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OCCURRENCE OF TWO SUGARCANE MOSAIC POTYVIRUS STRAINS IN SUGARCANE

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ABSTRACT:

The SCMV, the causal agent of SCMD and definite member of family *Potyviridae* are known to infect sugarcane, maize, sorghum and other poaceous plant species. In this study, antisera specific to SCMV-MDMV-A and SCMV-MDMV-B strains were used for ELISA detection of such strains in samples from different locations exhibited virus-like symptoms. Positive ELISA values belonging to SCMV-MDMV-A and SCMV-MDMV-B were found in samples collected from Sabahia (33.3 & 88.8%), Hawamdeih (3.4 & 13.8%), Nag-Hammadi (38.5 & 26.9%) Kom-Ombo (100 & 50%). SCMV-MDMV-A and SCMV-MDMV-B were found in samples collected from Abo-Korkas and SCRI with percentages of 11.4 and 32.3%, respectively. The results showed that *Zea mays* L. and *Danthonia pilosa* were successfully differentiated the two MDMV strains, in particularly, strain B. On the other hand, the later six grasses and grains were reacted with the MDMV-A strain. The infection of *Z. mays* L. and *S. bicolor* cv. Rio by the two strains initially resulted in a mosaic leaf symptoms, but later on in case of MDMV-A strain, a redding leaf followed by death of point was distinguished. Results showed the presence of cylindrical inclusions operated as pinwheels, scrolls and bundles in the cytoplasm of MDMV-A-infected *S. biocolor* cv. Rio cells. In transmission of SCMV-MDHV strains studies of aphids, *Myzus persicae* (Sulzer) transmitted the SCMV-MDMV-A only and was not able to transmit the second strain (SCMV-MDMV-B). Rigid particles in the purified virus preparation were found when stained with uranyl acetate and examined under the electron microscope. At the molecular levels, the purified viral RNA was used as a template for RT-PCR to amplify a part of the SCMV-MDMV-A-*cp* gene using specific primers. Results showed amplification of a DNA fragment with a size of about 400 bp. This fragment was A-tailed, cloned into pGEM®–T Easy vector, transformed in *Escherichia coli* (strain DHα) strain, miniprepared and purified DNA plasmid was confirmed by restriction endonuclease analysis.

INTRODUCTION

Sugarcane mosaic (SCMV) and maize dwarf mosaic (MDMV) viruses, definite members of family *Potyviridae* (Shukla *et al.* 1994), are known to infect sugarcane, maize, sorghum and other poaceous plant species (Pirone, 1972 and Teakle *et al.*, 1989). Occurrence of MDMV in Egypt has been recorded from maize (Allam *et al.*, 1987 and El-Morsi *et al.*, 2003).

Rao *et al.* (1998) reported that the sugarcane, maize, sorghum and jowar crops were found severely infected with mosaic disease, exhibited severe mosaic mottling and stunted growth. They also reported widespread of disease that caused serious damage

varied from 2 to 30% under field conditions. The virus had transmitted by some members of aphid vectors (Carmen *et al*., 1993 and Garrido *et al*., 1993).

SCMV is now known to be prevalent in many countries around the world (Teakle *et al*., 1989; Shukla *et al*., 1992 and Abd El Fattah *et al*., 2004). The abundance of MDMV-A infected johnsongrass appears to be the major contributing factor to the annual reappearance of MDMV in diverse areas as Argentina, Australia, France, Hungary, Italy, Morocco, Peru, Yugoslavia and the south edge of the corn belt southward in United States (Knoke *et al*., 1992).

Deng and Huang (1986) produced an antiserum specific to MDMV-B by intramuscular injection of purified virus into a rabbit. Teakle *et al.* (1989) reported that MDMV was different serologically from SCMV. Similarly JGMV was also serologically distant from MDMV and SCMV. Therefore, a major breaking in the identification and classification of the SCMV complex has achieved by Shukla *et al*. (1989b). They compared 17 strains from Australia and the United States on the basis of their reactions in western blotting with cross-absorbed PAbs directed towards surface-located, virus specific amino termini of *cp*. The results revealed that the 17 SCMV strains represent four polyviruses as Johnsongrass mosaic potyvirus (JGMV), MDMV, sorghum mosaic potyvirus (SrMV) and SCMV. (Shukla *et al*., 1989 a)

In this study, the presence of the SCMV-MDMV strains were screened in the germoplasms cultivated in six sugarcane experimental stations in Egypt *via* enzyme-linked immuno-sorbent assay (ELISA). Some biological and molecular characters of the identified strain(s) were also tested.

