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SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF *CUCURBIT APHID BORNE YELLOWS VIRUS* AFFECTING SQUASH, CUCUMBER AND ARABLE WEEDS IN SAUDI ARABIA

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ABSTRACT

Cucurbit aphid-born yellows virus (CABYV) is one of the primary plant viruses that cause yellowing disease of cucurbits that result in severe outbreaks leading to significant crop losses in Saudi Arabia's cucurbit crops. Implementing a comprehensive detection program to monitor the prevalence, distribution, and genetic diversity of CABYV populations in different cultivated and arable weed plant species would help implement management measures against this disease. This study aimed to identify CABYV infection in Saudi Arabia's cucumber, squash, and some arable weed hosts. A total of 50 cucumber, 38 of squash, and 27 samples of four distinct arable weeds, *Chenopodium murale, Malva parviflora, Solanum nigrum*, and *Sonchus oleraceous* were collected in the months of January to March of 2014-2015. Samples from several locations in the Riyadh region of Saudi Arabia were collected, and there were signs of a probable viral infection, such as interveinal chlorosis, yellowing symptoms, and chlorotic lesions resulting in total leaf yellowing.According to the obtained results, *Cucurbit aphid-borne yellows virus* (CABYV) was detected in cucumber (30 out of 50) and squash (29 out of 38), weed plant species (12/27) growing adjacent to cucumber fields using ELISA test. RT-PCR confirmed the occurrence of CABYV in cucumber, squash, and weed plants using a primer pair that is specific to the coat protein gene. The results revealed identical-sized CABYV CP gene segments (600 bp). Following partial nucleotide sequencing of the Saudi isolates of CABYV, the following accession numbers were uploaded to NBCI: MH192385 (CaBYV-SA-Cucumber), MH192386 (CaBYV-SA-Squash-1), MH192387 CaBYV-(SA-Squash-2), MH192388 (CaBYV-SA-Ch. Murale), MH192389 (CaBYV-SA-S. nigrum), MH192390 (CaBYV-SA-S. oleraceus), and MH192391 (CaBYV-SA-M. parviflora).BLAST and phylogenetic analysis showed that CABYV isolates from Riyadh region possess 98-100% identity among them, whereas their nucleotide sequence shows 93.3-100% identity with sequences of isolates deposited in NCBI.

Key Words: CABYV, Weeds, Symptoms, ELISA, RT-PCR, Sequence.

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INTRODUCTION

Cucurbits include cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* Thunb.), calabash (*Crescentia cujete* L.), squash (*Cucurbita pepo* L.), and pumpkin (*Cucurbita* moschata L.*)*, and they are divided into three different genera under the Cucurbitaceae family. According to Anderson *et al*. (2004), viruses are responsible for around half of all newly discovered plant diseases, which reduce the output of almost all vegetables and crops, including cucurbits. Cucurbit crops around the world are infected by about 96 viruses (Nagendran *et al*., 2017; Pozzi *et al*., 2020; Desbiez *et al*., 2020) among which *Cucurbit aphid-borne yellows virus* (CABYV) and Watermelon mosaic virus (WMV) are major plant pathogens that cause severe epidemics in cucurbit crops (De Moya-Ruiz *et al*., 2021; Rabadán *et al*., 2023). In cucurbits, CABYV causes yellow spotting on the lower leaves, which can become brittle and thickened, with interveinal chlorotic regions. Originally identified in France, where it has been the source of several outbreaks since 1982, CABYV is now found worldwide. *Myzus persicae* and *Aphis gossypii* transmit CABYV; it has not been transmitted

