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ORIGINAL ARTICLE

Association of tomato leaf curl Sudan virus with leaf curl disease of tomato in Jeddah, Saudi Arabia

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Abstract Tomato is an important vegetable crop and its production is adversely affected by leaf curl disease caused by begomovirus. Leaf curl disease is a serious concern for tomato crops caused by begomovirus in Jeddah, Kingdom of Saudi Arabia. Tomato leaf curl disease has been shown to be mainly caused either by tomato leaf curl Sudan virus or tomato yellow leaf curl virus as well as tomato leaf curl Oman virus. Many tomato plants infected with monopartite begomoviruses were also found to harbor a symptom enhancing betasatellites. Here we report the association of tomato leaf curl Sudan virus causing leaf curl disease of tomato in Jeddah, Kingdom of Saudi Arabia. The complete genome sequence analysis showed highest (99.9 %) identity with tomato leaf curl Sudan virus causing leaf curl disease in Arabian Peninsula. In phylogenetic relationships analysis, the identified virus formed closest cluster with tomato leaf curl Sudan virus. In recombination analysis study, the major parent was identified as tomato leaf curl Sudan virus. Findings of this study strongly supports the associated virus is a variant of tomato leaf curl Sudan virus causing disease in Sudan, Yemen and Arabian Peninsula. The betasatellites sequence analysis showed highest identity (99.8 %) with tomato leaf curl betasatellites-Amaranthus-Jeddah. The phylogenetic analysis result based on betasatellites formed closed cluster with tomato yellow leaf curl Oman betasatellites. The importance of these findings and occurrence of begomovirus in new geographic regions causing leaf curl disease of tomato in Jeddah, Kingdom of Saudi Arabia are discussed.

Keywords Tomato leaf curl disease · Begomovirus · Tomato leaf curl Sudan virus · Betasatellite · Saudi Arabia

Introduction

Tomato is an important vegetable crop grown all over the world. Fresh, manufactured, dry, canned or as juice, tomato is a very important source of vitamins (A and C) and minerals. The most important properties of vitamin A is to regulate immune system and maintain good bone growth by cell division and cell differentiations and to maintain surface linings of eye, urinary and intestinal tracts and eyes. Vitamin C is important in forming collagen which is main and important protein for bones muscles and blood vessels. Currently tomato is being consumed at higher rate in developed countries as luxury crop [30]. Saudi Arabia produced about 529.8 tons of tomatoes in 2013 for local consumption and considered as the biggest vegetable crop grown in green houses in the Kingdom of Saudi Arabia [13]. Tomato crops are affected by many diseases caused by various pathogens and among them begomoviruses belongs to family *Geminiviridae* are serious problems for tomato yield. Globally, tomato leaf curl and tomato yellow leaf curl are the most serious diseases caused by both mono and bipartite begomovirus in tropics and semi-tropics [11]. The family *Geminiviridae* has now been reported to have seven genera known as *Mastrevirus, Curtovirus, Begomovirus, Topocuvirus, Eragrovirus, Turncurtovirus, Becurtovirus* [7, 9, 24, 29]. Members of the genus

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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 and alphasatellites [6]. Betasatellites are small (~1.4 kb), highly diverse with circular ssDNA. The betasatellites replication, movement and transmission between plants are mediated by their helper virus. Betasatellites had an ORF beta C1, an adenine-rich region, and a satellite conserved region with 45–93 % sequence identity [26]. Alphasatellites, formerly known as DNA 1, are also approximately half the size of begomoviral genomes (~1375 nt) and show a conserved genome organization consisting of a single open reading frame (ORF) coding for a replication initiator protein (Rep) [31]. The dicotyledonous plants are infected by begomovirus transmitted by whitefly vector (*Bemisia tabaci)* in a persistent manner. Globally, the whiteflies have become serious pathogens for many dicotyledonous crops in tropical, subtropical and warmer temperate regions and mostly Solanaceous crops are being affected by begomovirus in East and Southeast Asia [14, 18]. The whitefly posing serious threat to global food security and there is an urgent need to develop effective strategies to control crop loss and manage begomoviral disease. Collectively, these begomoviruses causes significant loss to tomato crops in the Nile Basin, arid and semi-arid regions and southern part of the Arabian Peninsula [1, 19, 20]. Currently, the natural occurrence of mono-partite begomoviruses and their associated alpha and betasatellites (1.4 kb) are reported to be endemic into Eastern Hemisphere [4] and approximately 0.7 kb ssDNA satellite molecules as well as some smaller than unit size of helper genome or satellite sequences are also frequently detected together with begomoviruses PCR amplification in plant DNA extracts and these smallest satellites are shown to act as defective interfering (DI) agent [17] but the biological functions of DI sequences are not well known [15]. Recently, the association of few begomoviruses like *tomato leaf curl Sudan virus* (ToLCSDV), *tomato yellow leaf curl virus* (TYLCV), *tomato leaf curl Oman virus* (TLCOV), *chili leaf curl virus* (ChiLCV), *okra leaf curl Oman virus* (OLCOMV) infecting tomato, tobacco and okra has been reported from Nile Basin and in Southern region of Arabian Peninsula, Oman and Yemen [1–4, 15–17, 19–22]. One distinct strain of ToLCSDV without betasatellites was reported from Oman and designated as ToLCSDV-OM [20].

