COMPLETE NUCLEOTIDE SEQUENCE OF A POTYVIRUS CAUSING MAIZE DWARF MOSAIC DISEASE IN CENTRAL CHINA

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Summary. – The full-length nucleotide sequence of a potyvirus causing the maize dwarf mosaic (MDM) disease in Henan province, central China, was obtained by reverse transcription–polymerase chain reaction (RT-PCR) and rapid amplification of the cDNA 5'-end (5'-RACE). The viral genome comprised of 9596 nucleotides except the polyA tail and encoded a putative polyprotein of 3603 amino acids. The entire genomic sequence of this isolate shared identities of 94.2% and 98.3% with Sugarcane mosaic virus (SCMV) HZ isolate at the nucleotide and deduced amino acid levels, respectively, but only a 69.1% identity with MDM virus (MDMV) Bulgarian isolate (MDMV-Bg) at the nucleotide level. Phylogenetical tree analysis of the complete nucleotide sequences indicated that the Henan isolate of a potyvirus causing MDM disease is in fact a Henan strain of SCMV (SCMV-HN).

Key words: maize dwarf mosaic disease; Sugarcane mosaic virus; Henan isolate; Hangzhou isolate; complete nucleotide sequence; taxonomy; central China

Introduction

MDM disease was firstly reported in the United States in 1963, and the pathogen was identified as MDMV in 1965 (Janson and Ellett, 1963; Williams and Alexander, 1965). This disease is now one of major problems in maize growing all over the world. There are four potyviruses reported so far to cause dwarf mosaic disease in maize, namely MDMV, Johnson grass mosaic virus (JGMV), SCMV and Sorghum mosaic virus (SRMV) (Shukla *et al.*, 1994). The first report of MDM disease in China was from Huixian County, Henan Province in 1968 (Lin, 1976). Recently, this disease has spread widely in central and northern China; losses in maize production have been estimated at 20–30%, while more serious economic losses have been observed in some breeding fields (Zhou *et al.*, 1996). The pathogen of MDM disease has been identified firstly as MDMV B strain (MDMV-B) according to the host range and serological relationships (Shi and Xu, 1979). The studies of the pathogen biology and serological relationships in the last 20 years have shown that there might be three viruses or strains

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Abbreviations: BCMV = Bean common mosaic virus; CI = cylindrical inclusion; CP = coat protein; HC-Pro = helper component protease; JGMV = Johnsongrass mosaic virus; MDM = maize dwarf mosaic disease; MDMV = MDM virus; MDMV-B = MDMV B strain; MDMV-Bg = MDMV Bulgarian isolate; Nia = nuclear inclusion protein a; Nib = nuclear inclusion protein b; UTR = untranslated region; PVY = Potato virus Y; RACE = rapid amplification of cDNA end; RdRp = RNA-dependent RNA polymerase; RT-PCR = reverse transcription–polymerase chain reaction; SCMV = Sugarcane mosaic virus; SCMV-GD = SCMV Guangdong isolate; SCMV-HN = SCMV Henan isolate; SCMV-HZ = SCMV Hangzhou strain; SCMV-LP = SCMV Linping isolate; SCMV-XgS = SCMV Xiangshan isolate; SMV = Soybean mosaic virus; SRMV = Sorghum mosaic virus; SrMV-YH = SrMV Yuhang isolate; SrMV-C = SrMV Xiaoshan isolate; TEV = Tobacco etch virus; TVMV = Tobacco vein mottling virus; VPg = viral linked protein; YMV = Yam mosaic virus; ZYMV = Zucchini yellow mosaic virus

