Acta virologica **65**: 97 – 100, 2021 doi: 10.4149/av_2021_112

Identification of *Cucumber mosaic virus* from *Arisaema heterophyllum* Blume in China

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Received May 15, 2020; revised August 3, 2020; accepted February 6, 2021

Summary. – *Arisaema heterophyllum* Blume is a valuable medicinal plant in the *Araceae* family. The dried tuber of *A. heterophyllum* is used in the traditional Chinese medicine, Rhizoma Arisaematis, which is used to treat convulsions, inflammation and cancer. In 2017, typical mosaic virus-like symptoms were observed in *A. heterophyllum* in Jilin province, China. To further identify the pathogens, we conducted RT-PCR using virus- and genus-specific primers to amplify partial genome sequences of *Cucumber mosaic virus* (CMV), *Tobamovirus* and *Potyvirus*, respectively. The CMV primers showed specific amplification, but the *Tobamovirus* and *Potyvirus* primers did not. We further cloned and sequenced the 2b, MP and CP genes of the CMV-Ah isolate. Phylogenetic analysis showed the CMV-Ah isolate belonged to subgroup IB. To our knowledge, this is the first report of CMV infecting *A. heterophyllum* in China.

Keywords: Cucumber mosaic virus; Arisaema heterophyllum Blume; subgroup IB; phylogenetic analysis

Cucumber mosaic virus (CMV), a member of the genus *Cucumovirus* (the family *Bromoviridae*), has a high degree of diversity and a wide host range of over 1,200 species, including monocots and dicots, leading to many isolates being distributed worldwide (Jacquemond, 2012). The virus is transmitted via sap inoculation, grafting, aphid vectors and the seeds of some plant species (Palukaitis *et al*., 1992; Mcohizuki and Ohki, 2012). CMV isolates can be divided into subgroup I and II according to serological relationships, peptide mapping of the CP and nucleic acid hybridization (Palukaitis *et al*., 1992). Subgroup I can be further divided into subgroups IA and IB based on nucleotide variations of the 5'-non-coding region of RNA3 (Roossinck *et al*., 1999). Subgroups IA and II are distributed worldwide, but subgroup IB appears predominantly in East Asia (Roossinck, 2002). In 2009, subgroup III was proposed after the discovery of CMV isolates BX and PHz in *Pinellia ternata* in China (Liu *et al*., 2009; Jacquemond, 2012).

Arisaema heterophyllum Blume is a traditional medicinal plant in China. The dried root is used in the traditional Chinese medicine, Rhizoma Arisaematis, to treat convulsions, inflammation, and cancer (Shen *et al*., 2014; Dong *et al*., 2015). In 2017, virus-like symptoms were observed on *A. heterophyllum* in an herb garden at Jilin Agricultural University, Jilin province, China (43.49'N, 125.19'E). Compared with the healthy plants, the diseased *A. heterophyllum* showed severe mosaic symptoms and many dark green spots on the leaves (Fig. 1).

To identify the viral species infecting the diseased *A. heterophyllum*, we performed reverse-transcription (RT)-PCR to detect CMV, *Tobamovirus and Potyvirus.* Total RNA was extracted from 100 mg of the leaf tissue of naturally infected *A. heterophyllum* using a total RNA extraction kit (Tiangen, China). RT was then performed using M-MLV reverse transcriptase and a random 3' primer (10 μM) according to the manufacturer's instructions (Promega, USA). Subsequent PCR reactions were performed using the specific primer pairs to detect CMV (CMVCPf and CMVCPr) (Wang *et al*., 2013), *Tobamovirus* (Tob-Uni1 and Tob-Uni2) (Letschert *et al*., 2002) and *Potyvirus* (PotyF

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Abbreviations: CMV = *Cucumber mosaic virus*; CP = coat protein; MP = movement protein; RA = Rhizoma Arisaematis

Fig. 1

The virus-like symptoms of *A. heterophyllum* plants
(a) The leaf of *A. heterophyllum* showing severe mosaic symptoms. **(b)** Healthy *A. heterophyllum* plants.

and PotyR) (Marie-Jeanne *et al*., 2000). Only the CMV primers yielded an approximately 650 bp long fragment, the *Tobamovirus* and *Potyvirus* primers did not yield any products (Fig. 2). Nucleotide sequence analysis of the amplified fragment via BLASTn search on the NCBI website revealed high homology to the published sequences of CMV. This result was also confirmed via enzyme-linked immunosorbent assay with an OD_{405} of 1.257 in the diseased samples versus an OD_{405} of 0.178 in the healthy plant samples using anti-CMV antibody (Agdia, USA).

