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Original Article

Disease complex associated with begomoviruses infecting squash and cucumber in Saudi Arabia

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Abstract

During the field visits in growing season of 2022 in Dammam Region of Saudi Arabia, begomovirus-like symptoms including leaf curling, leaf cupping, leaf distortion, vein thickening and reduced leaf size were observed in squash and cucumber fields. Twenty-five samples were collected from each crop and PCR amplification was done using general diagnostic begomovirus primers (AC-1048/AV-494 and Begomo I/Begomo II). The obtained results showed desired sized amplified DNA fragments (550 bp and 1.1 kb) according to the primer sites. Sequencing results were analyzed using BLAST and revealed the presence of three different bipartite begomoviruses which include Squash leaf curl virus (SqLCV) isolated from squash and cucmber, Watermelon chlorotic stunt virus (WmCSV) and Tomato leaf curl Palampur virus (ToLCPalV) isolated from squash. The highest nucleotide identity found was 99.4% with Egyptian SqLCV isolated from squash and the lowest similarity was 93.3% found with a USA isolate isolated from wheel cactus. Sequencing results of two isolates of WmCSV showed 100% sequence identity with each other, eight isolates from Palestine isolated from watermelon, two isolates from Mexico isolated from prickly pear cactus and Watermelon, one isolate from each Lebanon and Jordan isolated from melon and wild mustard respectively. The lowest identity (87%) was found with a Saudi Arabian isolate isolated from papaya. For ToLCPalV isolate showed the highest identity (100 %) with an already reported isolate of same virus from melon in Saudi Arabia and two isolates isolated from cucumber and cantaloupe in Iran. However, the lowest identity (95.3%) was found with an Indian isolate isolated from eggplant. This is the first investigation of complex viral disease caused by SqLCV, WmCSV and ToLCPalV on the basis of molecular characterization from squash and a SqLCV isolate from Cucumber in Saudi Arabia.

Keywords: Begomovirus, Cucurbits, PCR, SqLCV, WmCSV, ToLCPalV

1. Introduction

Cucurbits contain a diverse group of plants in the family Cucurbitaceae which includes cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*), melon (*C. melo* L.), pumpkin (*Cucurbita maxima* L.), summer squash (Cucurbita pepo L.) and winter squash (Cucurbita moschata L.). These cucurbits produce many vital fruits and vegetables which play an important role in the diet globally [1]. According to the different estimates 3-5 % losses occur overall in production of these vegetables, sometimes losses are very high where the insect pests are not controlled efficiently especially in developing countries [2].

Squash and cucumber are important vegetable crops among the cucurbits in Saudi Arabia, and hydroponics and greenhouse technologies are increasing their production continuously. During the year 2010 production of squash was 14431 tons from 193 hectares which increased then to 16276 tons from 201 hectares in 2011. In year 2012 production was 15433 tons from 197 hectares which increased in 2013 that was 15309 tons from only 189 hectares, while for cucumber it reached 98387 tons cultivated on 1187 hectares [3].

Viral infections are a serious threat worldwide for the production of cucurbits which causes heavy economic losses and can incite a disease epidemic, while the seed-borne pathogens have more potential to disseminate around the globe through seed markets [4]. Geminiviridae is a family of plant viruses having single-stranded circular DNA genome encapsulated in semi-icosahedral twinned particles [5]. In this family, Begomovirus is a genus that is dispersed across the world infecting plants belonging to dicotyledonous species that are transmitted through whiteflies (*Bemisia tabaci*). Begomovirus is the largest genus in Geminiviridae family containing almost 424 species [6].

The genus Begomovirus is recognized for its wide diversity among its members. In the world, there are currently over 300 species of begomovirus known using species demarcation criteria defined by ICTV (such as DNA-A nucleotide sequence identity) [7, 8]. They are devastating pathogens that cause great losses throughout the world in

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several economically vital crops [9, 10].

