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Begomovirus infection on Cucumber in Saudi Arabia

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Abstract

Cucurbits are an important vegetable crops and among them Cucumber (*Cucumis sativus*) used mainly as vegetables and salad. The cucumber crop was found to exhibit yellow mosaic symptoms grown in the natural field in Saudi Arabia. We collected naturally infected samples and detected the begomovirus infection by polymerase chain reaction and full length as well as betasatellites viral genome was cloned and sequenced. The sequences of the full length viral genome had 2784 and betasatellites had 1377 nucleotides respectively. In a multiple sequences analysis, highest homology was observed with Tomato yellow leaf curl virus previously reported from Jizan and Al-Qasim, Saudi Arabia. The betasatellites sequences were also analyzed but, interestingly the highest homology was observed with Tomato yellow leaf curl betasatellites reported from Jeddah and Oman. In a phylogenetic tree analysis, the closest cluster was formed with begomovirus isolates identified earlier from Jizan and Al-Qasim. Based on the results obtained in this study, it is concluded that a variant of Tomato yellow leaf curl virus associated with yellow mosaic disease of cucumber in Saudi Arabia. These findings provide valuable information about the natural infection and disease spread caused by begomovirus in new geographic regions on new host.

Keywords: Begomovirus, betasatellites, Cucumber, Genetic diversity, Phylogenetic relationships Tomato yellow leaf curl virus.
Abbreviations: TYLCV_Tomato Yellow leaf Curl Virus.

Introduction

Cucumber (*Cucumis sativus)* belongs to the same botanical family as melons and squashes. Commercial production of cucumbers is usually divided into two types. "Slicing cucumbers" are produced for fresh consumption and widely cultivated crop around the world. Yellow mosaic, and leaf curl disease significantly limits the production of cucumber. Yellow mosaic disease of cucumber is caused by Tomato yellow leaf curl virus (TYLCV) belonging to the genus begomovirus of the family *Geminiviridae*. Begomovirus have circular single-stranded DNA (ssDNA) with either a mono- or bipartite genome. Bipartite begomovirus genome have two circular ssDNA molecules (~2.7 kb), well known as DNA-A and DNA-B while mono-partite begomovirus has only DNA-A mostly associated with satellite molecule known as betasatellites (Lazarowitz 1992; Briddon et al., 2003). The family *Geminiviridae* has now been reported to have seven genera known as *Mastrevirus, Curtovirus, Begomovirus, Topocuvirus, Eragrovirus, Turncurtovirus, Becurtovirus* (Muhire et al., 2014; Brown et al., 2012; Brown et al., 2015; Varsani et al., 2014). Members of the genus begomovirus have circular single-stranded DNA (ssDNA) with either a mono- or bipartite genome. The bipartite begomovirus genome has two ssDNA molecules (~2.7 kb), known as DNA-A and DNA-B while mono-partite begomovirus has only DNA-A with satellite molecule known as betasatellites (Briddon et al., 2004; Briddon et al., 2008). Betasatellites are small (~1.4kb), highly diverse with circular ssDNA. The betasatellites replication, movement and transmission between plants are mediated by their helper virus. Betasatellites had an open reading frame; beta C1, an adenine-rich region, and a satellite conserved region with 45-93% sequence identity (Sivalingam et al., 2010). Alphasatellites are capable of autonomous replication in host plant cells mediated by a nanovirus-like replication-associated protein (Saunders and Stanley, 1999; Cui et al., 2004; Saunders et al., 2008). Whitefly vector (*Bemisia tabaci)* has emerged as serious pathogens for most of the dicotyledonous crops across tropical, sub-tropical regions across the world. The emergence and spread of TYLCV from the Middle East to world and it is suspected that Iran could be the center for TYLCV diversity (Duffy and Holmes 2008; Lefeuvre et al., 2010; Kenyon et al., 2014).

