Molecular Detection of Begomoviruses Infecting Some Vegetable Crops in Saudi Arabia

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Abstract. Begomoviruses have a huge impact on crop production worldwide. Investigations of begomoviruses in four vegetable crops namely cucumber, squash, tomato, and watermelon were conducted by visual symptoms and molecular analysis using PCR with universal primers for begomoviruses. Obtained sequencing results of the core Coat Protein confirmed the presence of three already reported begomoviral species. TYLCV was detected on tomato samples exhibiting leaf curling and yellowing. Squash and greenhouse-grown cucumber plants were infected with the Oman strain TYLCV-OM. Watermelon chlorotic stunt virus (WmCSV) isolates were detected in watermelon and greenhouse cucumbers. The begomoviral species ToLCPMV shared 99% identity with the Iranian isolates that are known to be very destructive. Integrated pest management should be implemented against whitefly (*Bemisia tabaci*) to prevent the spread of begomoviruses on vegetable crops.

Keywords. Begomoviruses, cucumber, squash, tomatoes, watermelon, PCR.

1. Introduction

Weedy plants are widely dispersed and persist. Viral diseases caused by the Begomoviruses (family Geminiviridae) remain the most serious threat of global vegetable production. In Saudi Arabia, the first begomovirus was detected in the eastern province, which caused mosaic symptoms on tomato plants (Talhok, 1957). Since then, begomoviral diseases were reported on different crops in new geographical regions from Saudi Arabia. Symptoms appeared as dwarfism, leaves deformation, mosaic, vein yellowing, and stunting. Among those viruses, *Okra leaf curl virus* (OLCV) (Ghanem et al. 2003), *Tomato yellow leaf curl virus* (TYLCV) has been reported in Alhasa region on tomato and cultivated cucumber plants in Jeddah, Saudi Arabia (Alhudiab et al., 2014, Sohrab et al., 2017) and *Tomato leaf curl Sudan virus*

(ToLCSDV) was detected on squash (Sohrab, 2017), *Watermelon chlorotic stunt virus* (WmCSV) infection was reported on watermelon (Al-Saleh et al., 2014; Soliman et al., 2018).

There are 322 species within the genus *Begomovirus* detected by the International Committee on Taxonomy of Viruses (ICTV) in 2017 (Zerbini et al., 2017). So that, this genus is considered the largest genus in plant viruses, which contains one or two of circular single strand of DNA (ssDNA) (Zerbini et al., 2017). The first group containing Bipartite (DNA-A and DNA-B), which are prevalent in the New World, such as USA, Mexico and South America, while the monopartite group spreads in the Old world (OW), such as those countries in the continents of Asia, Africa, Europe and Australia. Monopartite begomoviruses contain particles associated

with the additional molecules called alphasatellites and betasatellites. These associated molecules are related to pathogenesis, symptom modulation and also act as silencing suppressors (Zhou, 2013). Despite the large number of viruses that have been detected around the world, all viruses in *Begomvirus* genus are transmitted by whitefly (*Bemisia tabaci*) biotype B (Gilbertson *et al.*, 2015) which is the dominant type in Saudi Arabia (Alhudaib *et al.*, 2014).

As the incidence of Begomoviruses increases in most areas of the Kingdom, with the increase in susceptible hosts, new strains are likely to be detected, further studies should be conducted to provide information on the various viruses of this genus. This study aimed to determine the status of begomoviruses infecting important vegetable crops e.g. tomato, cucumber, squash and watermelon using universal primers and characterization of these viruses via sequence of coat protein genes from detected viruses in order to benefit from these results in reducing the spread of these viruses.

2. Material and Methods

2.1 Field Visits and Collection of Samples

Vegetable fields were visited during the vegetable growing season in spring 2017-18 and leaf samples were collected from the symptomatic tomato, squash, cucumber and watermelon plants showing virus-like symptoms such as dwarf, wrinkle, veins yellowing, and mosaic. Ten samples from cucumber plants exhibited symptoms were selected from two greenhouses while tomatoes, squash and watermelon samples were collected from open fields in Alhasa area in the Eastern province where ten samples were collected with viral symptoms from tomatoes, squash and seven samples were collected from watermelon.

