Molecular evidence for the occurrence of TYLCV on *Mentha longifolia* in Jeddah, Saudi Arabia

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Abstract Begomoviruses are whiteflies transmitted virus causing serious disease in many important plants exhibiting variable symptoms with significant economic loss globally. *Mentha* is an important crop being grown here in Saudi Arabia for various purposes. The begomovirus associated disease was observed on *Mentha* crops during field survey which were growing near to tomato field. There is no published report available about the association of begomovirus on *Mentha* from this region. So, this work was conducted to identify the causative agent associated with yellow vein mosaic disease. Naturally infected samples were collected from various locations and causative agent was identified by PCR using begomovirus specific primers and further cloned and sequenced bidirectionally. The full genome had total 2785 nucleotides while betasatellite molecule had 1365 nucleotides. Based on full-genome sequence analysis, the identity matrix and phylogenetic analysis showed the highest identity (99.6%) with Tomato yellow leaf curl virus (TYLCV) reported from tomato in the Kingdom of Saudi Arabia. The identified begomovirus was observed as isolate of TYLCV.

Keywords *M. longifolia* · Begomovirus · TYLCV · Genetic diversity · Phylogeny

Mentha crop belongs to family *Lamiaceae* and extensively grown in Arabian region. More than twenty-nine *Mentha* species are being cultivated worldwide for many purpose. In Arabian region, two types of *Mentha* is being extensively grown and used in many herbal tea and spice mixture for aroma and flavor and they are known as *Mentha Longifolia* (ML) and *Mentha pulegium* (MP). The direct use of menthe and extracted essential oil are also being used as antiviral, antibacterial, cardiovascular and to inhibit LDL oxidation have been reported recently [1]. The begomovirus and their satellites are known to causes economic loss to economically important crops globally. Begomovirus belongs to the family *Geminiviridae* contains ssDNA with either monopartite (DNA- A) or bipartite (DNA- A and DNA- B genome of about 2.7 kb, encapsulated in twinned geminate particles. The family *Geminiviridae* have nine genera; *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Curtovirus*, *Eragrovirus*, *Grabovirus*, *Mastrevirus*, *Topocuvirus*, *Turncurtovirus*, [2, 5, 17]. They can be transmitted efficiently by whiteflies (*Bemisia tabaci*). The monopartite begomoviruses are known to have DNA- A and associated satellites (betasatellites-1.4 kb) or alphasatellites. Earlier, the alphasatellites (~ 1.3 kb) were known as DNA 1 containing a single ORF (Rep). Both satellites are well known to help in replication and symptom attenuation. The begomovirus associated disease transmitted by whiteflies causing severe economic loss and posing serious issue for food security globally [16]. It is well known that the Iran is an important center for emergence and spread of TYLCV to Middle East and World [3].

Recently, the occurrence of begomoviruses causing disease in many crops like *Amaranthus*, *Beans*, *Corchorus*, *Cucurbits*, *Okra*, *Tomato* has been reported from Saudi Arabia [9–14]. Globally, many viruses infecting mint species have been identified and published [6–8]. In this

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study, an association of begomovirus with yellow vein disease of *M. longifolia* in Saudi Arabia has been discussed. The cultivation of mentha crops are being conducted in approximately two hundred and fifty hectares in many locations of the kingdom like Al-Kharj, Al-Qassim, Dammam, Abha and Al-Madinah. A field survey was conducted in the month of March-2017 and naturally infected mentha crops were observed to exhibit yellow vein symptoms (Fig. 1a) with ~ 30–35% disease incidence. The presence of whiteflies was also observed near and on both mentha as well as tomato crop. Total eighteen samples were collected from multiple locations along with one healthy and further processed. To transmit the causative agent, a pure culture of whiteflies was maintained on Clitoria plants and allowed to feed on infected Mentha leaves for 24 h acquisition access period (AAP) and thirty healthy tomato seedlings and five mentha seedlings were inoculated by giving Inoculation access period (IAP) for 24 h. The associated virus was transmitted by whiteflies and developed similar yellow vein symptom (Fig. 1b) in 20 days in 27 plants with 8% transmission efficiency. The genomic DNA was purified by using DNAeasy plant mini kit (Qiagen Inc.) and begomovirus infection was confirmed

by using the in-house designed and developed specific primers from TYLCV reported earlier from Saudi Arabia (TYLCV-KT033715) (F) TAAGGGCCCGTGATTATGTTG (R) TTTATTAATTCGATATTGAATCAT. The PCR was conducted at 94 °C-120 s⁻¹ cycle, 94 °C-60 s, 50 °C-60 s, 72 °C-60 s, for 35 cycles with final extension of 5 min at 72 °C. PCR product was analyzed on 1% Agarose gel stained with ethidium bromide (0.5 lg ml⁻¹) and visualized on an Ultraviolet transilluminator.

