Identification of a monopartite begomovirus associated with yellow vein mosaic of *Mentha longifolia* in Saudi Arabia

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Abstract

Mentha is a very important crop grown and used extensively for many purposes in the Kingdom of Saudi Arabia. Begomoviruses are whitefly-transmitted viruses causing serious disease in many important plants exhibiting variable symptoms with significant economic loss globally. During farmers' field survey, yellow vein mosaic disease was observed in *Mentha longifolia* plants growing near tomato fields in Saudi Arabia. The causative agent was identified in 11 out of 19 samples using begomovirus-specific primers and the association of begomovirus with yellow vein mosaic disease in *M. longifolia* was confirmed. The full-length viral genome and betasatellite were amplified, cloned, and sequenced bidirectionally. The full DNA-A genome was found to have 2785 nucleotides with 1365 bp-associated betasatellite molecule. An attempt was made to amplify DNA-B, but none of the samples produced any positive amplicon of expected size which indicated the presence of monopartite begomovirus. The sequence identity matrix and phylogenetic analysis, based on full genome showed the highest identity (99.6%) with Tomato yellow leaf curl virus (TYLCV) and in phylogenetic analysis it formed a closed cluster with Tomato leaf curl virus infecting tomato and Corchorus crop in Saudi Arabia. The sequence analysis results of betasatellites showed the highest identity (98.9%) with Tomato yellow leaf curl betasatellites infecting tomato and phylogenetic analysis using betasatellites formed a close cluster with Tomato yellow leaf curl betasatellites infecting tomato and Corchorus crops, which has already been reported to cause yellow vein mosaic and leaf curl disease in many cultivated and weed crops growing in Saudi Arabia. The identified begomovirus associated with yellow vein mosaic disease in mentha could be a mutated strain of TYLCV and tentatively designated as TYLCV-Mentha isolate. Based on published data and latest information, this is the first report of identification of Tomato yellow leaf curl virus associated with yellow vein mosaic disease of *M. longifolia* from Saudi Arabia.

Keywords *Mentha longifolia* · Begomovirus · Betasatellite · TYLCV · Genetic diversity · Phylogenetic relationship

Introduction

Mentha is an important crop belonging to the family *Lamiaceae*. Till date, approximately 29–30 species of mentha are extensively grown globally and used for many purposes, especially for essential oil (Moreno et al. 2002). In Saudi Arabia, *Mentha longifolia* (ML) and *Mentha pulegium* (MP),

which are also known as Al-Madinah and Mugrabi mint, locally grow extensively and are used in many herbal tea and spice mixtures for aroma and flavor. Additionally, mentha species is found to have antibacterial properties and is traditionally being used against many diseases like throat infection, nausea and ulcerative colitis (Iskan et al. 2002; Daferera et al. 2003). Recently, the effect of an essential oil extracted from ML and MP in cardiovascular and throat health have been investigated to inhibit LDL oxidation against five bacterial pathogens in vitro. The results showed the ML contains very strong antioxidant and antibacterial activity than MP. The essential oil extracted from ML observed to contain high flavonoid contents can be used as an effective and active ingredient for cardiovascular and throat health (Ahmed et al. 2015).

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Begomoviruses and their satellites cause serious economic loss to both economically important as well as weed crops globally (Basak 2016). Currently, many weeds, as well as cultivated crops, are known to be infected with various begomoviruses globally (Paul et al. 2012; Mahatma et al. 2016; Sohrab 2016, 2017a, b; Sangeeta and Tiwari 2017). Begomoviruses belong to the family *Geminiviridae* and contain ssDNA with either monopartite (DNA-A) or bipartite (DNA-A and DNA-B) genome of ~ 2.7 kb, encapsulated in twinned geminate particles. Currently, the family *Geminiviridae* has seven genera known as *Mastrevirus*, *Curtovirus*, *Begomovirus*, *Topocuvirus*, *Eragrovirus*, *Turncurtovirus*, and *Becurtovirus* (Muhire et al. 2014; Varsani et al. 2014; Brown et al. 2015). They are well known to be efficiently transmitted by a whitefly vector (*Bemisia tabaci*). The monopartite begomoviruses have only DNA-A with either betasatellites (1.4 kb) or alphasatellites having only one gene known as beta C1 (Briddon et al. 2002; Sivalingam et al. 2010). Earlier, the alphasatellites were known as DNA-1 (~ 1375 nt) with a single ORF (Rep) and conserved genome organization (Xie et al. 2010). Helper virus is responsible for the replication and symptom attenuation of both alpha- and betasatellites (Idris et al. 2011). Currently, the begomovirus diseases have reached an epidemic status, posing severe threat to global food security and whitefly vector (*Bemisia tabaci*) has emerged as serious pathogens for most of the dicotyledonous crops across tropical and sub-tropical regions of the world (Varma et al. 2011).