Table 1. Primers used to amplify entire genomic nucleotide sequence of SCMV-HN

Fragment No.		Size (kbp)	Primer	Nucleotide sequence (5' to 3')
1	32 -UTR	0.3	F	AAT ACA GAG AGA CAC ACA GCT
	CP-C		R	GT CTA GAG GTA CCG GAT CC $(T)_{18}$
2	CP-C	0.9	F	GC CAT ATG TTC CAT CAA T
	CP-N		R	CC GGA ACT GCC GGA TCC TAG TGG TGC TGC TGC ACT CC
3	NIb-C	1.6	F	CT CAT ATG TGT GAG GTT ACA GAG ACA TGG
	NIb-N		R	CC GAA TTC CTA CAC AGT TCC GGA TTG ATG
4*	NIb-N	1.5	F	GAY CAR CTN CTN GAR TTY
	CI-mid		R	CCA TGT CTC TGT AAC CTC ACA
5*	CI-mid	1.7	F	GGN GCN GTN GGN TCN GGN
	CI-N		R	AAT AAC TGT GTT TAA AGC ACC
6*	CI-N	2.7	F	ATH GAR CAY TAY GCN GA
	HC-N		R	GTC GAC GGA TTT CCC TGA CCC GAC AGC
			N	CAA GAT GCT CGT GAA GTT CAC
7*	HC-N	0.9	F	GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG
	5'-UTR		R	TTA ATA GTT GGT GTG TGC TC
			N	TCT GTA TAG CCT TTC CAG

F = forward; R = reverse; N = nested.

* = degenerate primers designed from the conserved sequences of entire genomes of BCMV (U19287), JGMV (Z26920), MDMV (AJ001691), PVY (A0876), SMV (S42280), TEV (M15329), TYMV (X04083), YMV (U42596), and ZYMV (L92569). In degenerate primers H = A, C or T; N = A, C, G or T; R = A or G; Y = C or T; I = hypoxanthine.

causing this disease in China, namely MDMV-B, MDMV-G and SCMV (Shi *et al*., 1986; Zhu *et al*., 1986; Fan and Chiu, 1987). MDMV and SCMV are sap- and aphid-transmitted single-stranded positive-sense RNA viruses belonging to the genus *Potyvirus* and causing mosaic disease and yield losses in sugarcane, maize, sorghum, and other *Poaceous* plants (van Regenmortel *et al*., 2000).

Great efforts have been made in recent years to understand the molecular biology of potyviruses (Revers *et al*., 1999); these data have helped to resolve difficulties in classifying some members of the family *Potyviridae*. Complete genomic nucleotide sequences have been determined for some isolates of MDMV, SCMV and JGMV. Cheng *et al.* (2002) reported a complete genomic nucleotide sequence of a maize isolate collected from the city of Hangzhou, Zhejing province far from the main maize growing areas of China, and identified it as a Hangzhou strain of SCMV (SCMV-HZ, AJ297628). In this paper, we report the complete genomic nucleotide sequence of a potyvirus isolate causing MDM disease in the Henan province (SCMV-HN isolate), one of the major maize growing area and the region in China, in which MDM disease had first occurred.

Materials and Methods

Virus material. The isolate SCMV-HN was collected from diseased maize leaves expressing typical mosaic symptoms from maize fields in the Henan province in July 2000. For the virus purification and RNA isolation the isolate was inoculated mechanically into 10–14 day-old seedlings of susceptible maize inbred line Mo 17 in an insect-free greenhouse. Virus particles were purified by the method described by Chen *et al.* (1992). The genomic RNA was extracted from the purified virus by a sodium dodecyl sulfate-phenol method and collected by ethanol precipitation (Sambrook *et al*., 1989).

