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Capsid protein sequence gene analysis of *Apple mosaic virus* infecting pears

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Abstract

Apple mosaic virus (ApMV, genus Ilarvirus) was detected in pears, a previously non-reported virus host. No symptoms were visible on the host's leaves. Seventeen out of 22 randomly selected pear trees in Italy (Lombardy) and in three regions in the Czech Republic were ApMV-infected. All nine newly sequenced ApMV isolates from pears had a 15-nucleotide insertion in the capsid protein gene in identical position of that of apple isolates compared with isolates from hop and prunes. The insertion is the most prominent (but not essential) modification of the capsid protein gene, which results in a phylogenetic separation of ApMV isolates into three clusters. Sequence analysis data of an additional 15 isolates revealed a sequence correlation with kernelled fruit trees (apple and pear).

Introduction

Apple mosaic virus (ApMV) (genus Ilarvirus, family *Bromoviridae*) is an isometric virus with a segmented ssRNA genome. Its RNA3 codes for the movement protein and the capsid protein (CP). ApMV is named after the disease it causes in apple, the first host in which it was described (Bradford and Joly, 1933). At least 34 other plant species are susceptible hosts of ApMV (Brunt et al., 1996), including *Betula* (birch), *Corylus avellana* (filbert), *Fragaria* (strawberry), *Humulus lupulus* (hop), *Malus sylvestris* (crab-apple tree), *Malus pumila* (apple), *Prunus* (stone fruit), *Prunus armeniaca* (apricot), *Prunus avium* (sweet cherry), *Prunus dulcis* (almond), *Ribes rubrum* (red currant), *Rubus* (blackberry), *Rubus idaeus* (raspberry) and *Rubus occidentalis* (black raspberry) (Crop Protection Compendium, 2003). *Prunus domestica* (plum) and *Prunus persica* (peach) are secondary hosts, although no known vector has been reported. ApMV is not pollen-borne, does not occur in seedling rootstocks, and has not been identified in naturally infected weeds. However, its spread through soil in nurseries via root grafting has been observed (Hunter et al., 1958; Dhingra, 1972). The virus is graft-transmissible and it persists in propagative material, which is probably the main source of virus infection.

ApMV has been found in birch, hop, rose and other woody hosts and a number of synonyms of the virus have been assigned, e.g. European plum line pattern virus, Mountain ash variegation virus, Birch line pattern virus, Birch ringspot virus, Dutch plum line pattern virus, Hop A virus, Horsechestnut yellow mosaic virus, Rose mosaic virus, Hop virus A, Hop virus C, Mild apple mosaic virus and Severe apple mosaic virus. Some confusion regarding virus nomenclature continues in the present molecular era, when an isolate of *Prunus necrotic ringspot virus* (PNRSV) was published as an ApMV isolate (Sánches-Navarro and Pallás, 1994, AC No. U03857) and was later re-classified as a PNRSV isolate (Sánches-Navarro and Pallás, 1997). In addition, the G isolate of ApMV was published as PNRSV (Guo et al., 1995) and the first two published ApMV sequences of the CP gene (AMQCOATPA and AMU15608) varied greatly from a third sequence (S78319) at the amino acid level

due to frame-shift mutations identified later (Petrzik and Lenz, 2002).

In a previous paper, one CP sequence of an ApMV isolate from pear tree was analyzed (Petrzik and Lenz, 2002, AC No. AY054389). Although pear was known to be susceptible to ApMV (Kristensen and Thomsen, 1963) it was not known as a natural host. The aim of this study was to establish the occurrence of ApMV in pear trees and to determine if a correlation exists between the nucleotide sequence of the CP gene and the natural hosts of the virus.

Materials and methods

Flowering shoots and leaves were collected from pear trees growing in northern Italy (Lombardia) and in the Czech Republic (Southern, Northern and North-East region). Most of the trees were solitary but one location was a production orchard with several pear varieties. Several apple and plum trees from the same orchard were also tested for the presence of ApMV. Samples from three ApMV-infected apples were obtained from Belgium (Table 1).