The emergence and spread of TYLCV from the Middle East to World have taken place recently and it has been observed that Iran could be the center for TYLCV diversity (Duffy and Holmes 2008; Lefeuvre et al. 2010; Kenyon et al. 2014). Currently, the natural occurrence of various viruses infecting cultivated crops in the Kingdom have been reported (Ajlan and Ghanem 2007; Al-Saleh et al. 2014a, b; Al-Shahwan et al. 2017; Raza et al. 2017). Recently, the natural occurrence of begomovirus-caused disease in many crops like Amaranthus, Beans, Cucurbits, Okra, Tomato, and Corchorus has been reported in Saudi Arabia (Idris et al. 2012; Al-Saleh et al. 2014a, b; Sohrab 2016, 2017; Sohrab et al. 2016a, b, c, d, e, 2017a). Globally, many viruses have been reported from mint species (Tzanetakis et al. 2010) and the natural occurrence of begomoviruses on mint species has been reported as *Tomato leaf curl Pakistan virus* (Samad et al. 2008), *Tomato leaf curl Karnataka virus*, *Cotton leaf curl Multan virus* (Borah and Dasgupta 2012), *Chilli leaf curl India virus*, *Ageratum yellow vein virus* betasatellite (Saeed et al. 2014, 2017). Based on the published information on natural occurrence of begomoviruses on various crops in the Kingdom, this work was conducted to identify the association of virus with yellow vein mosaic disease of *M. longifolia*.

Materials and methods

Field survey, sample collection and virus detection

Mentha has been cultivated at very large scale in the Kingdom at multiple locations like Al-Kharj, Al-Qassim, Dammam, Abha and Al-Madinah, and pathogenic infection limits the cultivation and poses a serious threat of economic loss to Mentha production. To collect the sample a field survey was conducted, and a random sampling procedure was followed. Totally 19 symptomatic as well as non-symptomatic mentha leaf samples were collected from multiple locations of local field at Jeddah during the month of March 2017. The collected samples were brought to the lab and further processed. The causative agent was transmitted to mentha seedlings by whiteflies inoculation under protected condition in the glass house. Total genomic DNA was isolated from 100 mg symptomatic and non-symptomatic leaf samples using DNeasy plant mini kit following the manufacturer's instructions (Qiagen Inc.) and begomovirus infection was confirmed using the specific primers (F) TAAGGGCCCCGTGATTATGTTG (R) TTTATTAATTTCGATATTGAATCAT. The primers were designed using the sequence of tomato yellow leaf curl virus earlier reported from the Kingdom of Saudi Arabia (TYLCV-KT033715). 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), 5 µl 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. The successful amplification of betasatellites molecule was obtained using universal primer (Briddon et al. 2002). The PCR products were analyzed on 1% Agarose gel stained with ethidium bromide (0.5 lg ml⁻¹) and visualized on an Ultraviolet transilluminator.

Cloning, sequencing and phylogenetic analysis

The full-length begomoviral genome was amplified by rolling circle amplification using TempliPhi 100 amplification kit (GE Healthcare, Life Sciences, Piscataway, NJ, USA) as per the manufacturer's instructions. The amplified product was digested with *EcoRI*, *EcoRV*, and *HindIII* restriction enzymes and full-genome begomovirus (~ 2.7 kb) fragment was obtained and cloned into pUC-19 plasmid vector while betasatellite was cloned into pGEMT-easy vector. One clone of full genome and betasatellite was sequenced bidirectionally. The generated DNA

sequences were assembled and initially analyzed with full-length begomoviral genome using NCBI-BLAST. Sequences with maximum identities were selected for further pairwise multiple sequence alignment using BioEdit and ClustalW programme. The phylogenetic relationship was analyzed, and tree was constructed using MEGA 6.0 programme (Tamura et al. 2013) with selected begomovirus sequences.