In this study, we report the natural occurrence of begomovirus, begomovirus detection, viral genome cloning and sequencing, sequence identity matrix, genetic diversity and phylogenetic relationship of TYLCV associated with yellow mosaic disease of cucumber crops. During the field survey, it was observed that the naturally infected cucumber plants exhibit yellow mosaic symptoms in Jeddah, Saudi Arabia. The association of begomovirus and causes significant loss to various crops like tomato, tobacco, okra and chili crops in the Nile Basin, arid and semi-arid southern part of the Arabian Peninsula, Oman and Yemen have been published in many reports and identified as *Tomato leaf curl Sudan virus* (ToLCSDV),*Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl Al-Batinah virus* (ToLCABV),*Tomato leaf curl Oman virus* (ToLCOMV), *Chili leaf curl virus* (ChiLCV), *Okra leaf curl Oman virus* (OLCOMV), *Tomato leaf curl Sudan virus-Oman isolate* (ToLCSDV-Om) (Idris and Brown 2005; Ajlan et al., 2007; Khan et al., 2008; Fauquet et al., 2008; Idris et al., 2011; Idris et al., 2012; Khan et al., 2013a,b; Khan et al., 2014; Akhtar

et al., 2014; Al-Saleh et al., 2014; Idris et al., 2014). The present study was undertaken to identify and characterize the begomovirus causing yellow mosaic disease of cucumber in Jeddah, Saudi Arabia.

Results

Detection of Begomovirus and transmission by whiteflies on cucumber seedlings

During the field survey, total 7 samples were randomly collected from the naturally infected cucumber Plants. Begomovirus infection was confirmed by using specific PCR primers and ~856bp amplicon was visualized on 1% Agarose gel from infected cucumber leaf samples (Fig 2). The causative agent could be efficiently transmitted to young cucumber seedlings and the inoculated plants developed characteristic yellow mosaic symptoms in 70% plants by 6-14 days after inoculation and the disease symptoms produced in the experimental plants were approximately related to those observed in the field.

Cloning of viral genome, sequencing and Phylogenetic analysis with begomoviruses

The rolling circle amplification products obtained by using total DNA extracted from naturally infected cucumber plants were restricted with *Eco*RI and *Hin*d III restriction enzyme and only one clone was selected and obtained from an *Eco*RI restricted sample and one clone was experimentally confirmed and sequenced. The complete viral genome had 2784 nucleotides (nt) while betasatellites had 1377 nucleotides (nt). The full genome and betasatellites sequences have been submitted to NCBI-GenBank with accession numbers, KT033713-full length and KT180307-betasatellites and tentatively designated as TYLCV-Cucumber-Jeddah isolate.

The sequence analysis based on full revealed that the TYLCV-cucumber-Jeddah isolate shared greater identity ranged from 99.6% with TYLCV-Tomato-Jeddah isolate followed by 99.4-93.0% identity with TYLCV-Jizan 103 and Al-Qasim isolates and the lowest similarity (71.6%) were observed with TYLCV-Egypt isolate (EF107520) and TYLCV isolates from Oman and Iran showed 79-82% identity. The highest amino acid sequence identity was identified with TYLCV-Tomato-Jeddah isolates (KT033715) in all the 6 proteins (V2-98.9%, V1-98.3%, C3-98.1%, C2-98.6%, C1-98.2%, and C4-98.3%) respectively, with respective sequences of selected begomovirus isolate (Table 1). The sequence analysis based on betasatellites using Tomato yellow leaf curl betasatellites-Cucumber-Jeddah KT180307 isolate with selected begomovirus from various locations showed the highest identity (99.1-99.0 %) with Tomato yellow leaf curl betasatellites-Jeddah and Hadasham isolates (KT355021& KT728732) while isolates from Oman ranged from (88.2-97.8% identity) and Yemen isolates ranged from 45.2-45.9% identity and the lowest (44.3%) identity was found with Okra Leaf Curl virus-Jordan isolate (KJ396939) (Table 2). Based on the complete genome sequence and the phylogenetic analysis with selected begomoviruses, TYLCV-Cucumber-Jeddah isolate formed a closed cluster with TYLCV isolates from Jizan and AL-Qasim and this result indicates that identified TYLCV-Cucumber-Jeddah isolate is a variant of either Jizan and AL-Qasim (Fig 3). Interestingly, many isolates from Oman grouped together and formed a closed cluster and only one TYLCV-Oman isolate (FJ956706) clustered with Iran isolate (GU076448) while isolates from Egypt, Jordan and Iraq formed separate clusters. The other remaining isolates grouped on the basis of their geographical origin and formed separate clusters. The phylogenetic analysis result based on betasatellites sequences with selected begomovirus isolates formed a closed cluster with Tomato yellow leaf curl betasatellites from Jeddah, Hadasham, Tabuk and Hail. While isolates from Oman and Yemen formed separate clusters and isolates from Thailand, Vietnam, Jordan, Java and Mali formed completely separate clusters (Fig 4).