MATERIALS AND METHODS

Sample sources: Sets of sugarcane leaf samples, i.e., 9, 31, 58, 44, 26 and 4 from different germplasm varieties were collected from Sabahia, SCRI, Hawamdieh,Abo-Korkas,Nag-Hammadi and Kom-Ombo experimental stations, respectively. The samples that exhibited different systemic symptoms as described by Abd El Fattah, (2004) were screened for SCMV-MDMV strains *via* ELISA.

Antisera sources: Two antisera specific to some SCMV strains belonging to SCMV subgroup II (Shukla *et al*., 1992) were obtained from American Type Culture Collection (ATCC), Maryland, USA, through Prof. Dr. Mamdouh H. Abdel-Ghaffar, Ain Shams University, Cairo, Egypt were used for ELISA survey of SCMV-MDMV strains.

ELISA detection: The SCMV-MDMV strains in the collected samples were detected by I-ELISA technique as described by Koeing and Paul (1982).

Mechanical transmission and differential hosts: The plants in Table 1 were used for the determination of the

host range of the obtained SCMV-MDMV strain(s). In addition, some differential hosts were also tested. All these plants were subjected to artificial mechanical inoculation with the infectious sap as described by Allam *et al*. (1987).

Insect transmission: Data in Table 2 show the reaction of some differential hosts against two MDMV strains (Abd El Fattah, 1996).

Ultrastructural examination: Ultrathin sections of leaves of *S. bicolor* var. Rio seedlings (mechanically infected with SCMV-MDMV) were prepared and examined under the electron microscope according to the method of Abdel Ghaffar (1994).

Purification and negative staining: The leaves of seedlings of *S. bicolor* var. Rio infected with SCMV-MDMV-A as well as healthy plants (as a control) were used for purification as described by Abd El Fattah *et al.* (2004). On purification, a drop of the purified virus preparation was placed on the dark surface of the carbon-coated grid and subjected to negative staining as described by Abdel Ghaffar (1994).

Reverse transcription polymerase chain reaction (RT-PCR): SCMV-MDMV-A-infected leaf tissues were collected and viral RNA was isolated with the SV Total RNA Isolation System. Two oligonucleotides named: **P1**, 5'GTA TGG TGC ATC GAA AAT GGT3' and **P2**, 5'TGC TGC TGC TTT CAT CTG3' were designed according to the nucleotide sequence of the *cp* gene of SCMV as reported by Jeanne *et al*. (2001) and synthesized at AGERI, ARC, Giza, Egypt. The protocol using QIAGEN one step RT-PCR was followed for RT-PCR detection. The thermal cycle was programmed according to the profile as following: one cycle for 30 min at 50 ^{o}C; one cycle for 15 min at 95 ^{o}C; 40 cycles, each at 94, 50 and 72 ^{o}C for 30, 60 and 60 sec. respectively. The last cycle was extended for 5 min at 72 ^{o}C and stored at 4 ^{o}C. The PCR amplified product was detected and visualized using 1.2% agarose gel in 1 X TAE buffer at 80 volts for one hour.

Cloning, ligation, bacterial transformation and plasmid mini-preparation: The amplified RNA fragment from PCR, was tailed with adenine (A) and ligated in pGEM®–T easy vector (Promega) as described by Abd El Fattah *et al.* (2004). The competent cells of *Escherichia coli* (strain DHα) were prepared using calcium chloride as described by Hammond and Hammond (1989). The *E. coli* competent cells were transformed as mentioned by Abd El Fattah *et al.* (2004). The wizard® Plasmid Mini-preparation System (Promega) was used to isolate pure plasmid DNA with high yields. The plasmid yield and purity were checked by spectrophotometer at 260 and 280 nm (Sambrook *et al*., 1989).

Restriction digestion confirmation: The DNA insert was released from recombinant plasmid (RP) using the restriction endonuclease *Eco* R1 (Sambrook *et al.*, 1989), and was

resolved by electrophoresis using 1.2% agarose gel.

RESULTS AND DISCUSSION

The SCMV, the causal agent of SCMD, definite member of family Potyviridae (Shukla and Ward 1994), are known to infect sugarcane (Grisham and Legendre, 2000; Rao *et al*., 2003), maize (Wakman *et al.,* 2001), sorghum (Persley *et al*., 1985) and other poaceous plant species (Teakle *et al*., 1989). ELISA and antisera specific to MDMV strains A, B, and KS1 were used to determine relationship of such strains to SCMV strains I, J and M. (Viswanathan, 1997; Rao *et al*., 1998; Rao *et al*., 2003).

