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# Complete Deletion of *Wheat Streak Mosaic Virus* HC-Pro: a Null Mutant Is Viable for Systemic Infection

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# NOTES

## Complete Deletion of *Wheat Streak Mosaic Virus* HC-Pro: a Null Mutant Is Viable for Systemic Infection

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**A *Wheat streak mosaic virus* (WSMV) genome lacking HC-Pro was constructed and confirmed by reverse transcription-PCR to systemically infect wheat, oat, and corn. Coupled in vitro transcription/translation reactions indicated that WSMV P1 proteinase cleaved the polyprotein at the P1/P3 junction of the HC-Pro null mutant. The WSMV HC-Pro null mutant was competent for virion formation, but the virus titer was reduced 4.5-fold relative to that of the wild type. Collectively, these results indicate that WSMV HC-Pro is dispensable for replication and movement, two essential processes that are disrupted by point and small-insertion mutations introduced into potyvirus HC-Pro.**

Mutations in potyvirus HC-Pro affect multiple viral functions. Substitutions or small insertions in potyvirus HC-Pro disrupt polyprotein processing (6, 7, 18, 25), aphid transmission (1, 3, 4, 5, 13, 14, 16, 23, 26, 28), long-distance movement and maintenance of replication (11, 20, 21, 29), synergism with unrelated viruses (27), and suppression of posttranscriptional gene silencing (PTGS) (2, 8, 19, 22). *Wheat streak mosaic virus* (WSMV) is a tritimovirus distantly related to potyviruses (32) that encodes an HC-Pro homologue necessary for eriophyid mite transmission (33). However, the function(s) of WSMV HC-Pro required for infection remains undefined. To address this issue, we constructed and evaluated a null mutant in which the HC-Pro coding region was deleted from the WSMV genome.

To facilitate in-frame deletion of the HC-Pro coding region, the WSMV infectious clone pS81-SA (31) was modified by insertion of a SalI site immediately downstream of an engineered ApaI site in the 5′-proximal region of the P3 coding region (Fig. 1A). The resulting intermediate construct (pS81-SAS12) has two SalI sites flanking the HC-Pro coding region. Subsequent excision of the SalI fragment resulted in a genome (pS81ΔHC12-5) in which all but the 5′-proximal glycine codon of HC-Pro was removed. The deletion also removed the 5′-proximal glycine codon of P3. Thus, the P1 proteinase cleavage site (GLRWY/G) of the P1/P3 junction in the HC-Pro null mutant was identical to the P1/HC-Pro junction in wild-type WSMV (10).

Effects of introduced codons and/or deletion on polyprotein processing was assessed by coupled in vitro transcription/translation. Plasmids (0.5 μg) were linearized and used as templates in coupled reactions (SP6 RNA polymerase transcription and wheat germ extract translation). Translation products were labeled with [$^{35}$S]methionine, separated on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gradient gel (4 to 15%), and detected by autoradiography (Fig. 1B). SalI linearized pS81-SA immediately downstream of the P1/HC-Pro junction and resulted in the translation of P1 (41 kDa) but not HC-Pro (44 kDa). ApaI linearized pS81-SA immediately downstream of the HC-Pro/P3 junction such that both P1 and HC-Pro were translated, with P1 proteinase cleaving the polyprotein into mature P1 and HC-Pro. The presence of mature P1 and HC-Pro translation products for SnaBI-digested pS81-SA and pS81-SAS12 indicated that both *cis*-acting proteinases were functional. As pS81ΔHC12-5 lacks the HC-Pro coding region, the presence of mature P1 protein demonstrated the P1/P3 junction was processed by P1 proteinase. No discrete translation products were observed for coupled reactions lacking template DNA. These results indicated that deletion of HC-Pro and/or codon insertions did not interfere with polyprotein processing.

Plasmids were linearized with NotI and transcribed with SP6 polymerase, and the transcripts were inoculated into wheat as described previously (9). Transcripts derived from pS81-SA or pS81-SAS12 readily infected wheat (Table 1) and produced systemic symptoms typical of WSMV. Infectivity of transcripts derived from pS81ΔHC12-5 was reduced relative to that of the wild type or the intermediate construct (Table 1). However, passage of progeny virus derived from pS81ΔHC12-5 transcripts to wheat by sap inoculation resulted in infectivity similar to that of the wild type (Table 1) and obvious mosaic symptoms (Fig. 1C). This observation may indicate selection for adaptive mutations at one or more loci; however, progeny genomes were not examined in sufficient detail to address this hypothesis.

