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Identification of a Naturally Occurring Recombinant Isolate of *Sugarcane Mosaic Virus* Causing Maize Dwarf Mosaic Disease*

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Abstract. The complete nucleotide sequence of a potyvirus causing severe maize dwarf mosaic disease in Shaanxi province, northwestern China was determined (GenBank accession No. AY569692). The full genome is 9596 nucleotides in length excluding the 3′-terminal poly (A) sequence. It contains a large open reading frame (ORF) flanked by a 149 nt 5′-untranslated region (UTR) and a 255 nt 3′-UTR. The putative polyprotein encoded by this large ORF comprises of 3063 amino acid residues. Sequence comparisons and phylogenetic analyses showed that this potyvirus is an isolate of *Sugarcane mosaic virus* (SCMV). The entire sequences shared identities of 89.6–97.6% and 79.3–93.3% with 9 sequenced SCMV isolates at the nucleotide and deduced amino acid levels, respectively. But it showed much lower identities with *Maize dwarf mosaic virus* (MDMV), *Sorghum mosaic virus* (SrMV) and *Johnsongrass mosaic virus* (JGMV) isolates. The putative coat protein sequence is identical to that of a Chinese maize isolate SCMV-HZ. However, partition comparisons and phylogenetic profile analyses of the viral nucleotide sequences indicated that it is a recombinant isolate of SCMV. The recombination sites are located within the 6K1 and CI coding regions.

Key words: maize dwarf mosaic disease, phylogenetic analysis, potyvirus, *Sugarcane mosaic virus*, Virus recombination

Introduction

Maize dwarf mosaic disease (MDMD) is a worldwide disease causing severe yield losses in maize. In China, it was first reported in Henan province in 1968 [1]. Since then, it spreads widely and become a major disease of maize in most maize growing areas in China. *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) were identified as the pathogens causing this disease in China and other countries by serological investigations [1,2]. Both viruses are members of the SCMV subgroup in the *Potyvirus* genus of the *potyviridae* family [3]. Three other potyviruses, *Sorghum mosaic virus* (SrMV), *Johnson grass mosaic virus* (JGMV) and *Zea mosaic virus* (ZeMV) are also included in this subgroup [3,4]. These SCMV subgroup viruses are transmitted by aphids and cause mosaic and dwarf diseases in maize, sugarcane, sorghum and other poaceous plants [5]. Like other potyviruses, their genomes are single-stranded positive RNAs with a size of approximately 10 kb in length, containing single large open reading frames (ORFs). However, they are distinct to each other in the genome sequences, especially in the coat protein (CP) and 3′-untranslated region (UTR) sequences [3,6]. Based on the sequence comparisons and phylogenetic analyses of the full genome and the CP

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sequences, the pathogens causing MDMD in most maize-growing areas of China were identified as isolates of SCMV [5,7–9]. This was confirmed by the result of immunocapture RT-PCR that only SCMV was detected in all 62 diseased maize samples collected from eight maize-growing provinces in China [10]. These results suggest that SCMV is the most common potyvirus infecting maize in China.

In the 1990's, a severe outbreak of maize dwarf mosaic disease was recorded in Shaanxi province, northwestern of China [11]. To determine the causal potyvirus or potyviruses, samples were collected in a heavily diseased maize field in Shaanxi province. In the present study, we report the complete genome sequence of the potyvirus causing this disease. The sequence was compared with five SCMV isolates causing maize dwarf mosaic disease in the south (Guangdong province, AJ310105), north (Beijing, AY042184), southeast (Zhejiang province, AJ297628), northeast (Shandong province, AY149118) and the center (Henan province, AF494510) of China, and the relationships between this isolate and other SCMV subgroup potyviruses were discussed.

Materials and Methods

Virus Preparation and RNA Isolation

Severely infected maize leaves were collected in 1999 from Shaanxi province, northwestern of China. The virus was maintained by mechanical inoculation in a maize (*Zea mays*) susceptible variety, Mo 17. Total RNA was extracted from the infected maize leaves using the method of Chomezynski et al. [12].

cDNA Synthesis and Cloning

Total RNA was used as template for RT-PCR. The RNA was reverse transcribed by using M-MLV reverse transcriptase (Promega). Six overlapping cDNA fragments were amplified using RT-PCR (Fig. 1). The oligonucleotides used for RT-PCR were synthesized (Sangon) and their sequences were listed in Table 1. An enzyme mixture of *Taq* (Promega) and *Pfu* (Stratagene) DNA polymerase (4:1) was used for all PCR reactions. PCR products of the expected sizes were purified using Wizard® PCR Preps DNA Purification System (Promega). The purified PCR products were blunted with T_4 DNA polymerase and ligated to *Eco*RV-linearized pBluescriptII SK(+) (Stratagene), or ligated directly to pGEM-T vector (Promega), and transformed info *E. coli* DH5α cells. At least two independent clones for each cDNA fragment were sequenced from both directions.