The antisera specific to SCMV-MDMV-A and SCMV-MDMV-B strains were used for ELISA detection of such strains in samples collected from different locations exhibited virus-like symptoms as shown in Table-1. The Positive ELISA values belonging to SCMV-MDMV-A and SCMV-MDMV-B were found in samples collected from Sabahia (33.3 & 88.8%), Hawamdeih (3.4 & 13.8%), Nag-Hammadi (38.5 & 26.9%) and Kom-Ombo (100 & 50%). While, SCMV-MDMV-A Abo-Korkas (11.4 & 0.0% and SCRI (0.0 & 32.3%).

Table (1): Indirect-ELISA detection of SCMV-MDMV strains in samples of sugarcane experimental varieties showing virus-like symptoms.

Experimental Stations	TNTS	NPS	SCMV strains	
			MDMV-A (No./%)	**MDMV-B** (No./%)
Sabahia	11	9	3/33.3	8/88.8
SCRI	31	10	0/0	10/32.3
Hawamdeih	58	10	2/3.4	8/13.8
Abo-Korkas	44	5	5/11.4	0/0
Nag-Hammadi	26	17	10/38.5	7/26.9
Kom-Ombo	4	4	4/100	2/50
Total	174	55	24	35

TNTS: Total number of tested samples. NPS: Number of positive samples.

The data in Table 2 illustrated by Figures 1, 2, 3 and 4 showed the identification of MDMV strains based on the expressed symptoms or susceptibility of different plant species. The obtained results showed that *Zea mays* L. and *Danthonia pilosa* were successfully differentiated the two MDMV-B strains. On the other hand, the later six grasses and grains were

reacted with the MDMV-A strain. Similarly, it was also noted that, infection of *Z. mays* L. and *S. bicolor* cv. Rio by the two strains initially resulted in a mosaic leaf symptoms, but later on in case of MDMV-A strain, a redding leaf followed by death of point was distinguished. Our results disagreed with that reported by Gingary and Gordon (1981), who mentioned that *S. bicolor* cv. Atlas should, gave local lesions when inoculated with MDMV-B.

Table (2): Reaction of some differential hosts against two MDMV strains.

Differential hosts	MDMV strains	
	MDMV-A	MDMV-B
	Grasses	
Chloris canteral	M	-
Danthonia pilosa	-	M
Eragrostis bahiensis	M	-
Eriochloa punctata	M	-
	Grains	
Sorghum bicolor cv. Rio	M, R	M
Sorghum bicolor cv. Atlas	M	-
Sorghum helepense L.	M	-
TX 2786	M	-
Zea mays L.	M, R	M

M: Mosaic. R: Redding. - : No reaction.

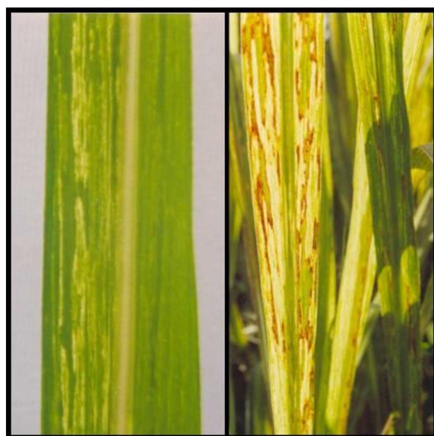

Figure 1: Naturally virus-infected sugarcane leaves of GT54-9 (right) and M57-531 (left) varieties exhibited virus-like symptoms (chlorotic streaks, severe mosaic and reddish necrotic streaks respectively.

Figure 2: *Z. mays* plant infected with MDMV-A and MDMV-B showing green mosaic.

Figure 3: Redding (left) and infection stage (right) shows reddish necrotic streaks on *S. bicolor* cv. Rio leaf infected with SCMV-MDMV-A strain.

Figure 4: *S. biocolor* cv. Rio infected with with MDMV-A strain showing green mosaic followed by redding and death of point.

The ultra structure of ultrathin sections of leaf showed the presence of cylindrical inclusions in the cytoplasm of MDMV-A-infected *S. biocolor* cv. Rio cells as shown in Figures 5, 6 and 7. These inclusions appeared as pinwheels, scrolls and bundles. In addition, ultrastructural examination of ultrathin sections prepared from *S. biocolor* cv. Rio leaf infected with MDMV-A strain, also showed the cytopathological effects of the applied strain on infected cells.

The disorganized chloroplast, degenerated mitochondria and deformed nucleus were found. Furthermore, a new kind of inclusion body was also found in the vacuoles of virus-infected cells. Lesemann *et al.* (1992) and Abd El Fattah *et al.* (2004) reported similar results when studied the presence of CI inclusions in the cytoplasm of SCMV-infected cells. Penrose (1974) studied the effects of an Australian isolate of SCMV on the type and location of inclusions associated with SCMV infection of sorghum and maize.

