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Comparison of the Nucleotide Sequence of the Coat Protein Open Reading Frame of Nine Isolates of Wheat Streak Mosaic Rymovirus

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Abstract. Wheat streak mosaic rymovirus (WSMV) is an important pathogen of wheat (*Triticum aestivum* L.). The coat protein region of nine isolates of WSMV were cloned by RT-PCR using primers that were inclusive of nucleotides 398-1825 (1) and sequence analysis indicated four regions of variability among the isolates.

Key words: wheat streak mosaic rymovirus, viral coat protein, nucleotide sequence, isolate comparison

Introduction

Serology (2) and RFLP mapping (3) have indicated heterogeneity among isolates of wheat streak mosaic rymovirus (WSMV), but the sequence of the 3′ end of only one isolate of WSMV has been reported (1). The sequence heterogeneity among an additional nine isolates of WSMV is presented in this report.

Materials and Methods

Sources of the WSMV isolates and the GenBank accession numbers for their respective nucleotide (nt) sequences are indicated in Table 1. PCR primers, provided by Dr. Roy French (USDA-ARS, Lincoln, NE), amplified the entire 1427 nt ORF for the coat protein gene inclusive of nts 398-1825. All nt and amino acid (aa) numbering for this study is according to Niblett et al. (1). Products from RT-PCR were cloned using a TA cloning kit (Invitrogen, San Diego, CA, USA) and directly sequenced using an ABI automated sequencer (Model 373, Forster City, CA, USA). Using a primer walk strategy, primers were designed to enable sequencing of both strands.

The Genbank accession numbers of the sequences reported in this paper are U53604, U54567, U54568, U54569, U54570, U54571, U54572, U54573, and U54574.

Results and Discussion

The nt and putative aa sequences were aligned and compared using MacVector™ software, which indicated the capsid region of WSMV strains is conserved at 98% and 96% identity at the nt and aa levels, respectively. Four variable regions were noted in aa alignments. In Region I, at positions 281-297 in the N-terminus of the capsid protein, there are three variable residues at positions 281, 293, and 297. Isolates TAMU, 964, Colo, PV106, Sidney, and 994 (designated Group I) have an alanine at position 281, while isolates OSU, PV91 and PV57 (designated Group II) have a threonine. Group I isolates have a glycine and a threonine at positions 293 and 297, while Group II isolates have a serine and a valine, respectively. Montana et al. (2) suggested that the different reaction of WSMV isolates to

Table 1. Origin and contributor of wheat streak mosaic virus isolates

Isolate designation	GenBank no.	Source	Contributor
OSU	U54571	USDA-ARS/OSU	Emil Sebesta
COLO	U54572	Fruita, CO	Bob Hammond
964	U54570	TX County, OK	OSU Diag. Lab
994	U54567	TX County, OK	OSU Diag. Lab
TAMU	U54574	Bushland, TX	Charles Rush
SIDNEY	U54573	Sidney, NE	Roy French
PV57	U53604	ATCC[1]	H.H. McKinney
PV91	U54568	ATCC	H.H. McKinney
PV106	U54569	ATCC	L.E. Williams

[1]American Type Culture Collection.

anti-WSMV monoclonal antibodies (Mabs) was a result of the antibody being produced to a specific discontinuous antigenic determinant (neotope) in the coat protein. In that study the isolates PV57, OSU, and PV91 reacted identically to the Mabs whereas other isolates did not. Sequence analysis indicated that the glycine versus serine at position 293 in Group I isolates is potentially highly antigenic. Thus, it may be part of a specific epitope and play a role in serological distinction of isolates.

Variable region II is located at aa position 354 where all isolates have an alanine except the previously published WSMV sequence (1) which contains a threonine. The other variable regions are located within the core region of the capsid protein (4) and unlikely influence the surface epitopes of the viral coat protein. Region III lies between residues 371 and 383 and region IV between residues 432 and 456. All isolates have an isoleucine at position 371 except PV106 which has a valine. Also, at positions 372 and 383, isolates 964, OSU, PV91 and PV57 have a glutamate residue where the other isolates have a lysine and an aspartate, respectively. At position 432, all isolates have a proline except for PV106 and PV57 which have an aspartate. Residue 437 is an alanine for all isolates except PV57 which contains a glycine. Isolate PV106 is unique at position 448 where it contains a three amino acid insertion (alanine-histidine-alanine) compared to other isolates. Isolate PV57 again varies from other isolates at positions 449 and 450, having an alanine and glutamine, respectively, instead of a glycine and glutamate. All isolates have a threonine at position 455 except for WSMV (1), TAMU, Sidney, and 994 which have a methionine. No variable residues were found in the putative C-terminus of the WSMV capsid protein.

Acknowledgments

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