Sequence Analyses

The full-length cDNA sequence was assembled from six overlapping clones and the primer sequences were corrected with the sequences from Shaanxi isolate. Multiple alignment and phylogenetic analyses were performed using software DNAMAN (Lynnon BioSoft, version 4.0). Recombination events were analyzed by comparing the phylogenies reconstructed from different genome regions. The recombination sites were found by using PHYLPRO (version beta 1.0) developed by Weiller [13]. Other viral sequences used in these analyses were obtained from GenBank/EMBL database (Table 2).

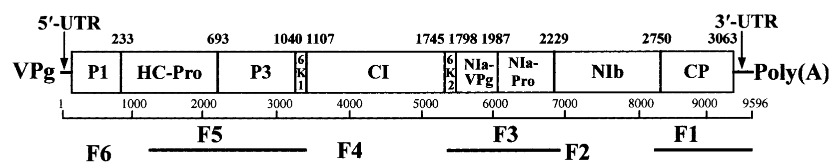

Fig. 1. cDNA cloning strategy and genome organization of a potyvirus isolated from Shaanxi, China. Untranslated regions and open reading frame were showed by a single line and an open bar, respectively. Vertical lines in the open bar indicated the putative cleavage sites within the polyprotein. The amino acid positions of the last residues of ten mature proteins were shown above the bar. Approximate nucleotide positions of six cDNA clones were shown below the genome map.

Table 1. Primers used in RT-PCR

Primer	Fragment	Direction	Primer sequence
Oligo(dT)	F1(8376-9596)	antisense	5′-TTT TTT TTT TTT TTT TTT-3′
P1		sense	5′-GGA TCT TCC CGG ATA CAT AGA AGA T-3′
P2	F2(6578-8403)	antisense	5′-GCC TTG TGC ACC CGC ATC AAC AGT TC-3′
P3		sense	5′-GGT GGC CAA TGT GGT TTA CCA TTG GT-3′
P4	F3(5309-6922)	antisense	5′-GCG GGC ACG GAC CTT TAA CAA CAT GT-3′
P5		sense	5′-GTG TTA GAG ACC AAC TGA TTG AGT T-3′
P6	F4(3163-5432)	antisense	5′-CAA GTC GCG TTG AAT GAG CGA AGC-3′
P7		sense	5′-CAT GCC AAT TTG ATG AAG GAG C-3′
P8	F5(1216-3323)	antisense	5′-GTG GGT CGA GTA TCA TAG TGA G-3′
P9		sense	5′-CCT TGC TAT GGC AAG TAG CAG-3′
P10	F6(1-1441)	antisense	5′-CCA TTT GAA TCG AGT TGA TTG TCG CAC-3′
P11		sense	5′-AAA AAC AAC AAG ACT CAA CAC AAC AC-3′

Note: P1 and P2 were designed base on the sequence of MDMV-B (D00949). P3, P4, P5, P10 and P11 were designed base on the sequence of MDMV-Bg (AJ001691) in the conserved regions. P7 and P8 were designed base on the sequence of SCMV-HZ. P6 and P9 were designed base on the sequence we determined in this study. Primer sequences were not counted in the positions of cDNAfragments except P11.

Table 2. Sources and GenBank accession numbers of virus sequences used in this paper