Begomoviruses consist of bipartite genomes with both segments' DNA-A and DNA-B while monopartite genomes have only DNA-A segment of genome [11]. Old World begomoviruses are primarily monopartite, while New World begomoviruses are primarily bipartite [12, 13]. In the past thirty years, numerous begomoviruses spread by whiteflies have become deadly infectious causing a lot of economic losses and posing a threat to crop production [14].

2. Material and methods

2.1. Sample collection, DNA extraction and PCR amplification

During the growing season of 2021, a field visit was conducted in squash and cucumber plants in Dammam region of Saudi Arabia. A total of 25 samples were collected from both crops showing begomovirus-like symptoms including leaf curling, yellowing occupied by mosaic and mottling patterns. Using the Thermo Scientific Gene JET Plant DNA Purification Mini Kit following the protocol instructions from the manufacturer, total DNA was extracted from the samples. General diagnostic begomovirus primers: AC1048: 5`-ggrttdgargcatghgtacatg-3`, AV494: 5`-gccyatrtayagraagccmag-3` (Wyatt and Brown,1996) and Begomo I: 5`-ccgtgctgctgcccccattgtccgcgtcac-3`; Begomo II: 5`- ctgccacaaccatggattcacgcacaggg-3` were used in PCR analysis [15-17]. PCR product was loaded on 1 % agarose gel and subjected to electrophoresis for analysis.

2.2. Nucleotide sequencing and phylogenetic tree analysis

From positive PCR amplified products, samples were selected (according to required concentration) and sent to Macrogen Inc. (Seoul, South Korea) for two-directional Sanger DNA sequencing. The obtained sequence results were analyzed with BLAST program. On MEGA-X, a phylogenetic tree was constructed using sequences that were aligned with ClustalW, using the Maximum-Likelihood method with 1000 boots-trap replications. DNA star software was used for nucleotide identity table of Saudi isolates with other reported isolates in GenBank database.

2.3. Statistical analysis

In order to create an SDT graph for our results comparison, the Sequence Demarcation Tool (SDT) analysis was performed using the SDTv1.2 application with the default parameter. This is a three-colored matrix based on the pairwise sequence identity (PSI) values.

3. Results

3.1. Field observations

Symptomatic squash and cucumber samples that were collected showed complex different viral-like symptoms including leaf curling, clear mottling on the leaf, narrowing of the leaf, deformed evidently, intermingled dark green, light green patches, vein chlorosis, and blistering symptoms causing deformation of leaves and stunted growth were observed and these plants exhibiting symptoms were suspected to be positively infected with the begomovirus. (Fig. 1).

The DNA fragments were purified from the agarose gel and sequenced in both directions. Sequencing results when analyzed using NCBI BLAST revealed the presence of three different viruses belonging to genus Begomovirus which include SqLCV, WmCSV and ToLCPalV.

3.2. PCR amplification and phylogenetic analysis

For further confirmation, universal primers. [AC1048/ AV494 (550bp) and Begom-I/ Begom-II (1.1Kb)] were used to amplify the begomoviruses' main CP gene, PCR was conducted using the extracted total DNA from plants that were exhibiting symptoms. **Fig. 2 A and B)**.

With the following accession numbers, these aligned sequences were deposited to the GenBank: OQ971715 (SqLCV from cucumber), OQ971716 and OQ971717 (SqLCV from Squash) using PCR product amplified with AV494/AC1048 primer. Whereas OQ971718, and OQ971719 (WmCSV from Squash) and OQ971720 (ToLCPalV from Squash) using PCR product amplified with Begomo-1 and Begomo-2 primer.