All the samples were processed and tested by PCR and only twelve samples were found to be positive based on an amplification of an amplicon approximately 750 bp in tested samples (Fig. 2). The betasatellite molecule was also amplified by using specific primers. The rolling circle amplification (RCA) using TempliPhi 100 Amplification Kit (GE Healthcare, Life Sciences, Piscataway, NJ, USA) was used to amplify the full genome as per manufacturer's instructions. The obtained product was further restricted by using *EcoRI*, *EcoRV*, and *Hind III* enzymes the full genome was cloned into pUC-19 while betasatellite was cloned into pGEMT-easy vector. After sequencing, the obtained sequences were edited, aligned and further analyzed, using NCBI-BLAST. The closely similar and

Fig. 1 Yellow vein mosaic symptoms on naturally infected (a), Inoculated (b) *M. longifolia* plants and Phylogenetic relationship of TYLCV-Mentha with selected begomoviruses (c)

Fig. 2 Detection of begomovirus infection by PCR in *M.longifolia* samples. M: 1 Kb ladder (MBI Fermentas), 1: Healthy sample, 2–19: Infected samples

Table 1 Sequence identity matrix of TYLCV- *Mentha* with selected begomovirus isolates

Accession nos.	Acronyms	Hosts	Locations	% Identity matrix						
				Full (nt)	V2 (aa)	V1 (aa)	C3 (aa)	C2 (aa)	C1 (aa)	C4 (aa)
KF31236	ChiLCV	Mentha	India	68.8	67.1	66.7	64.5	66.9	67.4	68.0
KR779820	ChiLCV	Mentha	India	68.5	67.5	65.9	64.8	67.5	64.9	68.1
KT033715	TYLCV	Tomato	Jeddah	99.6	97.9	98.3	96.1	96.6	98.2	98.3
KC845301	TYLCV	Tomato	Jizan	99.0	98.3	98.5	98.2	98.1	98.0	98.5
KF561125	TYLCV	Tomato	Al-Qasim	92.4	91.6	91.2	92.1	90.9	90.8	91.9
KF435137	TYLCV	Tomato	Alahsa	81.2	79.9	80.1	80.8	80.2	81.6	80.8
KF435136	TYLCV	Pepper	Alahsa	81.1	80.4	80.2	80.4	80.1	80.6	81.2
HE819240	TYLCV	Capsicum	Oman	78.6	77.8	77.0	78.0	77.6	79.2	78.0
KF229726	TYLCV	Tomato	Oman	77.5	76.9	76.6	75.0	76.7	76.5	76.8
KT033713	TYLCV	Cucumber	S. Arabia	98.9	97.5	97.0	97.8	97.1	98.7	97.0
KT355023	TYLCV	Corchorus	S. Arabia	99.5	98.4	98.0	98.8	98.1	98.7	98.0
KF040453	TYLCV	Tomato	Hail	93.7	92.5	92.4	92.9	93.1	91.7	92.0
KT728752	TYLCV	Tomato	Tabuk	98.6	97.2	97.0	97.7	96.6	97.8	97.6
KT728746	TYLCV	Tomato	Hail	98.5	97.5	97.8	97.6	98.8	98.7	98.1
JQ354991	TYLCV	Tomato	Iraq	78.6	77.2	77.0	76.7	78.0	77.5	77.9
KT728742	ToLCSDV	Squash	Jeddah	90.5	89.9	89.8	88.9	88.6	87.8	90.6
KT033711	ToLCSDV	Tomato	Jeddah	90.7	89.9	88.6	88.9	88.7	90.5	90.5
KT033710	ToLCSDV	Amaranth	Hadasham	90.8	89.0	88.8	88.3	88.1	89.6	90.0
JN591385	ToLCSDV	Tomato	Oman	92.2	91.0	89.9	88.3	90.1	91.6	91.0
JF919733	ToLCSDV	Tobacco	Yemen	93.7	92.5	92.3	92.9	93.1	92.7	92.0
DQ358913	TYLCMV	Tomato	Ethiopia	81.2	80.0	80.9	80.7	80.6	80.8	81.0
LN906594	CtoLCV	Cherry Tm.	Pakistan	69.1	68.9	67.9	68.8	68.9	69.8	68.6
DQ629102	ToLCNDV	Cherry Tm.	India	68.7	67.0	67.8	67.2	68.5	68.5	68.0
KF307208	CYVMV	Papaya	India	68.4	67.2	67.9	67.8	68.9	67.8	68.6
JF919731	ToLCSDV	Tomato	Yemen	89.2	88.0	88.8	87.9	88.0	88.8	88.0
KF471061	ChiLCV	Amaranth	India	71.0	70.2	69.8	69.7	70.6	70.8	70.5
EU867513	AgEV	Amaranth	India	70.6	69.2	69.3	69.6	70.0	70.0	70.5
KC106648	TYLCV	Tomato	Iran	78.3	77.2	78.0	76.7	76.9	77.5	77.6
GU076448	TYLCV	Tomato	Iran	82.2	81.0	81.9	81.9	81.0	81.6	81.7
AY594174	TYLCV	Tomato	Egypt	79.1	78.5	78.0	77.6	77.9	78.4	78.1
EF054894	TYLCV	Tomato	Jordan	82.3	80.5	81.8	81.8	81.5	81.5	81.0
JX131286	TYLCV	<i>S. arvensis</i>	Jordan	78.6	76.6	77.8	77.7	78.0	77.2	77.8
AY044138	TYLCV	Tomato	Sudan	81.7	80.0	80.7	81.0	80.5	80.0	81.5

