Cucumber mosaic virus RNA replicase is essential for systemic infection in Brassica juncea

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Research Article

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Abstract

Background

*Brassica juncea* belongs to the *Brassicaceae* family and is used as both an oilseed and vegetable crop. As only a few studies have reported on the cucumber mosaic virus (CMV) in *B. juncea*, we conducted this study to fill this gap.

Methods

CMV-Co6, for which systemic infection was confirmed in *B. juncea* and non-infectious CMV-Rs1 were used. To identify the determinants of systemic infection in *B. juncea*, we first constructed infectious clones of CMV-Co6 and CMV-Rs1 and used them as pseudorecombinants. Subsequently, the amino acids of the 2a and 2b proteins were compared, and a chimeric clone was constructed.

Results

RNA2 of CMV was identified as an important determinant in *B. juncea* because *B. juncea* were systemically infected with RNA2-containing pseudorecombinants, CMV-Co6, R/6/R, and R/6/6, were systemically infected *B. juncea*. The chimeric virus R/6Rns/R6cp, in which the C-terminal region of the 2a protein of CMV-Co6 is replaced by CMV-Rs1, still infects *B. juncea*.

Conclusions

It is the 2a protein that determines the systemic CMV infection in *B. juncea*, suggesting that conserved 160G and 214A play a role in systemic CMV infection in *B. juncea*.

Background

Cucumber mosaic virus (CMV) is a plant pathogenic virus of *Cucumovirus* genus belongs to the family *Bromoviridae* [1]. CMV is distributed worldwide and has the widest host range of over 1200 species in 500 genera and 100 plants families among plant pathogenic viruses[2, 3]. Thus, CMV is considered an economically important virus [4]. Recently, the occurrence of plant viruses transmitted mechanically was decreasing while the occurrence of plant viruses transmitted by insect vector was increasing [5]. CMV disease is transmitted in a nonpersistent manner by more than 90 species and CMV infection is increasing [6]. CMV particle had icosahedral form (~ 30 nm in length) and contains three positive-sense single stranded RNAs [6, 7]. Genome of RNA1, RNA2 and RNA3 of CMV encode five open reading frames (ORFs). 1a of RNA1 and 2a of RNA2 are involved in RNA-dependent RNA polymerase (RdRp) activity [6]. The role of 2b encoded by RNA2 is known to long-distance movement and RNA silencing suppressor [8–10]. The 3a protein and coat protein (CP) are encoded in RNA3, and CP is transcribed and translated from
Based on genetic diversity, CMV strains have different abilities to infect [2, 11, 12]. Also, CMV strains isolated from a species had genetic diversity and biological variation [13, 14].

The leaf mustard (Brassica juncea) belongs to the family Brassicaceae and is native to China but is now widely cultivated in India, Europe, Canada, Australia, Korea and Japan [15]. B. juncea has various common names such as brown mustard, Chinese mustard, Indian mustard, and oriental mustard. In Korea, B. juncea is eaten raw or used as a main or sub-ingredient for kimchi [16, 17]. In China and India, B. juncea is used as both an oilseed and vegetable crop. Viruses that infect B. juncea include turnip mosaic virus (TuMV, genus Potyvirus), youcai mosaic virus (YoMV, genus Tobamovirus), CMV and have been reported [18–22]. Among them, TuMV is a virus that causes great damage to B. juncea, and many studies have been conducted [19–21].

Many of the specific amino acids and nucleic acids of CMV genes involved in virulence have been discovered [2]. 3a protein determined systemic infection in soybean, and CP affected systemic infection in squash [23, 24]. In radish, 2b protein and C-terminal region of the 2a protein had essential functions for systemic movement [25]. CMV infecting B. juncea has been reported since the 1940s [22]. However, CMV infecting B. juncea is unknown in Korea, and studies on the interaction between B. juncea and CMV are unknown worldwide.

