

## First Report of *Cucumber mosaic virus* Isolated from Wild *Vigna angularis* var. *nipponensis* in Korea

Mi-Kyeong Kim<sup>1,6</sup>, Rae-Dong Jeong<sup>2</sup>, Hae-Ryun Kwak<sup>1,6</sup>, Su-Heon Lee<sup>3</sup>, Jeong-Soo Kim<sup>4</sup>, Kook-Hyung Kim<sup>5</sup>, Byeongjin Cha<sup>6\*</sup> and Hong-Soo Choi<sup>1\*</sup>

<sup>1</sup>Crop Protection Division, National Academy of Agricultural Science, Suwon 441-707, Korea

<sup>2</sup>Animal, Plant and Fisheries Quarantine and Inspection Agency, Pyeongtaek 451-821, Korea

<sup>3</sup>Department of Applied Biology, Kyungpook National University, Daegu 702-701, Korea

<sup>4</sup>Department of Plant Medicine, Andong National University, Andong 760-749, Korea

<sup>5</sup>Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

<sup>6</sup>Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea

(Received on January 21, 2013; Revised on March 25, 2014; Accepted on April 2, 2014)

A viral disease causing severe mosaic, necrotic, and yellow symptoms on *Vigna angularis* var. *nipponensis* was prevalent around Suwon area in Korea. The causal virus was characterized as *Cucumber mosaic virus* (CMV) on the basis of biological and nucleotide sequence properties of RNAs 1, 2 and 3 and named as CMV-wVa. CMV-wVa isolate caused mosaic symptoms on indicator plants, *Nicotiana tabacum* cv. Xanthi-nc, *Petunia hybrida*, and *Cucumis sativus*. Strikingly, CMV-wVa induced severe mosaic and malformation on *Cucurbita pepo*, and *Solanum lycopersicum*. Moreover, it caused necrotic or mosaic symptoms on *V. angularis* and *V. radiata* of *Fabaceae*. Symptoms of necrotic local or pin point were observed on inoculated leaves of *V. unguiculata*, *Vicia fava*, *Pisum sativum* and *Phaseolus vulgaris*. However, CMV-wVa isolate failed to infect in *Glycine max* cvs. 'Sorok', 'Sodam' and 'Somyeong'. To assess genetic variation between CMV-wVa and the other known CMV isolates, phylogenetic analysis using 16 complete nucleotide sequences of CMV RNA1, RNA2, and RNA3 including CMV-wVa was performed. CMV-wVa was more closely related to CMV isolates belonging to CMV subgroup I showing about 85.1–100% nucleotide sequences identity to those of subgroup I isolates. This is the first report of CMV as the causal virus infecting wild *Vigna angularis* var. *nipponensis* in Korea.

**Keywords :** *Cucumber mosaic virus*, *Vigna angularis* var. *nipponensis*, subgroup I

CMV is the type species of the genus *Cucumovirus* in the family *Bromoviridae* (Palukaitis and Garcia-Arenal, 2003; Roossinck et al., 1999). It is one of the most widespread and economically important plant viruses in the world and has a very extremely wide host range infecting approximately 1,000 plant species in 100 families (Douine et al., 1979; Kaper and Waterworth, 1981). Some of weed species are potential virus sources for aphid transmission into crop fields. These weed species are likely to be important role in the survival of CMV (Kaper and Waterworth, 1981). The CMV genome consists of three single-stranded RNAs, i.e. RNAs 1, 2, and 3 (Ding et al., 1994; Palukaitis and Garcia-Arenal, 2003; Palukaitis et al., 1992). Many CMV isolates have been described previously and classified into two subgroups, I and II, on the basis of serological properties and nucleotide sequence identity (Owen and Palukaitis, 1998; Palukaitis and Garcia-Arenal, 2003).

*Vigna angularis* var. *nipponensis* was an annual wild species belonging to the subgenus *Ceratotropis* in the genus *Vigna*. It is widely distributed in East Asian and Southeast Asian countries including Korea, Japan, China, Nepal, and Taiwan. *V. angularis* var. *nipponensis* was formerly treated as a progenitor of *Phaseolus* or *Azuki* (Tateishi, 1984; Lumpkin and McClary, 1994; Yamaguchi, 1992). *V. angularis* var. *nipponensis* is expected to be a valuable genetic source for breeding of azuki bean (Siriwardhane et al., 1991; Kaga et al., 2008). It is also very important species for the study of origin of Azuki bean.

Several virus are known to infect Azuki bean includ-

\*Co-Corresponding author.

Phone) +82-31-290-0431, FAX) +82-31-290-8488

E-mail) hschoi@rda.go.kr

Phone) +82-43-261-2557, FAX) +82-43-271-4414

E-mail) bjcha@chungbuk.ac.kr

ing *Alfalfa mosaic virus* (AMV), *Azuki bean mosaic virus* (AzMV), *Blackeye cowpea mosaic virus* (BICMV), *Bean common mosaic virus* (BCMV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), and *Cowpea mosaic virus* (CPMV) (Iizuka et al., 1990; Tsuchizaki et al., 1987). Among them, AMV (Heo et al., 1976; Jung et al., 2000), BCMV (Choi et al., 1989), and CMV (Choi et al., 1998; Heo et al., 1976) have been reported in Korea.

