Saudi Journal of Biological Sciences 27 (2020) 222–228

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Genetic diversity of begomoviruses infecting tomato plant in Saudi Arabia

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ARTICLE INFO

Article history:
Received 24 July 2019
Revised 21 August 2019
Accepted 22 August 2019
Available online 22 August 2019

Keywords:
S. lycopersicum
Viral disease
Begomoviruses
Genetic diversity
Saudi Arabia

ABSTRACT

Tomato is known as a highly valuable crop and grown worldwide for various uses. The cultivation and tomato production severely affected globally by several diseases caused by various pathogens. Begomoviruses causes yellow mosaic and leaf curl disease of tomato in the tropical, subtropical, temperate, and semi-arid regions. In Saudi Arabia, the tomato production adversely affected by disease caused by begomoviruses known as TYLCV and ToLCSDV. In this study, the pathogen was identified by Polymerase Chain Reaction using virus-specific primers and transmitted by whiteflies to healthy tomato seedlings. In a field survey, the tomato plants were exhibiting symptoms like viral infection. The infected leaf was randomly collected from various fields of tomato growing areas like Jeddah, Makkah, Tabuk, and Hail. The full-length viral genome was amplified by Rolling Circle Amplification technology (RCA) while betasatellites were amplified by PCR using universal betasatellites primers. The full-length viral genome (~2.7 kb) and betasatellites (~1.4 kb) 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 sequences showed the highest identity with an isolate of ToLCSDV and TYLCV. The nucleotide similarity and phylogenetic relationship showed the closest cluster with ToLCSDV and TYLCV. The data generated in this study elucidate that the causal organism is a variant of either TYLCV or ToLCSDV. The provided information from this study will be highly valuable for researchers and vegetable growers not only in Saudi Arabia but also in Arabian Peninsula.

1. Introduction

The disease of tomato caused by whitefly-transmitted begomoviruses has now become an important concern for tomato growers with significant economic loss globally (Moriones and Navas-Castillo 2000; Hanssen et al., 2010; Brown et al., 2012; Basak 2016). The begomoviruses fall under the family *Geminiviridae* with nine genera (Varsani et al., 2014, 2017; Zerbini et al., 2017). This is known as the largest group transmitted by whitefly vector which has now become a major group of viruses causing diseases in many crops worldwide (Varma et al., 2011). The begomovirus infection to multiple crops has already been reported from Asia and Southeast Asia and the Arabian Peninsula (Kenyon et al., 2014). Approximately forty different plant virus diseases have been described on more than thirty plant species in Arabian Peninsula (Al-Shahwan, 2003; Idris et al., 2012; Hosseinzadeh et al., 2014; Sohrab and Daur, 2018). The association of begomovirus with various crops such as, Amaranthus, Beans, Chili, Corchorus, Cucurbits, Mint, Okra, Pumpkin, Tobacco and Tomato have been reported so far from Arabian peninsula and the associated begomoviruses are known as TYLCV, ToLCOMV, ToLCSDV, ToLCSDV-Om, ChiLCV, OLCOMV, SqLCV, and BDMV-SA

Abbreviations: TYLCV, tomato yellow leaf curl virus; ToLCOMV, tomato leaf curl Oman virus; ToLCSDV, tomato leaf curl Sudan Virus; ToLCSDV-Om, tomato leaf curl Sudan Virus-Oman; ChiLCV, chili leaf curl virus; OLCOMV, okra leaf curl Oman virus; SqLCV, squash leaf curl virus; BDMV-SA, bean dwarf mosaic virus-Saudi Arabia.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.08.015
1319-562X/

(Al-Shahwan et al., 1997, 2002; Ghanem et al., 2003; Idris and Brown, 2005; Ajlan et al., 2007; Khan et al., 2008, 2014; Fazeli et al, 2009; Idris et al., 2011, 2012, 2014; Mohamed et al., 2012; Khan et al., 2013a, 2013b; Al-Saleh et al., 2014a, 2014b; Akhtar et al., 2014; Hosseinzadeh et al., 2014; Sohrab, 2016; Sohrab et al., 2016a-e; Sohrab, 2017; Sayed, 2017; Al-Shahwan et al., 2017; Sohrab and Daur, 2018a,b). In this study, the association of begomovirus infection to tomato disease has been provided based on virus identification, sequencing and genetic diversity. The information provided about the virus associated with tomato disease found to be the variant of either ToLCSDV or TYLCV from Saudi Arabia.

