Ahmed M. Soliman, *J. of Virol. Sci.*, Vol. 6: 46- 54, 2019
ISSN: 2636-2937

RESEARCH

Journal of Virological Sciences

Identification of *Cucumber Mosaic Virus* Infecting Some Vegetable Crops in Saudi Arabia

Ahmed M. Soliman

ABSTRACT

Background: *Cucumber mosaic virus* (CMV) is one of the widest spread viruses in the world and has the largest host range of plants.

Aim: The present study was conducted to identify four Saudi CMV isolates (cucumber, tomato, pepper and watermelon) using biological, serological and molecular assays.

Methods: Survey of some vegetable crops exhibiting mosaic, yellowing, blisters, shoestring leaf, mottling and stunting in different regions of Al-Ahsaa at Eastern Province in Saudi Arabia was conducted during the spring of 2016 and 2017. The viral isolates were biologically isolated by single chlorotic local lesion on *Chenopodium amaranticolor* and propagated in healthy *Nicotiana benthamiana*. Enzyme linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) with specific primers designed for the coat protein gene of CMV were used to detect the virus. RT-PCR products (657 bp) of coat protein gene of CMV were cloned and then sequenced.

Results: CMV was isolated from four vegetable crops (cucumber, tomato, pepper and watermelon) using ELISA and RT-PCR assays and the coat protein gene of the four isolates were submitted to GenBank. The CMV isolates showed nucleotide sequences similarity between them ranged from 97%-99%. **Conclusion:** This study revealed the existence of CMV in some vegetable crops in Al-Ahsaa at Eastern Province of Saudi Arabia. High nucleotide sequence similarity between the four isolates of CMV suggesting no genetic diversity. The four CMV isolates located in one cluster, belonging to subgroup IA.

Key words: *Cucumber mosaic virus*, RT-PCR, nucleotide sequences, diversity.

BACKGROUND

Cucumber mosaic virus, the type member of the genus *Cucumovirus* belonging to the family *Bromoviridae*, is one of the widest spread plant viruses causing disease in several plants all over world. CMV is transmitted in a non-persistent manner by more than 80 aphid species (Jacquemond, 2012; Kim *et al*., 2014). Typical symptoms of CMV include mosaic on leaves, mottling, stunting of plant, spike length reduction and very low filling of spikes leading to yield reduction (Revathy and Bhat, 2017). Symptoms differ depending on environment, host and age of the plant at infection time. Symptoms appear on younger leaves, which curl downward and become mottled, distorted and reduced in size. CMV is known to have a high potential for population diversity due to error-prone replication and short generation times (Ouedraogo *et al*., 2019). The genome of CMV contains three, positive-sense, single-stranded RNAs packaged in separate particles (Palukaitis *et al*., 1992). The genomic RNAs is classified as RNAs 1, 2 and 3. RNA 1 codes for the 1a protein, and is involved in virus movement. RNA 2 codes for the 2a protein, which is the viral RNA dependent RNA polymerase subunit of the CMV replicase, and for the 2b protein, which is translated from a subgenomic RNA and is involved in systemic spread and in the virulence of the virus (Gao *et al*., 2018). RNA 3 codes for two proteins, the 3a gene encodes the movement protein (MP) and 3b, the coat protein (CP). CMV can be divided into two major subgroups, I and II, based on the sequence similarity and serological relationships, and subgroup I can be further divided into subgroups IA and IB (Palukaitis, and Garcia-Arenal 2003; Jacquemond, 2012). RNA1 and RNA2 were placed in subgroup IB based on

Correspondence: amohamed@kfu.edu.sa
Full list of author information is available at the end of the article

