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Biological and Molecular Characterization of a Korean Isolate of Clover Yellow Vein Virus Infecting *Canavalia ensiformis*

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Jack bean (*Canavalia ensiformis*) is one of healthy products for fermented or functional food in Korea and is widely distributed and cultivated worldwide. During August 2022, Jack bean plants showing symptoms of yellow flecks, chlorosis, necrotic spots and mosaic were observed in Jangheung-gun, South Korea. By transmission electron microscopy, flexuous filamentous virus particles of approximately 750×13 nm in size were observed in the symptomatic leaf samples. The infection of a Korean isolate of clover yellow vein virus (ClYVV-Ce-JH) was confirmed using double antibody sandwich enzyme-linked sorbent assay, reverse transcription polymerase chain reaction and high-throughput sequencing. The complete genome sequence of ClYVV-Ce-JH consists of 9,549 nucleotides (nt) excluding the poly (A) tail and encodes 3,072 amino acids (aa), with an AUG start and UAG stop codon, containing one open reading frame that is typical of a potyvirus polyprotein. The polyprotein of ClYVV-Ce-JH was divided into ten proteins and each protein's cleavage sites were determined. The coat protein (CP) and polyprotein of ClYVV-Ce-JH were compared at the nt and aa levels with those of the previously reported 14 ClYVV isolates. ClYVV-Ce-JH shared 92.62% to 99.63% and 93.39% to 98.05% at the CP and polyprotein homology. To our knowledge, this is the first report of a Korean isolate of ClYVV from Jack bean plants and the complete genome sequence of a ClYVV Jack bean isolate in the world.

Keywords: ClYVV, High-throughput sequencing, Phylogenetic tree, RT-PCR, Sequence

Introduction

Clover yellow vein virus (ClYVV) is a member of the genus *Potyvirus* in the family (Hollings and Nariani, 1965) and causes severe damages that may reduce yields of many leguminous crops and ornamental plants in the world (Barnett et al., 1987; CABI, 2000; Crnov and Gilbertson, 2001; Dizadji and Shahraeen, 2011; Larsen et al., 2008; Ortiz et al., 2009; Provvidenti and Schroeder, 1973; Sasaya et al., 1997; Tu, 1988). ClYVV was first identified from *Triforlium repens* in England, UK (Hollings and Nariani, 1965). ClYVV was considered as a member of bean mosaic virus (BYMV) subgroups because ClYVV and BYMV have similar virion shapes under transmission electron microscope (TEM), serological reactions, and host ranges including productions of similar symptoms in some host species (Bos et al., 1977; Jones and Diachun 1977; Larsen et al., 2008; Sasaya et al., 1997). These taxonomic controversy has been resolved by comparing sequence information and multiple alignments of the coat proteins (CP) and 3′-untranslated regions (UTR) (Tracy et al., 1992; Uyeda et al., 1991).

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ClYVV virions are fluxous filamentous particles, approximately 750 nm in length and 15 nm in diameter (Hollings and Nariani, 1965). ClYVV contains a single molecule of linear, positive-sense, single-stranded RNA about 9.6 kb in size, which has a poly (A) tract and the 3'-end (Takahashi et al., 1997). Based on a few studies (Chang et al., 1998a, 1998b), the genome of potyvirus including ClYVV is initially translated into a single large open reading frame (ORF) which is processed by virus-encoded proteases, resulting in 10 mature proteins of P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP, and a small ORF which is produced by a frameshift in the P3 cistron and expressed as a fusion protein (P3N-PIPO) (Chung et al., 2008; Hisa et al., 2014). In Korea, the first detection of ClYVV has been reported in gladiolus (*Gladiolus hybridus*) (Park et al., 1998). Subsequently, the detection of ClYVV has been reported in soybean (*Glycine max)*, white clover (*Trifolium repens*), dwarf columbine (*Aquilegia buergeriana*), and orchid (*Dendrobium* sp.) in Korea (Park et al., 2014; Shin et al., 2014; Yoon et al., 2022). While complete (or near complete) nucleotide (nt) sequences of 14 ClYVV isolates have been published and/or deposited in Genbank database, little is known about the complete genome sequences of ClYVV isolates originated from Jack bean (*Canavalia ensiformis*) in the world. In this study, we report the complete genome sequence of a Korean isolate of ClYVV from Jack bean (*C. ensiformis*) and compare it with the sequences of other previously published ClYVV isolates.

