Arch Virol (2002) 147: 2437–2443
DOI 10.1007/s00705-002-0890-7

Archives of Virology
Printed in Austria

Maize dwarf mosaic disease in different regions of China is caused by *Sugarcane mosaic virus**

Brief Report

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Received April 23, 2002; accepted July 16, 2002
Published online October 7, 2002

Summary. *Sugarcane mosaic virus* (SCMV) was detected in all 62 maize samples collected from eight maize-growing provinces in China showing dwarf mosaic symptoms by immunocapture reverse-transcription polymerase chain reaction (RT-PCR). *Maize dwarf mosaic virus* (MDMV), *Sorghum mosaic virus* (SrMV) and *Johnsongrass mosaic virus* (JGMV), however, were not detected in any of the samples by RT-PCR. Eleven cDNA fragments of approximately 0.8 kilobases covering most of the coat protein (CP) gene of SCMV were sequenced and sequence analysis indicates that these eleven isolates share 98.1 to 100 % identity at the amino acid level. Sequence comparison and phylogenetic analysis of the CP genes from the eleven Chinese isolates as well as 21 SCMV subgroup virus isolates indicate that the eleven Chinese virus isolates were closely related to SCMV with 97.0 to 98.1 % sequence identity at the amino acid level, while relatively lower sequence identity was found with MDWV, SrMV or JGMV. The results indicate that the Chinese isolates are members of the SCMV species, and thus, SCMV can be considered as the most common and important potyvirus infecting maize in China.

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Maize dwarf mosaic disease (MDMD) is one of the most common and economically important virus diseases in maize (*Zea mays* L.) in many countries.

*The EMBL accession numbers of the sequences reported in this paper are AJ421456 to AJ421466.

The virus was firstly reported in 1962 in Ohio, U.S.A. [1]. For many years, potyviruses infecting poaceae were considered to be a single (*Sugarcane mosaic virus*, SCMV) or two virus species [SCMV and *Maize dwarf mosaic virus* (MDMV)] because of their similar particle morphology, host range, mode of transmission and physical and physicochemical properties [3]. Recently, Shukla et al. indicated that potyviruses of poaceae consisted of four different potyviruses including MDMV, SCMV, *Sorghum mosaic virus* (SrMV) and *Johnsongrass mosaic virus* (JGMV) [7]. The four viruses are listed together as a SCMV subgroup in the genus *Potyvirus* [7, 8]. MDMV and SCMV have been isolated and identified in maize throughout the world [1, 5]. JGMV has been found in Johnsongrass and maize in Australia and the United States and SrMV in maize in the United States [6, 7].

MDMD was first observed in maize in Henan province, China, in 1968 [5]. The disease causes significant economic losses in maize in many maize-growing regions in China [2]. To determine the causal virus or viruses of MDMD in China, we collected 62 leaf samples from maize plants showing dwarf mosaic symptoms in May through August in 2000 and 2001 in eight different maize-growing regions (e.g. Zhejiang, Shandong, Jiangsu, Henan, Hebei, Shanxi and Shannxi province and Shanghai suburb). The leaf samples were then analyzed immediately for virus infection by immunocapture RT-PCR as described by Nolasco et al. [4] or stored at $-70°C$ till use. Antibodies against SCMV, MDMV, SrMV or JGMV were kindly provided by J. Chen, Zhejiang Academy of Agricultural Sciences, China. First strand cDNA of each sample was synthesized using a reverse degenerate prime P_S (Table 1) specific for the 3′ terminal sequence of the coat protein (CP) gene of the SCMV subgroup viruses. Four different forward primers (P_{SC}, P_{MD}, P_{Sr} and P_{JG}) specific for the N-terminal part of the CP gene of the four SCMV subgroup

Table 1. Sequences of primers used in immunocapture RT-PCR

Primer name	Nucleotide sequence (5′-3′)[a]	Virus for detection	Source (accession no.) and position[b]
Reverse primer			
P_S	CAGCTGTGTGBCK STCTGTATT	SCMV MDMV SrMV JGMV	SCMV-BJ (S77088) 949–970
Forward primers			
P_{SC}	GGTAGTGGTACAGGAACAAGA	SCMV	SCMV-BJ (S77088) 148–168
P_{MD}	TGACGAAATTATAGATGTTAA	MDMV	MDMV-A (U07216) 876–896
P_{Sr}	AGGATGAATTAATTGACGTGC	SrMV	SrMV-SCH (U07219) 1535–1555
P_{JG}	AAACCAGCTAGTGGTGAAGGC	JGMV	MDMV-MDO (U07217) 148–168