Virus	Source	Accession number	Genomic region
JGMV		Z26920	Genomic
MDMV-Bg	Maize (Bulgaria)	AJ001691	Genomic
SCMV-MDB (1)	Maize (USA)	A34976	CP
SCMV-MDB (2)	Maize (USA)	D00949	CP
SCMV-BJ	Maize (Beijing)	AY042184	Genomic
SCMV-GD	Maize (Guangdong)	AJ310105	Genomic
SCMV-HN	Maize (Henan)	AF494510	Genomic
SCMV-HZ	Maize (Hangzhou)	AJ297628	Genomic
SCMV-LP	Sugarcane (Linping)	AJ310102	Genomic
SCMV-SD	Maize (Shandong)	AY149118	Genomic
SCMV-SX	Maize (Shaanxi)	AY569692	Genomic
SCMV-XgS	Sugarcane (Xiangshan)	AJ310103	Genomic
SCMV-YH	Sugarcane (Yuhang)	AJ310104	Genomic
SCMV-BOR	Maize (Germany)	X98167	CP
SCMV-Mx	Maize (Mexico)	AY195610	CP
SCMV-BRIS	Sugarcane (Brisbane)	AJ278405	Genomic
SCMV-USLA	Sugarcane (USA)	AF006736	CP
SCMV-SA	Sugarcane (South Africa)	AF006738	CP
SCMV-A	Sugarcane (USA)	U57354	CP
SrMV-XoS	Sugarcane (Xiaoshan)	AJ310197	Genomic
SrMV-YH	Sugarcane (Yuhang)	AJ310198	Genomic

Note: The abbreviations of virus names used in this table stand for Johnsongrass mosaic virus (JGMV), Maize dwarf mosaic virus (MDMV), Sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV).

Results and Discussion

Sequence and Genome Organization

The complete genome sequence of a potyvirus causing severe MDMD in Shaanxi province of China was determined (GenBank accession No. AY569692). The full genome is composed of 9596 nucleotides (nts), excluding the 3′-terminal poly(A) tail. The base composition of the genome is adenine 33.7%, cytosine 20.4%, guanine 21.5%, and uracil 24.4%, which is similar to that of most potyvirus genomes.

There are three in frame AUG codons within the first 500 nts of the long single open reading frame (ORF). But only the first one at nucleotide position 150–152 is in a context (C-G-A-G-A_{150}UG-G-C) in reasonable agreement with the consensus sequence (A-A-C-A-AUG-G-C) proposed for translation initiation in plants [14], so it is most likely to be the start codon of the polyprotein. The in frame termination codon UAG is located at position 9339–9342. Therefore, the predicted large single ORF consists of 9192 nts and encodes a putative polyprotein of 3063 amino acids (aa) with calculated Mr of 346,104. The 5′-UTR and 3′-UTR excluding the poly (A) tail are 149 and 255 nts, respectively. A 12 nts "potybox a" [8] (U_{15}CAACACAACAC_{26}) and a 7 nts "potybox b" [15] (C_{39}CAAGCA_{45}) that highly conserved in potyviruses were found in the 5′-UTR. However, "potybox a" was located in the sequence of primer P11, which was used in the RT-PCR cloning of the 5′-terminal sequence of the Shaanxi isolate. The P11 sequence is identical to the 5′-terminus of MDMV-Bg (AJ001691) and it has only one nucleotide difference to the conserved 5′-terminus of SCMV isolates.

Based on the comparisons of the amino acid sequence of the polyprotein encoded by the Shaanxi isolated potyvirus with that of other potyviruses, nine cleavage sites and the corresponding surrounding sequences for proteinases P1, HC-Pro and NIa-Pro were identified (Table 3). This indicated that the polyprotein encoded by the Shaanxi isolate was probably processed into ten putative proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP) in a manner similar to other potyviruses. The genome organization of the Shaanxi isolate was showed in Fig. 1.

Functional important motifs or conserved sequences were searched in the putative proteins encoded by the Shaanxi isolate. In P1 protein, the serine protease active site motif (G_{194}XSG_{197}) and proteolytic domain F_{207}IVRGR_{212} were found to be conserved in potyviruses. But the Shaanxi isolate, as well as SCMV subgroup potyviruses, contain shorter sequences between these two conserved blocks than other potyviruses. HC-Pro is a multifunctional protein that involved in the transmission by aphid vectors, protein processing, virus systemic movement, genome amplification and also functioned as a suppressor of gene silencing [16]. The N-terminal K_{287}ITC_{290} motif and the C-terminal P_{545}TK_{547} motif were probably involved in the binding of HC-Pro to the aphid stylet [17] and the D_{2755}AG_{2757} motif of coat protein [18,19]. Putative proteinase active site residues C_{579} and His_{652}, C_{527}CCVT_{531} motif which demonstrated to be involved in systemic movement and I_{552}GN_{554} motif which assumed to be important in genome amplification [20,21] were also found in HC-Pro of the Shaanxi isolate. The CI protein contains the NTP binding motif (G_{1195}XXGXGKST_{1203}) and helicase motifs [22]. The conserved blocks described by Kong and Steinbiss [23] in MDMV-Bg were also found in the CI protein of the Shaanxi isolate. The NIa-VPg protein contains a tyrosine residue in the Motif M_{1859}MY_{1861} which is probably required for the linking of VPg to 5′-end of the viral RNA [5]. The motif G_{2136}XCG_{2139} is the conserved protease active site of NIa-Pro. The NIb protein contains a G_{2581}DD_{2583} motif, which was found in many virus RNA dependent RNA polymerase [24]. In