First strand cDNA synthesis, PCR amplification and 5'-RACE. First strand cDNA was synthesized using the Super Script RNase H^- reverse transcriptase (GIBCO) and initial primer $1P_R$ (5'-GTC TAGAGGTACCGGATCC$(T)_{18}$-3') according to the manufacturer's instructions. Degenerate primers were designed from the conserved region of related potyviruses (Table 1). PCR amplification was conducted using TaKaRa EX Taq DNA polymerase. In order to reduce non-specific amplification, the first strand cDNA was synthesized using a primer designed from the 3'-terminal sequence mentioned above. For complete 5'-end, RT-PCR was performed using RACE with the cDNA. The latter primed with $7P_R$ (5'-TTAATAGTTGGTGTGTGCTC-3') was subsequently tailed with dA and terminal transferase (GIBCO). The resulting products were amplified using the forward primer $7P_F$ (5'-GGCCACGC GTCGACTAGTACGGGIIGGGIIGGGIIG-3'), an oligo $(dT)_{18}$ primer and Taq DNA polymerase (TaKaRa). The PCR products were detected by 1% (w/v) agarose gel electrophoresis.

Cloning and sequencing. The procedure of gene cloning, screening and plasmid extraction were done according to Sambrook *et al.* (1989). PCR fragments of the expected size were purified using the Gel Extraction Kit (QIAGEN), cloned into the pGEM-T Easy Vector (Promega), and used for transformation of *Escherichia coli* JM109 cells. Plasmid DNA was isolated from overnight cultures by alkaline lysis. The nucleotide sequence was determined by the dideoxynucleotide chain termination method in both directions in an automated sequencer (ABI377, Perkin Elmer) using the Ther-

Table 2. Alignment of putative cleavage sites and adjacent amino acid sequences in the polyproteins encoded by the members of the SCMV subgroup of potyviruses

Virus/strain/isolate	Cleavage site								
	P1/HC	HC/P3	P3/6K1	6K1/CI	CI/6K2	6K2/VPg	VPG/NIa	NIa/NIb	NIb/CP
MDMV-Bg	IEHY/A	YAVG/G	VIHE/G	VTHQ/S	VIHQ/G	VKHE/A	VEHE/A	VTEQ/G	VKHQ/A
SCMV-HN	IEHY/A	YIVG/G	VIHE/G	VTQQ/S	VIHQ/G	VSHQ/G	VAHE/S	VEEQ/C	VFHQ/S
SCMV-HZ	IEHY/A	YIVG/G	VIHE/G	VXQQ/S	VIHQ/G	VXHQ/G	VAHE/S	VEEQ/C	VFHQ/S
SCMV-GD	IEHY/A	YIVG/G	VIHE/G	VTQQ/S	VIHQ/G	VAHQ/G	VAHE/S	VEEQ/C	VFHQ/S
SCMV-LP	IEHY/A	YIVG/G	VIHE/G	VVQQ/S	VIHQ/G	VSHQ/G	VAHE/S	VEEQ/C	VFHQ/S
SCMV-XgS	IEHY/A	YIVG/G	VIHE/G	VVQQ/S	VIHQ/G	VSHQ/G	VAHE/S	VEEQ/C	VFHQ/S
SrMV-YH	IDHF/S	YIVG/G	VIHE/A	VTHQ/G	VIHQ/G	VCHQ/G	VEHE/S	VTEQ/G	VFHQ/A
JGMV	ICHY/S	YIVG/G	VEHE/R	VKHE/G	VIHE/N	VEHE/G	VEHE/G	ISNE/S	VEHQ/S

For the virus/strain/isolate abbreviations see their list on the front page.

mo Sequenase Dye Terminator Cycle Sequencing Kit (Amersham). The nucleotide and amino acid sequences were analyzed with DNSTAR2.5 Software (BioIT Co. Ltd.) and BioNumerics Software (Applied Maths Co.).

