PhD THESIS

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THE ROLE OF MOVEMENT PROTEIN IN THE DIFFERENT HOST RANGE AND SYMPTOM DEVELOPMENT OF CMV AND PSV

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1. BACKGROUND AND OBJECTIVES

Cucumber mosaic virus (CMV) and *Peanut stunt virus* (PSV) are members of the genus *Cucumovirus*, which is the genus with the broadest and most diverse host range. CMV threatens cultivated vegetable plants the most, including zucchini (Cucurbitaceae), peppers (*Capsicum annuum* L.) and tomatoes (*Solanum lycopersicum* L.), while PSV predominantly infects leguminous plants (Fabaceae). Despite their similarities in genomic structure, amino acid sequences, and protein functions, the host ranges of these two viruses differ considerably. To date, all protein-coding genes of cucumoviruses have been identified as genes responsible for pathogenicity. However, there is a lack of available information on the function of movement protein (MP) and the exact mechanism of MP's interaction with plant proteins. The aim of this thesis is to expand on this knowledge, as the detailed understanding of these mechanisms presents new opportunities in the development of plant virus control strategies, particularly through the creation of resistant plant varieties, with the aid of advanced biotechnological approaches.

Objectives of the study:

1. To investigate the host range and symptoms of *Cucumber mosaic virus* (CMV) and *Peanut stunt virus* (PSV) on horticultural crops and on test plants used in virological research.

2. To generate CMV and PSV RNA 3 reassortant and movement protein (MP) recombinant viruses using infectious *in vitro* virus transcripts, and to assess the infectivity of the resulting viruses.

3. To inoculate *Nicotiana benthamiana* Domin. and *Capsicum annuum* L. cv. Brody test plants with reassortant and recombinant viruses, characterize the symptoms, and observe differences in symptoms.

4. To examine the subcellular localization of CMV MP-eGFP and PSV MP-eGFP fusion proteins in epidermal cells of *N. benthamiana* Domin. and *C. annuum* L. cv. Brody.

5. To investigate the colocalization of CMV MP-eGFP and PSV MP-eGFP fusion proteins with plant plasmodesmata in *N. benthamiana* cells, and to study their binding to plasmodesmata in plasmolyzed cells.

6. To study the colocalization of CMV MP-eGFP and PSV MP-eGFP fusion proteins with plant plasmodesmata in epidermal cells of *C. annuum* cv. Brody plants.

2. MATERIALS AND METHODS

2.1. Materials Used in the Study

2.1.1. Plant material

For infection and intracellular localization studies, the following test plants were used: *Nicotiana benthamiana* Domin, *Nicotiana tabacum* L. cv. Xanthi-nc, *Nicotiana glutinosa* L., *Chenopodium murale* L., *Cucumis sativus* L. cv. Szenzáció, *Solanum lycopersicum* L. cv. Moneymaker, and *Capsicum annuum* L. cv. Brody. The *Nicotiana* species and *C. murale* were kept under long-day conditions, with 16 hours of light and 8 hours of dark at 24ºC during the light period and 16ºC during the dark period. Cucumber, pepper, and tomato plants were also grown under long-day conditions, but at 26ºC during the light period and 23ºC during the dark period.

2.1.2. Virus Isolates, Infectious Clones

The virus isolates used in this study: the Rp-PSV isolate was obtained by Dr. Pál Salamon in Gödöllő, from black locust (*Robinia pseudoacacia* L.), and its infectious clones pRP1-PSV, pRP2-PSV, pRP3-PSV were described by KISS et al. (2008). The Rs-CMV isolate was obtained by Dr. Pál Salamon in Gödöllő, from radish (*Raphanus sativus* L.), and its infectious clones pRS1-CMV, pRS2-CMV, pRS3-CMV were described by DIVÉKI et al. (2004). The MP recombinant infectious clones pCP3, pPC3, and pPΔC3 were generated using overlap PCR.