phylogenetic analysis while, RNA3 grouped with subgroup IA isolates (Moyle *et al*., 2018). In Saudi Arabia, CMV isolates have been described in several hosts: Bottlegourd (*Lagenaria siceraria* L.) (AL-Saleh and Al-Shahwan, 1997), Cucumber (*Cucumis sativus* L.) (AL-Besher and Salama, 1991; AL-Saleh and Al-Shahwan, 1997), Melon (*Cucumis melo* L.) (Salama *et al*., 1987; AL-Saleh and Al-Shahwan, 1997), Potato (*Solanum tuberosum* L.) (Al-Shahwan *et al*., 1998), Pumpkin (*Cucurbita maxima* Duch.) (Salama *et al*., 1987), Squash (*Cucurbita* spp) (AL-Saleh and Al-Shahwan, 1997), Tomato (*Lycopersicon esculentum* Mill.) (AL-Beshr and Salama, 1991; Salama *et al*., 1994), Watermelon (*Citrullus vulgaris* Schard) (AL-Saleh and Al-Shahwan, 1997) and Alfalfa (*Medicago sativa*) (Al-Shahwan *et al*., 2017). To the knowledge of the author, the nucleotide sequences of CMV isolates from Saudi Arabia has never been recorded in GenBank database. In this study, the four Saudi CMV isolates (cucumber, tomato, pepper and watermelon) were identified using biological, serological and molecular assays. In addition, Phylogenetic analyses were done using the nucleotide sequences of the coat protein gene of the four Saudi CMV isolates to confirm the CMV isolates and to determine to which subgroups the isolates belong.

MATERIALS & METHODS

Source of virus isolates

Several field visits were done in different vegetable growing areas (open fields and greenhouses) of Al-Ahsaa governorate at Eastern Province in Saudi Arabia during the spring (March to May) of 2016 and 2017. The naturally infected cucumber, tomato, watermelon and pepper plants showing viral symptoms including mosaic, yellowing, stunting, blister, shoestring leaf and distortion of leaves were collected. Infected vegetable leaf samples were kept in cool boxes after collecting from the field and stored at -80°C for later use.

Propagation of virus isolates

Infected young leaves of cucumber plants were grinding in phosphate buffer solution (pH 7.2). The infectious sap was mechanically inoculated on *Chenopodium amaranticolor* as an indicator host. The single local lesion assay was used for biological purification of the isolate and propagated on healthy *Nicotiana benthamiana* plants.

ELISA test

ELISA test was carried out to detect CMV in the collected samples using the method described by ELISA kits (Bioreba AG, USA) and according to Clark and Adams (1977). The ELISA test was carried out with several replicates, including positive and negative controls supplied with kits. Samples with an absorbency of at least twice the healthy controls were considered as a positive for the presence of the virus.

RNA extraction

RNA extraction from leaf samples was carried out using RNeasy Plant Mini Kit (QIAGEN) according to the instructions of manufacturer.

Primers for the coat protein gene of CMV

F4-forward primer (5'-TTGAGTCGAGTCATGGACAAATC-3') and F3-reverse primer (5'-AACACGGAATCAGACTGGGAG-3') designed by Lin *et al*. (2004) were used to amplify the full length of the coat protein gene of CMV.

One-step RT-PCR

One-step RT-PCR reactions were carried out using the " iScript One Step qRT-PCR Kit " (BIOMATIK) in 25 µL reaction volume. Each reaction contained 1 µL of the RNA extract (40 ng of total RNA), 12.5 µL iGreen Mastermix, 1.5 µL of 10 µM of each primer, 0.5 µL of qRT-PCR Enzyme Mix and 25 µL of nuclease-free water. Synthesis of cDNA was done at 40°C for 30 min and denaturation at 94°C for 4 min followed by 30 cycles of 94°C for 30 sec, 45°C for 2 min, 72°C for 2 min and a final cycle of 72°C for 5 min. 5 µL of PCR products were loaded in 1% agarose gels with 100 bp DNA ladder (BIOMATIK) and pictures were taken under UV light with digital imaging system gel doc (Syngene Bio Imagins, IN Genius).

Molecular cloning

DNA cloning was carried out to insert RT-PCR products (coat protein gene of CMV) into pGEM-T Easy vector (Promega) and according to manufacturer's instructions. The PCR products were ligated into the vector using ligase enzyme (provided with the kit). The recombinant plasmids were transformed into *Escherichia coli* DH5α strain.
Recombinant plasmids were isolated using Wizard Plus SV Minipreps DNA Purification System (Promega) from some colonies then digested with restriction enzyme *Eco*RI and fractionated on 1% agarose gel in 0.5X TBE buffer.

Nucleotide sequencing of the RT-PCR products

The nucleotide sequencing were carried out at Macrogen Company. The nucleotide sequences of the coat protein gene of CMV isolates were analyzed and compared using MEGA X (Kumar *et al*., 2018) and DNAMAN 8 Sequence Analysis Software (Lynnon, BioSoft) with different isolates from GenBank belonging to major subgroups I (IA, IB), II.

