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Investigation of Tissue-Specific Distribution and Genetic Variation of Alfalfa Mosaic Virus and Chinese Artichoke Mosaic Virus in Chinese Artichoke (*Stachys affinis* miq.)

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The Chinese artichoke (*Stachys affinis* syn. *S. sieboldii*) is a widely cultivated crop, and its rhizome is used as a medicinal vegetable. To investigate the causes of viral diseases in Chinese artichokes, the infection rates of four virus species infecting Chinese artichoke were investigated. Since the Chinese artichoke propagates through its tuber, this study aimed to determine whether viral transmission to the progeny is possible through the tuber, by identifying the virus present in the tuber and investigating its accumulation. First, reverse transcription polymerase chain reaction analysis was performed to detect viruses using total RNA extracted from the flowers, leaves, and tubers of Chinese artichoke plants. Alfalfa mosaic virus (AMV) and Chinese artichoke mosaic virus (ChAMV) had high infectivity in Chinese artichoke and most plants were simultaneously infected with AMV and ChAMV. These viruses were present in all tissues, but their detection frequency and accumulation rates varied across different tissues of the Chinese artichoke. Also, we sequenced the coat protein (CP) genes of AMV and ChAMV to investigate genetic variations of virus between the leaf and tuber. It provides information on CP gene sequences and genetic diversity of isolates identified from new hosts of AMV and ChAMV. This study offers valuable insights into the distribution and spread of the ChAMV and AMV within Chinese artichoke plants, which have implications for the management and control of viral infections in crops.

Keywords **:** Alfalfa mosaic virus, Chinese artichoke mosaic virus, *Stachys affinis*, tuber

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Stachys affinis, known as the Chinese artichoke, is a perennial herb native to China that is cultivated worldwide. The plant, which has an edible tuberous root, has several uses in traditional medicine and is believed to have many health benefits, such as improving digestion and strengthening the immune system (Baek et al., 2004; Kang et al., 2018; Lee and Lim, 2018). In recent years, Chinese artichokes have gained popularity as specialty crops; they are often grown by small-scale farmers in farmers' markets and gourmet restaurants. Studies have suggested that the Chinese artichoke is susceptible to viral diseases (Fuji et al, 2003; Kim et al, 2018).

In 2003, the Chinese artichoke mosaic virus (ChAMV) was isolated and identified in Japan (Fuji et al., 2003). Filamentous particles were observed in Chinese artichoke plants displaying mosaic symptoms, and these particles were initially classified within the potyvirus group based on serological properties. However, further analysis revealed differences in the amino acid sequence of the 3' terminal region of the viral genome, including the coat protein (CP) gene, distinguishing it from other distinct viruses within the potyvirus genus. As a result, a new potyvirus was proposed and named Chinese artichoke mosaic virus (ChAMV). In addition, researchers have detected mixed infections with alfalfa mosaic virus (AMV), cucumber mosaic virus

(CMV), and tobacco mosaic virus (TMV) in Chinese artichoke plants (Kim et al., 2018). These three viruses were identified using reverse transcription polymerase chain reaction (RT-PCR) assays, and their biological and molecular characteristics were analyzed through host response testing and nucleotide sequences of the virus genome. These viruses cause mosaic symptoms in the leaves of the infected plant, stunted growth, and reduced yields.

In the results of the survey on plant viruses infecting Chinese artichoke, we confirmed that AMV and ChAMV were the predominant causes of viral diseases in Chinese artichoke. AMV is a plant virus that infects a wide range of plant species, including alfalfa, tobacco, and tomato. It is known that this virus can naturally infect 698 species of 167 genera in 71 families (Moradi and Mehrvar, 2021). This virus is a type member of the genus Alfamovirus belongs to the Bromoviridae family and has a tripartite RNA genome. AMV is primarily transmitted by mechanical inoculation, such as through contaminated tools, but it can also be transmitted by aphids and other insects. The virus can also spread through infected seeds and plant materials (Kil et al., 2016; Kim et al., 2015; Mueller and Grau, 2007). ChAMV, a single-stranded RNA virus, belongs to the family Potyviridae. Potyvirus genomes are relatively long compared to other RNA viruses, about 10 kb. The genome contains a single large open reading frame that encodes a polyprotein. This polyprotein is later processed into individual functional proteins. Potyvirus genomes can exhibit high variability, leading to a wide range of strains and isolates within the genus. Such genetic variability has the potential to influence the spectrum of hosts, the manifestation of symptoms, and the virulence. Many potyviruses are transmitted by insect vectors, particularly aphids, in a non-persistent manner (Ng and Perry, 2004; Revers and García, 2015). This means the virus is acquired and transmitted by the vector during brief feeding periods. ChAMV, isolated from *S. affinis*, has been characterized through molecular and biological studies, which show its unique genetic and physical properties as a member of the Potyviridae family (Fuji et al., 2003). To date, there has been little discussion on the molecular characterization of ChAMV.