2. Materials and methods

2.1. Collection of leaf samples and virus transmission

A random survey was conducted in many tomato fields at various places like Jeddah, Makkah, Tabuk and Hail, Saudi Arabia for the collection of the samples. The top emerging tomato leaves were harvested by using hand gloves and kept in self-sealing plastic bags and immediately stored in ice. The collected samples were further processed and stored at −80 °C for further use. For whitefly transmission, the healthy whiteflies were raised on Clatoria plants and used for virus inoculation. A group of adult whiteflies (minimum 25) was feed on infected tomato leaf up to 24 h and released on 7–10 days old healthy tomato seedlings. The viruliferous whiteflies were killed by insecticidal spray to protect the spread of viral disease to other crops. The inoculated seedlings were daily observed for symptom appearance till thirty days post inoculation.

2.2. Virus Identification, Cloning, and sequencing

The infected tomato leaves (100 mg) were used to purify the DNA by using DNeasy plant mini kit as per kits instructions (Qiagen Inc.). The purified DNA was further used for virus detection by PCR. The causal organism was identified by PCR using TYLCV (F) TAAGGGCCCGTGATTATGTTG (R) TTTATTAATTCGATATTGA ATCAT(TYLCV-KT033715) and ToLCSDV (F)'GACTTGACGTCAGAG CTGGAT (R) CCAGCTCTGACGTCAAGTCAT (Sohrab and Daur, 2018a,b). The PCR was performed at 94 °C-120 s for 1cycle, 94 °C-60 s, 50 °C-60 s, 72 °C-60 s, for 35 cycles and the final extension was given for 5 min at 72 °C. The PCR mixture consisted of 2.5 units of *Taq* DNA polymerase (MBI;Fermentas),5μl of 10 × PCR buffer, 0.5 μl of 10 mM dNTPs and 0.5 μl (10 pmol) of forward and reverse primers. Total reaction volume was made up of 50 μl using sterile distilled water.

An amplicon of betasatellite (~1.4 kb) also obtained by using specific PCR primers (Briddon et al., 2002). The complete genome was amplified by RCA technology using with TempliPhi 100 Amplification Kit (GE Healthcare, USA) as per kit protocol. The amplicon was digested with restriction enzymes such as *Eco*RI and *Eco*RV. The restricted products were purified, cloned into PUC-18 cloning vector and sequenced bi-directionally and further analyzed to identify the genetic diversity.

2.3. Analysis of genetic diversity

The generated sequences in this study were assembled and aligned. The nucleotide sequence similarity was analyzed by BioEdit (v7.0.5) and CLUSTALW software program. The full-genome and betasatellites sequences were used to analyze the genetic diversity and phylogenetic relationship using MEGA7 (Kumar et al., 2016).

3. Results

3.1. Collection of samples and virus transmission

During survey, the infection of tomato crop was observed with typical leaf curl as well as yellow mosaic disease symptoms in various tomato field (Fig. 1A and B) which provided a clue about the begomoviral infection. Total eighteen samples were collected from tomato crops by using top emerging leaves at various locations in this study for virus detection, full-genome cloning, sequencing, analysis, and phylogenetic relationships. The virus inoculated tomato plants were transferred to screen house and daily observed for symptoms expression until thirty days. The causative organism was transmitted to inoculated heathy tomato seedlings and expressed comparable leaf curl symptoms as in the field after 18–23 days post-inoculation. Initially, the yellow dots appeared on infected tomato leaves and gradually fused and formed yellow mosaic followed by leaf curling symptoms in newly emerged top leaves and finally resulted in stunted plant growth.

3.2. Virus detection and sequencing

The begomovirus infection was identified in infected leaf samples from various locations by using specific PCR primers which

Fig. 1. (A)Natural infection of tomato plant with leaf curl symptom. (B) Natural infection of tomato plant with yellow mosaic symptoms.

produced an amplicon of ~750 bp (Fig. 2). Total of seventeen samples was found positive by specific primers. No positive amplification was observed in non-symptomatic samples. Restriction of Rolling Circle Amplified amplicon with *Eco*RI provided ~2.7 kb product and further analyzed by cloning into PUC-18 vector. Total eight full-length and eight betasatellites amplicons were cloned and completely sequenced from samples collected from various locations and a BLAST search was performed. Based on the blast result, the sequences showed the highest nucleotide sequences similarity with ToLCSDV, TYLCV, ToLCSDB, and TYLCB. Based on the sequence similarities they were tentatively designated as ToLCSDV-tomato-Jeddah isolates and TYLCV-tomato-Jeddah isolates (Table 1).