Materials and Methods

Virus source and transmission electron microscopy. Twenty leaf samples of Jack beans (*C. ensiformis*) plants showing virus-like symptoms were collected from different farms and stored at -80°C for mechanical inoculation, conventional reverse transcription polymerase chain reaction (RT-PCR), and high-throughput sequencing after quick freezing. Leaf samples of the symptomatic Jack bean plants were homogenized in 10 mM sodium phosphate buffer (pH 7.4) and centrifuged at 10,000 rpm for 3 min. Then, supernatant was subjected to identify a causal virus using TEM. All sample preparations were negatively stained on formvar-coated grids with 2.0% (w/v) sodium phosphotungstic acid solution. Virus particles were observed using a TEM (Carl Zeiss EM LEO 906E; Carl Zeiss, Jena, Germany).

Double antibody sandwich enzyme-linked sorbent assay (DAS-ELISA). A total of 20 symptomatic leaf samples of Jack beans plants were subjected to DAS-ELISA using *Potyvirus*-specific antisera (Agdia, Elkhart, IN, USA), according to manufacturer's instruction. In brief, leaf tissues were homogenized in 100 mM sodium phosphate buffer (pH 7.4) containing 0.02% NaN_3, 0.1% Tween 20, and 0.1% skim milk powder at a sample-to-buffer ratio of 1:3 (w:v), and 100 µl of extracted sap was loaded in duplicate onto micro-titer plates. The primary antibody specific to potyviruses, purchased from Agdia was diluted to 1:100 in carbonate buffer (50 mM sodium carbonate, pH 9.6) and the diluted antibody solution (1 µg/ml) was used for potyvirus detection in microtiter plates. Subsequently, goat anti-rabbit IgG conjugated alkaline phosphatase was used as a secondary antibody, according to the manufacturer's instructions (Promega, Madison, WI, USA). Substrate, 4-nitrophenyl phosphate (0.6 mg/ml), was allowed to react at room temperature for 1 h (SigmaAldrich, St. Louis, MO, USA). Plates were read with an automated plate reader (Titertek, Huntsville, AL, USA) at 405 nm. A sample was considered positive if the optical density (OD405) was greater than three times the mean of the healthy controls (Yoon et al., 2011).

Host range test. Leaf tissue exhibiting mosaic symptoms was ground using a mortar and pestle in 50 mM sodium phosphate buffer (pH 7.4). Subsequently, the extract then was used to inoculate on *Chenopodium quinoa* as a local lesion host. Local lesions were carefully excised from *C. quinoa* as soon as they became visible, ground as above, and inoculated to *C. quinoa*. After three times back-inoculation, *Nicotiana clevelandii* was mechanically inoculated with the sap of infected *C. quinoa* for virus propagation. A Korean isolate of ClYVV (named ClYVV-Ce-JH) selected was further confirmed by a biological method with some plant species as indicator plants by mechanical inoculation. The host range of ClYVV-Ce-JH was determined by mechanical inoculation onto several plant species using extracts from systemically infected *N. clevelandii* plants in 10 mM sodium phosphate buffer (pH 7.4). The diagnostic host species were as follows: *C. ensiformis, C. quinoa*, *C. amaranticola*, *Glycine max*, *N. benthamiana*, *N. clevelandii*, *N. tabacum* cv. Samsun NN, *Solanum lycopersicum*, *Vicia faba*, and *Vigna unguiculate* (Table 1). Five plants for each indicator species were inoculated with 50 mM sodium phosphate buffer (pH 7.4) as negative controls.