In this study, we used the CMV isolate, CMV-Co4, CMV-Co6 and CMV-Rs1 to determine the viral factors involved in systemic infection in B. juncea. First, to find which genomic RNA(s) determine(s) systemic infection, we investigate the systemic infection of the two CMV isolates and their pseudorecombinants in B. juncea. For this, the pseudorecombinants were constructed from the in vitro RNA transcripts derived from the cDNA clones of their genomic RNAs in Nicotiana benthamiana. Furthermore, we explored the systemic infection of CMV at the genetic level using chimeric virus. Our results revealed that the N-terminal of RNA replicase of CMV determine systemic infection in B. juncea.

Materials and Methods

Plant materials

All plant materials were grown in growth chamber at 27°C and 60% humidity. In growth chamber, a light-dark schedule is 16 hours of light and 8 hours of darkness (LD 16:8). B. juncea, Capsicum annuum, Cucumis sativus, Cucurbita pepo, Raphanus sativus and Vigna unguiculata seeds commercially sold in Korea were purchased and used.

Virus source and mechanical inoculation

CMV-Co4 and CMV-Co6 were isolated from Commelina communis in Chuncheon, Korea. The two viruses were isolated by single local isolation from Chenopodium quinoa and maintained in N. tabacum cv. Xanthi nc. CMV-Rs1 generated by the infectious clone obtained from CMV-Gn isolated from R. sativus [26].
All plants were ground with 0.01M phosphate buffer (PB, pH7.2) and the sap was inoculated onto at least three leaves of carborundum dusted target plants. In case of *B. juncea*, *C. annuum*, *C. sativus*, *C. pepo*, *R. sativus* and *V. unguiculata*, only the cotyledon was inoculated with the viruses. Both viruses were tested for infectivity by mechanically inoculating *B. juncea* and *R. sativus* at least eight each in one experiment and repeated three times.

**RNA extraction and RT-PCR**

Total RNA was extracted from two leaf disc. The leaf discs were collected from inoculated leaves or systemic leaves of plant materials. Collected leaf discs was homogenized with normally total RNA extraction buffer and isolated with phenol (Sigma, USA) and Phenol:Chloroform:isoamyl alcohol (25:24:1) (Bioneer, Korea) twice. Extracted total RNA was ethanol-precipitated and maintained in -70°C deepfreezer.

RT-PCR was carried out two-step. In RT reaction, cDNA was synthesized by M-MLV reverse transcriptase (Promega, USA) using CP specific 3’ primers with the first step at 42°C for 60 min, in second step at 94°C for 5 min. The cDNA was subjected to PCR using CP specific primer sets with the first step at 94°C for 5 min, in second step at 34 cycles at 94°C for 30 s, 50°C for 30 s and 72°C for 1 min. In last step at 72°C for 5 min and in PCR reaction, cDNA was amplified by i-Taq DNA polymerase (iNtRON, Korea). Amplified RT-PCR products were loaded in 1.2% agarose gel stained with MIDORI Green Advance (Nippon genetics, Japan). The primers used in this study are listed in Table 1.

**Table 1. List of primers used for CMV-detection and full genome sequence of CMV isolates**

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Nucleotide sequence (5’ → 3’)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV RNA1 5’ end <em>BamHI</em> T7</td>
<td>CGGGATCCGCGAATTAAGGTCTTCTCTTTTGAAGG</td>
</tr>
<tr>
<td>CMV RNA2 5’ end <em>BamHI</em> T7</td>
<td>CGGGATCCGCGAATTAAGGTCTTCTCTTTTGAAGG</td>
</tr>
<tr>
<td>CMV RNA3 5’ end <em>BamHI</em> T7</td>
<td>CGGGATCCGCGAATTAAGGTCTTCTCTTTTGAAGG</td>
</tr>
<tr>
<td>CMV RNA1 and 2 3’ end <em>PstI</em></td>
<td>GCCTGCAGTGGTTCTCCTTTTGAAGG</td>
</tr>
<tr>
<td>CMV RNA3 3’ end <em>SphI</em></td>
<td>GCCATGCTGGTTCTCCTTTTGAAGG</td>
</tr>
<tr>
<td>CMV-CP-5’</td>
<td>ATGGGACAAATCTGAATCAACCAG</td>
</tr>
<tr>
<td>CMV-CP-3’</td>
<td>TCAGACTGGAGCAGCCTCA</td>
</tr>
</tbody>
</table>

*The underlined primer sequences indicate restriction enzyme site; Italicized sequences indicate the T7 promoter.