Sequence of three isolates of SqLCV (accession no.: OQ971715, OQ971716, and OQ971717) on both strands of each isolate revealed that size of amplified products was 423 bp long. Sequence analysis showed that SqLCV isolates shared 100% nucleotide identity between themselves. The highest nucleotide identity found was 99.4% with Egyptian SqLCV (accession no.: MK284930) isolated from squash while the lowest similarity was 93.3% found with a USA isolate (accession no.; MW588395) isolated from wheel cactus (*Opuntia robusta*. Sequencing results of both isolates of WmCSV (accession no.: OQ971718 and OQ971719) showed that there was 100% similarity between themselves, eight isolates from Palestine (accession no.: JN673223, KJ854919, KJ854918, KJ854917, KJ854916, KJ854915, KJ854914, KJ854913 and KJ854912) isolated from watermelon, two isolates from Mexico (accession no.: MW588390 and KY124280) isolated from prickly pear cactus (*Opuntia auberi*) and Watermelon, one isolate from each Lebanon (accession

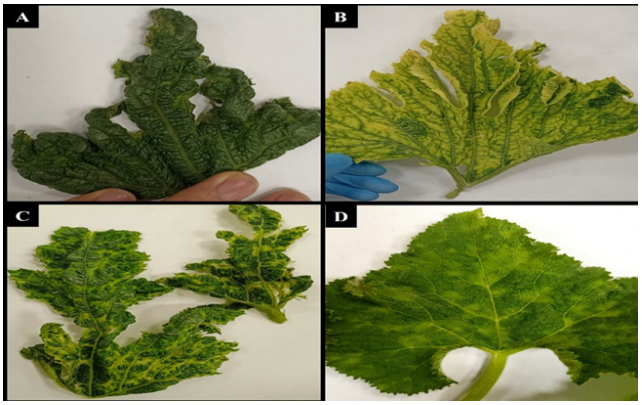

Fig. 1. Different symptoms of begomoviruses on squash plant (A, B, C) and Cucumber (D) infected with complex begomoviruses (SqLCV, WmCSV and ToLCPalV).

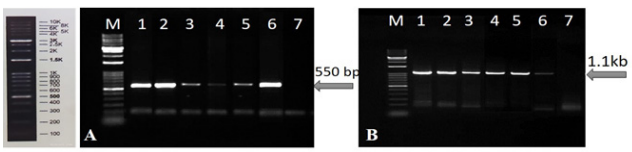

Fig. 2. PCR amplification from the infected squash and cucumber leaf samples with AV494/ AC1048 (A) and Begom-I/ Begom-II (B) primers. lane 1-5 are squash and lane 6 is cucumber sample, and lane M is 100bp ladder. Lane 7 is a negative control.

no.: HM368371) and Jordan (accession no.: JX131283) isolated from *C. melo* and wild mustard (*Sinapis arvensis)* respectively. The lowest identity (87%) was found with a Saudi Arabian isolate (accession no.: ON206052) isolated from papaya (*Crica papaya)*. For ToLCPalV isolate (accession no.: OQ971720) it showed highest identity (100 %) with an already reported isolate of same virus (accession no.: ON843661) from another host melon in Saudi Arabia and two other isolates from Iran (accession no.: FJ660444 and EU547683) isolated from *C. sativus* and cantaloupe respectively. However, the lowest identity (95.3%) was found with an Indian isolate (accession no.: OM315008) isolated from eggplant. Phylogenetic tree analysis and Distance matrix illustrating the pairwise nucleotide identity percentage showing nucleotide identity of Saudi Arabian isolates of SqLCV (Figure 3), WmCSV (Figure 4) and ToLCPalV (Figure 5) with other identified isolates worldwide. Closely related grouped isolates of each virus are highlighted with red color with highest identity, while different sequences are highlighted with blue color having lowest identity.

4. Discussion

Plant viruses cause agricultural losses of almost USD 30 billion a year [18]. Moreover, the economic effect of these infections will rise in the current state of global warming [19]. Four of the top twelve horticultural crops in the world are members of the Cucurbitaceae family, which is one of the biggest vegetable groups. *C. sativus*, *C. melo*, and watermelon (*C. lannatus*) are the three genera that contain the majority of cucurbits. Cucurbita spp. includes gourds, zucchini, pumpkin, and squash. Significantly more minor cucurbits are grown in several parts of the world; they are staple foods that are good for human health [20].

