.5% and 91.8-99.1%, respectively. Using 3'-CP conserved regions for nucleotide and amino acid sequence identity comparison among ASPV isolates provides better demarcating criteria for the taxonomy of ASPV. This is the first report of ASPV causing pear a disease in Taiwan.

Key words: ASPV, genetic diversity, phylogenetic analysis, pear disease, *Foveavirus.*

INTRODUCTION

Pear vein yellows (PVY) disease was believed to be induced by a virus without ultimate evidence until 1971 (Hibino and Schneider, 1971). PVY frequently occurs in pear-growing areas and is caused by *Apple stem pitting virus* (ASPV) (Cameron, 1989; Jelkmann, 1994; Leone *et al*., 1995).

ASPV, which was first reported from apple (*Malus sylvestris*) in 1954 (Smith, 1954), has a worldwide distribution in pome fruit trees (Sutic *et al*., 1999). ASPV is transmitted mechanically to a limited herbaceous host range and has no known vector (Brunt *et al*., 1996; Yanase *et al*., 1989). Although ASPV infections to commercial pome fruit trees are usually latent, susceptible apple (*Malus* spp.), pear (*Pyrus* spp.), quince (*Cydonia oblonga* and *Pyronia veitchii*), hawthorn (*Crataegus monogyna*) and rowan (*Sorbus discolor*) (Kundu and Yoshikawa, 2006; Martelli and Jelkmann, 1998; Sutic *et al*., 1999) may react with growth reduction and yield losses (Stouffer, 1989; Yanase *et al*., 1990). Symptoms in apple consist of green crinkle and star crack (Desvignes *et al*., 1999), stem pitting of Virginia crab, and epinasty and decline of Spy 277 (Stouffer, 1989). In addition to PVY, stony pit (PSP or red mottle disease) (Nemeth, 1986; Sutic *et al*., 1999) and necrotic spot (PNS) (Kishi *et al*., 1976) are induced by certain ASPV strains in various pear cultivars (Nemeth, 1986; Paunovic *et al*., 1999). Sooty ring spot and fruit deformation in quince have also been correlated to ASPV infection (Mathioudakis *et al*., 2009; Paunovic and Rankovic, 1998). A survey of ASPV incidence in Czech and Moravian apple orchards showed that 28% of the trees were infected (Kundu, 2003a). Samples collected from symptomless apple and pear trees in Greek orchards revealed a 96% and 43% incidence of the virus, respectively (Syrgianidis, 1988). In China, the largest pear-producing country, about 62% of the main pear cultivars are infected by ASPV in the pear-growing areas of northern districts (Wang *et al*., 1994).

ASPV is the type species of the genus *Foveavirus*, family *Flexiviridae* (Martelli and Jelkmann, 1998; Adams *et al*., 2004). It has flexous filamentous particles about 12×800 nm in size (Koganezawa and Yanase, 1990; Martelli *et al*., 2007), which contain a single-stranded positive sense RNA genome of *ca*. 9.3 kilobases with five open reading frames (Adams *et al*., 2004; Jelkmann, 1994; Martelli *et al*., 2007). High genetic variability among ASPV isolates/strains was observed in the nucleotide sequences of the replicase (Rodoni and Con-

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stable, 2008) and coat protein (CP) (Klerks *et al*., 2001) genes as well as the 3' untranslated regions (Kundu, 2008; Schwarz and Jelkmann, 1998). These variations were up to 20% in some cases (Yoshikawa *et al*., 2001).

In 2006, domestic Hengshen pear plants (*P. pyrifolia* cv. Hengshen) showing vein yellowing (Fig. 1a) were observed in central Taiwan. These symptoms were slightly different from those described previously for other pear species (Jelkmann *et al*., 1992; Jelkmann, 1994; Jelkmann and Keim-Konrad, 1997; Leone *et al*., 1995; Yanase *et al*., 1989). An ASPV isolate, designated as VY1, was recovered from a symptomatic Hengshen plant, characterized for its biological, serological and molecular properties, and confirmed by back-inoculation to be the causal agent of the disease. The CP gene of the isolate VY1 was compared molecularly with that of other ASPV isolates from pear and apple.

