The N-Terminal Region of *Wheat Streak Mosaic Virus* Coat Protein Is a Host- and Strain-Specific Long-Distance Transport Factor

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The N-Terminal Region of *Wheat Streak Mosaic Virus* Coat Protein Is a Host- and Strain-Specific Long-Distance Transport Factor

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Understanding the genetics underlying host range differences among plant virus strains can provide valuable insights into viral gene functions and virus-host interactions. In this study, we examined viral determinants and mechanisms of differential infection of *Zea mays* inbred line SDp2 by *Wheat streak mosaic virus* (WSMV) isolates. WSMV isolates Sidney 81 (WSMV-S81) and Type (WSMV-T) share 98.7% polyprotein sequence identity but differentially infect SDp2: WSMV-S81 induces a systemic infection, but WSMV-T does not. Coinoculation and sequential inoculation of SDp2 with WSMV-T and/or WSMV-S81 did not affect systemic infection by WSMV-S81, suggesting that WSMV-T does not induce a restrictive defense response but that virus-encoded proteins may be involved in differential infection of SDp2. The viral determinant responsible for strain-specific host range was mapped to the N terminus of coat protein (CP) by systematic exchanges of WSMV-S81 sequences with those of WSMV-T and by reciprocal exchanges of CP or CP codons 1 to 74. Green fluorescent protein (GFP)-tagged WSMV-S81 with CP or CP residues 1 to 74 from WSMV-T produced similar numbers of infection foci and genomic RNAs and formed virions in inoculated leaves as those produced with WSMV-S81, indicating that failure to infect SDp2 systemically is not due to defects in replication, cell-to-cell movement, or virion assembly. However, these GFP-tagged hybrids showed profound defects in long-distance transport of virus through the phloem. Furthermore, we found that four of the five differing amino acids in the N terminus of CP between the WSMV-S81 and WSMV-T isolates were collectively involved in systemic infection of SDp2. Taken together, these results demonstrate that the N-terminal region of tritimoviral CP functions in host- and strain-specific long-distance movement.

Plant viruses establish systemic infection by successful replication in initially infected cells, followed by cell-to-cell movement into adjacent cells through plasmodesmata and multistep long-distance movement through vascular tissue (8, 30, 31, 59). Cell-to-cell movement of plant viruses through plasmodesmata is potentiated with successful interactions between virus-encoded movement protein(s) (MP) and host proteins (59–61). In contrast, the long-distance movement of plant viruses is a more complex process, which involves movement through plasmodesmata connecting several cell types, followed by passive transport through phloem. Compatible virus-host interactions are crucial for viruses to reach and be loaded into the vascular transport system, followed by unloading into adjacent companion and mesophyll cells for further systemic invasion. Additionally, viruses encode long-distance transport factors (LTFs) required for efficient systemic infection of plants (15, 17, 35, 39, 43). However, the majority of these LTFs subsequently have been shown to function by suppressing the plant host RNA silencing defense pathway (21, 24, 40, 58) rather than direct involvement in viral long-distance transport. Moreover, the mechanism of LTFs of plant viruses is poorly understood.

*Wheat streak mosaic virus* (WSMV) is one of the more economically important viruses infecting wheat in the Great Plains of the United States because of its widespread distribution with persistent yield losses (7, 20). WSMV is the type species of the genus *Tritimovirus* within the family *Potyviridae*, with a 9,384-nucleotide (nt) [excluding the 3′ poly(A) tail] single-stranded positive-sense RNA genome (45). Similar to members of the *Potyvirus* and *Rymovirus* genera, the genomic RNA of WSMV is translated as a polyprotein that is subsequently processed by virus-encoded proteinases into at least 10 mature proteins (45). However, WSMV differs from members of the *Potyvirus* genus in the roles of P1 and helper component proteinase (HC-Pro) in the virus life cycle. HC-Pro of WSMV is dispensable for systemic infection of wheat (47) in contrast to the multifunctional roles of HC-Pro of potyviruses (1, 4, 15, 23, 25, 38, 57). Recently, P1 of WSMV, not HC-Pro as in potyviruses, was identified as the suppressor of RNA silencing (50, 51; R. French, unpublished data). WSMV is vectored by *Acercia tosichella* Keifer, the wheat curl mite, in a semipersistent manner (44), and HC-Pro was identified as a viral determinant for mite transmission (48, 49).

WSMV isolates Sidney 81 (WSMV-S81) and Type (WSMV-T) possess 98.7% polyprotein sequence identity (12; this study) and are representative members of WSMV genotypes in the United States (46). WSMV-S81 and WSMV-T differ in the ability to infect maize (*Zea mays* L.) inbred line SDp2 (here after SDp2): WSMV-S81 infects SDp2 systemically, eliciting chlorotic streaks and mosaic and chlorotic line pattern symptoms, while WSMV-T fails to infect SDp2 (Fig. 1). Viral determinants and the potential mechanism(s) behind differential infection of SDp2 by WSMV isolates, such as induction of a defense response by SDp2 upon WSMV-T infection or defects in cell-to-cell and/or long-distance movement in...
SDp2, are not known. Moreover, understanding the basis of host range differences among plant virus strains can provide valuable insights into viral gene functions and virus-host interactions.

Members of the family Potyviridae do not encode dedicated movement protein(s); rather, potyviral proteins involved in virus movement also have at least one other function in the virus life cycle, such as encapsidation, suppression of RNA silencing, and replication. HC-Pro, cylindrical inclusion protein (CI), and coat protein (CP) have been reported to have MP function (9, 18, 19, 25, 28), whereas P1, HC-Pro, 6K2, the proteinase domain of nuclear inclusion protein a (NIa-Pro), the viral genome-linked protein of NIa (NIa-VPg), and CP each have been identified with a role in host-specific infection (2, 10, 15, 16, 18, 19, 27, 29, 33, 36, 40–42). However, there is no information on the roles of tritimoviral proteins in virus movement except that mutations in the putative overlapping open reading frame at the C terminus of P3 (PIPO) (14) severely limited cell-to-cell movement without significantly affecting virus replication (13). Likewise, little is known about mechanisms of strain-specific differences in the host range of potyvirid species.

In this study, we examined viral determinants and mechanisms of differential infection of SDp2 by WSMV isolates. A series of hybrid viruses between WSMV-S81 and WSMV-T were created, and infectivity assays of these recombinant viruses revealed that the viral determinant for infectivity on SDp2 is located at the N-terminal region of CP, which contained five amino acid differences between the two isolates. We found that failure to infect SDp2 systemically by WSMV-T is not due to defects in replication, cell-to-cell movement, or virion assembly. However, WSMV-S81 with the CP cistron or CP amino acids (aa) 1 to 74 from WSMV-T showed profound defects in viral transport to upper noninoculated leaves. Taken together, these data indicate that the CP of WSMV functions as a host- and strain-specific LTF. Thus, here we demonstrate that determinants for host- and strain-specific infection of plant viruses can be restricted to differences in as few as four amino acids and that host range differences among the isolates of plant viruses might be due to defects in long-distance transport through the vasculature.