**Infectious clone construction and in vitro transcription**

The full-length RNAs of CMV-Co6, CMV-Co4 and CMV-Rs1 were amplified using *BamHI*-T7 polymerase sequence tagged 5’ primers and *PstI* or *SphI* tagged 3’ primers (Table 1). The plasmid vector pUC19 was digested with *BamHI*-*PstI* set for RNA1 and RNA3 or *BamHI*-*SphI* set for RNA2 and ligated with equally...
digested each RNAs. The primers used in this study are listed in Table 1. All clones constructed were sequenced and used in subsequent experiments. The complete genome sequences were deposited in Genbank with CMV-Rs1 (LC765220, LC765221 and LC765222 accession codes) and CMV-Co6 (LC765223, LC765224 and LC765225). Infectious full-length transcripts were in vitro synthesized using Bacteriophage T7 RNA polymerase (Thermo fisher scientific, USA) with Ribo m7G Cap Analog (Promega, USA).

For construction the RNA2 chimeric infectious clones, the restriction sites Ncol (1852) and Stul (2662) were digested in both RNA2 clones of CMV-Co6 and CMV-Rs1. Each fragment of Ncol to Stul cross-inserted into digested infectious clones.

In RNA3, the CP substitution clones were substituted with Apal (1142) and PstI digestion. Digested RNA3 fragment of CMV-Co6 with Apal and PstI inserted into digested CMV-Rs1 RNA3 infectious clone with Apal and PstI.

The transcribed RNAs were mixed with PB in ratio of 1:1:1:3 (RNA1:RNA2:RNA3:PB) and mechanically inoculated on three N. benthamiana plants. At 14 days post inoculation (dpi), viral propagation was confirmed by RT-PCR and mechanically back-inoculation on N. tabacum cv. Xanthi nc. Then, N. tabacum cv. Xanthi nc, which was confirmed to be infected with the virus, was used as a virus source.

Alignment analysis

Full-length RNA2 and RNA3 sequences of all CMV isolates were aligned using the MEGA7 tool. The two open reading frames, 2a protein and 2b protein, were translated with the MEGA7 tool and imaged using BioEdit software.

2a protein Structure Modeling

The 2a protein three-dimensional model was built using SWISS-MODEL Workspace (https://swissmodel.expasy.org/interactive) [27]. We applied the default setting for all parameters within the algorithms without any modifications.

Results

CMV-Co6 systemically infects B. juncea.

We examined the pathogenicity of CMV-Co6, CMV-Rs1, CMV-Co4, and CMV-Fny in the nine host plant species. The four CMV isolates were mechanically inoculated and detected in the upper leaves at 14 dpi by RT-PCR, and back inoculation of N. tabacum cv. Xanthi nc. All plant samples were inoculated in triplicate, and there was no difference in pathogenicity between individuals. The pathogenicity of the 4 CMV isolates in R. sativus was divided into two groups (See Supplementary Table 1). To confirm the infectivity of these 4 viruses in B. juncea, 8 B. juncea plants were mechanically inoculated with these four viruses, respectively. CMV-Co6 and CMV-Co4 induced systemic chlorosis in B. juncea and these viruses were detected by RT-PCR in all plants (See Supplementary Table 1). To exclude infection with other
Brassicaceae-infecting viruses such as TuMV and YoMV, back-inoculation was performed on *N. tabacum* cv. Xanthi-nc, which is consistent with the RT-PCR results. However, CMV-Rs1 and CMV-Fny did not infect any of the *B. juncea* plants (See Supplementary Table 1). CMV-Co6 and CMV-Rs1 were selected for subsequent experiments.