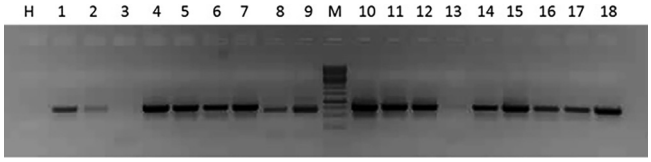

Fig. 2. PCR detection of begomovirus using Coat Protein gene specific primers. H: Healthy sample, M: 1 Kb ladder. 1–18 Field collected tomato leaf samples.

3.3. Analysis of genetic diversity

The generated nucleotide sequences were used for genetic diversity and phylogenetic relationship analysis by using BioEdit (v7.0.5) and MEGA 7 software. The multiple sequence alignment

Table 1
Sequence identity matrix of TYLCV and ToLCSDV.

TYLCV				ToLCSDV			
Accession No	Hosts	Locations	% Identity	Accession No	Hosts	Locations	% Identity
KF561125	Tomato	Al-Qasim	99.8	KT033707	Tomato	Jeddah	99.9
KT728746	Tomato	Hail	92.9	KT033711	Tomato	Jeddah	99.8
KF040453	Tomato	Hail	98.7	KT728747	Tomato	Hail	99.4
KT033715	Tomato	Jeddah	92.8	KT728748	Tomato	Hail	99.4
KT728752	Tomato	Tabuk	92.9	KT728749	Squash	Hail	99.4
KC845301	Tomato	Jizan	93.2	KT760556	Tomato	Tabuk	99.7
KT033706	Tomato	Hadasham	92.9	KT033708	Tomato	Hadasham	99.8
KT033709	Tomato	Hadasham	92.9	KT033714	Corchorus	Hadasham	99.8
KF561126	Tomato	Al-Qasim	98.9	KT760555	Squash	Hadasham	98.9
KF435137	Tomato	Al-Ahsaa	99.7	KT033712	Squash	Hadasham	98.8
KT033713	Cucumber	Hadasham	92.9	KT728750	Squash	Tabuk	99.4
KT355023	Corchorus	Jeddah	92.8	KT728751	Squash	Tabuk	99.3
MG571546	Mentha	Jeddah	92.3	KT033710	Amaranthus	Jeddah	99.9
KF435136	Pepper	Alahsaa	98.1	HG530539	Tomato	Usfan	99.8
HE819240	Pepper	Oman	79.5	KF444467	Bean	Hail	89.7
KF229725	Tomato	Oman	79.3	JF919733	Tobacco	Yemen	91.6
JN604488	Tomato	Oman	78.3	JF919734	Tobacco	Yemen	90.5
KC106648	Tomato	Iran	78.9	JN591386	Tomato	Oman	92.1
AJ132711	Tomato	Iran	79.9	HE819244	Tomato	Oman	91.2
AY594174	Tomato	Egypt	79.5	JN591386	Tomato	Oman	92.1
EF107520	Tomato	Egypt	76.1	AY044139	Tomato	Sudan	92.2
EF054894	Tomato	Jordan	82.4	JX483708	Tomato	Sudan	91.8
GQ861426	Tomato	Jordan	72.0	GU180085	Tomato	Sudan	88.9
JQ354991	Tomato	Iraq	78.8	JF919731	Tomato	Yemen	89.8
AY044138	Tomato	Sudan	81.5	EF110891	Tomato	Yemen	98.8
DQ358913	Tomato	Ethiopia	83.3	KT760555	Squash	Hadasham	99.4

Table 2
Sequence Identity matrix of TYLCB and ToLCSDB.