RESULTS

Sample collection for viruses

Field inspection of different vegetable growing areas of Al-Ahsaa governorate at Eastern Province in Saudi Arabia was done. The naturally infected cucumber, tomato, watermelon and pepper plants showed viral symptoms including mosaic, yellowing, stunting, blister, shoestring leaf and distortion of leaves were collected (Fig. 1).

ELISA test

The collected leaf samples were initially tested using ELISA kits (BIOREBA) and many samples gave positive reaction indicating the presence of the CMV in the collected samples. The ELISA tests were done on 220 samples (leaves of collected vegetables); 54 samples of cucumber, 58 samples tomato, 56 samples of pepper and 52 samples of watermelon plants (Table 1) using specific antibodies for CMV provided with kit (Bioreba, USA). Two independent replicates for each sample were made in order to minimize the variations in readings and normalize the data related to healthy leaves (negative control).

Table 1: The vegetable samples tested by ELISA for CMV and percentage of infection.

Crop	No. of samples	No. of positive (+) samples	No. of negative (-) samples	Percentage of infection (%)
Cucumber	54	15	39	27.78%
Tomato	58	14	44	24.14%
Pepper	56	13	43	23.21%
Watermelon	52	12	40	23.07%

Table (1) showed that, 15 samples of cucumber were CMV positive with 27.78% of infection, 14 samples of tomato were CMV positive with 24.14% of infection, 13 samples of

pepper were CMV positive with 23.21% of infection and 12 samples of watermelon were CMV positive with 23.07% of infection.

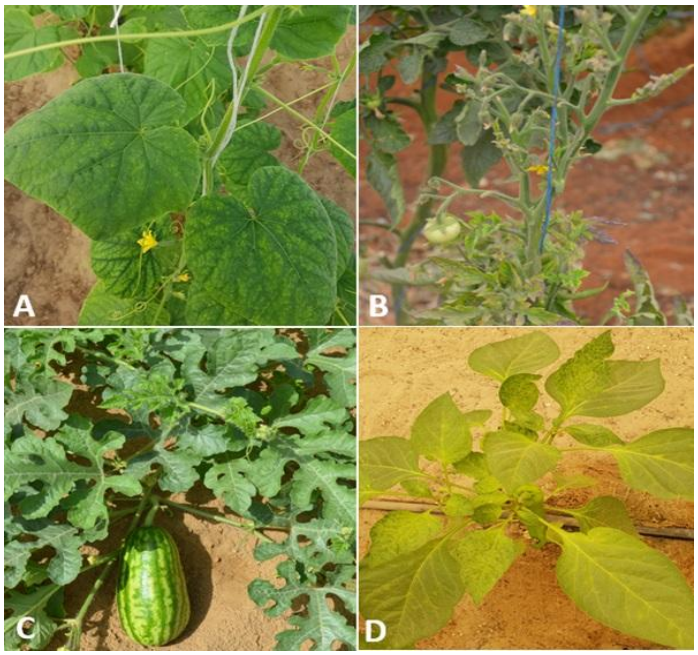

Fig. 1: Symptoms of viral diseases on vegetable plants during collecting of samples. A: blister and mosaic on cucumber; B: shoestring and yellowing on tomato; C: mild mosaic on watermelon; D: yellowing and mosaic on pepper.

One step RT-PCR and cloning

Vegetable leaf samples collected from growing areas (mentioned before) were subjected the RT-PCR assay. RT-PCR was carried out to amplify the coat protein gene of cucumber mosaic virus using set of primers F4 and F3, as described above. Those set of primers succeeded to amplify the expected size bands for the coat protein gene of CMV at 657 bp (Fig. 2). The samples were selected from previous positive ELISA tests.

RT-PCR products were succefully inserted into pGEM-T Easy vector and the recombinant plasmids were transformed into *Escherichia coli* DH5α strain. Recombinant plasmids were isolated successfully from different colonies using Wizard Plus SV Minipreps DNA Purification System. Digestion with restriction enzyme *Eco*RI and fractionation on 1% agarose gel in 0.5X TBE buffer was done ending with positive results.