In this article, we report the occurrence of a CMV on *Vigna angularis* var. *nipponensis* in Korea, characterized on the basis of biological and complete nucleotide sequence-based properties, and named it as CMV-wVa. To our knowledge, this is the first report of CMV on *V. angularis* var. *nipponensis* in the world.

*Vigna angularis* var. *nipponensis*, showing severe mosaic, chlorotic spots, and yellow symptoms were collected from fields around Suwon, Korea (Fig. 1). The collected isolate was identified by conducting Double Antibody Sandwich-Enzyme Linked Immune Sorbent Assay (DAS-ELISA) and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using CMV specific forward (5'-TGGTCGTC-CAACT ATTAACCAC-3'), Reverse (5'-TACTGATA-AACCAGTACCGGTGA -3') primers of the coding region of coat protein gene. Each cycle included a denaturing step at 94°C for 30 sec, an annealing step at 55°C for 30 sec, and extension step at 72°C for 90 sec, and finally kept at 72°C for 10 min. DAS-ELISA was performed using CMV polyclone antibody described by Clark and Adams (Agdia, USA). Shortly, CMV antibody and its conjugate were both diluted 1:200 and all incubations were carried out at 37°C for 1 h except for the substrate that was incubated for 30 min. Quantitative measurements were examined by absorbance at 405 nm ( $A_{405}$ ). To identify the virus isolate from *V. angularis* var. *nipponensis*, infected-leaf was macerated

in 0.01 M sodium phosphate buffer, pH 7.0 and the crude sap was rubbed to the leaves of indicator plants including *Chenopodium amaranticolor*. Single lesions were isolated and re-inoculated on healthy *Ch. quinoa* three consecutive times. The isolate obtained from the third re-inoculated *Ch. quinoa* was designated as CMV-wVa, propagated in *Nicotiana tabacum* cv. 'Xanthi-nc', and was subjected to biological and molecular analyses. To test the infectivity of virus isolate and the symptoms on indicator plants, seedlings of test plants at the 3–5 leaf stage were inoculated by sap extracted from *N. tabacum* cv. 'Xanthi-nc'. The plants were put in an insect-free greenhouse maintained at 20–25°C for 12–16 h light period. Disease symptoms were recorded four times a week for 30 days. CMV-wVa isolate caused mosaic or vein clearing symptom on the upper leaves of *N. tabacum* cv. 'Xanthi-nc', *N. glutinosa*, *Capsicum annum*, *Petunia hybrid*, *Cucurbita moschata*, *Cucumis sativus*, and *Cu. melo*. In addition, the CMV-wVa isolate induced severe mosaic, malformation or fern leaves on upper leaves of *Solanum lycopersicum* (Table 1) and *C. pepo* (Table 1 and Fig. 2D). CMV-wVa induced necrotic local lesion on inoculated leaves and chlorotic ring local, mosaic symptoms on upper leaves of *Vigna radiate* (Table 1 and Fig. 2G). Local lesions were observed on *Datura stramonium*, *Ch. amaranticolor*, and *Ch. quinoa*. However, no symptoms were observed on *Glycine max* (cvs. 'Sorok', 'Sodam', and 'Somyeong'), *Brassica pekinensis* L., *Raphanus sativus* L., and *Spinacia oleracea* L. (Table 1).

To compare the infectivity of CMV-wVa isolate with CMV isolates of various host, CMV-Z(zucchini), -RB(cone flower), -ZM(maize), -Pa(azuki bean), and CM-19(azuki bean) (Choi et al., 1998; Kim et al., 2010a, 2010b, 2011 and Iizuka et al., 1990) along with CMV-wVa were inoculated onto 6 different plant species belonging to *Fabaceae*.



**Fig. 1.** Natural symptoms on *Vigna angularis* var. *nipponensis* in field infected with *Cucumber mosaic virus* (CMV) isolate wVa. Chlorotic spots, mosaic, and leaf yellowing were observed.

**Table 1.** Symptoms developed on indicator plants inoculated mechanically with *Cucumber mosaic virus* isolate wVa (CMV-wVa)

Indicator plants	Symptoms <sup>a</sup> on the leaves	
	Inoculated	Upper
<b>Solanaceae</b>		
<i>Nicotiana tabacum</i> cv. 'Xanthi-nc'	cl	m
<i>N. glutinosa</i>	–	m
<i>Capsicum annuum</i>	–	m
<i>Solanum lycopersicum</i>	–	sm,f
<i>Datura stramonium</i>	cl	– <sup>b</sup>
<i>Petunia hybrida</i>	–	m
<b>Cucurbitaceae</b>		
<i>Cucurbita pepo</i>	cl	sm, mal
<i>C. moschata</i>	cl	vc
<i>Cucumis sativus</i>	cl	m
<i>Cu. melo</i>	cl	m
<b>Fabaceae</b>		
<i>Glycine max</i> cv		
'Sorok'	–	–
'Sodam'	–	–
'Somyeong'	–	–
<b>Brassicaceae</b>		
<i>Brassica pekinensis</i>	–	–
<i>Raphanus sativus</i>	–	–
<b>Chenopodiaceae</b>		
<i>Chenopodium amaranticolor</i>	p.p	–
<i>Ch. quinoa</i>	p.p	–
<i>Spinacia oleracea</i>	–	–

<sup>a</sup>cl, chlorotic local; f, fern leaves; m, mosaic; mal, malformation; p.p, pin point; sm, severe mosaic; vc, vein clearing; –, no symptom.