2.2. Methods Used in the Study

2.2.1. Generation of MP Recombinant Infectious Clones

The MP recombinant RNA 3s were constructed using overlap PCR with CMV and PSV RNA 3 (pRs3 and pRp3) cDNA clones. The infectious clone pCP3 was created by replacing the CMV MP and 5' non-coding region with the corresponding region of PSV RNA 3. The PCR product obtained from overlap PCR served as a template for in vitro transcription, which included the CMV 5' non-coding region and MP, as well as the non-coding region between PSV MP and CP, the CP, and the 3' non-coding region. The recombinant PCR product contained a T7 promoter and *Pst*I restriction site at the 5' end, and another *Pst*I site at the 3' end.

The infectious clone pPC3 was also created using the aforementioned cDNA clones and overlap PCR. The PSV MP and 5' non-coding region were replaced with the corresponding region of CMV RNA 3. After overlap PCR, the PCR product contained the PSV 5' non-coding region and MP, as well as the non-coding region between CMV MP and CP, the CP, and the 3' non-coding region. The recombinant PCR product contained a T7 promoter and *Bam*HI restriction site at the 5' end, and a *Bam*HI site at the 3' end.

The infectious clone pPΔC3 was constructed using the pRs3 and pRp3 cDNA clones via overlap PCR. The PSV MP and 5' non-coding region were replaced with the corresponding sequence of CMV RNA 3, and the nucleotide corresponding to the C-terminal 42 amino acids of the PSV MP was deleted from the sequence. After overlap PCR, the PCR product contained the PSV 5' non-coding region and the MP with a deletion of the 42 C-terminal amino

acids, as well as the non-coding region between CMV MP and CP, the CP, and the 3' non-coding region. The recombinant PCR product contained a T7 promoter and *Bam*HI restriction site at the 5' end, and a *Bam*HI site at the 3' end.

The MP recombinant PCR products (pCP3, pPC3, pPΔC3) generated by overlap PCR were transformed into *E. coli* DH5α strain according to the method of INOUE et al. (1990). The nucleotide sequence of the recombinant clones was determined at Biomi Ltd. (Gödöllő).

2.2.2. *In vitro* transcription, inoculation, and virion purification

Inoculums for the test plants were prepared using infectious *in vitro* transcripts. The previously mentioned infectious clones were used as templates. For CMV: pRs1-CMV, pRs2-CMV, pRS3-CMV; for PSV: pRp1-PSV, pRp2-PSV, pRp3-PSV; for the RNA3 reassortant viruses: pRs1-CMV, pRs2-CMV, pRp1-PSV, pRp2-PSV, pRS3-CMV, pRp3-PSV; and for the MP recombinant viruses: pRs1-CMV, pRs2-CMV, pCP3, pPC3, and pPΔC3 clones were used. The plasmids carrying the clones were linearized using *Bam*HI and *Pst*I restriction endonuclease enzymes. The transcript synthesis was carried out with T7 polymerase enzyme (SZILASSY et al., 1999).

In vitro RNA transcripts from the cDNA clones were mixed in equal amounts in different combinations, added to 40 µl of inoculation buffer so that the inoculum contained equal amounts of transcripts corresponding to RNAs 1, 2, and 3.

Virions were purified from upper leaves of *N. benthamiana* plants that showed symptoms of infection three weeks post-inoculation (LOT and KAPER,

1976). In the infection experiments, the purified virions were used to infect test plants at equal concentrations.

2.2.3. Preparation of MP-eGFP fusion proteins for intracellular localization studies

For intracellular localization studies, MP-eGFP fusion proteins were prepared. The gene segment encoding the CMV MP and PSV MP was fused to a sequence encoding eGFP at the C-terminal end, after the stop codon of the MPs was removed, allowing for the translation of the MP together with the GFP protein. The two fusion proteins were generated using overlap PCR with pRs3, pRp3, and peGFP cDNA clones. The PCR products obtained by overlap PCR contained *Sac*I restriction sites at the 5' end and *Bam*HI restriction sites at the 3' end. The PCR products were transformed into *E. coli* DH5α strain using the method described by INOUE et al. (1990). The nucleotide sequencing of the recombinant clones was performed by Biomi Ltd. (Gödöllő).

