Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

MOLECULAR CHARACTERIZATION OF CUCUMBER MOSAIC VIRUS SUBGROUP IB IN IRAQ

[a]Ahmed K. Ali, [b]Ibrahim A. Ahmed, [c]Nawres A. Al-Kuwaiti
[a] *Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq.*
[b] *College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.*
[c] *Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.*

ARTICLE INFO

Article history
Received: 11th July, 2023
Revised: 13th August, 2023
Accepted: 16th August, 2023

Keywords
Subgroup IB
Phylogenetic analysis
Cucumber mosaic virus

ABSTRACT

Cucumber mosaic virus (CMV) isolate was identified on cucumber and cowpea plants exhibiting mosaic, mottling and leaf distortion in local fields in the province of Baghdad. The virus was characterized by reverse transcriptase polymerase chain reaction (RT-PCR) using coat protein (CP) gene specific primers. Comparison of the coat protein sequence revealed up to 98-99% nucleotide identity with known CMV isolates. Phylogenetic analysis based on coat protein gene sequence clustered the Iraqi CMV isolate with members of subgroup IB. To the best of our knowledge, this is the first report of CMV subgroup IB in Iraq.

Corresponding Author: Ahmed K. Ali
Email: ahmed.ali83@uomustansiriyah.edu.iq

INTRODUCTION

Cucumber mosaic virus (CMV, family *Bromoviridae*) has broad host range, causing serious worldwide losses in economically important plant species (Palukaitis and García-Arenal, 2003; Scholthof et al., 2011). It has three positive-sense (+) RNA molecules, namely RNA1, RNA2, and RNA3 encapsidated in separate isometric particles (Palukaitis et al., 1992). Proteins encoded by RNA1 and RNA2 are mostly required for the replication of the viral genome, symptoms development, and gene silencing suppression (Palukaitis et al., 1992; Ding et al., 1994). RNA3-encoded proteins are essential for viral intercellular movement and particle assembly (Su et al., 2010). Based on sequence homology, geographical distributions, and serological characteristics, CMV isolates are classified into two subgroups, I and II (Verma et al., 2006; Tepfer et al., 2016). Isolates of subgroup I are further divided into two clusters, IA and IB. In Iraq, the occurrence of CMV has been reported by serological assays and indicator plants (Adhab and Al-Ani, 2013; Alfadhal and Zagier, 2017). Nevertheless, the subgroup affiliation and molecular characterization for any CMV isolate from Iraq have yet to be investigated. The current study analyzes partial nucleotide sequences of an Iraqi CMV isolate, and reveals its subgroup identity.

MATERIALS AND METHODS

Sample collection and identification of the pathogen

Symptomatic leaves of cucumber and cowpea were collected from some local fields in the province of Baghdad. RT-PCR was performed with RNA extracted from leaves using RNeasy Plant Mini Kit (Qiagen, Germany). Complementary DNA (cDNA) was synthesized in 20 µl reaction mixture at 50°C for 30 min with 1.0 µg RNA template, 1 X SSIV buffer, 0.5 mM dNTPs

each, 0.5 pmol reverse primer, 5 mM dithiothreitol (DTT), 1 µl Ribonuclease inhibitor, and 200 U of SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific, Lithuania). PCR amplification was carried out in 25 µl reaction mixture containing 2 µl cDNA template, 12.5 µl GoTaq G2 Green Master Mix (Promega, Madison, WI, USA), 9.5 µl nuclease-free H_2O, and 1 µl of 10 pmol of primer pairs CPTALLF (5'-YASYTTTDRGGTTCAATTCC-3') and CPTALLR (5'-GACTGACCATTTTAGCCG-3') specific to cucumovirus capsid protein (CP) encoding genes (Choi et al., 1999). The cycling conditions were 95°C for 2 min followed by 35 cycles of 30 s at 95°C, 30 s at 50°C, 1 min at 72°C and a final extension at 72°C for 10 min. PCR amplicons were visualized by 1.2% agarose stained with ethidium bromide using UV transilluminator (BioRad, USA). PCR products were Sanger sequenced (Macrogen, South Korea) in both directions using the same RCR primers used in the initial amplification. Sequences were edited, translated into putative amino acids using ClustalW algorithms (version 2.1) available at (http://www.ebi.ac.uk/). The phylogenetic analysis was carried out by comparing CP gene sequences of 21 CMV isolates belonging to subgroups (IA, IB and II) from different countries available in the GenBank using MEGA X software and the maximum likelihood (Kumar et al., 2018). The CMV sequences used for the phylogenetic analysis are described in Table 1.