Passage of progeny virus by sap inoculation from wheat to

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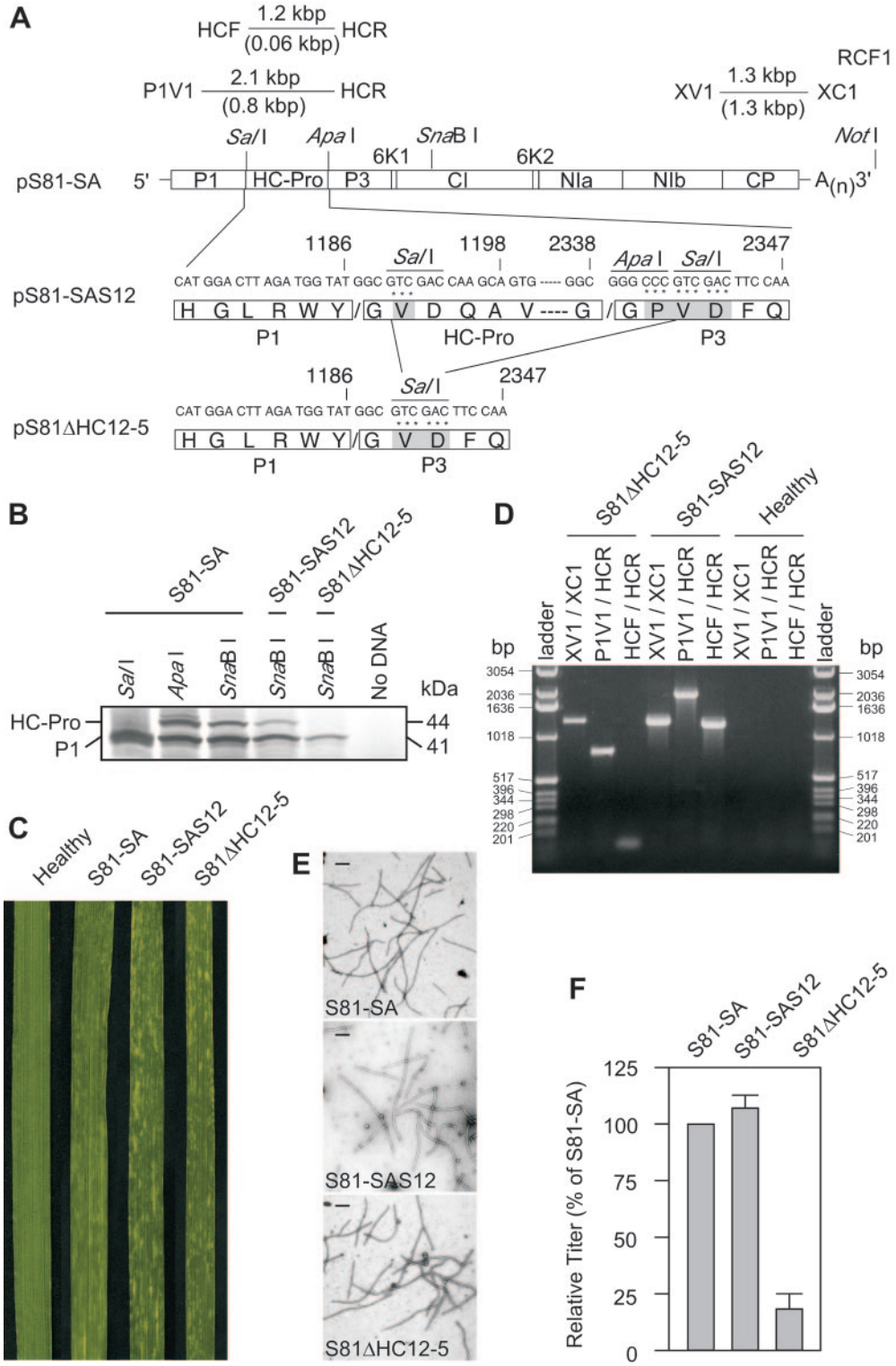