Discussion

Cucurbits are important vegetable crops and among them cucumber crops are mostly used as salad purposes. The cultivation and yield of cucumber are seriously affected by many viral diseases and globally, a yellow mosaic disease caused by begomovirus is a major limiting factor for cultivation of cucumber. Cucumber is an important vegetable crop in the Kingdom of Saudi Arabia for local consumption. In this study, we described the identification of virus infection; viral genome (full genome and betasatellites) cloning, sequencing, genetic variation and phylogenetic relationship of associated begomovirus and tentatively designated as TYLCV-cucumber-Jeddah. During the field survey, naturally infected plants were observed to exhibit yellow mosaic symptoms in a cucumber field. The emergences of many isolates of begomovirus during the last 20-30 years have become the most important groups of plant viruses. The cultivation and production of vegetable crop has been severely affected due to begomovirus and natural increase of more whiteflies population in the tropics and sub-tropics. The information about begomovirus genetic variability, virus evolution and host plant interaction will help to design and develop an effective and durable disease management strategy against viruses (Garcia et al., 2007). There are many important factors are known which enables virus adaptations and emergence in changed environments and climatic conditions and these factors are like mutation in coding and non-coding regions, recombination, reassortment, selection, genetic drift, interaction of virus host and virus vectors, mixed infection, high rate of replication, acquisition of satellite DNA molecules and extended host range of the whiteflies vector are known for genetic variability and evolution among the virus population (Seal et al., 2006). Recent studies have shown that Geminiviruses genomes are prone to DNA methylation in infected host plants (Yang et al., 2011; Zhang et al., 2011).

The sub-continental Southeast Asia could be a major center of diversity for begomoviruses and associated betasatellite molecules (Ha et al., 2008). But many reports have been published about the diversity and the emergence of begomovirus infecting Solanaceous crops in the East and South-East Asia (Lefeuvre et al., 2010, Yang et al., 2011; Kenyon et al., 2014). Since two decades, the cucurbits production has been seriously affected by yellow mosaic disease caused by many begomoviruses like Tomato Leaf curl Sudan Virus (ToLCSDV), TYLCV and Tomato yellow leaf curl virus-Oman, Okra leaf curl Oman Virus in Arabian Peninsula and Nile Basin (Ajlan et al., 2007; Khan et al., 2008; Idris et al., 2011, Khan et al., 2013a,b; Al-Saleh et al., 2014; Akhtar et al., 2014; Idris et al., 2014). In this study, the greater value of genetic diversity was observed based on full genome and betasatellites and phylogenetic relationships also showed the genetic diversity among begomovirus. The identified begomovirus was designated as TYLCV-cucumber-Jeddah isolate which represents a variant of

Table 1. Sequence identity matrix of TYLCV- Cucumber isolate (KT033713) with selected Begomovirus.