**RNA2 of CMV-Co6 determines systemic infection in *B. juncea***

To identify the RNA genome segment responsible for systemic CMV infection, infectious cDNA clones of CMV-Co6 and CMV-Rs1 and their pseudorecombinants were constructed by reassortment. RNA transcripts of pseudorecombinant viruses were generated using an in vitro transcription system and inoculated into *Nicotiana benthamiana*. Symptomatic leaves of the inoculated *N. benthamiana* were used as the inoculum for the experiments. Three pseudorecombinant viruses were named R/R/6, R/6/R, and R/6/6 using reassorted origin RNA segments, and were inoculated onto *B. juncea*. Although none of the recombinants induced symptoms in *B. juncea* or the parent virus, recombinant R/6/R and R/6/6 containing CMV-Co6 RNA2 spread to the upper leaves (Fig. 1, Fig. 2, and Additional File 1). Recombinant R/R/6 containing only RNA3 of CMV-Co6 was not infected in *B. juncea* (Fig. 1, Fig. 2, and Additional File 1). To clarify the systemic infection of *B. juncea* by RNA2, pseudorecombinant R/4/R containing RNA2 of CMV-Co4 was constructed and inoculated onto *B. juncea*. Similar to R/6/R, R/4/R were systemically infected with *B. juncea* (Fig. 1 and Additional File 2). These results suggest that RNA2 is involved in systemic CMV infection of *B. juncea*. Simultaneously, CMV-Co6, CMV-Rs1, and their pseudo-recombinant viruses were tested for infectivity in two *R. sativus* cultivars (Seoho-gold and Yeong-dong). Although there were differences in symptoms, systemic infection was confirmed in the two *R. sativus* cultivars when the virus contained RNA2 and RNA3 of CMV-Co6 (Table 2 and Additional File 3).

**Table 2**

Pathogenicity of pseudorecombinants and chimeric virus of CMV-Co6 and CMV-Rs1 in *Raphanus sativus*

<table>
<thead>
<tr>
<th>Host</th>
<th>Pathogenicity</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMV-Co6</td>
<td>CMV-Rs1</td>
</tr>
<tr>
<td><em>Raphanus sativus</em> cv. Seoho-gold</td>
<td>-/M (S)*</td>
<td>-/- (N)</td>
</tr>
<tr>
<td><em>R. sativus</em> cv. Yeong-dong</td>
<td>-/M (S)</td>
<td>-/- (N)</td>
</tr>
</tbody>
</table>

*Inoculated leaves/upper leaves symptoms (infectivity). M, mosaic; -, no symptoms or not infected. Infectivity was confirmed by RT-PCR and back-inoculation. L, locally infected; S, systemic infected; N, not infected.*

To determine the exact effect of RNA2 and RNA3 of CMV-Co6 on systemic infection in *R. sativus*, RT-PCR and back-inoculation were performed using the leaves of *R. sativus* inoculated with CMV-Rs1, R/6/R, and R/R/6. CMV-Rs1 and R/6/R were not infected with *R. sativus* and all, but R/R/6 was only locally infected (Table 2).
CMV RNA replicase (2a protein) determines systemic infection in B. juncea, but not 2b protein