In this study, we report the association of begomovirus with tomato leaf curl disease observed in open tomato field in Jeddah, Kingdom of Saudi Arabia. Naturally infected tomato leaf samples were collected from various locations exhibiting typical leaf curling symptoms in Jeddah, Kingdom of Saudi Arabia. The causative agent was detected by begomovirus specific PCR, efficiently transmitted by whitefly and characterized at molecular level by cloning and sequencing of full genome and analyzing the sequence identity, phylogenetic relationship and recombination pattern with selected begomovirus isolates from different locations. The sequence identity and phylogenetic analysis results strongly support the causative agent is a variant of tomato leaf curl Sudan virus (ToLCSDV), a virus that has been reported earlier to cause tomato leaf curl disease in Sudan, Oman and Yemen [2, 16, 20, 21].

Materials and methods

Field survey, sample collection and whitefly transmission

Field survey was conducted in end of April-2014 and during field survey, leaf curl disease was observed in tomato crop in open experimental plots of King Abdulaziz University, Jeddah, Saudi Arabia. Symptomatic and non-symptomatic top emerging leaves were collected from field infected tomato crops and immediately kept in ice with self-sealing plastic bags and brought to lab for further processing. Fresh culture of non-viruliferous whitefly was raised from their un-hatched eggs and maintained on healthy eggplant under insect proof condition. The adult whiteflies were allowed to feed on infected tomato leaves for 24 h for virus acquisition and then transferred onto healthy tomato seedlings (twenty whiteflies/seedling) to transmit the causal organism by giving 24 h inoculation access period under insect proof cages. Total 18 healthy seedlings were inoculated in three replicates and kept under insect proof condition for symptoms development for 25 days.

PCR amplification, cloning and complete genome sequencing

The infected tomato leaf samples (100 mg) were used to isolate total genomic DNA by using CTAB method with slight modifications and isolated DNA was re-suspended in sterile distilled water and stored at −20 °C until further use [25]. The PCR was performed by using purified DNA to detect the presence of virus by using begomovirus specific primers; TYC1F(GGGCCTAGAGACCTGCCCAC) and TYC1R(CCGGTAATATTATACGGATGGC) which amplify an 856-bp fragment of the 5' end of the C1 gene of TYLCV-IL by following standardized PCR conditions [12]. The presence of betasatellites was also confirmed by betasatellites specific primers and an amplicon of full-length betasatellites (~1.4 kb) were obtained from infected samples and cloned into pGEMT vector [5]. We

Table 1 Pair wise (%) sequence identities of DNA genome of begomovirus under study (ToLCSDV-tomato-Jeddah-KT033711) with selected begomoviruses at nucleotide (nt) and amino acid (aa) levels

Accession Nos.	Abbreviation	Host	Location	Percent identity matrix at nucleotides and amino acid level of KT033711-ToLCSDV-tomato-Jeddah with selected Begomovirus						
				DNA-A Full (nt)	V2 (aa)	V1 (aa)	C3 (aa)	C2 (aa)	C1 (aa)	C4 (aa)
KT033710	ToLCSDV	Amaranthus	Jeddah	99.9	99.6	99.8	99.7	99.8	99.9	99.8
HG530539	ToLCSDV	Tomato	S. Arabia	99.8	99.5	99.7	99.6	99.7	99.8	99.7
JF919733	ToLCSDV	Tobacco	Yemen	91.5	95.6	97.2	85.8	85.9	91.6	74.0
JF919734	ToLCSDV	Tobacco	Yemen	90.3	93.9	97.2	87.3	87.4	88.8	56.0
KC845301	TYLCV	Tomato	S. Arabia	91.6	62.3	98.1	85.8	88.1	91.6	69.0
KF561125	TYLCV	Tomato	S. Arbia	91.5	94.8	91.0	73.1	86.6	84.8	68.6
KF040453	TYLCV	Tomato	S. Arabia	91.2	98.2	91.0	74.6	89.6	82.8	67.6
JN591386	ToLCSDV	Tomato	Oman	92.2	96.5	98.0	88.0	88.1	86.2	77.0
FJ956700	TYLCV-OM[OM-Alb22-05]	Tomato	Oman	88.5	93.1	77.9	85.0	81.4	75.7	73.0
KF444467	ToLCSDV	green bean	S. Arabia	89.7	92.3	96.4	85.8	85.9	87.1	71.0
KF561125	TYLCV	Tomato	S. Arabia	91.5	98.2	91.0	73.1	86.6	84.8	68.6
HE819244	ToLCSDV	Tomato	Oman	91.5	98.2	91.8	74.6	88.8	85.7	70.0
JN591385	ToLCSDV	Tomato	Oman	91.2	98.2	92.2	75.3	87.4	85.9	77.0
JN591386	ToLCSDV	Tomato	Oman	92.1	98.2	98.0	88.0	88.1	86.2	77.0
AY044139	ToLCSDV-Sha[SD-Sha-96]	Tomato	Sudan	92.4	98.2	97.2	85.8	88.1	93.8	82.0
JX483708	ToLCSDV-Sha[SD-Sha-4.2]	Tomato	Sudan	91.9	95.6	97.2	85.8	81.4	93.3	75.0
JF919731	ToLCSDV-Tih:tom1:05	Tomato	Yemen	89.7	96.5	96.8	78.3	77.0	91.6	69.0
EF110891	ToLCSDV-YE[YE-05]	Tomato	Yemen	89.7	96.5	96.8	78.3	77.0	91.6	69.0
KC763630	ToLCSDV -[SD-WM-11]	Tomato	Sudan	88.3	90.5	96.1	86.5	87.4	91.0	72.0
AY044137	ToLCSDV-Gez[SD-Gez-96]	Tomato	Sudan	88.5	93.9	97.2	85.8	81.4	89.9	72.0
AY044138	ToLCSDV-Gez[SD-Gez	Tomato	Sudan	82.9	93.9	76.3	82.8	84.4	93.0	66.0
GU180085	ToLCSDV -[Wad Madani]	Tomato	Sudan	88.5	92.2	96.8	85.8	82.2	90.2	72.0
DQ358913	TYLCMLV-ET[ET-Mel-05]	Tomato	Ethiopia	83.2	94.8	76.7	85.0	86.6	90.2	72.0
DQ644565	TYLCV- Al-Batinah	Tomato	Oman	78.9	93.9	77.9	83.5	82.9	82.4	71.0
GU076448	TYLCV- [Kahnooj:Iran]	Tomato	Iran	83.7	92.2	77.9	83.5	82.2	81.2	66.0
KC106648	TYLCV-IL[IR:Boj:A1]	Tomato	Iran	79.1	92.2	78.2	82.0	80.0	80.0	38.0