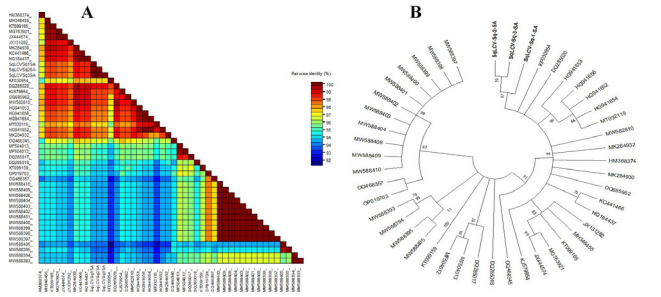

Fig. 3. Distance matrix illustrating (A) the pairwise nucleotide identity percentage of SqLCV Saudi isolates as compared with other most similar sequences. Phylogenetic tree (B) of evolutionary analyses between SqLCV Saudi isolates and other retrieved isolates from GenBank globally.

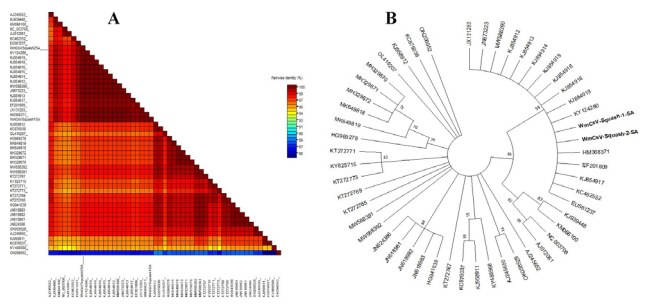

Fig. 4. Distance matrix illustrating (A) the pairwise nucleotide identity percentage of WmCSV Saudi isolates as compared with other most similar sequences. Phylogenetic tree (B) of evolutionary analyses between WmCSV Saudi isolates and other retrieved isolates from GenBank globally.

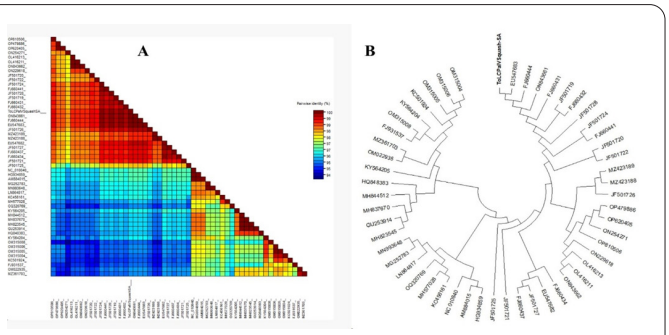

Fig. 5. Distance matrix illustrating (A) the pairwise nucleotide identity percentage of ToLCPalV Saudi isolates as compared with other most similar sequences. Phylogenetic tree (B) of evolutionary analyses between ToLCPalV Saudi isolates and other retrieved isolates from GenBank globally.

Cucurbit crops are susceptible to dozens of viruses that can spread globally through a variety of vectors or mechanical means [21, 22]. For the last thirty years, begomoviruses have become a major threat to cucurbit crops in Asia, Africa, America, and Europe. According to estimates, cucurbits are infected by 24 different species and variants of begomoviruses worldwide. Among the most often reported ones are squash leaf curl China virus, tomato leaf curl New Delhi virus, and ToLCPalV [23].