TYLCB				ToLCSDB			
Accession No	Hosts	Locations	% Identity	Accession No	Hosts	Locations	% Identity
KT760554	Cucumber	Jeddah	99.5	KT312999	Tomato	Jeddah	96.4
KT153252	Amaranthus	Hadasham	99.5	KT728731	Tomato	Hadasham	99.7
KT728740	Tomato	Tabuk	99.7	KT728735	Tomato	Hail	99.0
JF919721	Tomato	Yemen	94.1	KT728738	Tomato	Tabuk	99.2
JF919722	Tomato	Yemen	94.1	KT728729	Squash	Jeddah	99.3
DQ644567	Tomato	Oman	98.7	KT728730	Cucumber	Jeddah	99.6
KT728733	Cucumber	Hadasham	96.0	KT180308	Squash	Hadasham	96.5
KT180307	Cucumber	Jeddah	96.1	KT728736	Squash	Hail	99.7
JF919717	Tobacco	Yemen	94.1	KT728737	Squash	Hail	99.6
JF919718	Tobacco	Yemen	94.1	KT728739	Squash	Tabuk	99.0
NC_010126	Tomato	Oman	98.8	JF919717	Tobacco	Yemen	99.4
DQ644566	Tomato	Oman	98.7	JF919718	Tobacco	Yemen	98.6
HG969297	Papaya	Oman	90.1	JF919719	Tobacco	Yemen	98.8
HG969299	Ocimum	Oman	90.0	JF919720	Tobacco	Yemen	98.8
KT180306	Corchorus	Jeddah	90.9	JF919721	Tomato	Yemen	98.6
KT355022	Corchorus	Jeddah	98.3	JF919722	Tobacco	Yemen	98.6
KT355021	Tomato	Jeddah	98.5	KT199104	Amaranthus	Hadasham	98.6
DQ641714	Tomato	Vietnam	67.1	KJ396939	Tomato	Jordan	53.6
MG571547	Mentha	Jeddah	98.5	KC677734	Tomato	Japan	46.7
KU248483	R. Gourd	Jeddah	99.4	EU189147	Tomato	Vietnam	46.8

of the full-genome and associated betasatellites obtained from various clones; a high level of similarities was observed with selected begomovirus sequences from various locations. The betasatellites sequences generated in this study were highly similar to ToLCSDB-Oman and Yemen isolates. The complete genome sequences of TYLCV generated in this work was found to be more like TYLCV isolates.

3.4. Genetic variability of ToLCSDV and TYLCV infecting tomato plant

The complete genome of ToLCSDV and TYLCV and their associated betasatellites were used to identify the sequence identity/diversity with selected begomovirus isolates. Total eight full-genome (~2.7 kb) sequences were generated, assembled and analyzed from collected tomato samples. The sequences showed a greater identity with ToLCSDV and TYLCV. The full-genome nucleotide sequence similarity of an isolate of TYLCV-Tom-Jeddah was performed using with other begomovirus sequences and the identity was ranged from 99.8% to 72.0%. Five begomovirus isolates infecting tomato from Al-Qasim (99.8%) (TYLCV-KF561125), Al-Ahsaa (99.7%) (TYLCV-KF435137), Al-Ahsaa (98.9%) (TYLCV-KF561126), Hail (98.7%) (TYLCV-KF040453), and Al-Ahsaa (TYLCV-KF435136) showed high similarity and the lowest identity (76.1%) was observed with one begomovirus isolate from Egypt (TYLCV-EF107520) (Table 1).

The full-genome sequence identity matrix of ToLCSDV isolate was analyzed with selected begomoviruses and the similarity was varied from 99.9% to 90.5%. The highest similarity (99.9%) was observed with ToLCSDV-KT033707-Tomato and ToLCSDV-KT033710-Amaranthus-Jeddah and 99.8% with three isolates (KT033711-Tom-Jeddah, ToLCSDV-KT033711-Tom-Hadasham and, ToLCSDV-KT033714-Corchorus-Jeddah). The lowest similarity (90.5%) was observed with an isolate from Yemen (FJ919734-Tobacco). Interestingly; the identity matrix was varied from 89% to 92% in most of the previously identified isolates from, Sudan, Yemen, and Oman isolates.

