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The 3′ terminal sequence of RNA1 of wheat spindle streak mosaic virus canadian isolate (WSSMV-C)

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Abstract

The sequence of the 3′ terminal 1722 nucleotides (nts) of RNA1 of the type (Canadian) isolate of wheat spindle streak mosaic bymovirus (WSSMV-C) was determined. The sequence started within a single open reading frame (ORF), which was expected to encode the carboxyl terminus of the nuclear inclusion b protein (NIb) and the capsid protein (CP) of 294 amino acids, followed by a 3′ untranslated region (UTR) of 237 nucleotides. The NIb and CP of WSSMV-C share 99 and 100% amino acid sequence identity with the corresponding proteins of WSSMV-French isolate (WSSMV-F), but only 89 and 77% with wheat yellow mosaic virus (WYMV-J), respectively. The 3′UTR of RNA1 of WSSMV-C shares 94% nucleotide sequence identity with that of WSSMV-F but only 73% with WYMV-J and WYMV-Chinese isolate (WYMV-Chi). The results support the classification of WSSMV-C and WSSMV-F as strains of the same virus species which is distinct from WYMV.

For decades, mosaic diseases of wheat caused by filamentous soil-borne viruses have lead to yield losses of wheat crops in many areas in the world. The first record was from Japan in 1927 (Sawada, 1927), and later the causal agent was described as wheat yellow mosaic virus (WYMV) (Inouye, 1969). In Canada, mosaic symptoms attributed to a soil-borne virus were first noticed on winter wheat in southern Ontario in 1957, and these were designated as wheat spindle streak mosaic virus (WSSMV) (Slykhuis, 1969).

Wheat bymoviruses have been also recognized in China (Chen, 1993), France (Signoret et al., 1979), Italy (Rubies and Vallega, 1987), India (Ahlawat et al., 1976) and the U.S.A (Wiese et al., 1970), where they have either been called WSSMV (Proeseler and Stanarius, 1983; Rubies and Vallega, 1987; Signoret et al., 1979) or WYMV (Lapierre et al., 1985).

Both WSSMV and WYMV are transmitted by the soil-borne plasmodiophoraceous fungus *Polymyxa graminis* (Slykhuis and Barr, 1978; Usugi et al., 1989). *Triticum* spp. are the only known plant hosts in nature, but rye can be infected mechanically by both of them (Usugi and Saito, 1979). Particles of WSSMV and WYMV are slightly flexuous filaments with two modal lengths of ca. 550 and 275 nm for WYMV, whereas 600–625 and 275–300 nm for WSSMV; 13 nm in diameter, and contain two 3′-polyadenylated single-stranded RNA species, RNA1 (Mr 2.6×10^6(WYMV and WSSMV) and RNA2 (Mr 1.5×10^6 (WYMV), 1.4×10^6 (WSSMV)) (Usugi et al., 1989; Slykhuis and Polak, 1970).

Because they show many similar features and have only slight differences in symptomatology and serology, Usugi and Saito suggested that WYMV-J (Japanese isolate) and WSSMV-C (Canadian isolate) were strains of the same virus (1989). Recent serological analysis with monoclonal antibodies raised against WYMV-F (French isolate) also indicated that WYMV-F, WYMV-J and WSSMV-USA (American isolate) share a high epitopic homology (Hariri et al., 1996). However, comparison of the sequence of RNA1 of WYMV-J with that of the 3′ half of RNA1 of WSSMV-F shows that although they have a similar genetic organization, they share only 77% amino acid

