Saudi Journal of Biological Sciences 27 (2020) 3060–3064

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Molecular diagnosis of begomovirus associated with Chilli leaf curl disease in Jeddah, Saudi Arabia

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ARTICLE INFO

Article history:
Received 26 July 2020
Revised 6 September 2020
Accepted 6 September 2020
Available online 12 September 2020

Keywords:
Chilli leaf curl disease
Begomovirus
TYLCV
PCR
Saudi Arabia

ABSTRACT

Chilli (*Capsicum annum* L.) is well known as 'wonder spice'. This is a very valuable cash crop grown as a vegetable globally. Chilli leaf curl disease is a major threat and global concern for the cultivation of Chilli by farmers and growers. In this work, the molecular diagnosis, genetic diversity, phylogenetic relationship, and begomovirus association with Chilli leaf curl disease have been discussed. The infected leaves were randomly harvested from the Chilli field, at Jeddah, Saudi Arabia. A group of begomovirus vector, whiteflies were also observed on the Chilli crop and infected weeds growing in the neighboring field. The begomovirus was confirmed by coat protein gene specific primer, dot blot hybridization, sequencing and sequence analysis. The full coat protein gene was found to have 774 nucleotides. The nucleotide sequences analysis shared the highest identity with Tomato yellow leaf curl virus reported earlier infecting tomato from Saudi Arabia, and the lowest identity was observed with Tomato yellow leaf curl virus Oman isolate. The overall sequence identity ranged from more than ninety percent among the analyzed sequences. The phylogenetic relationship analysis formed the major three clusters and showed the closed clustering with Tomato yellow leaf curl virus isolates. The natural spread of the Tomato yellow leaf curl virus on the Chilli crop from other crops poses an important and serious threat to Chili cultivation in the Kingdom of Saudi Arabia. Based on the literature review and current evidence, this is the first report of leaf curl disease of Chilli from Saudi Arabia.

1. Introduction

Chilli (*Capsicum annum* L.) belongs to the family *Solanaceae* is known as important vegetable crop grown for multiple uses. Chilli crop is mainly cultivated for spice, vegetable, medicinal herb. Chilli contains vitamin A, C, B1 and B2, beta carotene, capsaicin, protein, calcium, and phosphorous. A chemical called chilli oleoresin-1, extracted from the dried chilli of *Capsicum annuum* is used in pain balms, plasters, and prickly heat powders. Chili peppers are rich in many minerals, vitamins, and amino acids essential for human health and growth. Peppers contain a wide array of phytochemicals such as vitamins, phenolics, and flavonoids that have antioxidants, antimicrobial and anti-degenerative properties (Jagtap et al., 2012; Omolo et al., 2014; Fattori et al., 2016; Ganguly et al., 2017; Saleh et al., 2018). The cultivation of Chilli is severely affected by many diseases caused by multiple pathogens including viruses, fungus, bacteria and nematodes. The Chilli leaf curl disease (ChiLCD) caused by a begomovirus known as *Chilli leaf curl virus* (ChiLCV) is an important limiting factor for Chilli cultivation worldwide (Shahid et al., 2019). Based on the current literature review, a total of sixty-five viruses are known to cause diseases in chilli crops (Thakur et al., 2018). The most important symptoms of ChiLCD includes; leaf curling and stunted plant growth. The begomovirus (*Geminviridae)* contains ss-circular DNA with a 2.5–2.8 kb genome. Currently, the family *Geminiviridae* are known to have nine genera such as; *Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragrovirus, Grablovirus Mastrevirus, Topocuvirus, Turncurtovirus* (Varsani et al., 2017; Zerbini et al., 2017). Recently, an additional satellite molecule known as delta satellites has been identified as novel satellites from the new word begomoviruses (Fiallo-Olive et al., 2016). The begomoviruses are reported as the

Abbreviations: ChiLCD, Chilli leaf curl disease; TYLCV, Tomato Yellow leaf Curl Virus; ToLCSDV, Tomato leaf curl Sudan virus; PCR, Polymerase Chain Reaction.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.09.009
1319-562X/

biggest genus and transmitted by a vector whitefly (*Bemisia tabaci*) in a very efficient manner. The incidence of the disease is directly correlated with the population of whiteflies vector and environmental factors. Many isolates of begomovirus and associated satellites have been reported globally infecting Chilli (Khan and Khan, 2017). The begomovirus causing ChiLCD to multiple crops has been identified and from the neighboring country, Oman (Khan et al., 2013, Shahid et al., 2019).