[a] B = A, G, C or T; K = G or T; S = C or T

[b] Numbering is from the 5′-extreme of the sequence

viruses (Table 1) and the primer P_S were used to amplify specific cDNA products from individual samples. Results of the experiments show that a RT-PCR product of approximately 0.8 kb is present in all 62 samples tested using the primers P_{SC} (specific for SCMV) and P_S, while no similar sized RT-PCR products are present in any of the 62 samples tested using the primers P_{MD} (specific for MDMV) and P_S, P_{Sr} (specific for SrMV) and P_S, and P_{JG} (specific for JGMV) and P_S (data not shown). This result indicates that all samples collected and analyzed are infected with SCMV and thus SCMV is likely to be the main causal virus of MDMD in maize-growing areas in China.

To determine the sequences of the virus isolates, 11 randomly selected RT-PCR products from eight provinces were cloned individually into the pGEM-T easy vector as instructed by the manufacturer (Promega) and sequenced in both directions with an automated DNA sequencing system (model 377, Perkin Elmer) as described [11]. Two independent cDNA clones were sequenced for each virus sample and the resulting sequence data were analyzed by the DNAMAN package Version 4 (Lynnon Biosoft, Quebec, Canada) or DNASTAR Program (DNASTAR Inc., Madison, WI, USA). Result of the analysis shows that each of the 11 cloned fragments (GenBank accession numbers AJ421456 to AJ421466) contains 802 nucleotides (nt) and encodes 267 amino acids (aa). Comparison of the sequences from the 11 cloned fragments revealed that the cloned fragments shared 98.8 to 99.8 % (nucleotide level) and 98.1 to 100 % (amino acid level) sequence identity suggesting that the eleven isolates were closely related to each other (Fig. 1).

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ZJ2       TPSTGTPAQGNTPPASGGSSRNNGGGQSGSNGTGGQAGSSGAGGQRDKDVDAGSTGKISVPKLKAMSKKMRLPKAKGKDVLHLDFLLTYK   90
HB        --------------------G-------------------------------------------------------------------   90
HN        --------------------G-------------------------------------------------------------------   90
NJ1       --------------------G-------------------------------------------------------------------   90
NJ2       --------------------G-------------------------------------------------------------------   90
SD        --------------------G-------------------------------------------------------------------   90
SH        --------------------G------A-------------------------------------------------------------   90
SNX       --------------------G-------------------------------------------------------------------   90
SX1       --------------------G-------------------------------------------------------------------   90
SX2       --------------------G-----------------------K-------------------------------------------   90
ZJ1       --------------------G-------------------------------------------------------------------   90

ZJ2       PQQQDISNTRATKEEFDRWYDAIKKEYEIDDTQMTVVMSGLMVWCIENGCSPNINGNWTMMDGDEQRVFPLKPVIENASPTFRQIMHHFS   180
HB        ----------------------------------------------------------------------------------------   180
HN        ----------------------------------------------------------------------------------------   180
NJ1       -------------K--------------------------------------------------------------------------   180
NJ2       ----------------------------------------------------------------------------------------   180
SD        ----------------------------------------------------------------------------------------   180
SH        ----------------------------------------------------------------------------------------   180
SNX       ----------------------------------------------------------------------------------------   180
SX1       ----------------------------------------------------------------------------------------   180
SX2       ----------------------------------------------------------------------------------------   180
ZJ1       ----------------------------------------------------------------------------------------   180

ZJ2       DAAEAYIEYRNSTERYMPRYGLQRNLTDYSLARYAFDFYEMTSRTPARAKEAHMQMKAAAVRGSNTRLFGLDGNVGETQENTERHTA   267
HB        ------------------------------------------------------------------------------S---   267
HN        ----------------------------------------A-----------------------------------------   267
NJ1       ----------------------------------------------------------------------------------   267
NJ2       ----------------------------------------------------------------------------------   267
SD        ----------------------------------------------------------------------------------   267
SH        ------------------------S---------------------------------------------------------   267
SNX       ----------------------------------------------------------------------------------   267
SX1       ---------------------------------------I----S-----------------------------------S---   267
SX2       ------------------------------------------------------------------------------D----   267
ZJ1       ------------------------------------------------------------------------------S---   267
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Fig. 1. Multiple alignment of the deduced amino acids sequences of the nearly full-length coat protein of the 11 Chinese isolates. Dashes indicate identical amino acid

