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Short Communication

Watermelon Chlorotic Stunt Virus is Associated with Cucumber Yellow Mosaic Symptoms in Oman

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Abstract

Cucumber (*Cucumis sativus*; family *Cucurbitaceae*) plants exhibiting begomovirus-like symptoms such as yellowing, mosaics and stunting were studied using cloning, sequencing, Species Demarcation Tool followed by phylogenetic clustering. The complete genome of DNA-A showed maximum sequence identity of 98.7% with the corresponding DNA-A of an isolate from "Iran" strain of *Watermelon chlorotic stunt virus* (WmCSV). The DNA-B displayed 97.5% nt identity with the component of DNA-B of WmCSV from Iran, too. Our results confirmed that yellowing and mosaic symptoms of cucumber are associated with a bipartite begomovirus (WmCSV). This study is the first characterization of WmCSV in association with described symptoms in cucumber from Oman.

Keywords: *Geminiviridae*; *Begomovirus*; *Cucumis sativus*; Watermelon chlorotic stunt virus

Introduction

Begomoviruses (family *Geminiviridae*) are circular single-stranded nucleic acids (ssDNA) viruses that are transmitted exclusively by whiteflies and cause severe viral diseases in several economic crops worldwide (Fauquet *et al*. 2008). In recent classification ssDNA viruses are divided in nine genera (*Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus, Turncurtovirus, Capulavirus, and Grablovirus*), depending on the genome structure, vectors, and susceptible host (Varsani *et al*. 2014). Begomoviruses are economically the most damaging among *Geminiviradae* family and are spread by a complex of whiteflies (*Bemisia tabaci* Genn.) (Navas-Castillo *et al*. 2011). Moreover, begomoviruses are distributed into two subgroups; monopartite begomoviruses (comprise of DNA-A) or bipartite begomoviruses comprises of two DNA components (DNA-A and DNA-B) about equal size (~ 2,800 bp). Both components share approximately 200 nucleotide (nt) DNA sequence with each other which share 80–100% identity known as the common region. The DNA-A encodes proteins essential for virus replication, influence of gene function and vector spreading, whereas the DNA-B molecule is responsible to provide genes which encodes proteins for virus movement and appearance of symptoms in plants (Rojas *et al*. 2005).

Since first detection and characterization of WmCSV in Iran and Sudan (Kheyr-Pour *et al*. 2000), it has been spreading into different plant species and transmitting to into diverse geographical areas (Domínguez-Durán *et al*. 2018). This study indicates the first confirmation of yellow mosaic symptoms of cucumber is associated with a bipartite begomovirus WmCSV in Oman.

Materials and Methods

Survey and sample collection

Survey was conducted (December 2016 and 2018) in cucumber fields in the Al-Batinah North (coordinates 23° 41' 26.69" N 57° 53' 30.78" E) region of Oman. Approximately 30–40% of cucumber plants exhibited symptoms like begomovirus infection such as yellowing, mosaics and stunting (Fig. 1). Cucumber plants were infested by medium to high (~5–10/leaf) whitefly (*Bemisia tabaci*; Middle East-Asia Minor I, MEAMI) populations. Sixteen different leaf samples were collected (ten symptomatic and six asymptomatic) and were tested for begomovirus infection.

Genomic DNA extraction and initial virus detection

Plant genomic DNA was extracted from all leaf samples (n=16) followed by CTAB-protocols (Porebski *et al*. 1997), and the resulting DNA was used in a PCR assay targeting the conserved region (~550 nt of the core coat protein gene) using universal detection primes AC1048/AV494 (Brown *et al*. 2001).

Rolling circle amplification and cloning

To further characterize the virus from cucumber samples,

four random selected PCR positive samples were used in Rolling Circle Amplification (RCA) as described earlier (Shahid *et al*. 2017). The concatamers of the RCA products were used in endonuclease reaction and monomer begomovirus fragments (~2.7 kb) were produced with *Hind*III and *Pst*I restriction enzymes (Shahid *et al*. 2019b). The linear DNA fragments were excised from 1% gel electrophoresis, purified and ligated into pUC19 vector at the compatible restriction enzyme sites.

Sequencing and sequence analysis

The putative complete components were confirmed and sequenced entirely by Macrogen, Korea. These full-length sequences were compared with related begomovirus sequences using BLASTn analysis at (https://www.ncbi.nlm.nih.gov/). Open Reading Frames (ORFs) in all genomes were discovered using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). Nucleotide sequence identity was determined by Species Demarcation Tool (SDT) with MUSCLE algorithm and phylogenetic analysis and tree construction was done in MEGA7 (Kumar *et al*. 2016). Recombination Detection Program (RDP 4.1) was run as described earlier (Martin *et al.* 2015).