MATERIALS AND METHODS

Virus isolation and maintenance. Symptomatic leaves form a cv. Hengshen pear growing in an orchard of the Miaoli County (central Taiwan) were collected in September 2006. Leaf samples were first tested by ELISA for the possible presence of viruses using commercially available antisera to *Apple stem grooving virus* (ASGV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV) (Bioreba, Switzerland), *Tomato bushy stunt virus* (TBSV) (ADI, LLC., USA), and *Apple mosaic virus* (ApMV) (ADGEN, UK). A positive reaction was obtained only with the antiserum to ASPV. A virus isolate, designated as VY1, was then recovered in *Chenopodium quinoa* via triple single-lesion isolations using 10 mM potassium phosphate buffer (pH 7.0) and propagated in the same host (Jan *et al*., 2000),

Host reactions and pathogenicity tests. VY1 was mechanically transmitted to at least three plants each of 25 herbaceous hosts of 9 different families using an inoculum prepared by grinding leaves of VY1-infected *C. quinoa* at 30-fold dilution (w/v) in 10 mM potassium phosphate buffer, pH 7.0. The same inoculum was used on four-week-old Hengshen pear seedlings, which received a booster inoculation two weeks after the first one. Two pear seedlings were mechanically mock-inoculated with potassium phosphate buffer as negative controls. All inoculated plants were kept in a greenhouse for at least 2 months for symptom development and were tested for the presence of the virus by indirect ELISA using a polyclonal antiserum to VY1. Infection of inoculated seedlings was verified by RT-PCR using the ASPV upstream primer FJJ07-21 [5'-TATGAACCCCAAGCTGG-3'] and downstream primer FJJ05-4 [5'-TAATY(C/T)ACTTCCTR(A/G)AT-3'], that yield an amplification product of about 0.5 kb.

Virus purification, electron microscopy and antiserum production. Virus was purified from VY1-infected *C. quinoa* plants as described by de Sequeira and Lister (1969) with minor modifications. Fresh leaves (100 g) were homogenized in 250 ml of 0.01 M potassium phosphate buffer (pH 7.0) followed by low speed centrifugation at 5,000*g* for 10 min. The supernatant was clarified by adding 1% bentonite, then 0.06 M Na_2HPO_4 and 0.03 M $CaCl_2$. It was centrifuged at 5,000*g* for 10 min and the clarification step was repeated without the addition of Na_2HPO_4 and $CaCl_2$. Virus particles were precipitated from the supernatant adding 8% polyethylene glycol (PEG 6000) and 0.02 M NaCl, followed by centrifugation at 10,000*g* for 10 min. The pellet was suspended in 0.01 M phosphate buffer and centrifuged in 10-40% sucrose gradient at 141,000*g* for 2.5 h. Purified virions were dialyzed and preserved in 10 mM potassium phosphate buffer, pH 7.0. Purified virus preparations and crude extracts of infected leaf tissue were placed separately on Formvar-coated carbon grids and stained with 2% uranyl acetate (pH 4.2) before examination with a JEOL 1200 EXII electron microscope. Purified virions were used to immunize a New Zealand white rabbit for production of a polyclonal antiserum following the procedure described previously (Jan and Yeh, 1995).

RT-PCR amplification, cloning, sequencing and sequence analyses. Total RNAs were extracted from healthy and virus-infected leaves of *C. quinoa* or pear plants as described by Napoli *et al*. (1990). RT-PCR primers for amplification of the viral CP region were designed on nucleotide sequences of different ASPV isolates retrieved from GenBank. RT-PCR was run according to Jan *et al*. (2000), using the upstream primer FJJ05-3 [5'-GTGY(C/T)GTTY(C/T)AR(A/G)TY(C/T)ATGR(A/G)CW(A/T)TCC-3'] and the downstream primer FJJ05-4 [5'-TAATY(C/T)ACTTCCTR(A/G)AT-3'] that yield an amplicon of about 1.2 kb. First strand cDNA was synthesized from total RNAs using a MMLV reverse transcriptase kit (Epicentre, USA), following the manufacturer's instructions, and PCR was performed using *Taq* DNA polymerase. Amplified DNA products were subsequently cloned into the pCRII-TOPO vector (Invitrogen-Life Technologies, USA), following the manufacturer's instructions. The inserted DNAs from selected clones were sequenced by an automatic DNA sequencer (Applied Biosystems, USA) at the Biotechnology Center, National Chung Hsing University, Taiwan. The CP sequence of ASPV from cv. Hengshen was compared with those of other ASPV isolates (Table 1) retrieved from GenBank. Sequence comparisons were performed with the DNASTAR Lasergene software (DNASTAR, Inc., USA). Sequence alignments were done using a CLUSTAL W algorithm (Thompson *et al*., 1994).