ToLCV tomato leaf curl virus, *ToLCSDV* tomato leaf curl Sudan virus, *TYLCV* tomato yellow leaf curl virus, *TYLCMLV-ET*[*ET-Mel-05*] tomato yellow leaf curl Mali virus, *S. Arabia* (Saudi Arabia)

used only positive samples for further experimental work and analysis.

The full length genomic components of begomovirus were amplified from purified DNA isolated from the symptomatic tomato plants by using rolling circle amplification technology (RCA) TempliPhi 100 Amplification Kit (GE Healthcare, Life Sciences, Piscataway, NJ, USA) following the manufacturer's instructions. The restriction enzyme *Eco*RI was selected and used for restriction of RCA products and further cloned into pGEM7Zf+ (Promega, Madison, WI). The confirmed clones were bi-directionally sequenced in our lab by using primer walking methods and analyzed by using NCBI BLAST.

Sequence, phylogenetic and recombination 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) [10] and genes were predicted using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Based on the BLAST results, the full-length sequences of selected ToLCSDV/TYLCV and tomato leaf curl virus isolates were retrieved from GenBank (Table 1) and multiple sequence alignment was performed by using CLUSTALW program (http://www.ebi.ac.uk/clustalw) with nucleotides (nt) sequences of selected begomoviruses from the GenBank.

The aligned nucleotide (nt) sequences of identified ToLCSDV along with selected begomovirus isolates were used to construct phylogenetic tree using maximum likelihood in MEGA6 program [27]. The recombination detection programme (RDP4) tool was used (darwin.uvigo.es/rdp/rdp.html) for detection of probable 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 [23].

Results

Field survey, sample collection and whitefly transmission

During field survey approximately 80 % plants were observed to be infected with leaf curl disease (Fig. 1a). Total six samples were collected from symptomatic and non-symptomatic tomato plants and processed for virus detection. Interestingly, the virus transmitting natural vector; whitefly (*B. tabaci*) was also observed in and around the field and under the leaves of infected tomato plants. The causative agent was successfully transmitted to 11/18 healthy tomato seedling by whiteflies inoculation which further developed similar leaf curl symptoms after 18–23 days post inoculation (dpi) as observed in the field.

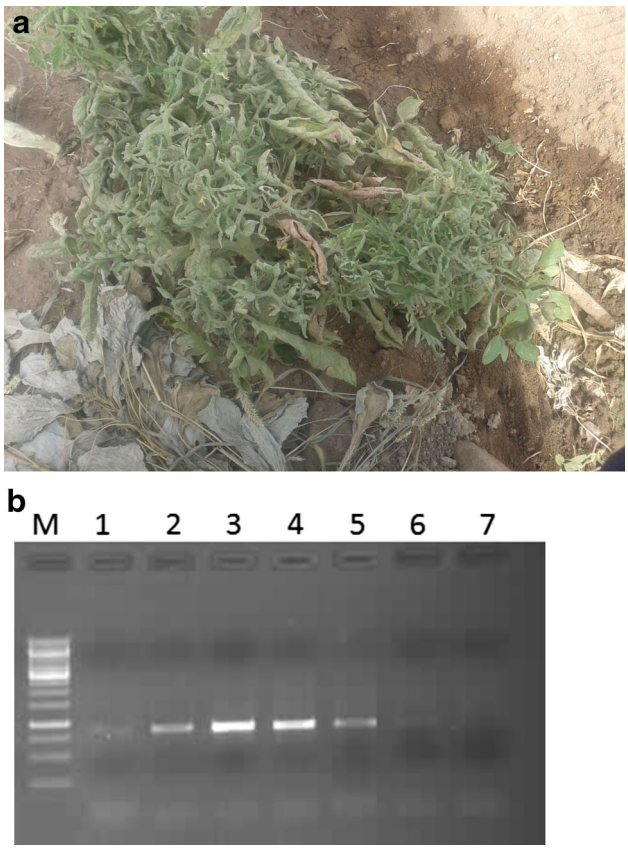

Fig. 1 **a** Naturally infected tomato plant exhibiting leaf curl symptoms. **b** Detection of begomovirus by polymerase chain reaction (M: 1 kb ladder, 1–6 infected tomato samples, 7—uninfected tomato sample