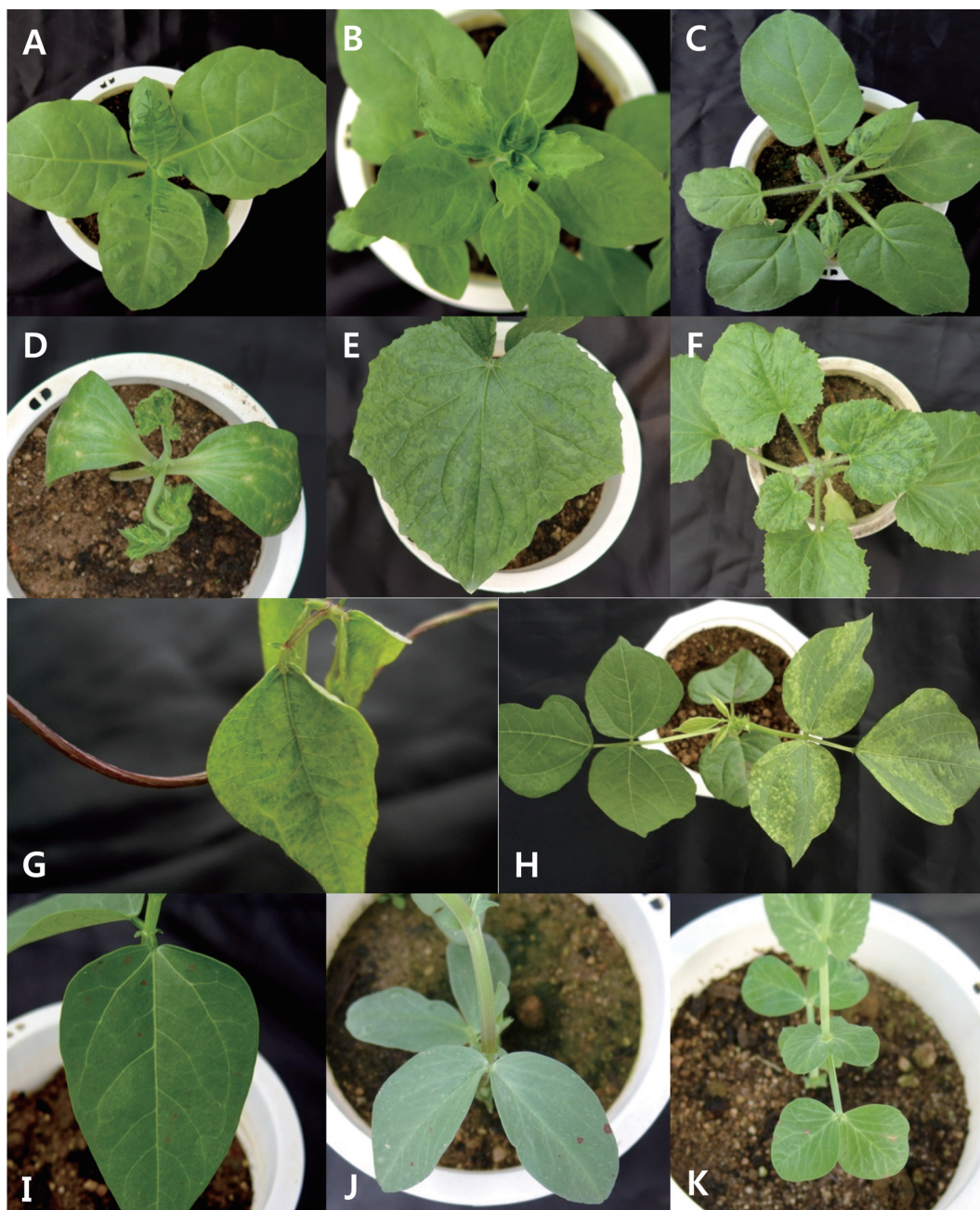
<sup>b</sup>Test results of symptomless leaves from ELISA was negative.

Four to 30 plant seedlings of each test plant listed in Table 2 were inoculated with sap extracted from *N. tabacum* cv. 'Xanthi-nc'. The CMV-wVa, -Z, -RB and -Pa induced similar chlorotic and necrotic symptoms as well as mottle and mosaic symptoms on inoculated or upper leaves of *Vigna angularis*. However, symptoms of necrotic local or pin point were observed on inoculated leaves of *V. unguiculata*, *Vicia fava*, *Pisum sativum*, and *Phaseolus vulgaris* with no systemic symptom. CMV-ZM isolate failed to infect in *V. angularis* and *Phaseolus vulgaris* (Table 2; Fig. 2). The experimental infectivity of CMV-wVa was similar to that reported for CMV isolates of zucchini, cone flower, maize and azuki bean in the host reaction of *Solanaceae* and *Cucurbitaceae* (Choi et al., 1998 and Kim et al., 2010a, 2010b, 2011). However, Japan CMV isolate (CM-19) from azuki bean did not systemically infect *V. radiata* (Iizuka et al.,

1990), only CMV-wVa isolate infected systemically causing local chlorotic ring or necrosis and systemic mosaic on *V. radiata* leaves. These differences are considered as unique characteristics of CMV-wVa.