Table 1. Experimental host range of a Korean isolate of clover yellow vein virus (ClYVV-Ce-JH) isolated from Jack bean (*Canavalia ensiformis*) in South Korea

Plant species	ClYVV-Ce-JH	
	Symptom[a]	RT-PCR[b]
Canavalia ensiformis	-/Yf, Ch, CS, M	-/+
Chenopodium amaranticola	NS/-	+/-
C. quinoa	CS/-	+/-
Glycine max	CS/Mo	+/+
Nicotiana benthamiana	CS/M, N	+/+
N. clevelandii	CS/Mo, M, NS	+/+
N. tabacum cv. Samsun NN	CS/-	+/-
Solanum lycopersicum	CS/mM	+/+
Vicia faba	NS/M, NS	+/+
Vigna ungucalata	CS/CS, mM	+/+

RT-PCR, reverse transcription polymerase chain reaction.
[a]Inoculated leaves/upper leaves. Symptoms were assessed by observations in the inoculated and upper leaves until 21 days after inoculation. The symptoms were briefly indicated as follows: Ch, chlorosis; CS, chlorotic spot; M, mosaic; Mo, mottle; mM, mild mottle; N, necrosis; NS, necrotic spot; Yf, yellow fleck.
[b]RT-PCR was performed from total RNA extracted from indicator plant species 21 days after inoculation. Positive symbol (+) indicates synthesis of RT-PCR product and negative symbol (-) indicates no synthesis of RT-PCR product.

All the inoculated plants were maintained in a greenhouse at 25±3°C with a 16 hr light period and were observed for symptom production until 21 days after inoculation.

RNA extraction, RT-PCR and RNA sequencing. One hundred mg of leaf tissue of infected Jack bean plants was frozen in liquid N_2 and ground to a fine powder. Total RNA was extracted using a RNeasy plant mini-kit, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Briefly, 0.1 g of leaf tissue of the diseased Jack bean plants was ground with lysis buffer from the kit in a 1.5 ml microcentrifuge tube using a bead beater. Subsequently, a contaminated DNA in the eluted total RNA solution was removed from the samples by on column DNase digestion with the RNase-Free DNase Set (Qiagen) according to the manufacturer's protocol. RNA was eluted from the columns with 50 µl nuclease-free water, and the concentration was measured using NanoDrop and a QuantiT RiboGreen RNA assay kit according to the manufacturer's instructions (Qiagen). Total RNA containing genomic RNA of ClYVV-Ce-JH was reverse-transcribed using SuperScript III® reverse transcriptase (ThermoScientific, Waltham, MA, USA) and the first cDNA was amplified using the oligonucleotide primers as shown in Table 2. The thermo-cycling conditions for all PCR amplifications were as follows; 3 min at 95°C (1 cycle), 94°C, 30 sec, 55°C, 30 sec and 72°C, 2 min (35 cycles), and a final extension at 72°C for 10 min. The synthesized RT-PCR products were purified using Qiaquick PCR purification (Qiagen) and cloned into pGEM-T easy vector (Promega), according to the manufacturer' instructions. We generated the RNA-seq libraries using the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions. Each library was paired-end (150 bp×2) sequenced by

Table 2. List of primers used for identification of a Korean isolate of clover yellow vein virus (ClYVV-Ce-JH) isolated from *Canavalia ensiformis* in South Korea in this study

Virus	Target[a]	Primer sequence (5′→3′)	Product size
BCMV	CP gene	(Forward) 5′-GATGATGACCAAATGTCAAT-3′ (Reverse) 5′-AAAGCGTAGCGAGCTAGATT-3′	290 bp
BCMNV	CP gene	(Forward) 5′-CCTATGGTGGGCGGTAGGAT-3′ (Reverse) 5′-AAGTCCATACCTAGGCATGT-3′	380 bp
BYMV	CP gene	(Forward) 5′-GAGTAGCAGGCAAATAGTAC-3′ (Reverse) 5′-TTATTTGGCGGAATGTTGGC-3′	410 bp
ClYVV	CP gene	(Forward) 5′-GGACTGCTGAACTTGGACCA-3′ (Reverse) 5′-TGAAGATCACCTGACGTGCC-3′	200 bp
SMV	CP gene	(Forward) 5′-ATGAATATGAGCTTGACGAT-3′ (Reverse) 5′-GCATGTTTTGATTCACATCC-3′	480 bp

BCMV, bean common mosaic virus; CP, coat protein; BCMNV, bean common mosaic necrosis virus; BYMV, bean yellow mosaic virus; ClYVV, clover yellow vein virus; SMV, soybean mosaic virus.
[a]Target means a part of CP gene of each virus, but not the full-length CP gene of each virus.