Results

Field survey and virus detection

During the field survey in March 2017, yellow vein mosaic disease was observed (Fig. 1a) for the first time in *M. longifolia* plant with ~ 30–35% disease incidence growing near naturally infected tomato crop exhibiting similar yellow vein mosaic and leaf curl symptoms. No other symptoms were observed in the healthy plant (Fig. 1b). The dense population of whiteflies (30–35%) was also observed in both mentha as well as tomato crops which indicated that it could be the whitefly-transmitted begomovirus-associated disease. The causative agent was successfully transmitted by whiteflies and developed similar yellow vein mosaic symptom on inoculated mentha seedlings. An amplicon of 750 bp was observed in total 11 samples out of 19 tested samples and full-genome was amplified in 9 samples as well as betasatellites were amplified in 12 samples. None of the samples produced either DNA-B amplicon by PCR. The presence

of betasatellite was also confirmed by amplifying the full-length betasatellite molecule using specific primers (Bridson et al. 2002). Only, the PCR-positive samples were used for further analyses.

Cloning, sequencing and phylogenetic analysis

Only one sample digested with *Eco*RI produced a ~ 2.7-kb fragment suggesting the association of a monopartite begomovirus. The DNA-A of the present isolate has all features of Old World begomoviruses. The complete viral genome found to have 2785 nt while betasatellite had 1365 nt and they have been submitted to GenBank under accession numbers MG571546 (full-genome) and betasatellites (MG571547). The full-genome sequence showed the highest similarity (99.6–99.5%) with TYLCV infecting tomato and Corchorus (KT033715; KT335023) reported from Saudi Arabia. Based on comparative sequence analysis, the betasatellite showed the highest similarity (98.8%) with TYLCB-Jeddah (KT355021). In a pairwise sequence alignment analysis, the full-genome DNA-A sequence of the present isolate (MG571546) was similar (99.6–70.6%) with selected begomovirus isolates (Table 1) while the betasatellite sequences showed 98.9–46.6% similarity with selected isolates (Table 2). The phylogenetic analysis of the present isolate with selected begomovirus isolates formed the closed cluster with TYLCV (KT033715-Tomato, KT355023-Corchorus, KT033713-Cucumber, KT728752-Tomato, KT728746-Tomato, KC845301-Tomato) earlier reported from Saudi Arabia (Fig. 2a). Interestingly, many other TYLCV isolates

Fig. 1 **a** Infected *Mentha longifolia* plant with yellow vein mosaic symptoms. **b** Healthy *M. longifolia* plant

Table 1 Sequence identity matrix of TYLCV-Mentha isolate (MG571546) with selected begomoviruses

Accession nos.	Acronyms	Hosts	Locations	% Identity matrix						
				Full (nt)	V2 (aa)	V1 (aa)	C3 (aa)	C2 (aa)	C1 (aa)	C4 (aa)
KT033715	TYLCV	Tomato	Jeddah	99.6	97.9	98.3	96.1	96.6	98.2	98.3
KT355023	TYLCV	Corchorus	Saudi Arabia	99.5	98.4	98.0	98.8	98.1	98.7	98.0
KC845301	TYLCV	Tomato	Jizan	99.0	98.3	98.5	98.2	98.1	98.0	98.5
KT033713	TYLCV	Cucumber	Saudi Arabia	98.9	97.5	97.0	97.8	97.1	98.7	97.0
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
KF040453	TYLCV	Tomato	Hail	93.7	92.5	92.4	92.9	93.1	91.7	92.0
JF919733	ToLCSDV	Tobacco	Yemen	93.6	92.5	92.3	92.9	93.1	92.7	92.0
KF561125	TYLCV	Tomato	Al-Qasim	92.4	91.6	91.2	92.1	90.9	90.8	91.9
JN591385	ToLCSDV	Tomato	Oman	92.2	91.0	89.9	88.3	90.1	91.6	91.0
KT033710	ToLCSDV	Amaranth	Hadasham	90.8	89.0	88.8	88.3	88.1	89.6	90.0
KT033711	ToLCSDV	Tomato	Jeddah	90.7	89.9	88.6	88.9	88.7	90.5	90.5
KT728742	ToLCSDV	Squash	Jeddah	90.5	89.9	89.8	88.9	88.6	87.8	90.6
JF919731	ToLCSDV	Tomato	Yemen	89.2	88.0	88.8	87.9	88.0	88.8	88.0
EF054894	TYLCV	Tomato	Jordan	82.3	80.5	81.8	81.8	81.5	81.5	81.0
GU076448	TYLCV	Tomato	Iran	82.2	81.0	81.9	81.9	81.0	81.6	81.7
AY044138	TYLCV	Tomato	Sudan	81.7	80.0	80.7	81.0	80.5	80.0	81.5
KF435137	TYLCV	Tomato	Alahsaa	81.2	79.9	80.1	80.8	80.2	81.6	80.8
DQ358913	TYLCMV	Tomato	Ethiopia	81.2	80.0	80.9	80.7	80.6	80.8	81.0
KF435136	TYLCV	Pepper	Alahsaa	81.1	80.4	80.2	80.4	80.1	80.6	81.2
AY594174	TYLCV	Tomato	Egypt	79.1	78.5	78.0	77.6	77.9	78.4	78.1
HE819240	TYLCV	Capsicum	Oman	78.6	77.8	77.0	78.0	77.6	79.2	78.0
JX131286	TYLCV	<i>S. arvensis</i>	Jordan	78.6	76.6	77.8	77.7	78.0	77.2	77.8
JQ354991	TYLCV	Tomato	Iraq	78.6	77.2	77.0	76.7	78.0	77.5	77.9
KC106648	TYLCV	Tomato	Iran	78.3	77.2	78.0	76.7	76.9	77.5	77.6
KF229726	TYLCV	Tomato	Oman	77.5	76.9	76.6	75.0	76.7	76.5	76.8
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
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