Table 1: Cucumber mosaic virus isolates retrieved from the GenBank used for phylogenetic analysis.

CMV isolate	Accession No.	Subgroup	Origin
LOR G	KT279571	IB	Iran
Adiyaman 73	MK704429	IB	Turkey
CMV-Rs	KP965807	IB	Russia
CMV-Banana	AY125575	IB	India
CMV-G10	AY541691	IB	Greece
TN TNAU SG1	KF891359	IB	India
Ixora	U20219	IB	Philippines
CN03	AJ810261	IB	China
BQ6	KF268463	IB	China
Fny	D10538	IA	USA
DI1	DQ002876	IA	Iran
LOR A	KT279565	IA	Iran
Lucknow	DQ295914	IA	India
D8	AB004781	IA	Japan
CMV-Ch	KP965806	IA	Iran
RT52	AJ810258	IA	USA
Sn	U22822	II	USA
Mb	GU002300	II	China
Trk7	L15336	II	Hungary
DKRD	U10922	II	-
RT67	AJ810253	II	Netherlands
TAV (outgroup)	AJ550020		India

RESULTS

Detection of CMV in cucumber and cowpea

Leaves of cucumber and cowpea showing virus-like symptoms such as mosaic, mottling and leaf distortion were collected from several fields in Baghdad governorate and screened for the presence of Cucumber mosaic virus (CMV) infection by RT-PCR (Figure 1 a, b).

Identification of CMV

Symptomatic leaf samples produced 930 bp PCR fragments corresponding to the coat protein (CP) region of CMV genome by using primers specific to cucumoviruses (Figure 2). Sequence comparison by BLASTn revealed that the obtained sequences were found to have the highest nucleotide (nt) identity with the CMV isolates in the GenBank. The isolates from

cucumber and cowpea were identical to each other at nucleotide and amino acid levels. Since no CMV sequence from Iraq has been reported to date, two isolates from different hosts (IRQ831 from cucumber and IRQ832 from cowpea) were selected and deposited in the GenBank (Accession numbers MW477481 and MZ267789, respectively), despite having the same nt identity. Nevertheless, we have selected IRQ831 isolate for analysis. To test the infectivity of both isolates, cucumber and cowpea test plants were mechanically inoculated by sap obtained from the naturally infected plants. CMV infections were detected in the inoculated leaves by symptoms development and RT-PCR (Figure 1 c, d; and Figure 2 lanes 3 and 4).