Illumina's HiSeq 4000 system (Macrogen, Daejeon, Korea). All raw sequence data were *de novo* assembled by the Trinity program with default parameters as described previously (Jo et al., 2022). The obtained contigs were subjected to BLASTX search with E-value $1e^{-10}$ as a cutoff against the plant viral database derived from the NCBI. The 5′/3′-terminal sequence of ClYVV-Ce-JH was amplified using SMARTer RACE 5′/3′ kit (TaKaRa Bio., Kyoto, Japan) according to the manufacturer's instructions. The 5′-terminal sequence was amplified by RT-PCR using a generic primer for the sequence ends of ClYVV (Takahashi et al., 1997) and an internal specific primer (5′-GGGTCCAGCCTCGATGTGAGTACTGG-3′). The cDNA synthesis was conducted using SMARTScribe transcriptase according to the manufacturer's instructions (TaKaRa Bio.). PCR was carried out using SeqAmp DNA polymerase (Takara Bio., Shiga, Japan) under the thermal cycling conditions as follows: pre-denaturation at 94°C for 2 min, 25 cycles of 94°C for 30 sec, 68°C for 30 sec, and 72°C for 3 min, and a final extension of 72°C for 10 min. The amplicons that were obtained were purified using Qiaquick PCR purification (Qiagen) and cloned into pGEM-T easy vector (Promega), according to the manufacturer' instructions. The cDNA clones were sequenced by the Sanger method. The assembled complete genome sequences of CSNV-Ce-JH has been deposited to NCBI GenBank (accession no. LC729726).

Sequence analysis and Phylogenetic tree analysis. Analysis of the nt and deduced amino acid (aa) sequences were done using BLAST search and DNASTAR Lasergene Genomics Suite software (DNASTAR Inc., Madison, WI, USA). All ClYVV-associated contigs were selected using the BLASTX results. Of the ClYVV-associated contigs, we selected viral contigs with sizes greater than 1,000 bp to identify ClYVV sequences that covered ORFs using the NCBI ORF-finder. For sequence comparison, 14 genomic sequences of ClYVV isolates available from the GenBank database were added to our data set (Table 3). The sequences from the database that were redundant, or smaller than the size of full-length ClYVV polyprotein were omitted. For phylogenetic analysis of ClYVV, nt and aa sequences of 15 ClYVV isolates including ClYVV-Ce-JH were aligned using MUSCLE implemented in MEGA 11 followed by manual modification (Tamura et al.,

Table 3. Pairwise sequence identities between ClYVV-Ce-JH (accession no. LC729726) isolated from Jack bean (*C. ensiformis*) and other isolates of ClYVV reported previously