To investigate sequence differences between the CMV-Ah isolate and other CMV isolates based on complete 2b, MP and CP sequences, flanking primers for specific amplification of the 2b (CMV2b-F: TTGAAATACARGAA GTCYGGG; CMV2b-R: AGCTGGATGGACAACCCGTTC), MP (CMVMP-F: GTCGTGTTGTCCGCACATTTG; CMVMP-R: CATCGCGTCACAGWTGTCTAC) and CP (CMVCP-F: GTAGA CAWCTGTGACGCGATG; CMVCP-R: CCATTTTAGCCGTAA GCTGG) genes of the CMV-Ah isolate were designed. PCR products of ~640 bp for 2b, ~1000 bp for MP and ~1000 bp for CP were obtained in the diseased samples. The PCR products were cloned and sequenced, and the complete sequences of the 2b, MP and CP genes of the CMV-Ah isolate were submitted to GenBank under accession numbers MK411766, MK411767 and MK411768, respectively. BLASTn analysis revealed that the 2b, MP and CP open-reading frames (ORFs) of the CMV-Ah isolate exhibited the highest sequence identity with the CMV-SD isolate, a tobacco isolate from China, at both the nucleotide (97.9%, 99.3%, and 98.8% identity, respectively) and amino acid (96.4%, 99.6%, and 100% identity, respectively) levels. Multiple sequence alignments of the CMV-Ah isolate with 14

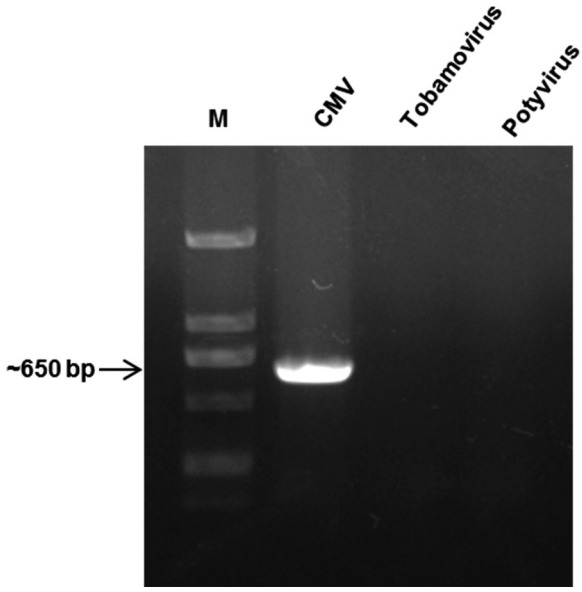

Fig. 2

RT-PCR detection of selected viruses in the diseased *A. heterophyllum* leaf samples

other isolates showed that the 2b, MP and CP ORFs of the CMV-Ah isolate had the highest nucleotide identities (91.1%–99.1%, 92.6%–99.3%, and 94.1%–98.8%, respectively) with CMV subgroup IB. Similarly, the 2b, MP and CP proteins of the CMV-Ah isolate also had the highest identities (83.8%–98.2%, 94.6%–99.6%, and 96.8%–100%, respectively) with the subgroup IB isolates (Table 1), suggesting that the CMV-Ah isolate belonged to CMV subgroup IB.

Phylogenetic analysis of the CMV-Ah isolate and 14 other isolates was performed using MEGA 7.0 software (Kumar *et al*., 2016). The neighbor-joining phylogenetic trees were constructed based on 1,000 bootstrap replicates, and all branches with bootstrap values <50% were collapsed. Phylogenetic trees of the 2b, MP, and CP ORFs based on nucleotide sequences showed that the 14 CMV isolates were divided into four clades corresponding to subgroups IA, IB, II and III (Fig. 3). The topologies of the CMV groups were similar based on the 2b, MP and CP genes in the phylogenetic trees. All three phylogenetic trees showed that the CMV-Ah isolate belonged to subgroup IB and was closely related to the CMV-SD isolate, which was consistent with the multiple alignment analysis results.

