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Phylogenetic relationships, recombination analysis and genetic variability of Tomato yellow leaf curl virus infecting tomato in Jeddah, Saudi Arabia

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Abstract

Tomato (*Solanum lycopersicum* L.) production is severely affected by many diseases caused by many pathogens and among them viruses are the most serious pathogen. Begomoviruses causes yellow mosaic and leaf curl disease of tomato in the tropical, subtropical, temperate, and even semiarid regions. Yellow leaf curl disease is caused by Tomato yellow leaf curl virus belonging to the genus Begomovirus of the family *Geminiviridae*. In this study, naturally infected tomato leaf samples were collected during field survey and causal virus was identified by PCR using tomato yellow leaf curl virus-specific primers and transmitted by whiteflies to healthy tomato seedlings. The full-length viral genome was amplified by rolling circle amplification technology while betasatellites from viral genome were amplified by PCR using universal betasatellites primers. The full-length viral genome (~2.7kb) and betasatellites (~1.4kb) were cloned and sequenced bi-directionally. The generated sequences were assembled and analyzed to find out the genetic variability by using bioinformatics tools and the genetic variability and phylogenetic relationships with selected begomoviruses were analyzed. The complete viral genome sequences showed highest (99.5%) similarity with an isolate of Tomato Yellow leaf curl virus-Jizan 103 isolate and 92.8% similarity with Tomato Yellow leaf curl virus-Al-Qasim isolate while the lowest (71.6%) were found with Tomato Yellow leaf curl virus-Egypt isolate. The newly identified virus formed the closest cluster with Tomato yellow leaf curl virus isolates from Jizan and Al-Qasim, Saudi Arabia. On the basis of sequence similarity and phylogenetic relationship and recombination analysis, it is concluded that the virus causing tomato yellow leaf curl disease is a variant of tomato yellow leaf curl virus either from Jizan or Al-Qasim isolate circulating in the Kingdom of Saudi Arabia.

Keywords: Complete genome; Phylogenetic relationships; Recombination analysis; Tomato, Tomato Yellow leaf curl virus.
Abbreviations: ChiLCV_Chili leaf curl virus; OLCOMV_Okra leaf curl Oman virus; TYLCV_Tomato Yellow leaf curl virus; ToLCOMV_ Tomato leaf curl Oman virus; ToLCSDV_ Tomato leaf curl Sudan virus.

Introduction

Leaf curl disease of tomato (*Solanum lycopersicum*) is a major limiting factor for tomato production globally. Tomato crops are severely affected by leaf curl and yellow mosaic disease in arid, semi-arid and temperate regions. Begomoviruses belongs to family *Geminiviridae* are ssDNA circular viruses with either monopartite (DNA-A genome of about 2.7 kb, encoding six ORFs) or bipartite genomes (DNA A and DNA B genomes of 2.5-2.6 kb) encapsulated in twinned particles (Lazarowitz 1992). Whitefly (*Bemisia tabaci)* has emerged as serious pathogens for most of the dicotyledonous crops across tropical, subtropical and warmer temperate regions worldwide. Tomato yellow leaf curl disease has become the most serious viral disease worldwide. Tomato yellow leaf curl virus (TYLCV) is a monopartite begomovirus, efficiently transmitted by the vector whitefly (*Bemisia tabaci*) to dicotyledonous plants and responsible for higher threat to tomato crop production. (Hanssen et al., 2010; Brown et al., 2012). Monopartite begomoviruses are associated either alphasatellites or betasatellites (Mansoor et al., 1999; Briddon et al., 2004; Briddon et al., 2008). Alphasatellites are capable of autonomous replication in host plants cells mediated by a nanovirus-like replication-associated protein (Saunders and Stanley, 1999) while the replication of betasatellites is dependent on their helper virus (Cui et al., 2004; Saunders et al., 2008). Recently, the emergence and spread of TYLCV from the Middle East to world infecting Solanaceous crops in East and Southeast Asia have been reported 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).The genetic variability and new host adaptation with extended host range in the new environment and expanded geographical region is a result of frequent mutation and recombination occurs during replication (Padidam et al., 1999).Generally, Polymerase chain reaction using sequence-specific primers and rolling circle amplification by phi29 DNA polymerase

with random primers followed by genome cloning and sequencing methods are being used frequently to identify and detect the extent of genetic diversity and its associated alpha and betasatellites molecules among the begomoviral genomes (Dean et al., 2001).