CMV has one of the broadest host ranges among plant viruses, and its numerous strains have been differentially characterized (Palukaitis et al., 2003). Most CMV typically infects *Solanaceae* and *Cucurbitaceae*, but either produce local lesions or do not infect legume species (Whipple et al., 1941). CMV-wVa isolate infected not only *V. angularis* and *V. radiata* but also *Solanaceae* and *Cucurbitaceae*. Despite no ordinary occurrence of CMV in wild *V. angularis* var. *nippensis*, CMV-wVa has a broad host ranges including red bean, mung bean, tomato, pepper and cucumber. So it is very important role in CMV sources for aphid transmission into commercial fields.

Total RNAs were extracted from the *N. tabacum* leaves that were inoculated with CMV-wVa by easy-spin™ total RNA kit (iNtRON, Korea). Sequence specific full-length primers were designed based on previously reported genomic sequences of CMV isolates available in GenBank of National Center for Biotechnology (NCBI) (Kim et al., 2010b). RT reaction was carried out at 42°C for 30 min, and was denatured by heating at 95°C for 5 min in 5× RT buffer containing 5 µg of total RNA, 10 pmoles of the downstream primer, 2.5 mM each of four dNTPs, and 2.5 units AMV reverse transcriptase (Promega, USA). The RT reaction mixture was mixed with 1.25 units GoTaq™ DNA polymerase (Promega, USA), 5× Green PCR buffer and 2.5 mM MgCl<sub>2</sub>, and 10 pmoles of the upstream primer followed by amplification in a Bio-Rad Thermal Cycler (USA). The amplified PCR products were purified using PCR gel/direct extraction kit (iNtRON, Korea) and sequenced. End sequences of the genomes were obtained with the 5'/3' rapid amplification of cDNA ends (RACE) protocol (Frohman et al., 1988). cDNA clones containing the 5' end of the genomes were obtained using Xec primers (5' AAAGAATTCCCCCCCCCCCC-3') and CMV 5'-RNA1 (5'-TCCTTTATCGCCGTGGGAGGCTAC-3'); for CMV 5'-RNA2 (5'-TCCTCGGGAGTGTGACACC-3'); and for CMV 5'-RNA3 (5'-CCTACTGGTACCTTG-GAAAGCCAT-3') primers complementary to nucleotides 151–128, 159–140, and 144–121, respectively, of CMV strain. In addition, cDNA clones containing the 3' end of the genomes were obtained using CMV 3'-RNA1 (5'-TTGCTCGTGCTT AGCGG3'); for CMV 3'-RNA2 (5'-ACAAAGTCCCAGCGAGAG-3'); for CMV 3'-RNA3 (5'-TTGATTCTACCGTGTGGGTGACAGT-3'), and anchor primers (5'-GACCACGCGTATCGATGTC-GACTTTTTTTTTTTTTTTTTT-3').



**Fig. 2.** Symptoms on test plants inoculated with CMV-wVa. CMV-wVa caused mosaic symptom on the upper leaves of *Nicotiana tabacum* cv. 'Xanthi-nc' (A), *Petunia hybrida* (B) and *N. glutinosa* (C) while causing severe mosaic and/or malformation on *Cucubita pepo* (D), *Cucumis sativus* (E), and *Cu. melo* (F). CMV-wVa induced mosaic symptom on the upper leaves of *Vigna radiate* (G) and *V. angularis* (H). CMV-wVa caused necrotic spot symptoms on the of *V. unguiculata* (I), *V. fava* (J) and *Pisum sativum* (K); however, the symptoms were not prevalent on the upper leaves.

The lengths of CMV-wVa RNAs 1, 2, and 3 were consisted of about 3.3 kb, 3 kb and 2.2 kb, respectively. CMV-wVa RNAs 1, 2, and 3 nucleotide sequences were deposited at the GenBank under accession codes JX014246, JX014247, and JX014248, respectively. So far full length three RNAs segment sequence of wild azuki bean of CMV

have been not reported. This is the first record on *Vigna angularis* var. *nipponensis*. The sequences were then compared with corresponding regions of the other CMV isolates along with previously reported Korean CMV isolates (-Z, -RB, -ZM and -Pa) retrieved from the GenBank database (Choi et al., 1998; Kim et al., 2010a, 2010b, 2011 and

**Table 2.** Comparison of the infectivity of CMV-wVa isolate with CMV isolates on test plants belonging to *Fabaceae*

Indicator	Symptoms <sup>a</sup> on the leaves (inoculated/upper)				Reference		
	CMV-wVa	CMV-Z <sup>b</sup>	CMV-RB <sup>c</sup>	CMV-ZM <sup>d</sup>	CMV-Pa <sup>e</sup>	CM-19 <sup>f</sup>	
<i>Vigna angularis</i>	cl,nl/mo	14/30 <sup>g</sup>	nl/m	nl/m	-/- <sup>h</sup>	/m	/m
<i>V. unguiculata</i>	nl/-	20/25	nl/-	pp/-	pp/	nt	l/-
<i>V. radiate</i>	nl/crl,nl,m	4/6	nt	nt	nt	nt	l/-
<i>Vicia fava</i>	nl/-	5/15	nl/-	nl/-	nl/-	nt	l/-
<i>Pisum sativum</i>	nl/-	7/20	nl/-	nl/-	nl/-	nt	l/n
<i>Phaseolus vulgaris</i>	nl/-	4/26	nl/-	nl/-	-/-	nt	l/-

<sup>a</sup>cl, chlorotic local; m, mosaic; mo, mottle; l, local; nl, necrotic local; pp, pin point; -, no symptom; nt, not tested.