Table 3. Putative cleavage sites and adjacent sequences of the viral proteases in the genome of the Shaanxi isolate

	P6	P5	P4	P3	P2	P1	P1′
P1/HC-Pro†	M	E	I	E	H	Y	A
HC-Pro/P3‡	R	E	Y	I	V	G	G
P3/6K1*	T	G	V	I	H	E	G
6K1/CI*	P	P	V	T	Q	Q	S
CI/6K2*	N	T	V	I	H	Q	G
6K2/NIa -VPg*	T	E	V	S	H	Q	G
NIa-VPg/NIa-Pro*	T	G	V	A	H	E	S
NIa-Pro/Nrb*	M	S	V	E	E	Q	C
NIb/CP*	E	D	V	F	H	Q	S

†*P1 protease*
‡*HC protease*
**NIa protease*

the *potyvirus* genus, NGDD was found to be conserved in this motif. However, an A to T transversion in the Shaanxi isolate causes the substitution of N with Y residue. The N-terminus of CP of the Shaanxi isolate is rich in glycine and serine, like SCMV isolates [2,5], and contains four nearly exactly GGQSG repeats and some shorter repeats such as TPPA, GSSG and GSGT.

Phylogenetic Analyses

The primary sequence analyses of the Shaanxi isolate indicated that it is closely related to the SCMV subgroup potyviruses. To examine the relationships of this isolate to other potyviruses, the complete nucleotide and amino acid sequences of the Shaanxi isolate were compared with isolates of SCMV subgroup potyviruses. The results showed that this isolate shared identities of 79.3–93.3% and 89.6–97.6% with nine sequenced SCMV isolates at the nucleotide and deduced amino acid levels, respectively (Table 4). But it only shared much lower identities with MDMV-Bg (AJ001691), JGMV (Z26920), SrMV (AJ310197 and AJ310198) either at nucleotide (less than 70.1%) or deduced amino acid (less than 76.3%) levels. Phylogenetic tree analyses based on the multiple alignments of the entire nucleotide and amino acid sequences showed that the Shaanxi isolate was grouped into the cluster of SCMV isolated from maize (Fig. 2). CP sequence comparisons showed that the Shaanxi isolate is identical to SCMV-HZ (data not shown). These results indicated that the potyvirus isolated from Shaanxi is an isolate of SCMV and shared highest identities with SCMV-HZ (Table 4). Thus it was referred to as SCMV-SX.

The variable N-terminus of the potyvirus CP is necessary for aphid transmission and systemic infection, and is important for the adaptation of the virus to its host [25]. Differences in this region are important for the potyvirus discrimination. Based on the N-terminal coat protein (CP) serology, strains of SCMV and MDMV were assigned into four distinct potyviruses: SCMV, MDMV, SrMV and JGMV [3]. To examine the relationships among different SCMV isolates, multiple alignments of N-terminal CP sequences were performed (Fig. 3). At least 3 types of N-terminal CP sequences were detected, similar results was also obtained by Alegria et al. [26]. The sugarcane