**MATERIALS AND METHODS**

**Virus isolates.** pACYC-WSMV-S81 and pSP6-WSMV-S81, infectious cDNA clones of WSMV-S81, were described previously (11) and were used as base clones to exchange sequences with WSMV-T. The latter isolate was activated from dried wheat leaves that had been stored at 4°C under anhydrous calcium sulfate since 1976. WSMV-T RNA was isolated from partially purified virions as described previously (53).

**Development of an infectious cDNA clone of WSMV-T.** The genomic RNA sequence of WSMV-T (12) (GenBank accession number AF285169) was used to design primers to develop an infectious cDNA clone. Two conserved restriction endonuclease sites in the genomic RNAs of WSMV-S81 and WSMV-T at nt 3905 (AflII) and at nt 6319 (BstEI) were used to progressively replace WSMV-S81 sequences with those of WSMV-T. Reverse transcription-PCR (RT-PCR)-amplified WSMV-T cDNA fragments from nt 1 to 3909, 3910 to 6319, and 6320 to...
the 3' end were exchanged into psP6-WSMV-S81 (11). A minus-strand primer (W-1) complementary to nt 3960 to 3925 was used for reverse transcription, followed by PCR with oligonucleotides W-1 and W-124 (a plus-sense primer, 5'-GGTTGCGGACCCGGCCTATAGTGATCACTAGAAATACACCAACCCAAATCTGCTG-3', containing the SaII [in italics] and NgOMV [in boldface] sites) and an SP6 RNA polymerase promoter [underlined] immediately upstream of the WSMV 5' terminus sequence. The resulting PCR product was digested with SaII and AvrII and ligated into similarly digested psP6-WSMV-S81 to obtain psP6-WSMV-S81-5.3kb-T. Positive clones were identified based on the presence of the plus-sense primer-derived NgOMV site and differential restriction sites between the cDNAs of WSMV-S81 and WSMV-T.

We next exchanged the WSMV-T cDNA fragment between the AvrII (nt 3905) and BstEII (nt 6319) sites into psP6-WSMV-S81-5.3kb-T. A pair of oligonucleotides, W-2 (a plus-sense primer corresponding to nt 3898 to 3933) and W-6 (a minus-sense primer complementary to nt 6339 to 6303), was used to amplify a cDNA fragment from nt 3898 to 6339, which was digested with AvrII and BstEII and ligated into similarly digested psP6-WSMV-S81-5.3kb-T to obtain psP6-WSMV-S81-5.6kb-T. Finally, a cDNA fragment corresponding to 6.3 kb to the 3’ end was amplified by RT-PCR with a pair of primers, W-3 (corresponding to nt 6303 to 6548) and W-8 (complementary to nt 9584 to 9535, followed by 105 thymidine residues and SpeI and NotI restriction sites). The RT-PCR product was digested with BstEII and NotI restriction enzymes and ligated into similarly digested psP6-WSMV-S81-5.6kb-T to obtain a full-length cDNA clone of the WSMV Type isolate, psP6-WSMV-T.

Construction of hybrid viruses. WSMV-T cDNA fragments between AvrII and BstEII (nt 3905 to 6319) and between the 3’ end (nt 6319 to the 3’ end) were amplified as described above and ligated into psP6-WSMV-S81 between the AvrII and BstEII sites and between BstEII and NotI sites (Fig. 1) to obtain psP6-WSMV-S81-3.9kb-T and psP6-WSMV-S81-6.3kb-3’-T, respectively. A WSMV-T cDNA fragment between the AvrII and BstEII sites (nt 3905 to 6319) was ligated into similarly digested psP6-WSMV-S81-6.3kb-3’-T to obtain psP6-WSMV-S81-3.9kb-3’-T.

The Nb and CP cistrons and CP 3' end sequences from WSMV-T were precisely exchanged into psP6-WSMV-S81 by overlap extension PCR (22), and PCR fragments were ligated into psP6-WSMV-S81 between the BstEII and NotI restriction sites. Similarly, the CP cistron from WSMV-S81 was amplified using pACYC-WSMV-S81 as a template and precisely exchanged into psP6-WSMV-T by overlap extension PCR.

The sequences encoding CP aa 1 to 74, 135 to 194, and 75 to 194 from WSMV-T were precisely exchanged into psP6-WSMV-S81 by overlap extension PCR amplification between BstEII and the 3’ end with oligonucleotides W-3 and W-59 to obtain psP6-WSMV-S81-CPaa1-74-T. psP6-WSMV-S81-CPaa135-194-T, and psP6-WSMV-S81-CPaa75-194-T, respectively. cDNA encoding CP aa 1 to 74 from WSMV-S81 was exchanged into psP6-WSMV-T by overlap extension PCR to obtain psP6-WSMV-T-CPaa1-74-S81. The change of CP aa 1 to 74 from WSMV-S81 was exchanged into psP6-WSMV-T by overlap extension PCR to obtain psP6-WSMV-T-CPaa1-74-S81, as described above.

Plasmid DNA preparation. Escherichia coli strain JM109 harboring pUC18-based psP6-WSMV was propagated at 30°C overnight to minimize possible toxicity to the bacteria, whereas E. coli strain JM109 with pACYC177-based pACYC-WSMV was propagated at 37°C overnight. Plasmid DNA was prepared using the Bio-Rad plasmid midiprep kit (Bio-Rad, Hercules, CA), according to the manufacturers’ instructions.

Nucleotide sequencing. Nucleotide sequencing of the full-length cDNA clone of WSMV-T, junctions of hybrid cDNAs between WSMV-S81 and WSMV-T, and the presence of introduced mutations in the CP cistron were verified by sequencing plasmid DNAs or PCR amplicons at the University of Florida ICBR Core DNA Sequencing Facility using an Applied Biosystems 3730xl model sequencer.

In vitro RNA transcription and inoculation of wheat seedlings. In vitro transcription was carried out in 40 µl-reaction mixtures consisting of 1.0 µg of NotI- or SpeI-linearized plasmids, 40 mM Tris-HCl, pH 7.9, 20 mM dithiothreitol (DTT), 8.5 mM MgCl2, 2 mM spermidine, 1.2 mM concentration each of ATP, CTP, UTP, and cap analog (m7G[5’ppp][5’G]; Epicentre Biotechnologies, Madison, WI), 0.008 mM GTP, 20 U of λ RNasin RNase inhibitor (Promega Corporation, Madison, WI), and 50 U of SP6 RNA polymerase (Epicentre Biotechnologies). Transcription reaction mixtures were incubated at 37°C for 15 min, followed by increasing the GTP concentration to 0.5 mM and further incubation for 2 h at 37°C. One microliter of transcription reaction was analyzed on 1% native agarose gels in 1× TAE buffer to examine the integrity and quality of in vitro transcripts.

Freshly prepared in vitro transcripts were mixed with an equal volume of 2% sodium pyrophosphate, pH 9.0, containing 1% baked Celite and directly inoculated to 12 to 18 wheat seedlings at the single-leaf stage. Inoculated wheat seedlings were incubated in a greenhouse at 24 to 30°C maximum and 18 to 20°C minimum temperatures with a 14-h photoperiod. Wheat seedlings were observed for symptom development from 5 days postinoculation (dpi) onwards. Three independent clones per each hybrid/mutant were inoculated to wheat or SDp2 (see below), and the results of one representative clone per construct were given.