To investigate the viral factors of CMV involved in B. juncea infection, we compared and analyzed the amino acid sequences of two genes, 2a and 2b, encoded by RNA2. There were 6 amino acid differences between CMV-Co6 and CMV-Rs1 in compound 2a (Table 3). Among them, four amino acids, 160G, 214A, 805I, and 832 L, were significantly different between CMV isolates infected with B. juncea and CMV isolates not infected with B. juncea (Table 3). In the 2b protein, CMV-Co6 and CMV-Rs1 showed no significant differences. In a previous study, the systemic infectivity of CMV to R. sativus was determined by the 2b protein and the C-terminal region of the 2a protein [25]. Therefore, we constructed an RNA2 infectious clone, 6Rns, in which the overlapping region of the 2a and 2b proteins were replaced by CMV-Rs1 in CMV-Co6 (Fig. 3). An RNA2 infectious clone, 6Rns in which the overlapping region of the 2a and 2b proteins was substituted from CMV-Co6 to CMV-Rs1 was constructed (Fig. 3). Therefore, the chimeric clone 6Rns of RNA2 has the two amino acids (805th and 832nd) of 2a of CMV-Rs1 (Fig. 3). At the same time, an RNA3 R6 clone in which CP, which is a major determinant of systemic CMV infection in R. sativus (family Brassicaceae), was substituted, was constructed (See Additional File 4). The chimeric pseudorecombinants consisted of R/R/R6cp, R/6/R6cp, and R/6Rns/R6cp, and their infectivity was confirmed in N. benthamiana by in vitro transcription. Three chimeric viruses were maintained in N. tabacum cv. Xanthi ncs were inoculated into B. juncea and the two R. sativus cultivars, and systemic infection was identified by RT-PCR and back-inoculation at 15dpi. R/6/R6cp and R/6Rns/R6cp were systemically infected with B. juncea (Fig. 1 and Additional File 1). In contrast, in the two R. sativus cultivars, only the case containing RNA2 and CP of CMV-Co6 (R/6/R6cp) was systemically infected, and R/6Rns/R6cp lost its ability to infect systemically (Table 2). These results suggest that two amino acids, 805I and 832 L, located in the C-terminal region of the 2a protein of CMV-Co6, may be involved in the systemic infection of CMV-Co6 in R. sativus, but there are differences in the determinants involved in systemic CMV infection in B. juncea.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amino acid position</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>160* Gly</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td>214* Ala</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td>313 Thr</td>
<td>Ile</td>
</tr>
<tr>
<td></td>
<td>449 Val</td>
<td>Asp</td>
</tr>
<tr>
<td></td>
<td>805* Ile</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td>832* Leu</td>
<td>Pro</td>
</tr>
</tbody>
</table>

Asterisks indicate that common amino acid residues of CMV-Co4 and CMV-Co6 that differ from CMV-Rs1.
To estimate the differences between these 2a proteins from the protein structure, the 2a proteins of CMV-Co6, CMV-Rs1, and chimeric 6Rns were modeled using SWISS-MODEL. The four 2a proteins matched the template nonstructural protein 4 RNA-directed RNA polymerase. In the predicted three-dimensional model, some changes were observed because of the substitution of the C-terminal region of the 2a protein (Fig. 4). However, the N-terminal region of 2a significantly changed its structure (Fig. 4).

Additionally, local infection of chimeric viruses in *R. sativus* was analyzed. The chimeric viruses R/R/R6cp and R/6Rns/R6cp, which contain the CP of CMV-Co6, only locally infected *R. sativus* and R/R/6 (Table 2).

**Discussion**

To date, few studies have analyzed the determinants of CMV infectivity in *Brassicaceae* crops [25, 28]. CMV-Co4 and CMV-Co6, isolated from the weed *C. communis*, can infect various host plants and induce distinct symptoms (See Supplementary Table 1). In addition, CMV-Co4 and CMV-Co6 infected in *R. sativus* and *B. juncea* were systemically, although only mild symptoms were observed (See Supplementary Table 1). These results suggest that showing to the wide host range of CMV, cross-infection between various crops occurs, and CMV hides in some plants.