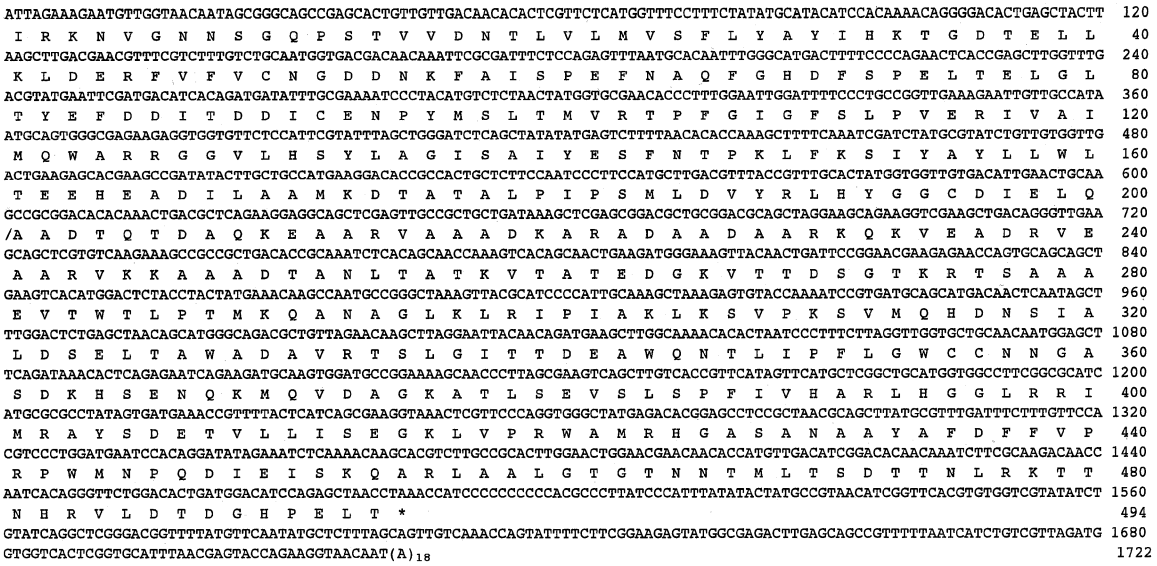

Figure 1. The 3′ terminal nucleotide and translated amino acid sequences deduced from the WSSMV-C cDNA clone. The proteolytic cleavage site between the NIb and CP sequences is shown by an oblique slant in the amino acid sequence. The polyprotein stop codon is denoted by an asterisk.

sequence identity in their CPs and 74% nucleotide sequence identity in their 3′ untranslated regions, suggesting that they are different species (Namba et al., 1998).

To better understand the taxonomic relationship between WSSMV and WYMV, we cloned and sequenced the 3′ terminus of RNA1 of WSSMV-C, which was described as the type strain of WSSMV by Slykhuis (1976).

WSSMV-C particles were purified as described by Usugi and Saito (1976) from the infected leaves of wheat plants (*Triticum aestivum* L. cvs. Hatakedakomugi or Norin 61) in which WSSMV-C was propagated by mechanical inoculation. Viral RNA was isolated from purified virus particles as described by Kashiwazaki et al. (1989).

2μg of a mixture of WSSMV-C RNA1 and RNA2 suspended in water was heated at 70 °C for 5 minutes before quenching on ice. cDNA synthesis was done following the manufacturer's protocol with the cDNA synthesis kit (cDNA Synthesis System Plus, Amersham, Little Chalfont, U.K.). The first strand cDNA was synthesized by oligo (dT) priming followed by second strand cDNA synthesis as described by Gubler and Hoffman (Gubler and Hoffman, 1983).

Double stranded cDNA was cloned into *Sma* I-cut dephosphorylated, pBluescript II SK (+), and one clone with 2.5Kb insertion (pBSWSSMV56) was selected for sequence analysis.

The nucleotide and amino acid sequences were analyzed with DNASIS software (Ver.3.6 Hitachi Soft Engineering, Yokohama, Japan), and CLUSTAL V was used to align the nucleotide sequences.

Comparisons of the restriction map and partial sequences from both ends of pBWSSMV56 with the sequence reported for WSSMV-F RNA1 (Sohn et al., 1994) revealed that it may be from RNA1. The sequence of this clone was analyzed by the dideoxynucleoside chain termination reaction (Sanger et al., 1977) using either universal primers or internal primers designed from determined sequences in an automated DNA sequencer (ABI PRISM^{TM} 310 Genetic Analyzer, Perkin Elmer, USA). All parts of the cDNA were sequenced in both orientations.

Complete sequencing of pBWSSMV56 clone provided 1722 nucleotides, upstream of the 3′ poly(A) tail (Figure 1). This sequence starts within a long open reading frame (ORF) which terminates with an UAA ochre codon at position 1483, leaving 237 untranslated nucleotides at the 3′ terminus (3′UTR). A possible polyadenylation signal UAUGA (Zaret and Sherman, 1982) was found on the 136 nucleotides upstream of the poly(A) tail.