reported from Arabian Peninsula formed a separate cluster. The phylogenetic analysis based on sequences of present betasatellite isolate (MG571547) with selected begomoviruses formed a closed cluster with TYLCB reported from Saudi Arabia and Oman infecting tomato and cucumber (Fig. 2b).

Discussion

Mint is an important essential oil crop and globally used for multiple purposes. In this study, an association of begomovirus with yellow vein mosaic disease of *M. longifolia* in Saudi Arabia has been discussed. In Saudi Arabia, mint is extensively used for flavor and spices mixture. Although the begomovirus infection has been reported earlier in mentha species from other locations (Saeed et al. 2017; Saeed

and Samad 2017), currently no published report is available from Saudi Arabia. Based on recently published reports, the incidence of begomoviral disease in new crops and new regions are increasing at an alarming rate and causing huge economic loss to cultivated crops in the Kingdom. In this study, the findings provided a convincing evidence about the spread of TYLCV to mentha crops. In this work, during field survey, we observed a dense population of whiteflies to both tomato and mentha crops which indicated that the virus causing yellow vein mosaic disease to mentha crops could be a whitefly-transmitted begomovirus. The detection of begomovirus using begomovirus-specific primers in tested samples provided strong amplification of a begomoviral genome of 750 bp size in 11 out of 19 samples tested. We further extended our experimental work by full-genome as well as betasatellite molecules cloning and sequencing. The

Table 2 Sequence identity matrix of TYLCB-Mentha isolate (MG571547) with selected betasatellites