2.2.4. *Agrobacterium* Transformation and Agrobacterium-Mediated Transient Gene Expression

The MP-eGFP clones, prepared by overlap PCR and cloned in *E. coli*, were transformed into *Agrobacterium tumefaciens* C53C1 strain after being ligated into a pBin expression plasmid vector using the integrated restriction sites (*Sac*I, *Bam*HI).

For Agrobacterium-mediated transient gene expression, the concentration of *Agrobacterium* cultures carrying the MP-eGFP plasmids was adjusted prior to agroinfiltration. The bacterial cultures carrying the MP-eGFP construct were adjusted to an OD600 of 0.4. Additionally, for each construct, an

Agrobacterium strain carrying the P19 silencing suppressor protein was added to the suspension (JAY et al., 2023), with the P19 strain concentration set to OD600 0.2. The Agrobacterium suspensions were injected into the abaxial surface of 4–5 leaf stage *Nicotiana benthamiana* and *Capsicum annuum* plants using a syringe (NEMES et al., 2014).

2.2.5. Confocal Laser Scanning Microscopy

The intracellular localization of eGFP-tagged MPs was observed using confocal laser scanning microscopy, 24 hours after agroinfiltration.

To label the plasmodesmata aniline blue staining was used, which specifically stains the callose present at the plasmodesmata (KUMARI et al., 2021). For plasmolyzed cell studies, leaf disk samples were soaked in a 10% NaCl solution for 15 minutes.

2.2.6. Statistical Analysis and Image Processing

To quantify the plasmodesmata colocalization of MPs, we used the Pearson correlation coefficient (PCC). Fifty plasmodesmata were measured from five different microscopic images using the colocalization plugin of the Fiji ImageJ (1.52) software (LEASTRO et al., 2021). PCC values between 0.2 and 0.4 indicate weak correlation, while values above 0.5 indicate strong correlation. Negative values represent inverse correlation (DUNN et al., 2011; LEASTRO et al., 2018). To determine significant differences in plasmodesmata colocalization, a one-way ANOVA model was applied ($P<0.05$). For pairwise comparisons of CMV MP-eGFP and PSV MP-eGFP in *C. annuum* cells, a two-sample t-test was performed, with a significance level of $P < 0.05$.

2.2.7. Virus and eGFP Detection

To confirm the success of infection, CMV, PSV, RNA 3 reassortant, and MP recombinant viruses were detected from inoculated *N. benthamiana* and *C. annuum* plants using RT-PCR, cDNA was synthesized from the extracted RNA by performing reverse transcription. PCR analysis was then conducted using the cDNAs. Specific oligonucleotide primers for the CP were used for virus detection.

For the samples from plants infiltrated with MP-eGFP fusion proteins, protein extraction was carried out and the protein samples were separated in a 12% denaturing (SDS-containing) polyacrylamide gel and then transferred onto a nitrocellulose membrane. To block the membrane, Western Blocker Solution (Sigma) was used. The eGFP fusion protein associated with MPs was detected with a rabbit polyclonal anti-GFP primary antibody (Agrisera) at a dilution of 1:3,000. The secondary antibody was HRP-conjugated anti-rabbit antibody (Agrisera) at a dilution of 1:5,000. For visualization, the Pierce ECL HRP detection reagent kit (Thermo Fisher Scientific) was used.

3. RESULTS

3.1. Host Range and Symptoms of CMV and PSV on Different Host Plants

According to previous studies, despite the similar genome organization and properties of CMV and PSV, their host ranges differ significantly (EDWARDSON and CHRISTIE, 2018; MINK et al., 1969). Commonly used test plants and agriculturally significant plants were inoculated with Rp-PSV and Rs-CMV to compare the host ranges and symptoms caused by the two viruses.