Virus detection, cloning and complete genome sequencing

The causative agent was identified and confirmed by PCR using begomovirus specific primers in both symptomatic as well as non-symptomatic samples as ~856 bp amplicon was visualized on 1 % Agarose gel (Fig. 1b). A fragment of ~2.8 kb from infected samples was obtained after restriction of RCA products by using *EcoRI* restriction enzyme. The restricted RCA products and betasatellites were cloned into separate vectors and confirmed clones were sequenced bi-directionally at our special infectious agents unit (SIAU). The aligned complete genome was found to have 2791 nucleotides (nt) and full betasatellites had 1375 nt. The full length genome and betasatellites sequence have been submitted to GenBank under accession numbers KT033711 (full genome) and KT312999 (betasatellites).

Sequence and phylogenetic analysis

The comparisons of full genome nucleotide (nt) sequence with selected begomovirus sequences collected from different locations revealed that ToLCSDV-tomato-Jeddah isolate (KT033711) shared identity ranged from 78.4 to 99.8 % with other selected ToLCSDV isolates (Table 1). The highest identity (99.9 %) was found with ToLCSDV-Amaranth-Jeddah (KT033710) followed by (99.8 %) with ToLCSDV-KSA46 (HG530539) and lowest (79.1 %) identities with TYLCV-Iran isolate (KC106648). Based on the ICTV guidelines for species demarcation, at <91 % nt identity [7], the ToLCSDV-tomato-Jeddah isolate was considered as a variant of ToLCSDV-KSA46 isolate. The ToLCSDV-tomato-Jeddah isolate (KT033711) showed a range of diversity among the four main types of isolates that also group as the ToLCSDV including the Oman isolate (ToLCSDV-Mir-JN591385), at 91.2–92.1 %, the ToLCSDV-Yemen isolate (ToLCSDV-Ye-JF919733) from tobacco and tomato ranged from 89.7 to 91.5 % identity, ToLCSDV-Gezira-isolate-AY044137, at 82.9–88.5 % nt identity and ToLCSDV-Shambat-isolate AY044139 at 91.9–92.4 % identity. The highest amino acid sequence identity was observed with ToLCSDV-KSA46-isolate-HG530539 isolate in all the six proteins (V2-99.6 %, V1-99.8 %, C3-99.7 %, C2-99.8 %, C1-99.9 %, and C4-

Table 2 Pair wise (%) sequence identities of betasatellites under study (ToLCSDB-tomato-Jeddah-KT312999) with selected betasatellites at nucleotide (nt) levels

Accession Nos.	Acronyms	Location	Host	% identity
KT199104	ToLCSDB	Hadasham-Jeddah	Amaranthus	**99.8**
JF919717	ToLCYEB	Yemen	Tobacco	99.1
JF919718	ToLCYEB	Yemen	Tobacco	99.2
JF919719	ToLCYEB	Yemen	Tobacco	99.3
JF919720	ToLCYEB	Yemen	Tobacco	99.1
JF919721	ToLCYEB	Yemen	Tobacco	99.1
JF919722	ToLCYEB	Yemen	Tobacco	99.0
NC010126	TYLCβ-Om	Oman	Tomato	49.5
DQ644566	TYLCβ01-Om	Oman	Tomato	49.5
HG969297	TYLCβ-Om	Oman	Papaya	49.0
HG969296	TYLCβ-Om	Oman	Papaya	48.0
HE800552	TYLCβ-Om	Oman	Tomato	49.1
HE800551	TYLCβ-Om	Oman	Tomato	49.1
HE800550	TYLCβ-Om	Oman	Tomato	48.4
HE800549	TYLCβ-Om	Oman	Tomato	49.1
HE800548	TYLCβ-Om	Oman	Tomato	49.1
HE800547	TYLCβ-Om	Oman	Tomato	49.1
HE800546	TYLCβ-Om	Oman	Tomato	49.5
HE800545	TYLCβ-Om	Oman	Tomato	48.6
HE800544	TYLCβ-Om	Oman	Tomato	48.7
HE800543	TYLCβ-Om	Oman	Papaya	47.8
HE800542	TYLCβ-Om	Oman	Capsicum	49.3
HE800541	TYLCβ-Om	Oman	Capsicum	49.4
HE800540	TYLCβ-Om	Oman	Capsicum	49.3
KJ396939	OkLCV satDNA 10	Jordan	Tomato	46.0
NC004903	TYLCTHVβ	Thailand	Tomato	47.2
DQ641714	TYLCVVβ	Vietnam	Tomato	48.5
NC007485	TYLCMVβ	Mali	Tomato	47.6
KC677734	ToLCJaB	Japan	Tomato	47.5

ToLCYEB tomato leaf curl Yemen betasatellites, *TYLCβ-Om* tomato yellow leaf curl Oman betasatellites, *TYLCTHVβ* tomato yellow leaf curl Thailand betasatellites, *ToLCJaB* tomato leaf curl Java betasatellites

99.8 %) with respective sequences of selected begomovirus isolates from different locations (Table 1).