This study was conducted to identify the viruses that infect the Chinese artichoke and to investigate their infection rates. Chinese artichoke is a perennial herbaceous plant that can be propagated through tubers. The viruses, infected with the Chinese artichoke plant, can be transmitted to the next generation through tubers, potentially impacting the growth and yield of Chinese artichokes. Therefore, the distribution and abundance of the viruses within the plant were investigated to confirm the possibility of transmission by tubers. Additionally, we also performed molecular analysis of the nucleotide sequence of the CP gene of AMV and ChAMV isolated from Chinese artichoke in Korea.

Materials and Methods

Plant selection and growth. Chinese artichoke seedlings exhibiting viral symptoms, such as a mosaic pattern, were observed at a garden shop located in Chuncheon-si, Gangwon-do, Korea. Most commercially available seedlings of Chinese artichoke show viral symptoms. A total of 300 seedlings that showed viral disease-like symptoms

Fig. 1. Seedlings and tissues (flower, leaf, and tuber) of Chinese artichoke (*Stachys affinis*) showing viral symptoms. The mild mosaic was observed in the leaves of most of the seedlings. The seedlings were grown for three months until flowers and tubers formed. Viral symptoms were not observed in the flowers and tubers.

were examined, and 30 samples with severe mosaic symptoms were selected for testing to investigate the incidence of pathogenic viruses. The selected plants were brought to the laboratory, transplanted into soil, and grown for approximately 3 months in an outdoor greenhouse environment until flowers and tubers were formed. We divided the selected plants into three parts: flowers, leaves, and tubers (roots) and observed the symptoms in each part (Fig. 1).

Identification of viruses and their distribution. We investigated the incidence of the four viruses known to infect Chinese artichoke (AMV, ChAMV, CMV, and TMV) in seedlings with viral symptoms. The seedlings of Chinese artichoke were grown until flowers and tubers were formed, and RT-PCR was performed by dividing them into flowers, leaves, and tubers (Fig. 2A). We performed RT-PCR using species-specific primers for AMV, ChAMV, and CMV, and genus-specific primers for TMV. To investigate the distribution and abundance of viruses within the plant, total RNA was extracted from the leaves of young seedlings showing mosaic symptoms. RNA templates were prepared using the BCS Plant RNA Prep Kit (Biocube System Inc., Suwon, Korea), following the manufacturer's instructions. We synthesized cDNA from total RNA, including viral genomic RNA, using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and random hexamer primers (Thermo Fisher Scientific Inc., Waltham, MA, USA).

RNA preparation and cDNA synthesis. In this study, we designed specific primers for the RT-PCR assay by referring to the known CP gene sequence information of ChAMV and sequenced Korean isolates using PCR amplification products verified using these primers. PCR was performed using a T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) in a final reaction volume of 50 µl containing cDNA, rTaq polymerase (Takara Bio Inc., Shiga, Japan), and specific primers for AMV, ChAMV, CMV, and TMV. Nucleotide sequences of the primers used in this study are listed in Table 1. The PCR conditions were as follows: an initial denaturation at 94°C for 3 min followed by 35 cycles (denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and an extension at 72°C for 30 s), and a final extension at 72°C for 5 min. The amplified DNA fragments were analyzed and electrophoresed on a 1% agarose gel.