Results and Discussion

Viral genome organization

The genome of SCMV-HN (Acc. No. AF494510) was composed of 9596 nucleotides (nts) except the 3'-poly(A) tail. Computer analysis revealed a single long ORF of 9192 nts in one of the ORFs of the positive strand, beginning with AUG at nts 150–152 and terminating with UGA at nts 9339–9341. There were 149 nts in the 5'-untranslated region (UTR), identical with that of other SCMV isolates from China (SCMV-HZ, AJ297626; SCMV-LP, AJ310102; SCMV-XgS, AJ310103; SCMV-YH, AJ310104; SCMV-GD, AJ310105), but different from that of MDMV-Bg (Kong and Steinbiss, 1998; AJ001691). The 5'-UTR had a high similarity with SCMV-HZ (96.7%) and a low similarity with MDMV-Bg (53.3%), SrMV-C (NC004035) (52.3%) and JGMV (NC003606) (42.8%). The conserved motif UCAACACAACAU was also found at the 5'-terminus (nts 15–26) only with a replacement of the last nucleotide from U to C. There were 255 nts in 3'-UTR. The latter had a high similarity with SCMV-HZ (99.2%) and a low similarity with MDMV-Bg (54.1%), SrMV-C (53.6%) and JGMV (53.7%). The predicted translation product (polyprotein) of this ORF contained 3063 amino acids (aa) with a calculated M_r of 346.1 K.

Nine putative protease cleavage sites were identified by comparing the putative coding region of SCMV-HN with the consensus protease recognition motifs in other potyviruses (Shukla *et al.*, 1994). Hence, 10 standard potyvirus proteins were identified, namely P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP from N- to C-terminus, respectively. Among the nine putative protease cleavage sites, the HC-Pro/P3 junction (G_{693}-G_{694}) for SCMV-HN was identical with those of other potyviruses sequenced to date, while the NIa/NIb junction (Q2229-Q2230) was distinct from those of other potyviruses except that of the SCMV isolates infecting sugarcane and maize (Table 2). The deduced coat protein (CP) of this isolate was the only structural protein; it consisted of 313 aa (except UGA) with a calculated M_r of 33.7 K. There were some conserved aa sequences in the SCMV-HN isolate identical with that of other potyviruses, such as C_{432}DNQLD$_{437}$ and L_{643}VDH$_{646}$ in HC-Pro; E_{1219}PTRPL$_{1224}$, L_{1362}VYV$_{1365}$, V_{1413}ATNIIENGVT$_{1423}$ and G_{1464}RVGR$_{1468}$ in CI; L_{2255}VTKH$_{2259}$, F_{2419}TAAP$_{2423}$, A_{2477}DGS$_{2480}$, G_{2537}NNSGQPSTVV$_{2547}$ and W_{2623}FMS$_{2627}$ in NIb; A_{2962}EAYI$_{2966}$, Q_{3014}MKAA$_{3018}$ and T_{3041}ERH$_{3045}$ in CP. A nucleotide-binding site (G_{1195}AVGSGKST$_{1203}$) and the RNA helicase motif D_{1284}-E-X-H$_{1287}$ were found in CI protein, while a putative RdRp motif (C_{2475}DADGS$_{2480}$ and S_{2450}-G-X_3-T_{2545}-X_3-N_{2549}-T-X_{30}-G_{2581}-D-D$_{2583}$) was found in NIb protein. The conserved sequence motifs K_{287}ITC$_{290}$ P_{545}TK$_{547}$ in HC-Pro and D_{2755}AG$_{2757}$ in CP were considered essential in the transmission of the virus by aphids and the motifs C_{527}CCVT$_{531}$ in HC-Pro and R_{2952}-X_{43}-D$_{2996}$ in CP were presumably involved in long-distance movement of SCMV-HN as other potyviruses (Doljia *et al.*, 1994; Revers *et al.*, 1999). The NIa-VPg protein contained a tyrosine at the conserved aa 186, which was required for the VPg linking to potyviral RNA (Shukla *et al.*, 1994).

