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Identification of *Sugarcane Mosaic Potyvirus* Strains in Egypt

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Sugarcane mosaic potyvirus (SCMV) is the causal agent of mosaic disease which is reported to be one of the most important viral diseases attacking sugarcane crop in Egypt. Therefore, the present work was designed to screen the SCMV strains in Egypt based on biological, serological and molecular characterization. Data of the use of *Sorghum bicolor* vars. Rio and Atlas show successful detection of 7 SCMV strains (A, B, D, E, H, I, and M). The indirect-enzyme-linked immunosorbent assay (I-ELISA) detection of SCMV strains showed the presence of five strains, i.e., SCMV-A, SCMV-B, SCMV-D, SrMV-H and SrMV-I. The electron microscopy of SCMV-E-infected sorghum plant cells proved the presence of the cytoplasmic inclusions characterized to the potyviruses. The virus was purified and an antiserum containing polyclonal antibodies specific to SCMV-E strain was raised. Western blot immunoassay (WBIA) was successfully used to study the specificity of the produced antiserum to SCMV-E strain. On the level of the molecular studies of SCMV-E strain, a single protein band with a size of about 35-37 KDa was obtained after SDS-PAGE analysis of the purified virus preparation. The SCMV-E-RNA was isolated from virus-infected sugarcane tissues and then used as a template for reverse transcription-polymerase chain reaction (RT-PCR) to amplify the c-DNA that directly used to amplify the coat protein (CP) gene of SCMV-E strain. The amplified cp gene was used as a template using the internal primer combination in PCR for confirmation its specificity to the SCMV-cp gene as a PCR product with a size of about 400 bp was amplified. The cp gene PCR product was cloned into the pGEM-T easy vector and introduced into *Escherichia coli* strain DH5_ and the plasmid was then isolated, purified for sequencing the nested PCR product. The similarity between the present sequence (SCMVEgy1) and that of the 14 overseas strains belonging to different SCMV groups was determined.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is usually cultivated in tropical and subtropical regions, northern and southern of the equator and up to 31 tatitude south and 37 north (Abbott, 1961, Farrag and Nour, 1983 and Abd El Fattah, 1996). This crop is grown for different purposes to face the needs of various countries. In Egypt it is cultivated to obtain raw sugar, which is then refined for the production of crystallized sugar, known as "sucrose". The area cultivated with sugarcane on 2002-2003 for sugarcane production was 323400 feddans yielding about 16.46 million tons cane, 938381 tons sugar (represents 73 % of the sugar productivity in Egypt (Annual Report for Sugar Crops, Egypt, 2003).

Most of the sugarcane plantations are concentrated in Upper Egypt (El-Menia, Souhog, Qena and Aswan Governorates) where climatic conditions are most suitable for its cultivation. Sugarcane is considered to be the principal crop for sugar industry and the main purpose, newly, fresh

syrup and using the remaining by-products after crushing as raw materials for wood and upper industries. It is also used in the industrial fermentation that necessary for the alcohol, active yeast (Bread yeast), citric acid, acetic acid, and dextrin. In addition, it provides many industries with the raw materials required (Higgy, 1966 and Abd El Fattah, 1996).

There are many factors affecting sugarcane productivity, among those factors virus diseases, which play a major role in reducing reading cane yield. Mosaic, a typical virus disease of leaves, was undoubtedly a major cause of the decline of many varieties. It has a greater influence on the sugar industry of the world than any other disease with possible exception of Sereh (Farrag, 1978 and Gillaspie *et al.*, 1978). *Sugarcane mosaic potyvirus* (SCMV) is the causal agent of mosaic disease which reported to be one of the most important viral diseases attacking sugarcane crop and transferred by seed cuttings or sap inoculation and many aphid species such as corn leaf aphid (*Rhopalisphum maidis*, Fitch) (Zummo and Charpentier, 1965). A number of 17 SCMV strains are reported and did not represent one *potyvirus* but in fact belonged to four distinct potyviruses, namely, *Johnsongrass mosaic potyvirus* (JGMV), *Maize dwarf mosaic potyvirus* (MDMV), *Sorghum mosaic potyvirus* (SrMV) and SCMV (Shukla and Ward, 1994).

Sugarcane mosaic disease (SCMD) is undoubtedly a major cause of the decline of world industry (Abbott, 1961 and Rao *et al.*, 2003). Therefore, the present work designed to study screens of the SCMV strains in Egypt, followed by biological, serological and molecular identification of SCMV strain(s).

MATERIALS AND METHODS

Source of samples

In this experiment, 9, 31, 58, 44, 26 and 4 sugarcane leaf samples with different symptoms, belonging to different germplasm varieties, cultivated in Sabahia, Sugar Crops Research Institute (SCRI), Hawamdieh, Abo-Korkas, Nag-Hammadi and Kom-Ombo experimental stations, respectively, were collected.

Source of antisera

Five antisera containing polyclonal antibodies (PAbs) specific to SCMV strains, i.e., SCMV-A (PVAS-181), SCMV-B (PVAS-186), SCMV-D (PVAS-52), SCMV-H (PVAS-51a) and SCMV-I (PVAS-323) obtained from American Type Culture Collection (ATCC), Maryland, USA and kindly provided by Sugar & Integrated Industries, El-Hawamdieh, Giza, Egypt were used for ELISA survey of SCMV strains.

Serological detection of SCMV strains using indirect-enzyme linked-immunosorbent assay (ELISA) technique

For detection of SCMV strains in virus-infected samples, the indirect-enzyme linked immunosorbent assay (I-ELISA) technique was used as described by Koeing and Paul (1982).

Biological detection of SCMV strains

Virus-infected leaves were collected and ground separately in a mortar and a pestle using acid washed sand, carborandum 600 mesh, and potassium phosphates buffer 0.1 M pH 7.2 at rate 1:2 w/v was used for extracting the sap as given by Abd El Fattah (1996). The sap was rubbed

immediately on leaves of sweet sorghum (*S. bicolor* vars. Rio and Atlas) as recommended by Dean (1970) and Koike (1976) in the presence of carborandum twice with seven days intervals (Tosic and Ford, 1972). Four subsequent transmissions were carried out till the symptoms produced by the isolates became constant and identical. The development of symptoms on each variety recorded for a period of 28 days post virus inoculation.

Characterization of SCMV-E strain

Leaves of CP31-294 sugarcane variety naturally infected with SCMV-E strain subjected to electron microscopy as given by the method of Abdel-Ghaffar (1994). The ultrathin sections were then examined in the Electron Microscope Unit, Specialized Hospital, Ain Shams University, Cairo, Egypt.

The infectious sap was extracted from sugarcane plant infected with SCMV-E. Young leaves of seedling of *S. bicolor* var. Rio showing severe mosaic symptoms were harvested 15 days post inoculation with the infectious sap and was frozen overnight at -20°C. The procedure of Handojo and Noordam (1971) for virus purification was carried out and the final pellet was resuspended in a volume of 0.1 ml suspension buffer and examined by the electron microscope. According to the method given by Abdel-Halim *et al.* (2000), the purity of the purified virus preparation of this study and its yield were determined using the extinction coefficient, i.e., 2.4-2.8 of potyviruses.