Both *N. glutinosa* and *N. benthamiana* test plants were systemically infected by both viruses, although the symptoms were milder in the case of PSV. The *N. tabacum* cv. Xanthi-nc plant was systemically infected by CMV, but PSV did not caused systemic symptoms on this plant. *Chenopodium murale* served as a local lesion host for both viruses, but we observed a difference in lesion size; PSV caused smaller lesions on the inoculated leaves than CMV. The cucumber plant (*Cucumis sativus*) was systemically infected by CMV, causing leaf deformation and severe mosaic symptoms on the systemic leaves, while PSV did not infected the plant. Tomato (*Solanum lycopersicum*) proved to be a systemic host for CMV, causing strong mosaic symptoms, dwarfing, and leaf narrowing. No symptoms were observed on tomato plants infected with PSV. Since the host range of PSV mainly includes legumes, we also studied the symptoms caused by both viruses in beans (*Phaseolus vulgaris*). CMV did not cause systemic symptoms in the infected plants. However, PSV caused severe dwarfing, curling, leaf deformation, twisting, and mild mosaic patterns on the foliage.

3.2 Generation of Infectious RNS3 Reassortant and MP Recombinant Viruses and Inoculation of *Nicotiana benthamiana* Test Plants

To investigate the differences observed in the host range and symptoms caused by the two cucumoviruses, we generated various RNS3 reassortant and MP recombinant viruses. The infectivity of these viruses was tested on a common host plant, *Nicotiana benthamiana*. Three-leaf stage *N. benthamiana* plants were inoculated with CMV, PSV, C12P3 and P12C3 RNS3 reassortants, as well as C12CP3 and C12PC3 MP recombinant viruses. The development of symptoms was monitored over the next three weeks.

Except for the C12PC recombinant virus, all constructs were able to infect *N. benthamiana* systemically, although the intensity of symptoms varied significantly. The CMV MP-containing viruses caused severe leaf deformation, strong chlorotic mosaic, and stunting of the entire plant. Symptoms appeared four days post-inoculation. In contrast, the PSV MP-containing viruses (PSV, C12P3) induced symptoms later, appearing on day seven, and these were milder, mainly consisting of a mild mosaic and slight leaf deformation.

According to previous studies, there are examples within the Bromoviridae family where the MP of two viruses can function together with the CP of the other virus only when a C-terminal 33 amino acid deletion is present (NAGANO et al., 2001; SALÁNKI et al., 2004). Since the C12PC3 recombinant was not infectious, we identified the homologous region in the PSV MP corresponding to the C-terminal 33 amino acids of the CMV MP, which was the C-terminal 42-amino acids. We created a PSV MP C-terminal 42 amino acid deletion mutant (pPΔC3). Upon inoculating *N. benthamiana*,

we observed systemic symptoms seven days post-inoculation, which, like the other PSV MP-containing constructs (PSV, C12P3), were milder and developed more slowly.

The dynamics of *N. benthamiana* infection were also examined using RT-PCR with CP-specific primers for all viral constructs, alongside visual observations. Two days post-inoculation, viral RNA was not detectable in any of the constructs. Four days post-inoculation, the CMV MP-containing constructs, where symptoms had already appeared, showed detectable viral RNA (CMV, P12C3, and C12CP3). Seven days post-inoculation, viral RNA was detectable in all virus constructs (CMV, PSV, C12P3, P12C3, C12CP3, and C12PΔC3), even in those with later symptom onset.

In subsequent experiments, we used the deletion mutant for the infections. Virions were purified from infected plants of all virus constructs and used for further inoculation experiments.

3.3 Inoculation of *Capsicum annuum* with RNS3 Reassortant and MP Recombinant Viruses

We continued our investigations using *Capsicum annuum* cv. Brody, which is a host for CMV but not for PSV. Inoculated plants exhibited strong symptoms of CMV infection, including severe leaf deformation, characteristic chlorotic mosaic, and stunting of the plant. Symptoms appeared two weeks post-inoculation and progressively intensified over time. In contrast, PSV did not induce any symptoms in the plants.