Comparison of SCMV-HN with other members of the SCMV subgroup

The potyviruses JGMV, MDMV, SCMV, SrMV, and the newly identified Zea mosaic virus (ZeMV, Seifers *et al.*, 2000) were included in the SCMV subgroup of potyviruses

Table 3. Nucleotide and amino acid similarity (%) of SCMV-HN and other eight members of the SCMV subgroup of potyviruses

Region	Virus/strain/isolate							
	SCMV-HZ	SCMV-LP	SCMV-XgS	SCMV-YH	SCMV-GD	MDMV-Bg	SrMV-C	JGMV
5'-UTR	96.7	86.7	85.9	85.2	80.7	53.3	52.3	42.8
P1	95.3(98.6)	68.0(67.4)	67.9(67.4)	68.0(67.4)	73.9(80.9)	56.9(54.3)	53.1(45.9)	42.3(27.4)
HC	89.2(98.5)	81.7(94.8)	81.8(94.8)	81.6(94.6)	82.6(95.4)	72.9(82.9)	72.8(83.5)	56.4(47.7)
P3	87.6(95.4)	84.3(91.7)	84.0(91.7)	83.8(91.4)	83.1(88.5)	69.9(70.1)	72.6(72.4)	50.5(33.9)
6 K 1	89.6(100)	78.7(89.7)	76.7(89.7)	77.7(89.7)	79.7(91.2)	65.3(70.6)	68.8(73.5)	54.1(45.3)
CI	90.7(99.1)	79.2(95.3)	79.0(95.5)	79.1(95.8)	79.9(95.9)	71.8(81.2)	73.8(82.8)	59.2(54.5)
6 K 2	98.8(100)	76.9(87)	76.2(87)	75.6(87)	72.5(81.5)	63.1(68.5)	62.5(74.1)	48.8(38.9)
VPg	99.8(100)	79.0(87.8)	78.0(88.4)	78.3(87.8)	76.8(87.9)	68.0(72.6)	72.5(80.0)	58.0(53.7)
Nia	99.0(99.2)	79.6(95.1)	80.9(96.3)	80.5(95.5)	83.6(95.5)	64.9(68.7)	67.5(72.4)	54.8(44.6)
Nib	98.9(99.4)	92.9(92.9)	92.9(92.9)	92.9(92.9)	80.1(92.9)	70.7(76.6)	71.2(78.7)	61.7(60.9)
CP	99.0(99.3)	73.4(73.2)	84.1(89.1)	84.2(89.8)	90.2(94.3)	70.4(74.7)	71.6(72.8)	57.9(58.9)
3'-UTR	99.2	88.6	89.0	89.5	93.8	54.1	53.6	53.7
Entiregenome	94.2(98.3)	80.0(90.7)	79.9(91.1)	79.9(91.1)	81.6(91.5)	68.5(74.6)	69.5(75.8)	56.2(49.6)

The homology matrix was derived from multiple alignment of entire genomic nucleotide and deduced amino acid sequences of SCMV-HZ and other eight viruses/strains/isolates of the phylogenetic subgroup of SCMV potyviruses. The similarity in amino acids is shown in brackets.

identified by phylogenetic analysis (Shukla *et al*., 1994; Chen *et al.,* 2002; Fan *et al.,* 2003). The complete nucleotide sequences of JGMV, MDMV-Bg, SrMV (U57358), SCMV-HZ and SCMV-GD (AJ310105), all causing MDM disease in China, have been reported recently (Cheng *et al*., 2002).

A comparison of SCMV-HN with other eight members of the SCMV subgroup (Table 3) showed that between entire genomes of SCMV-HN and MDMV-Bg and SrMV-C there was a 68.5% and 69.5% similarity at nucleotide level and a 74.6% and 75.8% similarity at amino acid level.

SCMV-HN was less similar to JGMV with only 56.2% and 49.6% similarities for nucleotide and corresponding amino acids, respectively. For several SCMV isolates infecting sugarcane (SCMV-LP, AJ310102; SCMV-XgS, AJ310103; SCMV-YH, AJ310104), there were intermediate nucleotide similarities (79.9–81.6%). The entire genome of SCMV-HN was more similar to that of SCMV-HZ with 94.2% for nucleotides and 98.3% similarity for amino acids, respectively. In 5'-UTR, CI, 6K2 VPg, NIa, NIb, CP and 3'-UTR nucleotide similarities were increasing from 95.3% to 99.4%, respectively, while amino acid similarities were of 98.2% to 100%.