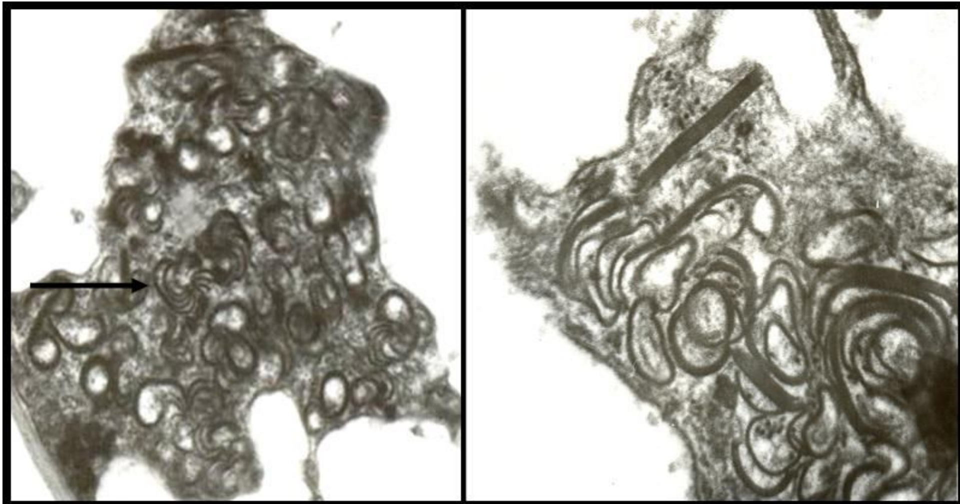

Figure 5: Ultrastructure of ultrathin sections prepared from leaves of *S. biocolor* cv. Rio infected with MDMV-A strain. Note the presence of CI inclusions as pinwheels (arrow), bundles and scrolls (X-46000).

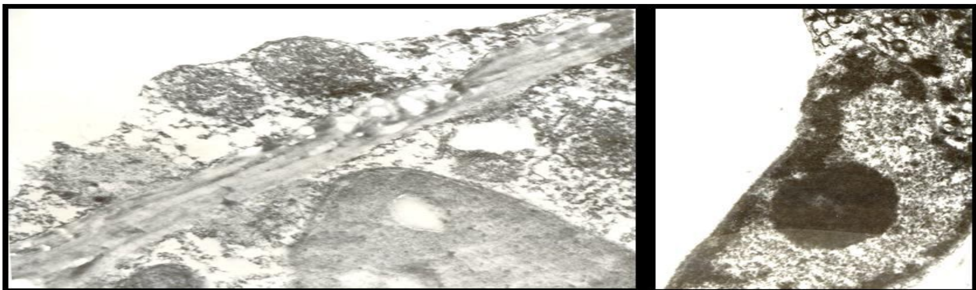

Figure 6: Ultrastructure of ultrathin sections prepared from leaves of *S. biocolor* cv. Rio infected with MDMV-A strain showing the cytopathological effect on virus-infected cells found on chloroplast, mitochondria and nucleus (X-17000).

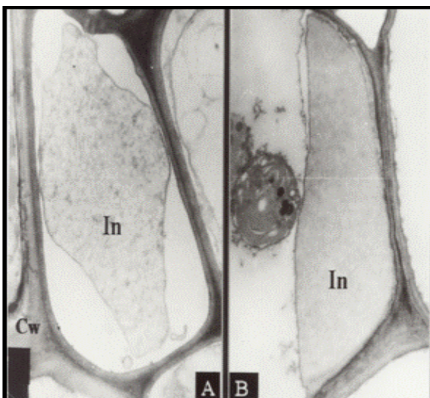

Figure 7: Ultrastructure of ultrathin sections prepared from leaves of *S. biocolor* cv. Rio infected with MDMV-A strain showing the presence of new kind of virus like inclusions (X-17000).

Aphids-like insects were successfully collected from insect-infected maize from the open field. These colonies were identified by Institute of Plant Protection, ARC, Giza, the results showed the presence of five different genera (*Aphis craccivora* (Koch), *Macrosiphum avenae* (F.), *Myzus persicae* (Sulzer), *Rh. maidis* (Fitch), *Rhopalosiphum padi* (L.), and *Schizaphis graminum* (Ronddani)) separately and was grown in a single special insect cadge. Their efficiency in transmission of SCMV-MDMV strains under investigation was determined. The results in Table 3 and Figure 8 showed that *Aphis craccivora* (Koch), and *Macrosiphum avenae* (F.) were failed to transmit the two SCMV-MDMV strains. As interestingly, *Myzus persicae* (Sulzer) could be as a differential vector, as it transmits the SCMV-MDV-A and was not able to transmit the second strain (SCMV-MDMV-B). *Rhopalosiphum padi* (L.) and *Schizaphis graminum* (Ronddani) were found to be more effective in transmission of SCMV-MDMV-A strain (36.6 & 73.3%) than SCMV-MDMV-B strain (26.6 & 56.6%) respectively. On the other hand, *Rh. maidis* (Fitch) showed 33.3% transmission of SCMV-MDMV-B.