In this study, the betasatellites were also identified, cloned and sequenced from infected tomato plants. The sequences obtained from TYLCB and ToLCSDB were used for sequence identity matrix analysis and one isolate from tomato (TYLCB-KT728740) showed the highest (99.7%) similarity followed by three isolates from Cucumber, Amaranthus and Ridge gourd (TYLCB-KT760554, KT153252, KU248483) showed 99.5–99.4% similarities. The isolate from Oman (DQ644566, DQ644567, NC010126, HG969297, and HG969299) showed 98.7–90.0% similarity while the isolates from Yemen showed 94.1% similarity. The lowest similarity (67.1%) was observed with an isolate from Vietnam (DQ641714). The sequence similarity of ToLCSDB-Tom-Jeddah isolate with other begomovirus was found to be 99.7–46.7%. (Table 2). The highest similarity (99.7099.6%) was observed with an isolate from Hadasham (KT728731, KT728736, KT728730 and KT 728737). The sequence similarity with an isolate from Yemen found to range from 99.4% to 98.6%. The lowest (46.7–46.8%) similarity was observed with an isolate from Japan (KC677734) and Vietnam (EU189147).

The results of sequence diversity and phylogenetic relationship from complete nucleotide sequences of TYLCV and ToLCSDV were analyzed with selected begomoviruses sequences. The TYLCV isolate from Jeddah identified from tomato closely clustered with TYLCV-Corchorus (KT335023) and TYLCV-Mentha isolates (MG571546). Interestingly, one begomovirus isolates isolated from Hail infecting green bean (ToLCSDV-KF44467) formed the closed cluster with TYLCV isolates reported from Al-Qasim. An extra cluster was also observed with begomovirus isolates reported from Saudi Arabia, Sudan, Ethiopia, Egypt, Iran, and Jordan. Interestingly, one isolate of TYLCV from Al-Ahsaa formed a closed cluster with Sudan, Ethiopia, and Jordan (Fig. 3). The ToLCSDV-tomato-Jeddah isolate clustered with ToLCSDV-KSA46 (HG530539), ToLCSDV-Corchorus from Jeddah (KT033714) and ToLCSDV-Amaranthus (KT033710). Interestingly, four isolates from Yemen and three from Oman formed a separate cluster while one isolate from Hail (KT728747) and further clustered with begomovirus reported from Squash from Tabuk and Hail (Fig. 3). The phylogenetic analysis results based on the selected TYLCB and ToLCSDB formed multiple clusters with various isolates. The TYLCB formed closed clusters with an isolate from Cucumber, Corchorus, Mentha and tomato crops reported from Saudi Arabia. Interestingly, an isolate from Japan and Vietnam clustered to an isolate identified of Amaranthus and Ridge gourd crops from Saudi Arabia (Fig. 4).