Preparation of SCMV-E specific antiserum

The antiserum of SCMV-E was raised as described by Abdel-Halim *et al.* (2000) using two New Zealand white rabbits (5 kg each) and three intramuscular injections with 7 days interval three weeks post the first injection. The antiserum collected 10 days after the last injection from the ear that was not used for injection and stored at 4°C for further studies. The specificity of SCMV-E antiserum *via* western blot analysis described by Towbin *et al.* (1979) was determined.

Molecular characterization of SCMV-E strain

The molecular weight of virus coat protein (subunits) of the virus strain has been determined by SDS-PAGE using 4% staking gel on a 12% resolving gel the buffer system as described by Laemmli (1970) and Shukla and Ward (1988).

The viral RNA was isolated from virus-infected fresh tissues via the SV Total RNA Isolation System. The protocol of QIAGEN one-step RT-PCR kit was followed for isolation of the *cp* gene of the SCMV-E strain. Four oligonucleotides used through this study as shown in Table (1) were designed according to the nucleotide sequence of the *cp* gene of SCMV as given by Handley *et al.* (1998) and Jeanne *et al.* (2001) and synthesized at AGERI, ARC, Giza, Egypt. The thermal cycle was programed according to the profile as follows: RT step: one cycle for 30 min at 50°C. To activate the hot start *Taq*: one cycle for 15 min at 95°C was done. Amplification: 40 cycles, each of 94, 50 and 72°C for 30, 60 and 60 sec, respectively. The last cycle extended for 5 min at 72°C and stored at 4°C. The PCR amplified product was detected by electrohporesing as described by Sambrook *et al.* (1989) using 1.2% agarose gel in 1 X TAE buffer at 80 volts for one h.

Table (1): The nucleotide sequence of the primers used for PCR analysis.

Primer	Size	Sequence (5'----------3')
Ncp+ve	21	GTA TGG TGC ATC GAA AAT GGT
Ncp-ve	18	TGC TGC TGC TTT CAT CTG
Cp1+ve	21	GAC GAA GTT TTC CAC CAA GCT
Cp6-ve	21	CTG TGC TGT CCG TCA CGT GGC

In nested-PCR confirmation, the DNA insert detected by PCR using Ncp+ve and Ncp-ve primers. Amplification reaction was carried out in a volume of 50 µl as mentioned before using 100 ng purified plasmid as a template, *Taq* DNA polymerase and $MgCl_2$ buffer.

Sequencing the nested-PCR product of SCMV-E-cp gene

The nested-PCR fragment was tailed with adenine (A) to be ligated in pGEM®–T Easy vector (Promega). Wizard® DNA Clean-up System kit (Promega # A7280) was used to purify PCR product after A-tailing. A-tailed PCR fragment was lighted into the pGEM-T easy vector in a volume of 20 µl. Competent cells of *Escherichia coli* (strain DHJ) were prepared using calcium chloride ($CaCl_2$) as described by Hammond and Hammond (1989). Transformation was performed with *E. coli* competent cells as described by Nour El-Din (2003). Detection of recombinants was achieved by blue (non-transformed) or white (transformed) colonies screening. The wizard® Plasmid Mini-preparation System (Promega) was used to isolate pure super-coiled plasmid DNA with high yields. Plasmid yield and purity checked by spectrophotometer at 260 and 280 nm. To confirm the cloning, DNA insert was liberated from 1 µg recombinant plasmid (RP) using the restriction endonuclease *Eco* R1 as mentioned by Sambrook *et al.* (1989). The nested-PCR was also used as a second confirmatory test as mentioned before.

One recombinant clone was sequenced using ABI Prism 310 Genetic Analyzer (at AGERI, ARC, Giza, Egypt). The DNA sequence of the interested plasmid was determined by cycle sequence using the fluorescent dideoxy chain terminator technology. BigDye Terminator kit and an Applied Biosystsem 373 A Sequencer (Perkin-Elmer Applied Biosystsem Crop., Foster city,CA.). Using the NUC directory, European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany and site http://www.ncbi.nlm.nih.gov/). DNA sequences related to SCMV *cp* gene of different strains, i.e., SCMV-A (U57354), B (U57355), D (U67356), E (U57357), SrMV-H (U57358), SrMV-I (U57359), SrMV-M (U57360), MDMV-A (A34974), MDMV-B (D00949), MDMV-CHI (S77088), JGMV-Aust1(Z26920), JGMV-Aust (D00094), JGMV-USA (U07217) and JGMV-USA1 (U07216) were aligned with the nucleotide sequence of the PCR product in the plasmid using the DNA Star Software Package (Expert Sequence Analysis Software, USA).

DNA sequence translated to protein using EditSeq program. DNA and protein sequences of our viral isolate and overseas SCMV strains were alignment using MegaAlign program. Both DNA and protein alignment produced a multiple sequence alignment. The program allowed the translation of the nucleic acid sequence to a polypeptide sequence followed by forming a

phylogenetic tree (dendogram) by clustering the amino acid sequences based on overall similarity.

RESULTS AND DISCUSSION

Serological survey of SCMV strains

SCMV occurring on sugarcane plants under natural conditions have been described under different names. A survey of available literature reveals that the identification and classification of these viruses are mainly based on host range, insect transmission, biological properties, particle morphology and serological relationships (Shukla *et al.*, 1989). Symptoms may be a useful guide in diagnosing sugarcane viruses, Louie *et al.* (1974) showed that symptoms may vary with location, plant age and genotype, as well as environmental factors. Therefore, the symptoms should be supported with other tests. Technique like indirect-ELISA was standardized for detection of SCMV for effective quarantine in sugarcane (Viswanathan, 1997).

Data in Table (2) showed that all samples obtained from Sabahia, Abo-Korkas, Nag-Hammadi and Kom-Ombo Experimental Stations gave positive ELISA values, and indicated the presence of SCMV strain(s) in such samples. On the other hand 27 samples from each of SCRI (87%) and Hawamdieh (46.6%) Experimental Stations were infected. Data also show that the five detected SCMV strains were found in Abo-Korkas. While, the SrMV strains were not detected in samples from Sabahia, SCRI, Hawamdieh and Nag-Hammadi Experimental Stations. The strains SCMV-A did not detected in those samples obtained from Nag-Hammadi, while, SrMV-H strain was detected only in Abo-Korkas Experimental station. In other words, the presence of SCMV strains (ranging from 54.75 to 86.13%) was proved to be the higher than those strains belonging to SrMV (ranging from 10.22 to18.98%). In addition, the samples (varieties) which showed single infection, i.e., infected with one SCMV strain were reported as shown in Table (3). It is of importance to point to that all single-infection varieties (SIV) were found to be cultivated in SCRI and Hawamdieh Experimental Stations (Table 3).