2.2 Extraction of Genomic DNA

Nucleic acid extractions and PCR amplifications were done in the Pests and plant diseases unit, College of Agricultural and Food Science, King Faisal University. Total DNA was extracted according to the method described by Doyle (1987) with some modification. About 100 mg leaf tissue was grinded with sand in 700 μ l of CTAB buffer (2% CTAB, 1.42 M NaCl, 20 mM EDTA and 100 mM Tris pH 8, 2% Polyvinylpyrrolidone (PVP40) and 1.5% β -mercapto ethanol) then the mix was vortexed and incubated for 30 min at 65 °C. the mixture was centrifuged for 1 min at 10,000 rpm then 500 μ l was transferred to a new 1.5 ml tube then 500 μ l of chloroform was added and mixed by vigorously vortexing. The mix was spun for 10 min at 10,000 rpm then the supernatant was removed to a new 1.5 ml tube and the DNA was precipitated by adding 300 μ l cold isopropanol. The mix was centrifuged for 10 min at 13000 rpm then the precipitated DNA pellet was washed with 70% cold ethanol two times. Then the pellet was air-dried and dissolved in 50 μ l free nuclease water.

2.3 PCR Amplification, Agarose Gel Electrophoresis and Sequencing

The PCR amplification of the begomovirus was carried out using the degenerate primer pair, AV494 (5'-GCCYATRTAYAGRAAGCCMAG-3') and AC1048 (5'-GGRTTDGARGCATGHGTACATG-3') (Wyatt and Brown, 1996)). The PCR reaction was done in a 25 μ l reaction containing 1 μ l of the DNA extract (~40 ng of genomic DNA), 2 mM MgCl₂, 2.5 of 10x PCR buffer, 1.5 μ L of 10 μ M of each primer, 2.5 μ l of 10 mM dNTPs, 0.3 μ l of 5U Taq DNA Polymerase (Biomatik, USA) to a final volume of 25 μ L with Nuclease-free water. PCR was conducted in the ESCO Swift Maxi Thermal Cycler. The PCR conditions were as follow:

Initial denaturation at 95°C for 2 min and 30 cycles of denaturation at 95°C for 1 min, primer annealing at 58°C for 1 min, extension at 72°C for 1 min follow by a final extension cycle at 72°C for 10 min. The PCR products were resolved to be analyzed on an agarose gel electrophoresis using TBE buffer.

The confirmed PCR products were purified using PCR purification kit (Qiagen) and the purified PCR products were sequenced by Macrogen Inc., (Korea) in both directions using AV494/ AC1048 primer pair.

2.4 Sequence Comparisons and Phylogenetic Analysis

The nucleotide sequences of the core coat protein (CP) were initially analyzed using Blastn in online database (<http://www.ncbi.nlm.nih.gov>) with the already submitted sequences. The top hits were downloaded and used to compare the sequences obtained in this study. The pair-wise nucleotide sequence comparison was carried out using Muscle algorithm available in species demarcation tool (SDT) (Muhire *et al.*, 2014). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and evolutionary analyses were conducted in MEGA7 software (Kumar *et al.*, 2016).

3. Results and Discussion

Plants showing different viral like symptoms including leaf curling, yellowing, distortion, and stunting were collected. The symptomatic plants were suspected to be positive for the presence of begomovirus infection (Fig. 1).

For further confirmation PCR analysis were carried out using degenerate primers (AC1048/AV494), which are universally designed to amplify the core CP gene of the begomoviruses. The PCR amplicons when electrophoresed on the agarose gel showed that

cucumber (10/10), squash (10/10), tomato (09/10) and watermelon (04/07) plants were positive and produced the expected ~550bp amplification (Fig. 2).

The bands were purified from the gel and directly sent for sequencing in both directions from Macrogen (Korea). Aligned sequences were deposited in the GenBank databases and their respective accession numbers were obtained (Table 1).

The sequencing results showed that the virus isolates belonged to 4 different begomovirus species. TYLCV-OM isolates were more prevalent on the squash crop while TOLCPMV isolates were detected on cucumber plants. On the other hand, one sample from cucumber was infected by WmCSV, which was the only detected virus in all watermelon plants and the most prevailing begomovirus detected from the cucurbits in Saudi Arabia (Soliman *et al.*, 2018). All tomato samples were infected with TYLCV. TYLCV-OM was reported previously from Al-Batinah region in Oman (Khan *et al.*, 2008). Data present in Fig. 3 and Table 2 reveals that all the begomovirus isolates from squash plants were 98 to 100% identical to each other while, shared their nt sequence identities 95 to 96% to the TYLCV-OM strain. The clone Cu20 from Cucumber were 98 to 100 % nt sequence identity to other TYLCV-OM from squash and showed 96% nucleotide (nt) sequence identity to the TYLCV-OM strain reported from Oman. The clone To5 from tomato shared 98% identity to other TYLCV reported previously from Saudi Arabia and Kuwait and 95% to TYLCV-OM strain. All TYLCV and TYLCV-OM were placed in group I, clade1 with identity ranged from 95 to 100%, which indicated that TYLCV-OM strain was originated from TYLCV and jumped from solanaceous crops to cucurbits. International Committee on Taxonomy of Viruses (ICTV) has classified