Accession Nos.	Acronyms	Hosts	Locations	% Identity matrix						
				Full (nt)	V2 (aa)	V1 (aa)	C3 (aa)	C2 (aa)	C1 (aa)	C4 (aa)
KT033715	TYLCV	Tomato	Jeddah	99.6	98.9	98.3	98.1	98.6	98.2	98.3
KC845301	TYLCV	Tomato	Jizan	99.4	99.3	99.5	99.2	99.1	99.0	99.3
KF561125	TYLCV	Tomato	Al-Qasim	93.0	92.6	92.2	92.5	91.9	91.8	90.9
KF435137	TYLCV	Tomato	Alahsaa	81.7	81.0	81.1	80.9	81.2	80.6	80.7
KF435136	TYLCV	Pepper	Alahsaa	81.3	81.4	81.2	80.5	81.1	81.6	80.9
HE819240	TYLCV	Capsicum	Oman	79.1	78.8	79.0	78.1	78.6	78.2	78.2
KF229726	TYLCV	Tomato	Oman	78.0	77.9	77.6	75.9	77.7	76.9	76.6
KF229725	TYLCV	Tomato	Oman	79.5	78.5	78.0	78.9	78.1	78.7	77.0
KF229724	TYLCV	Tomato	Oman	79.5	78.4	78.0	78.8	78.1	78.7	76.0
KF229723	TYLCV	Tomato	Oman	79.5	78.5	78.0	78.9	78.1	78.7	77.0
KF229722	TYLCV	Tomato	Oman	79.2	78.2	79.0	78.7	76.6	77.8	78.6
KF229721	TYLCV	Tomato	Oman	79.3	78.5	78.8	78.6	78.8	78.7	77.1
HE819245	TYLCV	Tomato	Oman	79.2	78.2	79.0	78.7	76.6	77.8	78.5
HE819243	TYLCV	Tomato	Oman	79.8	78.2	78.0	78.7	78.1	77.2	77.7
HE819242	TYLCV	Tomato	Oman	79.2	78.6	79.1	78.7	76.6	77.8	78.6
HE819241	TYLCV	Tomato	Oman	78.1	77.9	77.6	75.9	77.7	76.9	76.5
JN604488	TYLCV	Tomato	Oman	78.2	78.0	77.8	77.3	77.1	77.6	76.0
JN604487	TYLCV	Tomato	Oman	78.2	78.0	77.8	77.3	77.1	77.6	76.0
JN604486	TYLCV	Tomato	Oman	79.7	79.5	79.0	78.9	78.1	78.7	78.0
JN604485	TYLCV	Tomato	Oman	79.5	78.2	79.0	78.7	76.6	77.8	78.5
JN604484	TYLCV	Tomato	Oman	79.2	78.9	79.9	78.8	76.9	77.8	78.6
DQ644565	TYLCV	Tomato	Oman	82.2	82.0	81.8	81.2	81.5	80.9	80.0
FJ956706	TYLCV	Tomato	Oman	79.2	78.9	79.9	78.8	76.9	77.8	78.6
FJ956705	TYLCV	Tomato	Oman	79.1	78.0	78.8	78.2	79.0	78.8	79.0
FJ956704	TYLCV	Tomato	Oman	79.2	78.2	79.0	78.7	76.6	77.8	78.5
FJ956703	TYLCV	Tomato	Oman	79.2	78.2	79.1	78.6	78.6	79.0	78.5
FJ956702	TYLCV	Tomato	Oman	78.8	78.2	77.8	78.7	78.1	76.2	77.6
FJ956701	TYLCV	Tomato	Oman	79.2	78.1	78.9	78.4	77.6	77.8	77.6
KC106648	TYLCV	Tomato	Iran	79.2	78.2	79.0	78.7	76.6	77.8	78.6
AJ132711	TYLCV	Tomato	Iran	79.6	78.0	78.5	78.9	78.6	78.8	78.1
EU085423	TYLCV	Tomato	Iran	81.1	79.9	80.8	80.7	81.0	80.4	80.7
GU076448	TYLCV	Tomato	Iran	82.2	81.0	81.1	80.9	81.2	80.6	80.7
AY594174	TYLCV	Tomato	Egypt	79.3	78.5	78.3	78.6	78.9	78.8	76.1
EF107520	TYLCV	Tomato	Egypt	71.6	71.5	70.8	70.6	70.8	70.7	70.1
EF054894	TYLCV	Tomato	Jordan	82.7	82.5	81.8	81.9	81.4	81.9	80.0
GQ861426	TYLCV	Tomato	Jordan	72.1	72.0	70.9	69.9	68.0	68.7	70.1
JX131286	TYLCV	*S. arvensis*	Jordan	78.8	78.2	77.8	78.7	78.1	76.2	77.6
JQ354991	TYLCV	Tomato	Iraq	78.7	78.3	77.6	78.1	78.0	77.2	77.9
AY044138	TYLCV	Tomato	Sudan	81.9	80.9	80.8	81.2	81.0	80.4	81.7