<sup>b</sup>CMV-Z, *Cucumber mosaic virus* - Zucchini (Kim et al., 2010b).

<sup>c</sup>CMV-RB, *Cucumber mosaic virus* - *Rudbeckia bicolor* (Kim et al., 2010a).

<sup>d</sup>CMV-ZM, *Cucumber mosaic virus* - *Zea mays* (Kim et al., 2011).

<sup>e</sup>CMV-Pa isolated from *Phaseolus angularis* (Choi et al., 1998).

<sup>f</sup>CM-19 isolated from *Phaseolus angularis* (Iizuka et al., 1990).

<sup>g</sup>Number of plants infected/number of plant mechanically inoculated.

<sup>h</sup>Test results of symptomless leaves from ELISA was negative.

**Table 3.** Nucleotide/amino acid sequence identities (%) between CMV-wVa and the other previously reported CMV strains and/or isolates

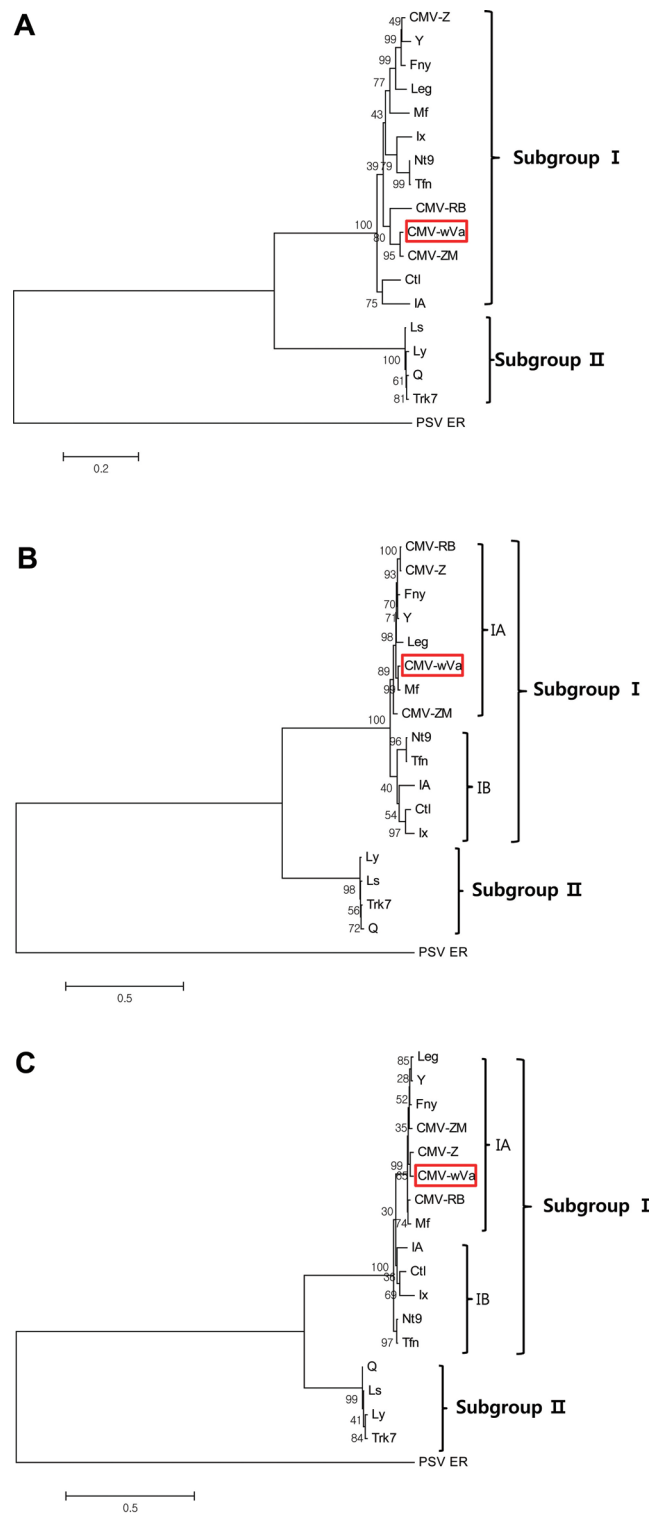
Isolate	Group	Isolates <sup>a</sup>	Nucleotide and amino acid identity (%)				
			RNA1		RNA2		RNA3
			1a	2a	2b	3a	CP
wVa	I	CMV-ZM	98.8/99.2	96.6/97.7	97.3/98.2	96.8/98.9	96.5/98.6
		CMV-RB	92.6/95.7	96.6/97.7	100/100	96.7/98.9	97.3/98.2
		CMV-Z	92.5/97.7	96.6/97.7	96.4/95.5	97.3/98.9	98.0/99.1
		CMV-Fny	91.8/97.5	96.9/97.9	97.3/97.3	97.1/98.9	97.0/98.6
		CMV-Y	91.2/95.5	97.2/98.5	97.6/96.4	97.6/99.3	96.8/97.7
		CMV-Leg	91.9/96.7	96.3/97.3	91.9/87.3	97.0/99.3	96.8/98.6
		CMV-Mf	92.4/96.6	98.6/98.8	98.8/99.1	96.7/98.9	97.3/98.2
		CMV-NT9	91.1/96.9	91.9/93.2	86.3/99.1	94.1/97.1	94.7/98.2
		CMV-Tfn	90.9/96.8	92.0/93.0	86.3/79.3	94.1/97.1	95.0/98.6
		CMV-Ix	90.8/96.1	90.0/91.4	86.3/74.4	92.5/94.6	91.8/95.4
		CMV-CTL	90.6/95.7	90.8/93.4	87.2/81.1	94.8/96.1	92.2/98.2
		CMV-IA	89.1/94.2	89.7/90.7	85.1/73.9	93.7/96.4	92.5/97.2
		CMV-Pa	-	-	-	91.3/92.2	97.0/99.1
		CM-19	-	-	-	-	-
		Outgroup	II	CMV-Q	77.8/84.1	71.9/73.1	54.4/47.7
CMV-Trk7	77.9/84.6			71.9/74.4	55.0/47.7	78.8/83.9	76.1/80.4
CMV-Ly	77.7/84.3			72.0/74.2	55.3/46.8	78.9/83.5	76.5/82.2
CMV-Ls	77.9/85.0			71.9/74.7	55.0/47.7	78.8/83.2	76.8/81.7
Outgroup	PSV-ER	66.7/71.7	56.9/51.9	44.3/32.7	63.4/64.9	51.2/44.8	