TYLCV-OM as an isolate of TYLCV from Iran. The group II in clade 1 includes all WmCSV isolates which shared identity with other reported WmCSV from Saudi Arabia and Palestine ranged from 97 to 99%. The nt sequence identities between the begomovirus isolates from group I and group II were 71 to 76%. The clade 2 includes all ToLCPMV isolates obtained from cucumber samples Cu7 and Cu9 which were 99% identical to Iranian isolates from cucumber and cantaloupe. These two isolates shared 94% identity with Indian isolates from musk melon, papaya, and also ToLCPMV obtained from tomato in Pakistan. ToLCPMV was first detected on tomato crops in India (Kumar *et al.*, 2008) since then the virus has spread dramatically in cucurbit plants including melon and cucumber in Iran resulted in severe symptoms, which caused up to 100% losses in these two crops (Heydarnejad *et al.*, 2009).

Global migration of begomoviruses represents major threat to the vegetable

production in the Middle Eastern countries. However, newly and more aggressive strains of begomoviruses may not be detected yet in Saudi Arabia. Extensive survey should be done to widen our knowledge about these newly emerging begomovirus species to protect vegetable production from these devastating viruses in Saudi Arabia.

4. Conclusion

Begomoviruses are major and serious threat to vegetable production under either open field or green houses-grown vegetable crops. New aggressive strains may be introduced to Saudi Arabia but not detected yet. ToLCPMV was detected only in India and Iran but not reported in the Arabian Peninsula. This requires further study to monitor these new introductions into the Saudi Arabia vegetable ecosystem and to benefit from the study in the development of control strategies against begomoviruses.

Fig. 1. Different symptoms of begomoviruses infected plants. (A) Cucumber infected with Tomato leaf curl Palampur virus (ToLCPMV); (B) Squash infected with Tomato yellow leaf curl virus-Oman (TYLCV-OM); (C) Tomato infected with Tomato yellow leaf curl virus (TYLCV); and (D) Watermelon infected with Watermelon chlorotic stunt virus (WmCSV).

Fig. 2. PCR amplification of the core CP from the infected plant leaf samples in this study with AV494/ AC1048. . From lane 1-10 are cucumber samples, 11-20 are squash samples, 21- 30 are tomato samples while 31-37 are watermelon samples and M is 100bp ladder. -Ne is non-template control.

Table 1. Deposited accession numbers of different begomoviruses in this study.

Accession number	Isolate name	Host	Virus name	Type	Acronym
MG970132	Sq4	Squash	Tomato yellow leaf curl virus-Oman	Monopartite	TYLCV-OM
MG970133	Sq7	Squash			
MG970134	Sq8	Squash			
MG970135	Sq9	Squash			
MG970136	Sq10	Squash			
MG970137	Sq12	Squash			
MG970138	Sq13	Squash			
MG970139	Cu20	Cucumber	Tomato leaf curl Palampur virus	Bipartite	ToLCPMV
MG970140	Sq22	Squash			
MH025640	Cu7	Cucumber	Watermelon chlorotic stunt virus	Bipartite	WmCSV
MH025641	Cu9	Cucumber			
MH025643	Cu1	Cucumber	Tomato yellow leaf curl virus	Monopartite	TYLCV
MH025644	Wm10	Watermelon			
MH025645	To5	Tomato

Fig. 3. Phylogenetic tree of evolutionary analyses between begomovirus isolates. Accession numbers obtained in this study are highlighted with white text on a black background.