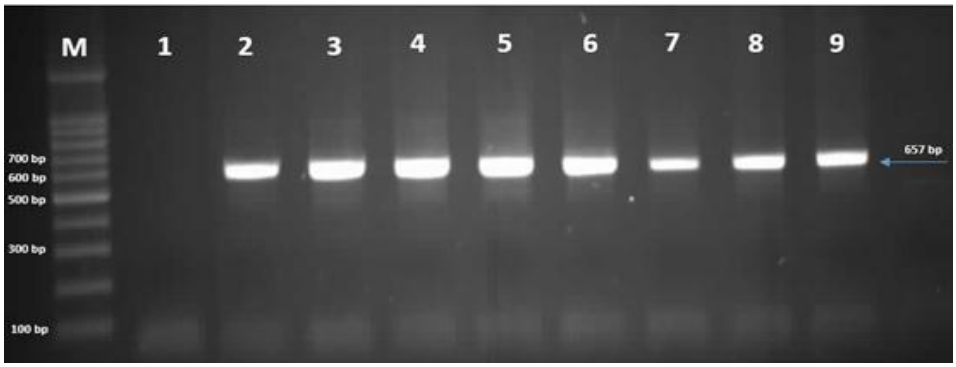

Fig. 2: Agarose gel electrophoresis of RT-PCR amplified products. M: 100 bp DNA ladder (Promega); 1: healthy sample; 2, 3: two infected samples of cucumber; 4, 5: two infected samples of watermelon; 6, 7: two infected samples of tomato; 8, 9: two infected samples of pepper.

Nucleotide sequencing

Coat protein gene for the four isolates of CMV were sequenced in Macrogen Company (South Korea). Then submitted to the GenBank under the accession numbers MK579335, MK579336, MK579337 and MK579338 for cucumber, pepper, tomato and watermelon isolates respectively. The sequenced coat protein gene for the four isolates were used in phylogenetic analysis using MEGA X (Kumar *et al*., 2018) and the Optimal Alignment Method of DNAMAN 8 software (Lynnon BioSoft) to study the relationship between the CMV isolates used in this study and those isolates available in GenBank belonging to major subgroups I (IA, IB) and II (Table 2, Fig. 3).

Table 2: The selected isolates of CMV coat protein gene from GenBank belonging to subgroup I (A and B) and II used for comparison.

Subgroup	Strain	Accession Number	Country
IA	FT	D28487	Japan
	OL	AJ890464	India
	Mf	AJ276481	Korea
	GPP	AJ131623	Netherlands
	Ny	U22821	Australia
	FNY	D10538	USA
	I17F	Y18137	France
	Kor	L36251	Korea
IB	C7-2	D42079	Japan
	NT9	D28780	Taiwan
	Ixora	U20219	USA
	Tfn	Y16926	Italy
	M48	D49496	Taiwan
	Phym	X89652	India
	Oahu	U31220	USA
	D	AF281864	India
II	M2	AB006813	Japan
	AL	AJ585086	India
	BKD	AJ131621	Netherlands
	Q	M21464	Australia
	R	Y18138	France
	LS	AF127976	USA
	Trk7	L15336	Hungary
	Kin	Z12818	UK

The coat protein gene of the four isolates showed a very high nucleotide sequences similarity between the four Saudi isolates ranged from 97%-99% suggesting no genetic diversity. It is obvious from figure (3) that, the four Saudi isolates present in one cluster and belonging to the minor subgroup IA.