<sup>a</sup>The Genbank accession number of the reference CMV isolates : Va (JX014246, JX014247 and JX014248); ZM (JN180309, JN180310, and JN180311); RB (GU327363, GU327364, and GU327365); Z (GU327366, GU327367, and GU327368); Fny (D00356, D00355, and D10538); Y (D12537, D12538, and D12499); Leg (D16403, D16406, and D16405); Mf (AJ276479, AJ276480, and AJ276481); NT9 (D28778, D28779, and D28780); Tfn (Y16924, Y16925, and Y16926); Ix (Y20220, U20218, and U20219); CTL (EF213023, EF213024, and EF213025); IA (AB042292, AB042293, and AB042294); Pa (AB290913, and AB290152); Q (X02733, X00985, and M21464); Trk7 (AJ007933, AJ007934, and L15336); Ly (AF198101, AF198102, and AF198103); Ls (F416899, AF416900, and AF127976); PSV (NC002038, NC002039, and NC002040); - (not reported).

Roossinck, 2002). The sequences of Japan CM-19 isolate from azuki bean have been not reported.

Multiple nucleotide/amino acid sequence alignment was performed by using Clustal W (MEGA 5.0). Comparative sequence analysis disclosed that the 1a, 2a, 2b, 3a, and CP ORFs of CMV-wVa had 89.1–98.8%, 89.7–98.6%, 85.1–100%, 91.3–97.6%, and 91.8–98.0% nucleotide sequence identity, respectively, with CMV subgroup I isolates at the nucleotide level (Table 3). In contrast, identities of 54.4 to 79.0% were observed with subgroup II isolates at the nucleotide level. Database comparisons of the deduced amino acid sequences showed 94.2 to 99.2% sequence identity with 1a ORF gene of CMV subgroup I. In CMV-wVa, 1a ORF showed highest sequence identity at nucleotide level with the CMV-ZM isolate (98.8%) and 2a and 2b ORFs of CMV-wVa RNA2 showed 98.6 and 100% sequence identity with the CMV-Mf and -RB isolates, respectively. Similarly, the 3a and CP ORFs of CMV-wVa showed 97.6 and 98.0% sequences identity with the CMV-Y and -Z isolates, respectively. To compare the sequence identity of CMV-wVa isolate with CMV-Pa from azuki bean in Korea showed lowest sequence identity (91.3%) of 3a ORF (Table 3). In general, nucleotide and the deduced amino acid sequences of five ORFs of CMV-wVa showed highest sequence identity with CMV subgroup I isolates. Phylogenetic analysis were performed employing the Maximum likelihood method packaged in the MEGA 5.1 software and with the complete nucleotide sequences of three segments of CMV isolates from the GenBank database. The bootstrap maximum-likelihood trees for these genomic regions are shown (Fig. 3A, B, and C). Phylogenetic analysis of RNAs 1, 2, and 3 showed that 17 CMV isolates were divided into two subgroups such as subgroup I and II. CMV-wVa isolate was belonged to subgroup I and was closely related to the CMV-ZM and -Rb in analysis with the RNA1 sequence. Analysis of nucleotide sequences with RNAs 2 and 3 showed that CMV-wVa was most closely related to the members of subgroup IA. Especially, CMV-wVa RNA1 was divided in subgroup I. Along with CMV-wVa RNA1, RNA2 and RNA3 of CMV-wVa were also assigned to subgroup I based on phylogenetic analysis (Fig. 3). Therefore, results of phylogenetic analyses of the RNA segments 1, 2, and 3 suggested that CMV-wVa belongs to subgroup I of CMV.