Table 2. Pair-wise nucleotide sequence comparison 26 nucleotide sequences conducted in species demarcation tool (SDT).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1-MH025645_TYLCV_To5	100																									
2-KF435137_TYLCV_SA	98	100																								
3-HG969254_TYLCVOM_OM	95	96	100																							
4-KF229723_TYLCVOM_OM_To	95	96	99	100																						
5-KJ830842_TYLCV_KU	98	90	96	96	100																					
6-MG970135_TYLCVOM_Sq9	96	97	96	97	97	100																				
7-MG970137_TYLCVOM_Sq12	96	97	96	97	97	100	100																			
8-MG970138_TYLCVOM_Sq13	96	97	96	96	97	100	100	100																		
9-MG970139_TYLCVOM_Cu20	95	96	96	96	96	99	99	98	100																	
10-MG970140_TYLCVOM_Sq22	95	96	96	96	96	99	99	98	100	100																
11-MG970134_TYLCVOM_Sq8	95	96	96	97	96	98	98	98	100	100	100															
12-MG970132_TYLCVOM_Sq4	95	96	96	97	96	98	98	98	100	100	100	100														
13-MG970133_TYLCVOM_Sq7	95	96	96	97	96	98	98	98	100	100	100	100	100													
14-MG970136_TYLCVOM_Sq10	95	96	96	97	96	98	98	98	100	100	100	100	100	100												
15-MH025643_WmCSV_Cu1	74	75	76	76	76	76	76	76	75	75	76	76	76	76	100											
16-KJ958912_WmCSV_SA	75	71	77	76	72	76	76	76	75	75	76	76	76	76	99	100										
17-MH025644_WmCSV_Wm10	75	76	77	76	76	76	76	76	76	76	76	76	76	76	100	99	100									
18-KC462552_WmCSV_PL_Wm	74	71	76	76	71	75	75	76	75	75	75	75	75	75	98	98	98	100								
19-KJ939448_WmCSV_SA	75	71	76	75	72	76	76	76	76	76	76	76	76	76	97	97	97	98	100							
20-MH025641_ToLCPMV_Cu7	71	71	71	71	71	71	71	71	72	72	72	72	72	72	70	71	70	70	70	100						
21-MH025642_ToLCPMV_Cu9	71	71	71	71	71	71	71	71	72	72	72	72	72	72	70	71	70	72	70	100	100					
22-FJ660444_ToLCPMV_IR_Cu	71	72	71	71	71	70	70	70	71	71	71	71	71	71	70	70	70	69	70	99	99	100				
23-EU547683_ToLCPMV_IR_Can	71	72	71	71	71	70	70	70	71	71	71	71	71	71	70	69	70	69	70	99	99	100	100			
24-AM884015_ToLCPMV_IN_To	72	72	71	71	72	71	71	70	71	71	71	71	71	71	71	70	70	69	70	94	95	97	97	100		
25-LN864817_ToLCPMV_PK_Pap	72	72	71	71	71	71	71	70	71	71	71	71	71	71	71	70	70	69	70	94	94	97	97	99	100	
26-KY564204_ToLCPMV_IN_Mmel	73	72	72	72	73	72	72	72	72	72	72	73	73	73	71	69	70	69	69	94	94	93	93	94	93	100

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الكشف الجزيئي لفيروسات البيجمو التي تصيب بعض محاصيل الخضر في المملكة العربية السعودية

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المستخلص. فيروسات البيجمو لها تأثير كبير على إنتاج المحاصيل في جميع أنحاء العالم. تم إجراء دراسة حول فيروسات البيجمو على أربعة محاصيل للخضر، وهي: الخيار، والكوسة، والطماطم، والبطيخ، عن طريق الأعراض الظاهرية والتحليل الجزيئي باستخدام تفاعل البلمرة المتسلسل مع البادئات العالمية لفيروسات البيجمو. وأكدت نتائج التسلسل في المنطقة الأساسية في الجين الذي يشفر للغلاف البروتيني وجود ثلاثة أنواع من فيروسات البيجمو، والتي تم تسجيلها بالمملكة العربية السعودية سابقاً. تم الكشف عن فيروس تجعد واصفرار أوراق الطماطم (TYLCV) على عينات الطماطم التي تظهر تجعد في الأوراق والاصفرار. وكانت الكوسة وخيار البيوت المحمية مصاب بفيروس اصفرار وتجعد أوراق الطماطم-سلالة عمان (TYLCV-OM). ووجدت نباتات البطيخ وخيار البيوت المحمية مصابة بفيروس النقرم الأصفر في البطيخ (WmCSV). وكانت عزلات فيروس تجعد أوراق الطماطم بالامبور تتطابق بنسبة ٩٩٪ مع العزلة الإيرانية من هذا الفيروس والمعروفة بشراستها. لذلك فإن برامج مكافحة المتكاملة يجب تطبيقها ضد الذبابة البيضاء لمنع انتشار فيروسات البيجمو على محاصيل الخضر.

الكلمات المفتاحية: فيروسات البيجمو، الخيار، كوسة، طماطم، بطيخ، تفاعل البلمرة المتسلسل، السعودية.