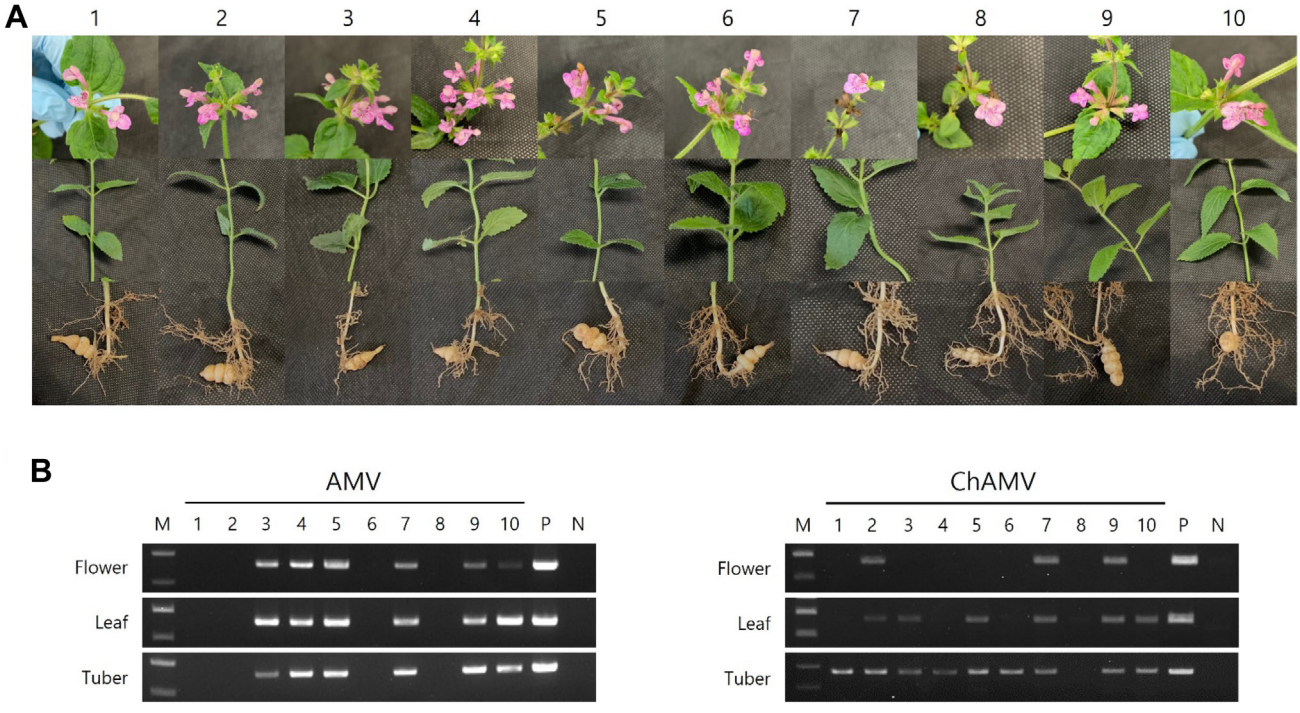

Fig. 2. Distribution of artichoke mosaic virus (AMV) and Chinese artichoke mosaic virus (ChAMV) in Chinese artichoke. (A) Ten Chinese artichoke plants were selected, each showing distinct mosaic symptoms on their leaves. (B) Reverse transcription polymerase chain reaction (RT-PCR) was performed using total RNA extracted from the flowers, leaves, and tubers of each plant. The RT-PCR results showed that both AMV and ChAMV were detected in flowers, leaves, and tubers. The flowers and tubers developed normally and did not exhibit any viral symptoms. Lanes 1-10, samples 1 to 10 of Chinese artichoke plants.. P and N refer to the positive and negative controls of the virus in RT-PCR assay, respectively.