S. no.	Accession nos.	Acronyms	Hosts	Locations	% Identity
1.	KT355021	TYLCB	Tomato	Jeddah	98.9
2.	NC_010126	TYLCB-Om	Tomato	Oman	98.6
3.	KT728732	TYLCB	Tomato	Hadasham	98.5
4.	DQ644566	TYLCB-Om	Tomato	Oman	98.5
5.	KT728734	TYLCB	Tomato	Hail	98.3
6.	KT728740	TYLCB	Tomato	Tabuk	98.2
7.	KT180307	TYLCB	Cucumber	Hadasham	98.1
8.	KT355022	TYLCB	Corchorus	Jeddah	98.1
9.	KT312999	TYLCB	Tomato	Jeddah	97.9
10.	KU248483	TYLCB	Ridge gourd	Hadasham	95.1
11.	HE800545	TYLCB-Om	Tobacco	Oman	95.0
12.	HE800546	TYLCB-Om	Tobacco	Oman	94.1
13.	HE800541	TYLCB-Om	Papaya	Oman	93.3
14.	HE800551	TYLCB-Om	Tobacco	Oman	93.3
15.	HE800547	TYLCB-Om	Tobacco	Oman	93.3
16.	HE800549	TYLCB-Om	Tobacco	Oman	93.0
17.	HE800552	TYLCB-Om	Tobacco	Oman	93.0
18.	HE800548	TYLCB-Om	Tobacco	Oman	92.9
19.	HE800542	TYLCB-Om	Papaya	Oman	92.8
20.	HE800540	TYLCB-Om	Papaya	Oman	92.6
21.	HE800544	TYLCB-Om	Tobacco	Oman	92.1
22.	HE800550	TYLCB-Om	Tobacco	Oman	91.0
23.	HG969297	TYLCB-Om	Papaya	Oman	90.2
24.	HE800543	TYLCB-Om	Papaya	Oman	90.2
25.	HG969296	TYLCB-Om	Papaya	Oman	88.1
26.	JQ710745	AgeLCB	Amaranthus	India	65.1
27.	DQ641714	TYLCVVß	Tomato	Vietnam	61.6
28.	KF471033	TYLCTHB	Amaranth	India	55.4
29.	NC_004903	TYLCTHVß	Tomato	Thailand	54.9
30.	KC677734	ToLCJaB	Tomato	Java	54.3
31.	JF919720	ToLCYEB	Tobacco	Yemen	49.9
32.	JF919721	ToLCYEB	Tomato	Yemen	49.8
33.	JF919719	ToLCYEB	Tobacco	Yemen	49.7
34.	JF919718	ToLCYEB	Tobacco	Yemen	49.6
35.	JF919722	ToLCYEB	Tomato	Yemen	49.5
36.	JF919717	ToLCYEB	Tobacco	Yemen	49.2
37.	KT728729	ToLCSDB	Squash	Jeddah	49.1
38.	KT199104	TYLCB	Amaranthus	Hadasham	49.1
39.	NC_007485	TYLCMVß	Tomato	Mali	46.6
40.	KJ396939	OkLCV	Okra	Jordan	46.0

generated sequences showed the highest sequence identity (99.6–70.6%) with DNA-A genome while the betasatellite sequences showed 98.9–46.6% similarity with selected isolates. The close clustering of begomovirus isolates with previously reported begomovirus isolate TYLCV (KT033715-Tomato, KT355023-Corchorus) from Saudi Arabia provided another supporting information that the virus is closer to TYLCV infecting many cultivated crops in the Kingdom (Sohrab 2017a). The tomato fields nearby mentha crop fields

were also infected with similar strain of TYLCV as reported in mentha crop.

This 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 findings in this work based on PCR detection, sequence similarity

Fig. 2 **a** Phylogenetic relationships of TYLCV-Mentha isolate (MG571546) with selected begomoviruses based on full DNA-A genome. **b** Phylogenetic relationships of TYLCB-Mentha isolate (MG571547) with selected begomoviruses based on betasatellite genome

and phylogenetic relationships provided a convincing evidence that the begomovirus is expanding their host range and causing disease to a new host known as *M. longifolia*. Currently, very few cultivated and weed crops are reported to be infected with begomovirus in the Kingdom (Sohrab 2017a, b). It is well known that whiteflies play a significant role in virus disease spread to new hosts and new locations (Varma et al. 2011). 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 (Ajlan and Ghanem 2007; Duffy and Holmes 2008; Khan et al. 2008; Nawaz-ul-Rehman and Fauquet 2009; Lefeuvre et al. 2010; Paprotka and Jeske 2010; Sivalingam et al. 2010; Idris et al. 2011, 2012; Huang et al. 2013; Al-Saleh et al. 2014a, b; Hosseinzadeh et al. 2014; Kenyon et al.

2014; Khan et al. 2014; Paz-Carrasco et al. 2014; Yang et al. 2014; Tahir et al. 2015; Sohrab 2016, 2017; Al Rwahnih et al. 2017). The natural infection of TYLCV in *M. longifolia* showed the gradual increase of begomovirus host range which may cause heavy economic losses to the other cultivated as well as medicinal and weed crops in the near future in the Kingdom. 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.

Conclusion

Based on results obtained from this study, it is concluded that the begomovirus associated with yellow vein mosaic disease could be a mutated strain of TYLCV reported earlier to cause yellow vein mosaic and leaf curl disease in

many cultivated and weed crops in the Kingdom. So, in the future, there is an urgent need to conduct detailed studies about the presence of any other biotype of whitefly vectors, begomovirus disease epidemiology and incidence on other crops with the viral genome diversity and identification of any newly emerged virus strain so that an effective disease management strategy to protect the crops can be designed and developed in the Kingdom.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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