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Identification and molecular analysis of watermelon chlorotic stunt virus infecting snake gourd in Saudi Arabia

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Abstract

Snake gourd (*Trichosanthes cucumerina*) plants exhibiting typical begomovirus-like symptoms of stunted growth, leaf yellowing and mottling were observed at an open field in the Eastern region of Saudi Arabia. Sequencing analysis of the amplified complete DNA molecules revealed that the plants were infected with watermelon chlorotic stunt virus (WmCSV), which is a bipartite begomovirus prevalent mostly in the Old World and a serious threat to cucurbit production in the Arabian Peninsula, Middle East and Africa. The two WmCSV DNA-A isolates (SG31A and SG52A) were 98.9% identical and showed their highest nucleotide (nt) sequence identities (98.7%) with the isolates from Iran and Saudi Arabia. The DNA-B isolates (SG31B and SG52B), on the other hand, were 97.4% identical and exhibited their highest nt sequence identities (99.5 and 97%) with isolates reported from Iran and Oman. In the phylogenetic dendrograms the identified isolates clustered closely with previously reported WmCSV isolates from Iran and Saudi Arabia. Infectivity assays revealed that the DNA-A components alone could not induce infection in *Nicotiana benthamiana* plants however, together with DNA-B these isolates successfully caused typical begomovirus symptoms and both components were detected successfully using Southern blot hybridization. This study highlights the importance of conducting extensive future begomovirus surveillance to detect spillover events that could threaten native vegetable production in Saudi Arabia. This is crucial as begomoviruses pose a serious threat to vegetable cultivation throughout the Middle East.

Keywords: agroinoculation; bipartite begomovirus; *Nicotiana benthamiana*; snake gourd; WmCSV

Introduction

Begomoviruses, with over 500 identified species, represent the largest genus within the *Geminiviridae* family, primarily infecting dicotyledonous crops worldwide (Brown *et al.,* 2015; Zerbini *et al.,* 2017). These single-stranded DNA (ssDNA) viruses, encapsulated in twinned-icosahedron protein coats, are responsible for significant diseases in tropical, subtropical, and temperate regions (Hanley-Bowdoin *et al.,* 2013). Based on their genome organization, begomoviruses are categorized as monopartite or bipartite. Bipartite begomoviruses possess two nearly equal-sized DNA components, DNA-A and DNA-B, each encapsulated within the protein coat. These components share a region known as the common region (CR). This region spans approximately 200-400 base pairs and harbors a distinctive stem-loop structure containing the nona-nucleotide sequence TAATATTAC (Zerbini *et al.,* 2017). Monopartite begomoviruses, in contrast, have a single DNA-A

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component equivalent to the bipartite DNA-A. Five to six open reading frames (ORFs) encoded within the begomovirus genome govern replication, encapsidation, and pathogenesis (Iqbal *et al.,* 2012; Saeed *et al.,* 2018). DNA-A in bipartite viruses harbors these ORFs in opposite orientations: two in the virion-sense strand (AV1 and AV2) and four on the complementary strand (AC1, AC2, AC3, and AC4) (Hanley-Bowdoin *et al.,* 2013). These proteins play a multifunctional role throughout the viral life cycle including: viral replication and encapsidation, regulation of viral gene expression, viral DNA accumulation, symptom determination, and suppression of transcriptional and post-transcriptional gene silencing (Hanley-Bowdoin *et al.,* 2013). Within the genome's CR, a single promoter initiates transcription for all ORFs (Ashraf *et al.,* 2014). Beyond the well-characterized six ORFs, breakthrough studies have revealed a collection of novel, miniature ORFs (V3, C5, C6, and C7) encoded by the monopartite begomovirus genome (Gong *et al.,* 2021; Liu *et al.,* 2023). Bipartite DNA-B encodes only two ORFs (BV1 and BC1) in opposite orientations (Fondong, 2013). Notably, DNA-B plays a crucial role in regulating viral movement across cellular compartments within the infected plant (Briddon *et al.,* 2010).