As per the current status and published reports; the information about the natural infection of the begomovirus on Chilli crop is lacking from the kingdom of Saudi Arabia. So far, viral diseases including begomovirus on various crops have been identified from the Arabian region and neighboring countries like; Nile Basin, Oman, Yemen, and Iran (Al-Shahwan et al., 1997; Ghanem et al., 2003; Ajlan et al., 2007; Idris et al., 2012, 2014; Khan et al., 2013; Hosseinzadeh et al., 2014; Al-Saleh et al., 2014; Sohrab, 2016; Khan and Khan, 2017; Shahid et al., 2019). The begomovirus and their associated satellites infecting other crops like Amaranthus, Beans, Cucurbits, Corchorus, Mentha, Tomato and weeds have been identified earlier from the Arabian region (Al-Shahwan et al., 1997; Ghanem et al., 2003; Ajlan et al., 2007; Idris et al., 2012, 2014;; Hosseinzadeh et al., 2014; Al-Saleh et al., 2014; Sohrab, 2016, 2017a, 2017b, 2020;; Sohrab et al., 2016; Sohrab and Daur, 2018a, 2018b).

Based on the current status, the detailed work about the natural spread, diagnosis and genetic diversity of ChiLCD is urgently needed. In this work, the natural infection of chilli crops, molecular diagnosis, sequencing, and the association of begomovirus with ChiLCD have been discussed. The causative agent was identified as TYLCV. The transmission and spread of TYLCV to Chilli crop from other crops and weeds poses a severe risk to Chilli cultivation in Saudi Arabia.

2. Materials and methods

2.1. Sample collection and virus detection

A field survey was performed to collect the leaves from Chilli growing farmer's field in Jeddah, Saudi Arabia. The chilli plants exhibiting yellowing and leaf curling symptoms were randomly selected and total six samples were collected from a different fields. The whiteflies populations were also observed in and around the Chilli crops and also on weeds crops which provided an indication that could be the begomovirus infection. The genomic DNA was purified from the infected leaf samples by using DNAeasy plant mini kit (QIAGEN) as per kit protocol. The virus infection was initially confirmed by Polymerase chain reaction (PCR) using begomovirus specific coat protein gene primers. The PCR condition was followed as per earlier published paper (Sohrab and Daur, 2018a, 2018b). The PCR reaction mixture was set up with purified DNA, Taq polymerase (2.5 units) (MBI; Fermentas, USA), 10X PCR buffer (5 µl), 10 mM dNTPs (0.5 µl), forward and reverse primers (0.5 µl) (10 pmol each) and final volume was made to 50 µl by using sterile distilled water. The causal organism was identified by using PCR primers TYLCV (F) TAAGGGCCCGTGATTATGTTG (R) TTTATTAATTCGATATTGAATCAT (TYLCV-KT033715). The virus infection was further confirmed by using the dot blot hybridization assay. The purified DNA samples were directly blotted on the nylon membrane and further hybridized with a begomovirus coat protein gene-specific probe.

2.2. Viral genome cloning, sequencing

The PCR amplified product was eluted, gel extracted, purified, cloned, sequenced and further used for sequence and phylogenetic relationship analysis. Only one PCR amplicon was used for cloning into a pGEM-T easy vector and finally sequenced.

2.3. Analysis of genetic diversity

The nucleotide sequences were aligned, initially searched and compared for the similarity with other begomoviruses using BLASTn. The sequences showed the high similarities were further selected and retrieved from GenBank for sequence identity matrix and phylogenetic relationship analysis. The nucleotide sequences identity matrix was analyzed by using BioEdit and ClustalW software (v7.0.5). Initially, the coat protein gene sequences were analyzed with other selected begomovirus sequences retrieved from GenBank. The genetic diversity was determined based on the percent identity matrix and the phylogenetic relationship was analyzed by using MEGA7 software (Kumar et al., 2016).