Here, we report for the first time that the medicinal plant, *A. heterophyllum*, was infected with a CMV-Ah isolate belonging to subgroup IB according to the sequencing analysis of the 2b, MP and CP genes. The CMV-Ah isolate had the highest sequence identity with a CMV-SD isolate previously isolated from tobacco in China. Identification of CMV from *A. heterophyllum* in Jilin province suggests

Table 1. Nucleotide (nt) and amino acid (aa) identities of CMV-Ah isolate with the selected strains of CMV and other members of the genus *Cucumovirus*

Strains	RNA2	RNA3	Subgroups	% identity at					
				2b		MP		CP	
				nt	aa	nt	aa	nt	aa
CMV-Fny	D00355	D10538	IA	88.1	82.9	94.2	95.7	94.4	98.2
CMV-Mf	AJ276480	AJ276481	IA	86.0	79.7	94.3	95.7	94.1	97.7
CMV-O	D10209	D00385	IA	87.8	80.2	93.7	95.7	93.5	97.7
CMV-Y	D12538	D12499	IA	87.8	80.2	93.9	96.1	94.2	97.3
CMV-SD	D86330	AB008777	IB	97.9	96.4	99.3	99.6	98.8	100.0
CMV-Ctl	EF213024	EF213025	IB	92.9	89.2	94.5	95.0	94.1	98.6
CMV-Ix	U20218	U20219	IB	91.1	83.8	92.6	94.6	94.2	96.8
CMV-As	AF033667	AF013291	IB	99.1	98.2	97.0	97.1	97.7	99.1
CMV-Ls	AF416900	AF127976	II	58.3	47.3	77.3	83.2	76.1	81.3
CMV-Q	X00985	M21464	II	57.7	47.3	77.5	83.2	76.4	82.2
CMV-Trk7	AJ007934	L15336	II	58.3	47.3	77.7	83.9	75.3	79.9
CMV-TN	AB176848	AB176847	II	54.5	46.9	76.6	82.4	75.8	81.7
CMV-BX	DQ399549	DQ399550	III	78.9	73.9	90.6	94.3	92.2	97.3
CMV-PHz	EU723570	EU723569	III	79.2	75.7	90.2	93.9	90.9	97.3
PSV-ER	U15729	U15730	Out group	47.8	33.0	62.6	63.7	51.3	44.9

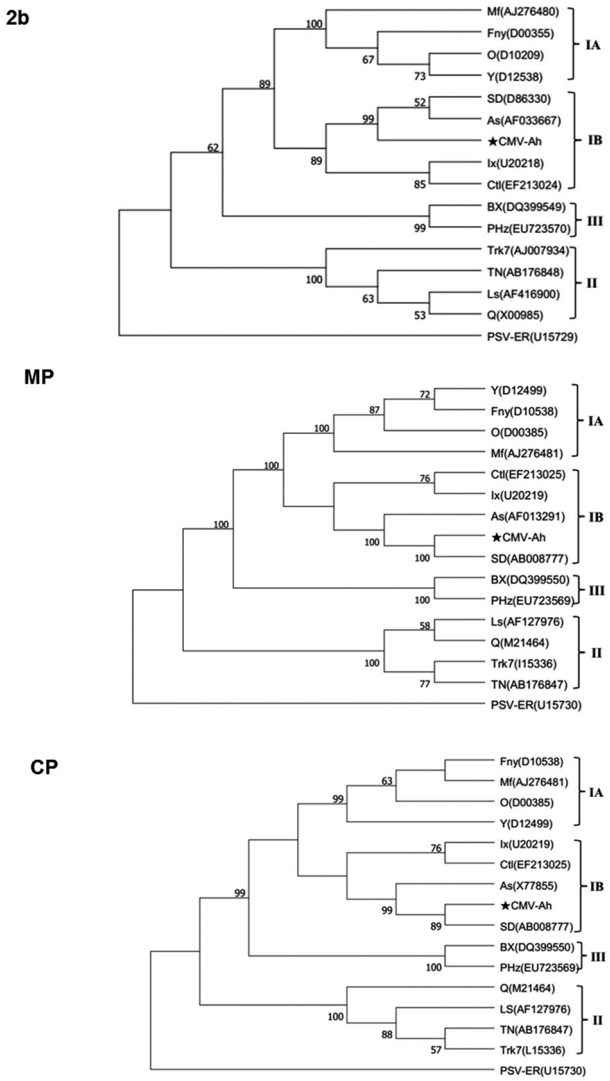

Fig. 3

Phylogenetic analysis of the CMV-Ah isolate and other CMV isolates based on nucleotide sequences of the 2b, MP and CP genes The trees were generated using neighbor-joining method in MEGA7.0 software with 1000 bootstrap replications. The *Peanut stunt virus* (PSV) strain ER was used as an outgroup. The CMV-Ah isolate is marked by a star (★). The numbers on the branches indicate bootstrap values and branches with <50% bootstrap value were collapsed.