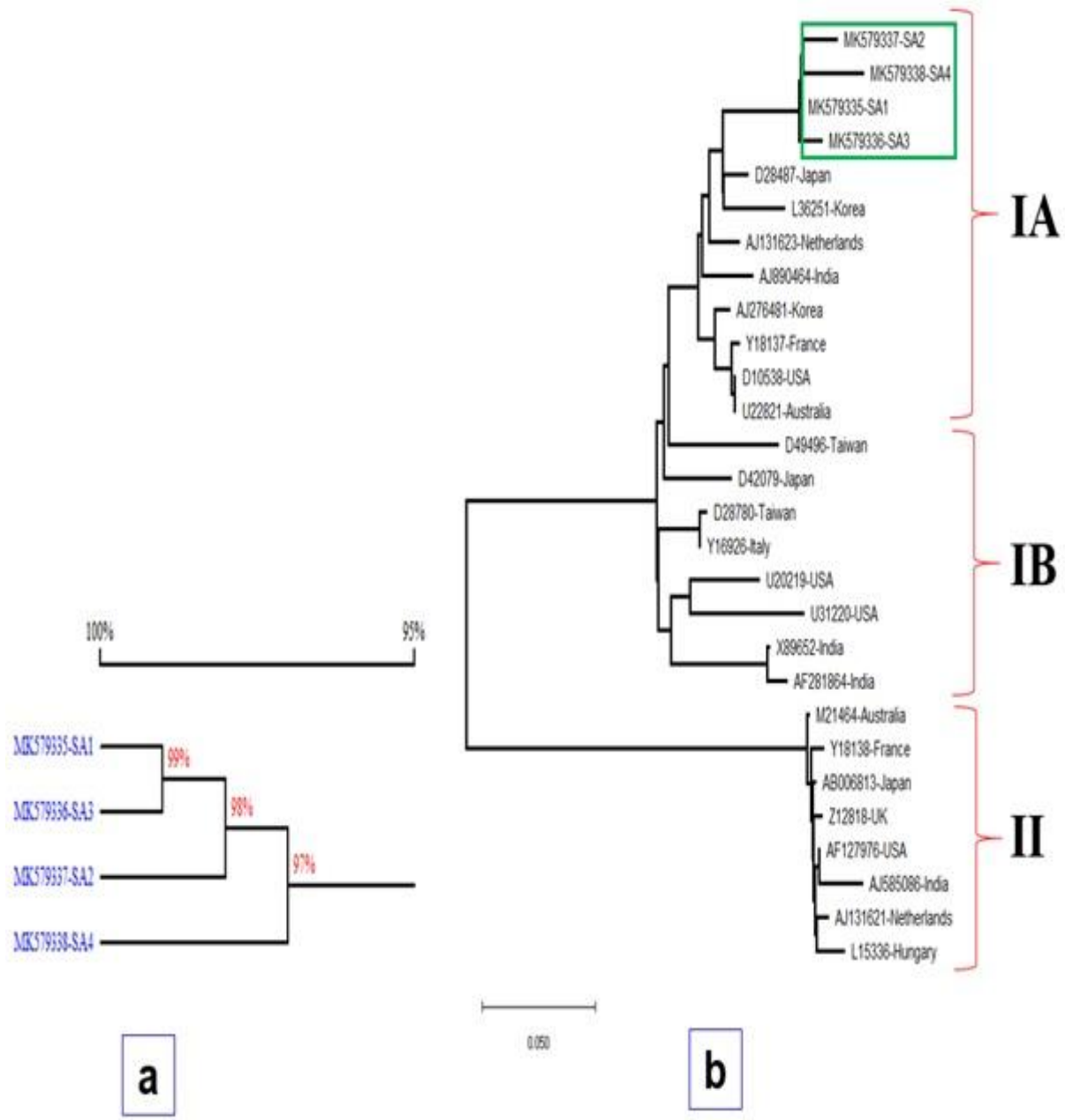

Fig. 3: Phylogenetic tree showing relationships (a) between the four CMV Saudi isolates (b) among several isolates of CMV belonging to the major subgroups I (IA, IB) and II and CMV of Saudi isolates (green box) based on the nucleotide sequences of their CP genes.

DISCUSSION

The current investigation was carried out to identify the subgroups of CMV isolated from different vegetable crops including cucumber, tomato, pepper, and watermelon. Further, studying the similarity of CMV isolated from previous mentioned vegetable crops in Saudi Arabia was targeted. In the present study, the CMV of four Saudi isolates was detected in naturally infected cucumber, tomato, watermelon, and pepper plants and the infection percentages of the CMV in the collected samples were in a range between 23-27% using ELISA technique and also confirmed by RT-PCR technique. Therefore, the infection with CMV causes significant losses reach up to one quarter of the yield. The intensive economic and yield loss nominated CMV to be one of the top 10 plant viruses (Scholthof *et al*., 2011; Sinha and Samad, 2019). Understanding genetic structures of virus populations and their evolutionary mechanisms is an extremely important step to control viral diseases and to