Despite a distinct geographic pattern in begomovirus genome architecture, fascinating anomalies exist. Notably, bipartite begomoviruses are surprisingly scarce in the Old World (OW), while monopartite ones are rarely encountered in the New World (NW) (Romay *et al.,* 2019). A significant number of devastating plant diseases in the OW originate from the association between monopartite begomoviruses and single-stranded DNA-satellites such as alphasatellites, betasatellites, and recently discovered deltasatellites (Fiallo-Olivé and Navas-Castillo 2020). Betasatellites, in particular, impact disease progression by encoding the symptom determinant protein βC1 (Yang *et al.,* 2019). Alphasatellites and deltasatellites play pivotal roles in monopartite begomovirus infections by furnishing supplementary functions that enhance the complexity of the infection mechanism (Briddon *et al.,* 2018). Importantly, while bipartite begomoviruses are less common in the OW, exceptions exist. Several studies have identified bipartite begomoviruses in the Old World (AlHudaib *et al.,* 2022; Rezk *et al.,* 2019; Shahid *et al.,* 2021). Begomoviruses are widely spread due to several factors. First, a cryptic group of whitefly species (*Bemisia tabaci* complex) is prevalent worldwide and transmits the virus. Second, begomoviruses can infect wild plants, creating a reservoir for the virus. Third, human activities like travel and agriculture can unknowingly move the virus (Rojas *et al.,* 2018). Finally, begomoviruses can change and adapt by combining their genetic material and acquiring new parts, allowing them to infect a wider range of plants. Watermelon chlorotic stunt virus (WmCSV), a begomovirus with bipartite genomic architecture, was initially documented in Yemen during the late 1980s (Alhubaishi *et al.,* 1987), and from hereon it quickly spread to neighboring Middle Eastern and North African countries (Kheyr-Pour *et al.,* 2000). Subsequently, it was reported in Israel, Jordan, Lebanon, Oman, and Palestine (Abudy *et al.,* 2010; Al-Musa *et al.,* 2011; Ali-Shtayeh *et al.,* 2014; Khan *et al.,* 2012; Samsatly *et al.,* 2012; Shafiq *et al.,* 2021). Most recently, WmCSV has been found in Mexico (Dominguez-Duran *et al.,* 2018) and USA (Fontenele *et al.,* 2021). In Saudi Arabia, WmCSV was first detected in watermelon crops in 2014 (Al-Saleh *et al.,* 2014). A recent study also characterized it from zucchini and watermelon plants in the Eastern region (Rezk *et al.,* 2019).

Begomoviruses, a constant threat to vegetable crops in tropical and subtropical regions (Zerbini *et al.,* 2017), pose a particular challenge in Saudi Arabia. Snake gourd (*Trichosanthes cucumerina*), a popular annual vegetable in many Asian countries with known pharmacological and therapeutic properties, is no exception. A recent survey in Saudi Arabia's Al Ahsa region identified symptomatic snake grourd plants. Subsequent testing confirmed begomovirus infection (Sattar, 2018), prompting a detailed investigation into the complete genome of the dominant begomovirus strain affecting these crops.

Materials and Methods

Sample collection

During the 2016-17 growing season, the snake gourd plants in a field located in the Al-Ahsa region of Saudi Arabia were observed exhibiting stunted growth, leaf yellowing, and mottling (Figure 1). The youngest leaves from three symptomatic and one asymptomatic plant were collected and stored in plastic bags for further analysis.

Figure 1. Snake gourd plants in the Al-Ahsa region, Saudi Arabia, exhibiting signs of a possible viral infection. The symptoms included leaf yellowing and mottling **(A)**, contrasting a healthy plant **(B)**

Genomic DNA Isolation and Agarose Gel Electrophoresis

Genomic DNA was extracted from all leaf samples using CTAB extraction method as described in previous literature (Doyle *et al.,* 1990). To ensure purity and minimize contamination risks, the isolated DNA was eluted in autoclaved, double-distilled water. Its quality and integrity were then evaluated using 1% agarose gel electrophoresis with 0.5 × TBE buffer.

Rolling circle amplification (RCA) and PCR amplifications

Rolling circle amplification (RCA) was performed on isolated DNA from all plant samples using Phi-29 DNA polymerase from a commercially available ThermoScientific kit, as described previously (Qurashi *et al.,* 2017). The RCA product was quantified using gel electrophoresis. Successful products were then diluted 10× for use in a subsequent PCR-based screening with specific degenerate primers (Table 1) to amplify the coat protein (CP) gene of the begomovirus genome (Wyatt and Brown 1996). Successful PCR amplicons were visualized on an agarose gel stained with ethidium bromide. Attempts to amplify DNA-satellites using established universal primers for alphasatellites (Bull *et al.,* 2003) and betasatellites (Briddon *et al.,* 2002) were unsuccessful. The amplified partial CP products were sequenced at Macrogen, Korea.

Cloning and sequencing

The RCA products were analyzed for restriction fragment length polymorphism (RFLP) with EcoRV, EcoRI, SmaI, XhoI, and SacII endonucleases. The enzymes EcoRI, SmaI, and SacII generated RFLP products corresponding to the full-length monomeric begomovirus genome (~2.7-2.8 kb). The restricted fragments were then recovered from the gel using the ThermoScientific GeneJet Gel Extraction kit and cloned into the pBluescript cloning vector. Selected recombinant clones containing DNA fragments inserted from EcoRI and SacII digestions were sequenced completely using Sanger sequencing at Macrogen, Korea.

Table 1. List of primers used for the detection of begomovirus and/or DNA-satellites

Primers	Primer sequence (5'-3')	Nucleotide position	PCR product
For amplification*			
AC1048	GGRTTDGARGCATGHGTACATG		Coat Protein (Wyatt and Brown, 1996)
AV494	GCCYATRTAYAGRAAGCCMAG		
Beta01	GGTACCACTACGCATCGCAGCAGCC		CLCuMuB (Briddon *et al.*, 2002)
Beta02	GGTACCTACCCTCCCAGGGGTACAC		
DNA101	CTGCAGATAATGATGTAGCTTACCAG		Alphasatellite (Bull *et al.*, 2003)
DNA102	CTGCAGATCCTCCACGTGTATAG		
For detection and confirmation**			
WCp-F	AGGAGATATTCTCATTTCCAC	327-820	Partial Cp
WCp-R	GTCGCAGTGCTGGGCTCGTTGT		
WBV1-F	TACGCGTGGACGAAAGCGGAAG	528-1222	Partial BV1
WBV1-R	CACTTCACATTTCGAACGAACTGT		