In this study we report the natural occurrence, identification of virus by PCR, cloning and sequencing of full length and associated betasatellites viral genomes, sequence analysis, genetic diversity, phylogenetic relationship and recombination pattern of Tomato yellow leaf curl virus causing tomato leaf curl disease in Jeddah, Saudi Arabia. The naturally infected tomato plants develop yellowing; leaf curling and stunting resulted in significant yield loss in tomato production in Jeddah, Saudi Arabia. Many isolates of begomoviruses infecting and causes significant loss to tomato, tobacco and Okra crops in the Nile Basin, arid and semi-arid southern part of the Arabian Peninsula like Oman and Yemen have been reported 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 (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; Khan et al., 2013b; Khan et al., 2014; Akhtar et al., 2014; Al-Saleh et al., 2014).The present study was undertaken to identify the genetic variability and recombination pattern of Tomato yellow leaf curl virus infecting tomato crops in Jeddah, Saudi Arabia.

Results

Confirmation of begomovirus infection and transmission by whiteflies

Begomovirus infection was confirmed by PCR and ~856bp amplicon visualized on 1% Agarose gel from all infected samples**.** Total 10 samples of tomato leaves were randomly collected from the field. The causal virus causing yellow leaf curl disease on tomato could be efficiently transmitted to young tomato seedlings and the inoculated plants developed characteristic yellow leaf curl symptoms in 80% plants by 6–12 days after inoculation, (DAI) and the disease symptoms produced in the experimental plants were approximately related to those observed in the tomato field.

Cloning, sequencing and Phylogenetic analysis

The rolling circle amplification products obtained by using total DNA extracted from naturally infected tomato plants were restricted with E*co*RI restriction enzyme. The separated ~2.7 kb fragments purified by gel purification kit (Qiagen) and cloned in pUC19. The betasatellites (~1.4 kb) were PCR amplified and cloned into a pGEMT-easy vector. One full length (~2.7 kb) and betasatellites (~1.4 kb) clones from tomato were experimentally confirmed sequenced in both directions. The sequences of the complete viral genome had 2789 nucleotides while betasatellites had 1368 nucleotides. The full genome and betasatellites sequences have been submitted to NCBI GenBank with accession numbers, KT033715-full length and KT355021-betasatellites and tentatively designated as TYLCV-tomato-Jeddah isolate.