Isolate	Accession no.	Host	Country	Polyprotein identities (%)		CP identities (%)	
				Nucleotide	Amino acid	Nucleotide	Amino acid
JS	ON456384	*Vicia faba*	China	92.11	97.56	95.08	99.63
SS	OP868578	*Senna septemtrionalis*	China	92.21	98.05	94.10	98.16
YC	OP296252	*Vicia faba*	China	92.11	97.56	95.08	99.63
TZ	OP296251	*Vicia faba*	China	91.51	97.79	94.59	99.26
Kash7	MW675690	*Phaseolus vulgaris*	India	92.02	97.85	95.45	99.63
NGSTPS18	MW848532	*Vicia faba*	Germany	81.92	93.39	81.92	92.62
BH	LC643587	*Aquilegia buergeriana*	South Korea	91.30	97.30	94.34	99.26
IA-2016	MK292120	*Glycine max*	USA	92.02	97.85	96.43	98.89
IA-2017	MK318185	*Glycine max*	USA	91.92	97.72	96.31	99.26
Dendrobium	LC506604	*Denderobium* spp.	South Korea	92.04	97.92	94.22	99.63
No. 30	AB011819	*Vicia faba*	Japan	92.57	97.56	95.20	98.89
Ca	MW287328	*Centella asiatica*	USA	93.34	97.43	96.93	99.26
Hefei	KU922565	*Vicia faba*	China	91.45	97.46	93.85	98.16
DSMZ-PV-0848	OR607765	*Limonium sinuatum*	Germany	93.62	97.75	96.06	98.89
DSMS-PV-0367	MW854270	*Phaseolus vulgaris*	Germany	83.78	93.34	81.96	92.48

CP, coat protein.

2021). The deduced full-length polyprotein or CP sequences of ClYVV were manually adjusted using CLUSTAL W (Tamura et al., 2021) for calculation of sequence identities. Phylogenetic trees were constructed based on the neighbor-joining method and Maximum likelihood method. Bootstrap resampling (1,000 replications) was used to measure the reliability of individual nodes in each phylogenetic tree.

Results and Discussion

During August 2022, virus-like symptoms including yellow flecks, chlorosis, necrotic spots and mosaic symptoms were observed from Jack beans (*C. ensiformis*) on farms in Jangheung-gun, Jeollanam-do, South Korea (Fig. 1). Flexuous filamentous virus particles of approximately 750 nm in length and 13 nm in width were observed using TEM in the sap of the symptomatic leaf samples of the Jack bean plants (Fig. 2), suggesting that a causal virus is a member of potyviruses. To confirm this speculation, the samples were subjected to DAS-ELISA using antibody specific to potyviruses. Of 20 samples tested, 18 samples showed positive reactions in the DAS-ELISA (data not shown) when absorbance values (A_{405} nm) of four times the healthy control reading were used as the positive threshold. These results suggest that the symptomatic Jack bean plants cultivated in farms are infected by one or a few species of the genus *Potyvirus*.

Fig. 1. Systemic symptoms of Jack bean (*Canavalia ensiformis*) infected naturally with clover yellow vein virus isolate Ce-JH. The infected Jack bean plants showed yellow fleck, vein chlorosis, mosaic (left) or necrotic spots and mosaic (right) symptoms in the leaves.

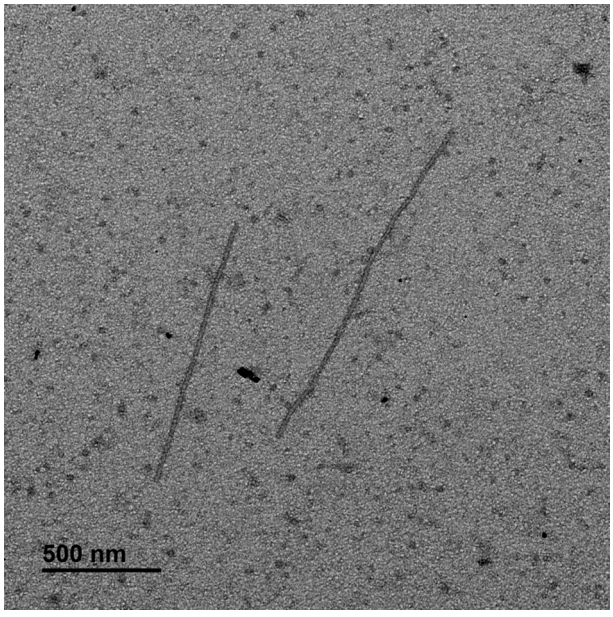

Fig. 2. Transmission electron micrograph of virus particles of clover yellow vein virus isolate Ce-JH negatively stained with 2.0% (w/v) sodium phosphotungstic acid solution from crude extracts of the symptomatic leaves of Jack bean (*Canavalia ensiformis*). Virus particles were observed using a transmission electron microscope (Carl Zeiss EM LEO 906E; Carl Zeiss, Jena, Germany).