The P12C3 and C12CP3 constructs, which contain the CMV MP, caused severe leaf distortion, mosaic symptoms, and stunting similar to the CMV

infection. The PSV MP-containing viruses, C12P3 and C12PΔC3, remained asymptomatic during the observation period, similar to PSV infection. The visual observations were supported by RT-PCR.

3.4 Subcellular Localization of CMV MP-eGFP and PSV MP-eGFP Fusion Proteins in Nicotiana benthamiana Cells

During the infection of test plants, we identified the MP as a key factor influencing symptom type and host range. The primary function of MP is to facilitate viral movement from cell to cell, which requires localization of the MP at the plasmodesmata (NAVARRO et al., 2019). We compared the subcellular localization of CMV MP and PSV MP in *Nicotiana benthamiana* epidermal cells, as well as their colocalization with plasmodesmata, using CMV MP-eGFP and PSV MP-eGFP fusion proteins.

Both PSV MP-eGFP and CMV MP-eGFP were observed at the cell periphery in punctate formations via confocal microscopy. However, CMV MP-eGFP clearly localized at the plasmodesmata, while PSV MP-eGFP was more homogeneously distributed throughout the cytoplasm. The ratio of MP-eGFP signal localization at plasmodesmata versus plasma membrane was quantified, and it was found to be significantly higher for CMV MP-eGFP compared to PSV MP-eGFP. This supports the finding that CMV MP predominantly localizes at the plasmodesmata, whereas PSV MP is more evenly distributed between the plasma membrane and plasmodesmata.

To quantify the colocalization of the MPs with plasmodesmata, we used the Pearson correlation coefficient (PCC), and statistical analysis revealed significant differences between the two proteins. A strong colocalization with

plasmodesmata was observed for CMV MP-eGFP ([PCC] = 0.67), whereas PSV MP-eGFP showed weaker colocalization with plasmodesmata ([PCC] = 0.42). This difference was statistically significant.

We also examined the binding of CMV MP-eGFP and PSV MP-eGFP to plasmodesmata in plasmolyzed *N. benthamiana* epidermal cells. In this experiment, only the MP-eGFP proteins bound to plasmodesmata remained at the cell wall, while unbound proteins detached from the cell wall along with the detached plasma membrane due to plasmolysis. This experiment confirmed that PSV MP exhibited weaker binding to plasmodesmata.

Our results indicate that while PSV MP can localize to plasmodesmata, it does not integrate into the structure as effectively as CMV MP.

3.5 Subcellular Localization of CMV MP-eGFP and PSV MP-eGFP Fusion Proteins in *Capsicum annuum* Cells

Since in *Capsicum annuum* cv. Brody infection experiments, we identified the MP as a limiting factor for infection, further investigations were conducted in this plant regarding the subcellular localization of the two MPs. CMV MP-eGFP formed punctate structures around plasmodesmata, while PSV MP-eGFP was primarily observed in the cytoplasm, with minimal overlap with plasmodesmata signals. The ratio of CMV MP-eGFP at plasmodesmata versus plasma membrane was significantly higher than the ratio for PSV MP-eGFP, that presence was more pronounced in the cytoplasm.

To measure the colocalization of the MPs with plasmodesmata, we used the Pearson correlation coefficient (PCC). CMV MP-eGFP showed a strong colocalization with plasmodesmata ([PCC] = 0.74), which was higher than the

correlation observed in *Nicotiana benthamiana* cells. PSV MP-eGFP exhibited very little colocalization with plasmodesmata ([PCC] = 0.25), which was much lower than the correlation observed in *N. benthamiana* cells ([PCC] = 0.42).

We concluded that while PSV MP can be detected at plasmodesmata, it does not bind to them as effectively as CMV MP. This difference in the MP's ability to associate with plasmodesmata likely causes the differences in host range between the two viruses in *C. annuum*, as well as the differences in symptom development in *N. benthamiana*.