*Vigna angularis* var. *nippensis* is considered to be the wild counterpart of azuki bean (Lumpkin and McClary, 1994; Tateishi, 1984; Yamaguchi, 1992) and is distributed widely in Asian countries including Korea. Several azuki bean crops naturally infected by CMV have been reported (Choi et al., 1998; Heo et al., 1976; Iizuka et al., 1990).



**Fig. 3.** Phylogenetic trees derived from nucleotide sequences of the CMV RNA segments 1, 2, and 3 of CMV-wVa with the other previously reported CMV isolates (panels A, B, and C, respectively). *Peanut stunt virus* (PSV) was used as an outgroup. Phylogenetic analyses were performed employing the maximum likelihood method packaged in the MEGA 5.1 software.

However, there is no report of natural infection of *V. angularis* var. *nippensis* (wild azuki bean) by CMV in the world. CMV-wVa isolate causing severe mosaic, necrotic, and yellow symptoms on *Vigna angularis* var. *nipponensis* identified by serological test (DAS-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR). Results of this study indicated that CMV-wVa isolate infected systemically causing local chlorotic ring or necrosis and systemic mosaic on *V. radiata* and economically plants including pepper, tomato. CMV-wVa was more closely related to CMV isolates belonging to CMV subgroup I. This is the first reported show the occurrence and identification of CMV subgroup I on *V. angularis* var. *nippensis* on the basis of biological and complete sequence analyses. Further studies of comparison analysis between CMV-wVa (wild azuki bean, Korea) and CM-19 (azuki bean, Japan) will be required for identification of gene involved in pathogen and geographical relation.

### Acknowledgements

This study was carried out with the support from the Research Program (PJ 007755) for Agricultural Science & Technology Development, Rural Development Administration, Korea.

### References

- Choi, G. S., Kim, J. H., Kim, J. S. and Choi, J. K. 2004. Characterization of *Cucumber mosaic virus* Isolated from Water Chickweed (*Stellaria aquatica*). *Plant Pathol. J.* 20:131–134.
- Choi, J. K., Kim, H. J., Hong, J. S., Kim, D. W. and Lee, S. Y. 1998. Identification and differentiation of *Cucumber mosaic virus* isolates in Korea. *Plant Pathol. J.* 14:7–12.
- Choi, Y. M. and Lee, S. H. 1989. Identification of *Azuki bean mosaic virus* on *Phaseolus angularis*. *Plant Pathol. J.* 5:49–53.
- Ding, S. W., Anderson, B. J., Haase, H. R. and Symons, R. H. 1994. New overlapping gene encoded by the cucumber mosaic virus genome. *Virology* 198:593–601.
- Douine, L., Quiot, J. B., Marchoux, G. and Archange, P. 1979. Recensement des especes vegetales sensible au virus de la mosaïque du concombre (CMV). *Etude bibliographique. Ann. de Phytopathologie* 11:439–475.
- Frohman, M. A., Dush, M. K. and Martin, G. R. 1988. Rapid production of full-length cDNA from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA* 85:8998–9002.
- Gallitelli, D. 2000. The ecology of *Cucumber mosaic virus* and sustainable agriculture. *Virus Res.* 71:9–21.
- Gal-On, A., Kaplan, I. B., Roossinck, M. J. and Palukaitis, P. 1994. The kinetics of infection of zucchini squash by *Cucumber mosaic virus* indicates a function for RNA 1 in virus movement. *Virology* 205:280–289.
- Hampton, R. O. and Francki, R. I. B. 1992. RNA-1 dependent seed transmissibility of *Cucumber mosaic virus* in *Phaseolus vulgaris*. *Phytopathology* 82:127–130.
- Hayes, R. J. and Buck, K. W. 1990. Complete replication of a eukaryotic virus RNA in vitro by a purified RNA-dependent RNA polymerase. *Cell* 63:263–368.
- Heo, N. K., Kang, M. S. and Ha, K. S. 1976. Identification of virus from azuki bean plant. *Korean J. Crop Sci.* 42:160–165.
- Iizuka, N. 1990. Studies on virus diseases of adzuki bean (*Vigna angularis* Wight) in Japan. *Bull. Tohoku Natl. Agr. Exp. Station* 82:77–113.
- Jung, H. W., Jung, H. J., Yun, W. S., Kim, H. J., Hahm, Y. I., Kim, K. H. and Choi, J. K. 2000. Characterization and partial nucleotide sequence analysis of *Alfalfa mosaic Alfamoviruses* isolated from potato and azuki bean in Korea. *Plant Pathol. J.* 16: 269–279.
- Kaga, A., Isemura, T., Tomooka, N. and Vaughan, D. A. 2008. The genetics of domestication of the Azuki bean (*Vigna angularis*). *Genetics* 2:1013–1036.
- Kaper, J. M. and Waterworth, H. E. 1981. *Cucumoviruses*. In: Kurstak E, ed. *Handbook of Plant Virus Infections and Comparative Diagnosis*. Amsterdam, The Netherlands: Elsevier/North Holland, 257–332.
- Kim, M. K., Kwak, H. R., Ko, S. J., Lee, S. H., Kim, J. S., Kim, K. H., Cha, B. J. and Choi, H. S. 2010a. Characteristics of *Cucumber mosaic virus* infecting pin wood coneflower in Korea. *Plant Pathol. J.* 26:93–98.
- Kim, M. K., Kwak, H. R., Jeong, S. G., Ko, S. J., Lee, S. H., Kim, J. S., Kim, K. H., Choi, J. K., Choi, H. S. and Cha, B. J. 2010b. First report of *Cucumber mosaic virus* infecting from Zucchini in Korean. *Plant Pathol. J.* 26:139–148.
- Kim, M. K., Kwak, H. R., Lee, S. H., Kim, J. S., Kim, K. H., Cha, B. J. and Choi, H. S. 2011. Characteristics of *Cucumber mosaic virus* isolated from *Zea mays* in Korea. *Plant Pathol. J.* 27:372–377.
- Lee, H. G., Kim, S. R., Jeon, Y. W., Kwon, S. B., Ryu, K. H. and Choi, J. K. 2008. Identification and characterization of three isolates of *Cucumber mosaic virus* isolated from weed hosts. *Res. Plant Dis.* 14:15–20.
- Lumpkin, T. A. and McClary, D. C. 1994. Azuki bean: Botany, Production and Uses. CAB International, Cambridge. 268.
- Marechal, R., Mascherpa, J. M. and Stainier, F. 1978. Etude taxonomique dun group complexed especes des genres *Phaseolus* et *Vigna* (*Papilionaceae*) sur la base de donnees morphologiques et polliniques, traitees par lanalyse informatique. *Boissiera* 28:1–237.
- Nitta, N., Takanami, Y., Kuwata, S. and Kubo, S. 1988. Comparative studies on the nucleotide sequence of *Cucumber mosaic virus* 3 between Y stain and Q strain. *Ann. Phytopathol. Soc. Jpn.* 54:516–522.
- Owen, J. and Palukaitis, P. 1998. Characterization of *Cucumber mosaic virus*. I. Molecular heterogeneity mapping of RNA 3 in eight CMV strains. *Virology* 166:495–502.