Fig 1. Naturally Infected cucumber plant exhibiting yellow mosaic symptoms.

Table 2. % Identity matrix of Cucumber (KT180307) Begomovirus with selected isolates.

Accession Nos	Acronyms	Hosts	Locations	%Identity
KT355021	TYLCB	Tom	Jeddah	99.1
KT728732	TYLCB	Tom	Hadasham	99.0
KT728734	TYLCB	Tom	Hail	98.5
KT728740	TYLCB	Tom	Tabuk	98.2
JF919717	ToLCYEB	Tob	Yemen	45.2
JF919718	ToLCYEB	Tob	Yemen	45.6
JF919719	ToLCYEB	Tob	Yemen	45.7
JF919720	ToLCYEB	Tob	Yemen	45.9
JF919721	ToLCYEB	Tom	Yemen	45.8
JF919722	ToLCYEB	Tom	Yemen	45.5
NC_010126	TYLCß-Om	Tom	Oman	97.8
DQ644566	TYLCß-Om	Tom	Oman	97.4
HG969297	TYLCß- Om	Pap	Oman	90.2
HG969296	TYLCß- Om	Pap	Oman	88.2
HE800552	TYLCß- Om	Tob	Oman	93.1
HE800551	TYLCß- Om	Tob	Oman	93.5
HE800550	TYLCß- Om	Tob	Oman	91.4
HE800549	TYLCß- Om	Tob	Oman	92.0
HE800548	TYLCß- Om	Tob	Oman	92.7
HE800547	TYLCß- Om	Tob	Oman	93.7
HE800546	TYLCß- Om	Tob	Oman	94.2
HE800545	TYLCß- Om	Tob	Oman	95.1
HE800544	TYLCß- Om	Tob	Oman	92.1
HE800543	TYLCß- Om	Pap	Oman	90.5
HE800542	TYLCß- Om	Pap	Oman	93.2
HE800541	TYLCß- Om	Pap	Oman	93.6
HE800540	TYLCß- Om	Pap	Oman	92.9
KJ396939	OkLCV	Okra	Jordan	44.3
NC_004903	TYLCTHVß	Tom	Thailand	58.4
DQ641714	TYLCVVß	Tom	Vietnam	61.0
NC_007485	TYLCMVß	Tom	Mali	46.8
KC677734	ToLCJaB	Tom	Java	55.3

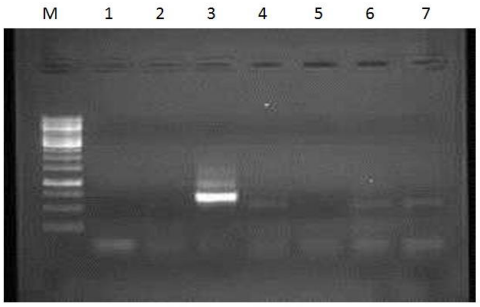

Fig 2. PCR detection of begomovirus. M: 1kb Ladder, 1: Healthy, 2-7-Field collected cucumber leaf samples.