Infectivity assays on SDp2. Wheat leaves infected with wild-type WSMV isolates and hybrid viruses were grown in 20 mM sodium phosphate buffer, pH 7.0 (1:20, wt/vol), and mechanically inoculated to 10 to 20 Carborundum-dusted SDp2 seedlings at the two-leaf stage. Inoculated SDp2 seedlings were incubated in a growth chamber at 22°C maximum and 18°C minimum temperatures, with a 14-h photoperiod. Symptom development on SDp2 plants was observed from 9 dpi onwards, and leaves were photographed at 17 dpi.

Real-time RT-PCR and quantification of genomic RNA copies. Real-time PCR was performed to quantify the genomic RNA copies as described previously (54), except in vitro transcripts from gel-eluted PCR product were used to make a standard curve. The RNA template used to make a standard curve was converted into picomoles using the following formula: number of µg of RNA × 10^4 pg per 1 µg × 1 pmol per 340 pg × 1 per number of bases of the transcript × Avogadro’s constant (6.023×10^23 molecules/mol). The absolute number of genomic RNA copies of WSMV was calculated from the threshold cycle (C_T) values of real-time RT-PCR using G-Gene software (32, 34). The normalized expression of the target gene from each sample was calculated based on the C_T value of 188 RNA of the corresponding sample.

Observation of GFP fluorescence. The expression of green fluorescent protein (GFP) in inoculated and upper noninoculated leaves was observed with a Zeiss Stereo Discovery V12 fluorescence microscope (Carl Zeiss Microlmaging, Inc., New York, NY) using GFP narrow-band filters. Images were captured using an AxioCam MRc5 camera attached to the microscope.

RESULTS

Development of an infectious cDNA clone of WSMV-T and sequence homology with WSMV-S81. Three cDNA fragments of WSMV-T were progressively exchanged into psP6-WSMV-S81, an infectious cDNA clone of the WSMV Sidney 81 isolate (11), using conserved restriction endonuclease sites. Several independent full-length cDNA clones of WSMV isolate Type psP6-WSMV-T were obtained, and the infectivity of these clones was assayed on wheat and SDp2 to ensure that the cDNA clones retained the same phenotype as that of wild-type virus. In vitro transcripts from psP6-WSMV-T induced chlorotic streaks and spots at 5 to 7 dpi, followed by severe chlorotic streaks and mosaic, mottling, and leaf yellowing symptoms on wheat cv. Tomahawk by 14 to 21 dpi, similar to those induced by transcripts of WSMV-S81 (Fig. 1B). However, when SDp2 seedlings were inoculated with crude sap from psP6-WSMV-T transcript-inoculated wheat, no visible symptoms were observed at 18 dpi (Fig. 1A and C), suggesting that cloned virus retained a phenotype similar to that of the wild-type WSMV-T. Both cloned and wild-type viruses of WSMV-T induced a few chlorotic spots/streaks on the upper noninoculated leaves of SDp2 at 30 dpi (data not shown). In the same experiment, SDp2 seedlings inoculated with WSMV-S81 initiated chlorotic streaks and mosaic and chlorotic line pattern symptoms at 9 to 11 dpi (Fig. 1A and C).

The genomic RNA sequence of WSMV-T, deduced from the sequence of an infectious cDNA clone, was 9,384 nt [excluding the poly(A) tail located at the 3’ end of the genomic RNA], identical in size to that of WSMV-S81. At the nucleotide level, WSMV-T possesses 97.6%, 98.5%, and 98.6% identities with the genomic RNA, 5’ nontranslated region (NTR),
TABLE 1. Amino acid differences between the polyproteins of WSMV Sidney and Type isolates

<table>
<thead>
<tr>
<th>Cistron</th>
<th>No. of amino acid differences</th>
<th>Differing amino acids (Sidney vs Type)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>6</td>
<td>D118N; T162A; L184M; T270S; F272L; L348F</td>
</tr>
<tr>
<td>HC-Pro</td>
<td>5</td>
<td>V360A; H132Y; S407A; T603I; M501T</td>
</tr>
<tr>
<td>P3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6K1</td>
<td>1</td>
<td>N106K</td>
</tr>
<tr>
<td>CI</td>
<td>6</td>
<td>T1145A; R1196K; E1242D; I1357V; I1480T; M1583I</td>
</tr>
<tr>
<td>6K2</td>
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<td></td>
</tr>
<tr>
<td>NLa</td>
<td>3</td>
<td>R1768S; T1833A; A1859S</td>
</tr>
<tr>
<td>Nlb</td>
<td>6</td>
<td>T2235V; G2242E; H2427Y; D2461V; E2462G; N2589S</td>
</tr>
<tr>
<td>CP</td>
<td>11</td>
<td>A20E; S21P; Q30L; A50V; G52E; A76T; D79G; G88S; T92V; K167E; D178E</td>
</tr>
</tbody>
</table>

* Positions of differing amino acids between the two polyproteins are shown as "Sidney vs Type," except for the CP. The locations of differing amino acids in the CP cistron are represented by relative amino acid positions (aa 1 to 349) within the CP cistron. Both WSMV Sidney and Type isolates contain the same number of amino acids in the respective polyproteins.

and 3’ NTR, respectively, of WSMV-S81. The polyproteins of WSMV-T and WSMV-S81 share 98.7% sequence identity, differing only by 38 aa out of 3,035 aa (Table 1). The amino acid sequences of P3 and 6K2 of WSMV-S81 and WSMV-T are identical, whereas the other mature proteins possess a variable number of amino acid differences, from 1 aa in 6K1 to 11 aa in CP (Table 1). The cloned WSMV-T differs with previously described wild-type WSMV-T (12) in five amino acid residues at positions 143, 152, 1480, 2168, and 2589.

Failure to infect SDp2 systemically by WSMV-T is not due to induction of a defense response. It is possible that failure to infect SDp2 by WSMV-T could have been due to a defense response elicited upon infection without inducing a hypersensitive response, as no visible symptoms were detected on inoculated SDp2 leaves. If WSMV-T elicited a local- or long-distance movement-restricting (LDMR) defense response, it was predicted that such response might also restrict WSMV-S81 in coinfection experiments. To examine such a possibility, WSMV-S81 and WSMV-T inocula were prepared from 14-dpi wheat leaves at a 1:10 dilution to obtain a high virus titer to infect as many cells as possible with both viruses in coinfection experiments. The presence (in WSMV-T) or absence (in WSMV-S81) of a SacI restriction site at nt position 8309 was used as a marker to screen the infection status of WSMV-S81 and/or WSMV-T in doubly inoculated SDp2 (Fig. 2A and B).