Total RNA was isolated from 100 mg of plant tissue (flowers or leaves) by the RNeasy Plant Mini Kit (Qiagen). Reverse transcription and PCR was done with the Access RT-PCR kit (Promega) and detection primers: 92D9up 5′-GGCCATTA GCGACGATTAGTC and 1425re 5′-ATCGGC AAAGTCAATGTTGAC (Generi Biotech, Czech Republic). The thermal cycling scheme was: 45 min at 48 °C, 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 90 s at 68 °C followed by a final incubation of 10 min at 68 °C. The detection primers amplify a region corresponding to nucleotide (nt) 1044–1425 (numbered according to the AMU15608 sequence) which includes the movement protein – CP intergenic region and the first 300 nt of the CP gene. The complete CP gene was amplified with the 92D9up and 92E0re 5′-ATGCTTTAGTTCCCTCTCGG primers as above and it was sequenced with the same primers and a BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems,

Table 1. Apple mosaic virus isolates used in this study

Source	Isolate	Origin	AC number	Reference
Almond	Venza	Italy	AY054388	Petrzik and Lenz (2002)
Apple	I	USA	AMQCOATPA	Alrefai et al. (1994)
Apple		Korea?	AF548367	Lee et al. (2002) unpublished
Apple	A	USA	AMU15608	Shiel et al. (1995)
Apple		Korea?	AY125977	Kim et al. (2002) unpublished
Apple	H	CZ	AY054385	Petrzik and Lenz (2002)
Apple	B1a	Belgium	AY542540	this work
Apple	C1	Belgium		this work – partial sequence
Apple	C3a	Belgium	AY542541	this work
Apple	Iv J1	CZ		this work – partial sequence
Hop		Australia	AF473580-96	Crowle et al. (2003)
Hop	ZC	CZ	AY054387	Petrzik and Lenz (2002)
Mahaleb	G	Germany	S78319	Guo et al. (1995)
Pear	It 1	Italy	AY542546	this work
Pear	It 2	Italy		this work – partial sequence
Pear	It 3	Italy		this work – partial sequence
Pear	It 4	Italy		this work – partial sequence
Pear	Iv 10	CZ	AY542542	this work
Pear	Iv 3	CZ		this work – partial sequence
Pear	Iv 8	CZ		this work – partial sequence
Pear	Cerin	CZ	AY542544	this work
Pear	Roz 144	CZ	AY542545	this work
Pear	Kravare	CZ	AY542543	this work
Pear	HC1	CZ	AY054389	Petrzik and Lenz (2002)
Plum	Iv S1	CZ		this work – partial sequence
Prune	TC1	CZ	AY054386	Petrzik and Lenz (2002)

Warrington, England) on an automated DNA AB710 sequencer.

Multiple sequence alignments were determined using the www service CLUSTALW, (http://www.ebi.ac.uk/clustalw/) (Thompson et al., 1994). Phylogenetic analyses were done by the PHYLIP package (Felsenstein, 1993). Topology of trees was inferred by means of DNAPARS program and bootstrap analyses (100 replicates) were performed to check the statistical significance. Additional data of previously sequenced ApMV isolates were used for the phylogenetic analysis (Table 1). Because of the lack of the first 24 nt in AF80-96 sequences (Crowle et al., 2003), all the remaining sequences were trimmed at the 5′end to give sequences of 644 nt in length corresponding to the region of nt position 1150–1794 of the AMU15608 sequence. Putative recombinant sequences were searched for by computing the phylogenetic profiles with the programme PhylPro ver. 1.0 (Weiller, 1998).

Results and discussion

None of the data sources has previously mentioned pears as a primary or secondary host species of ApMV. The only report of ApMV transmission by inoculation to pear variety Beurré Hardy and the development of yellow-greenish ringspot symptoms is that of Kristensen and Thomsen (1963). However, the authors were not able to retransmit the virus from pear and they expressed doubts that ApMV was the cause of the symptoms. Forty years later, ApMV was detected by chance in a solitary growing pear in Southern region of the Czech Republic by RT-PCR amplification (Petrzik and Lenz, 2002).

The leaves of ApMV infected pears analysed here were mostly symptomless. Sporadically faint yellow ringspots were observed on some leaves. However, the virus was not transmitted further to other hosts and thus we cannot exclude the presence of other pathogens in the examined samples. The presence of ApMV in pears was determined by RT-PCR in four of six samples from Italy and in 13 of 16 samples from the Czech Republic, representing a 77% (17 out of 22) incidence of ApMV in these samples. Sequence analysis of the 5′-end of the CP gene of all four Italian isolates and six Czech isolates revealed that all isolates possess a 15 nt insertion at the nucleotide position 141. In addition, the sequences of the 5′- third of the CP gene of all pear isolates are identical (Figure 1).