Table 4. Percent identities of the complete nucleotide sequences (top right) and amino acids sequences of polyproteins (bottom left) of potyviruses in the subgroup of *Sugarcane mosaic virus*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 SCMV-SX		**91.8**	**90.5**	**93.3**	**90.0**	82.7	80.8	80.9	80.7	79.3	70.0	70.1	69.8	56.3
2 SCMV-BJ	**97.2**		**95.1**	**96.9**	**94.5**	81.0	79.8	80.0	79.8	79.1	70.0	70.2	69.4	56.7
3 SCMV-HN	**97.0**	**98.5**		**94.2**	**98.2**	81.6	80.0	79.9	79.9	79.2	70.2	70.4	69.3	56.7
4 SCMV-HZ	**97.6**	**98.7**	**98.3**		**93.7**	80.9	79.8	79.9	79.8	79.1	70.1	70.2	69.5	56.7
5 SCMV-SD	**96.5**	**97.7**	**98.5**	**97.5**		81.3	79.8	79.8	79.8	79.0	70.2	70.5	69.2	56.6
6 SCMV-GD	92.1	91.3	91.5	91.3	91.3		83.1	83.1	83.2	79.0	70.1	70.1	69.6	56.6
7 SCMV-LP	91.3	90.6	90.7	90.5	90.3	93.6		**97.2**	**96.2**	79.3	69.4	69.5	69.3	56.3
8 SCMV-XgS	91.8	91.0	91.1	91.0	90.9	93.8	**98.6**		**96.4**	79.5	69.7	69.9	69.3	56.3
9 SCMV-YH	91.7	90.9	91.1	90.9	90.8	93.7	**98.2**	98.6		79.3	69.4	69.6	69.0	56.3
10 SCMV-BRIS	89.6	89.4	89.6	89.3	90.1	89.5	89.8	90.0	90.0		69.8	69.9	68.8	55.0
11 SrMV-XoS	75.6	75.9	76.1	75.7	76.0	75.6	75.3	75.5	75.7	76.2		**95.8**	71.9	56.2
12 SrMV-YH	76.3	76.6	76.8	76.4	76.7	76.1	76.0	76.1	76.2	76.9	**98.0**		71.8	56.5
13 MDMV-Bg	75.5	75.5	75.7	75.2	75.5	74.7	74.8	75.0	75.0	75.4	77.9	78.4		55.9
14 JGMV	49.6	49.8	49.9	49.9	49.8	49.8	49.9	49.8	49.9	49.3	49.8	50.2	49.9	

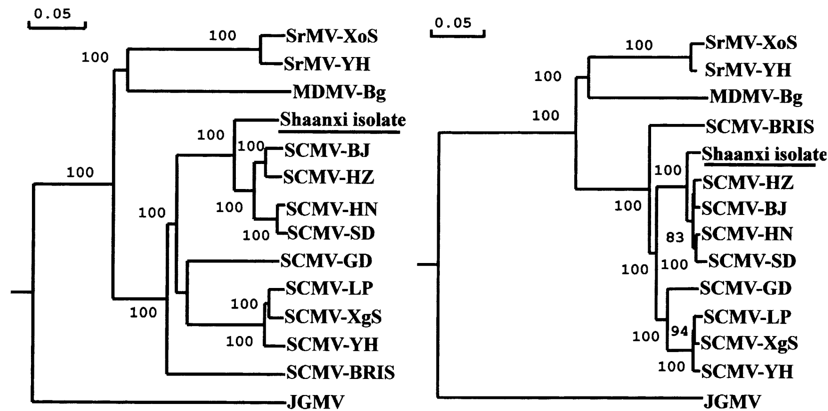

Fig. 2. Phylogenetic tree analyses of the SCMV subgroup potyviruses. The tree was constructed from multiple sequence alignment of genome RNA sequences (left tree) or polyprotein sequences (right tree) using DNAMAN 4.0. Bootstrap values noted indicated the times each branching was found in 100 bootstrap analyses. Bars showed the genetic distances. The sequences used in the analyses were obtained from GenBank/EMBL (see Table 2).

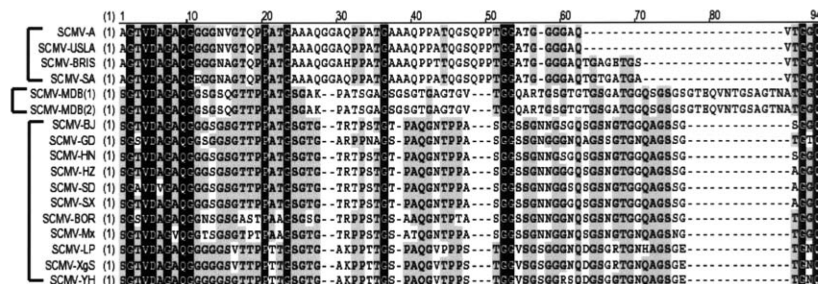

Fig. 3. Alignment of the amino terminal portions of the coat proteins of SCMW. Three distinct groups were indicated with black lines.