Serological analyses. Indirect ELISA tests were per-

formed as described by Clark and Adams (1977) with minor modifications (Wu *et al*., 2010). Leaf samples, extracted with coating buffer (0.05 M sodium carbonate, pH 9.6, 0.02% sodium azide) at 50-fold dilution, were placed in polystyrene microtitration plates (Greiner Bio-One, Germany). The VY1 antiserum was used at 5,000-fold dilution in conjugate buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.2% ovalbumin) to react with test samples, followed by addition of alkaline phosphatase (AP)-conjugated goat anti-rabbit immunoglobulin (Jackson Immuno Research Laboratory, USA) at 5,000-fold dilution in conjugate buffer. AP substrate tablets (Sigma-Aldrich, USA) were dissolved in substrate buffer at 1 mg/ml (9.7% diethanolamine, and 0.02% sodium azide, pH 9.8). Absorbance values were measured at 405 nm with a Labsystems Multiskan EX microplate reader (Labsystems, Finland).

Western blotting was done as described by Jan and Yeh (1995). Crude leaf sap of healthy and virus-infected pear or *C. quinoa* was separated by 10% SDS-PAGE, then transferred to a PVDF membrane (Perkin Elmer, USA). Proteins were incubated with the polyclonal antiserum to isolate VY1 at 5,000-fold dilution in TSW buffer (10 mM Tris base, 0.9% sodium chloride, 0.25% Gelatin, 0.1% Triton X-100, and 0.02% SDS), followed by incubation with 5,000-fold dilution of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin in TSW buffer. The reaction was revealed by treating the membrane with NBT/BCIP (Amresco, USA).

RESULTS

Virus isolation. Leaf extracts from symptomatic pear plants consistently reacted only with the commercial ASPV antiserum in indirect ELISA. Chlorotic local lesions developed on the leaves of *C. quinoa* 5 to 7 days after inoculation, but no systemic infection followed.

Host reaction and symptomatology. Of the 25 inoculated herbaceous hosts, nine proved susceptible to VY1: *Chenopodium murale* (local infection, I), *C. quinoa* (I), *Nicotiana glutinosa* (systemic infection, S), *N. edwardsonii* (S), *N. occidentalis* (S), *N. rustica* (S), *Celosia argentea* (S), *Ocimum basilicum* (S) and *Lactuca sativa* (S). Like *C. quinoa*, *C. murale* reacted with chlorotic local lesions, whereas necrotic lesions were observed in *N. rustica, N. glutinosa*, *N. edwardsonii*. These *Nicotiana* species and *N. occidentalis* were also invaded systemically showing chlorotic mottling and/or deformation of the the leaves. Systemic chlorosis developed in both *C. argentea* and *L. sativa*, but only the former host showed

Table 1. ASPV isolates used in this study and sequence identity in the CP gene of isolate VY1 and 19 other ASPV isolates.

GenBank Accession no.	Host/Country	CP size (nt) and type[a]	Complete CP nt/aa identity[b]	Virus group[c]
D21828	Pear/Germany	1245/ Type 1	83.9/85.9	A
D21829	Apple/Germany	1245/ Type 1	84.0/85.9	A
NC_003462	Apple/Germany	1245/ Type 1	84.0/85.9	A
AF345894	Pear/Poland	1125/ Type 3	81.8/86.1	B
AF345895	Pear/Poland	1125/ Type 3	84.3/86.7	B
AF438522	Apple/Poland	1191/ Type 2	79.2/84.4	C
AF495382	Apple/Poland	1191/ Type 2	78.0/83.9	C
AF345892	Pear/Poland	1191/ Type 2	77.7/83.6	D
AF345893	Pear/Poland	1128/ Type 3	83.7/84.0	D
AF491929	Pear/Poland	1185/ Type 2	79.6/86.1	D
AF491930	Apple/Poland	1191/ Type 2	75.1/82.9	D
AF491931	Apple/Poland	1191/ Type 2	73.8/81.1	D
AF438521	Apple/Poland	1191/ Type 2	75.1/81.1	D
AB045371	Apple/Japan	1191/ Type 2	73.5/81.1	D
AY572458	Pear/Brazil	1131/ Type 3	83.6/86.7	D
DQ003336	Apple/Czech	1185/ Type 2	74.6/80.5	D
EU708018	Pear/China	1185/ Type 2	80.0/86.0	D
EU314950	Apple/ China	1191/ Type 2	78.7/84.3	D
FJ619187	Apple/China	1185/ Type 2	77.1/83.5	D
VY1	Pear/Taiwan	1233/ Type 1	100/100	D