3. Results

3.1. Field survey and virus detection

During the field survey, total of six samples were collected from Chilli plants growing in the field. The natural infection of the virus on weeds was also observed growing near to Chilli crops and approximately 80–90% disease incidence was observed on Chilli crops (Fig. 1). The begomovirus infection was confirmed by PCR using specific primers which produced 750 bp amplicon in all tested samples excluding healthy one (Fig. 2A). The dot blot assay produced strong hybridization signals with coat protein specific probes in all the six samples tested including positive (Fig. 2B). The strong hybridization of the CP gene probe confirms the begomovirus infection in the naturally infected Chilli leaf samples.

3.2. Viral genome sequencing and analysis

The coat protein (CP) gene of begomovirus was PCR amplified purified, cloned and finally sequenced. The full-CP gene was found to have total of 774 nucleotides (nt). The generated sequence was assembled and analyzed by using BioEdit and tentatively designated as TYLCV-Chilli-Jeddah and finally submitted to GenBank with accession number (MK541892). The highest nucleotide sequence identity (99.9%) was observed with the TYLCV isolate reported earlier from tomato crops in Saudi Arabia.

Fig. 1. Field infected Chilli plant with leaf curl symptoms and whiteflies.

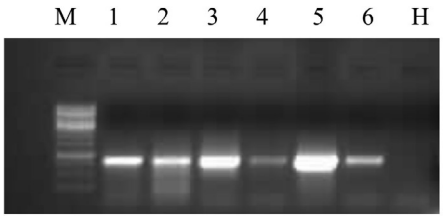

Fig. 2A. PCR detection of begomovirus using Coat Protein gene primers. H: Healthy sample, M: 1 Kb ladder. 1–6 Field collected leaf samples.

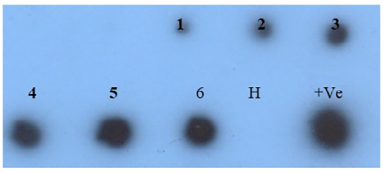

Fig. 2B. Detection of begomovirus infection by Dot Blot hybridization assay. H: Healthy sample; 1–6 field collected leaf samples.

3.3. Sequences and phylogenetic relationship analysis

The CP gene sequences of TYLCV-Chilli-Jeddah were used to analyze the sequence identity/diversity with other begomoviruses. The multiple sequence alignment of the coat protein showed the significant identity with other selected begomovirus genome collected from multiple locations and different crops. The highest (99.9%) sequence identity matrix was observed with the TYLCV-Tomato-Jeddah isolate infecting tomato crops in the Kingdom of Saudi Arabia. The average sequence identity were ranged from 98.2 to 99.7% with the other selected begomoviruses reported earlier from the different crop of Arabian Peninsula. Additionally, the CP gene sequences were also analyzed with other begomoviruses infecting different crops from Arabian Peninsula and the sequence identity was varied from 75.0 to 75.9%. This similarity showed that the virus associated with leaf curl disease of chilli is less different from the TYLCV causing disease in tomato and other crops in the Kingdom of Saudi Arabia. Interestingly, the sequence analysis of TYLCV-Chilli-Jeddah with other selected *Tomato leaf curl Sudan Virus* (ToLCSDV) isolates from multiple crops ranged from 96.0 to 97.4% identity. The percent identity matrix of begomovirus sequences has been presented in Table 1.

The phylogenetic relationship of TYLCV-Chilli-Jeddah was analyzed with selected begomoviruses from the Kingdom of Saudi Arabia and the Arabian Peninsula. The TYLCV-Chilli-Jeddah formed the closest cluster with TYLCV-Tomato-Jeddah isolate along with the other begomovirus isolates reported to cause disease in many different crops like Tomato, Corchorus, Cucumber, and Mentha in Saudi Arabia. The phylogenetic relationship formed there major clusters with the begomoviruses infecting different crops from the various locations. The first cluster consists most of the TYLCV isolates from various crops reported from Saudi Arabia and the second cluster had ToLCSDV isolates of many crops from the Arabian Peninsula, while the third cluster consists only of TYLCV isolates from the Gulf countries as well as African regions (Fig. 3). The phylogenetic relationship analysis clearly indicates that the TYLCV-Chilli-Jeddah is the same isolates reported earlier to infect tomato crops from the Kingdom. The molecular diagnosis, sequence analysis and phylogenetic analysis confirms that the virus has moved from tomato to chilli crops by whiteflies transmission. The natural spread of TYLCV and ToLCSDV by whiteflies from weeds and other