Table 1. Primers used for RT-PCR and qRT-PCR in this study

Virus	Primer name	Sequence (5′-3′)[a]	Reference
For RT-PCR			
Alfalfa mosaic virus	AMV-CP-F	ATGAGTTCTTCACAAAAGAAAGC	This study
	AMV-MS-CP-R	TCAAAGATCGTCAGCTTCGTC	
Chinese artichoke mosaic virus	ChAMV-CP-F	GCAGATGAAACAATTGATGCTGG	This study
	ChAMV-CP-R	TCARCCGTGGCGMACCCCAAG	
	Poty-CI-F	GGIVVIGTIGGIWSIGGIAARTCIAC	Ha et al. (2008)
	Poty-CI-R	ACICCRTTYTCDATDATRTTIGTIGC	
Cucumber mosaic virus	CMV-R3-561-Fw	TTGGGAATCGTAAGCGGTGTTTTG	Phan et al. (2014)
	CMV-R3-1476-Rv	TTACAACGTTCACTCCCCACAAAG	
Tobacco mosaic virus	TobamodF	TKGAYGGNGTBCCNGGNTGYGG	Li et al. (2018)
	TobamodR	ACNGAVTBNABCTGTAATTGCTAT	
For qRT-PCR			
Alfalfa mosaic virus	AMV-CP-F	ATGAGTTCTTCACAAAAGAAAGC	This study
	qAMV-CP-R	CCCGTCTGTGGCAGTATAGT	
Chinese artichoke mosaic virus	qChAMV-F	TAGAGCAAAGGAGGCACAC	This study
	qChAMV-R	TGCTGTATGACGTTCTGTTC	
Endogenous control	Actin-F	ATCGGAATGGAAGCTGCTG	Kim et al. (2018)
	Actin-R	GTACCACCACTGAGGACAATG	

RT-PCR, reverse transcription polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction.
[a]I: inosine, Y: C/T, R: A/G, D: G/A/T, M: A/C, N: A/C/G/T, W: A/T, K: T/G.

Quantitative reverse transcription polymerase chain reaction analysis. For the comparison of viral accumulation in each tissue of Chinese artichoke, the total RNA was extracted from flowers, leaves, and tubers of Chinese artichoke plants co-infected with AMV and ChAMV using BCS Plant RNA Prep Kit (Biocube System Inc.). The RNA was extracted from 0.3 g of leaf and tuber tissue confirmed to be co-infected with AMV and ChAMV, and then resuspended in 50 µl of RNase-free water. The concentration of total RNA was measured using the Colibri Microvolume Spectrometer (Berthold Titertek Instruments, Inc., Pforzheim, Germany) and adjusted to 100 ng/µl. Three biological replicates (plants) were performed. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted using the Step One Plus real-time PCR system (Applied Biosystems, Foster City, CA, USA). Specific primers for qPCR targeting AMV and ChAMV were designed (Table 1). Each reaction contained 100 ng of total RNA, EzAmp HS One-Step RT-qPCR 2× Master Mix (ELPIS Biotech Inc., Daejeon, Korea), and 1 µM of each forward and reverse primer. RT-qPCR conditions included reverse transcription at 50°C for 10 min, pre-denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing/extension at 60°C for 30 s. A non-template control (D.W.) was included as a negative control. The cycle threshold (Ct) values were calculated using Step One Software version 2.3 (Applied Biosystems). The levels of viral RNAs were determined using the ΔΔCt method relative to the expression level of actin in Chinese artichoke, which served as stable and suitable internal controls. The change in gene expression was calculated using the equation $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001).

Sequence analysis and phylogenetic analysis. For sequence analysis of CP gene of the AMV and ChAMV, the PCR products were ligated into a pGEM-T easy vector system (Promega) and sequenced (Macrogen Inc., Seoul, Korea). Sequences were analyzed using the Basic Local Alignment Search Tool (BLAST in NCBI) and MEGA10 software. We selected a sequence of 32 potyviruses for phylogenetic analysis. The sequences were aligned and subjected to phylogenetic analysis using the neighbor-joining method with MEGA10, with a bootstrap value of 1,000. All positions with gaps and missing data were eliminated, and the remaining 867 bases were used. Subsequently, a BLASTn search was conducted to determine sequence identity. Then, a phylogenetic tree was constructed based on the CP of ChAMV and the 38 potyvirus species.