The comparison of full genome sequence revealed that TYLCV-tomato-Jeddah isolate shared similarity ranged from 99.5%-71.6% with selected TYLCV isolates followed by 99.5% identity with TYLCV-isolate Jizan103 (KC845301) and 92.8% with TYLCV Al-Qasim isolates (KF561125) while the lowest similarity (71.6%) were found with TYLCV-Egypt isolate (EF107520). Even so, the TYLCV-Oman-tomato isolate showed a range of diversity ranged from 78-79% and the TYLCV isolates from Iran ranged from 79-82%, along with isolates from Jordan varied from 78-82% similarity. The highest amino acid sequence identities was identified with TYLCV-KT033715 in all the 6 proteins (V2-99.5%, V1-99.7%, C3-99.4%, C2-99.2%, C1-99.8%, and C4-99.0%) respectively, with respective sequences of selected begomovirus isolates (Table 1). Following the guidelines from ICTV published (Brown et al., 2012) for species demarcation (<91% nucleotides similarity), this TYLCV-tomato-Jeddah isolate could be considered as a variant of TYLCV-Jizan 103 isolate. The presence and identification, amplification of betasatellites molecules with TYLCV isolates from tomato and other crops have been reported earlier by many researchers (Briddon et al., 2002; Idris et al., 2012; Khan et al., 2013a; Khan et al., 2014; Akhtar et al., 2014). The comparative sequence analysis of the betasatellites generated from tomato yellow leaf curl betasatellites-tomato-Jeddah (KT355021) showed highest similarity (99.4%) with tomato yellow leaf curl betasatellites-Oman isolates (NC_010126 & DQ644566) and the lowest similarity (46.5%) was found with tomato yellow leaf curl betasatellites-Mali isolate (NC007485) (Table 2). Based on the complete genome sequences aligned with selected begomoviruses sequences a phylogenetic tree was constructed and the phylogenetic analysis results showed that the TYLCV-tomato-Jeddah isolate formed two separate clades with other selected begomovirus isolates from different locations. The TYLCV-tomato-Jeddah isolate formed the closest cluster with TYLCV-Jizan 103 isolate (KC845301) followed by TYLCV-tomato-Al-Qasim isolates (KF561125). Interestingly, most of the TYLCV isolates from Oman formed separate cluster while other isolates from Iran, Egypt and Jordan clustered together. One more separate cluster was formed with mixed isolates from Saudi Arabia, Sudan, Iran and Jordan (Fig.2). The phylogenetic analysis results based on selected betasatellites indicated that the isolate from TYLCV-Tomato-Jeddah (KT355021) while tomato yellow leaf curl betasatellites isolates from Oman and Yemen formed a separate clusters (Fig. 3).

Recombination analysis

Recombination analysis was carried out to find out the recombination pattern among the selected TYLCV isolates using in RDP4 programme (Martin et al., 2015). The algorithms of, RDP (P 1.653×10^{-12}), GENCONV (P 1.274×10^{-13}), MaxChi (P $7.392\times^{10-11}$), Chimaera (P 7.756×10^{-11}), Si Scan (P $2.510\times^{10-35}$) and 3 Seq (P $1.225\times^{10-15}$) predicted that the genome of the herein newly described TYLCV-Tomato-Jeddah exhibit evidence of intra-specific recombination. The bootscan and RDP analysis of the TYLCV-Tomato-Jeddah genome together indicated definite evidence of recombination within the viral genome. Two recombinant fragments (co-ordinates 2041 to 2742 nucleotides positions in the replicase gene and 1944 to 2671 nucleotides positions toward the 3′ end of the Replicase gene) were detected for TYLCV-Jeddah-tomato isolate and these fragments shared high levels of sequence identity with TYLCV (KC845301; 99.5% and 92.8% similarity with TYLCV Al-Qasim isolate (KF561125). TYLCV-SA-(KF435136) was indicated as the minor and TYLCV-SA

Table 1. Percent identity matrix of TYLCV- Tomato -Jeddah isolate (KT033715) with selected Begomoviruses.

Accession Nos.	Abbreviation	Hosts	Locations	% Identity matrix at nucleotides (nt) and Amino acids level (aa)						
				Full genome (nt)	V2 gene (aa)	V1 gene (aa)	C3 gene (aa)	C2 gene (aa)	C1 gene (aa)	C4 gene (aa)
KF435136	TYLCV	Pepper	Saudi Arabia	81.7	81.4	81.2	80.5	81.1	81.6	80.9
KF561125	TYLCV	Tomato	Saudi Arabia	92.8	92.6	92.2	92.5	91.9	91.8	90.9
KF435137	TYLCV	Tomato	Saudi Arabia	81.3	81.0	81.1	80.9	81.2	80.6	80.7
KC845301	TYLCV	Tomato	Saudi Arabia	99.5	99.3	99.5	99.2	99.1	99.0	99.3
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 Tomato plant exhibiting severe leaf curl symptoms.