[a]Type identification is based on the size of ASPV CP genes: type 1 includes isolates with a length greater than 1,200 nt; type 2 includes isolates with a length between 1,185 and 1,191 nt; type 3 includes isolates with a length shorter than 1,140 nt.
[b]Comparisons of the CP gene of ASPV isolate VY1 with those of other ASPV isolates available in database; nt = nucleotide; aa = amino acid.
[c]The A, B or C grouping was based on the sequence comparison of CP gene shown in Table 2.

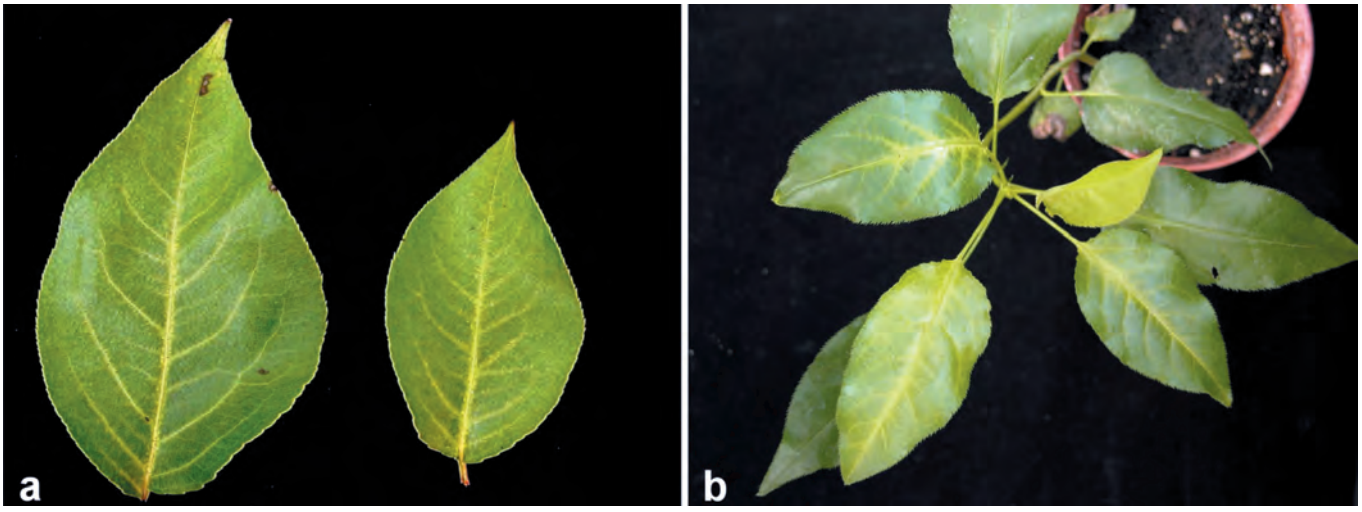

Fig. 1. Leaves showing vein yellows-associated symptoms on diseased *Pyrus pyrifolia* cv. Hengshen in orchards in central Taiwan (a). Pear seedling displaying vein yellows on emerging leaves after inoculation with ASPV isolate VY1 (b).

also leaf deformation. Latent systemic infection was found in *O. basilicum.* All the above reactions were due to virus infection as confirmed by ELISA. *Amaranthus gangeticus*, *Gomphrena globosa, Chrysanthemum coronarium, Brassica chinensis, Dianthus caryophyllus*, *D. chinensis, Cucumis melo*, *Citrullus lanatus, Capsicum annuum*, *C. frutescens*, *Datura stramonium*, *N. tabacum* (cvs Vam-Hicks, Hicks, Xanthi and Samsun) and *Solaum esculentum* were apparently not infected.