Results and Discussion

Virus detection and infection rate in Chinese artichoke. The majority of commercially available Chinese artichoke plants showed mosaic symptoms, which are representative of the plant virus, on their leaves; however, the severity of these symptoms was weak. As the plants grew, the symptoms that appeared on the leaves were almost invisible, and only a few Chinese artichokes exhibited mosaic symptoms. No symptoms induced by the virus were observed in the petals or tubers, and it was confirmed that tubers formed normally in the roots (Fig. 1).

To investigate the viruses that infected Chinese artichokes showing viral symptoms, an RT-PCR assay was performed using specific primers for AMV, ChAMV, CMV, and TMV. In the current study, the results indicate that AMV, ChAMV, and CMV were identified in the leaves of Chinese artichoke seedlings, whereas TMV was not detected (Table 2). ChAMV was detected in 65 of 80 samples with the highest infection rate of 81.3%. The AMV infection rate was 58.8%, with 47 of the 80 samples testing positive for the virus. These results indicate that ChAMV and AMV are the major viruses infecting Chinese artichoke. CMV was detected in only one sample. CMV is a virus commonly found in various crops due to its wide host range, but its occurrence rate in Chinese artichoke was very low.

Furthermore, we confirmed that most virus-infected plants were co-infected with AMV and ChAMV. The rate of co-infection with AMV and ChAMV was 56.3% (45 of 80 samples), whereas mixed infections were not observed in CMV-infected plants. Co-infection with more than two different viruses is known to cause more severe symptoms in plants (Mascia and Gallitelli, 2016; Murphy and Bowen, 2006; Singhal et al., 2021). However, in this study, despite co-infection with AMV and ChAMV, we confirmed that the symptoms caused by both viruses were mild mosaic. It suggested that the co-infection of AMV and ChAMV does not significantly impact the growth and tuber formation of Chinese artichoke plants, nor does it affect the severity of virus symptoms.

Table 2. Detection of Chinese artichoke-infecting viruses

Virus	Detection frequency (positive/tested samples)	Detection rate (%)
AMV	47/80	58.8
ChAMV	65/80	81.3
CMV	3/80	3.8
TMV	0/80	0.0
AMV single	2/80	2.5
ChAMV single	20/80	25.0
AMV + ChAMV co-infection	45/80	56.3

AMV, alfalfa mosaic virus; ChAMV, Chinese artichoke mosaic virus; CMV, cucumber mosaic virus; TMV, tobacco mosaic virus.

Distribution and abundance of AMV and ChAMV in Chinese artichoke tissues. The Chinese artichoke is a perennial plant capable of propagating through tubers, which is the plant's main method of vegetative reproduction. Recently, research has also been conducted on the efficient cultivation methods of artichokes via tubers (Ikemoto et al., 2024). The presence of viruses in tubers indicates that the virus can be transmitted to progeny plants. The re-infection of viruses when propagating virus-free seed tubers is a major problem in growing Chinese artichoke. Therefore, we targeted AMV and ChAMV, which showed a high infection rate in the virus incidence survey and confirmed the presence of viruses in each part (flowers, leaves, and tubers) to determine whether the virus was distributed in tubers. RT-PCR results showed that both AMV and ChAMV were present in the flowers, leaves, and tubers (Fig. 2B). In Chinese artichoke plants infected with AMV, the virus is present simultaneously in flowers, leaves, and tubers. However, in the case of ChAMV, there were instances where the virus was detected only in the tubers and not in the flowers and leaves. Our findings indicate that ChAMV exhibits a higher prevalence in tubers compared to AMV.

To ascertain whether the high prevalence of ChAMV compared to AMV is associated with the abundance within the plant, we investigated the viral accumulation within Chinese artichoke co-infected with AMV and ChAMV. Additionally, we conducted qRT-PCR analysis for each flower, leaf, and tuber to determine if there were differences in viral accumulation among plant tissues (Fig. 3). As a result, the viral accumulation within Chinese artichoke co-infected with AMV and ChAMV showed higher levels of AMV than ChAMV across flowers, leaves, and tubers. Contrary to our expectations, it was observed that AMV exhibits a higher abundance within the Chinese artichoke compared to ChAMV. We confirmed that the viral accumulation of AMV and ChAMV within Chinese artichoke varies among tissues. Both AMV and ChAMV showed the highest accumulation in the leaves. However, the accumulation of AMV in leaves was approximately 55 times higher than that of ChAMV. The accumulation of AMV followed the order of leaf, tuber, and flower, but significant