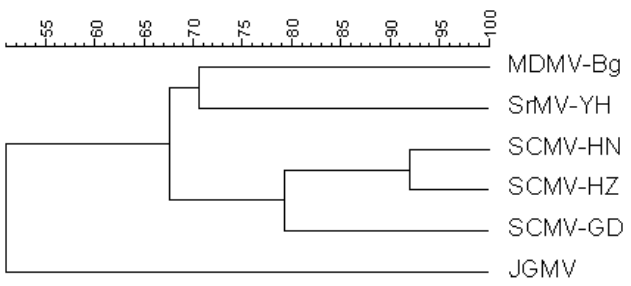

Fig. 1
Position of SCMV-HN among 6 members of the SCMV subgroup of potyviruses

The tree was constructed from multiple sequence alignment of entire genomic nucleotide sequences using BioNumerics (Applied Maths Co.). The scaling ruler represents the percentage of identity

Classification status of SCMV-HN

The nucleotide sequence of viral genome has been a main criterion for virus classification, Shukla and Ward (1988) proposed that the similarity of CP gene might represent the similarity of entire genome. In analyzing CP nucleotide sequences of 18 potyviruses they have suggested the following criterion for classification: a 38–71% similarity indicates different virus, a 90–99% similarity indicates different strain of the same virus, and a similarity above 99.0% indicates the same strain. The classification criterion for different virus in the genus *Potyvirus* in the Seventh Report of the International Committee on Taxonomy of Viruses (van Regenmortel *et al*., 2000) is (i) the similarity ≤80% for CP gene or the similarity ≤85% for entire genome, and (ii) with different cleavage site of polyprotein.

Phylogenetical (tree) analysis for the complete nucleotide sequence illustrated the position of the Henan isolate of SCMV (SCMV-HN) among 6 members of the SCMV subgroup of potyviruses (Fig. 1). The results revealed that SCMV-HN is a definite member of the SCMV subgroup, clustered along with SCMV-HZ, SCMV-LP, SCMV-XgS, SCMV-YH and SCMV-GD.

At this point it should be stressed that the SCMV subgroup of potyviruses as well as the goup of potyviruses are phylogenetic but not taxonomic terms. On the other hand it

is generally believed that phylogenetic clustering helps in identification of new virus strains, new viruses, new virus species and perhaps even new higher virus taxons.

There was the nearest relationship between the SCMV-HN and SCMV-HZ, the similarities of 94.2% for entire genome and 98.3% for the polyprotein, respectively. They had also identical polyprotein cleavage sites (Table 2). All of these results suggested that they are the same strain of SCMV. It was surprising to obtain the nucleotide similarity of the conserved HC-Pro region of 89.2%, but the amino acid similarity of 98.5%. As the similarities between SCMV-HN and SCMV-GD (an isolate from sweet maize in Guangdong province) were 91.5% in the polytprotein, 94.3% in CP gene and 90.2% in CP these two isolates should belong to different strains of SCMV.

Several maize potyviruses collected from different provinces of China (SCMV-BJ, AY042184; SCMV-HuB, AJ310110; SCMV-HeB, AJ310117; SCMV-JS, AJ310107; SCMV-DY, AJ310106; SCMV-SD, AJ310117) have been sequenced for the complete genome or 3'-region of the genome including the CP sequence. As the similarities between their CP genes were above 99.0%, these isolates originated apparently from the same strain of SCMV. Therefore, the maize potyvirus occurring in China represents a strain of SCMV (SCMV-HN), which had played a role in the MDM epidemics.

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