Results

Detection of begomovirus

PCR results confirmed the amplification of amplicon with an expected size (~550 nt) of the conserved coat protein (CP) gene, from all symptomatic cucumber leaf samples, whereas all asymptomatic leaf samples were uniformly negative. The initial sequence analysis of the PCR product confirmed WmCSV (a bipartite begomovirus) is associated with yellowing and mosaic symptoms in cucumber plants. None of the samples were positive for alphasatellite, betasatellite or other begomovirus using universal amplification primers (Bull *et al*. 2003; Shahid *et al.* 2019a) either in PCR or by RCA.

Characterization of DNA-A of bipartite begomoviruses

Full-length monomer molecules of DNA-A were produced with *Hind*III restriction of RCA product. After sequencing all the sequences contigs were assembled and full-length genome sequences were produced. The resultant full-length assembled sequences shared 99.1–100% nucleotide (nt) identity with each other and out of those two complete nt sequences were deposited to GenBank (acc no. MK649818 and MK649819). Each sequence was 2,752 nt long and displayed the composition of genes attributes typical to the DNA-A component of Old World (OW) of bipartite begomoviruses. Consisting of four ORFs [Replication associated protein (Rep), Trans-replication associated protein (TrAp), Replication enhancer protein (REn), and

Fig. 1: *Cucumis sativus* plants showing yellow mosaic symptoms associated with WmCSV (**X**) in comparison with healthy *C. sativus* plant (**Y**)

AC4 protein] in the complementary-sense strand. Whereas two ORFs were found [Coat protein (AV1) and AV2 protein] in the virion-sense strand (Table 1). Sequence Demarcation Tool (SDT) using pairwise sequence alignments analysis (Muhire *et al*. 2014) revealed highest nt identity (98.3%) with the sequences of DNA-A of an isolate of the "Iran" strain of WmCSV (AJ245652) earlier identified in Iran (Kheyr-Pour *et al*. 2000). A phylogenetic tree further confirms that the WmCSV isolates cluster with WmCSV isolates reported from Iran and Saudi Arabia (Fig. 2A). No evidence of recombination events among bipartite begomovirus complex was found using different algorithms in RDP 4.1 program (Martin *et al*. 2015).

Characterization of DNA-B of bipartite begomoviruses

Four full-length cognate sequences of DNA-B components were obtained with *Pst*I restriction of RCA product which shared 99–100% nt identity with each other and two representative sequences were submitted to GenBank (accession numbers MK649820 and MK649821). Both the DNA-B components displayed genome organizations typical to the DNA-B of earlier discovered bipartite begomovirus, containing the two ORFs; MP: movement protein and NSP: Nuclear shuttle protein in the complementary and virion-sense strand respectively (Table 1). Each component was 2,728 nt in length and exhibited 97.3% nt identity with the DNA-B segment of WmCSV (AJ245653) from Iran (Kheyr-Pour *et al*. 2000). In phylogenetic analysis the DNA-B of WmCSV isolates cluster with the cognate DNA-B of WmCSV isolates (Fig. 2B).

Discussion

Mainly bipartite begomoviruses are originated from the New World (central and South America) with the exception of merely a slight number that occurs in the OW (Australia, Japan, China, Indian subcontinent, Africa, Mediterranean and European region) (Zerbini *et al*. 2017). After the first detection of WmCSV in Iran (Middle East) and Sudan

Table 1: Features of WmCSV isolated from natural infection of cucumber plants in Oman

Isolate	Watermelon chlorotic stunt virus (DNA-A)								DNA-B				
	Accession#	Size (nt)	Position of genes (coordinates)/no. of amino acids [predicted coding capacity in kDa]						Clone	Accession#	Size (nt)	Position of BV1 gene(coordinates)/ no. of amino acids [predicted coding capacity in kDa]	Position of BC1 gene(coordinates)/ no. of amino acids [predicted coding capacity in kDa]
			CP	V2	Rep	TrAP	REn	C4					
18P	MK649818	2,752	315- 1091 257 (28.7)	155-514 119 (13.9)	1540 – 2625 361 (41.30)	1233 – 1640 135 (15.7)	1088 – 1492 134 (15.7)	2328 – 2471 47 (5.6)	P2	MK649820	2,728	500-1255 251 (27.9)	1295-2215 306 (34.04)
20P	MK649819	2,752	315- 1091 257 (28.7)	155-505 116 (13.1)	1540 – 2625 361 (41.30)	1233 – 1640 135 (15.7)	1088 – 1492 134 (15.7)	2328 – 2471 47 (5.6)	P7	MK649821	2,728	500-1255 251 (27.9)	1295-2215 306 (34.04)