These results close to that found by Viswanathan (1997), Rao *et* al. (1998a&b) and Rao *et* al. (2003). Jarjees and Uyemoto (1984) used the ELISA and antisera specific to MDMV strains A, B, and KS1 and others specific to SCMV strains A, B, D and H to determine their relationship to SCMV strains I, H and M.

Biological detection of SCMV strains

Tosic *et al.* (1990) and Shukla and Ward (1994) pointed to that differentiation of viruses of SCMV subgroup on the basis of differential hosts has been shown to be as effective as biochemical, biophysical and serological characterization of different potyviruses of sugarcane potyvirus subgroup. Khurana and Singh (1972) reported that *S. bicolor* cvs. Atlas and Rio were useful in differentiation between SCMV isolates. Therefore, in this study, these two sorghum cultivars as also recommended by Tippett and Abbott (1968), Dean (1970), Koike and Gillaspie (1989) and Tosic *et al.* (1990) were used for detecting the SCMV strains.

Due to lacking the antisera specific to all SCMV strains, two sorghum varieties, .i.e., *Sorghum bicolor* var. Atlas and *S. bicolor* var. Rio were used for detecting the SCMV

strains. Data in Figures (1), (2), (3), (4), (5), (6) and (7) showed that the use of the Rio and Atlas differential hosts were successful in detecting the SCMV strains. As 7 strains named A, B, D, E, H, I, and M were detected in sugarcane samples (varieties) exhibited virus-like symptoms. The characteristic symptoms for each strain were as described below:

Variations in development of the induced symptoms were obtained based on the temperature. It was also noted that no symptoms were obtained on the *S. bicolor* var. Atlas when inoculated with sap extracted from sugarcane plant infected with SCMV-M as well as SCMV-I strains. The results close to that carried out by Farrag and Nour (1983) in Egypt, who reported the presence of 9 strains of SCMV based on the use of some differential hosts.

The characteristic symptoms for each strain were described. In Egypt, Farrag and Nour (1983) succeeded in differentiation between 9 SCMV strains based on a differential host (*S. biocolor* cv. Co18). Ford *et al.* (1989), Shukla and Teakle (1989), Teakle *et al.* (1989) and Tosic *et al.* (1990) showed that the four viruses in SCMV (SCMV, SrMV, JGMV and MDMV) subgroups were differentiated on the basis of their reactions on some plant species, such as Johnsongrass, oat, sorghum lines OKY8 and SA 8735, Atlas sorghum.

These results also agreed with that reported by Gillaspie *et al.* (1986), who showed that seven types of SCMV from Java were distinguished using symptomatology on the cane varieties BM396, Co281, CP31-294 and Ps 41. They also revealed that on CP 31-294, CP 31-588 and sweet sorghum cv. Rio, SCMV strains A, B, and E were differentiated. Tosic *et al.* (1974) differentiated four viruses, i.e., SCMV, MDMV, SrMV and JGMV on the basis of differential host reaction.

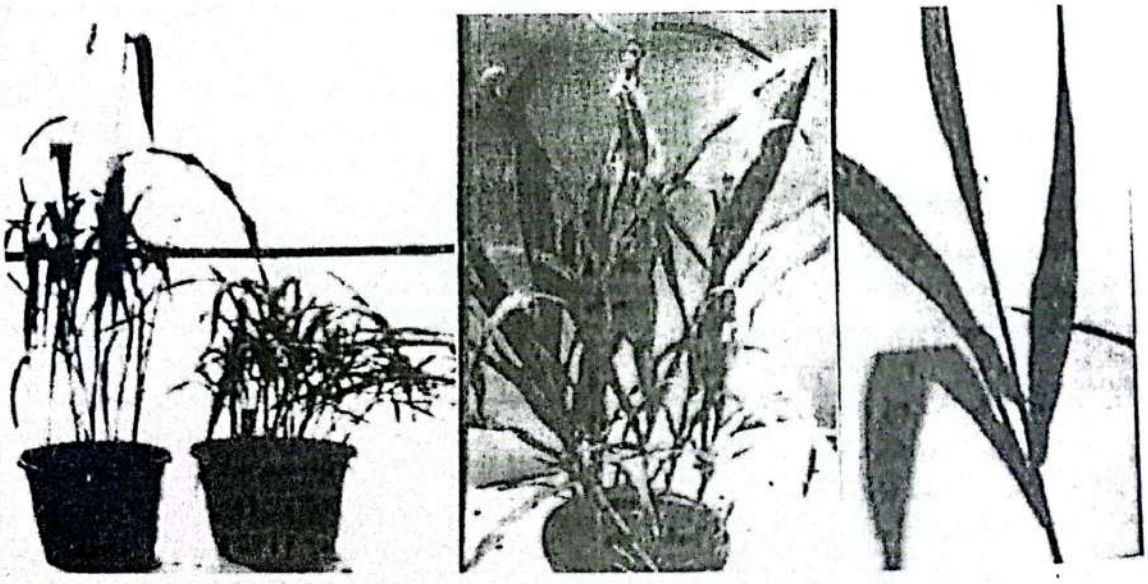

Fig. (1): Symptoms produced by SCMV-A strain on *S. bicolor* vars. Atlas (a and c) and Rio (b).
SCMV-A: On Rio: Severe mosaic. On Atlas: Mosaic, stunting and severe necrosis.

Table (2): Indirect-ELISA detection of SCMV strains in samples of sugarcane experimental varieties showing virus-like symptoms, and collected from six Experimental Stations in July of the 1999/2000 season

ES	Common symptoms	TNTS	PS		ELISA detection of SCMV strains				
					SCMV			SrMV	
					A	B	D	H	I
Sabahia	M,MM,C, N, LR,St	9	No.	9	7*	5	9	0	0
			%	100					
SCRI	M,MM,St, Y, LR,N, Mot.	31	No.	27	14	24	22	0	0
			%	87.1					
Hawamdeih	M,MM,Mo t,LR,St,D, C,LD	58	No.	27	12	22	15	0	0
			%	46.6					
Abo-Korkas	M,MM ,Mot,St ,N,C.	44	No.	44	41	40	44	14	25
			%	100					
Nag-Hammadi	M,MM ,St	26	No.	26	0	23	21	0	0
			%	100					
Kom-Ombo	M,MM ,St. LR,Y	4	No.	4	1	4	4	0	1
			%	100					
Total		172	137 (79.7%)		75	118	115	14	26

Note: Each sample represents a variety.
* = Number of infected samples.
C = Chlorosis.
D = Death of growing point.
Es = Experimental station.
LD = Leaf deformation
LR = Leaf Redding.
M =Mosaic
MM = Mild mosaic.
Mot = Mottling
PS = Number of positive samples.
N = Necrosis.
TNTS = Total number of tested samples.
St = Severe stunting.
Y = Yellowing.