reduce the risk of emerging new viruses (Kim *et al*., 2014). To achieve this goal, the isolated CMV from Saudi four infected vegetable crops was transmitted mechanically into *C. amaranticolor* by single local lesion assay as reported by many investigators and propagated in healthy *N. benthamiana* (Osman *et al.,* 2004). Then, the CP gene of CMV (657 bp) was successfully amplified using RT-PCR using specific primers for the full length of the coat protein gene of CMV according to Lin *et al*. (2004). Sequenced coat protein gene for the four isolates and submitting the data to the GenBank under the accession numbers MK579335, MK579336, MK579337 and MK579338 showed a very high nucleotide sequences similarity between the four isolates ranged from 97%-99% suggesting no genetic diversity. The phylogenetic analysis showed that, the four CMV isolates of Saudi Arabia is located in the same cluster belonging to subgroup IA. According to the sequence similarity and serological relationships, CMV was divided into two major subgroups, I and II, and subgroup I was divided further into subgroups IA and IB (Palukaitis, and Garcia-Arenal 2003; Jacquemond, 2012). Furthermore, Moyle *et al*. (2018) stated that, RNA1 and RNA2 were placed in subgroup IB based on phylogenetic analysis while, RNA3 grouped with subgroup IA isolates. In addition, several isolates of CMV have been classified according to the sequences of RNA3 (Deyong *et al.,* 2005). Lin *et al*. (2004) used phylogenetic analyses of different CMV genomic regions to detect natural reassortment between subgroup IA and IB isolates and possible reassortment between subgroup IA isolates but no evidence for recombination was obtained. In the contrary, Nouri *et al*. (2014) detected a recombination between subgroups I and II as well as IA and IB in RNA 3 based on phylogenetic and computational analysis. Balad-Blume *et al*. (2017) developed the detection of CMV using the Luminex xTAG technology based on nucleic acid test. The advantage of this technology is allowing the simultaneous detection of several targets. The presence of CMV and differentiation between the two subgroups I and II for which significant differences with regard to virulence and severity of symptoms have been proved by this method. Generally, Survey in this study found the existence of the CMV subgroup IA and no CMV subgroup II isolates in Al-Ahsaa governorate-Saudi Arabia. The identification of further studies on CMV subgroups is important to develop an effective control of CMV in the field and better understanding of CMV epidemics. An improved understanding of genetic structure and associated factors driving CMV evolution can help to design improved disease management strategies (Nouri *et al*., 2014).

CONCLUSION

In the current study the author concluded that, CMV could infect several vegetable crops in Saudi Arabia causing significant economic loss. The coat protein gene of the four CMV isolates were submitted to GenBank under the accession numbers MK579335, MK579336, MK579337 and MK579338 for cucumber, pepper, tomato and watermelon isolates respectively. Based on nucleotide sequences, the study confirmed that, high similarity of CMV isolates suggesting that, the need of spending more efforts to control this virus. In future, mutation could occurs which will cause serious epidemic with different strains of the virus. In addition, the four CMV isolates in this study were found located in one cluster and belonging to subgroup IA.

ACKNOWLEDGMENT

The author gratefully acknowledges Pests and Plant Diseases Unit (PPDU) at College of Agricultural and Food Sciences, King Faisal University for giving access to all their facilities. Also, many thanks to Dr. Sherif M. El-Ganainy for his valuable assistance during the preparation of this manuscript.

COMPETING INTERESTS

The author declares that there is no conflict of interests.

AUTHOR DETAILS

Dept. of Arid Land Agric., College of Agricultural & Food Sciences, King Faisal Uni., Saudi Arabia.
Virus & Phytoplasma Res. Dept., Plant Pathol. Res. Institute, Agricultural Research Center, Egypt.

RECEIVED: Jan, 2019; **ACCEPTED:** March 2019; **PUBLISHED:** July 2019

REFERENCES

Al-Besher, A.A. and Salama, E.A. (1991). Economic effect of diseases and cultivars tolerance of tomato and cucumber grown in greenhouses in the Eastern province of Saudi Arabia. *Saudi Journal of Biological Sciences*, 1: 169-187.

Al-Saleh, M.A. and Al-Shahwan, I.M. (1997). Viruses infecting cucurbits in Riyadh, Gassim and Hail regions of Saudi Arabia. *Arab Gulf Journal of Scientific Research*, 15: 223-254.

Al-Shahwan, I.M., Abdalla O.A. and Al-Saleh, M.A. (1998). Potato viruses in central Saudi Arabia. *Journal of King Saud University*. *Agricultural Sciences*, 10: 45-53.

Al-Shahwan, I.M., Abdalla, O.A., Al-Saleh, M.A. and Amer, M.A. (2017). Detection of new viruses in alfalfa, weeds and cultivated plants growing adjacent to alfalfa fields in Saudi Arabia. *Saudi Journal of Biological Sciences*, 24: 1336-1343.