Table 2 Percent identity matrix of TYLC betasatellites- Tomato -Jeddah isolate (KT355021) with selected betasatellites.

Accession Nos in NCBI-Pub Med	Abbreviations	Location	Host	% identity
JF919717	ToLCYB	Yemen	Tobacco	49.4
JF919718	ToLCYB	Yemen	Tobacco	49.4
JF919719	ToLCYB	Yemen	Tobacco	49.4
JF919720	ToLCYB	Yemen	Tobacco	49.5
JF919721	ToLCYB	Yemen	Tobacco	49.4
JF919722	ToLCYB	Yemen	Tobacco	49.4
NC010126	TYLCOB	Oman	Tomato	99.4
DQ644566	TYLCOB	Oman	Tomato	99.4
HG969297	TYLCOB	Oman	Papaya	90.7
HG969296	TYLCOB	Oman	Papaya	88.7
HE800552	TYLCOB	Oman	Tomato	93.6
HE800551	TYLCOB	Oman	Tomato	93.9
HE800550	TYLCOB	Oman	Tomato	91.9
HE800549	TYLCOB	Oman	Tomato	93.4
HE800548	TYLCOB	Oman	Tomato	93.3
HE800547	TYLCOB	Oman	Tomato	94.3
HE800546	TYLCOB	Oman	Tomato	94.8
HE800545	TYLCOB	Oman	Tomato	95.8
HE800544	TYLCOB	Oman	Tomato	92.6
HE800543	TYLCOB	Oman	Papaya	91.0
HE800542	TYLCOB	Oman	Capsicum	93.7
HE800541	TYLCOB	Oman	Capsicum	94.2
HE800540	TYLCOB	Oman	Capsicum	93.5
KJ396939	OkLCB	Jordan	Tomato	45.2
NC004903	TYLCB	Thailand	Tomato	58.5
DQ641714	TYLCB	Vietnam	Tomato	62.0
NC007485	TYLCB	Mali	Tomato	46.5
KC677734	ToLCJaB	Japan	Tomato	55.5

Table 3. Recombination analysis of Tomato Yellow leaf curl virus-Tomato-Jeddah using the RDP 4.0.

Break points at nucleotides position				Detection Methods					
Start point (nt)	End point (nt)	Minor Parent	Major Parent	RDP	GENCONV	MaxChi	Chimaera	SiScan	3 Seq
2041	2742	TYLCV-SA (KF435136)	TYLCV-Jizan-(KC845301)	1.653×10^{-12}	1.274×10^{-13}	$7.392\times^{10-11}$	7.756×10^{-11}	$2.510\times^{10-35}$	$1.225\times^{10-15}$
1944	2671	TYLCV-Iran (GU076448)	TYLCV-Al-Qasim (KF561125)	2.151×10^{-13}	1.247×10^{-14}	$5.135\times^{10-11}$	7.412×10^{-12}	$2.294\times^{10-35}$	$1.336\times^{10-13}$

Abbreviations : RDP: Recombination detection programme; SiScan : Sister-Scanning

(KF435136) major parent and TYLCV-Al-Qasim isolate (KF561125) was found as recombinant isolate but the actual recombinant may be the TYLCV-Iran isolate (GU076448). The recombination results suggest that the TYLCV-Jeddah-tomato strain evolved either from TYLCV Iran or Al-Qasim isolate during recombination (Table 3).