4. DISCUSSION

Cucumber mosaic virus (CMV) is known to infect more than 1200 host species across more than 100 plant families, whereas *Peanut stunt virus* (PSV) primarily infects legumes, despite their taxonomic similarities and functional similarities in genome organization and protein function. The host range and symptom development are complex phenomena influenced by different virus-plant interactions. In the case of cucumoviruses, the role of all five viral proteins in host range determination has been established, but detailed information about these interactions is still lacking (SALÁNKI et al., 2018).

We investigated the diversity of host range and symptoms of CMV and PSV using commonly used test plants in plant virology and important crops in vegetable production. As reported in the literature, PSV was unable to infect *Nicotiana tabacum* cv. Xanthi systemically, while CMV was able to cause systemic symptoms in this plant. On *Nicotiana benthamiana* test plants, both viruses caused systemic symptoms, but differences were observed in the intensity and phenotype of these symptoms (KISS et al., 2008). In cucumber and tomato, CMV caused systemic symptoms, while PSV did not. However, in legumes such as bean (a member of the Fabaceae family), PSV infected systemically, whereas CMV did not.

To investigate the differences in host range and symptoms of CMV and PSV, we generated RNS3 reassortants (C12P3, P12C3) and MP recombinants (C12CP3, C12PC3). Previous studies that involved *Groundnut rosette virus* (GRV), and *Cymbidium ringspot virus* (CymRSV) MP have also demonstrated the role of the CMV movement protein (MP) in determining the host range, (HUPPERT et al., 2002; RYABOV et al., 1999).

In our study, after inoculating *N. benthamiana* with recombinant and reassortant viruses, all constructs, except for the C12PC recombinant, caused systemic infection. Based on previous studies, it is known that within the Bromoviridae family, the MP of two viruses only functions together with the coat protein (CP) when the C-terminal 33 amino acids are deleted (NAGANO et al., 2001; SALÁNKI et al., 2004). Therefore, based on this information, we created a C12PC recombinant with an MP C-terminal deletion mutant (C12PΔC), where the CMV MP C-terminal 33 amino acids were removed from the PSV MP sequence. The C12PΔC mutant was able to cause systemic symptoms in *N. benthamiana*. These results suggest that the PSV MP, similar to the TAV MP, is less flexible than the CMV MP, which may explain CMV's much broader host range. In infection experiments, viruses containing the CMV MP (P12C3, C12CP3) caused typical CMV symptoms such as dwarfing, severe chlorotic mosaic, and pronounced leaf deformation, with symptoms detectable in systemic leaves as early as four days post-inoculation. In contrast, PSV MP-containing constructs (C12P3, C12PΔC3) caused milder symptoms such as slight leaf deformation and weaker mosaic patterns, with systemic symptoms detectable only seven days post-inoculation.

We extended the infection studies to *Capsicum annuum* cv. Brody, which is a host of CMV but not of PSV. The P12C3 and C12CP3 reassortant and recombinant viruses, containing the CMV MP, caused strong leaf distortion, pronounced mosaic symptoms, and dwarfing in the pepper plant, similar to CMV infections, whereas PSV MP-containing viruses (C12P3, C12PΔC3) were unable to infect *C. annuum* systemically. Based on our infection experiments in *N. benthamiana* and *C. annuum*, we conclude that the

differences in host range and symptoms between CMV and PSV are associated with the MP of each virus.