*Primers used for initial detection

**Primers used to detect and confirm the presence of DNA-A and DNA-B components of WmCSV through Southern blot hybridization

Comparative sequence analysis and phylogenetic dendrograms

The CP sequences from the samples were initially screened using the BLASTn similarity-searching tool against a public sequence database. For detailed analysis, the obtained full-length nucleotide (nt) sequences of DNA-A and DNA-B components were aligned separately with the closest related begomovirus sequences. The sequences retrieved with the highest similarity were then used for further analysis in Mega-11 software (Tamura *et al.,* 2021). Pairwise nt sequence identities were determined using sequence demarcation tool (SDT v1.2) as suggested by Muhire *et al.,* (2013). Phylogenetic relationships were inferred using MEGA-11 software with robust statistical support obtained through bootstrapping.

The ORF architecture and nt position of the ORFs were inferred using ORF finder tool (*https://www.ncbi.nlm.nih.gov/gorf/gorf.html*). Additionally, MEGA-11 software was employed to calculate genetic distances and infer phylogenetic dendrograms with a set value of 1000 boostraps and the best-fit Maximum-likelihood method within MEGA-11 software.

Determination of recombinant hot spots

The presence of any recombination hot spots was confirmed with Recombination Detection Program (RDP4) using six different algorithms and a p-value set at < 0.05. Only recombination events supported by at least three algorithms among SiScan, RDP, MaxChi, GENECONV, Chimera and BootScan were accepted.

Agro-inoculations and plant infectivity analysis

Rrecombinant pBluscript clones containing monomeric copies of the full-length DNA-A and DNA-B sequences were used to construct infectious partial-dimeric clones. For DNA-A of WmCSV a partial copy was obtained by double digestion with SacII and BamHI restriction endonucleases. The resulting ~2.5 kb fragment was excised and cloned into SacII/BamHI digested pGreen-II vector to produce pG31A-0.9. Next, a SacII-digested full-length fragment was ligated to the pG31A-0.9 construct, which had also been digested with Sac-II, to produce pG31A-1.9 partial tandem repeat construct (WmA1). Similarly, a partial fragment of ~0.3 kb for DNA-B component was obtained with an EcoRI and ClaI digestion. The resulting product was ligated into the pGreen-II vector to produce pG31B-0.1. The full-length DNA-B component, obtained by EcoRI digestion, was ligated into similarly digested pG31B-0.1 to obtain pG31B-1.1 (WmB1). The same strategy was followed to construct the partial tandem repeat constructs pG52A-1.9 (WmA2) and pG52B-1.1 (WmB2), respectively. All ligation reactions were performed using the ThermoScientific T4 DNA ligase enzyme. The

partial tandem repeat constructs were separately electroporated into the *Agrobacterium tumefaciens* strain as described previously (Sattar *et al.,* 2019). The integrity of all clones was verified at each step of the experiment through colony PCR and digestion reactions. Details concerning the specific endonucleases and plasmids used in this study can be found in Supplementary Table 1.

Agro-infectious clones of WmCSV DNA-A and DNA-B were introduced into 3–4-week-old *Nicotiana benthamiana* plants, following the procedure outlined previously (Just *et al.,* 2017). Plants receiving the inoculum were kept in insect-free chambers under controlled conditions with 18 hours of light cycle, a 24/22 °C day/night temperature cycle of, and 70% relative humidity. Control plants included health plants and plants mock-inoculated with *A. tumefaciens* GV3101 containing an empty pGreen-II plasmid. Apical leaves were collected from 15-day-old inoculated plants for total plant genomic DNA extraction. PCR and Southern blot hybridization were employed to confirm the presence of each component, following established protocols (Leke *et al.,* 2013). Specific primers for detection are listed in Table 1. Equal amounts of DNA were loaded and visualized on an agarose gel.

Results

Sequence comparison and evolutionary relationships

The expected fragment size of the partial CP amplicons (~550 bp) from the three symptomatic snake gourd plants (SG-3, SG-5 and SG-9) were amplified using AC1048/AV494 primers, cloned and subsequently sequenced. Analysis of the core CP sequences using BLASTn identified WmCSV in all three snake gourd samples. Two full-length clones for DNA-A (SG31A and SG52A) from plants SG-3 and SG-5 were each 2752 nt in length. These sequences have been deposited in GenBank with the accession numbers PP320240 and PP320241, respectively. The organization of each isolate resembled typical OW bipartite begomoviruses DNA-A with two ORFs: CP (258 aa) and pre-coat protein (Pre-Cp) (119 aa) in the virion-sense orientation and four ORFs in the complementary-sense orientation: replication associated protein (Rep; 361 aa), replication enhancer protein (REn; 134 aa), transcriptional activator protein (TrAp; 135 aa), AC4 protein (47 aa) and AC5 protein (255 aa). These clones shared 98.9% identity with each other. Clone SG31A exhibited the highest nt sequence identity (98.7%) with Iranian WmCSV isolates (KT272765 and KT272769) (Esmaeili *et al.,* 2015), while the clone SG52A showed the highest nt sequence identity (98.7%) with a WmCSV isolate (KJ958912) identified from Al-Ahsa, Saudi Arabia (Rezk *et al.,* 2019) (Figure 2A). The phylogenetic dendrogram demonstrated that the WmCSV isolates clustered closely with previously reported WmCSV isolates (Figure 3A). Further resolution of the phylogenetic dendrogram focusing solely on WmCSV isolates (Figure 3A1) revealed two separate groups: Asian and African. The Asian group was further subdivided into three subgroups based upon the evolutionary relatedness. Isolates SG31A and SG52A formed a distinct sub-group 1 with high confidence (99% bootstrap value), closely related to WmCSV isolates found in Iran, Oman, and Saudi Arabia. Therefore, based on the ICTV criteria for begomovirus species demarcation (>92%), the identified begomovirus isolates represent new members of WmCSV infecting snake gourd in Saudi Arabia.