Mechanical inoculation of ASPV VY1 to 20 cv. Hengshen seedlings resulted in the infection of 11 of them, all of which showed within 3 weeks leaf symptoms (Fig. 1b) similar to those observed in the field. No symptoms developed in negative controls. The presence of ASPV was ascertained in all symptomatic seeedlings by serology (Fig. 2) and RT-PCR.

Electron microscopy and Western blot. Purified virus preparations contained filamentous particles of a size (*ca*. 12 × 800 nm) comparable to that of ASPV. In SDS-PAGE of purified virus preparations, a single protein with M_r of *ca*. 43 kDa was resolved (not shown), i.e. a size comparable to that of ASPV CP subunits. A protein of the same size was detected in Western blots from VY1-infected *C. quinoa* and pear plants (Fig. 3).

Molecular characterization of ASPV isolates. A cDNA fragment of about 1,200 bp was amplified from total RNA isolated from VY1-infected *C. quinoa* tissues by RT-PCR with degenerated primers FJJ05-3 and FJJ05-4 designed for amplifying the ASPV CP gene. The analysis of this sequence determined from four different clones, showed that it matched the CP sequence of ASPV isolates from database. The VY1 CP (accession No. HM352767) was 1,233 nt in size and encoded a predicted protein of 410 amino acids with an estimated M_r of 43.2 kDa, a figure tallying with those obtained in SDS-PAGE

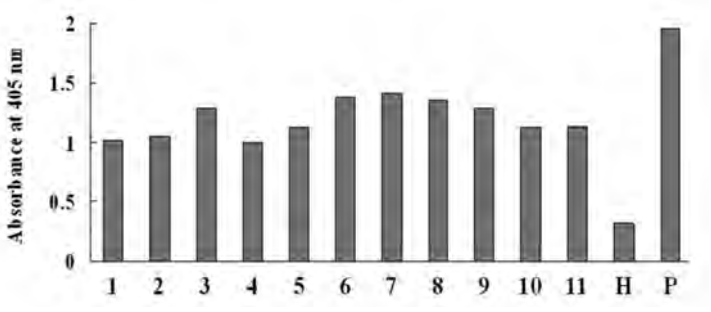

Fig. 2. ELISA detection of ASPV isolate VY1 in eleven mechanically inoculated Hengshen pear plants (number 1-11). Absorbance values were measured at 405 nm. H, negative control (mock-inoculated pear plant). P, positive control (VY1-inoculated *Chenopodium quinoa* plant).

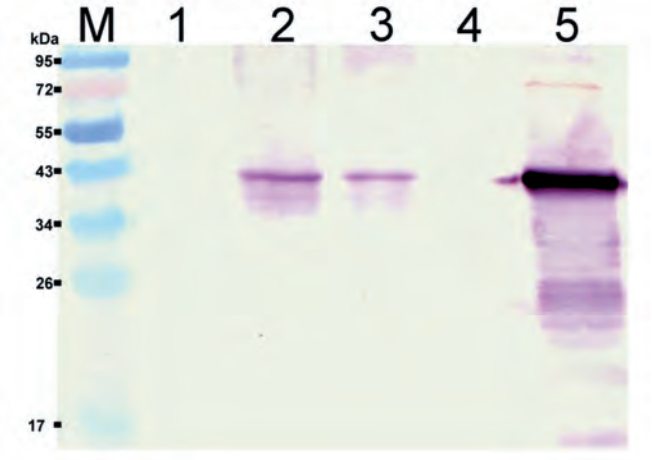

Fig. 3. Western blotting analysis of *Apple stem pitting virus* (ASPV). A 43 kDa protein band was detected in leaf crude sap extracted from ASPV VY1-infected pear (lanes 2 and 3) and *Chenopodium quinoa* (lane 5) but not from healthy pear (lane 1) and *C. quinoa* (lane 4) after reaction with the polyclonal antiserum to VY1. Lane M, protein markers (ProSieve color protein markers, BioWhittaker, USA).

and Western blots. Comparisons of VY1 CP with those of nineteen ASPV isolates from GenBank revealed that the nucleotide and amino acid identity ranged between 73.5-84.3% and 80.5-86.7%, respectively (Table 1).