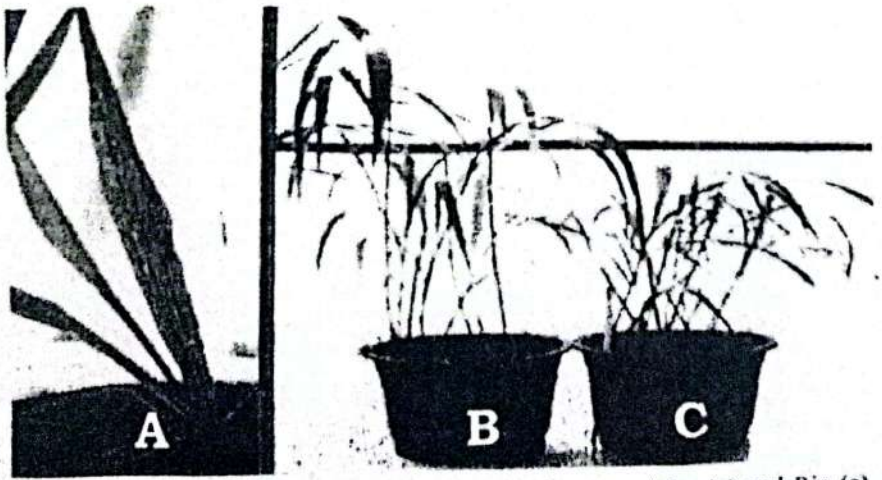

Figure (2): Symptoms produced by SCMV-B strain on *S. bicolor* vars. Atlas (c) and Rio (a) b) healthy. SCMV-B: On Rio: Green mosaic On Atlas: Severe mosaic and stunting.

Table (3): Single SCMV strains in some sugarcane experimental varieties cultivated in different Sugarcane Experimental Stations in Egypt during 1999/2000 season judged by I-ELISA

ES	Varieties	*SCMV strains*					H
		SCMV -A	SCMV – B	SCMV – D	SrMV – H	SrMV - I	
SCRI	CP270-1210	0.088	**0.184**	0.090	0.00	0.00	0.080
	CP29-116	**0.370**	0.084	0.082	0.00	0.00	0.082
Hawamdieh	89/60-14	0.081	0.102	**0.231**	0.151	0.055	0.081
	84112	0.058	0.027	**0.528**	0.210	0.015	0.133
	Co 419	0.091	0.082	**0.289**	0.124	0.069	0.093
	87/15-1	0.085	**0.208**	0.105	0.095	0.003	0.098
	HG	0.133	**0.287**	0.188	0.141	0.041	0.130
	88/18-3	0.148	**0.267**	0.118	0.158	0.162	0.098
	85/19-2	0.126	**0.181**	0.130	0.139	0.021	0.090
	Co 842	0.120	**0.370**	0.038	0.154	0.036	0.092
Total	**10**	**1**	**6**	**3**	**0**	**0**	**0**

Note: The bold ELISA values show twice as much as the ELISA value of the healthy samples.
ES= Experimental stations. H= Healthy.

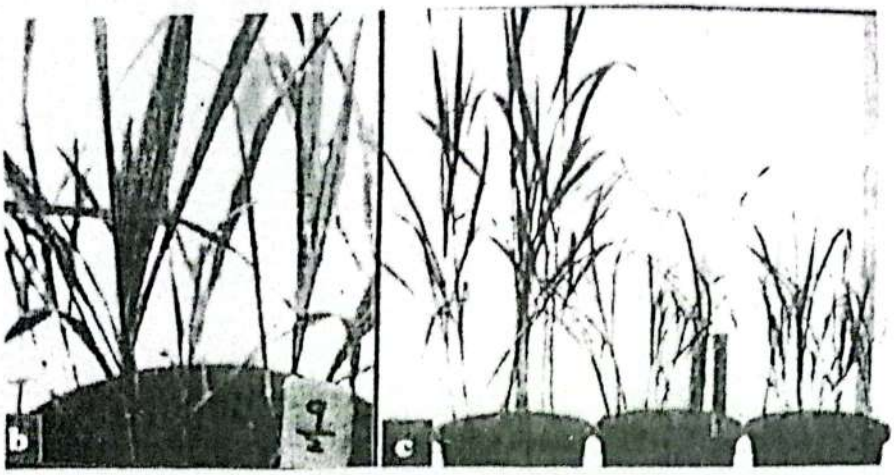

Figure (3): Symptoms produced by SCMV-D strain on *S. bicolor* vars. Atlas (c) and Rio (a and b). The healthy is also involved in c. SCMV-D: On Rio: Severe mosaic and severe stunting. On Atlas: Mosaic. death of tip.

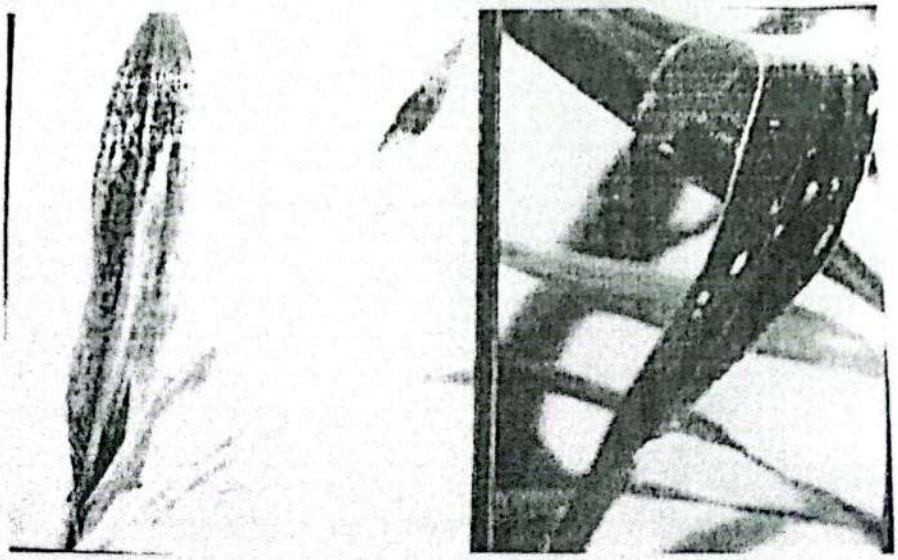

Figure (4): Symptoms produced by SCMV-E strain on *S. bicolor* vars. Atlas (b) and Rio (a). SCMV-E: On Rio: Green mosaic. On Atlas: Spindle shaped local lesions.