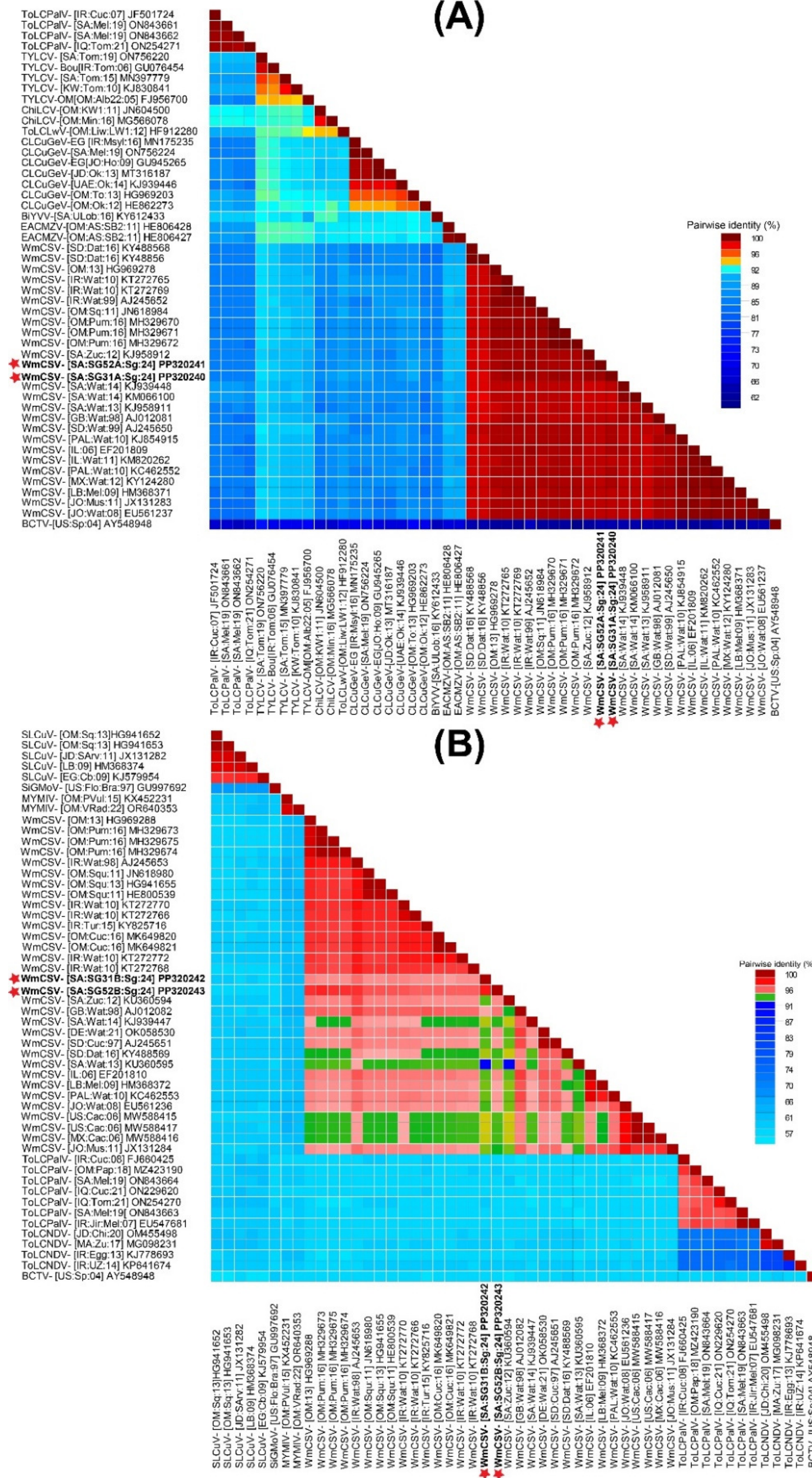

Figure 2. A pairwise nucleotide sequence identity matrix to compare the full-length genomes of all identified WmCSV DNA-A (A) and WmCSV DNA-B (B) sequences

This analysis utilized the Species Demarcation Tool (SDT v. 1.2). To effectively visualize the results, we constructed a color-coded matrix. The color scheme (red, green, and blue shades) reflects established species and strain demarcation thresholds for begomovirus genomic components, as defined by Muhire *et al.* (2013). Isolates from this study are highlighted in bold and marked with a red star for easy identification.