To identify further a causal member of potyviruses that can infect Jack bean cultivars, we analyzed the virus-infected Jack bean samples using RT-PCR analysis highly specific to CP genes of bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), BYMV, ClYVV, and soybean mosaic virus (SMV). RT-PCR analysis showed specific amplification of ClYVV using primers of ClYVV CP gene. nt of the amplified RT-PCR product was determined by Sanger sequencing, confirming the authentic infection of ClYVV. Amplification of RT-PCR products was not observed using primers specific to the CP genes of BCMV, BCMNV, BYMV, and SMV, respectively (data not shown). These results suggest that a causal virus from the symptomatic Jack bean plants is an isolate of ClYVV (named ClYVV-Ce-JH).

To characterize further pathological properties of ClYVV-Ce-JH, the virus was serially inoculated to *C. quinoa* plants and *N. clevelandii* plants were inoculated with a single local lesion excised from leaves of *C. quinoa* after the fourth passages. Then, 10 plant species were mechanically inoculated with ClYVV-Ce-JH. Host range and symptoms of ClYVV-Ce-JH were summarized in Table 1. Both the inoculated leaves and the systemic leaves developed different viral disease symptoms at 7 to 21 dpi, depending on plant species. Briefly, *C. amaranticola* and *C. quinoa* showed the symptoms of necrotic spots and chlorotic spots, respectively. *N. benthamiana* and *N. clevelandii* showed different systemic symptoms including mosaic, necrotic spots, and mottle, suggesting good propagation host species. The symptoms of yellow flecks, vein chlorosis, chlorotic spots and mosaic were observed on

the tested Jack bean plants (*C. ensiformis*) similar to the original source Jack bean plants (Table 1).

These results were similar to results of host range tests with other ClYVV isolates (Bos et al., 1977; Jones and Diachun, 1977; Larsen et al., 2008; Sasaya et al., 1997), suggesting that ClYVV-Ce-JH has typical pathological properties similar to those of other isolates.

To further molecularly characterize ClYVV-Ce-JH, total RNA isolated from the symptomatic Jack bean plants was sequenced by high-throughput sequencing. A total of 14,257,459 raw reads were obtained from the samples. After trimming adapter sequences, a total of 12,586,417 clean reads with length of 110-150 nts remained for further analyses. Using Velvet software, the clean reads were assembled

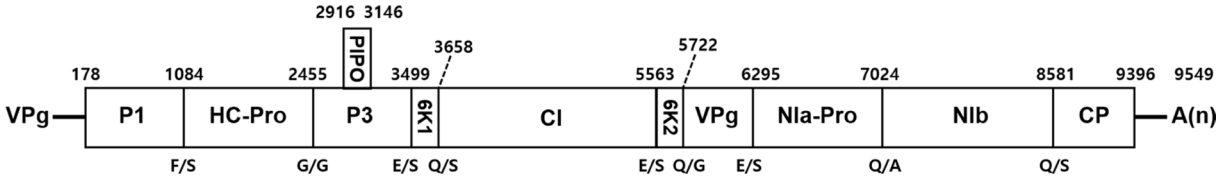

Fig. 3. The predicted genome structure of a Korean isolate of clover yellow vein virus (ClYCC-Ce-JH) isolated from Jack bean (*Canavalia ensiformis*). The first nucleotide position of each open reading frame (ORF) is indicated on the schematic genome of ClYVV-Ce-JH. The last nucleotide position (nt 9,396) of stop codon of polyprotein was also indicated on the coat protein (CP) ORF of ClYVV-Ce-JH. Amino acid sequences of the cleavage sites are shown on the below of schematic genome of ClYVV-Ce-JH. The ORFs of ClYVV-Ce-JH are as follows: P1, the first protein; HC-Pro, helper component-proteinase; P3, the third protein; 6K1, 6 kDa protein 1; CI, cytoplasmic inclusion protein; 6K2, 6 kDa protein 2; VPg, viral genome-linked protein; NIa-Pro, nuclear inclusion a protease; NIb, nuclear inclusion b protease; PIPO, pretty interesting potyvirus ORF.