identical sequences were isolated and used for further analysis to generate sequence identity matrix and phylogenetic relationship by BioEdit and MEGA 7.0 [4]. The EcoRI digested sample produced ~ 2.7-kb product. The full genome had 2785 nt and 1365 bp found in betasatellites molecule. Both the sequences have been deposited into GenBank with accession numbers MG571546 (Full genome) and betasatellites (MG571547). The full genome sequence analysis result showed 99.6–99.5% identity with TYLCV reported earlier from tomato and Corchorus (KT033715; KT335023) associated with yellow vein mosaic disease in Saudi Arabia while betasatellite sequence analysis observed to be identical (98.8%) with TYLCB-Jeddah (KT355021). The full genome sequence of TYLCV-mentha (MG571546) found to similar (99.6–68.5%) with selected begomovirus isolate (Table 1) while the betasatellite sequences showed 98.9–46.6% similarity with selected isolates. In a phylogenetic analysis, based on full genome with selected begomovirus isolates; a closed cluster formation was observed with begomovirus isolates like TYLCV (KT033715-Tomato, KT355023-Corchorus, KT033713-Cucumber, KT728752-Tomato, KT728746-Tomato, KC845301-Tomato) (Fig. 1c). Additionally, other begomovirus isolates reported from Arabian region grouped into a separate cluster.

Globally, the full plant of mint or essential oil extracted is being used for various purposes including Saudi Arabia. Currently, many begomoviruses infecting mint have been reported earlier from multiple locations [6–8, 15] but no report from Arabian Peninsula. Many cultivated and weed crops are reported to be infected with begomovirus in the kingdom with high disease incidence rate. Based on the results obtained in this study, it is expected that the begomovirus is increasing and moving towards other crops and causing severe disease in menthe and it is expected that some more crops can be further infected by begomovirus in this region. The genetic diversity of full length as well as betasatellites genome with selected begomoviruses reported from Arabian Peninsula also provided evidence for emergence and spread of begomoviral disease to many crops in many other locations due to many factors including sequences variation by frequent recombination and mutation of viral genome, vectors, climatic conditions, change in cropping system are the most significant factors for the emergence and spread of new begomoviruses as a serious threat to economically important crops in the Arabian peninsula. The results of this study provided the sufficient evidence that the TYLCV is causing yellow vein disease in menthe in Jeddah, Saudi Arabia. The accumulated results require further extended studies so that an effective disease management strategy can be designed and developed in the Kingdom.

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