Since association of a betasatellites molecule with ToLCSDV has been reported earlier [17]. Separate PCR was performed to amplify the betasatellites using betasatellites specific primers [5]. An amplicon of 1.4 kb was amplified from infected samples indicating the presence of betasatellites. Comparative sequence analysis of the betasatellites of ToLCSDB-tomato-Jeddah (KT312999) with previously reported begomovirus isolates showed the highest identity (99.8 %) with tomato leaf curl betasatellites-Amaranthus-Hadasham-Jeddah-(KT199104) followed by tomato leaf curl betasatellites-Yemen (99.3 %) (JF919719) and the lowest (46.0 %) identity were found with okra leaf curl betasatellites-Jordan isolate (KJ396939) (Table 2).

The phylogenetic analysis results based on complete genome sequences with selected begomovirus isolates indicates that ToLCSDV-tomato-Jeddah isolate is a variant of ToLCSDV (Fig. 2). The ToLCSDV-tomato-Jeddah isolate formed closest cluster with an isolates of ToLCSDV-KSA46 (HG530539) and ToLCSDV-Amaranth-Jeddah (KT033710). All ToLCSDV isolates grouped on the basis of their geographical origin, e.g. either the Nile Basin or Asia and the other group was further divided by having an origin in Oman or Yemen. The phylogenetic analysis result based on betasatellites nt sequences with selected begomovirus isolates formed closed cluster with Tomato yellow leaf curl Oman betasatellites (NC_010126 and DQ644566). They are divided into two main clusters while other isolates from Yemen formed separate cluster with Oman isolates (Fig. 3). The findings suggest that

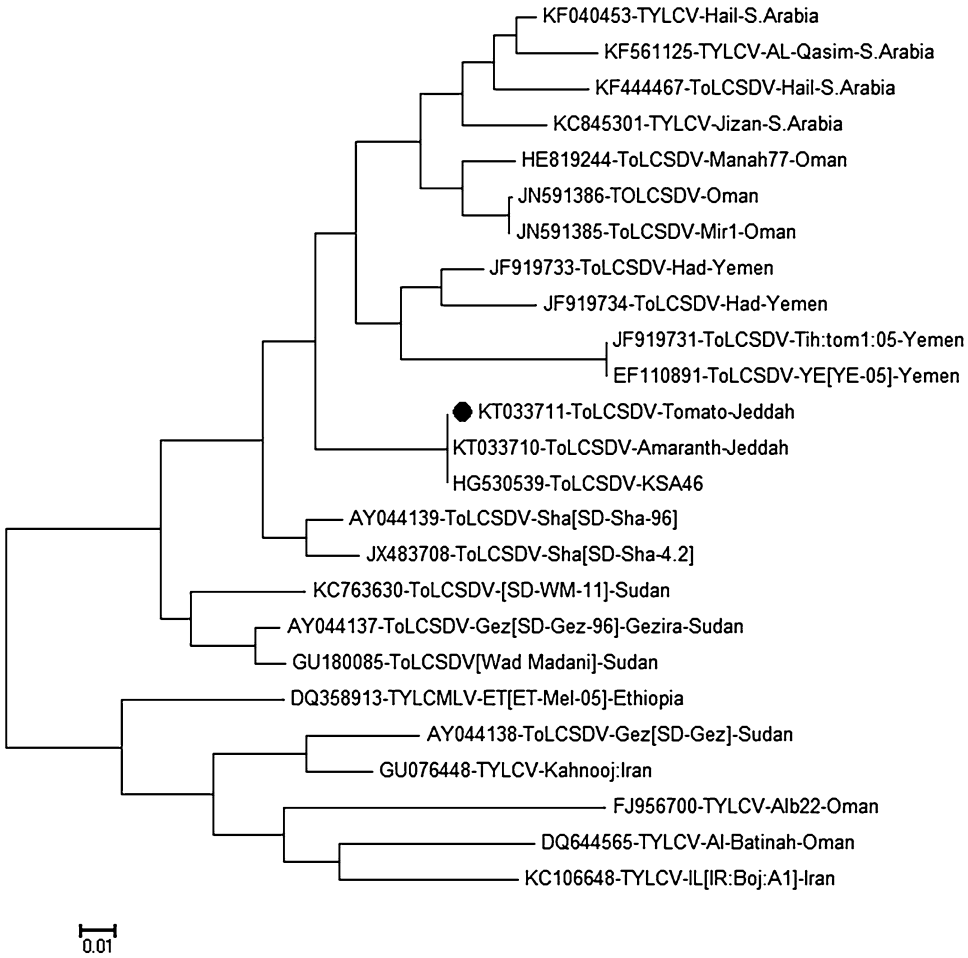

Fig. 2 Phylogenetic relationships of begomovirus based on full DNA-A genome with selected isolates

ToLCSDV isolate is endemic to the Southern region of Arabian Peninsula [16].

Recombination analysis

Recombination analysis was carried out with full genome sequences of selected begomovirus isolates using the RDP4 program [27]. Three of these algorithms, RDP (P 1.657 $\times$ 10^{-14}), GENCONV (P 1.274 $\times$ 10^{-15}), Max-Chi (P 7.395 $\times$ 10^{-14}), Chimaera (P 8.767 $\times$ 10^{-13}), Si Scan (P 3.509 $\times$ 10^{-35}) and 3 Seq (P 1.328 $\times$ 10^{-12}) showed the interspecific recombination and identified as a ToLCSDV variant. Two recombinant fragments (coordinates 2052–2752 in the C1 gene and 1954 to 2688 toward the 3′ end of the Replicase gene) were detected for ToLCSDV-Jeddah-tomato isolate, and these fragments shared high levels of sequence identity with ToLCSDV (GU180085; 93.1 % similarity). ToLCSDV-WM-Sudan-GU180085 was indicated as the major parent, and ToLCSDV-WM: 11-Sudan (KC763630) minor parent and TYLCV-Al Batinah-Oman (DQ644565) was found as recombinant isolate but the actual recombinant may be the ToLCSDV-WM-Sudan-GU180085. The recombination analysis results suggest that the ToLCSDV-tomato-Jeddah isolate evolved either from ToLCSDV or TYLCV isolate by recombination (Table 3).

