Genetic diversity of begomoviruses infecting tomato plant in Saudi Arabia

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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; Hansen 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, ToLCMV, ToLCSDV, ToLCSDV-Om, ChiLCV, OLCOMV, SqLCV, and BDMV-SA.
(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 fed 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 (Qia-gen Inc.). The purified DNA was further used for virus detection by PCR. The causal organism was identified by PCR using TYLCV (F) TAAGGGCCCTGATTATGTTG (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 1 cycle, 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 EcoRI and EcoRV. 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 healthy 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
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 EcoRI 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).

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 | Accession No | Hosts  | Locations | % Identity |
|-------|--------------|--------|-----------|------------|
| KF561125 | Tomato | Al-Qasim | 99.8 |
| KT728746 | Tomato | Hail | 92.9 |
| KT040453 | Tomato | Hail | 98.7 |
| KT033715 | Tomato | Jeddah | 92.8 |
| KT037827 | Tomato | Tabuk | 92.9 |
| KC845301 | Tomato | Jizan | 93.2 |
| KT033706 | Tomato | Hadasham | 92.9 |
| KT033709 | Tomato | Hadasham | 92.9 |
| KT051126 | Tomato | Al-Qasim | 98.9 |
| KT051137 | Tomato | Al-Ahsaa | 99.7 |
| KT033713 | Cucumber | Hadasham | 92.9 |
| KT355023 | Corchorus | Jeddah | 92.8 |
| MG571546 | Mentha | Jeddah | 92.3 |
| KT435136 | Pepper | Alahsaa | 98.1 |
| HE819240 | Pepper | Oman | 79.5 |
| KT229725 | Tomato | Oman | 79.3 |
| JN604488 | Tomato | Oman | 78.3 |
| KC165648 | Tomato | Iran | 78.9 |
| AJ122711 | Tomato | Iran | 79.9 |
| AY594174 | Tomato | Egypt | 79.5 |
| EF107520 | Tomato | Egypt | 76.1 |
| EF054894 | Tomato | Jordan | 82.4 |
| Q861426 | Tomato | Jordan | 72.0 |
| JQ34991 | Tomato | Iraq | 78.8 |
| AY944138 | Tomato | Sudan | 81.5 |

| ToLCSDV | Accession No | Hosts  | Locations | % Identity |
|---------|--------------|--------|-----------|------------|
| KT033707 | Tomato | Jeddah | 99.9 |
| KT033711 | Tomato | Jeddah | 99.8 |
| KT040457 | Tomato | Hail | 99.4 |
| KT037848 | Tomato | Hail | 99.4 |
| KT037849 | Tomato | Square | 99.4 |
| KT760556 | Tomato | Tabuk | 99.7 |
| KT033708 | Tomato | Hadasham | 99.8 |
| KT033714 | Tomato | Hadasham | 99.8 |
| KT040458 | Tomato | Hail | 99.4 |
| KT037844 | Tomato | Square | 99.8 |
| KT228750 | Tomato | Square | 99.4 |
| KT228751 | Tomato | Square | 99.3 |
| KT3037110 | Amananthus | Jeddah | 99.9 |
| HE819246 | Pepper | Oman | 98.7 |
| KT919319 | Pepper | Oman | 98.9 |
| JT919314 | Pepper | Oman | 98.9 |
| KT919312 | Pepper | Oman | 98.8 |
| JT9193114 | Pepper | Oman | 98.8 |
| HE819245 | Pepper | Oman | 98.8 |
| KT9193115 | Pepper | Oman | 98.8 |
| KT9193116 | Pepper | Oman | 98.8 |
| JT9193117 | Pepper | Oman | 98.8 |
| HE819247 | Pepper | Oman | 98.8 |

Table 2
Sequence identity matrix of TYLCB and ToLCSDB.

| TYLCB | Accession No | Hosts  | Locations | % Identity |
|-------|--------------|--------|-----------|------------|
| KT760554 | Cucumber | Jeddah | 99.5 |
| KT153252 | Amananthus | Hadasham | 99.5 |
| KT728740 | Tomato | Tabuk | 99.7 |
| JF919721 | Tomato | Yemen | 94.1 |
| JF919722 | Tomato | Yemen | 94.1 |
| DQ644567 | Tomato | Oman | 98.7 |
| KT728733 | Cucumber | Hadasham | 96.0 |
| KT180307 | Cucumber | Jeddah | 96.1 |
| JP919717 | Tobacco | Yemen | 94.1 |
| JF919718 | Tobacco | Yemen | 94.1 |
| NC_010126 | Tomato | Oman | 98.8 |
| DQ644566 | Tomato | Oman | 98.7 |
| HG696297 | Papaya | Oman | 90.1 |
| HG696298 | Ocimum | Oman | 90.0 |
| KT180306 | Corchorus | Jeddah | 90.9 |
| KT355022 | Corchorus | Jeddah | 98.3 |
| KT355021 | Tomato | Jeddah | 98.5 |
| DQ641714 | Tomato | Vietnam | 67.1 |
| MG571547 | Mentha | Jeddah | 98.5 |

| ToLCSDB | Accession No | Hosts  | Locations | % Identity |
|---------|--------------|--------|-----------|------------|
| KT312999 | Tomato | Jeddah | 96.4 |
| KT728731 | Tomato | Hail | 99.4 |
| KT728735 | Tomato | Hail | 99.4 |
| KT728738 | Tomato | Tabuk | 99.5 |
| KT728729 | Cucumber | Jeddah | 99.9 |
| KT872730 | Cucumber | Jeddah | 99.6 |
| KT872732 | Cucumber | Jeddah | 99.3 |
| KT180308 | Cucumber | Jeddah | 96.5 |
| KT872736 | Cucumber | Jeddah | 99.7 |
| KT872737 | Cucumber | Jeddah | 99.6 |
| KT872739 | Tobacco | Jeddah | 99.0 |
| JT919717 | Tobacco | Yemen | 99.4 |
| JT919718 | Tobacco | Yemen | 98.6 |
| JF919719 | Tobacco | Yemen | 98.8 |
| JF919720 | Tobacco | Yemen | 98.7 |
| JF919721 | Tobacco | Yemen | 98.6 |
| JF919722 | Tobacco | Yemen | 98.6 |
| KT199104 | Amananthus | Hail | 98.6 |
| KT965399 | Tomato | Jordan | 53.6 |
| KC277734 | Tomato | Japan | 46.7 |
| 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 ToLCSDV 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).
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

Fig. 4. Phylogenetic relationships of TYLCB and ToLCSDB based on betasatellite genome.
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 (Briddion 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 (García-Andrés 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 whitefly vector are known for genetic variability and evolution among the virus populations 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 identification 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 whitefly’s 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.

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