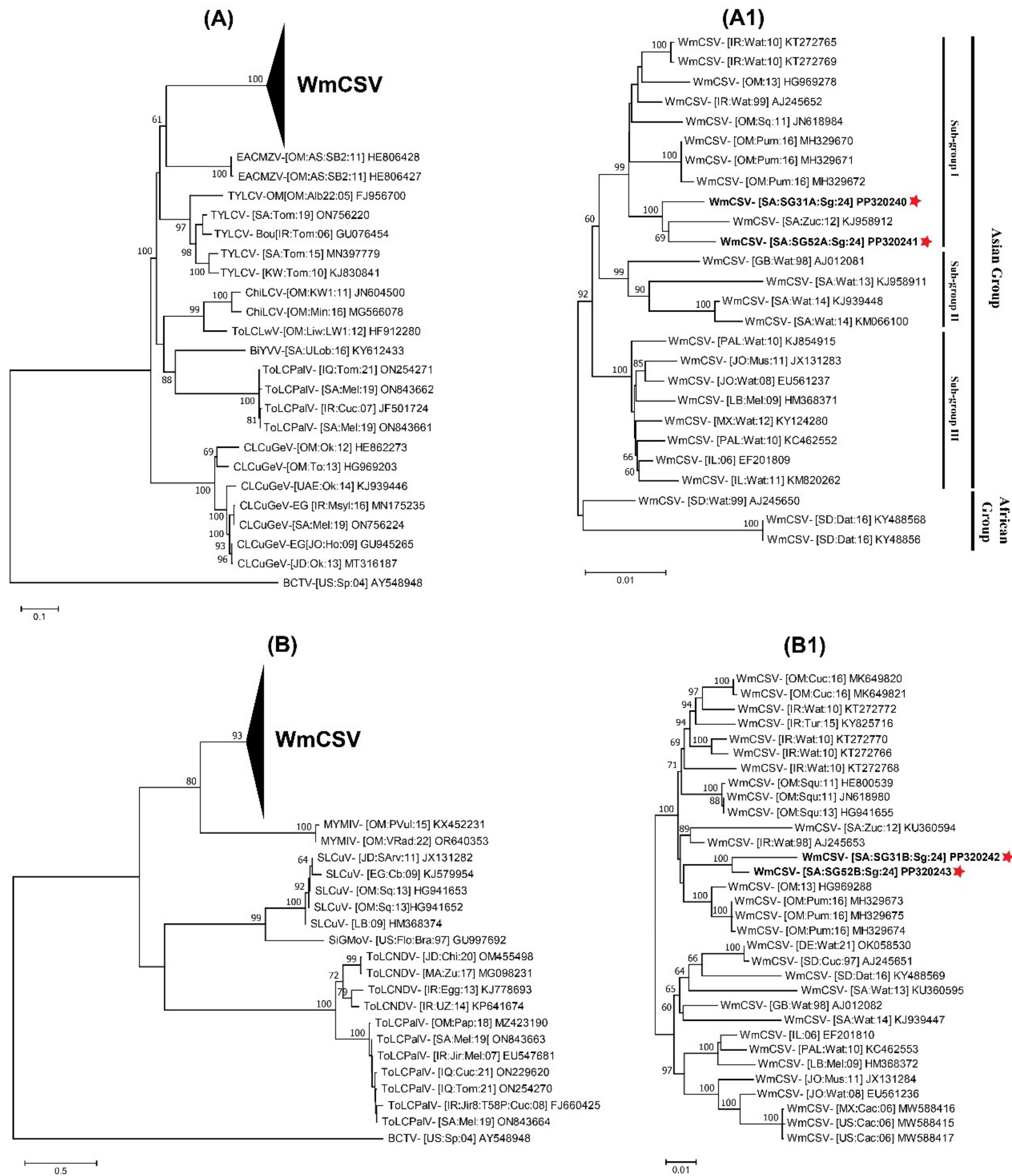

Figure 3. Maximum likelihood phylogenetic dendrograms to visualize the evolutionary relationships between (A) full-length genomes of identified WmCSV DNA-A and (B) DNA-B isolates

For comparative purposes, full-length sequences of closely related begomoviruses were retrieved from the NCBI GenBank database. The WmCSV DNA-A and DNA-B clades were further delineated as A1 and B1, elucidating the diversity among WmCSV isolates in this investigation. Each isolate's information, including host plant species, geographic origin, and GenBank accession number, is displayed within the dendrograms. Isolates from this study are highlighted in bold and marked with a red star for easy identification. To assess branch support, the trees were constructed with 1000 bootstrap replicates. Taxonomic abbreviations for begomovirus species follow the guidelines outlined by Zerbini *et al.,* (2017).