isolates from South Africa (SCMV-SA, AF006738), Australia (SCMV-BRIS, AJ278405) and USA (SCMV-USLA, AF006736; SCMV-A, U57354) formed one type. They have been assigned to the strain of SCMV-A [27]. Two SCMV-MDB isolates (A34976 and D00949) showed significant differences to other isolates and formed a second type. It was suggested that SCMV-MDB should be considered as a new potyvirus species [7,27]. All Chinese maize, sugarcane isolates and the maize isolates from Germany (SCMV-BOR, X98167) and Mexico (SCMV-Mx, AY195610) formed the third type. However, based on the results of the genome sequence comparisons (Table 4), the Chinese isolates could be further divided into three groups, that is: (1) sugarcane isolates, (2) a maize Guangdong isolate, and (3) maize isolates from other provinces including the Shaanxi isolate. The similar comments was also suggested by Chen et al. [5]. The CP sequences of the last group isolates showed about 95% identities with SCMV-Mx and SCMV-BOR, suggesting that these maize isolates should be classed as a new strain but not geographical isolates.

RNA recombination analyses

Phylogenetic relationships among SCMV isolates (SCMV-BJ, -BRIS, -GD, -HN, -HZ, -LP, -SD, -SX, -XgS and -YH) were also analyzed using the nucleotide sequences of different region of the genomes (5′-UTR, P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb, CP and 3′-UTR). Different clustering properties were revealed for SCMV-SX and SCMV-GD in the phylogenetic trees (data not shown). In most cases, SCMV-SX was clustered together with SCMV-BJ,-HN, -HZ and -SD isolates. But in regions 6K1 and

CI, SCMV-SX was clustered together with SCMV-GD. SCMV-GD shows significant similarities to three Chinese sugarcane isolates (SCMV-LP, -XgS, -YH) in most regions of the viral genome. But in the regions of CP and 3′-UTR, it is more similar to SCMV-BJ. In the region of NIa-Pro, however, SCMV-GD showed similar distances to all other SCMV isolates (data not shown). These results indicated that the genomes of SCMV-SX and SCMV-GD were probably originated from two or more different parent isolates. They could be natural recombinant isolates. Alternatively, it is possible that the original maize sample was co-infected by a mixture of two different SCMVs and cDNA sequences from these two viruses were improperly assembled.

To discriminate between these two possibilities for SCMV-SX, PHYLPRO analysis was performed. Only five genome sequences (SCMV-BRIS, SCMV-GD, SCMV-HZ, SCMV-SX and SCMV-YH) were used in this analysis for minimal noise. The variable sites and a window of 100 bp were used for maximal phylogenetic profile signals (Fig. 4). Two possible recombination sites were revealed at positions 3281 and 5015 for SCMV-SX. Both sites were located in the sequence of cDNA fragment F4 (3163-5432). Pairwise comparisons indicated that the F4 fragment was probably originated from two or more different parent isolates. The central region (3281–5015) of it showed highest homology with SCMV-GD. However, the 5′-terminus (3163–3280) showed highest identity (98.3%) with SCMV-HZ and the 3′-terminus (5016–5432) showed high identities to four Chinese maize isolates (SCMV-BJ, -HN, -HZ, -SD) (Table 5). Therefore, different origination of

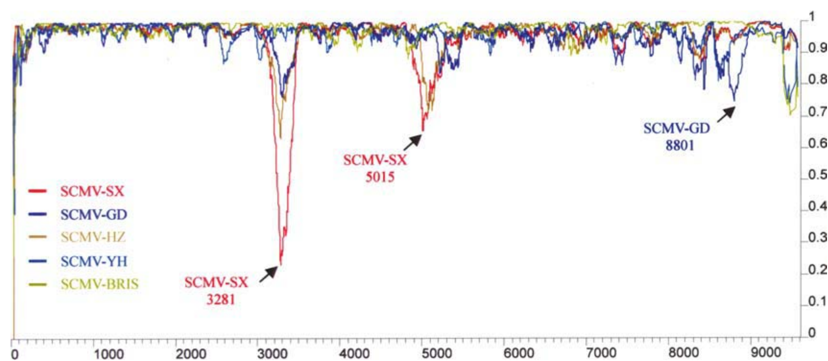

Fig. 4. Phylogenetic profile of the five SCMV sequences. The profile used the variable sites and a fixed window size of 100 bp. The phylogenetic correlation (y-axis) was obtained at each informative site from pairwise distance analysis of all aligned sequences and ranges from +1 (perfectly correlated) to 0 (unrelated). Recombination signals appear as areas of low phylogenetic correlation, visualized by single sharp-pointed downward peaks in the graph. Strongest recombination signals were revealed in positions 3281 and 5015 of SCMV-SX and in position 8801 of SCMV-GD.