Sequence variability of ASPV. Comparative analysis of the CP sequence of 20 ASPV isolates allowed the identification of three types based on the size of their CP gene: type 1 (>1,200 nt) consisting of 4 isolates, VY1, D21828, D21829 and NC_003462; type 2 (between 1,185 and 1,191 nt), comprising 12 isolates, AB045371, AF345892, AF438521, AF438522, AF491930, AF491931, AF495382, EU314950, DQ003336, FJ619187, AF491929 and EU708018; and type 3 (<1,140 nt), including 4 isolates, AF345893, AF345894, AF345895 and AY572458. These isolates shared 73.4-100% and 77.8-100% nucleotide and amino acid identity, respectively (Table 2). Three German isolates (D21828, D21829 and NC_003462), two Polish pear isolates (AF345894, AF345895), two Polish apple isolates (AF495382 and AF438522) and the 13 additional isolates were further divided into groups A, B, C and D, respectively. The intra-group identity of CP sequences was high, i.e. 99.8-100% and 99.5-100%, 93.0 and 97.3%, and 93.8 and 98.7% at the nucleotide and amino acid level for groups A, B and C, respectively (Table 2), whereas the variability at the inter-group level was higher, i.e. 74.8-91.2% and 79.4-93.7% at the nucleotide and amino acid level, respectively (Table 2). The 5' terminus of the CP gene (from amino acid position 1 to 194 corresponding to that in the longest CPs of D21828, D21829 and NC_003462) of the 20 ASPV