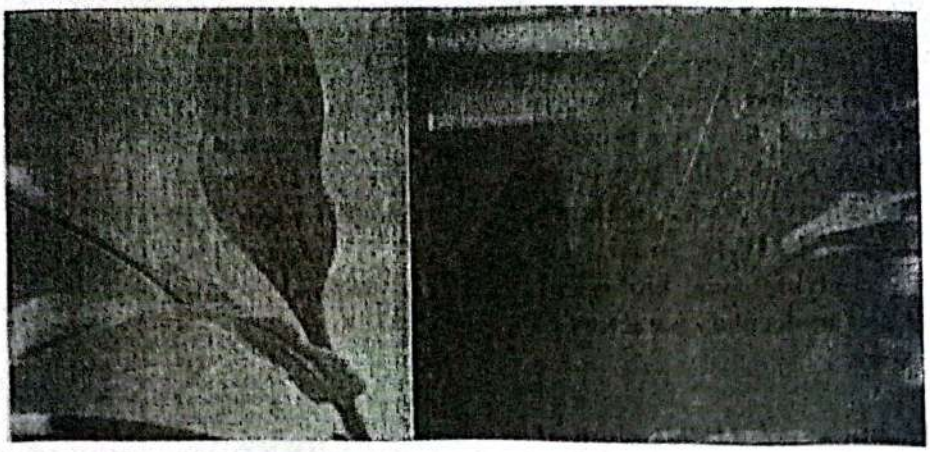

Figure (5): Symptoms produced by SCMV-H strain on *S. bicolor* vars. Atlas (b) and Rio (a). SCMV-H: On Rio: Mild mosaic. On Atlas: Green mosaic and little necrosis on the top of leaves.

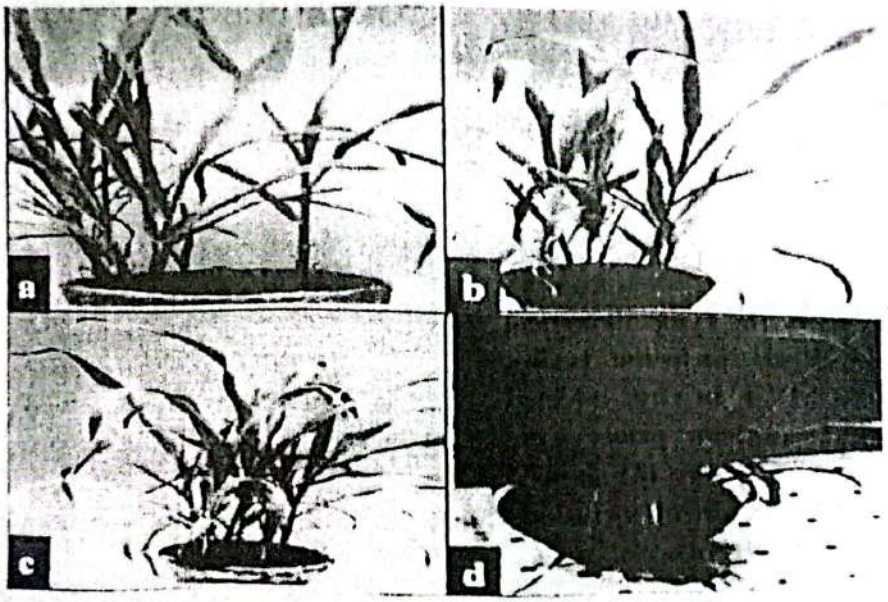

Figure (6): Symptoms produced by SCMV-I strain on *S. bicolor* var. Rio (a, b, c and d). SCMV-I: On Rio: Pale yellow leaf, severe mosaic, reddings in the midrib that extended down the leaf sheath, and necrotic lesions in a broken pattern or in broad bands.

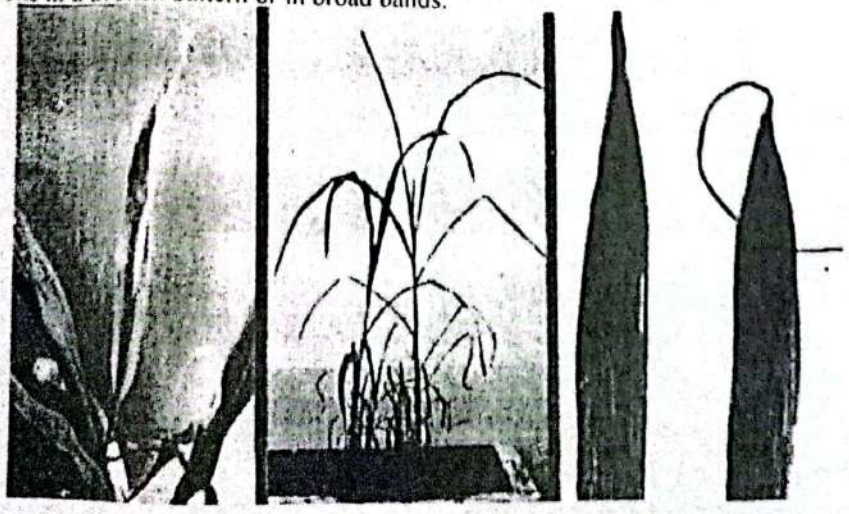

Figure (7): Symptoms produced by SCMV-M strain on *S. bicolor* var. Rio (a, b and c). SCMV-M: On Rio: Cross mosaic extensive reddish-brown necrotic area, mosaic and browning of the tip extending downwards and completely brown plant.

Characterization of SCMV-E strain.

Based on the review of the literature (Farrag and Nour, 1983, in Egypt), differential host reactions as well as ELISA results, the sugarcane (CP31-294 variety) plants naturally infected with SCMV-E strain was selected for studying some biological, serological and molecular characters of a SCMV strain.

Lesemann *et al.* (1992) showed that the cytoplasmic cylindrical inclusions (CIs) induced by JGMV, MDMV, SrMv and SCMV, together with other cytological alterations allow the four viruses to be distinguished. They showed that pinwheels, scrolls and laminated aggregates were produced only by SCMV whereas the other three viruses produced only pinwheels and scrolls.

In this work the cytopathological effects as well as inclusion bodies induced by SCMV-E in virus-infected sugarcane and sorghum plants were studied. Results in Figure (8) showed the presence of cylindrical inclusions which appeared as pinwheels, scrolls and laminated aggregates in the cytoplasm of virus-infected cells of *S. bicolor* var. Rio 15 days post inoculation with sap extracted from SCMV-E-infected sugarcane plants. In addition a large number of virus particles aggregated side by side (Figure 9) was also found.

These results are in full agreement with that reported by Herlod and Weible (1963) and Lesemann *et al.* (1992). Penrose (1974) also agrees with the obtained results, he studied the effects of an Australian isolate of SCMV on the type and location of inclusions associated with SCMV infection of sorghum and maize.

A virus yield of about 7-10 mg/kg virus-infected tissues with a good purity was obtained (with a dilution factor 10^{-1}). These results are in full agreement with that reported by Pirone and Anzalone (1966), Handojo and Noordam (1971), Langenberg (1973), Joshi (1989), Rao *et al.* (1998b) and El-Morsi *et al.* (2003).

Figure (8): Electron micrograph of ultrathin section of leaf from SCMV-E-infected *S. bicolor* var. Rio (15 days post inoculation), showing scrolls (S) and pinwheels (PW) inclusions in the cytoplasm close to the chloroplast (Ch) and the mitochondria (M) (X-28000).