FIG. 1. Construction and phenotypic evaluation of a WSMV HC-Pro null mutant. (A) Schematic representation of modifications made to an infectious clone of WSMV (pS81-SA) to generate an intermediate construct (pS81-SAS12) and a WSMV HC-Pro null mutant (pS81ΔHC12-5) in which the HC-Pro coding region was completely deleted. The locations of polyprotein coding regions are indicated for pS81-SA, with regions of the genome modified in the intermediate and null mutant constructs expanded. Nucleotide coordinates listed are for WSMV Sidney 81 (GenBank accession no. AF057533); positions of relevant restriction sites are shown. Codons inserted to generate endonuclease restriction sites are indicated by asterisks, inserted amino acids are indicated by gray shading, and proteinase cleavage sites are indicated by slashes (/). The locations of annealing sites for RT primers (HCR and RCF1), three primer sets used for PCR (XV1 and XC1, HCF and HCR, and P1V1 and HCR), and sizes of RT-PCR products expected for the intermediate (shown above horizontal lines) and null mutant (shown below horizontal lines and in parentheses) constructs are indicated. (B) Proteinase activities of P1 and HC-Pro for the three WSMV constructs. DNA templates were linearized by digestion

wheat, oat, and corn indicated that the host ranges of the intermediate and null mutant constructs were essentially unaltered from that of the wild type (Table 1). All three constructs produced typical streak mosaic symptoms on SDp2 corn. Symptoms on oat for each construct ranged from mild mosaic to no symptoms, with the null mutant producing a higher percentage of symptomless infections than the wild type or the intermediate construct. For all three host species, no symptoms were observed on uninoculated control plants.

WSMV genotypes present in infected wheat following inoculation with transcripts were verified by reverse transcription-PCR (RT-PCR) of total RNA extracted (24) from systemically infected leaves 21 days postinoculation (Fig. 1D). The location of each primer and sizes of expected RT-PCR products are indicated in Fig. 1A. RT reactions employed a mixture of primers RCF1 (24) and HCR (31) complementary to WSMV RNA. WSMV infection was confirmed by amplification of the coat protein coding region with primers XV1 and XC1 (15). Viral genotypes derived from pS81-SAS12 and pS81ΔHC12-5 could be distinguished based on product size by using primers HCR and HCF, which amplify HC-Pro (31), or primers HCR and P1V1 (5′-CTTCATTGAGGATCACTTTGCTGC-3′), which amplify the 3′-proximal two-thirds of P1 and all of HC-Pro. For primer sets that flank HC-Pro, the 1.2-kbp difference in product size (Fig. 1D) corresponded to the length of the HC-Pro coding region. For all primer sets, RT-PCR products from wheat plants infected with transcripts of pS81-SAS12 were indistinguishable from those of wheat plants infected with transcripts of pS81-SA (data not shown). RT-PCR assays (primers HCR and P1V1) verified that all three constructs were able to systemically infect wheat, oat, and corn following sap inoculation with progeny virus produced in transcript-inoculated wheat (data not shown). No products were amplified by RT-PCR of total RNA samples extracted from uninoculated healthy plants (Fig. 1B and data not shown). Due to differences in size of expected RT-PCR products obtained using primers HCR and HCF (Fig. 1D), preferential amplification of the null mutant could have masked detection of WSMV genotypes bearing HC-Pro. To verify that the null mutant was competent to systemically infect wheat, corn, and oat in the absence of coinfection with a genotype bearing HC-Pro, cDNA generated using primer HCR also was used as the template for another PCR containing primer HCR and an upstream primer (HCV580) annealing to sequences within HC-Pro (nucleotides 1737 to 1762). The expected ~640-bp product was amplified from plants infected with S81-SA or the intermediate construct but not from plants infected with the null mutant or uninoculated healthy plants (data not shown).

Virions were extracted from wheat leaves systemically infected with progeny virus derived from pS81-SA, pS81-SAS12, or pS81-ΔHC12-5 and visualized by electron microscopy as described previously (32). Virions with flexuous rod-shaped morphology were observed for all three viral genotypes (Fig. 1E) but not from samples extracted from uninoculated healthy plants. A commercially available enzyme-linked immunosorbent assay kit (Agdia, Elkhart, IN) was used to detect WSMV coat protein and to determine relative virus titers in systemically infected leaves, as described previously (33). The titers of virus derived from pS81-SA (designated 100%) and pS81-SAS12 (relative titer of 109%) were similar (Fig. 1F). The relative titer of the HC-Pro null mutant was 4.5-fold less than (22%) that of virus derived from pS81-SA. These results indicated that the null mutant was competent for virion formation but that virus accumulated to lower levels in the null mutant than in the wild type. A similar reduction in virus titer has been observed for *Tobacco etch virus*, in which the N-terminal aphid transmission domain of HC-Pro was deleted (12).