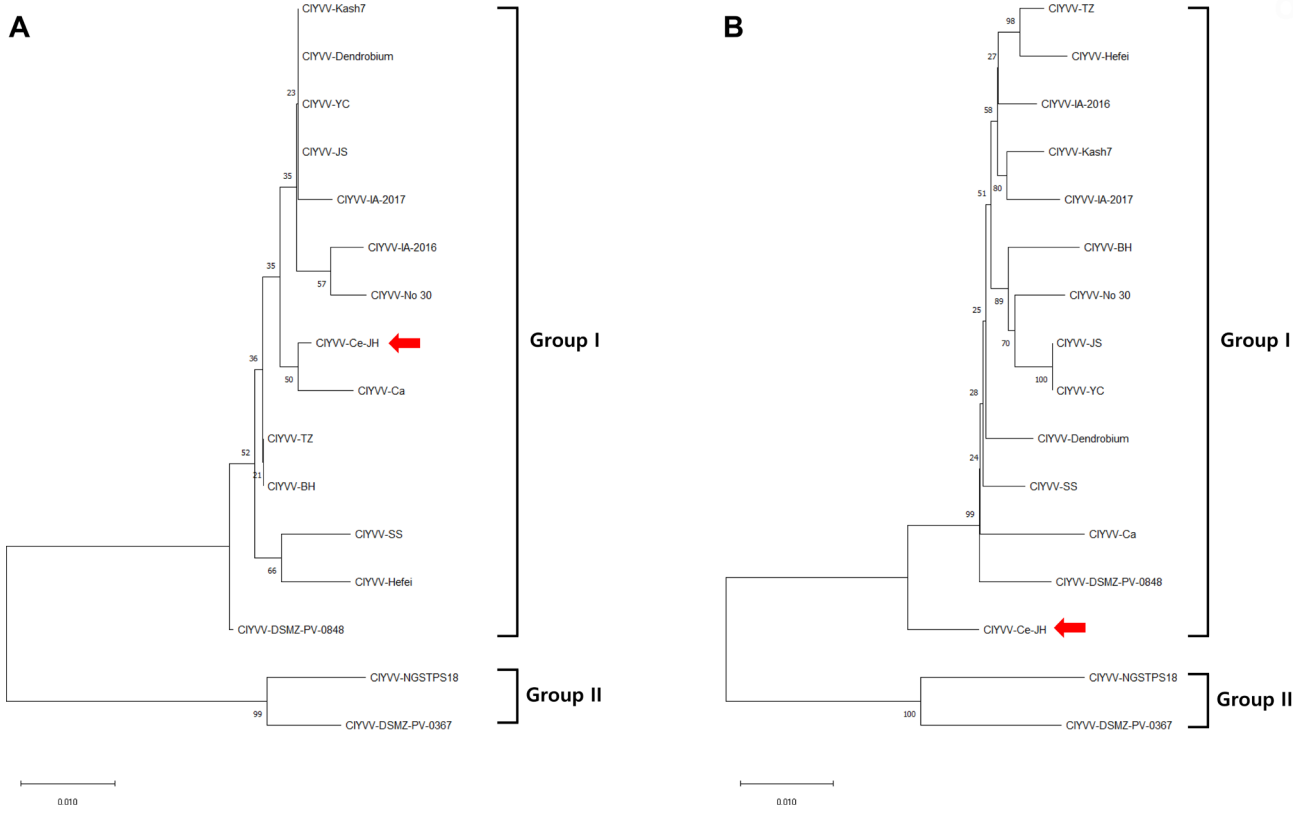

Fig. 4. Rooted trees showing phylogenetic relationship of 16 clover yellow vein virus isolates based on amino acid sequences of the coat proteins (A) and polyproteins (B). Multiple sequence alignments were generated with MEGA 11 software (Tamura et al., 2021), and the tree was constructed by the neighbor-joining algorithm based on calculations from pairwise amino acid sequence distances. The horizontal branch lengths are proportional to the genetic distance, and numbers shown at branch point indicate bootstrap values. The data set was subjected to 1,000 bootstrap replicates. Sequences of ClYVV isolates for comparisons were obtained from Genbank Database. Accession numbers are shown on Table 3. Scale bar indicates 0.01 substitutions per nucleotide position.