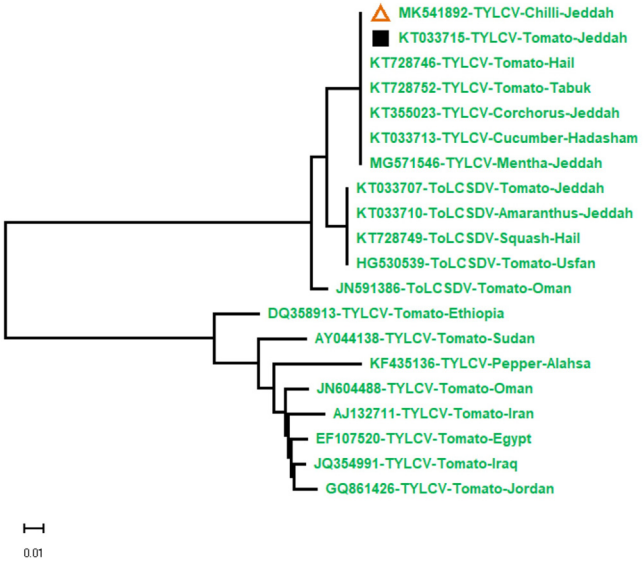

Fig. 3. Phylogenetic relationships of TYLCV-Chilli-Jeddah with selected begomoviruses based on Coat Protein gene sequences.

Table 1
Percent identity matrix of TYLCV-Chilli-Jeddah (MK541892) with selected Begomoviruses.

S. No	Accession No	Hosts	Location	% Identity
1	KT033715-TYLCV	Tomato	Jeddah	99.9
2	KT728746-TYLCV	Tomato	Hail	99.7
3	KT728752-TYLCV	Tomato	Tabuk	98.8
4	KT355023-TYLCV	Corchorus	Jeddah	99.4
5	KT033713-TYLCV	Cucumber	Hadasham	98.2
6	MG571546-TYLCV	Mentha	Jeddah	92.9
7	KF435136-TYLCV	Pepper	Alahsaa	92.9
8	JN604488-TYLCV	Tomato	Oman	75.0
9	AJ132711-TYLCV	Tomato	Iran	75.8
10	JQ354991-TYLCV	Tomato	Iraq	75.6
11	EF107520-TYLCV	Tomato	Egypt	75.1
12	GQ861426-TYLCV	Tomato	Jordan	75.9
13	AY044138-TYLCV	Tomato	Sudan	75.1
14	DQ358913-TYLCV	Tomato	Ethiopia	75.6
15	KT033707-ToLCSDV	Tomato	Jeddah	96.2
16	KT033710-ToLCSDV	Amaranthus	Jeddah	96.0
17	KT728749-ToLCSDV	Squash	Hail	96.9
18	HG530539-ToLCSDV	Tomato	Usfan	97.4
19	JN591386-ToLCSDV	Tomato	Oman	96.7

crops to other economically important crops indicates an alarming situation for the cultivation of vegetables in the Kingdom of Saudi Arabia.