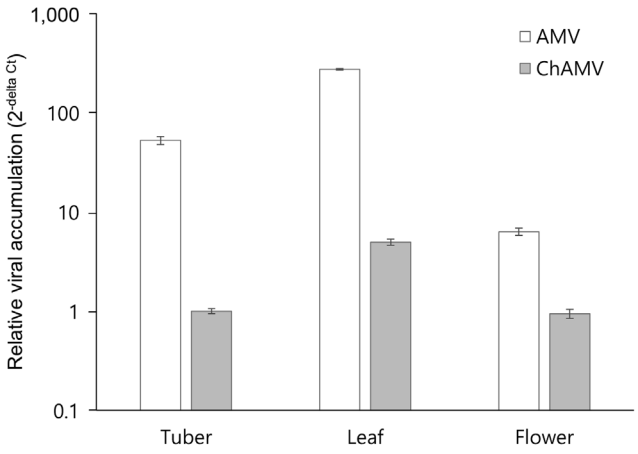

Fig. 3. Comparison of viral accumulation in flowers, leaves, and tubers of Chinese artichoke co-infected with artichoke mosaic virus (AMV) and Chinese artichoke mosaic virus (ChAMV). quantitative reverse transcription polymerase chain reaction was performed on specimens confirmed to be co-infected with AMV and ChAMV through reverse transcription polymerase chain reaction. Each experiment was conducted in triplicate, and error bars were represented as standard errors. To relatively quantify the viral accumulation within the plant body, the housekeeping gene actin of Chinese artichoke was utilized as an endogenous control.

differences in accumulation were observed among tissues within the same individual. Relative to the flower with the lowest accumulation, the tuber was approximately 8 times higher, while the leaf was approximately 43 times higher. In the case of ChAMV, accumulation in flowers and tubers was nearly similar, with leaves showing approximately five times higher accumulation than flowers and tubers.

The investigation into the occurrence rates of viral infections revealed that ChAMV infection was more frequent than AMV. However, viral accumulation analysis within a single plant co-infected with both viruses confirmed the dominance of AMV. Contrary to our expectation that the accumulation within tubers would be higher due to the characteristic propagation of Chinese artichoke, both AMV and ChAMV showed the highest viral accumulation in the leaves. Therefore, our findings suggest that there is no correlation between the viral occurrence rates and distribution and abundance within the plant. AMV and ChAMV are representative aphid-transmitted viruses (Ng and Perry, 2004), making them likely primary sources of horizontal transmission of virus from plant to plant. It is speculated that the presence of insect vectors may have a greater impact on prevalence than the level of viral accumulation.

Molecular characterization of AMV and ChAMV isolates from Chinese artichoke. In the above experiment, we confirmed differences in viral accumulation between the leaves and tubers of Chinese artichoke, leading us to investigate the genetic diversity in virus genome sequences. Furthermore, to analyze the molecular characteristics of AMV and ChAMV isolated from Chinese artichoke in Korea, we conducted nucleotide sequencing and phylogenetic analysis of the CP genes of AMV and ChAMV. To investigate the genetic diversity between leaves and tubers, the CP gene of each virus was amplified through RT-PCR from the total RNA of Chinese artichoke leaves and tubers infected with AMV and ChAMV and used for sequencing analysis. We named the viral CP nucleotide sequences obtained from each leaf and tuber of five infected Chinese artichoke plants with AMV and ChAMV as the isolate "CAL1-CAL5" (Leaf) and "CAR1-CAR5" (Rhizome), respectively.

As a result of comparing the CP gene sequences in leaves and tubers within a single plant, the sequence variations and amino acid substitutions were identified in only one Chinese artichoke plant: for AMV between CAL4 and CAR4, and ChAMV between CAL2 and CAR2. No differences were observed in the remaining samples. Our results show very little tissue-specific sequence diversity in Chinese artichoke for both AMV and ChAMV. Interestingly, in the case of ChAMV CP, only one nucleotide change, and amino acid substitution were confirmed between the CAL2 and CAR2, however, in the case of AMV CP between the CAL4 and CAR4, differences in 11 nucleotides and substitutions of seven amino acids were confirmed.