Figure 8: Adults of *Rhopalosiphum padi* collected from Upper Egypt and subjected to identification under a control greenhouse conditions.

Table 3: Efficiency of five different aphid genera in transmission of SCMV-MDMV strains under investigation.

Aphids	% of transmission of MDMV strains	
	MDMV-A	MDMV-B
Aphis craccivora (Koch)	0.0	0.0
Macrosiphum avenae (F.)	0.0	0.0
Myzus persicae (Sulzer)	16.6	0.0
Rh. maidis (Fitch)	23.3	33.3
Rhopalosiphum padi (L.)	36.6	26.6
Schizaphis graminum (Ronddani)	73.3	56.6

The Results in Figure 9 showed the presence of rigid particles in the purified virus preparation when stained with 2% uranyl acetate and examined with the electron microscope.

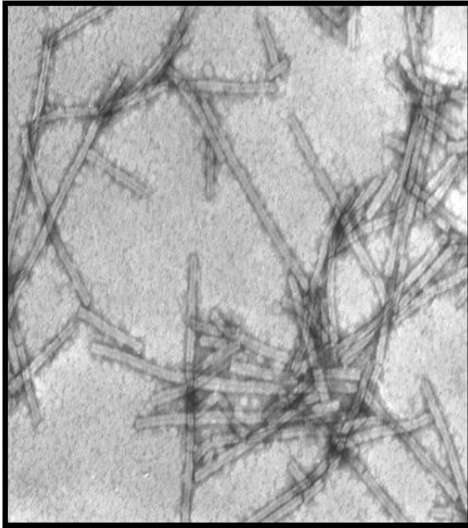

Figure 9: Morphology of MDMV-A strain negatively stained with 2% uranyl acetate and examined under the TEM (X-46000).

At the molecular levels, the purified virus preparation was used for extraction of the viral RNA which directly used as a template for RT-PCR and to amplified a part of the SCMV-MDMV-A-*cp* gene (400 bp) using primers as in Figure 10.

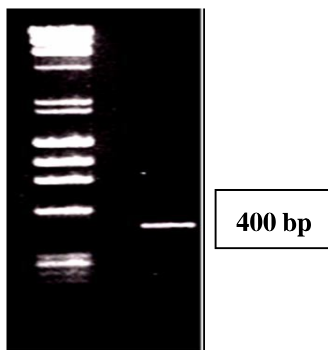

Figure 10: Agarose gel (1.2%) electrophoresis shows RT-PCR isolation of the viral *cp* gene of SCMV-MDMV-A strain and nested-PCR confirmation. Note: A fragment with sizes of 400 bp was amplified.

On A-tailing and purification of the amplified fragment it was successfully ligated into pGEM®–T Easy vector and transformed in *E. coli* (strain DHα) strain. The pure plasmid DNA was isolated by wizard® Plasmid Mini-preparation System (Promega). The results in Figure 11 showed the restriction endonuclease analysis using *Eco* RI confirmation of the mini-prepared and purified DNA plasmid. As result of Eco R1 digestion, a fragment with size of about 400 bp was released.

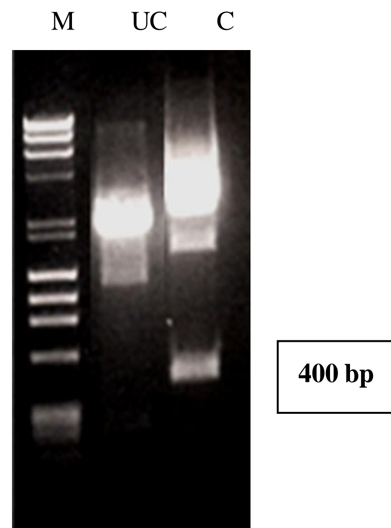

Figure 11: Agarose gel (1.2%) electrophoresis shows restriction endonuclease analysis confirmation of the mini-prepared and purified DNA plasmid. Note: A fragment with size of about 400 bp was released. M: DNA standard marker. UC: uncut. C: Cut.

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