Two full-length clones for DNA-B (SG31B and SG52B) from symptomatic snake gourd plants SG-3 and SG-5 were also completely sequenced. The nt sequences were deposited in the publicly available GenBank database with accession numbers PP320242 and PP320243. The clones were 2728 and 2727 nt in length and displayed a typical genome organization of bipartite begomovirus DNA-B, having two ORF nuclear shuttle protein (NSP; 251 aa) and movement protein (MP; 306 aa) in the opposite orientations, respectively. The

DNA-B isolates exhibited 97.4% identity to each other. Isolate SG31B shared its highest nt sequence identity (95.5%) with WmCSV DNA-B (AJ245653) reported from Iran (Kheyr-Pour *et al.,* 2000), while isolate SG52B shared its highest nt sequence identity at 97% with WmCSV isolates (MH329673 and MH329675) identified from Oman (Shafiq *et al.,* 2021) (Figure 2B). In the phylogenetic analysis the DNA-B sequences of WmCSV isolates identified in this study clustered together (100% bootstrap value) with other WmCSV isolates reported previously (Figure 3B and 3B1).

A detailed examination of the sequences from all DNA-A and DNA-B clones revealed a recurring sequence (TGGAGAC) resembling an iteron located upstream of the TATA box, as deciphered by Ali-Shtayeh *et al.,* (2014) at their respective nt coordinates (Table 2). Furthermore, recombination analysis did not detect any potential recombination breakpoint within any WmCSV DNA-A and DNA-B isolates.

Table 2. Characteristic iteron sequences present in the common regions of WmCSV DNA-A and DNA-B components

Clone	Genomic component	Iteron	Nucleotide Position
SG31A	DNA-A	TGGAGAC	2639-2646
SG52A			2675-2681
			2682-2688
SG31B	DNA-B		2614-2619
SG52B			2649-2655
			2656-2662

Agro-infectivity assay

The partial tandem repeat constructs for WmCSV DNA-A (WmA1 and WmA2) and DNA-B isolates (WmB1 and WmB2) were constructed from plants SG-3 and SG-5, respectively, to test their pathogenicity. The results showed that WmA1 alone did not induce any symptoms in all five inoculated *N. benthamiana* plants. However, when co-inoculated with WmB1, all five plants developed typical begomovirus symptoms of leaf curling and mottling at 10 days post-inoculation (dpi) (Figure 4). Similar results were obtained with WmA2 and WmB2. The Southern blot hybridization assays also supported these findings (Figure 5). WmCSV DNA-A was not detected in the plants inoculated with WmA1 or WmA2 alone. However, in plants co-inoculated with either WmA1+WmB1 or WmA2+WmB2, both WmCSV DNA-A and DNA-B molecules were successfully accumulated and detected (Figure 5).

