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A New Natural Host of Apple mosaic virus : Berberis thunbergii atropurpureum

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Abstract

Apple mosaic virus (ApMV), a pathogen of stone and pome fruit trees in the world , shows great variability in its biological and molecular properties. Berberis thunbergii atropurpureum plants with systemic symptoms consisting of light and dark red mosaic patterns were collected in Ukrainee in 2008 and 2009 and subjected to DAS-ELISA and RT-PCR tests. They were all positive against ApMV antiserum in DAS-ELISA and isolated RNAs were all amplified with ApMV primer set. RT-PCR amplified products of all isolates were subjected to restriction enzyme treatment and the results used to generate similarity coefficient dendrograms. B. thunbergii atropurpureum isolates were shown as a separate and new ApMV genotype among the other genotypes tested therefore, B. thunbergii atropurpureum was a new host of the pathogen for the world.

Keywords: Apple mosaic virus, Berberis thunbergii atropurpureum

INTRODUCTION

Berberis thunbergii atropurpureum (red barberry) is a ornamental shrub with dark red color and was planted commonly in the recreation parks of Turkey and Kiev (Ukraine). It belongs to Berberidaceae family and is native to Japan and eastern China. Cucumber mosaic virus (CMV) and Berberis phyllody (16SrV-B group phytoplasma [1] are the infections detected on red barberry.

Apple mosaic ilarvirus (ApMV) is one of the most common virus infection of pome and stone fruit species in the world [2]. The virus is a member of family Bromoviridae, genus Ilarvirus , subgenus III. The virus genome is tripartite consisting of positive sense, single-stranded RNA. ApMV particles are isometric and 25-29 nm in diameter. Major hosts of the virus are pome and stone fruits, hop, horse chestnut , hazelnut, raspberry , birch, rose and some herbaceuos plants in the world and causes symptoms such as systemic mosaic, mottle, oak leaf pattern and circular rings on those [2].

In genomic organisation of ApMV, RNA is divided to three segments and the largest RNA segment RNA1, is 3476 nucleotides long and encodes a unique large open reading frame (ORF), RNA 2 is 2979 nucleotides in lenght and also encodes a single open reading frame [3], ApMV RNA 3 is 2056 nucleotides long and encodes 2 open reading frames: first ORF encodes the putative movement protein and the second encodes capsid protein [4] . A small RNA, RNA 4 also has been found in the infected cells which is also responsible for capsid protein synthesis [5]. Capsid protein of ApMV plays important role on the initiation of the infection besides its role on the protection of the viral RNA and is 25000 daltons [6]. No similarity has been detected in coat protein gene sequences of other Bromoviridae family members [6]. Virus has been detected on several hosts such as hazelnut [7], apple [8,9,10], cherry [11] and rose [12] by ELISA in Turkey and apple in Ukraine. RT-PCR detection of ApMV Turkish apple isolates have been done by Ulubas and Ertunc [8]. During the survey programme in Kiev, Berberis thunbergii atropurpureum plants showing systemic mosaic symptoms, shortening of internodes , reduction in leaf size and area were collected and subjected to DAS-ELISA and RT-PCR analysis .

MATERIALS and METHODS

Virus source

Berberis thunbergii atropurpureum plants with systemic mosaic symptoms were collected during the surveys done in 2008 and 2009 from the recreation gardens of Kiev. One Ukranian and one Turkish apple isolates of ApMV were used as positive control .

DAS-ELISA

Standart DAS-ELISA test was performed in order to determine if there is a mix infection of apple viruses on the Berberis thunbergii atropurpureum (red barberry) plants, using ApMV, CMV, ACLSV, ASGV polyclonal antisera [13].

Nucleic acid isolation

Nucleic acids were isolated from 300 mg of fresh leaves according to the method of Menzel et al. [14] for apple and 300 mg of bark and leaf tissue for Berberis thunbergii atropurpureum by Rott and Jelkman [15].

Primer and RT-PCR amplification:

Spesific primers of coat protein gene region of ApMV were used for RT-PCR amplification and were ApMV-Sense (5'-ATC CGA GTG AAC AGT CTA TCC TCT AA-3') ApMV-Antisense (5'-GTA ACT CAC TCG TTA TCA CGT ACA A-3') [14]. The expected amplified product was 262 bp long which represented partial of capsid protein open reading frame (ORF). The RT and PCR reactions reactions were performed as a single non–interrupted thermal cycling programme. A modified PCR mix was designed and used with the selected primer set . Total volume of the reaction was 25 µl and each reaction contained: Oligonucleotide primers (up and down stream) 1 µl of each, 1.5 µl dNTP mix, 2.5 µl 10 X Taq buffer, 0.4u MMLV-RT (Fermentas), 0.1u RNase inhibitor (Promega), 1.5µl MgCl2, 1 u Taq DNA Polymerase (Fermentas), 15.7 µl RNase-free water, 2 µl RNA sample. The amplification protocol was similar to Menzel et al [14]. To ensure the absence of contaminations, negative control (water) and positive control (Ukranian and Turkish apple isolates) were also included to the assays.