The RNA2 of CMV-Co6 is involved in systemic infection in *B. juncea* and acts in a dose-dependent manner, despite differences in the genes encoded by RNA1 and RNA3. In the present work, recombinants R/6/6 and R/6/R containing RNA2 of CMV-Co6 were systemic infected in *B. juncea*, but no symptoms were observed compared to CMV-Co6 (Fig. 2). These results indicate that RNA2 is required for systemic infection, whereas RNA1 is required for symptom induction. R/R/6 containing RNA3 of CMV-Co6 caused local infection in *R. sativus*. In addition, *R. sativus* was locally infected with the chimeric virus R/R/R6, which contains only the CP of CMV-Co6 (Table 2). These results suggest that the gene involved in host adaptation to CMV in *R. sativus* is CP, and that systemic infection with the 2a protein occurs sequentially after local infection with CP. Host infection by plant pathogenic viruses requires a series of processes such as plant penetration, host recognition, and uncoating [29, 30]. The CP of CMV is involved in cell-to-cell and long-distance movement in cowpeas and tobacco, as well as in determining host adaptation to maize [31, 32]. Plants protect themselves against viruses through various mechanisms. Resistance at the single-cell level, termed extreme resistance, is a condition in which viral replication does not occur or occurs at essentially undetectable levels in the inoculated cells [33]. The inoculated leaves of *R. sativus* back-inoculation showed that the viruses containing the CP of CMV-Co6 were still biologically active at 14 dpi. Therefore, at least in *R. sativus*, CP of CMV-Co6 is thought to overcome the extreme resistance of *R. sativus*. This was determined by six amino acids (24A, 28S, 179F, 156A, 188Y, and 205V) in the CMV-Co6 CP. The CMV CP has been studied for functional changes caused by single amino acid changes. CMV CP amino acids 129 and 214 determine cell-to-cell movement in squash, and 129 determines local symptom expression in some plant species [23, 34]. Another CMV CP amino acid, 148, affects symptom recovery through phosphorylation, and 162nd affects aphid transmission [35, 36]. In *R. sativus*, the systemic infection factors of CMV CP are suggested to be 17 and 129 prolines [25]. However, amino acids
17 and 129 of CMV-Co6 and CMV-Rs1 were identified as proline. These results suggest that the function of the CMV CP in R. sativus may be the result of another interaction beyond a single amino acid level. Similar to the 2a protein, changes in CP were specific to R. sativus, highlighting the specific interaction of the plant species with the virus. In addition, previous studies on CMV infectivity in R. sativus have used CMV-Y and CMV-D8 [28]. Because CMV-Rs1 is non-infectious, whereas CMV-Y is a locally infecting strain, this study is the first to identify the local determinants of CMV infection in R. sativus.