Discussion

Tomato leaf curl disease is a major limiting factor for cultivation of tomato in tropical and sub-tropical areas globally. Tomato is an important vegetable crop in the Kingdom of Saudi Arabia for local consumption. In this study, we report the PCR identification, cloning, complete genome sequencing, genetic variability and phylogenetic relationship of TYLCV-Tomato-Jeddah isolate with other selected begomoviruses from different locations. During the field survey, naturally infected plants were observed to exhibit yellow leaf curl symptoms in tomato field. The strategies for development of durable disease management against viruses require the information about genetic variability, virus evolution and host plant interaction (Garcia et al., 2007). The most important factors 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 and extended host range of the whiteflies vector are known for genetic variability and evolution among the virus population which enables virus adaptations and emergence in changed environments and climatic conditions (Seal et al., 2006). Although, novel distinct species of begomoviruses were mostly identified in the early 2000s and this happens due to more interest of begomovirus research which enhanced the identification and determination of begomovirus emergence and evolution of novel species by viral genome sequencing. Ha et al., (2008) suggested that sub-continental South-East Asia could be a major center of diversity for begomoviruses based on the great diversity of local strains and species of monopartite begomoviruses and associated betasatellite molecules identified in these regions.

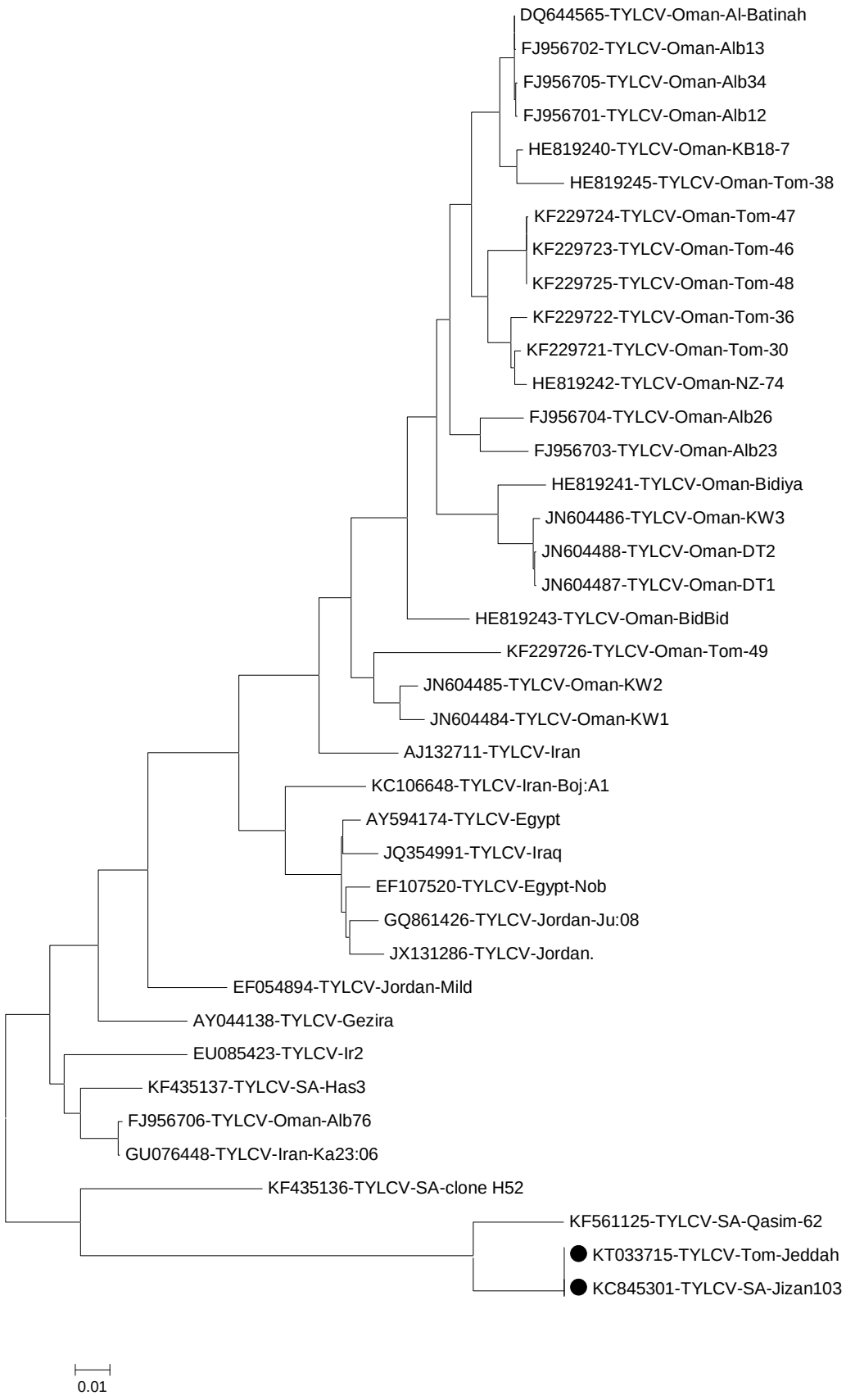

Fig 2. Phylogenetic relationships of begomovirus genome under study with selected strains (selected based on full genome BLASTn analysis) determined by NJ method within MEGA v6.1 with 1000 bootstrap replicates. Explain cluster more.