The unusually high sequence identity of pear isolates originating from geographically distant localities points to a hypothesis of a host-conditioned modification and a fixation of nucleotide substitutions in pear host compared to apple and plum hosts. To test this hypothesis we selected an orchard with a mixed culture of pear, apple and plum trees where a high incidence of ApMV was shown. Ten samples were collected from pears, five from plums and three from apples from this orchard. All the trees grew in the neighbourhood for more than 10 years. All pears (Iv 1–10), two plums (Iv S1, Iv S5) and one apple tree (Iv J1) were ApMV infected, as detected by RT-PCR using primers 92D9 and 1425re.

Sequence analysis of samples collected from the orchard revealed the presence of a 15 nt insertion in all samples. The insertion itself was identical independent of the source tree of the isolate. Furthermore, the 198 nt long segment of the plum Iv S1 isolate was indistinguishable from the sequences of the pear isolates. Also the nt similarity of the same length segment of the apple Iv J1 isolate is higher with the pear isolates (98%) than with the apple isolates (max. 94.4%). None of the newly sequenced isolates was detected to be a recombinant by the PhylPro programme.

Six branches can be clearly recognised on the phylogenetic tree (Figure 2a). The ApMV isolates from pear trees formed a new branch close to but distinct from the branch containing the isolates from apple and the branch of isolates from almond. All these branches contained isolates with some kind of insertion at the hot spot after the nucleotide 141 (Petrzik and Lenz, 2002). With four exceptions, there are correlations of the tree and the original host of the isolates: there are three isolates from the Czech Republic found in prune, hop and pear, which formed a distinct branch outside the clusters of hop and pear isolates, and the isolate from mahaleb classified close to isolates from hop.

On the other hand, the correlation with the geographical origin is better for ‘sub-branches’ than for the higher ones. This is evident for apple isolates from USA, apple C1, B1 and C3 isolates

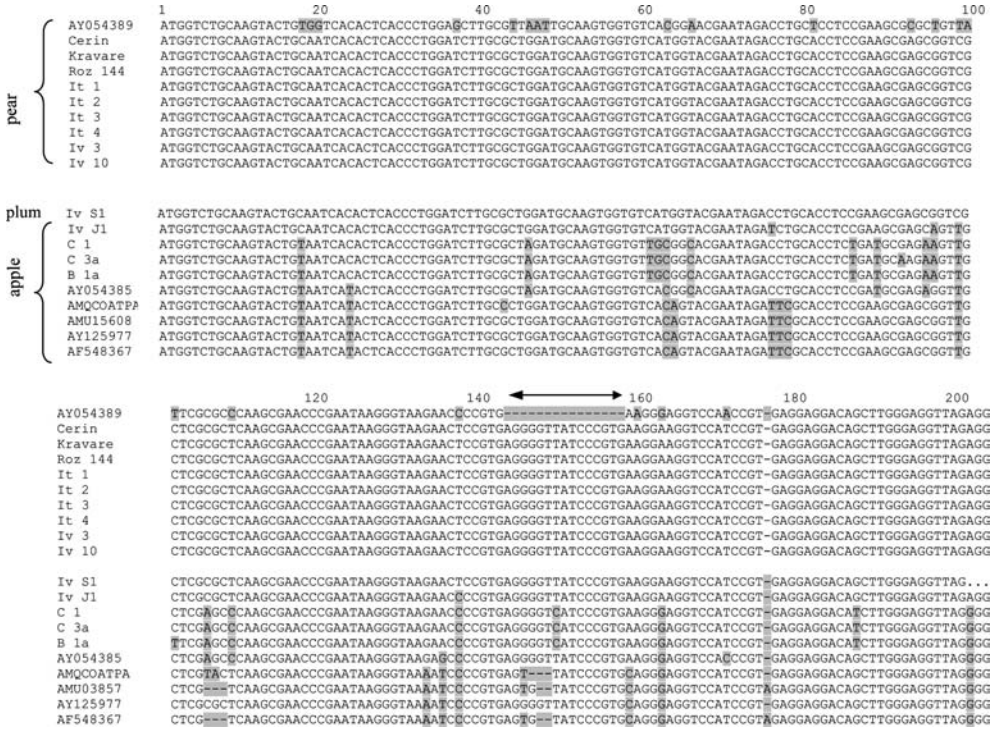

Figure 1. Alignment of the 5′-terminal part of the ApMV capsid protein gene of isolates from the pear and apple cluster. The double-headed arrow encloses position of the insertion after nt 141. Shown as DNA, counted from the first nt of the CP gene.

from Belgium, and also for hop isolates from Australia. However, the European isolates straggle about five clusters and the Czech isolates itself about three clearly distinguished clusters.