SDp2 seedlings coinnoculated with WSMV-S81 and WSMV-T, or singly inoculated with either WSMV-T or

FIG. 2. Analyses of doubly and sequentially inoculated SDp2 plants for systemic infection by WSMV-S81 and/or WSMV-T. (A) The method for screening SDp2 plants for systemic infection with WSMV-S81 and/or WSMV-T is depicted. A unique SacI restriction site at nt 8309 in the WSMV-T genome, which is absent in that of WSMV-S81, was used as a marker to screen for the presence of WSMV isolates in inoculated SDp2. (B) Agarose gel electrophoresis of RT-PCR products of total RNAs from WSMV-S81- and WSMV-T-infected wheat. RT-PCR product from WSMV-S81-infected wheat (lane 1), followed by SacI digestion (lane 2); RT-PCR-amplified product from WSMV-T-infected wheat (lane 3), followed by SacI digestion (lane 4). Note that the RT-PCR product from WSMV-T was digested into 561- and 315-bp fragments, whereas the RT-PCR product from WSMV-S81 is not cleaved by the SacI restriction enzyme, as expected. No RT-PCR products were obtained from total RNA of healthy wheat or water controls, which are not included in the picture shown. (C) Infectivity of SDp2 plants inoculated with WSMV-S81, WSMV-T, WSMV-S81 plus WSMV-T, WSMV-S81 followed by WSMV-T, and WSMV-T followed by WSMV-S81. The number of plants infected with WSMV-S81 or WSMV-T based on RT-PCR analyses of total RNA from SDp2 plants are presented.
WSMV-S81, were reinoculated 4 days later with WSMV-S81 or WSMV-T, respectively. Coinoculation with WSMV-S81 and WSMV-T, or prior inoculation of SDp2 with WSMV-T, did not prevent infection of WSMV-S81, as 100% of SDp2 plants were infected with WSMV-S81 (Fig. 2C). These data together with formation of infection foci on inoculated leaves of SDp2 with GFP-tagged WSMV-S81 containing WSMV-T CP (see below) suggested that SDp2 plants did not exhibit induced resistance upon infection with WSMV-T. Conversely, WSMV-T failed to infect WSMV-S81-inoculated SDp2 (Fig. 2C), suggesting that WSMV-S81 proteins did not complement systemic infection defects of WSMV-T in SDp2. Taken together, these data suggested that failure of WSMV-T to infect SDp2 might be due to incompatibility between WSMV-T and SDp2 proteins.

**WSMV-S81 with the sequence between 6.3 kb and the 3′ end from isolate WSMV-T failed to infect SDp2.** Potential WSMV genetic determinants involved in differential infection of SDp2 were mapped by systematic exchanges of WSMV-S81 sequences with those of WSMV-T or vice versa, followed by examining the ability of hybrid viruses to infect SDp2.

We utilized conserved AflII (nt 3905) and BstEII (nt 6319) restriction sites in the genomes of WSMV-S81 and WSMV-T to exchange WSMV-T sequences into pSP6-WSMV-S81 (Fig. 3A). The cDNAs of WSMV-T encompassing nt 1 to 3905, nt 3906 to 6319, nt 1 to 6319, nt 3906 to the 3′ end of the genome, and nt 6320 to the 3′ end of the genome were exchanged into pSP6-WSMV-S81 to obtain pSP6-WSMV-S81-3′-6.3kb-T, pSP6-WSMV-S81-3′-6.3kb-T, pSP6-WSMV-S81-3′-6.3kb-T, and pSP6-WSMV-S81-6.3kb-3′-T, respectively (Fig. 3A, panels c to g). pSP6-S-WSMV-S81-5′-3.9kb-T comprised the 5′ NTR through the N terminus of CI from WSMV-T (Fig. 3A, panel c), pSP6-WSMV-S81-3′-6.3kb-T contained most of the CI protein 6K2 and most of the Nla sequence from WSMV-T (Fig. 3A, panel d), and pSP6-WSMV-S81-5′-6.3kb-T comprised the 5′ NTR through most of the Nla from WSMV-T (Fig. 3A, panel e).

All chimeric viruses of WSMV-S81 bearing segments from isolate WSMV-T produced chlorotic streaks, and mosaic, mottling, and leaf yellowing symptoms on wheat cv. Tomahawk similar to those produced by the full-length virus clones of WSMV-S81 and WSMV-T (data not shown). Progeny virus in crude sap, derived from wheat leaves infected with in vitro transcripts of hybrid cDNA clones, were inoculated to SDp2 seedlings and examined for symptom development and accumulation of genomic RNA copies in upper noninoculated leaves at 18 dpi by real-time RT-PCR, as described previously (54). WSMV-S81-5′-3.9kb-T, WSMV-S81-3′-6.3kb-T, and WSMV-S81-5′-6.3kb-T infected >90% of SDp2 plants with chlorotic streaks and mosaic and chlorotic line pattern symptoms similar to those infected by WSMV-S81 (Fig. 3A and B, panels c to e). Accumulation of genomic RNAs in the upper noninoculated leaves of SDp2 by these hybrid viruses was comparable to that by WSMV-S81 (Table 2). These data suggested that the WSMV-T sequence between nt 1 to 6319 did not contain any determinants negatively affecting systemic infection of SDp2.

We next examined WSMV-S81-3′-6.3kb-3′-T and WSMV-S81-6.3kb-3′-T, which contained a major part of CI through the 3′ end and the C terminus of Nla through the 3′ end from WSMV-T, respectively (Fig. 3A, panels f and g). Crude sap from WSMV-S81-3′-6.3kb-3′-T and WSMV-S81-6.3kb-3′-T-infected wheat leaves failed to induce visible symptoms following inoculation of SDp2 at 18 dpi (Fig. 3A and B, panels f and g). These data indicated that the WSMV-T sequence between 6.3 kb and the 3′ end contained the determinants that affected systemic infection of SDp2.

**Viral determinants for systemic infection of SDp2 mapped to the CP.** The above-described experiments suggested that WSMV-S81 with minimal WSMV-T sequence that failed to infect SDp2 systemically contained the C-terminal regions of Nla, Nlb, CP, and 3′ NTR with differences in 0, 6, and 11 aa (Table 1) and 2 nt, respectively, compared to corresponding sequences of WSMV-S81. To further delineate WSMV determinants that affected systemic infection of SDp2, we precisely exchanged Nlb and CP plus 3′ NTR sequences from isolate WSMV-T into those of pSP6-WSMV-S81 to obtain pSP6-WSMV-S81-Nlb-T and pSP6-WSMV-S81-CP-3′-T, respectively (Fig. 4d and e). Infection of SDp2 by WSMV-S81-Nlb-T was similar to that by WSMV-S81 (Fig. 4d; Table 2), suggesting that viral determinants of SDp2 infectivity are not located in the Nlb cistron. However, WSMV-S81-CP-3′-T failed to induce visible symptoms on SDp2 at 18 dpi (Fig. 4e; Table 2), suggesting that the CP and/or 3′ NTR sequence from the WSMV-T affected the ability to infect SDp2. The 3′ NTR of WSMV-T differs from that of WSMV-S81 in 2 nt, and to examine the role, if any, of these 2 nt in differential infection of SDp2, pSP6-WSMV-S81-CP-T was generated in which the CP cistron of isolate WSMV-T was precisely exchanged into that of pSP6-WSMV-S81 (Fig. 4f). WSMV-S81-CP-T failed to infect SDp2 systemically at 18 dpi (Fig. 4f, Table 2), indicating that the CP of WSMV-T is responsible for the lack of infectivity on SDp2.