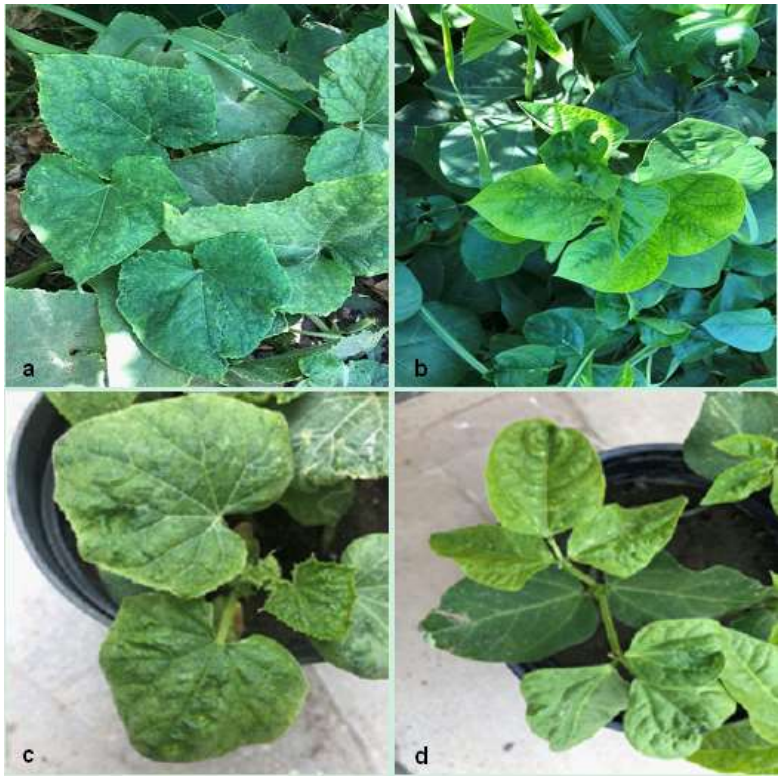

Figure 1: (A-D) Symptoms of CMV infected cucumber and cowpea plants. Naturally infected (A-B), mechanically inoculated by sap (C-D)

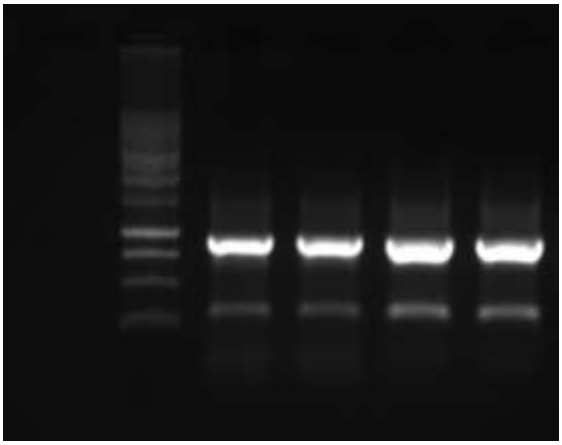

Figure 2: RT-PCR detection of cucumber mosaic virus in cucumber and cowpea. The expected size of DNA bands was (~ 930 bp). –ve, water control; M, 1 Kb DNA marker; lanes 1-2 (naturally infected cucumber and cowpea, respectively); lanes 3-4 (mechanically inoculated cucumber and cowpea, respectively).

Phylogenetic analysis

Maximum likelihood (ML) tree constructed by aligning the CP gene sequence of IRQ831 with 21 CMV isolates clustered the Iraqi isolate (IRQ831) in subgroup IB (Figure 3). IRQ831 showed high nt (98.5-99.1%) and aa (99-100%) identities with isolates LORG (KT279571) and Adiyaman 73 (MK704429) from Iran and Turkey, respectively. Within the same subgroup, the Iraqi isolate showed nt identity (93-96%) with some representative isolates such as BQ6, Ixora, TN TNAU SG1 and CMV-Rs obtained from China, Philippines, India, and Russia, respectively. With the isolates of subgroup II, the isolate IRQ831 showed great divergence which is represented by low identity (77-81%).