Table 5. Percentage identities between genome regions of SCMV-SX and other SCMV isolates

			F4			
	Full length	1–3162	3163–3280	3281–5015	5016–5432	5433–9596
SCMV-BJ	91.8	**94.7**	**97.4**	78.8	**92.6**	**94.8**
SCMV-HN	90.5	90.9	84.6	78.7	**92.8**	**95.0**
SCMV-HZ	93.3	**98.8**	**98.3**	79.4	**92.1**	**95.0**
SCMV-SD	90.0	89.5	81.2	79.1	**92.8**	**94.8**
SCMV-GD	82.7	79.3	71.8	**89.2**	83.0	83.0
SCMV-LP	80.8	78.8	78.6	83.2	81.5	81.2
SCMV-XgS	80.9	78.9	79.5	83.5	80.3	81.4
SCMV-YH	80.7	78.4	78.6	83.4	80.1	81.3
SCMV-BRIS	79.3	78.4	78.6	80.7	75.3	79.8

SCMV-SX genome is not a result of improperly assembling. It is more likely that SCMV-SX is a natural recombinant isolate. SCMV-HZ or its analogue might be one parent isolate, however, SCMV-GD or its analogue was not certainly to be the other parent isolate because only about 90% of identity was found between the closest alike regions of SCMV-SX and SCMV-GD.

The phylogenetic profile also revealed one possible recombination site at position 8801 for SCMV-GD. Pairwise comparisons suggested that region 1–8801 of SCMV-GD showed low identities (about 80%) with other SCMV isolates, while region 8802–9596 showed significant high identities (more than 96.0%) with other Chinese maize isolates (Table 6). SCMV-GD is also a possible natural recombinant isolate.

However, the recombination sites showed in the phylogenetic profile may not surely be the natural recombination sites. When the parameters changed or more sequences were added to the analysis, the sites changed with a range of about 100 nts. The best way to figure out the recombination site may need the sequences of the certain parent isolates.

Recombination has been reported in many positive-sense, negative-sense or double-stranded RNA viruses infecting animals, plants and bacteria [28]. It has played a significant role in the evolution of RNA viruses by generating genetic variation, reducing mutational load, and producing new viruses [28]. In the genus *Potyvirus*, recombination has been reported for a number of different species such as *Plum pox virus* (PPV) [29], *Potato virus Y* (PVY) [30], *Bean common mosaic virus* (BCMV) [30], *Yam mosaic virus* (YMV) [31] and *Lettuce mosaic virus* (LMV) [32]. Oertel et al. suggested that an isolate of SCMV (Se26) was a possible recombinant [2]. In the present study, SCMV-SX and SCMV-GD were demonstrated to be possible intra-specific recombinants. These suggested that recombination events might also play an important role in the evolution of SCMV.

SCMV-SX was collected in 1999 in a heavily infected maize field in Shaanxi province. In the 1990's, susceptible maize varieties were widely grown in Shaanxi province [11]. Therefore, two or more different SCMVs might have chances to co-infect maize plants in some places. Then, recombination events might occur in some plants. Survived recombinants were proliferated and spread in the susceptible maize plants. The recombinants that have higher fitness than both of their parents would spread quickly and caused more severe disease. Therefore, the recombinant viruses might contribute greatly to the heavily maize dwarf mosaic disease and the severe losses in Shaanxi province of China in the 1990's.

Table 6. Percentage identities between genome regions of SCMV-GD and other SCMV isolates

	Full length	1–8801	8802–9596
SCMV-BJ	80.9	79.6	**96.4**
SCMV-HN	81.6	80.3	**96.1**
SCMV-HZ	80.9	79.5	**96.4**
SCMV-SD	81.2	79.9	**96.0**
SCMV-SX	82.7	81.5	**96.4**
SCMV-LP	83.0	82.5	89.5
SCMV-XgS	83.1	82.5	90.2
SCMV-YH	83.2	82.7	89.5
SCMV-BRIS	79.0	78.1	89.7