To further confirm the role of CP as the determinant of SDp2 infection, the CP cistron was exchanged from isolate WSMV-S81 into that of pSP6-WSMV-T to obtain pSP6-WSMV-T-CP-S81 (Fig. 4g). WSMV-T containing the CP cistron from WSMV-S81 was able to systemically infect SDp2 (Fig. 4g, Table 2) and induced mosaic and chlorotic line pattern symptoms. Taken together, these results identified WSMV CP as a genetic determinant of host-specific infection.

**The N-terminal region of CP is involved in host- and strain-specific infection of WSMV.** The Cps of WSMV-S81 and WSMV-T differ in 11 aa, mostly located in the N-terminal half of the CP (Fig. 5B; Table 1). To delineate the role of these amino acids of CP in differential infection of SDp2, subsets of codons from WSMV-T CP were exchanged into those of pSP6-WSMV-S81 and examined for the ability of these hybrid viruses to infect SDp2. Sequences encoding amino acids 1 to 74 with five different residues (Fig. 5C), aa 135 to 194 with two different residues (Fig. 5D), and aa 75 to 194 with six different residues (Fig. 5E) were precisely exchanged into the CP of WSMV-S81. Amino acid differences were not observed in the C-terminal half of the CP (aa 194 to 349) between the isolates; thus, no exchanges were made. WSMV-S81 that contained the CP aa 135 to 194 or 75 to 194 from isolate WSMV-T infected SDp2 systemically (Fig. 5D and E; Table 2). In contrast, WSMV-S81 that contained CP aa 1 to 74 from WSMV-T failed to induce visible symptoms on SDp2 and did not accumulate genomic RNA, as revealed by real-time RT-PCR (Fig. 5C;
To further confirm these results, the sequence encoding WSMV-T CP aa 1 to 74 was replaced with that encoding WSMV-S81 to generate WSMV-T-CPaa1-74-S81. This chimeric virus infected SDp2 similar to that infected by the wild-type WSMV-S81 (Fig. 5G; Table 2). Thus, one or more of these five differing amino acids in the first 74 residues of CP were found to be involved in systemic infection of SDp2 by WSMV isolates.

The CP or CP aa 1 to 74 from isolate WSMV-T does not affect cell-to-cell movement or virion assembly of WSMV-S81 in SDp2. We examined the mechanistic basis for differential infection of SDp2 by WSMV isolates, including whether it is...
TABLE 2. Quantification of genomic RNA copies of chimeric viruses between *Wheat streak mosaic virus* isolates Sidney and Type in systemic upper noninoculated leaves of *Z. mays* line SDp2

<table>
<thead>
<tr>
<th>Construct</th>
<th>Plant 1 (copies ± SE)</th>
<th>Plant 2 (copies ± SE)</th>
<th>Plant 3 (copies ± SE)</th>
</tr>
</thead>
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<tr>
<td>WSMV-S81</td>
<td>3.63 × 10^4 ± 8.0 × 10^2</td>
<td>3.28 × 10^4 ± 6.5 × 10^2</td>
<td>6.07 × 10^4 ± 4.5 × 10^3</td>
</tr>
<tr>
<td>WSMV-T</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>WSMV-S81-5’3.9 kb-T</td>
<td>3.41 × 10^4 ± 2.2 × 10^2</td>
<td>1.63 × 10^4 ± 1.4 × 10^3</td>
<td>5.88 × 10^4 ± 1.5 × 10^3</td>
</tr>
<tr>
<td>WSMV-S81-3.9-6.3kb-T</td>
<td>1.47 × 10^4 ± 6.0 × 10^2</td>
<td>8.17 × 10^3 ± 1.3 × 10^3</td>
<td>1.36 × 10^4 ± 8.5 × 10^3</td>
</tr>
<tr>
<td>WSMV-S81-6.3kb-3’T</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
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<tr>
<td>WSMV-S81-5’6.3kb-T</td>
<td>5.85 × 10^4 ± 4.9 × 10^2</td>
<td>5.73 × 10^4 ± 7.4 × 10^2</td>
<td>9.36 × 10^4 ± 3.7 × 10^3</td>
</tr>
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<td>WSMV-S81-3.9kb-3’T</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>WSMV-S81-Nib-T</td>
<td>5.82 × 10^4 ± 1.8 × 10^3</td>
<td>4.81 × 10^4 ± 8.3 × 10^2</td>
<td>1.30 × 10^4 ± 6.5 × 10^3</td>
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<tr>
<td>WSMV-S81-CP-3’T</td>
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<td>UD</td>
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<td>WSMV-S81-CP-T</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
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<tr>
<td>WSMV-S81-CP-S81</td>
<td>2.92 × 10^4 ± 8.6 × 10^2</td>
<td>2.15 × 10^4 ± 3.4 × 10^2</td>
<td>7.09 × 10^4 ± 8.3 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-CP-aa1-74-T</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>WSMV-S81-CP-aa135-194-T</td>
<td>3.21 × 10^4 ± 1.1 × 10^3</td>
<td>3.24 × 10^4 ± 8.8 × 10^2</td>
<td>6.00 × 10^4 ± 7.7 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-CP-aa75-194-T</td>
<td>4.44 × 10^4 ± 5.1 × 10^3</td>
<td>3.90 × 10^4 ± 2.4 × 10^3</td>
<td>9.04 × 10^4 ± 2.9 × 10^3</td>
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<tr>
<td>WSMV-T-CP-aa1-74-S81</td>
<td>4.28 × 10^4 ± 7.8 × 10^3</td>
<td>5.09 × 10^4 ± 2.0 × 10^3</td>
<td>4.15 × 10^4 ± 0.4 × 10^3</td>
</tr>
<tr>
<td>WSMV-S81-ASpop3 EP</td>
<td>2.5 × 10^4 ± 0.98 × 10^2</td>
<td>UD</td>
<td>5.17 × 10^4 ± 2.1 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-QL</td>
<td>5.07 × 10^4 ± 1.4 × 10^3</td>
<td>8.82 × 10^4 ± 2.3 × 10^3</td>
<td>2.14 × 10^4 ± 1.0 × 10^3</td>
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<tr>
<td>WSMV-S81-AG30032VE</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>Healthy</td>
<td>UD</td>
<td>UD</td>
<td>UD</td>
</tr>
</tbody>
</table>

The number of genomic RNA copies determined at 18 days postinoculation using real-time RT-PCR from fully expanded leaves with triplicate samples. The number of genomic RNA copies was quantified from three independent plants, with the average number of genomic RNA copies per 2.5 ng of total RNA ± standard error (SE) shown. UD, undetected.

due to defects in cell-to-cell and/or long-distance movement or virion assembly. Cell-to-cell movement of WSMV-S81 and its variants WSMV-S81-CP-T or WSMV-S81-CP-aa1-74-T in inoculated SDp2 leaves was monitored by examining the infection foci in inoculated leaves using the GFP-tagged viruses and by quantifying the genomic RNA copies by real-time RT-PCR at 4, 7, and 10 dpi as described previously (54).