that this virus may be an emerging threat to *A. heterophyllum* in this region. In addition to CMV, the diseased *A. heterophyllum* could have been infected with other undetected viruses. High-throughput sequencing, such as siRNA sequencing, will be further used to detect more viruses. The existence of CMV and its potential damage to other medicinal plant species should be evaluated. Our results will be useful for controlling the viruses infecting *A. heterophyllum* and other medicinal plants.

Acknowledgments. This work was supported by National Key R & D Program of China (2018YFD0201100), Doctor Startup Foundation of Jilin Agricultural University (201705), China Postdoctoral Science Foundation (2017M621223) and 111 Project (D17014).

References

Dong G, Wang JS, Guo PP, Wei DD, Yang MH, Kong LY (2015): Toxicity assessment of Arisaematis Rhizoma in rats by a 1H NMR-based metabolomics approach. Mol. Biosyst. 11, 407–417. https://doi.org/10.1039/C4MB00583J

Jacquemond M (2012): Chapter 13-Cucumber Mosaic Virus. In Loebenstein G, Lecoq H (Eds): Advances in Virus Research. Vol 84. Academic Press, pp. 439–504. https://doi.org/10.1016/B978-0-12-394314-9.00013-0

Kumar S, Stecher G, Tamura K (2016): MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054

Letschert B, Adam G, Lesemann DE, Willingmann P, Heinze C (2002): Detection and differentiation of serologically cross-reacting tobamoviruses of economical importance by RT-PCR and RT-PCR-RFLP. J. Virol. Methods 106, 1–10. https://doi.org/10.1016/S0166-0934(02)00135-0

Liu YY, Yu SL, Lan YF, Zhang CL, Hou SS. Li XD, Chen XZ, Zhu XP (2009): Molecular variability of five Cucumber mosaic virus isolates from China. Acta Virol. 53, 89–97. https://doi.org/10.4149/av_2009_02_89

Marie-Jeanne V, Ioos R, Peyre J, Alliot B, Signoret P (2000): Differentiation of Poaceae potyviruses by reverse transcription-polymerase chain reaction and restriction analysis. J. Phytopathol. 148, 141–151. https://doi.org/10.1046/j.1439-0434.2000.00473.x

Mcohizuki T, Ohki ST (2012): Cucumber mosaic virus: viral genes as virulence determinants. Mol. Plant Pathol. 13, 217–225. https://doi.org/10.1111/j.1364-3703.2011.00749.x

Palukaitis P, Roossinck MJ, Dietzgen RG, Francki R (1992): Cucumber mosaic virus. Adv. Virus Res. 41, 281–348. https://doi.org/10.1016/S0065-3527(08)60039-1

Roossinck MJ (2002): Evolutionary history of Cucumber mosaic virus deduced by phylogenetic analyses. J. Virol. 76, 3382–3387. https://doi.org/10.1128/JVI.76.7.3382-3387.2002

Roossinck MJ, Zhang L, Hellwald KH (1999): Rearrangements in the 5' nontranslated region and phylogenetic analyses of Cucumber mosaic virus RNA 3 indicate radial evolution of three subgroups. J. Virol. 73, 6752–6758. https://doi.org/10.1128/JVI.73.8.6752-6758.1999

Shen LN, Zhang Y, Wang Q, Xu L, Feng NP (2014): Preparation and evaluation of microemulsion-based transdermal delivery of total flavone of rhizoma arisaematis. Int J. Nanomed. 9, 3453–3464. https://doi.org/10.2147/IJN.S66524

Wang R, Wang N, Ye T, Chen H, Fan ZF, Zhou T (2013): Natural infection of maize by Cucumber mosaic virus in China. J. Phytopathol. 161, 880–883. https://doi.org/10.1111/jph.12141