We also investigated the genetic variation of AMV and ChAMV between different plants of Chinese artichoke. Pairwise comparisons analysis showed that a total 666 bp of AMV CP between CAL1-CAL5 shared 99.25-100% (99.19-100%) identities at the nucleotide and amino acid levels, respectively. Partial sequences (843 bp) of ChAMV CP between CAL1-CAL5 shared 99.04-100% (99.02-100%) identity, respectively. Considering that the CP homology of AMV isolates reported worldwide is between 93.89-99.95% (Moradi and Mehrvar, 2021), the genetic diversity among AMV isolates from Chinese artichoke is not considered substantial. This means that AMVs isolated from Chinese artichoke are genetically highly conserved. A BLASTn search revealed that the AMV CA isolates showed the highest homology (99.1-99.4% identities) with the isolate "AMV/China_Wenxian_1/H5 (OL706260.1)" isolated from *Medicago sativa* in China. In phylogenetic analysis, AMV CA isolates were not closely related to AMV isolated from other host plants in Korea.

Since ChAMV was first reported in Japan in 2003, its complete genome sequence has not been revealed. Only

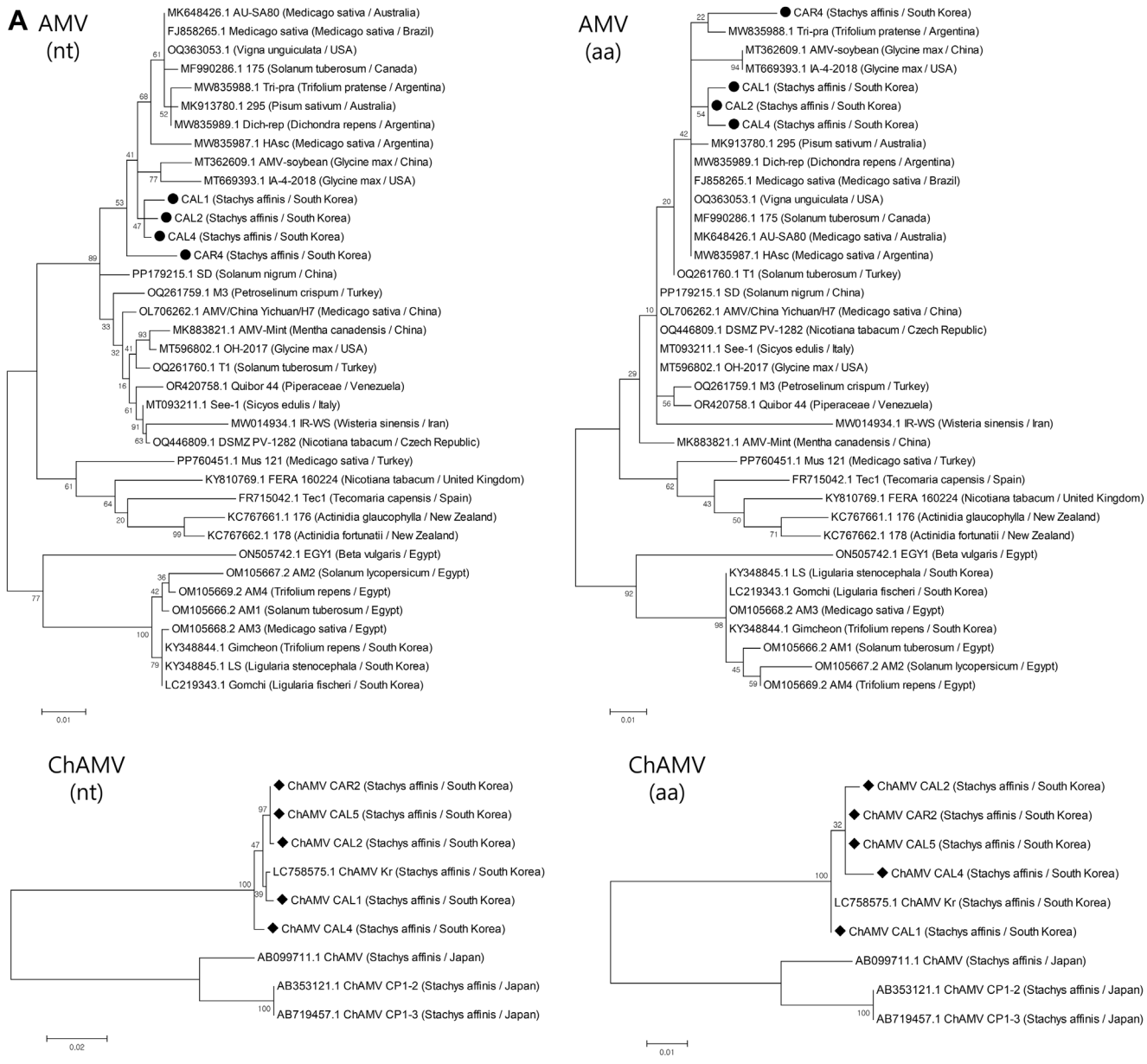

Fig. 4. Phylogenetic analysis of coat protein (CP) gene of artichoke mosaic virus (AMV) and Chinese artichoke mosaic virus (ChAMV) isolated from Chinese artichoke (A) and genetic variation in CP between the ChAMV Japan and Korea isolates (B). The phylogenetic tree constructed based on alignments of the nucleotide (nt) sequence and deduced amino acid (aa) sequence of the CP gene of AMV and ChAMV. The phylogenetic analysis was performed using the maximum likelihood method in the bootstrap test (1,000 replicates) by MEGA10 software. The horizontal branch lengths are proportional to genetic distance. The isolates of AMV and ChAMV isolated from Chinese artichoke (CA isolates) is marked with black circle or black diamond.

the CP gene and partial sequences of the NIb region on polyproteins identified from Chinese artichoke in Japan and Korea have been registered in the NCBI GenBank. We used the partial sequence of 867 nucleotides encoding the CP in the ChAMV polyprotein obtained from cDNA through RT-PCR for the following analysis. Alignments of the nucleotide sequences of ChAMV CP revealed that our sequence shared 86.34% identity with the CP of ChAMV identified in Japan. The amino acid sequence of CP was predicted