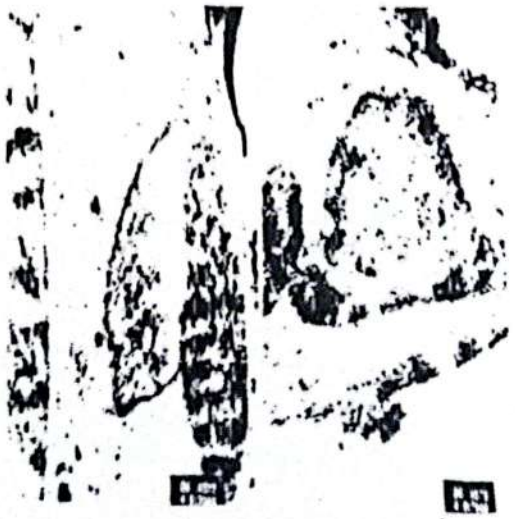

Figure (9): Electron micrograph of ultrathin section of leaf from SCMV-E-infected *S. bicolor* var. Rio (15 days post inoculation), showing a large number of virus-like particles side by side in the cytoplasm (X-13000).

They showed that a range of the virus yield was from 10 to 110 mg/kg virus-infected tissue. The electron micrograph of purified virus preparation negatively stained with 2% uranyl acetate showed the presence of filamentous virus-like particles measuring 750 X 12-13 nm. These results agreed with that reported by Rishi and Rishi (1985), Abd El Fattah (1996) and El-Morsi *et al.* (2003).

Production of specific antiserum

Serology is the most widely used practice for detecting viruses in infected material and also for establishing the identity of the causal virus (Moghal and Franki, 1976). Jarjees and Uyemoto (1984) produced antisera to MDMV (Strains, A, B and KS1) and SCMV (Strains A, B, D and H). These antisera were then used to determine their relationship to SCMV strains (I, J and M). In this work, an antiserum contains polyclonal antibodies specific to SCMV-E was raised. The specificity of the produced antiserum to SCMV-E was confirmed using the raised antiserum via western blot immunoassay (WBIA).

The raised antiserum with a titer of 1/128 that determined by I-ELISA as described by Hampton *et al.* (1990) (un shown data) was successfully used in western blot immunoassay (WBIA), to confirm its specificity to SCMV-E strain using sap from SCMV-E-infected and healthy sugarcane plants. In Egypt, Abdel-Ghaffar (1994) and El-Morsi *et al.* (2003) produced antisera specific to MDMV-A strain. Results proved the presence of one band with a molecular weight of about 35-37 KDa (Figure 10). Viswanathan (1997) produced polyclonal antibodies specific to the SCMV N strain and successfully performed for virus detection via the ELISA technique.

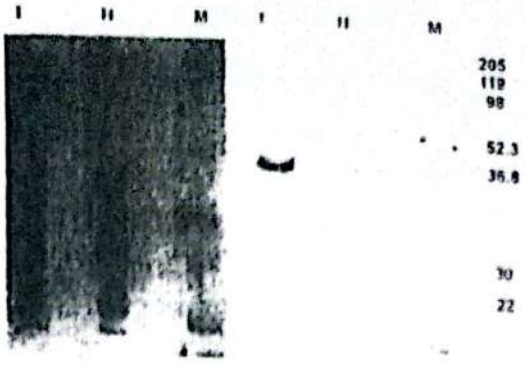

Figure (10). SDS-PAGE analysis (Left) of saps obtained from SCMV-E-infected (lane, 1) and healthy (lane, 2) sugarcane plants. WBIA (Right) of the same previous saps using the produced antiserum. M: Protein marker.

Molecular characters of SCMV-E strain.

Jensen *et al.* (1988) compared virus-induced proteins for 12 isolates of MDMV and four isolates of SCMV. They found that the capsid protein was 37 KDa and varied in size among isolates from 34.4 to 39.7 KDa. The relative molecular weights of capsid protein were 36.9, 36.7 and 37.3 KDa for SCMV-A, B and MDMV-B, respectively. On the other hand, in Australia, Gough and Shukla (1981) estimated the SCMV isolate as 33.7 KDa. Results in Figure (11), showed the presence of a single protein band with a size of about 35-37 KDa. No similar band was observed from the healthy purified preparation. These results agreed with that obtained by Chen (1978), who estimated the MW of capsid protein of SCMV-A, SCMV-B, SCMV-D and MDMV-B as 39, 39, 38 and 36 kDa.

Recently, the use of RT-PCR to amplify DNA transcribed from genomic RNA has been successfully applied to a range of pathogens (Henson and French, 1993 and Smith and van de vadle, 1994 and Gibbs and Mackenzie, 1997). The SCMV-E-RNA was isolated from virus-infected sugarcane tissues using the SV Total

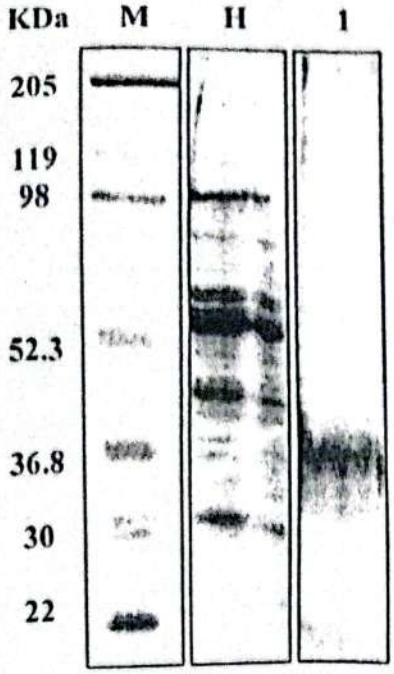

Figure (11): 12% SDS-PAGE of purified virus prepared from SCMV-E-infected *S bicolor* var. Rio (lane 1). H:healthy. M: Protein marker.

RNA Isolation System with high quality and substantially frees of genomic DNA contamination. The RNA was then used as a template for RT-PCR to amplify the c-DNA *via* the QIAGEN one step RT-PCR kit by use of an oligo (dT), as a common primer, nearly full-length c-DNA could be synthesized. Data in Figure (12) show that the *cp* gene (a size of about 700 nt) was amplified using two oligonucleotides (CP1+ve and Cp6-ve) as recommended by Handley *et al.* (1998). The amplified *cp* gene was used as a template using the internal primer combination (Ncp+ve and Ncp-ve) as described by Jeanne *et al.* (2001) in PCR for confirmation its specificity to the SCMV-*cp* gene as a PCR product with a size of about 400 bp was amplified (Figure 13).