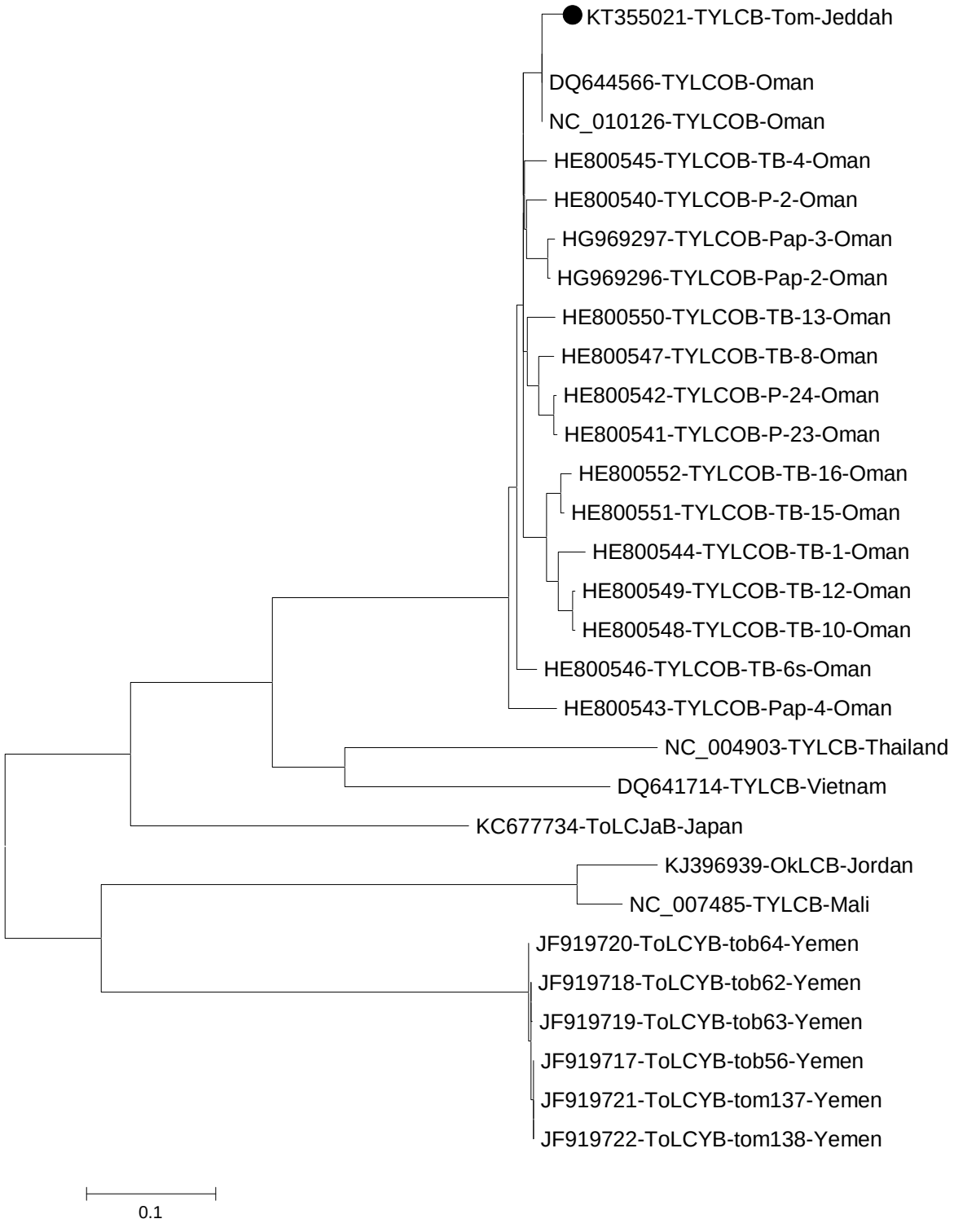

Fig 3. Phylogenetic relationships of betasatellites under study with selected strains (selected based on betasatellites BLASTn analysis) determined by NJ method within MEGA v6.1 with 1000 bootstrap replicates.

It is also assumed that Iran could be the center for diversity, emergence and spread of TYLCV from the Middle East to worldwide (Lefeuvre et al., 2010; Kenyon et al., 2014). Globally, due to increasing importance of leaf curl disease in tomato, enormous efforts and research work has been made to identify and introduce the resistance genes into many tomato cultivars to improve the resistance against tomato leaf curl disease. Currently, many resistance genes (Ty-1to Ty-5) have been identified from wild relatives and successfully introduced into many commercial tomato cultivars (de la Pena et al., 2010).Recently, there is a report has been published about the diversity and emergence of begomovirus infecting Solanaceous crops in the east and south-east Asia (Yang et al., 2014; Kenyon et al., 2014) but earlier it was also reported that the emergence of TYLCV started in between 1930-1950 in Middle East and Iran could be a center for TYLCV diversity based on inter- and intra-species recombination patterns and spread globally around 1980 just immediately emergence of two strains *i.e.* TYLCV-Mld and-IL (Lefeuvre et al., 2010). Since two decades, the tomato production is seriously affected by leaf curl and yellow mosaic disease caused by several 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., 2013; Al-Saleh et al., 2014; Akhtar et al., 2014; Idris et al., 2014; Hosseinzadeh et al., 2014). The genetic variability of begomoviruses infecting tomato could be result of many factors like, climatic changes, change in the cropping systems, intensified and expanded crop production system, more whiteflies population, introduction of tolerant and susceptible tomato