4. Discussion

In Saudi Arabia, the cultivation of Chilli crops is being conducted on a small scale for the local consumptions. In this work, the field survey, sample collection, molecular diagnosis, genetic diversity, phylogenetic relationships association of begomovirus with ChiLCD caused by TYLCV, and the have been discussed. The genetic diversity of TYLCV-Chilli-Jeddah showed the highest identity with TYLCV were reported from tomato crops in the Kingdom. The lowest identity was observed with TYLCV-Tomato-Oman isolate. Interestingly, the significant identity was also observed with the other TYLCV isolates from multiple selected regions. An analysis of ToLCSDV of selected isolates from multiple locations with TYLCV-Chilli-Jeddah isolate also showed the identity at a higher level. The phylogenetic relationship analysis of TYLCV-Chilli-Jeddah with other selected begomovirus isolates formed major three clusters. The closest cluster had mostly TYLCV isolates of different crops from Saudi Arabia. The ToLCSDV clustered separately containing many isolates from different locations. The third cluster was formed only with TYLCV isolates infecting tomato and Pepper isolates from other regions of the Arabian Peninsula. The genetic diversity of and variability of TYLCV and Chilli leaf curl virus from Solanaceous crops have been reported earlier from the Kingdom of Saudi Arabia, Arabian Peninsula and South East Asia (Idris et al., 2012, 2014;; Khan et al., 2013; Hosseinzadeh et al., 2014; Kenyon et al., 2014). So far, only a few begomoviruses in the Kingdom such as TYLCV and ToLCSDV causing disease in Tomato, Cucumber, Sponge gourd, Squash, Mentha, Corchorus, Amaranthus, and Okra have been reported (Idris et al., 2012, 2014;; Al-Saleh et al., 2014; Sohrab, 2016, 2017a, 2017b, 2020;; Sohrab et al., 2016; Sohrab and Daur, 2018a, 2018b). The Association of ChiLCV and betasatellite infecting crops has been reported from Oman (Khan et al., 2013; Shahid et al., 2019). The introduction of begomovirus causing disease in the different crops in the kingdom has been introduced from the surrounding countries.

In this study, based on the sequencing and sequence analysis and similarity, the virus causing leaf curl disease of Chilli has been identified as TYLCV. The phylogenetic relationship also showed closed clustering of TYLCV isolates reported to cause leaf curling and yellowing diseases to other crops in the Kingdom. The association of TYLCV has already been reported from various crops in the kingdom. The identification of TYLCV from Chilli crop and sequence identity as well as phylogenetic analysis clearly indicates the association of TYLCV to ChiLCD. The transmission and spread of TYLCV from tomato to Chilli crops shows an alarming situation for vegetable growers in the kingdom of Saudi Arabia. It is well reported that the weeds and whiteflies play an important role as an alternative host and to spread of begomovirus from one region to others with extended hosts range (Sohrab, 2017a, 2017b, 2020;; Sohrab and Daur, 2018a). The genetic diversity is a common phenomenon in both plants and humans and favors the emergence of new virus strains/ species and causing diseases in both plants and humans (Ahmad et al., 2009, 2018). The identification of many other viruses including begomoviruses causing diseases to economically important crops is increasing gradually in the Kingdom. There are many factors involved in the emergence and spread of begomoviruses (Seal et al., 2006). The change in the genomic sequences, presence of whiteflies vector, climatic conditions, changing cropping system, frequent recombination and mutation of the 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. The emergence and spread of plant viruses and their associated vectors in the Kingdom posing an alarming situation for the cultivation of vegetable and other economically important crops as it is well reported that the TYLCV has become the serious threat for tomato production globally (Basak, 2016; Hanssen et al., 2010; Thakur et al., 2018). The strategies for the development of durable disease management against viruses require information about the genetic variability, virus evolution, and host plant interaction. The role of whiteflies in the transmission and global emergence of begomovirus has already been described in the published paper (Varma et al., 2011). Based on the current scenario, there is an urgent need to conduct a detailed study of plant viruses infecting known or unknown plants with symptomatic or asymptomatic as well as weed crops in the kingdom. The generated information will be highly useful for researchers, vegetable growers, and agriculture ministry to design and develop the disease management strategies in the Kingdom of Saudi Arabia.

5. Conclusion

The data generated from this work provided a piece of preliminary information about the spread of TYLCV to Chilli crops from tomato as well as suspected weed crop. This information concluded that the begomovirus identified is the TYLCV earlier reported from Tomato in Jeddah, Saudi Arabia. This indicates that the virus has spread from infected tomato and weeds by whiteflies and caused leaf curl disease to Chilli crops. The identification of begomovirus from the whiteflies vector is urgently needed to confirm the role of vector for disease transmission to other economically important crops. It is well reported that the neighboring countries have also contributed to disease spread to other crops in the Kingdom of Saudi Arabia. The movement and export and import of plant materials from other countries and their screening of pathogens; especially for the viruses and bacteria including fungus is urgently needed to protect the vegetable and economically important crops in the Kingdom. Based on the literature and generated information, this is the first report of TYLCV infection on Chilli crops in the Kingdom of Saudi Arabia.

Acknowledgements

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under the grant number G: 210-141-41.The author therefore acknowledge with thanks DSR 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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