The first begomovirus in Saudi Arabia was found in the eastern province [24] infecting tomatoes. After that, begomoviral infections on various crops have been reported. Dwarfism, deformed leaves, mosaic, vein yellowing, and stunting were the symptoms that first manifested. Among those viruses are the WmCSV infection and the okra leaf curl virus (OLCV) [25]. Tomato yellow leaf curl virus (TYLCV) has been documented on Jeddah's farmed cucumber and tomato plants in the Eastern region, Saudi Arabia, and tomato leaf curl Sudan virus (TLCSDV) was detected on squash [26-28]. Recently three viruses had been reported from papaya: TYLCV, WmCSV and Cotton leaf curl Gezira betasatellite (CLCuGB) [6].

There are reports of mixed infection of begomoviruses in various crops and cucurbits previously throughout the world [7, 29-31]. In this study, we have reported three bipartite begomoviruses isolated from Squash (SqLCV, WmCSV and ToLCPalV) and this is the first report of SqLCV infection in cucumber and ToLCPalV in squash in Saudi Arabia. SqLCV was discovered in Saudi Arabia and recognized by immunosorbent electron microscopy in a partially purified extract of squash samples [32], but there were no molecular evidences for the presence of this virus. SqLCV was previously documented in the region on cucurbits including Palestine [33] and Egypt [34]. Further studies are needed to understand the etiology and pathogenesis of mixed infection of three bipartite begomoviruses. The complete genome of DNA A and DNA B are needed to study their synergistic or antagonistic effects and their recombination and pseudo recombination effects.

Watermelon was first found to be infected by WmCSV in Yemen [35] but has been thoroughly addressed in a number of Middle Eastern nations and regions (Iran, Israel, Jordan, Lebanon, Oman, West Bank, Palestine and Saudi

Arabia) and Sudan in Africa [33, 36-43]. Although WmCSV can infect other cucurbits as well, it primarily causes significant harm to watermelon crops [36]. Recently, it has been reported to infect watermelon in Sonora, Mexico [44] resulting in the characteristic leaf curling and yellowing symptoms, marking the first instance of this begomovirus species being identified in the Western Hemisphere. Since WmCSV has been found in Saudi Arabia in zucchini and other hosts in nearby nations, it appears that this virus is a major concern to cucurbits in the area [40].

ToLCPalV was first identified in tomato fields in India [45], the virus has spread rapidly among cucurbit and other plants, which include watermelon, chiles, cucumber, muskmelon, bitter gourd, pumpkin, squash, and common beans [15, 46-51-52].

It is crucial to understand the diversity and distribution of begomoviruses in order to develop effective disease control methods. This study focused on begomovirus infection in squash and cucumber crops grown in Saudi Arabia. Begomovirus sequences from the hosts squash and cucumber were successfully amplified by PCR employing universal begomovirus primers for begomovirus detection.

5. Conclusion

This work provides insight into begomovirus infection in squash and cucumber plants in Saudi Arabia. ToLCPalV and SqLCV are reported for the first time to infect squash and cucumber plants respectively. WmCSV has been reported from watermelon and melon before in Saudi Arabia while it is reported for the first time from squash in Saudi Arabia.

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Author contributions

Conceived the project and designed the studies: Mahmoud A Amer. Sample collection and execution of experiments in the lab: Mohammed A. Al-Saleh. Analysis of data and drafting of the manuscript, Zaheer Khalid, Khadim Hussain, Mahmoud Amer. Critical revision of the manuscript for important intellectual content: Mohammed A. Al-Saleh, Ibrahim M Al-Shahwan. All authors read and approved the final version of the manuscript.

Availability of data

Partial genome sequences of one isolate of SqLCV from cucumber (OQ971715), two isolates of SqLCV from Squash (OQ971716 and OQ971717), while two isolates of WmCSV from Squash (OQ971718, and OQ971719) and one isolate of ToLCPalV from Squash (OQ971720) were used in this study and all the data is available in Genbank and accession numbers are provided in the parentheses.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Consent for publications

All authors have read and approved the final manuscript for publication.

Ethical approval and consent to participate

The authors declare that they did not use humans or animals in the research presented in this manuscript.

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