cultivars, higher rate of disease incidence and virus spread, introducing other begomovirus species by humans through infected plant materials. Apart from that other important factors are also contributes in the genetic variability, emergence and spread of begomovirus which includes; high rate of mutation in coding and non-coding sequences during recombination, pseudo-recombination and acquisition of satellite DNA molecules etc. In order to better understand the current situation and genetic variations among the circulating begomoviruses in this region and to develop an effective sustainable management strategies for the tomato leaf curl diseases, there is an urgent need not only to prepare a fine distribution map of begomovirus genotypes and whiteflies types across the region but also the resistance pattern and reactions of cultivated tomato lines carrying different combinations of resistance genes against different begomovirus genotypes causing leaf curl disease of tomato in this region. In this study, the TYLCV-tomato-Jeddah isolate represents a variant of circulating begomovirus that have spread throughout the western region and Arabian Peninsula. The analysis of the full-length genome and phylogenetic relationships revealed that the level of genetic variation observed in the natural population of TYLCV and it is suspected that identified virus isolate present in Saudi Arabia has moved either from Yemen or from Oman or Al-Qasim. Recent studies have shown that Geminiviruses genomes are prone to DNA methylation in infected host plants (Yang et al., 2011; Zhang et al., 2011). In Oman, TYLCV is associated with a betasatellites that have only been identified in Yemen (Tomato yellow leaf curl Oman betasatellite) (Khan et al., 2008).On the other side Yemen is separated from Oman and Saudi Arabia by a vast harsh desert, collectively constituting potential barriers to virus and whitefly movement (Idris et al., 2012).