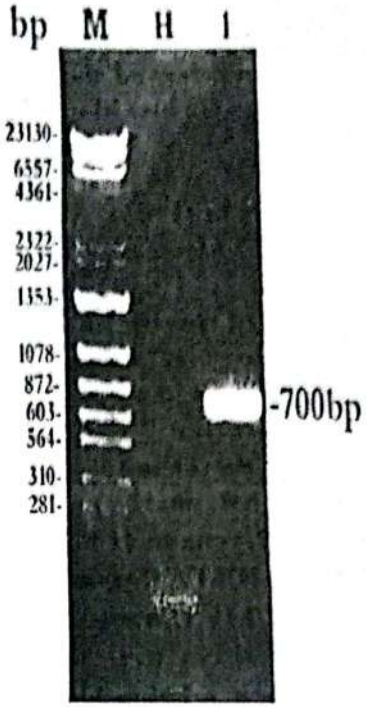

Figure (12): 1.2% agarose gel in TAE buffer stained with ethidium bromide showing RT-PCR amplification of the SCMV-E-*cp* gene using external primers (700 bp) (Lane 1). M: λ DNA marker digested with *Hind* III and ΦX174 digested with *Hae* III (23130, 9416, 6557, 4361, 2322, 2027, 1353, 1078, 872, 603, 564, 310 and 281 bp). H: healthy (as a negative control).

Partial sequence of SCMV-E-*cp* gene.

In order to partial sequence the SCMV-E-*cp* gene. The PCR product (700 bp) was extracted from the agarose gel using the Agarose Gel DNA Extraction Kit and cloned into the pGEM-T easy vector. The recombinant plasmid was then introduced into *E. coli* strain DH5α The white colonies were subcultured in LB ampicillin containing broth. The plasmid was then isolated and purified using the. Wizard® Plasmid Minipreparation System (Promega)

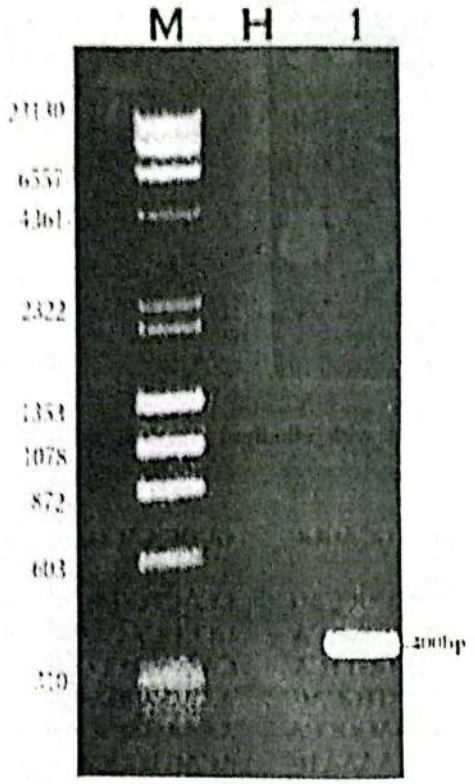

Figure (13): 1.2% agarose gel in TAE buffer stained with ethidium bromide showing nested-PCR amplification of the SCMV-E-*cp* gene using internal primers (400 bp) (Lane 1). M: Refer to Figure 12. H: healthy.

(Figure 14). The presence of the insert was detected via nested-PCR and digestion analysis (Figure 15). The relationships between different isolates of SCMV has recently been examined at a phylogenetic level using sequences derived from the NIB (or replicase) and coat protein-encoding regions (Yang and Mirkov, 1997 and Handley *et al.*, 1998). The nucleotide sequences of the PCR fragment, which appeared to be containing 399 nucleotides (Figure 16) and its deduced amino acids (133 amino acids) was studied. The DAG motif important for potyvirus transmission by aphids (Atreya *et al.*, 1990) was found in the CP.

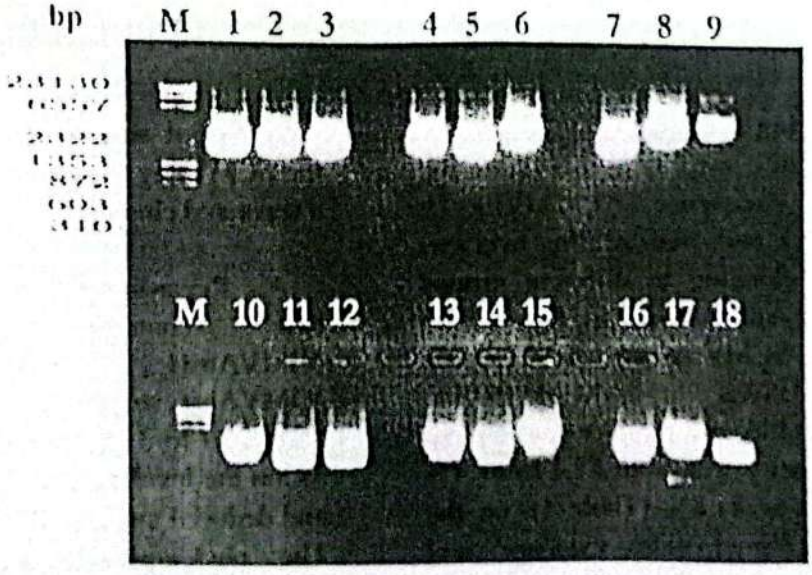

Figure (14): 1.2% agarose gel in TAE buffer stained with ethidium bromide showing plasmid minipreparation of 18 clones containing the *cp*-PCR fragment. M: refer to Figure 12.

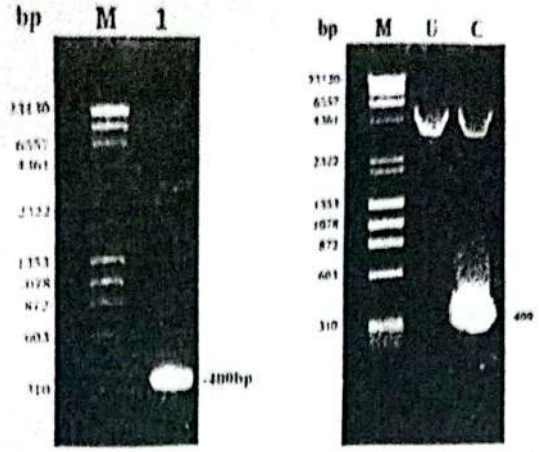

Figure (15): 1.2% agarose gel in TAE buffer stained with ethidium bromide showing PCR confirmation of a selected clone. M: refer to Figure 12.

Nucleotide sequences	CTGGAACAGT	CGATGCAGGC	GCGCAAGGAG
GAGATGGGAA	AGCCGGAACT	CAGCCGCCAG	CCACTGGAGC
GGAGGAGCTC	AACCACCAGT	TTCTGGAGCA	GCCGCACAAC
AGCAGCTCAA	CACCCGCGAC	TCAAGGTTCA	CAACCACCCA
CAGGGGGAGC	TACTGGTGGA	GTTGGTGCAC	AAACGGGAGC
TGGTGAAATT	GATTCAGTCA	CAGGAGGCCA	AAAAGACAAG
GATGTAGATG	CTCGTACGAC	AGGCAAAATC	ACAGTGCCAA
AACTTAAAGC	CATGTCGAAG	AAGATGCGCT	TGCCAAAAGC
AAAAGGAAAA	GATGTTTTGC	ATCTAGACTT	TTCTGTTAAC
ATACAAACCG	CAACAACCAA	GACATTATCA	AACACAAGA
Deduced amino acids			
AGTVDAGAQG	GDGKAGTQPP	ATGAAAQGGA	QPPVSGAAAQ
PPATQGSQPP	TGGATGGVGA	QTGAGEIDSV	TGGQKDKDVD
AGTTGKTTVP	KEKAMSKKMR	LPKAKGKDVL	TLDESVNIQT
ATTKTLSNP	ATS		

Figure (16). DNA sequences and deduced amino acids of 399 bp fragment amplified from the SCMV E-cp gene.