Although the complete nucleotide sequence of the ipomovirus *Cucumber vein yellowing virus* (CVYV) lacks HC-Pro (17), viability of an axenic culture of HC-Pro-deficient CVYV has not been conclusively demonstrated. This study represents the first report of an HC-Pro null mutant in the context of an infectious clone. The null mutant was viable, indicating that WSMV HC-Pro was dispensable for the establishment and maintenance of systemic infection. Therefore, HC-Pro was not required for replication, cell-to-cell movement, long-distance movement, or encapsidation of WSMV, although HC-Pro may be involved in optimization of one or more of these processes. Since the titer of the null mutant was reduced relative to that of the wild type, WSMV HC-Pro likely performs a function (perhaps suppression of PTGS) necessary for optimal levels of virus accumulation. Indeed, WSMV P1/HC-Pro appears to act

TABLE 1. Infectivities of a wild-type construct (pS81-SA), an intermediate construct (pS81-SAS12), and a full HC-Pro deletion construct (pS81ΔHC12-5) of WSMV on three hosts[a]

| Construct | No. of plants infected/no. of plants inoculated[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat | | | | Oat | | SDp2 corn | |
| | Transcripts | | Progeny virus | | Progeny virus | | Progeny virus | |
| pS81-SA | 10/10 | 9/10 | 10/10 | 6/6 | 10/10 | 9/10 | 8/10 | 8/9 |
| pS81-SAS12 | 9/9 | 10/10 | 16/16 | 12/12 | 10/10 | 10/10 | 5/10 | 7/9 |
| pS81ΔHC12-5 | 3/10 | 1/10 | 16/16 | 13/13 | 8/10 | 6/10 | 9/10 | 6/9 |
| None | 0/10 | 0/10 | 0/16 | 0/10 | 0/10 | 0/10 | 0/10 | 0/9 |

[a] Infectivities and genotypes were verified by RT-PCR.
[b] Plants were mechanically inoculated with RNA derived from cloned WSMV genomes (transcripts) or with sap extracted from infected wheat plants (progeny virus) previously inoculated with transcripts.

with SalI, ApaI, or SnaBI and used to program coupled in vitro transcription/translation reactions. For simplicity, only the translation products corresponding to mature P1 (41 kDa) or HC-Pro (44 kDa) are shown. (C) Systemic mosaic symptoms in wheat inoculated with progeny virus of the three constructs. An uninoculated (Healthy) plant leaf is shown for comparison. (D) RT-PCR products of total RNA extracted (21 days postinoculation) from leaves of uninoculated plants (Healthy) or plants systemically infected with virus derived from transcripts of the intermediate construct pS81-SAS12 or the HC-Pro null mutant pS81ΔHC12-5. Lanes are labeled according to primer sets employed during PCR, and DNA standards (ladder) appear in the outer lanes with sizes in base pairs (bp) indicated. Sizes of RT-PCR products expected are indicated in panel A. (E) Electron micrographs of negatively stained virions extracted from plants infected with virus derived from the three constructs. Bar, 100 nm. (F) Relative virus titers (means ± standard deviations, expressed as percentages of pS81-SA) in systemically infected wheat leaves, as estimated for the three constructs by enzyme-linked immunosorbent assay.

as a suppressor of PTGS in transient assays conducted with immature wheat embryos (30). However, a "recovery" phenotype associated with PTGS was not observed in the absence of HC-Pro, as wheat infected with the null mutant produced symptomatic leaves for at least 3 months postinoculation (data not shown).

As with potyviruses, mutations in WSMV HC-Pro also abolish long-distance movement (D. C. Stenger and R. French, unpublished data). Since the WSMV HC-Pro deletion mutant was infectious, interference by mutant HC-Pro likely prevented long-distance movement. Host range restriction upon replacement of WSMV HC-Pro with potyvirus or rymovirus HC-Pro (31) also may be due to interference. Viability of the WSMV HC-Pro null mutant further indicates that a reassessment of potyvirus HC-Pro function may be warranted. A similar evaluation of null mutants could determine whether debilitating phenotypes associated with potyvirus HC-Pro mutants result from the loss of a required function or, alternatively, from interference by mutant HC-Pro.

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Mentions of proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval to the exclusion of others that also may be suitable.

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