circulating begomovirus that have spread throughout the western region and the Arabian Peninsula. The identified begomovirus isolate causing yellow mosaic disease of cucumber in Jeddah, Saudi Arabia has moved either from Yemen or from Oman. The genetic variability of begomovirus infecting cucumber in Jeddah, Saudi Arabia also could be the result of many factors like, climatic changes, change in the cropping systems, expanded crop production system, increased whiteflies population with a high disease incidence rate and virus spread to new hosts and the introduction of other begomovirus species by humans through infected plant materials. In order to better understand the current situation and genetic variability among the circulating begomoviruses and to develop an effective, sustainable management strategy for the yellow mosaic diseases, there is an urgent need not only to prepare a fine distribution map of begomovirus genotypes and types of whiteflies types across the region but also the resistance pattern and reactions of cucumber lines carrying different combinations of resistance genes against different begomovirus genotypes causing mosaic disease of cucumber in Jeddah, Saudi Arabia.

Materials and Methods

Sample collection, virus detection and whiteflies transmission

Naturally infected leaf samples from symptomatic cucumber plants displaying characteristic yellow mosaic symptoms (Fig 1) were collected during field survey in April 2014 from farmers field Jeddah, Saudi Arabia. Total genomic DNA was

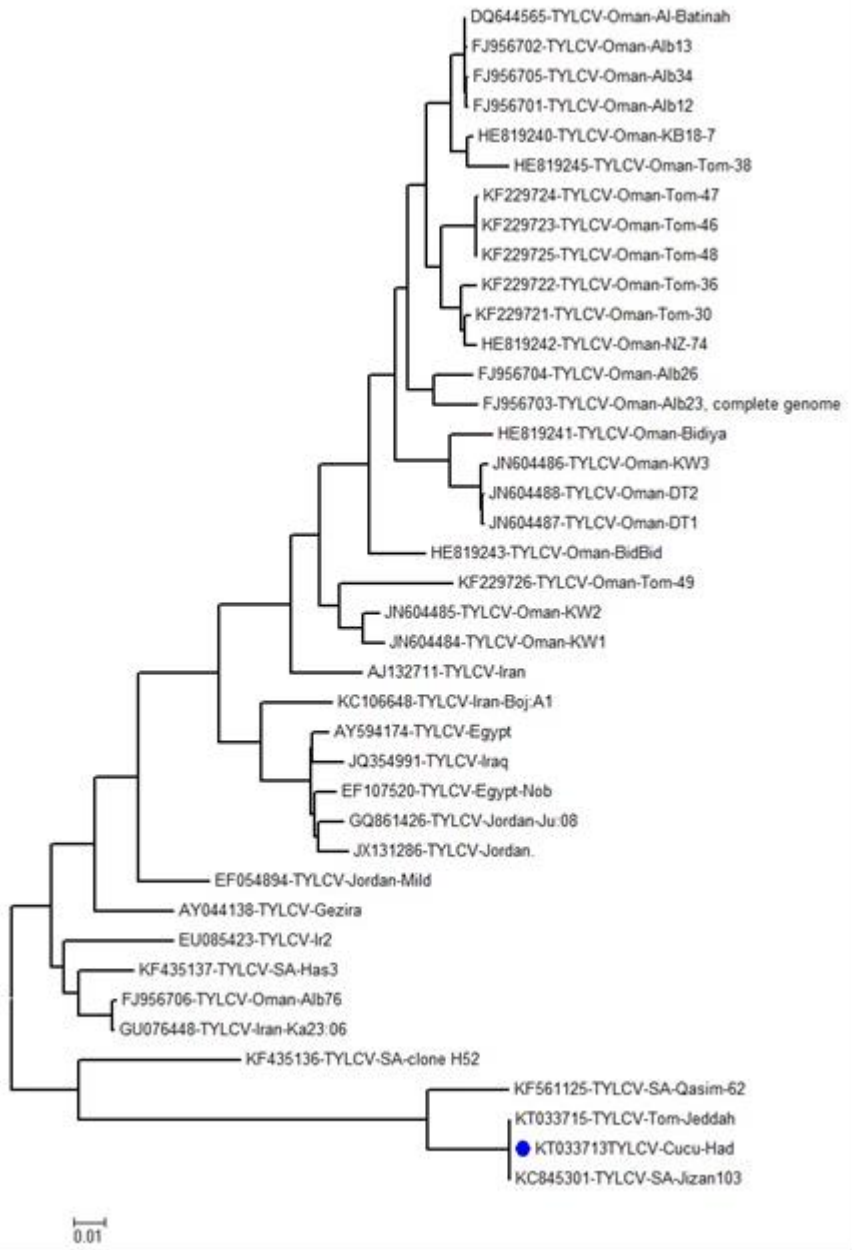

Fig 3. Phylogenetic relationships of begomovirus genome under study with selected strains (selected based on full genome BLASTn analysis) determined by NJ method within MEGA v6.1 with 1000 bootstrap replicates.