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References

1. Gao W. and Wei N., Acta Univ Agric Boreali-occidentalis *28*, 31–34, 2002.
2. Oertel U., Schubert J., and Fuchs E., Arch Virol *142*, 675–687, 1997.
3. Shukla D.D., Tosic M., Jilka J.M., Ford R.E., Toler R.W., and Langham M.A.C., Phytopathology *79*, 223–229, 1989.
4. Seifers D.L., Salomon R., Marie-Jeanne V., Alliot B., Signoret P., Haber S., Loboda A., Ens W., She Y.M., and Standing K.G., Phytopathology *90*, 505–513, 2000.
5. Chen J., Chen J., and Adams M.J., Arch Virol *147*, 1237–1246, 2002.
6. Frenkel M.J., Jilka J.M., Mckern N.M., Strike P.M., Clark Jr J.M., Shukla D.D., and Ward C.W., J Gen Virol *72*, 237–242, 1991.
7. Fan Z., Chen H., Liang X., and Li H., Arch Virol *148*, 773–782, 2003.
8. Liu X., Wang X., Zhao Y., Zheng C., and Zhou G., Acta Virologica *47*, 223–227, 2003.

9. Wang S., Shang Y., Zhao J., Yang L., Chen J., Zheng T., Lu X., Sun H., and Chen J., Chin J Virol. *19*, 159–163, 2003.
10. Jiang J. and Zhou X., Arch Virol *147*, 2437–2443, 2002.
11. Chen Z., Zhang S., Zhang M., and Wu Y., Acta Phytopathologica Sinica *29*, 333–338, 1999.
12. Chomezynski P. and Sachi N., Analyt Biochem *162*, 156–159, 1987.
13. Weiller G.F., Mol Biol Evol *15*, 326–335, 1998.
14. Lütcke H.A., Chow K., Mickel F.S., Moss K.A., Kern H.F., and Scheele G.A., EMBO J *6*, 43–48, 1987.
15. Turpen T., J Gen Virol *70*, 1951–1060, 1989.
16. Plisson C., Drucker M., Blanc S., German-Retana S., Le Gall O., Thomas D., and Bron P., J Biol Chem *278*, 23753–23761, 2003.
17. Blanc S., Ammar E.D., Garcia-Lampasona S., Dolja V.V., Llave C., Baker J., and Pirone T.P., J Gen Virol *79*, 3112–3122, 1998.
18. Blanc S., Lopez-Moya J.J., Wang R., Garcia-Lampasona S., Thornbury D.W., and Pirone T.P., Virol *231*, 141–147, 1997.
19. Peng Y.H., Kadoury D., Gal-On A., Huet H., Wang Y., and Raccah B., J Gen Virol *79*, 897–904, 1998.
20. Cronin S., Verchot J., Haldeman-Cahill R., Schaad M.C., and Carrington J.C., Plant Cell *7*, 549–559, 1995.
21. Kasschau K.D., Cronin S., and Carrington J.C., Virol *228*, 251–262, 1997.
22. Laín S., Riechmann J.L., and Garcia J.A., Nucleic Acids Res *18*, 7003–7006, 1990.
23. Kong P. and Steinbiss H.H., Arch Virol *143*, 1791–1799, 1998.
24. Argos P., Nucleic Acids Res *16*, 9909–9916, 1988.
25. Ullah Z., Chai B., Hammar S., Raccah B., Gal-On A., and Grumet R., Physiol Mol Plant Pathol *63*, 129–139, 2003.
26. Alegria O.M., Royer M., Bousalem M., Chatenet M., Peterschmitt M., Girard J-C., and Rott P., Arch Virol *148*, 357–372, 2003.
27. Handley J.A., Smith G.R., Dale J.L., and Harding R.M., Arch Virol *143*, 1145–1153, 1998.
28. Worobey M. and Holmes E.C., J Gen Virol *80*, 2535–2543, 1999.
29. Cervera M.T., Riechmann J.L., Martin M.T., and Garcia J.A., J Gen Virol *74*, 329–334, 1993.
30. Revers F., Le Gall O., Candresse T., Le Romancer M., and Dunez J., J Gen Virol *77*, 1953–1965, 1996.
31. Bousalem M., Douzery E.J.P., and Fargette D., J Gen Virol *81*, 243–255, 2000.
32. Krause-Sakate R., Fakhfakh H., Peypelut M., Pavan M.A., Zerbini F.M., Marrakchi M., Candresse T., and Le Gall O., Arch Virol *149*, 191–197, 2003.