Sequencing analysis

The similarity between the present sequence (SCMVEgy1= SCMV-E) and that of the overseas strains belonging to different groups could be summarized as follows: In case of SCMV group, that contain SCMVAUSA, SCMVBUSA, SCMVDUSA and SCMVEUSA strains, the similarity was from 82.4 to 88.4% and 77.3 to 81.8% (Table 4), on the level of nucleotide sequence and deduced amino acids, respectively. The phylogenetic analysis of the present nucleotide sequence (Figure 17) and the deduced amino acids showed that the SCMVEgy1 strain was classified in a separated cluster.

Regarding the second group, that contains JGMVUSA1, JGMVAust1, JGMVUSA and JGMVAust strains, the identity was from 20.7 to 31.7% and 24.1% (Table 5), on the level of nucleotide sequence and deduced amino acids, respectively. The phylogenetic analysis of the present nucleotide sequence (Figure 18) and the deduced amino acids showed that the SCMVEgy1 strain was

classified in a separated subcluster.

Considering the third group, that contains MDMV-A, MDMV-B and MDMV-china strains, the homology was from 20.9 to 52.3% and 32.3 to 47.5% (Table 6), on the level of nucleotide sequence and deduced amino acids, respectively. The phylogenetic analysis of the present nucleotide sequence (Figure 19) showed that the SCMVEgy1 strain was classified in a separated subcluster. While, the deduced amino acids phylogenetic tree showed the presence of the Egyptian and the Chinese strains in one cluster.

Finally, the sequence analysis of the applied SCMV strain and those belonging to the subgroup of SrMV, that contains SrMVHUSA, SrMVIUSA and SrMVMUSA showed a identity from 40.1 to 49.3% and 36.1 to 36.8 % (Table 7), on the level of nucleotide sequence and deduced amino acids, respectively. The phylogenetic analysis of the present nucleotide sequence (Figure 20) and the deduced amino acids showed that the SCMVEgy1 strain was classified in a separated cluster.

As a conclusion, the SCMVEgy1 strain was found to be too close to the SCMV subgroup and in particularly, the SCMVE-USA. As similarities of about 88.4 and 81.8% based on the DNA sequence and deduced amino acids, respectively, was obtained.

Table (4): Similarity between SCMVEgy1 strain and some overseas SCMV strains

Strains	SCMVAUSA		SCMVBUSA		SCMVDUSA		SCMVEUSA	
	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid
SCMVBUSA	86.7	87.9	*	*				
SCMVDUSA	86.9	96.9	96.3	89.5	*	*		
SCMVEUSA	88.1	92.2	90.4	94.4	90.4	92.2	*	*
SCMVEGY1	82.6	75.8	82.4	79.8	82.9	77.3	88.4	81.8

Table (5): Similarity between SCMVEgy1 strain and some overseas JGMV strains

Strains	JGMVAUSTI		JGMVAUSTI		JGMVUSA		JGMVAUST	
	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid
JGMVAUSTI	83.0	95.0	*	*				
JGMVUSA	99.0	99.3	82.5	95.7	*	*		
JGMVAUST	21.2	95.0	20.7	100.0	21.0	95.7	*	*
SCMVEgy1	30.2	24.1	31.7	24.1	30.5	24.1	20.7	24.1

Table (6): Similarity between SCMVEgy1 strain and some overseas MDMV strains

Strains	MDMV-A		MDMV-B		MDMV-CHI	
	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid
MDMV-B	21.8	37.0	*	*		
MDMV-CHI	22.4	41.8	56.2	65.6	*	*
SCMV-Egy1	20.9	32.3	46.1	45.1	52.3	47.5

Table (7): Similarity between the DNA sequence of SCMVEgy1 strain and some overseas SrMV strains

Strains	SrMV-H		SrMV-I		SrMV-M	
	DNA	Amino acid	DNA	Amino acid	DNA	Amino acid
SrMV-I	97.3	97.3	•	•		
SrMV-M	97.0	97.3	97.3	97.3	•	•
SCMVEgy1	40.1	36.8	39.5	36.1	49.3	36.1

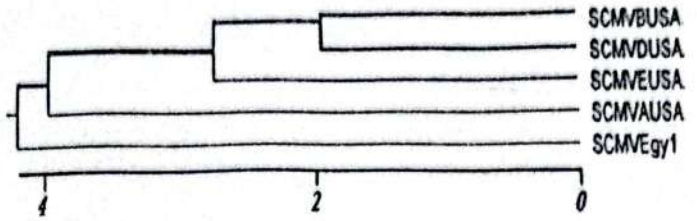

Figure (17): Phylogenetic analysis based on the nucleotide sequences showing the genetic relationship between the SCMV strains group and SCMVEgy1 strain.

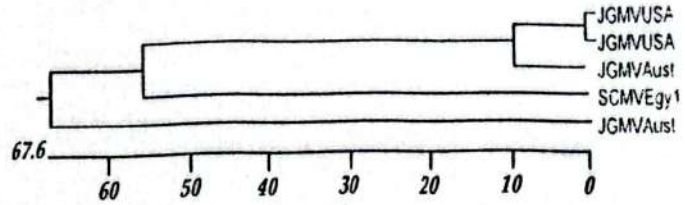

Figure (18): Phylogenetic analysis based on the nucleotide sequences showing the genetic relationship between the JGMV strains group and SCMVEgy1 strain.

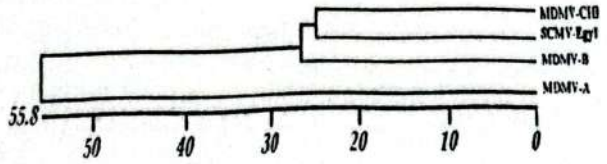

Figure (19): Phylogenetic analysis based on the nucleotide sequences showing the genetic relationship between the MDMV strains group and SCMVEgy1 strain.

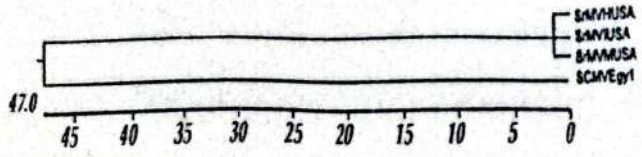

Figure (20): Phylogenetic analysis based on the nucleotide sequences showing the genetic relationship between the SrMV strains group and SCMVEgy1 strain.

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