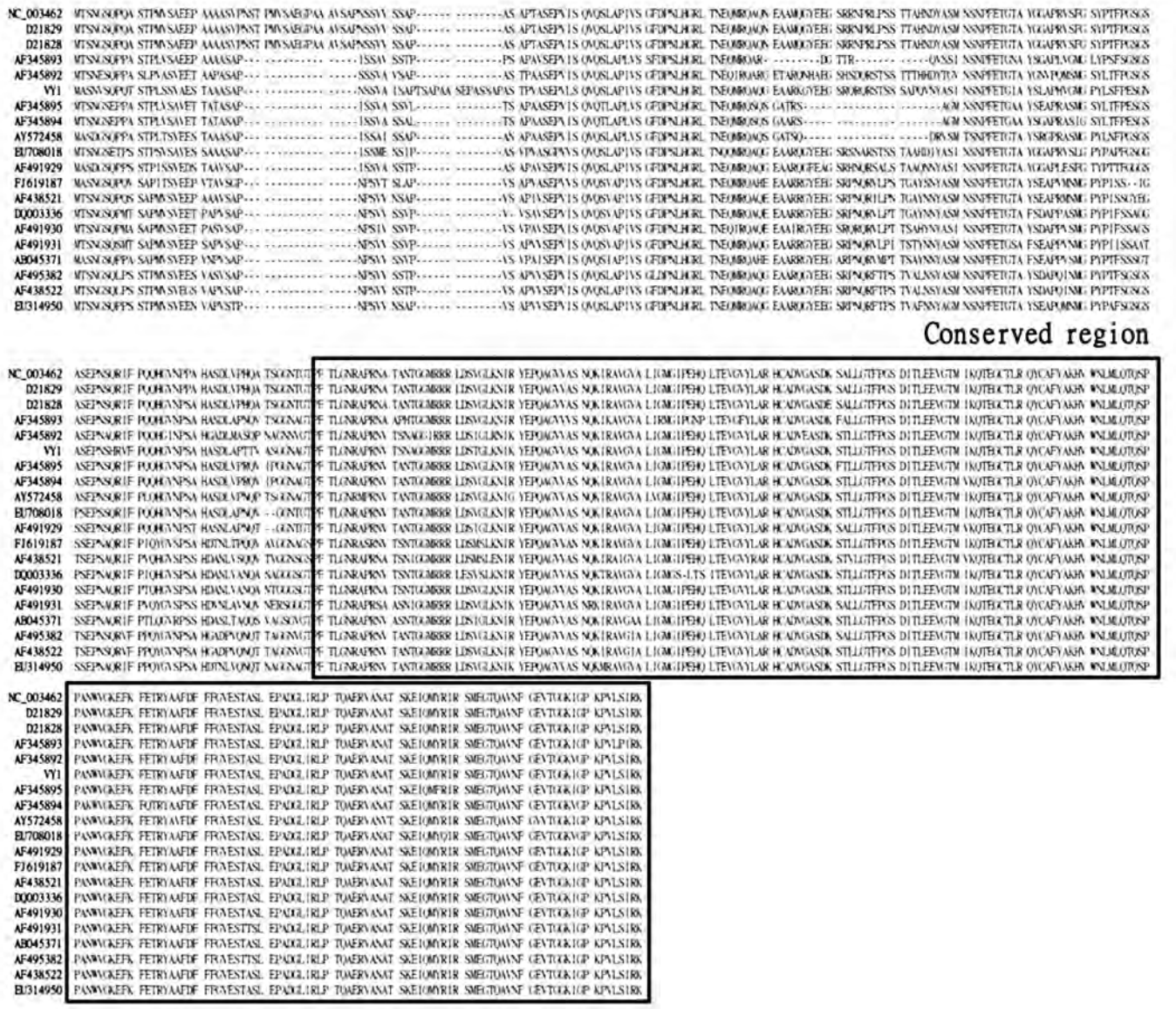

Fig. 4. Multiple alignment of CP amino acid sequences of 20 ASPV isolates from different hosts and geographic origins. A conserved region 220 amino acid in size identified in the C terminal sequence of the ASPV CP gene is boxed out.

Table 2. Intra- and inter-group CP gene sequence comparisons at the nucleotide and amino acid level among ASPV isolates.

	A[a]	B	C	D
A	99.8-100/99.7-100[b]	84.4-87.3/86.9-92.3	79.9-81.3/85.2-87.9	75.6-87.5/82.1-92.2
	99.5-100/99.5-100[c]	89.0-89.1/96.8-98.2	87.6-87.7/98.2-98.6	82.3-88.6/94.1-99.1
B		93.0/88.8	79.4-81.8/82.8-87.0	74.8-87.1/81.1-95.5
		97.3/97.3	84.2-85.6/96.8-98.6	79.4-88.3/91.8-98.2
C			93.8/90.0	77.3-91.2/82.2-93.5
			98.7/99.1	82.1-93.7/93.2-98.6
D				73.4-91.0/79.8-93.9
				77.8-92.9/90.5-98.2

[a]A, B and C grouping is based on at least 97% amino acid identity within the CP gene: group A comprises three German isolates (D21828, D21829 and NC_003462); group B contains two isolates from Poland (AF345894 and AF345895); group C contains two isolates from Poland (AF495382 and AF438522); group D consists of the other 13 ASPV isolates.
[b]The nucleotide identity (%) of the complete CP gene /conserved CP gene region (660 nt at the 3' terminal end) of ASPV isolates.
[c]The amino acid identity (%) of the complete CP gene/conserved CP gene region (220 aa in the C terminal end) of ASPV isolates.

isolates was highly variable while the 3' portion was relatively conserved (Fig. 4). A conserved region containing 220 amino acids (660 nts) was observed in the C terminus of the CP gene (3'-conserved region) with 81.1-95.5% and 91.8-99.1% sequence identity at the nucleotide and amino acid level, respectively (Table 2).

DISCUSSION

Our results have shown that the virus (VY1) isolated from a diseased cv. Hengshen pear exhibiting vein yellowing is an isolate of ASPV that reproduced the field symptoms when inoculated to healthy seedlings. Sequence analyses of the CP gene showed that VY1 shared more than 73.5% and 80.5% nucleotide and amino acid identity, respectively, with known ASPV isolates. The demarcating criteria for discrimination of foveavirus species include natural host range, mechanical transmissibility, serological specificity, and CP size and less than 72% nucleotide identity or 80% amino acid homology in the CP or the replicase genes (Adams *et al*., 2005). In accordance with these criteria, VY1 was identified as an isolate of ASPV and the causal agent of pear vein yellows disease in Taiwan. Nonetheless, differences in the type of symptoms were observed with respect to those reported elsewhere (Nemeth, 1986) which may depend on genotypic differences of pear cultivars or virus isolates.