Amplified fragments of ApMV CP genes were all subjected to electrophoresis in 1 % agarose slab gels at 100V for 1 h and then stained with ethidium bromide and examined by image analysis system of Sygene.

Restriction digestion of PCR Products

Restriction enzymes used for digestion of RT-PCR products were Hinf I (Genemark), BamH I (Genemark), Ava I (Genemark), EcoRI (Sigma), SspI (Fermentas), RsaI (Fermentas), HindIII (Genemark), HincII (Genemark).8 µl of PCR product , 1µl enzyme buffer and 0,5µl spesific restriction enzyme were all incubated overnight at 37 oC in an incubator and then subjected to 6 % polyacrilamide gel electrophoresis. Gells were stained with ethidium bromide and visualised. Phylogenetic analysis was performed according to the Jaccord's similarity matrix analysis (Numerical Taxonomy and Multivariate Analysis System, Version 2).

RESULTS and DISCUSSION

During the surveys on ApMV infection on apple to Ukrainee, Berberis thunbergii atropurpureum plants with systemic mosaic symptoms, shortening of the internodia , reduced in leaf size and area were collected (Figure 1) and subjected to ELISA and RT-PCR amplification with the primer set of capsid protein gene of ApMV. The systemic mosaic symptoms on red barberry plants were quite clear and sharp and only two isolates were collected with those symtoms from the recreation gardens of Kiev.

They were all positive against the ApMV antiserum but failled to react with the other antisera tested. The obtained absorbans value were thrice high of controls thus, DAS-ELISA results revealed that those symptoms on Berberis thunbergii atropurpureum plants were spesific to ApMV. There was no mix infection on the plants tested. Therefore, in order to confirm these results, they were subjected to one-step RT-PCR amplification with ApMV capsid protein gene primer set of Menzel et al [14] . PCR amplified products were 262 bp long as shown in Figure 2. The PCR mix designed in our lab and used in this research worked better than the original PCR mix of Menzel et al. [14]. The bands obtained from Berberis plants were at the same level with ApMV infected apple isolates (Figure 2). It was quite clear the virus infection on Berberis thunbergii atropurpureum plants were ApMV .

There is not much information on serotypes and pathotypes of ApMV. In order to obtain genetic polymorphism between ApMV isolates capsid protein genes, all of the RT-PCR products were (even hazelnut and other apple isolates) subjected to enzymatic digestion with restriction endonuclease enzymes of AvaI (Genemark), Bam HI (Genemark), Hinf I (Genemark), Ssp I (Fermentas), EcoR I(Sigma), Hinc II (Genemark), Hind III (Genemark) ve Rsa I (Fermentas) . First 4 enzymes digested the PCR products whereas other 4 enzymes had no effect and failled to digest the amplified product.

The length of ApMV is variable due to either deletions and insertations and some variations were detected depending on host and geographic region [16]. As seen in the dendogram, 15 different serotypes were obtained among all of the isolates tested in the research. Ukranian apple isolates were 85% similar to each other but the genotype belonging to Berberis thunbergii atropurpureum was unique and different from the other variants at the ratio of 62 %.

According to the results of DAS-ELISA and RT-PCR, Berberis thunbergii atropurpureum is a new host of Apple mosaic virus for the world.

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Figure 1. Systemic mosaic symptom on *Berberis thunbergii atropurpureum* plant.

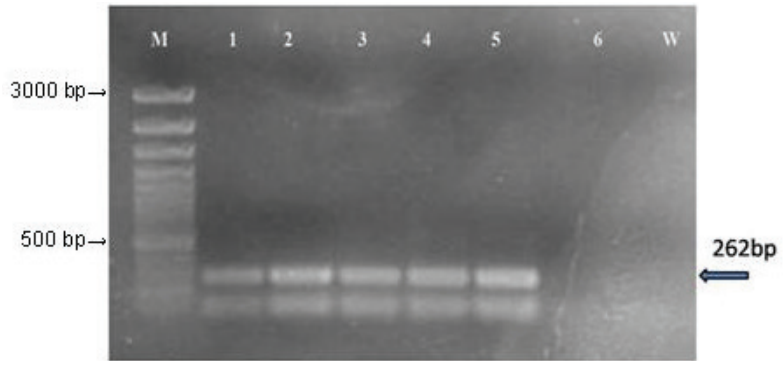

Figure 2. RT-PCR amplification of Turkish and Ukranian ApMV isolates.
1-2: Ukrainean Berberis thunbergii isolates , 3: Ukrainean rose isolate,
4: Ukrainean apple isolate, 5: Turkish apple isolate, 6: Healthy apple,
W: Water control, M: 100 bp plus ladder (Fermentas).