The partial ORF encodes a polypeptide of 494 amino acids. Comparison with the sequence reported for WSSMV-F suggests that this polypeptide represents the C-terminal part of a polyprotein, including part of the nuclear inclusion protein (NIb) followed by

Table 1. The relationships between the capsid proteins and 3′ untranslated regions of bymoviruses

	WSSMV-C	WSSMV-F	WYMV-Chi	WYMV-J	BaYMV-J	BaYMV-G	BaMMV-Kal	BaMMV-Nal	RNMV
WSSMV-C		94	73	73	70	65	50	51	49
WSSMV-F	**100**		74	74	71	66	51	51	49
WYMV-Chi	**76**	**76**		98	69	64	52	52	49
WYMV-J	**77**	**77**	**97**		68	64	52	52	49
BaYMV-J	**76**	**76**	**68**	**68**		66	50	52	51
BaYMV-G	**74**	**74**	**68**	**68**	**96**		51	70	51
BaMMV-Kal	**34**	**34**	**35**	**35**	**34**	**34**		92	44
BaMMV-Nal	**33**	**33**	**34**	**34**	**33**	**34**	**94**		43
RNMV	**40**	**40**	**37**	**37**	**38**	**38**	**55**	**53**	

Percentage of CP identical amino acids are below the diagonal. Percentage of 3'UTR identical nucleotide acids are above the diagonal. Virus acronyms and references of their sequence data in alphabetical order as follows: BaMMV-Kal, and -Nal, barley mild mosaic bymovirus -Kal and -Nal strains (Kashiwazaki et al., 1992; 1996); BaYMV-J and -G, barley yellow mosaic bymovirus Japanese (Kashiwazaki et al., 1990) and German (Peerenboom et al., 1992) isolates; RNMV, rice necrosis mosaic bymovirus (Badge et al., 1997); WSSMV-C and -F, wheat spindle streak mosaic bymovirus Canadian (Slykhuis, 1969) and French (Sohn et al., 1994) isolates; WYMV-J and -Chi, wheat yellow mosaic bymovirus Japanese (Namba et al., 1998) and Chinese (Yu et al., 1995) isolates.

the capsid protein (CP). The Q/A at position 200/201 (amino acid) is thought to be the cleavage site between the NIb and CP regions, as reported for WSSMV-F.

The consensus motif GDD, which has been found in all RNA dependent RNA polymerases (Koonin and Dolja, 1993), is present in the NIb protein region at amino acid positions 52–54.

The predicted capsid protein of WSSMV-C consists of 294 amino acids with calculated Mr values of 31.7K. This value is consistent with that estimated by SDS-PAGE of purified virus particles (Usugi et al., 1989).

This paper reports the nucleotide sequence of the 3′ region of RNA1 of the Canadian type strain of WSSMV (WSSMV-C). Comparison with the sequence reported for WSSMV-F (French isolate) indicates that WSSMV-C and WSSMV-F have the same genetic organization. The amino acid sequences of the C-terminal part of the NIb and whole of the CP of WSSMV-C determined here and the corresponding sequences of WSSMV-F are aligned without gaps. There are only two amino acid differences between their NIb regions (99% identity), and no amino acid variation in their CPs (100% identity). Although the 3'UTR of RNA1 of WSSMV-C is 6 nucleotides longer than that of WSSMV-F, they show 94% nucleotide sequence identity when aligned with gaps (data not shown). These high amino acid and nucleotide sequence identities confirm that WSSMV-C and WSSMV-F are strains of the same virus.

WSSMV-C and WYMV-J have 89% and 77% amino acid sequence identity, in the NIb and CP regions, respectively, and 73% nucleotide sequence identity in the 3′UTRs of RNA1. These identities are significantly lower than those between WSSMV-C and -F, BaYMV-J and -G, and BaMMV -Ka1 and -Na1. However, they are similar to those between WSSMV (-C and -F) and BaYMV (-J and G), whereas much higher than those between WSSMV (-C and -F) and BaMMV (-Ka1 and Na1), and between WSSMV (-C and -F) and RNMV (Table 1). Nucleotide sequence has recently been reported for the 3′ region of RNA1 of WYMV-Chi (Yu et al., 1995). WYMV-J and WYMV-Chi share high sequence identity in their CPs and 3′UTRs (Table 1). Thus, classification of wheat bymoviruses into two distinct species, WSSMV and WYMV, is justified. The sequence data indicate the occurrence of WSSMV in Canada and France, and WYMV in Japan and China, but more isolates from different locations need to be sequenced for better understanding of the taxonomy of wheat bymoviruses and their distribution in the world.

The nucleotide sequence data reported here will appear in the DDBJ Nucleotide Sequence Databases under the accession number (AB010578).

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