Based on these results, the cause of observed unusual high sequence identity in the pear isolates remains obscure. The effects of the host as well as the effect of closely related viruses in neighbourhood could both contribute. This could be the case of isolates from the orchard analysed here. We were not able to discriminate the original host and source of the virus in the orchard, but we propose that some yet unidentified kind of transmission could exist between nearby growing (even unrelated) trees as an identical sequence was detected in plum and pear isolates and the apple Iv J1 isolate possesses sequence intermediate between the pear and apple isolates (Figure 2b). The mechanism of transmission from pear to these non-related hosts remains obscure, as the virus has no known vector and it is not known to be mechanically or pollen-transmissible. An unknown vector or a contact in soil in nearby growing trees is supposed to be the probable mechanism of ApMV transmission in this case.

In this report, pear tree is presented as an often (77%) infected, mostly symptomless host of ApMV. The epidemiological impact of this infection as a gene-pool reserve and a source of virus should be evaluated yet as well as the existence of selection pressure of the host on the virus genome. If true, the present day observed sequence correlation with host may not be valid when another host is prevalent in neighbourhood.

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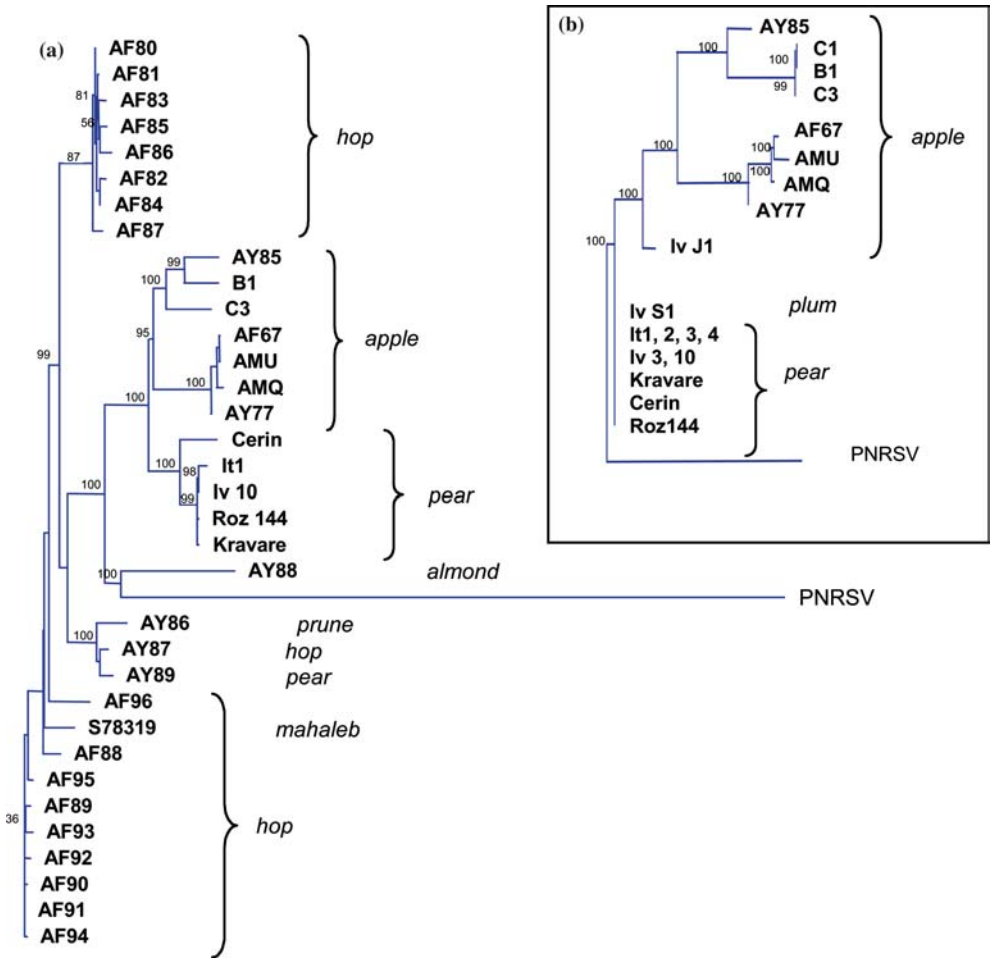

Figure 2. Phylogenetic tree obtained from the alignment of nucleotide sequences of the whole (a) CP gene and the 5′-terminal part of the pear and apple cluster (b). Abbreviations of the GenBank AC No. (characters plus last two digits) and names of the new sequenced isolates are used. Sequence Y07568 of the *Prunus necrotic ringspot virus* (PNRSV) was used as an outgroup. Bootstrap values (% replication) are shown.

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