The primary role of the cucumoviral MP is to facilitate the virus's movement from cell to cell through plasmodesmata, which requires proper localization of the MP at the plasmodesmata (NAVARRO et al., 2019). We compared the subcellular localization of CMV MP-eGFP and PSV MP-eGFP fusion proteins in *N. benthamiana* epidermal cells. CMV MP-eGFP was clearly localized at the plasmodesmata, with a higher plasmodesma/plasma membrane ratio, while PSV MP-eGFP was more homogeneously distributed in the cytoplasm with less clear localization at the plasmodesmata, resulting in a lower plasmodesma/plasma membrane ratio. The Pearson correlation coefficient (PCC) analysis confirmed strong colocalization with plasmodesmata for CMV MP-eGFP ([PCC] = 0.67), whereas PSV MP-eGFP showed weaker colocalization with plasmodesmata ([PCC] = 0.42). In plasmolyzed *N. benthamiana* epidermal cells, we confirmed that while PSV MP was detectable at the plasmodesmata, it did not integrate into their structure as efficiently as CMV MP.

Since *C. annuum* cv. Brody is susceptible to CMV but not to PSV, we conducted further studies on this plant. The CMV MP-eGFP showed strong colocalization with plasmodesmata ([PCC] = 0.74), higher than that observed in *N. benthamiana*. In pepper epidermal cells, PSV MP-eGFP showed very weak colocalization with plasmodesmata ([PCC] = 0.25), much lower than the correlation observed in *N. benthamiana* ([PCC] = 0.42).

The restriction of cell-to-cell movement could limit infection or delay symptom development. The structure of PSV MP belongs to the 30K

superfamily, and the main domains show strong homology with CMV MP. The largest difference between them is observed in the C-terminal region, which is longer (by 7 amino acids) than the CMV or TAV C-terminal regions. Previous studies have shown that the CMV MP's integration into plasmodesmata is facilitated by a phosphorylatable serine residue at position 28 (SÁRAY et al., 2021), and this residue is also present in PSV MP. In the 30K superfamily, a plasmodesma localization signal was recently identified at the N-terminal 50th amino acid in TMV (YUAN et al., 2016). A key interacting partner of this plasmodesma localization signal, the plant synaptotagmin (SYTA), has also been identified (YUAN et al., 2018).

There is limited data available regarding cucumoviruses in this context, and further research is needed to identify the factors required for cell-to-cell movement and to analyze these interactions in different host-virus relationships.

5. NEW SCIENTIFIC FINDINGS

1. We confirmed that a 42 amino acid deletion in the C-terminal region of PSV MP is required for proper interaction with CMV CP.

2. Viruses containing CMV MP (C12CP3, P12C3) induced stronger symptoms similar to CMV, such as stunting, strong chlorotic mosaic, and severe leaf deformation, whereas viruses containing PSV MP (C12PΔC3, C12P3) caused milder leaf deformation and weaker mosaic symptoms on *Nicotiana benthamiana*. Furthermore, the CMV MP-containing viruses were detectable in the upper systemic leaves three days earlier than the PSV MP-containing constructs, which showed delayed symptom development.

3. CMV MP-containing viruses (C12CP3, P12C3) caused strong leaf distortion, typical mosaic symptoms, and stunting in *C. annuum* cv. Brody plants, similar to CMV infection. In contrast, PSV MP-containing viruses (C12PΔC3, C12P3) did not induce symptoms and were unable to systemically infect the pepper plants.

4. In *N. benthamiana* cells, CMV MP-eGFP fusion protein showed strong colocalization with plasmodesmata, while PSV MP-eGFP fusion protein showed weaker colocalization. In plasmolyzed cells, PSV MP-eGFP exhibited weaker binding to plasmodesmata.

5. The CMV MP-eGFP fusion protein showed stronger plasmodesmata colocalization in *C. annuum* cv. Brody plants compared to *N. benthamiana*. In contrast, PSV MP-eGFP fusion protein showed minimal colocalization with plasmodesmata in *C. annuum*. This reinforces the observation that PSV is incapable of effective cell-to-cell movement in pepper plants, and this is likely the key reason why PSV cannot infect *C. annuum*.

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7. PUBLICATIONS RELATED TO THE DISSERTATION SUBJECT

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8. PUBLICATIONS NOT CLOSELY RELATED TO THE DISSERTATION SUBJECT

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