Bald-Blume, N., Bergervoet, J.H.W. and Maiss, E. (2017). Development of a molecular assay for the detection of *Cucumber mosaic virus* and the discrimination of its subgroups I and II. *Journal of Virological Methods*, 243: 35-43.

Clark, M.M. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, 34, 475-483.

Deyong, Z., Willingmann, P., Heinze, C., Adam, G., Pfunder, M., Frey, B. and Frey, J.E. (2005). Differentiation of *Cucumber mosaic virus* isolates by hybridization to oligonucleotides in a microarray format. *Journal of Virological Methods*, 123: 101-108.

Gao, S., Lu, J., Cheng, X., Gu, Z., Liao, Q. and Du, Z. (2018). Heterologous replicase from Cucumoviruses can replicate viral RNAs, but is defective in transcribing subgenomic RNA4A or facilitating viral movement. *Viruses*, 10, 590.

Jacquemond, M. (2012): *Cucumber mosaic virus*. *Advances in Virus Research*, 84: 439-504.

Kim, M.-K., Seo, J.-K., Kwak, H.-R., Kim, J.-S., Kim, K.-H., Cha, B.-J. and Choi, H.-S. (2014). Molecular genetic analysis of *Cucumber mosaic virus* populations infecting pepper suggests unique patterns of evolution in Korea. *Virology*, 104(9): 993-1000.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35(6): 1547-1549.

Lin, H.X., Rubio, L., Smythe, A.B. and Falk, B.W. (2004). Molecular population genetics of *Cucumber mosaic virus* in California: Evidence for founder effects and reassortment. *Journal of Virology*, 78(12): 6666-6675.

Moyle, R., Pretorius, L.S., Shuey, L.S., Nowak, E. and Schenk, P.M. (2018). Analysis of the complete genome sequence of *Cucumber mosaic virus* Strain K. *Genome Announcement*, 6(7) e00053-18.

Nouri, S., Arevalo, R., Falk, B.W. and Groves, R.L. (2014). Genetic structure and molecular variability of *Cucumber mosaic virus* isolates in the United States. *PLoS ONE*, 9(5): e96582.

Osman, M., El-Dougdoug, K.A., Abd El-Salam, E.T., Taha, R.M. and El-Hamid, Reham M. (2004). Histocytopathological effect of *Cucumber mosaic virus* on squash leaves. *Egyptian Journal of Virology*, 1: 343-353.

Ouedraogo, R.S., Pita, J.S., Somda, I.P., Traore, O. and Roossinck, M.J. (2019). Impact of cultivated hosts on the recombination of *Cucumber mosaic virus*. *Journal of Virology*, doi: 10.1128/JVI.01770-18. [Epub ahead of print].

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G. and Francki, R.I.B. (1992). *Cucumber mosaic virus*. *Advances in Virus Research*, 41: 281-348.

Palukaitis, P. and Garcia-Arenal, F. (2003). Cucumoviruses. *Advances in Virus Research*, 62: 241-323.

Revathy, K.A. and Bhat, A.I. (2017). Complete genome sequencing of *Cucumber mosaic virus* from black pepper revealed rare deletion in the methyltransferase domain of 1a gene. *Virus Disease*, 28(3): 309-314.

Salama, E.A., Abdulsalam, K.S. and Khan, M. (1987). Occurrence of cucurbit viruses in the Eastern Province of Saudi Arabia. *Proceedings of Saudi Biological Society*, 10: 257-271.

Salama, E.A., AL-Beshr, A.A. and Abou-Ghadir, M.F. (1994). Identification and characterization of tomato viruses in the Eastern Province of Saudi Arabia. *Egyptian Journal Phytopathology*, 22: 125-141.

Scholthof, K-B.G., Adkins, S.; Czosnek, H., Palukaitis, P., Jackquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P. Hemenway, C. and Foster, G.D. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12(9): 938-954.

Sinha, S. and Samad, A. (2019). First report of *Cucumber mosaic virus* associated with yellowing mosaic disease of African basil (*Ocimum gratissimum*) in India. *Plant Disease*, 103(1): 167.

Cite this article as:
Ahmed M. Soliman (2019): Identification of *Cucumber Mosaic Virus* Infecting Some Vegetable Crops in Saudi Arabia. *Journal of Virological Sciences*, Vol. 6: 46- 54.