ASPV has a narrow host range, which was confirmed in the present study since isolate VY1 had also a limited host range which, however, differed somewhat from that reported in the literature. For instance, *C. murale* and *N. occidentalis* can be infected by most ASPV isolates (Koganezawa and Yanase, 1990; Kundu and Yoshikawa, 2006; van der Meer, 1986) including VY1. However, isolates from apple (isolate ASP) (Koganezawa and Yanase, 1990; Kundu, 2003b) and pear (isolates PSP and PNS) (Paunovic *et al*., 1999; Yanase *et al*., 1989) induce systemic vein and leaf necrosis in *N. occidentalis* subsp. *obliqua* and systemic vein yellowing in *N. occidentalis* 37B, whereas VY1 induced systemic mottling and chlorosis. ASP (Koganezawa and Yanase, 1990), PNS (Yanase *et al*., 1989) and VY1 can infect *Celosia cristata*. VY1 can infect *N. glutinosa* and *N. rustica*, but ASP (Koganezawa and Yanase, 1990) and PNS (Yanase *et al*., 1989) do not. By contrast, ASP (Koganezawa and Yanase, 1990) and PNS (Yanase *et al*., 1989) infect *G. globosa* whereas VY1 does not.

ASPV isolates show molecular divergence in CP genes and differences in their size, which ranges from about 42 to 48 kDa. High genetic variation in the 5' portion of the CP gene was reported by Nemchinov and Hadidi (1998) and confirmed in this study (Fig. 4). The isolates in each of the three groups, A, B, or C, shared high nucleotide and amino acid identity even though the value was slightly higher in the complete CP gene as compared with that in the conserved region in the 3'-terminus of the same gene. In group D, which comprises 13 ASPV isolates, a wider range of nucleotide and amino acid sequence identity was found in both the complete CP and in its conserved CP gene region. However, both nucleotide and amino acid sequence identities were higher in the conserved region than in the complete CP gene. For example, the nucleotide sequence identify of the complete CP gene was at 73.4-91.0% whereas that of the conserved region was 79.8-93.9%; the amino acid sequence identity of the complete CP was 77.8-92.9% while that of the conserved region was 90.5-98.2% (Table 2).

CP gene size had apparently no influence on the extent of molecular divergence of the gene. For example,

a Chinese apple isolate (EU314950, 1,191 bp) shared 80.7% nucleotide identity with another Chinese apple isolate (EU708018, 1,185 bp) of type II, but shared 91.2% nucleotide identity with a Polish pear isolate (AF438522, 1,191 bp). A Polish pear isolate (AF491929, 1,185 bp) of type II shared 87.1% nucleotide identity with another pear isolate from the same country (AF345894, 1,125 bp) in type III, but shared 75.3% nucleotide identity with a Polish apple isolate (AF491931, 1,191 bp) in type II.

The inter-group sequence identity values changed when the 3' CP conserved region and the complete CP gene were compared (Table 2). For example, a Chinese apple isolate (EU314950) was more closely related to a Polish pear isolate (AF491929) with 86.8% amino acid identity than to a Chinese pear isolate (EU708018) with 85.0% identity in their complete CP gene, but a Chinese apple isolate (EU314950) was more closely related to a Chinese pear isolate (EU708018) with 98.2% than to a Polish pear isolate (AF491929) with 97.7% amino acid identity when sequences of the 3'-CP conserved regions were compared. Isolate VY1 was closer to two Polish pear isolates (AF345894 and AF345895) with 87.2-87.5% amino acid identity than to the Chinese apple isolates (FJ619187 and EU314950) with 83.5-84.3% identity in their complete CP gene, but VY1 isolate was closer to Chinese apple isolates (FJ619187 and EU314950) with 97.3-98.2% than to Polish pear isolates (AF345894 and AF345895) with 96.4-97.3% amino acid identity in the 3' CP conserved region. The taxonomy of the ASPV species is shaky when their complete CP genes are used because of the high variations showed in the 5' terminus of CP gene. Therefore, the conserved region of the ASPV CP gene can provide a better demarcation criteria for assessing ASPV phylogenetic relationships.

Collectively, the results of pathogenicity and serological assays, host reactions and molecular characterization provide substantial evidence that VY1 is a genuine ASPV isolate and the causal agent of the vein yellows disease of pear in Taiwan.

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