into 27 contigs and the assembled sequences were aligned with viral reference genomes through searches performed using the BLASTn tool. Eleven contigs (1,195–9,367 nts) revealed 95–99% nt identities with ClYVV genome. Analysis of other assembled contigs was not matched with other viral genomic sequences in the Genbank, suggested that it excluded the contamination of another potyvirus that can infect Jack bean plants. Taken together, it confirms that the viral disease of the symptomatic Jack bean plants was caused by the sole infection of ClYVV-Ce-JH. The complete genome sequence of ClYVV-Ce-JH was assembled from the longest contig consisted of 9,367 nts (near full-genome of ClYVV-Ce-JH) and virus-termini sequences obtained from RACE analysis. The complete genomic sequence of ClYVV-Ce-JH is 9,549 nts with 177 nts at the 5'UTR and 153 nts at 3'UTR excluding the poly (A) tail. The viral genome encodes a large polyprotein of 3,072 aa from nt 178 to nt 9,396, which is cleaved into ten mature proteins of P1 (302 aa), HC-Pro (457 aa), P3 (348 aa), 6K1 (53 aa), CI (635 aa), 6K2 (53 aa), VPg (191 aa), NIa-Pro (243 aa), NIb (519 aa), and CP (271 aa) (Fig. 3). The P3-PIPO is located within the P3 cistron from nt 2916 to nt 3,146, which starts at the conserved motif G2A6 and is expressed as a P3N-PIPO fusion product (Fig. 3). The cleavage sites of each protein were indicated below the schematic genome of ClYVV-Ce-JH (Fig. 3).

Pairwise alignments revealed that the polyprotein nt sequences of ClYVV-Ce-JH shared genome sequence identities ranging from 81.92% with ClYVV-NGSTPS18 isolated from broad bean (*Vicia faba*) to 92.02% with ClYVV-Kash7 isolated from common bean (*Phaseolus vulgaris*) (Table 3). The polyprotein of ClYVV-Ce-JH shared 93.39–98.05% with those of other ClYVV isolates available in GenBank at aa level. The CP of ClYVV-Ce-JH shared 81.92–96.93% and 92.62–99.63% with those of other ClYVV isolates available in GenBank at nt and aa levels, showing ClYVV CP is the most highly conserved (Table 3), similar to results reported from other potyvirus comparisons (Hammond and Hammond, 2003; Parrella and Lanave, 2009; Takahashi et al.,1990; Wylie et al., 2002, 2008). Phylogenetic tree analysis of aa of ClYVV CP and polyprotein sequences revealed a similar result the 16 ClYVV isolates were clustered indicating two groups in Fig. 4. The two isolates from Germany (ClYVV-NGSTPS18 and ClYVV- DSMZ-PV-0367) were clustered into one group, and the other 13 isolates, including ClYVV-Ce-JH in this study, were clustered into another group I (Li et al., 2023). However, it remains to be determined relationship between pathological properties and genetic taxonomy because of high sequence identities of ClYVV CPs. In addition, we did not find out any relationships between ClYVV strains originating from different geographical regions and isolation host species (Table 3, Fig. 4). These results were coincident with results of the previous phylogenetic tree analysis with ClYVV isolates though the authors divided into two groups using polyproteins of ClYVV isolates in the world (Li et al., 2023). It will be interesting if the pathological properties of ClYVV-Ce-JH is different from those of other ClYVV isolates (i.e., a Japanese isolate, No. 30) using infectious cDNA clones of the isolates. To our knowledge, this is the first of the complete genome sequence of an isolate of ClYVV from Jack bean in the world.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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