Table 2. Percentage nucleotide and amino acid sequence identities between the nearly full-length coat protein gene of 11 Chinese isolates and the four SCMV subgroup viruses

	SCMV-Hoe		MDMV-A		SrMV-H		JGMV-MDO	
	nt	aa	nt	aa	nt	aa	nt	aa
HB	95.8	97.8	69.7	78.4	69.0	73.8	55.6	60.6
HN	95.8	97.8	69.5	78.8	68.7	73.8	55.5	61.0
NJ1	95.9	97.8	69.5	79.2	68.8	74.2	56.1	61.0
NJ2	95.6	98.1	69.7	79.2	69.1	74.2	56.0	61.0
SD	96.0	98.1	70.0	79.2	69.1	74.2	56.1	61.0
SH	95.4	97.4	69.7	78.8	69.0	74.2	56.0	60.2
SNX	95.8	98.1	70.1	79.2	69.2	74.2	56.1	61.0
SX1	95.3	97.0	69.7	78.8	68.7	73.8	55.9	60.6
SX2	95.8	97.4	69.7	79.2	69.0	74.2	56.0	61.0
ZJ1	95.8	97.8	69.8	78.8	69.1	73.8	55.9	60.6
ZJ2	95.6	97.8	70.0	78.8	69.1	74.2	56.0	61.0

Table 3. Potyvirus CP gene sequences used for comparison and phylogenetic analysis

Virus species	Isolate	Abbreviation	Plant source	Country	GenBank accession number
SCMV	MDB	SCMV-MDB	maize	USA	D00949
	Beijing	SCMV-BJ	maize	China	S77088
	Hubei	SCMV-HuB	maize	China	AJ310110
	Dongyang	SCMV-DY	maize	China	AJ310106
	Zhejiang	SCMV-ZJ	maize	China	AJ297628
	Bulgaria	SCMV-Bg	maize	Bulgaria	AJ006201
	S15	SCMV-S15	maize	Spain	AJ311169
	Boetzingen	SCMV-Boe	maize	Germany	X98168
	Hoenstedt	SCMV-Hoe	maize	Germany	X98169
	Seehausen	SCMV-Se	maize	Germany	X98165
	A	SCMV-A	sugarcane	USA	U57354
	B	SCMV-B	sugarcane	USA	U57355
	D	SCMV-D	sugarcane	USA	U57356
	E	SCMV-E	sugarcane	USA	U57357
	Usla	SCMV-Usla	sugarcane	USA	AF006736
	Isis3	SCMV-Isis3	sugarcane	Australia	AF006728
	SC	SCMV-SC	sugarcane	Australia	D00948
	SA	SCMV-SA	sugarcane	South Africa	AF006738
MDMV	A	MDMV-A	maize	USA	U07216
JGMV	MDO	JGMV-MDO	maize	USA	U07217
SrMV	H	SrMV-H	sugarcane	USA	U57358

It is generally accepted that different potyvirus species have less than 85 % identity in the CP sequence [8] and that the amino acid sequence of the CP gene can be used to determine the taxonomic status of individual potyviruses [9].