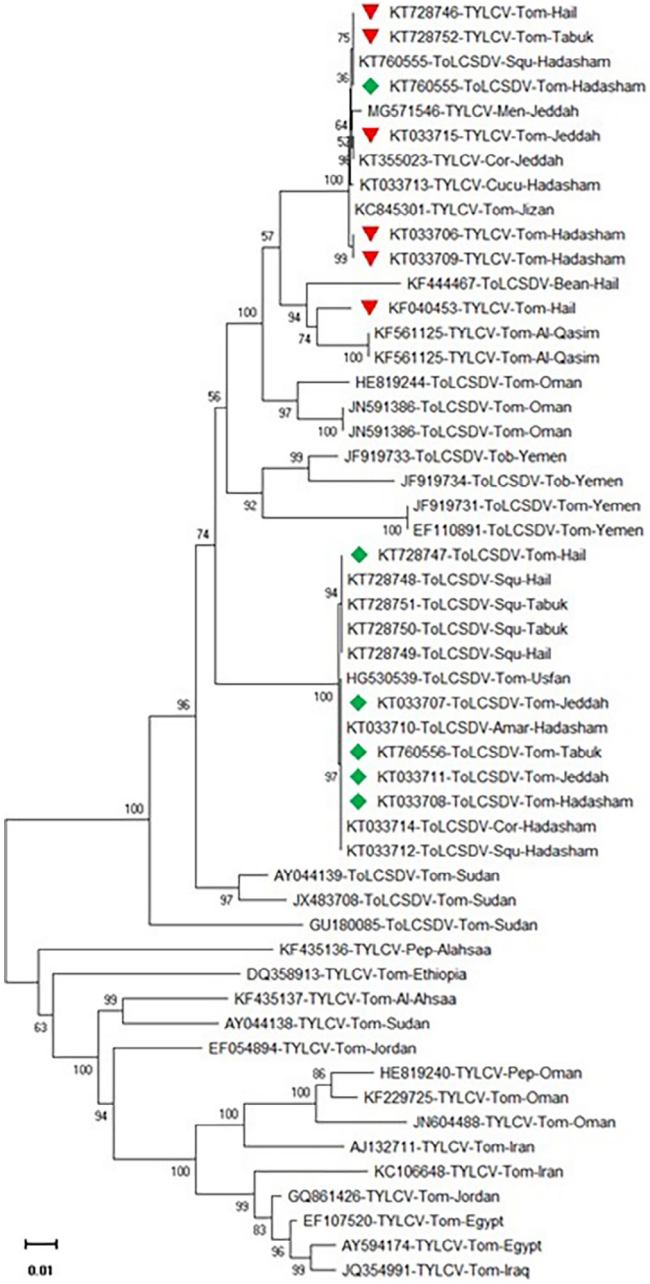

Fig. 3. Phylogenetic relationships of TYLCV and ToLCSDV based on full genome.