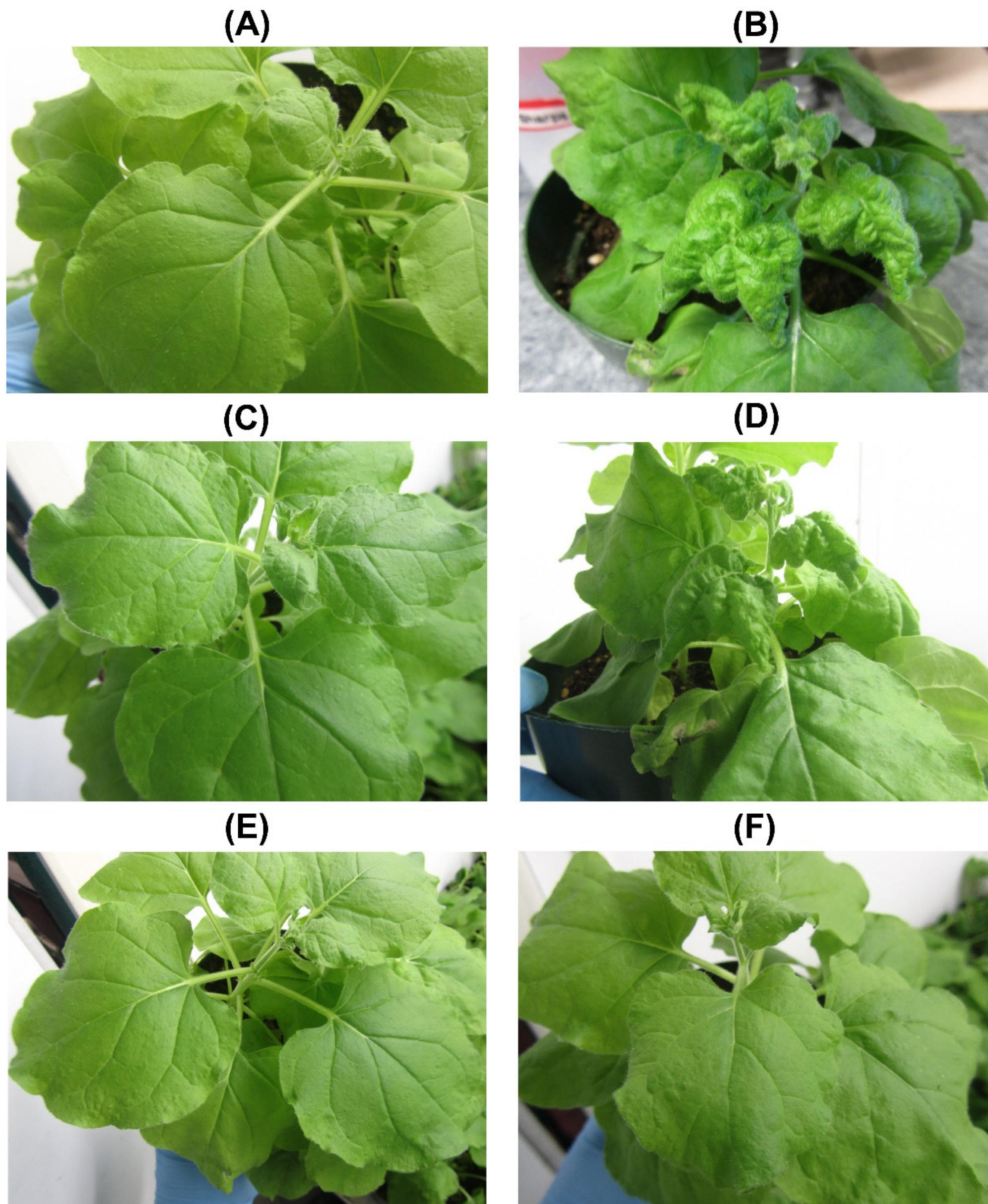

Figure 4. Symptoms observed in *N. benthamiana* plants following inoculation. The plants were inoculated with partial tandem repeats of watermelon chlorotic stunt virus DNA-A and DNA-B isolates as follows: **(A)** WmA1, **(B)** WmA1+WmB1, **(C)** WmA2, **(D)** WmA2+WmB2. Healthy, non-inoculated **(E)**, and mock-inoculated **(F)** *N. benthamiana* plants are included for comparison. Photographs were captured at 15 days post-inoculation (dpi)

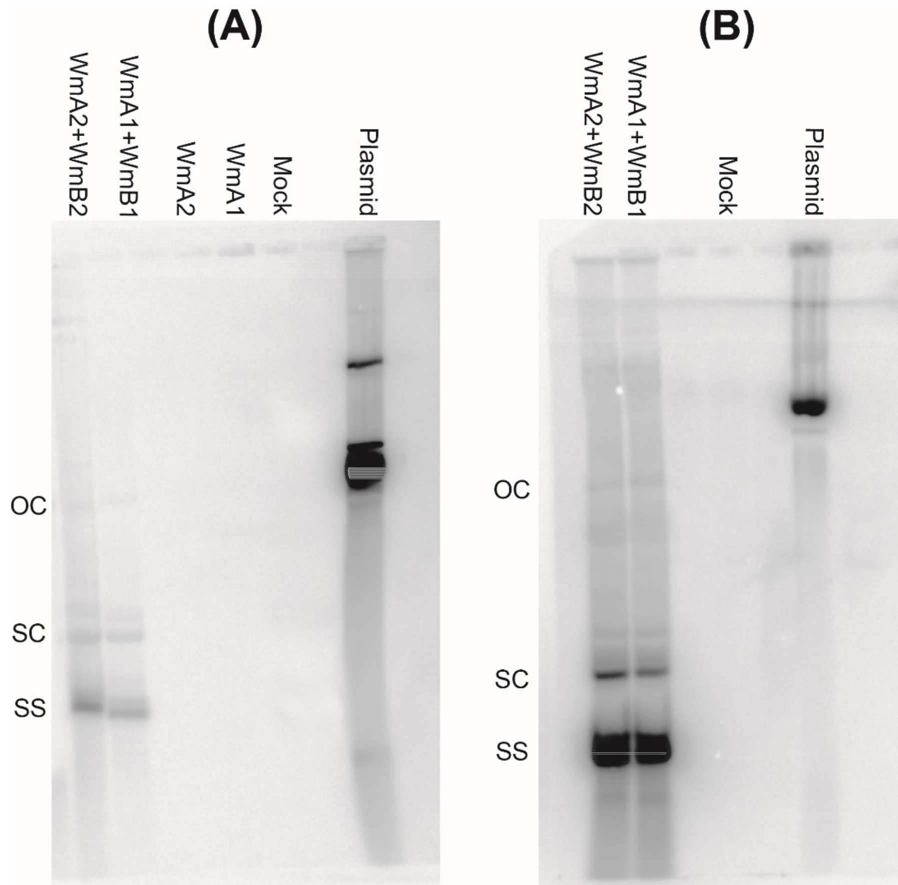

Figure 5. Southern blot analysis was performed on DNA extracted from *N. benthamiana* plants inoculated with agrobacteria. Blots were probed to detect the presence of two components of watermelon chlorotic stunt virus (WmCSV) DNA-A **(A)** and DNA-B **(B)**

Samples corresponding to each lane were extracted from the specified plants (as mentioned above each lane), with mock inoculated plants (Mock) serving as negative controls. Leaf samples were taken from the youngest, newly expanding leaves of the plants. Additionally, plasmid containing the full-length clone was included as positive control. The positions of viral open circular (OC), supercoiled (SC) and single-stranded (ss) DNA forms are marked. Samples were extracted at 15 days post-inoculation (dpi).