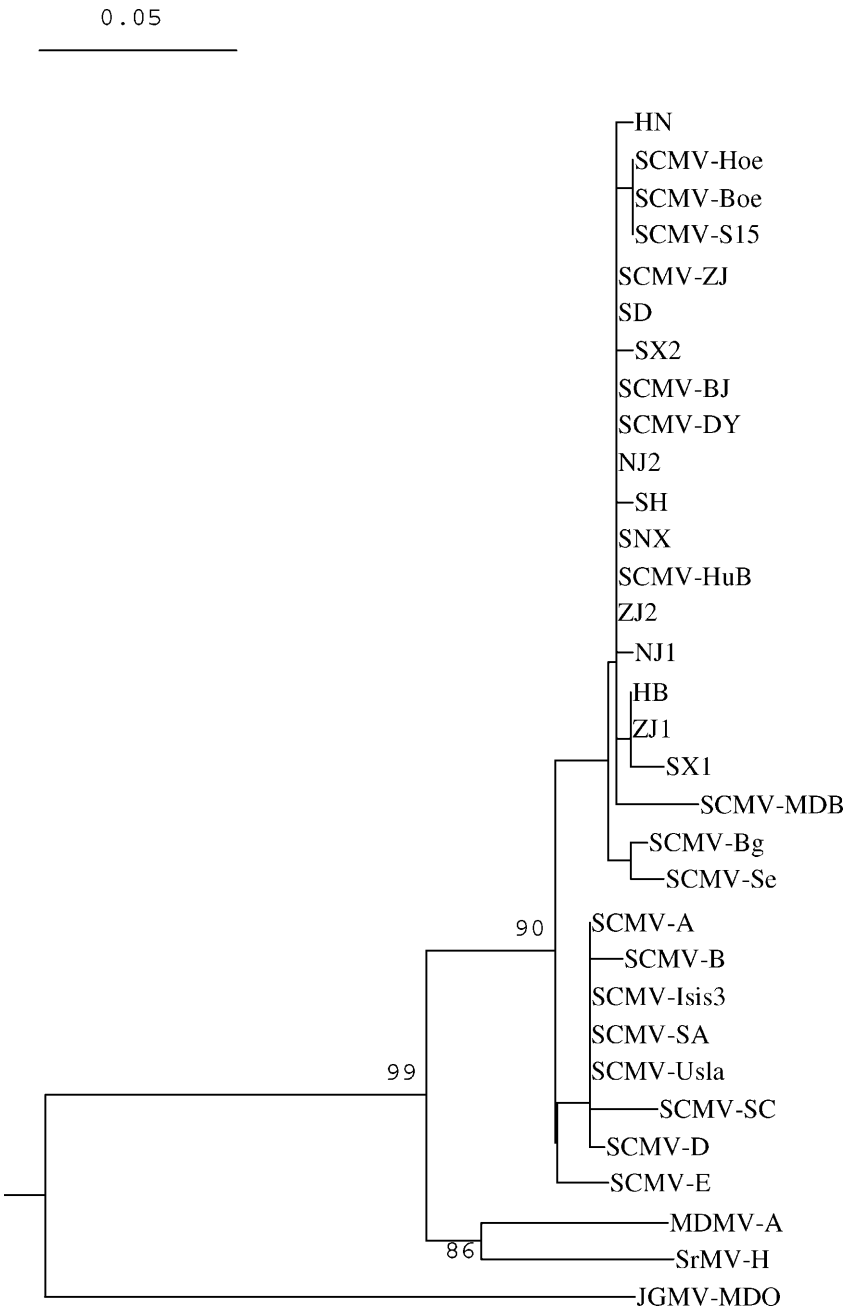

Fig. 2. Phylogenetic tree of amino acid sequence of the conserved core region of CP gene of the 11 Chinese isolates and 21 SCMV subgroup virus isolates. The CP core regions correspond to D70-R285 in JGMV-MDO. The tree was produced using Neighbor-joining method in DNAMAN. Vertical distances are arbitrary; horizontal distances are proportional to genetic distances (see scale bar). The numbers at each branch indicate the percentage of 1000 bootstrap, which supports the grouping at that node

The percentages of nucleotide and amino acid sequence identity of the eleven Chinese isolates and the CP genes of the four SCMV subgroup viruses are shown in Table 2. A high amino acid sequence identity (97.0 to 98.1 %) was found between the Chinese isolates and the SCMV-Hoe isolate, whereas low amino acid sequence identities were found between the Chinese isolates and MDMV-A (78.4 to 79.2 %), SrMV-SCH (73.8 to 74.2 %) and JGMV-MDO (60.2 to 61.0 %) (Table 2).

The phylogenetic tree was constructed based on the amino acid sequence alignment of the core regions of CP genes of the 11 Chinese isolates and 21 known SCMV subgroup virus isolates. The accession number, plant source and country of origin of the 21 SCMV subgroup virus isolates are listed in Table 3. As expected, the 11 Chinese isolates and the SCMV isolates are clustered together in the same branch, while MDMV, SrMV and JGMV are clustered in independent branches (Fig. 2). Phylogenetic analysis also shows that SCMV isolates can be separated into two groups. Group 1 contains isolates from China, Germany, Bulgaria, Spain and United States. All isolates in this group were originally isolated from maize. Group 2 contains isolates from United States, Australia and South Africa, and all isolates in this group were originally isolated from sugarcane. A correlation of CP sequence patterns of SCMV with hosts has been reported [10], and indicates that infected hosts may have exerted a selection pressure for virus gene evolution.

Our results indicate that MDMD in major maize growing area in China is caused by SCMV, while no other SCMV subgroup viruses are detected.

Acknowledgments

We thank Dr. Xinshun Ding, Samuel Roberts Noble Foundation, Ardmore, USA for critical reading of the manuscript. This project was supported by China National Key Basic Research and Development Program (G2000016204) and the National Outstanding Youth Foundations from China National Natural Science Foundation (3996005).

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