The genomic RNA copies from three independent plants at

![FIG. 4. Analyses of Nb and CP cistrons of WSMV for differential infection of SDp2. (a to g) The genomic organization of WSMV-S81 (a), WSMV-T (b), recombinant WSMV-S81 viruses with 6.3 kb to the 3’ end (c), the Nb (d), CP to the 3’ end (e), or CP (f) from WSMV-T, and WSMV-T with the CP cistron from WSMV-S81 (g). Regions of the WSMV-S81 and WSMV-T genomes are denoted by gray shading and stippling, respectively. The number of wheat or SDp2 plants infected out of the number of plants inoculated with viruses that are shown in panels a to g are indicated next to the respective genomic maps.](image-url)
4, 7, and 10 dpi, using one inoculated leaf per plant, were quantified by real-time RT-PCR. The WSMV variants WSMV-T, WSMV-S81-CP-T, and WSMV-S81-CP-aa1-74-T, which all failed to systemically infect SDp2, accumulated in inoculated leaves at levels comparable to those of WSMV variants which do infect SDp2 systemically (Table 3). WSMV genomic RNA was detected in all the samples tested at 4 dpi (1.12 × 10^3 to 6.39 × 10^3 per 2.5 ng of total RNA), and

FIG. 5. Analyses of subsets of WSMV coat protein (CP) for differential infection of SDp2. (A) WSMV-S81 genomic organization with an enlarged view of WSMV-S81 CP, with the location of finer exchanges between WSMV-S81 and WSMV-T denoted by relative amino acid residue coordinates of CP. (B) The CP cistron from WSMV-T in WSMV-S81 is indicated with stippling. (C to E) The following subsets of CP amino acids of WSMV-S81 that were exchanged with those of WSMV-T are indicated with stippling: amino acids 1 to 74 (C), amino acids 135 to 194 (D), and amino acids 75 to 194 (E). (F) The genomic organization of WSMV-T is indicated. (G) Amino acids 1 to 74 in the CP of WSMV-T were replaced with those of WSMV-S81. Asterisks indicate the locations of differential amino acids between the CPs of WSMV-S81 and WSMV-T. Data from infectivity assays of recombinant hybrid viruses on wheat or SDp2 are indicated next to the genomic organizations, with the number of plants infected out of the number of plants inoculated shown.

4, 7, and 10 dpi, using one inoculated leaf per plant, were quantified by real-time RT-PCR. The WSMV variants WSMV-T, WSMV-S81-CP-T, and WSMV-S81-CP-aa1-74-T, which all failed to systemically infect SDp2, accumulated in inoculated leaves at levels comparable to those of WSMV variants which do infect SDp2 systemically (Table 3). WSMV genomic RNA was detected in all the samples tested at 4 dpi (1.12 × 10^3 to 6.39 × 10^3 per 2.5 ng of total RNA), and

TABLE 3. Detection of the number of genomic RNA copies of recombinant viruses between Wheat streak mosaic virus isolates Sidney and Type in inoculated leaves of Zea mays line SDp2a,b

<table>
<thead>
<tr>
<th>Construct</th>
<th>Ability to infect SDp2 systemically</th>
<th>No. of genomic RNA copies ± SE a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSMV-S81</td>
<td>Yes</td>
<td>3.72 × 10^3 ± 6.5 × 10^2</td>
</tr>
<tr>
<td>WSMV-T</td>
<td>No</td>
<td>6.39 × 10^3 ± 8.9 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-6.3kb-3'-T</td>
<td>No</td>
<td>3.28 × 10^3 ± 1.53 × 10^3</td>
</tr>
<tr>
<td>WSMV-S81-5' 6.3kb-T</td>
<td>Yes</td>
<td>6.03 × 10^3 ± 6.5 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-CP-T</td>
<td>No</td>
<td>1.31 × 10^3 ± 5.7 × 10^2</td>
</tr>
<tr>
<td>WSMV-T-CP-S81</td>
<td>Yes</td>
<td>1.12 × 10^3 ± 4.6 × 10^2</td>
</tr>
<tr>
<td>WSMV-S81-CPaa1-74-T</td>
<td>No</td>
<td>4.81 × 10^3 ± 2.5 × 10^3</td>
</tr>
<tr>
<td>WSMV-T-CPaa1-74-S81</td>
<td>Yes</td>
<td>1.84 × 10^3 ± 3.0 × 10^2</td>
</tr>
<tr>
<td>Healthy</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a The numbers of genomic RNA copies of WSMV and its hybrid viruses were determined from inoculated leaves of SDp2 by real-time RT-PCR.
b Average number of genomic RNA copies per 2.5 ng of total RNA and standard errors. ND, not detected.
accumulation of viral RNAs increased over time, with a maximal RNA accumulation with WSMV-T at 10 dpi (9.43 \times 10^4 per 2.5 ng of total RNA) (Table 3). WSMV-T accumulated in inoculated leaves to nearly twice the level of WSMV-S81, which infects SDp2 systemically. These data revealed that WSMV-T and WSMV-S81 genotypes accumulated in inoculated leaves regardless of the ability of viruses to invade noninoculated leaves of SDp2 (Table 3). Sequence analysis of RT-PCR products of CP from total RNAs extracted at 10 dpi from inoculated SDp2 leaves with WSMV-S81-CP-T and WSMV-S81-CP-aa1-74-T revealed that no reversions or other nucleotide substitutions were present in the introduced sequences (data not shown).

Next, we examined the accumulation of WSMV RNA from upper noninoculated leaves of SDp2 at 18 dpi by real-time RT-PCR. WSMV-S81 variants with the CP or with the N-terminal region of the CP from WSMV-T failed to accumulate to detectable levels (Table 2), whereas all other

FIG. 6. Effect of CP or CP amino acids (aa) 1 to 74 from WSMV-T on cell-to-cell movement and virion formation of WSMV-S81 in SDp2. (A) Genomic organization of WSMV-S81-GFP-6K1/CP(7aa). The GFP cistron was inserted between the P1 and HC-Pro cistrons of WSMV-S81 (55). The arrow and arrowhead indicate the locations of predicted cleavages performed by P1 and Nia-Pro at the 6K1/CI heptapeptide sequence, respectively. (B) The CP or CP aa 1 to 74 from WSMV-T was transferred to WSMV-S81-GFP-6K1/CI(7aa). The GFP-tagged WSMV-S81 variants efficiently infected wheat cv. Tomahawk, and the expression of GFP in upper noninoculated wheat leaves is shown (panels a to c). The infection foci (shown with arrows) from WSMV-S81-GFP (panel d) and its variants with the entire WSMV-T CP (panel e) or with only aa 1 to 74 (panel f) inoculated the leaves of SDp2 at 7 days postinoculation. The number of infection foci per inoculated leaf is indicated at the bottom of each picture. The GFP fluorescence was observed under a stereo fluorescence microscope with GFP narrow-band filters. Leaves that are not expressing GFP fluorescence appear black. Bars represent 200 \( \mu \)m. Virus particles from SDp2 leaves at 10 dpi inoculated with WSMV-S81-GFP-6K1/CI(7aa) (panel g) and its variants with complete CP (panel h) or CP aa 1 to 74 (panel i) from WSMV-T. Bars represent 200 nm.
WSMV hybrid viruses between the WSMV-S81 and WSMV-T isolates accumulated as much RNA as wild-type virus WSMV-S81 (Table 2).