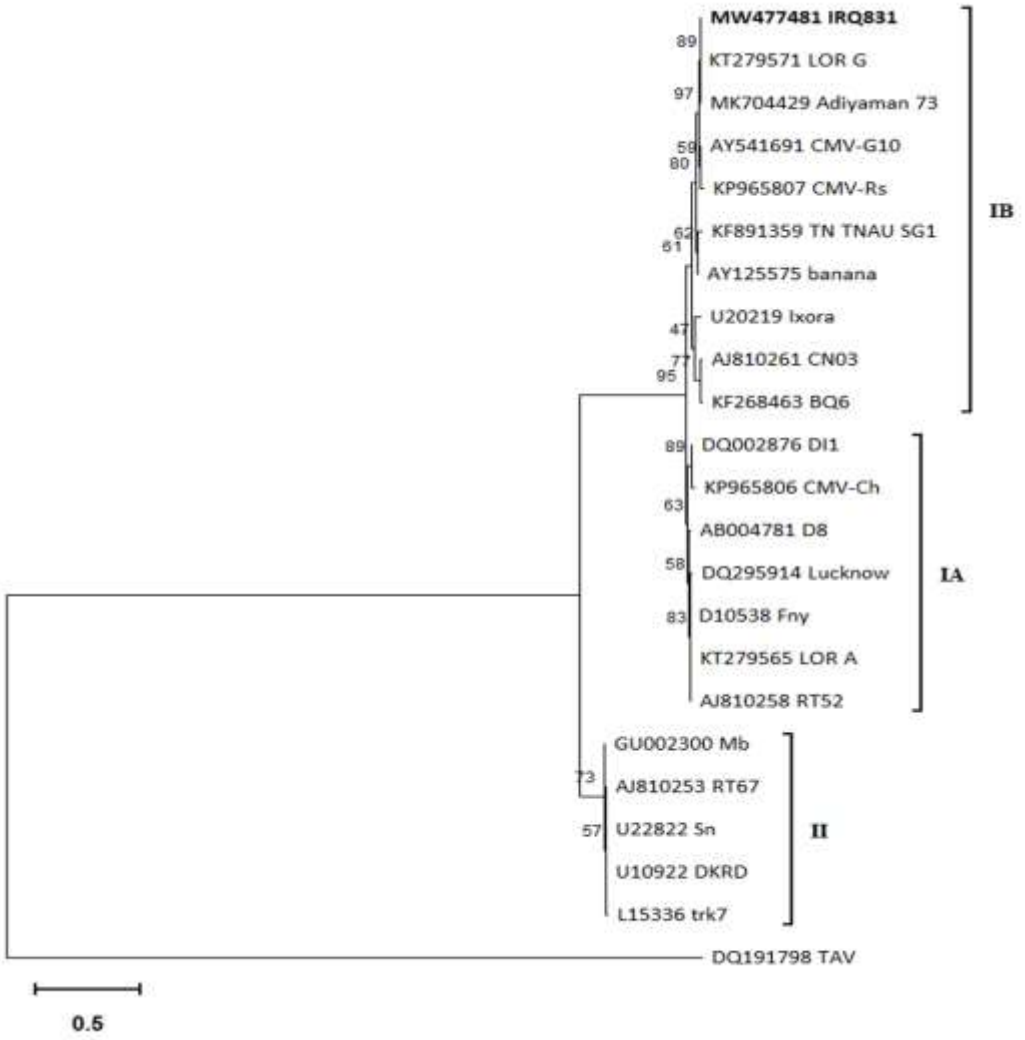

Figure 3: Phylogenetic analysis of CMV coat protein (CP) gene sequences. Maximum likelihood (ML) phylogenetic tree was calculated using MEGA X software (Kumar et al., 2018). The names and accession numbers of the isolates of subgroups (IA, IB, and II) were indicated at the branches. The Iraqi CMV isolates is highlighted. Values at each node indicate percentage of 1000 bootstrap replicates. Tomato aspermy virus (TAV) was used as outgroup.

DISCUSSION

Sequence availability and subgroup affiliation for Iraqi CMV isolates were lacking. Earlier reports that detected CMV in Iraq were based on serological assays, indicator plants (Adhab and Al-Ani, 2013), and RT-PCR (Alfadhal and Zagier, 2017). To date, no CMV sequence from Iraq, other than that of isolates described here, has been available in the Genbank.

The discrimination among CMV isolates mostly depends on sequence variability, hence dividing them into three

subgroups (IA, IB, and II) (Roossinck, 2002). The phylogenetic tree generated by aligning the CP gene sequence clustered the Iraqi isolate IRQ831 with representative isolates of subgroup IB. In addition, sharing maximum nucleotide identity (98-99%) with isolates from Iran and Turkey suggests that IRQ831 CMV isolate could have been introduced to Iraq by imported agricultural products. The occurrence of this virus in numerous geographic areas including Iraq indicates the wide distribution of CMV isolates belonging to subgroup IB (Roossinck, 2002; Koundal et al., 2011; Rabie et al., 2017).