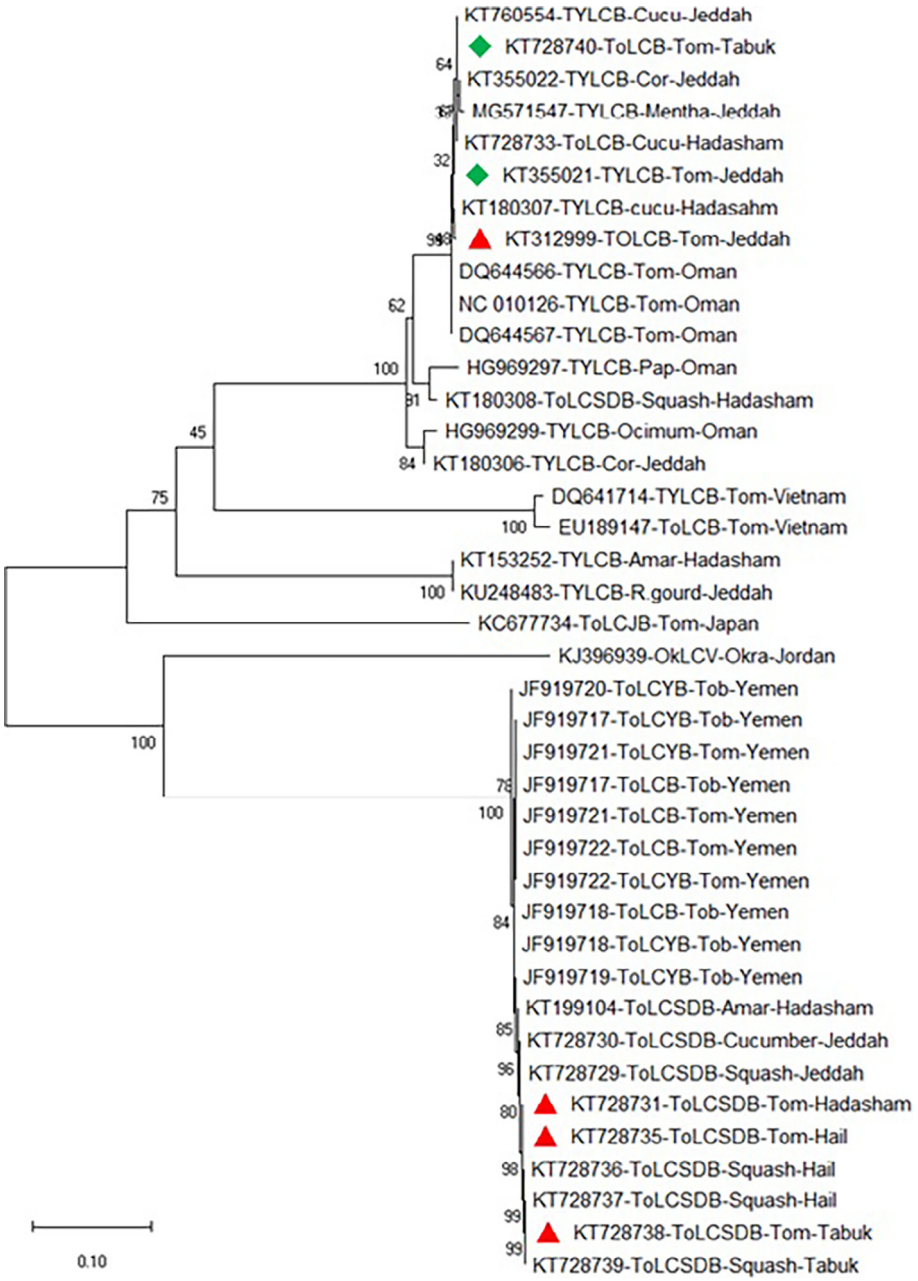

Fig. 4. Phylogenetic relationships of TYLCB and ToLCSDB based on betasatellite genome.

4. Discussion and conclusion

Tomato is well known as a vegetable crop globally. The tomato cultivation adversely affected by multiple diseases. Viral diseases are the most common including mosaic and leaf curling followed by severe stunting disease. Many cultivated and weed crops are known to be infected with begomoviruses in the Kingdom with high disease incidence rate. In Saudi Arabia and Arabian peninsula, the tomato cultivation takes place at smaller scale for local consumption and their cultivation is severely affected since two decades by begomovirus associated disease (Hosseinzadeh et al., 2014; Ajlan et al., 2007; Khan et al., 2008, 2013a; Idris et al., 2011, 2012, 2014; Al-Saleh et al., 2014a; Akhtar et al., 2014).

In this study, an information has been provided about the genomic diversity of begomovirus associated disease of tomato in the Kingdom. The generated information was resulted from field survey, virus identification, full-length viral genome amplification, sequencing followed by analysis of genetic variability and phylogenetic relationship of TYLCV and ToLCSDV isolates. The diversity and homology also reflected in the phylogenetic relationship analysis as different clusters were formed with selected begomovirus isolates. There are some begomovirus isolates formed separate clusters even though they were identified from Oman, Yemen, Sudan, Ethiopia, and Iran. A similar pattern was also observed when full-genome nucleotide sequences of betasatellites from TYLCB and ToLCSDB were analyzed by sequence identity matrix and phylogenetic relationship. The molecular diversity and role of betasatellites in disease severity and symptoms expression, as well as emergence of new virus strains/isolates and causing disease to multiple crops, have already been reported from many regions. It is well recognized that genetic recombination plays a significant role in the diversification and evolution of Geminiviruses. Recombination has been documented to occur between Geminivirus, between betasatellites, alphasatellites and between

helper viruses and betasatellites (Hosseinzadeh et al., 2014; Sohrab et al., 2016c; Sohrab and Daur, 2018b).

The results generated in this work indicate that there are some variant or recombinant strains of begomoviruses have emerged due to frequent recombination in the Kingdom and have introduced either from Yemen or Oman as it was observed in the genome size variations, sequence similarity in either full-genome or betasatellites. The betasatellites genome diversity has been reported earlier (Briddon et al., 2004). The begomovirus can cause disease to the new crops in broader region with their extended hosts. The genetic diversity of full genome as well as betasatellite genome with selected begomoviruses reported from Arabian Peninsula also provided evidence for emergence and spread of begomoviral disease to many crops in multiple locations (Idris et al., 2012).

The strategies for development of durable disease management against viruses require the information about genetic variability, virus evolution and host plant interaction (Garcia-Andres 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 Southeast 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. The change in the genomic sequences, presence of whiteflies' vector, climatic conditions, changing cropping system, frequent recombination and mutation of viral genome are the most significant factors for the emergence and spread of new begomovirus strains/isolates which are a serious threat to economically important crops in the Kingdom of Saudi Arabia and Arabian peninsula. As per data generated in this work, it is concluded that the causal organism is a variant of either ToLCSDV or TYLCV circulating in the Kingdom. This requires detailed genetic diversity analysis and recombination pattern study by collecting more samples from multiple locations during different cropping seasons.

Declaration of Competing Interest

Author declares no conflict of interest.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Under the grant number G:61-141-1439. The author therefore acknowledge with thanks for technical and financial support. The author is also thankful to Special Infectious Agents Unit, King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia for providing necessary research facilities.

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