isolated from 100 mg leaf tissue using a DNAeasy plant mini kit (Qiagen Inc.) and begomovirus infection was also confirmed by PCR using specific primers TYC1F (5'-GGGCCTAGAGACCTGGCCCAC-3') and TYC1R (5'-CCGGTAATATTATACGGATGGC-3') (Hosseinzadeh et al., 2014). The presence of betasatellites were also confirmed by amplifying the full-length betasatellites genome by using specific primers; beta01/beta02 (Briddon et al., 2002). Fresh culture of non-viruliferous whiteflies were raised from the whitefly eggs and maintained in a healthy Clatoria plant in insect-proof cages. Total twenty four hours of acquisition access period (AAP) were given to acquire the virus from infected leaf samples by healthy whiteflies followed by 24 hours inoculation access period (IAP) to transmit the causative agent on healthy cucumber seedlings using 15 whiteflies/cucumber plant and inoculated seedlings were further maintained and monitored for symptom development under insect-proof cages up to five-six weeks.

Viral genome cloning and sequencing

Total genomic DNA was isolated and full-length begomovirus genome was amplified by rolling circle amplification (RCA) technique using TempliPhi 100 Amplification Kit (GE Healthcare, Life Sciences, Piscataway, NJ, USA) following the manufacturer's instructions. The amplified products were digested with *Eco*RI and *Hin*d III and full-length begomovirus (~2.7 kb) fragment was cloned into the plasmid vector pUC-19 and pGEMT-easy vector was used to clone betasatellites genome. One clone of a full genome (~2.7kb) and betasatellites (~1.4kb) from cucumber plant sample were obtained and