Materials and Methods

Field survey and detection of the virus

Naturally infected leaf samples from symptomatic tomato plants displaying characteristic tomato yellow leaf curl disease symptoms (Fig. 1) were collected during field survey from experimental plots of King Abdulaziz University, Jeddah, Saudi Arabia. The begomovirus infection was confirmed by using total DNA isolated from 100 mg leaf tissue using DNAeasy plant mini kit (Qiagen Inc.) following the manufacturer's instructions. The begomovirus specific primers were used in PCR reaction which amplify an amplicon of the 856-bp product from the 5' end of the C1 gene of TYLCV-IL (Hosseinzadeh et al., 2014).The PCR products were analyzed on 1% Agarose gel stained with ethidium bromide (0.5 ug ml^{-1}) and visualized on a UV transilluminator. The betasatellites were also confirmed by amplifying the full-length betasatellites using specific primers; beta01/beta02 produced an amplicon of (~1,350 bp) (Briddon et al., 2002). Fresh culture of Non-viruliferous whitefly was raised from the whitefly eggs and maintained on eggplant (*Solanum melongena*) in insect-proof cages. Fully mature whiteflies were given an acquisition access period (AAP) of 24 h on infected leaves. After the required AAP, the viruliferous whiteflies were given for an inoculation access period (IAP) of 24 h on healthy test seedlings (15 whiteflies/ tomato plant) and the inoculated seedlings were monitored for symptom development under insect-proof cages up to five weeks. The virus isolates were maintained on the respective hosts by whitefly transmission.

Cloning and complete genome sequencing

The rolling circle amplification technology (RCA) was used to amplify the full-length genomic components of begomovirus from purified DNA isolated from the infected tomato plants by 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 full-length begomovirus (~2.7 kb) fragments were cloned in the plasmid vector pUC19. The betasatellites were PCR amplified and cloned into a pGEMT-easy vector. One clone of a full genome (~2.7kb) and betasatellites (~1.4kb) from tomato were obtained and sequenced in both directions using a primer walking strategy. The DNA sequence was determined for full length begomoviral genomic clones by primer walking methods and analyzed using BLAST (NCBI).

Sequence, phylogenetic and recombination analysis

The obtained sequences of complete gnome and betasatellites were assembled, analyzed and aligned for percentage similarity matrix determination by using the software programme, BioEdit (version 5.0.9) and genes were predicted using ORF Finder (NCBI) and multiple sequence alignments were performed by using CLUSTALW program (http://www.ebi.ac.uk/clustalw) using nucleotides sequences of selected begomoviruses. The phylogenetic tree was constructed using MEGA6 program from the aligned nucleotide sequences with neighbor-joining methods (Tamura et al., 2013). The recombination detection programme (RDP4) tool was used (darwin.uvigo.es/rdp/rdp.html) for detection of potential recombinant sequences, identification of likely parental sequences and localization of possible recombination

breakpoints. The analysis was performed with default settings using a 0.05 P-value cutoff and standard Bonferroni corrections for multiple testing (Martin et al., 2015).

Conclusion

The results obtained from full length viral genome and associated betasatellites sequences, phylogenetic and recombination analysis concludes that the identified virus causing tomato yellow leaf curl disease in Jeddah, Saudi Arabia is a variant of tomato yellow leaf curl virus reported from Arabian Peninsula and Nile basin circulating in the Kingdom of Saudi Arabia.

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Competing Interests

The authors have declared that no competing interest exists.

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