Accurate phylogenetic affiliation greatly contributes in exploring the geographic distribution and biodiversity of viral populations. Rigorous testing of imported plant products can minimize the occurrence of viral infections and prevent possible disease outbreaks.

CONCLUSION

In this work, CMV was detected on cucumber and cowpea plants in Iraq and characterized by RT-PCR. The phylogenetic position based on CP gene sequence placed the Iraqi CMV isolate with members of subgroup IB, particularly, with isolates reported from bordering countries, Iran and Turkey. This suggests that this virus migrated between counties through the exchange of plant materials. The information provided by this report will be fundamental in surveillance efforts and tracking the spread of the virus. Rigorous testing of imported plant products can minimize the occurrence of viral infections and prevent possible disease outbreaks. In addition, identification of new isolates may shed more light on the genetic diversity and the evolution of CMV in Iraq.

AUTHORS' CONTRIBUTIONS

AKA conceived the study, carried out the research and wrote the manuscript; IAA and NAA assisted in the research work, data analysis and proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adhab, M.A., Al-Ani, R.A., 2013. Characterization of an isolate of *Cucumber mosaic cucumovirus* from Radish (*Raphanus sativus*) in Iraq. The Plant Pathology Journal 12(2), 115-119.

Alfadhal, F., Zagier, S.S., 2017. Identification of Cucumber Mosaic Virus (CMV) on eggplant by using some of indicator plants, Immunostrip assay and reverse transcriptase - polymerase chain reaction (RT-PCR). Kufa Journal for Agricultural Sciences 9(4), 92-107.

Choi, S.K., Choi, J.K., Park, W.M., Ryu, K.H., 1999. RT-PCR detection and identification of three species of cucumoviruses with a genus-specific single pair of primers. Journal of Virological Methods 83, 67-73.

Ding, S.W., Anderson, B.J., Haase, H.R., Symons, R.H., 1994. New overlapping gene encoded by the cucumber mosaic virus genome. Virology 198, 593-601.

Koundal, V., Haq, Q., Praveen, S., 2011. Characterization, genetic diversity and evolutionary link of Cucumber mosaic virus strain New Delhi from India. Biochemical Genetics 49, 25-38.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology Evolution 35, 1547-1549.

Palukaitis, P., García-Arenal, F. 2003. Cucumoviruses. Advances in Virus Research. 62, 241-323.

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

Rabie, M., Ratti, C., Calassanzio, M., Abdel Aleem, E., Fattouh, F.A., 2017. Phylogeny of Egyptian isolates of *Cucumber mosaic virus* (CMV) and *Tomato mosaic virus* (ToMV) infecting *Solanum lycopersicum*. European Journal of Plant Pathology 149, 219-225.

Roossinck, M.J., 2002. Evolutionary history of *Cucumber mosaic virus* deduced by phylogenetic analysis. Journal of Virology 76, 3382-3387.

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

Su, S., Liu, Z., Chen, C., Zhang, Y., Wang, X., Zhu, L., Miao, L., Wang, X.C., Yuan, M., 2010. Cucumber mosaic virus movement protein severs actin filaments to increase the plasmodesmal size exclusion limit in tobacco. Plant Cell 22, 1373-1387.

Tepfer, M., Girardot, G., Fénéant, L., Tamarzizt, H.B., Verdin, E., Moury, B., Jacquemond, M., 2016. A genetically novel, narrow-host-range isolate of cucumber mosaic virus (CMV) from rosemary. Archives of Virology 161(7), 2013-2017.

Verma, N., Mahinghara, V.K., Ram, R., Zaidi, A.A., 2006. Coat protein sequence shows that *Cucumber mosaic virus* isolate from geranium (*Pelargonium* spp.) belongs to subgroup II. Journal of Biosciences 31, 47-54.