We confirmed that the 2a protein plays a decisive role in the systemic infection of CMV in B. juncea using a chimeric infectious clone (Fig. 1, Fig. 2 and Additional File 1). It is well known that mainly 3a and 2b proteins play a role in the systemic infection of host plants by CMV [24, 37, 38]. Although the 2a protein is mainly responsible for RNA replication, some studies have reported that the N-terminal region or the GDD motif contributes to systemic infection [39, 40]. Particularly, in R. sativus, which belongs to the Brassicaceae family, CMV requires overlapping regions 2a, 2b, and CP for systemic infection [25, 28]. In our study, unlike CMV-Rs1, CMV-Co6 was systemically infected in R. sativus, and chimeric R/6/R6cp containing RNA2 and the CP of CMV-Co6 (Table 2). However, the chimeric virus R/6Rns/R6cp, in which the C-terminal region of 2a was substituted, lost its ability to infect R. sativus (Table 2). This is consistent with previous results showing that the CMV requires the CP and C-terminal region of 2a for systemic infection in R. sativus [25]. At the amino acid level, 805I and 832L of the 2a protein were shown to be essential for systemic infection of CMV in R. sativus but differed from the previously targeted 2a protein of CMV-D8 (AB179765). These results are thought to be due to structural differences in proteins and specific matches between viruses and hosts. Simultaneously, changes caused by 805I and 832L in the CMV 2a protein did not affect systemic infection in B. juncea (Fig. 1). Alignment analysis of the 2b and 2a proteins indicated that the N-terminal region of the 2a protein is essential for the CMV to systemically infect B. juncea, while the 2b protein can be excluded. 2a protein interacts with 1a protein to form a replicase complex and is negatively modulated by the phosphorylation of its N-terminal region [41]. When compared with the 2a protein sequence of R/4/R infected systemically in B. juncea, 160G and 214A of the 2a protein, which are targeted as factors for systemic infection in B. juncea, are located at potential phosphorylation sites (125–335). Phosphorylation is a post-translational modification that alters protein function and has been shown to play various roles in several viruses, such as the bamboo mosaic virus, brome mosaic virus, cauliflower mosaic virus, and potato virus A [42–46]. We modeled four 2a proteins, CMV-Co6, CMV-Rs1, chimeric 6Rns, and chimeric R6ns, using a protein prediction model to structurally analyze changes in amino acids. The four 2a proteins were modeled as viral RdRp but were structurally changed significantly by the four N-terminal amino acids. Changes in the two amino acids of the C-terminus of the 2a protein appeared to be key for determining systemic infection in R. sativus, although the changes were very small in the prediction model. These results suggest that changes, such as phosphorylation of the N-terminus of RNA replicase, affect the systemic infection of CMV, specifically in B. juncea. However, the results caused by the C-terminal changes in the 2a protein in R. sativus were not interpreted. Therefore, future research is necessary to create point mutants, analyze actual phosphorylation, and identify host counterparts.
Brassicaceae includes major economic crops such as Chinese cabbage, radish, and mustard. The genus Brassica comprises 37 species. Many Brassicaceae crops are consumed as leafy vegetables and are damaged by viruses [15, 17, 47, 48]. CMV does not cause significant damage to Brassicaceae crops but is mostly found as a co-infection with other viruses. The 2b protein of CMV is a viral suppressor of RNA silencing (VSR), which is known to intensify symptoms due to a synergistic effect when co-infected with heterogeneous viruses [49, 50]. In N. benthamiana and Arabidopsis thaliana, CMV induced more severe symptoms when co-infected with TuMV [51, 52]. Even in R. sativus, CMV has been reported to cause systemic infection through co-infection with TuMV [12, 52, 53]. In addition, CMV-Co6 used in this study induced symptoms in both B. juncea and R. sativus following a single infection (Fig. 2, and Additional File 3). Therefore, a study of the interaction between CMV and Brassicaceae crops is necessary. In this study, we identified the determinants of systemic infection by CMV infecting B. juncea; however, further studies on CMV pathogenicity in the Brassica genus including bok choy, cabbage, Chinese cabbage and turnip are needed.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests: The authors declare that they have no competing interests.

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References


34. Kobori T, Miyagawa M, Nishioka K, Ohki ST, Osaki T. Amino acid 129 of Cucumber mosaic virus coat protein determines local symptom expression and systemic movement in Tetragonia expansa,


**Figures**

**Figure 1**

Schematic diagram of used viruses with different RNA and their host response in *N. benthamiana* and *B. juncea*. The three boxes indicated that origin RNA. The black box represents CMV-Co6 or CMV-Co4 and
the white box represents CMV-Rs1. Infectivity was confirmed by RT-PCR and back-inoculation. *infected plants/inoculated plants.

**Figure 2**

Host response of *Brassica juncea* inoculated with CMV-Co6, CMV-Rs1 and their pseudorecombinants and chimeric virus. Photographs were taken 14 days post inoculation. Only CMV-Co6 induced systemic
chlorosis (Chl). – indicated that no symptoms. Black arrows indicated that light green island by chlorosis.

**Figure 3**

Schematic diagram of genome structure for chimeric RNA2. (a) Structure of the chimeras constructed between CMV-Co6 and CMV-Rs1. R, 6 and 6Rns indicate that their origin genome type. The chimeric RNA2 6Rns was generated by exchange of *NcoI*/*Stul* fragment. Asterisks indicated that the six different amino acids. Infectivity was confirmed by RT-PCR and back-inoculation. \(^1\)Infected plants/inoculated plants.
Figure 4

Three-dimensional model of 2a protein. (a) CMV-Co6. (b) CMV-Rs1. (c) Chimeric 6Rns. (d) Chimeric R6ns. Blue arrows indicate N-terminal. Red arrows indicate C-terminal.

Supplementary Files

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- Additionalfile4.pptx