using a nucleotide sequence derived from clones of the ChAMV PCR product. The amino acid homology of the CP gene between the two isolates was 89.93%. The results of the comparison of the CP amino acid sequences between the Japanese and Korean isolates are shown in Fig. 4. We confirmed that 29 of the 288 amino acids were different from each other, and variations in the amino acid residues were concentrated in the N-terminal of the coding region of CP gene. As the results of sequence comparison and phylogenetic analysis, our results show that the ChAMV CA isolates in Korea have high homology to each other, however, ChAMV between Japan and Korea showed significant genetic variations, even though they are isolated from the same plant species. ChAMV, identified in Japan, has only 1,675 nucleotides known out of about 10 kb, the average genome size of potyviruses, and nothing is known about the pathogenicity or biological characteristics. Therefore, further research is needed to determine whether the sequence differences between Korea isolates, and Japan isolate are related to the viral pathogenicity.

In summary, we identified the plant viruses infecting *S. affinis* exhibiting mosaic symptoms and investigated the incidence of these viruses. AMV and ChAMV were identified as the main viruses that infect *S. affinis*, and these two viruses generally coinfect plants. We also confirmed that the virus could be evenly distributed across all tissues, including reproductive organs such as flowers and tubers (roots). Despite ChAMV exhibiting a higher infection rate than AMV, viral accumulation within co-infected plants showed significantly higher levels of AMV than ChAMV, with the highest accumulation observed in leaves rather than flowers and tubers. Our results indicate that AMV and ChAMV have high infectivity in Chinese artichoke and can be transmitted to progeny plants through tubers. However, viral symptom expression and distribution were not correlated with viral accumulation levels. This information provides valuable insights into the distribution and spread of these viruses within plants, which has implications for the management and control of plant virus infection, and for producing virus-free Chinese artichoke.

Conflicts of Interest

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

Acknowledgments

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