Discussion

Tomato is an important agricultural crop grown globally and Saudi Arabia produces about 529.8 tons of tomatoes per year for local consumption and tomato is considered as the biggest vegetable crop grown under green houses in the Kingdom of Saudi Arabia [13]. Globally, the begomovirus emergence over the last 20–30 years has become the most important groups of plant viruses affecting vegetable crop production due to increase of whitefly vector population in the tropics and subtropics and tomato leaf curl or tomato yellow leaf curl has become the most devastating viral disease worldwide [11]. The expansion and intensification of tomato cropping favored the increased populations of whiteflies (*B. tabaci)* with wider expansion of leaf curl disease incidence in tomato crops. For the past two

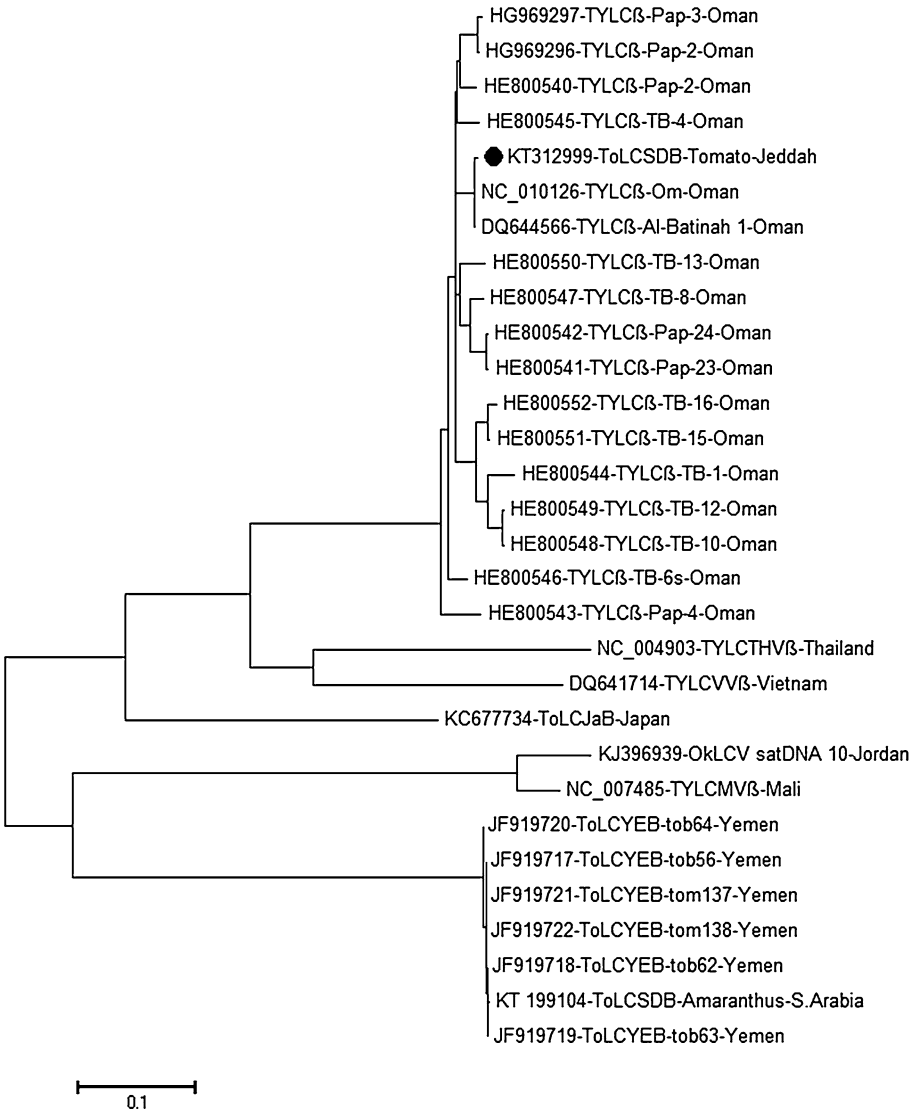

Fig. 3 Phylogenetic relationships of begomovirus based on full betasatellites genome with selected isolates

decades, tomato production in Arabian Peninsula and Nile Basin has been affected by severe leaf curl disease caused by begomovirus [1, 8, 16]. The presence of begomovirus and association of ToLCSDV causing leaf curl disease of tomato in Gezira, Sudan has been cloned and sequenced in 1996 [14]. Currently, many begomoviruses have been reported to be associated with leaf curl disease of tomato in Arabian Peninsula and Nile Basin such as ToLCSDV, TYLCV, ToLCV-OM and OLCOMV, associated with betasatellites [1, 2, 4, 15–17, 19–22].