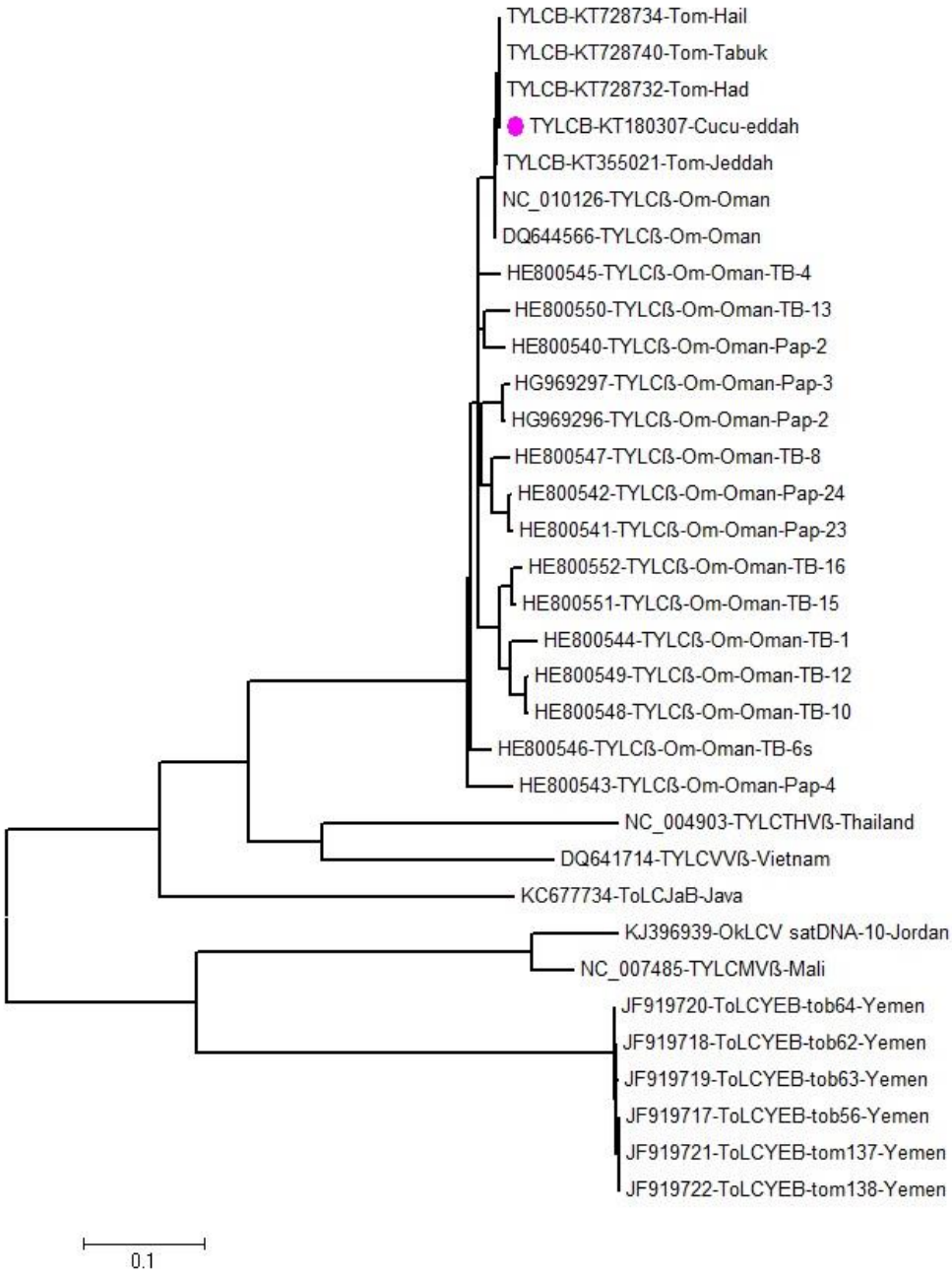

Fig 4. Phylogenetic relationships of betasatellites under study with selected strains (selected based on betasatellites BLASTn analysis) determined by NJ method within MEGA v6.1 with 1000 bootstrap replicates.

sequenced into bidirectional way. The DNA sequence was assembled and determined for full length begomoviral genomic clones by primer walking methods and analyzed by using NCBI-BLAST.

Sequence and phylogenetic analysis

The full length sequences were assembled and initially BLAST for sequence homology and percentage similarity was determined by using the software programme, BioEdit (version 5.0.9) and multiple sequence alignments were performed by using CLUSTALW program (http://www.ebi.ac.uk/clustalw) using nucleotides (nt) sequences of selected begomovirus. The phylogenetic tree was constructed by using MEGA 6 program from the aligned nucleotide sequences with neighbor-joining method (Tamura et al., 2013).

Conclusion

The results obtained in this study from full length viral genome and associated betasatellites sequences; phylogenetic analysis concludes that the identified virus causing yellow mosaic disease of cucumber in Jeddah is a variant of Tomato yellow leaf curl virus reported from the Arabian Peninsula and Nile Basin circulating in the Kingdom of Saudi Arabia.

Competing Interests:

The authors have declared that no conflict of interest exists.

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