Recently, we developed several GFP-tagged WSMV-S81 constructs as marker viruses to examine the movement of WSMV in wheat (55). These GFP-tagged WSMV-S81 vectors were able to infect SDp2 systemically, with a slight delay (1 to 4 days) in symptom expression compared to that of the wild-type virus. Hence, GFP-tagged WSMV [WSMV-S81-GFP-6K1/CI(7aa)] (Fig. 6A) was used to examine the effect of CP or CP aa residues 1 to 74 from WSMV-T on cell-to-cell and/or long-distance movement and virion assembly of WSMV-S81 in SDp2. The GFP cistron with the 6K1/CI cleavage site was engineered between the P1 and HC-Pro cistrons of WSMV-S81-CP-T and WSMV-S81-CPaa1-74-T to obtain WSMV-S81-GFP-CP-T and WSMV-S81-GFP-CPaa1-74-T, respectively. In vitro-generated transcripts from pSP6-WSMV-S81-GFP-CP-T and pSP6-WSMV-S81-GFP-CPaa1-74-T infected wheat with symptoms and expression of GFP fluorescence similar to those of WSMV-S81-GFP-6K1/CI(7aa) (Fig. 6B, panels a to c). SDp2 seedlings were inoculated at the two-leaf stage with crude sap from wheat leaves infected with WSMV-S81-GFP-CP-T or WSMV-S81-GFP-CPaa1-74-T and produced 17.0 ± 5.5 and 18.5 ± 9.5 foci per leaf, respectively, at 7 dpi on inoculated leaves (Fig. 6B, panels e and f), while the wild-type GFP-tagged virus WSMV-S81-GFP-6K1/CI(7aa) produced 19.0 ± 9.5 foci per leaf (Fig. 6B, panel d). These data suggested that similar numbers of infection foci were produced by WSMV-S81-GFP-6K1/CI(7aa) and its variants, WSMV-S81-GFP-CP-T and WSMV-S81-GFP-CPaa1-74-T (Fig. 6B, panels d to f). Infection foci produced by WSMV variants with the CP or with the N-terminal region of the CP from WSMV-T were similar in size to those induced by WSMV-S81-GFP-6K1/CI(7aa) (Fig. 6B, panels d to f). Furthermore, the ability to form virions by WSMV-S81-GFP-6K1/CI(7aa) and its variants with CP or CP amino acids 1 to 74 from WSMV-T was examined under a Hitachi H-7500 transmission electron microscope using partially purified preparations from inoculated SDp2 leaves at 10 dpi. All three of these viruses produced similar-quality virions in inoculated SDp2 leaves (Fig. 6B, panels g to i). These data together with the comparable numbers of genomic RNA copies that accumulated in leaves inoculated with WSMV-S81, WSMV-T, and WSMV-S81 with CP sequences from WSMV-T suggested that the CP cistron from WSMV-T did not appreciably affect replication, cell-to-cell movement, or virion assembly of WSMV-S81 in SDp2.

![FIG. 7. The CP or CP aa 1 to 74 from isolate WSMV-T displayed profound defects in systemic transport of WSMV-S81 in SDp2. (A to I) WSMV-S81-GFP-6K1/CI(7aa), WSMV-S81-GFP-CP-T, or WSMV-S81-GFP-CPaa1-74-T were inoculated to SDp2 and examined for GFP fluorescence in upper noninoculated leaves at 14 days postinoculation (dpi) (A to C), 20 dpi (D to F), and 30 dpi (G to I). Note that leaves without GFP expression appear black. Bars represent 200 μm.](image-url)
WSMV-S81 with CP or CP aa 1 to 74 from WSMV-T displays a profound defect in systemic transport in SDp2. The above-described experiments suggested that failure to infect SDp2 systemically by WSMV-T or WSMV-S81 with CP or CP aa 1 to 74 from WSMV-T is not due to defects in replication, cell-to-cell movement, or virion assembly. We next examined what prevented WSMV-S81 from systemic invasion of SDp2 using GFP-tagged viruses. WSMV-S81-GFP-6K1/CI(7aa) and its variants, WSMV-S81-GFP-CP-T and WSMV-S81-GFP-CPaa1-74-T, were inoculated to SDp2 at the two-leaf stage with crude sap derived from transcript-inoculated wheat, followed by examination of long-distance movement of viruses in upper noninoculated leaves by observing green fluorescence at 14, 20, and 30 dpi (Fig. 7).

WSMV-S81-GFP moved systemically into upper noninoculated leaves by 14 dpi with a series of virus unloading sites along major and minor parallel veins, and from there the virus moved from cell to cell and formed several clusters of cells expressing green fluorescence, which appeared as strings of beads along the veins (Fig. 7A). By 20 dpi, further cell-to-cell movement of the virus occurred from unloading sites and formed larger strips of cells expressing GFP along the veins (Fig. 7D). In contrast, WSMV-S81-GFP-CP-T and WSMV-S81-GFP-CPaa1-74-T produced only a few clusters of cells expressing GFP at 30 dpi in upper noninoculated leaves along a few veins, which appeared as dispersed strings of beads (Fig. 7H and I). Moreover, we did not observe a significant increase in
the size or the number of green fluorescent clusters, even at 45 dpi. However, the clusters of cells observed expressing GFP in upper noninoculated leaves suggested that WSMV-S81-GFP-CP-T and WSMV-S81-GFP-CPaa1-74-T were competent in exiting from phloem cells, albeit inefficiently, and were capable of cell-to-cell movement in upper noninoculated leaves upon exiting from vascular tissue, though it occurred very late in the infection cycle. Our data suggested that the CP of WSMV-T, precisely the N-terminal 1 to 74 aa, impedes systemic invasion of WSMV in SDp2 by affecting the timing and extent of virus vascular transport to upper noninoculated leaves. Thus, WSMV CP may function in virus loading into, exiting from, and/or transport through the phloem of SDp2 plants.

**DISCUSSION**

The differential infection of SDp2 by two related WSMV isolates, WSMV-S81 (infests SDp2) and WSMV-T (does not infect SDp2), could have been due to selective induction of defense response induced by these isolates. Under this scenario, the expectation would be that WSMV-T would induce the LDMR defense response and that WSMV-S81 would not induce such a response. Coinoculation of SDp2 with WSMV-S81 and WSMV-T and sequential inoculation of SDp2 with WSMV-T and WSMV-S81 did not prevent systemic infection of SDp2 by WSMV-S81. These data suggest that WSMV-T did not induce the LDMR defense response to restrict the virus to inoculated leaves. These data also indicated that the systemic infection defect of WSMV-T in SDp2 was not complemented in trans by WSMV-S81, but it might be a cis-preferential function.