In this study, the characterization of begomovirus association with leaf curl disease of tomato based on virus detection, whitefly transmission, sequence analysis, phylogenetic relationships, recombination analysis and genetic diversity were performed and causative agent was identified as a variant of ToLCSDV circulating and causing leaf curl disease of tomato in the Kingdom of Saudi Arabia. The ToLCSDV-tomato-Jeddah-isolate analyzed here that have been spread throughout the western region and Arabian Peninsula either through plant material moved by human activities and/or by the endemic whitefly vectors transmission. The analysis included field isolates collected from Jeddah, Saudi Arabia together with selected full-length begomovirus genome sequences available in the GenBank database that includes ToLCSDV, TYLCV and ToLCV respectively from various regions.

The causative agent was identified by PCR and efficiently transmitted to healthy tomato seedlings and the full genome nucleotide (nt) and betasatellites nt identity ranged from 78.9 to 99.9 % with tomato leaf curl Sudan Virus isolates reported earlier from Saudi Arabia, Oman and Yemen. The phylogenetic analysis results based on full genome formed closed cluster with ToLCSDV isolates while betasatellites formed close cluster with TYLCV. The

Table 3 Recombination analysis of tomato leaf curl Sudan virus isolate-tomato-Jeddah using the RDP 4.0

Break point positions		Minor parent	Major parent	Detection methods					
Start	End			RDP	GENCONV	MaxChi	Chimaera	SiScan	3 Seq
2052	2752	ToLCSDV-WM:11-Sud (KC763630)	ToLCSDV-WM-Sud-GU180085	1.657×10^{-14}	1.274×10^{-15}	7.395×10^{-14}	8.767×10^{-13}	3.509×10^{-35}	1.328×10^{-12}
1954	2688	TYLCV-Boj: Al-Iran (KC106648)	ToLCSDV-WM-Sud-GU180085	2.154×10^{-14}	1.247×10^{-15}	5.145×10^{-14}	8.429×10^{-13}	3.183×10^{-35}	1.239×10^{-12}

recombination pattern analysis results by using RDP also support the findings as ToLCSDV-tomato-Jeddah isolate genome showed evidence of recombination within ToLCSDV-WM-Sudan-GU180085 as major parent, and ToLCSDV-WM:11-Sudan (KC763630) as minor parent and TYLCV-AlBatinah-Oman (DQ644565) was found as recombinant isolate. On the basis of recombination pattern analysis using published sequences of tomato-infecting begomoviruses, the mean genomic substitution rate was found to be 2.88×10^{-4} nucleotide substitutions per site per year (subs/site/year) which could be the result of frequent recombination within the viral genomes [28]. However, as indicated, there is evidence for considerable recombination between the members of the distinct far East Asian clade of begomoviruses in Japan, such that tomato-infecting isolates have been detected within each of the sub-clades [28]. It is more than likely that ToLCSDV isolate circulating in Kingdom of Saudi Arabia has originated either from Yemen or Oman, as the studied virus being the closest relative to the Saudi Arabian, Oman and Yemen isolates. In Oman tomato leaf curl disease was first identified in 1993 but the etiology of disease was confirmed recently and the natural occurrence of ToLCSDV has been reported in Oman [20, 22]. In Yemen, tomato leaf curl virus is associated with a betasatellites that has only been identified and known as Tomato yellow leaf curl Yemen betasatellites [16]. On the other side vast harsh desert condition separates the Yemen from Oman and Saudi Arabia and this is important and strong barriers for whiteflies and virus movement. It is known that TYLCV and ToLCSDV isolates has been spread and circulating in the Kingdom [17].

It is obvious that the expansion and intensification of cropping systems favors the emergence and increase level of whitefly population and emergence of begomovirus variants and acquisition of satellite DNA molecules with more aggressive or crop-adapted characteristics due to mutation, recombination, pseudo-recombination, movement of infected plant materials, introduction of tolerant and susceptible tomato cultivars and climatic condition in the localized regions also promoted the favorable condition for the whiteflies vector and allowed the spread of the viruses [17, 18]. Based on results obtained from sequence identity, phylogenetic and recombination analysis using full genome, this study concluded that the identified virus is a variant of ToLCSDV reported from Sudan, Yemen and Arabian Peninsula causing severe leaf curl disease of tomato in Jeddah, Kingdom of Saudi Arabia.