Discussion

WmCSV, a typical bipartite OW begomovirus, was first identified infecting watermelon plants in Yemen in the late 1980s (Alhubaishi *et al.,* 1987). Since its initial discovery, the virus has spread throughout the Middle East, the Arabian Peninsula, and northwestern Africa. More recently, introduction of WmCSV has been reported in Mexico (Dominguez-Duran *et al.,* 2018) and the USA (Fontenele *et al.,* 2021). Based on previous reports, WmCSV appears to be expanding its presence in cucurbit fields across Saudi Arabia (Al-Saleh *et al.,* 2014; Alhudaib *et al.,* 2018; Rezk *et al.,* 2019). This unique evolutionary suggests WmCSV has adapted and diversified within the region, potentially becoming a long-term threat to Saudi Arabian agriculture.

The current field study identified snake gourd plants exhibiting characteristic signs of begomovirus infection, such as yellowing and mottled leaves. Initial CP-based analysis identified the presence of begomovirus, which was further confirmed through RFLP analysis of the RCA products from the symptomatic plants. Detailed comparative sequence analysis and phylogenetic dendrograms confirmed that the snake gourd

plants were infected with WmCSV DNA-A and DNA-B. The DNA-A isolates SG31A and SG52A exhibited the closest nt sequence identity to WmCSV isolates previously identified in Iran (Esmaeili *et al.,* 2015) and Saudi Arabia (Rezk *et al.,* 2019), respectively. The phylogenetic dendrogram revealed a distinct cluster for these isolates, alongside other WmCSV isolates from Iran, Oman and Saudi Arabia (Figure 3A1). Similarly, the DNA-B isolates SG31B and SG52B showed their highest nt sequence identities with WmCSV DNA-B reported from Iran (Kheyr-Pour *et al.,* 2000) and Oman (Shafiq *et al.,* 2021), respectively. Phylogenetic analysis suggests that there have been at least two independent introductions of WmCSV into Saudi Arabia. Isolates found in eastern Saudi Arabia are more closely related to Iranian isolates, while isolates from western Saudi Arabia and Yemen form a distinct group (Figure 3A1). This is consistent with previous research showing that most begomoviruses in Saudi Arabia have been introduced across borders (Al-Saleh *et al.,* 2014; AlHudaib *et al.,* 2022; Rezk *et al.,* 2019). Based on the current investigation and previous studies, Iran is a potential source for the introduction of these viruses into the Saudi Arabian agroecosystem. However, more data are required to confirm this speculation. The overland introduction of such begomoviruses into Saudi Arabia poses a challenge. Potential factors contributing to this introduction may include extensive trade, cross-border travel of a significant expatriate population, and various means of transportation.

Similar to studies of many other bipartite begomoviruses (Ranjan *et al.,* 2014; Sattar *et al.,* 2021; Xiao *et al.,* 2023), our study found that WmCSV DNA-A alone did not induce systemic infection in *N. benthamiana* plants. This aligns with previous findings for WmCSV infectivity, where co-inoculation with DNA-B is crucial (Kheyr-Pour *et al.,* 2000; Shafiq *et al.,* 2021). The reason for WmCSV's unique behavior in *N. benthamiana* remains unclear. Perhaps the virus lacks optimal adaptation to *Solanaceae* family. Iqbal *et al.,* (2017) identified CP and AV2 proteins as essential for systemic spread of DNA-A in begomoviruses without DNA-B. This suggests potential deficiencies in how WmCSV's CP or AV2 proteins interact with *N. benthamiana*, limiting its capacity for systemic spread without DNA-B.

International trade of fruits, vegetative plant parts (like cuttings or tubers), and whiteflies significantly contributes to the spread of viral diseases across vast regions. This is exemplified by the movement of begomovirus-related infections between countries (Sattar *et al.,* 2013) and even continents (Dominguez-Duran *et al.,* 2018; Sattar *et al.,* 2017). While WmCSV's emergence poses a potential risk to cucurbit cultivation in the Middle East, detailed data on factors promoting its dissemination is lacking. Based on the identification of WmCSV from Africa (Kheyr-Pour *et al.,* 2000), Middle East (Rezk *et al.,* 2019; Shafiq *et al.,* 2021) and Americas (Dominguez-Duran *et al.,* 2018; Fontenele *et al.,* 2021), we can hypothesize that it may become a world-wide pandemic for cucurbit production. This necessitates an in-depth investigation into the genetic makeup of WmCSV, variations in the viral sequence and factors responsible for the spread of WmCSV in the Middle East. New genome editing tools can be employed to explore resistance sources against begomoviruses within cucurbit crops (Verma *et al.,* 2023). Additionally, alternative methods like next-generation sequencing can offer promising avenues for begomovirus detection (Shahid *et al.,* 2021).

Conclusions

This study identifies WmCSV DNA-A and DNA-B in snake gourd plants from the Eastern region of Saudi Arabia. Building on previous reports, it provides evidence of WmCSV actively circulating in vegetable-growing regions of Saudi Arabia. The findings suggest two separate introductions of WmCSV, with Iran as a possible source for its spread into Saudi Arabia. These results imply a broad distribution of WmCSV across Saudi Arabia's vegetable-growing areas. Therefore, more critical and focused surveillance studies are necessary to determine the exact distribution and forecast potential future epidemics of this virus in the region.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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