As there are 38 aa differences (out of 3,035 aa) between the WSMV-T polyprotein and that of WSMV-S81, we reasoned that failure to infect SDp2 by the WSMV-T isolate might be due to incompatibility of some of the viral proteins with one or more host factors and, consequently, may affect virus replication and/or translocation. A reverse-genetics approach was used by exchanging the SDp2-resistance-breaking isolate (WSMV-S81) sequences with those of the SDp2 resistant isolate (WSMV-T) and vice versa and examining the ability of progeny virus to infect SDp2. Chimeras of WSMV-S81 with different portions of WSMV-T sequence, except the CP cistron, were able to systemically infect SDp2 similar to that by wild-type WSMV-S81, indicating that WSMV-T cistrons encompassing P1 through N1b were not responsible for the restrictive SDp2 infection phenotype. However, WSMV-S81 with the CP sequence from WSMV-T failed to infect SDp2 systemically at 20 to 25 dpi. This result, together with the finding that WSMV-T bearing the CP cistron from WSMV-S81 was able to establish a systemic infection in SDp2, provided unequivocal evidence that a tritimoviral CP is the key factor involved in host- and strain-specific infection. Several potyviral proteins, including CP, have been reported to be involved in differential infection of host plants. For example, CP, P1, and HC-Pro of Plum pox virus (16, 40, 41), VPg of Tobacco etch virus (TEV) (42), the 6K2 of Potato virus A (36), N1a-Pro of Papaya ringspot virus (10), and P3 of Turnip mosaic virus (52) were reported to be involved in host-specific infection.

The CPs of WSMV-S81 and WSMV-T differ in 11 of 349 aa, and the majority of these amino acid differences are conserved among WSMV-T-like isolates (46), suggesting that viral determinants for differential infection of SDp2 might reside within these differential amino acid residues. Finer-scale exchanges between CP cistrons allowed the SDp2-null phenotype of WSMV-T to be mapped to the first 74 amino acid residues in which the two isolates have five amino acid differences. Using site-directed mutagenesis of WSMV-S81, we demonstrated that WSMV-S81 bearing amino acid changes AS20/21, EP to AS20/21, EP and AG50/52, VE to AS20/21, EP and AG50/52, VE, but not Q30, L to Q30, L, had reduced capacity to infect SDp2. However, infectivity to SDp2 was abolished only when the four substitutions (AS20/21, EP and AG50/52, VE) were combined, which may be due to either additive or multiplicative effects. We also observed similar SDp2-null phenotypes with point mutations in the C-terminal region of WSMV-S81 CP (S. Tatineni, unpublished data) and WSMV-S81 with the CP cistron from SDp2-null isolate WSMV-E1 Batán 3 (12; data not shown).

In addition to genome protection, the CP of potyviruses is involved in multiple functions such as vector transmission (3, 5), cell-to-cell (19, 38) and long-distance (2, 19) movement, and host-specific infection (16). The central core domain of TEV CP is involved in cell-to-cell movement, and the variable
N- and C-terminal regions are essential for long-distance transport (18, 19). Similarly, failure of WSMV-T or WSMV-S81 with WSMV-T CP to infect SDp2 could be due to defects in virion assembly/disassembly, replication, or cell-to-cell or long-distance movement in SDp2. WSMV-S81 and its variants with CP from WSMV-T accumulated comparable numbers of genomic RNA copies in inoculated leaves, produced similar numbers of infection foci per inoculated leaves, and formed virus particles, suggesting that the failure to infect SDp2 by WSMV-T is not due to defects in replication, cell-to-cell movement of the virus, or virus assembly/disassembly. Collectively, these results identified WSMV CP as a long-distance transport factor in SDp2, but whether WSMV CP functions in long-distance movement in wheat is not known. However, a WSMV mutant expressing a truncated CP was unable to move from initially infected cells (13), so it is likely that CP is a required MP in wheat.

The long-distance movement of GFP-tagged WSMV with the entire WSMV-T CP or only aa 1 to 74 was greatly affected, with little or no effect on cell-to-cell movement. WSMV-S81 variants with WSMV-T CP induced initial signs of systemic infection at 30 dpi, with a few isolated clusters of cells expressing GFP. In contrast, WSMV-S81 developed several clusters of cells expressing GFP by 14 dpi, followed by invasion by the virus throughout most of the upper noninoculated fully expanded leaves by 20 dpi. These data suggest that the extent and timing of vascular transport of WSMV-S81 with WSMV-T CP were different from those of WSMV-S81. It is possible that WSMV-S81 with WSMV-T CP failed to enter into vascular tissue from local infection foci, followed by long-distance passive transport to upper noninoculated leaves. As mentioned above, WSMV-S81 variants with WSMV-T CP infrequently invaded parallel veins of upper noninoculated leaves by 30 dpi, with GFP fluorescence observed in both vascular tissue and adjacent mesophyll cells. Thus, it seems that gaining entry into or transport through the phloem is a low-probability event. However, the ability of virus to exit from veins into adjacent mesophyll cells may also be adversely affected.

What is the mechanism of WSMV CP function in the host-specific LTF? The differing amino acids of the N-terminal regions of the CPs between WSMV-S81 and WSMV-T probably are crucial for interactions with SDp2 proteins for efficient long-distance movement, and in the absence of compatible interactions between the CP and host proteins, virions either inefficiently enter into and/or are transported through the phloem, as mentioned above. The fact that WSMV-S81 with CP or CP amino acids 1 to 74 from WSMV-T CP formed virus particles similar to those of WSMV-S81 demonstrated that CP from WSMV-T did not affect virion formation of WSMV-S81 in SDp2. The majority of LTFs are reported to have a role in suppression of host RNA silencing (15, 35, 37). However, using transient expression of GFP by infiltrating *Nicotiana benthamiana* 16C leaves with *Agrobacterium*-harboring WSMV CP and mGFP, we found that the CP is not involved in the suppression of RNA silencing (50, 51; R. French, unpublished data), thus differing with the majority of LTFs. However, the possibility that WSMV CP is involved in some other steps of the host RNA silencing pathway in SDp2 cannot be excluded. Our data suggest that WSMV CP is a distinctive LTF, in that it affects host-specific long-distance transport of virus through vascular structure and that a minimum of four differing amino acids play an important role in host- and strain-specific LTF function.

In summary, we demonstrated that a tritimoviral CP functions in differential infection of SDp2 by WSMV isolates and that four specific amino acid residues at the N terminus of CP are responsible for this WSMV phenotype. Failure to infect SDp2 was due to a block in systemic infection by affecting entry into, or exit from, and/or transport through the phloem but not due to defects in replication, cell-to-cell movement, or virion assembly. Because interactions between viral and host proteins are crucial for successful systemic infection of plants (6, 26, 51, 56), identification of SDp2 host factors that differentially interact with CPs of WSMV isolates may lead to insights into the nature and mechanisms of virus long-distance transport in monocot plants.

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