- Palukaitis, P., Roossinck, M. J., Dietzgen, R. G. and Francki, R. I. 1992. *Cucumber mosaic virus*. *Adv. Virus Res.* 41:281–348.
- Palukaitis, P. and Garcia-Arenal, F. 2003. *Cucumoviruses*. *Adv. Virus Res.* 62:241–323.
- Roossinck, M. J., Bujorski, J., Ding, S. W., Hajimorad, R., Hanada, K., Scott, S. and Tousignant, M. 1999. Family *Bromoviridae*. 923–935 in: *Virus Taxonomy Seventh Report of the International Committee on Taxonomy of Viruses*.
- Roossinck, M. J. 2001. *Cucumber mosaic virus* a model for RNA virus evolution. *Mol. Plant Pathol.* 2:59–63.
- Roossinck, M. J. 2002. Evolutionary history of *Cucumber mosaic virus* deduced by phylogenetic analyses. *J. Virol.* 76:3382–3387.
- Shi, B. J., Miller, J., Symons, R. H. and Palukaitis, P. 2003. The 2b protein of *Cucumoviruses* has a role in promoting the cell-to-cell movement of pseudorecombinant viruses. *Mol. Plant-Microbe Interact.* 16:261–267.
- Siriwardhane, D., Egawa, Y. and Tomooka, N. 1991. Cross-compatibility of cultivated adzuki bean (*Vigna angularis*) and rice bean (*V. umbellata*) with their wild relative. *Plant Breed.* 107: 320–325.
- Smartt, J. 1990. Grain legumes: evolution and genetic resources. Cambridge University Press, Cambridge, UK. 392.
- Stevens, P. F. 2001. Angiosperm Phylogeny Website Version 9. <http://www.mobot.org/MOBOT/research/APweb/>.
- Tateishi, Y. 1984. Contributions to the Genus *Vigna* (*Leguminosae*) in Taiwan *I. Sci. Rep. Tohoku Univ. 4th ser. (Biology)* 38: 335–350.
- Tomooka, N., Vaughan, D. A., Moss, H. and Maxted, N. 2002. The Asian *Vigna*: Genus *Vigna* subgenus *Ceratotropis* genetic resources. *Kluwer, Dordrecht.* 92–184.
- Tsuchizaki, T. and Toshihiro, O. 1987. Relationship among *Bean common mosaic virus*, *Blackeye cowpea mosaic virus*, *Azuki bean mosaic virus* and *Soybean mosaic virus*. *Ann. Phytopath. Soc. Japan.* 53:478–488.
- Verdcourt, B. 1970. Studies in the *Leguminosae-Papilionoideae* for the Flora of Tropical east Africa. IV. *Kew Bull.* 24:507–569.
- Whipple, O. C. and Walker, J. C. 1941. Strains of *Cucumber mosaic virus* pathogenic on bean and pea. *J. Agric. Res.* 62:0027–0060.
- Yamaguchi, H. 1992. Wild and weed azuki beans in Japan. *Econ. Bot.* 46:384–394.