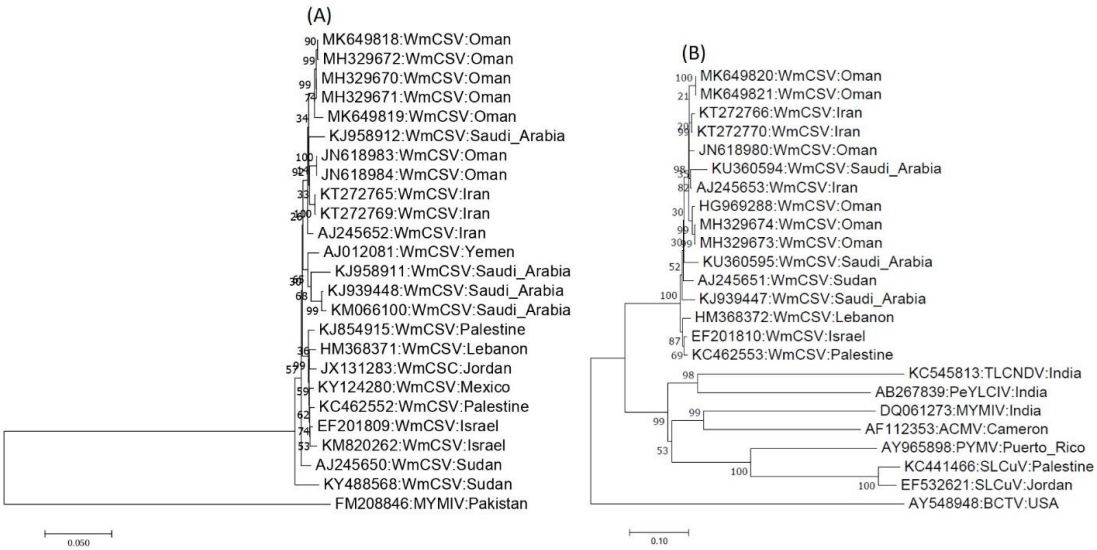

Fig. 2: Phylogenetic dendrograms was constructed in MEGA7 using selected sequences of DNA-A (**A**) and DNA-B (**B**) of WmCSV components (Kumar *et al*., 2016) with 1000 bootstrap values at the nodes, where vertical and horizontal branches are arbitrary and proportional respectively (Felsenstein 1985). The trees were rooted on the sequence of MYMIV-FM208846 and BCTV-AY548948, respectively. The GenBank acc. no and country of origins for each DNA are indicated in each case

(North Africa) (Kheyr-Pour *et al*. 2000), it has been increasing its host range and transmitting to diverse hosts and geographical areas. For instance, in the past WmCSV has been reported to infect watermelon, cucurbits, squash, pumpkin in Jordan, Lebanon and Oman respectively (Al-Musa *et al*. 2011; Khan *et al*. 2012; Samsatly *et al*. 2012; Shafiq *et al*. 2020). More recently, WmCSV has been reported in South America *i.e*., Mexico (Domínguez-Durán *et al*. 2018). Cucumber is an extensively grown as a greenhouse vegetable crop in Oman as well as in other countries. However, recently it has been reported as the host for different viruses like squash leaf curl virus (SLCV), mungbean yellow mosaic virus (MYMIV) in Palestinian and Oman (Ali-Shtayeh *et al*. 2010; Shahid *et al*. 2018). Al-Batinah region is the main agriculture-cropping area where several diverse crops are grown in the winter season in Oman. Thus, there is possibility that whitefly vector; containing the WmCSV particles can transmit them to other crops. Further genetic diversity including different plant species needs to be investigated to determine geographical distribution of this bipartite begomovirus in the country. Different options are available to control these begomoviruses, for instance by targeting different coding genes (Replication protein and/or Coat protein *etc*.) and intergenic regions of cotton leaf curl virus (CLCuV) and tomato yellow leaf curl virus (TYLCV), respectively (Ji *et al*. 2015; Khan *et al*. 2019). However, CRISPR-Cas9 proved to be more efficient tool in *Nicotiana benthamiana* genome editing (Mubarik *et al*. 2016, 2019), which can be used to develop resistance in different plant species in near future.

Conclusion

This is the first evidence of WmCSV infecting cucumber in Oman. The findings of another host infected with WmCSV demonstrate increasing host range of this virus in Arabian Peninsula.

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Author Contributions

MSS design, perform experiment and wrote the manuscript. MS prepared of samples for sequencing and AMA edited the manuscript.

Conflict of Interest

The authors of this article have no conflict of interest

Data Availability Declaration

The authors declare that data reported in this article are available with the corresponding author and will be provided on reasonable demand

Ethics Approval

Not applicable

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