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References

1. Ajlan AM, Ghanem GAM, Abdulsalam KS. Tomato yellow leaf curl virus (TYLCV) in Saudi Arabia: identification, partial characterization and virus–vector relationship. Arab J Biotech. 2007;10:179–92.
2. Akhtar S, Khan AJ, Singh AK, Briddon RW. Identification of a disease complex involving a novel monopartite begomovirus with beta-and alpha satellites associated with okra leaf curl disease in Oman. Arch Virol. 2014;159:1199–205.
3. Al-Saleh MA, Al-Shahwan IM, Brown JK, Idris AM. Molecular characterization of a naturally occurring interspecific recombinant begomovirus with close relatives widespread in southern Arabia. Virol J. 2014;11:103.
4. Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K, Stanley J, Zafar Y, Malik KA, Markham PG. Identification of DNA components required for induction of cotton leaf curl disease. Virology. 2001;285:234–43.
5. Briddon RW, Bull SE, Mansoor S, Amin I, Markham PG. Universal primers for the PCR-mediated amplification of DNA b, a molecule associated with some monopartite begomoviruses. Mol Biotech. 2002;20:315–8.
6. Briddon RW, Bull SE, Amin I, Idris AM, Mansoor S, Bedford ID, Dhawan P, Rishi N, Siwatch SS, Abdel-Salam AM. Diversity of DNA β: a satellite molecule associated with some monopartite begomoviruses. Virology. 2003;312:106–21.
7. Brown JK, Zerbini FM, Castillo JN, Moriones E, Sobrinho RR, Silva JCF, Olive EF, Briddon RW, Zepeda CHN, Idris A, Malathi VG, Martin DP, Bustamante RR, Ueda S, Varsani A. Revision of begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol. 2015;60:1593–619.
8. Duffy S, Holmes EC. Phylogenetic evidence for rapid rates of molecular evolution in the single-stranded DNA begomovirus Tomato yellow leaf curl virus. J Virol. 2008;82:957–65.
9. Group GS. New species and revised taxonomy proposal for the genus Begomovirus (*Geminiviridae*): phylogenetic and pairwise distance analysis using the same approach as implemented for the genera *Mastrevirus* and *Curtovirus* in the same family, vol. 2014; ICTV.
10. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.
11. Hanssen IM, Lapidot M, Thomma BPHJ. Emerging viral diseases of tomato crops. Mol Plant-Microbe Interact. 2010;23:539–48.
12. Hosseinzadeh MR, Bakhsh MS, Osaloo SK, Brown JK. Phylogenetic relationships, recombination analysis, and genetic variability among diverse variants of tomato yellow leaf curl virus in Iran and the Arabian Peninsula: further support for a TYLCV center of diversity. Arch Virol. 2014;159:485–97.
13. http://www.factfish.com/statistic-country/saudi+arabia/tomatoes,+production+quantity. Accessed 30 Dec 2015.
14. Idris AM, Brown JK. Evidence for interspecific-recombination for three monopartite begomoviral genomes associated with the tomato leaf curl disease from central Sudan. Arch Virol. 2005;150:1003–12.
15. Idris AM, Shahid MS, Briddon RW, Khan AJ, Zhu JK, Brown JK. An unusual alpha satellite associated with monopartite begomoviruses attenuates symptoms and reduces betasatellite accumulation. J Gen Virol. 2011;92:706–17.
16. Idris AM, Abdullah NM, Brown JK. Leaf curl diseases of two Solanaceous species in Southwest Arabia are caused by a monopartite begomovirus evolutionarily most closely related to a species from the Nile Basin and unique suite of betasatellites. Virus Res. 2012;169:296–300.
17. Idris AM, Al-Saleh Piatek MJ, Al-Shahwan I, Ali S, Brown JK. Viral metagenomics: analysis of begomoviruses by illumina high-throughput sequencing. Viruses. 2014;6:1219–36.
18. Kenyon L, Tsai WS, Shih SL, Lee LM. Emergence and diversity of begomoviruses infecting Solanaceous crops in East and Southeast Asia. Virus Res. 2014;186:104–13.
19. Khan AJ, Idris AM, Al-Saady NA, Al-Mahruki MS, Al-Subhi AM, Brown JK. A divergent isolate of tomato yellow leaf curl virus from Oman with an associated DNA beta satellite: an evolutionary link between Asian and the Middle Eastern virus-satellite complexes. Virus Genes. 2008;36:169–76.
20. Khan AJ, Akhtar S, Singh AK, Briddon RW. A distinct strain of tomato leaf curl Sudan virus causes tomato leaf curl disease in Oman. Plant Dis. 2013;97:1396–402.
21. Khan AJ, Akhtar S, Al-Zaidia AM, Singh AK, Briddon RW. Genetic diversity and distribution of a distinct strain of Chili leaf curl virus and associated betasatellite infecting tomato and pepper in Oman. Virus Res. 2013;177:87–97.
22. Khan AJ, Mansoor S, Briddon RW. Oman: a case for a sink of begomoviruses of geographically diverse origins. Trends Plant Sci. 2014;19:67–70.
23. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol. 2015;1:vev003.
24. Muhire BM, Varsani A, Martin DP. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One. 2014;9:e108277. doi:10.1371/journal.pone.0108277.
25. Porebski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep. 1997;15:8–15.
26. Sivalingam PN, Malathi VG, Varma A. Molecular diversity of the DNA-beta satellites associated with tomato leaf curl disease in India. Arch Virol. 2010;155:757–64.
27. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA, 6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9.
28. Ueda S, Onuki M, Hanada K, Takanami Y. Unique grouping of the Far East Asian begomovirus complex based on sequence analyses of the DNA-A genome and associated DNA satellite molecules isolated from tomato, honeysuckle and Eupatorium plants in Japan. Arch Virol. 2008;153:417–26.
29. Varsani A, Navaz-Castillo J, Moriones E, Hernández-Zepeda C, Idris A, Brown JK, Zerbini FM, Martin DP. Establishment of three new genera in the family *Geminiviridae*: *Becurtovirus*, *Eragrovirus and Turncurtovirus*. Arch Virol. 2014;159:2193–203.
30. Wener ZH. Importance of the tomato. http://www.agrisupportonline.com/Articles. Accessed 30 Dec 2015.
31. Xie Y, Wu P, Liu P, Gong H, Zhou X. Characterization of alphasatellites associated with monopartite begomovirus/betasatellite complexes in Yunnan, China. Virol J. 2010;7:178.