mechanically or by seedborne, and no effect of virus infection on seed quality has been observed (Keinath *et al*., 2017; Radouane *et al*., 2021). In addition, CABYV infects several arable weeds, which can act as primary sources of infection and reservoirs (Mnari-Hattab *et al*., 2009; Knierim *et al*., 2010). CABYV is a phloem-restricted virus that mainly infects cucurbits, but it can also infect some non-cucurbit species, including chickpeas (Kumari *et al*., 2018; Mayo and D'Arcy, 1999), passionfruit (Zhang *et al*., 2020), lettuce, several arable weeds (Mnari-Hattab *et al*., 2009), *Vicia faba* (Buzkan *et al*., 2017) and melons (Schoeny *et al*., 2019). A novel strain of CABYV has been identified to be causing severe symptoms and several outbreaks of the yellowing disease. It has also been reported that CABYV is predominating the yellowing disease as a single infection of zucchini plants grown in Spain (Rabadán *et al*. 2021). Few other isolates have been reported to infect peanuts in Kenya (Mabele *et al*., 2023), spiny gourd in Srilanka (Abeykoon *et al*., 2018), and passion fruit in Brazil (Vidal *et al*., 2023), cucurbits in Saudi Arabia have been known to be infected by more than 15 viruses in the last 15 years (Al-Saleh *et al*., 2014, 2015a; Ahmad *et al*., 2018; Santosa *et al*., 2018). The economic importance of cucurbits is well understood in Saudi Arabia and worldwide, and the mechanisms underlying the disease caused by CABYV are unstated specifically in a unique set of environmental conditions prevailing in Saudi Arabia. Moreover, the role of arable weed species is evident as a reservoir during the off-season, making both aspects a prime research gap that must be understood to develop a basis for devising an effective strategy to mitigate the problem. Therefore, the current work aims to unfold the molecular characters and to determine the phylogenetic relationship with other isolates that have been reported worldwide, as well as to detect and verify the presence of CABYV in cucumber, squash, and other arable weeds using molecular and serological approaches.

MATERIALS AND METHODS

Sampling and serological detection: A total of 115 different samples were collected during 2014-2015 from symptomatic and asymptomatic cucumber (50), squash plants (38) and 27 various weed samples (*Chenopodium murale*, *Malva parviflora*, *Solanum nigrum* and *Sonchus oleraceous*). These samples show overall yellowing symptoms, growth reduction, interveinal chlorosis, and yellowing of the basal leaves (Figure 1, 2). These samples were collected from five greenhouses and open fields in the Al-Kharj, Riyadh region of Saudi Arabia. The collected samples were tested against the critical cucurbit viruses including *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucurbit chlorotic yellows virus* (CCYV), *Papaya ringspot virus* (PRSV), *Melon necrotic spot virus* (MNSV), *Cucumber green mottle mosaic virus* (CGMMV), and *Watermelon chlorotic stunt virus* (WmCSV), ZYMV, CMV, WMV, SqMV and CABYV using the specific antibodies provided by the manufacturer, LOEWE®, Biochemica, and DSMZ, Germany, using the BioTek ELx808 ELx808IU Absorbance Microplate Reader according to Clark and Adams (1977) instructions at Plant Virology Lab, Department of Plant Protection, King Saud University, Saudi Arabia.

Total RNA Extraction and RT-PCR: Total RNA was isolated from the obtained samples using the Isolate Plant RNA Mini Kit, Meridian, Biosience, UK (Cat no. BIO-52077), and the RNA was then subjected to The QIAGEN One-Step RT-PCR Kit (Cat. No. / ID: 210212) following the manufacturer's instructions. The RT-PCR reaction was set up in 50 µl as follows using the primers 5'-gaatacggtcgcggctagaaatc-3' (CE9) and 5'-ctatttcgggttctggacctggc-3' (CE10) that will amplify the complete (600 bp) coat protein (CP) gene for CABYV (Juarez et al., 2004; Aarabe et al., 2018, Topkaya and Desbiez, 2020). In an Eppendorf Mastercycler gradient, amplifications were performed, the Gene Direx DNA Marker DNA Ladder (1kb) was utilized, and the DNA documentation gel analysis equipment (IN genius, Cambridge, UK) was used to document the RT-PCR amplified product.

Nucleotide sequence and analysis: The obtained RT-PCR fragments of CP (598bp) region of the Saudi Arabian CABYV isolates were submitted to the Macrogen Company in South Korea for a sequence in two directions with each viral-specific primer. BLAST program was used to analyze the sequences obtained for the coat protein region in CABYV genome. The MEGA-X program generated a phylogenetic tree using the Maximum Likelihood and 1000 bootstrap repeats with default parameters. DNASTAR was then used to determine the percentage of nucleotide identity between CABYV isolates and other isolates that have been reported globally (Table 1). Graphs comparing Saudi isolates to other NBCI database isolates were created using sequence demarcation tools (SDT).

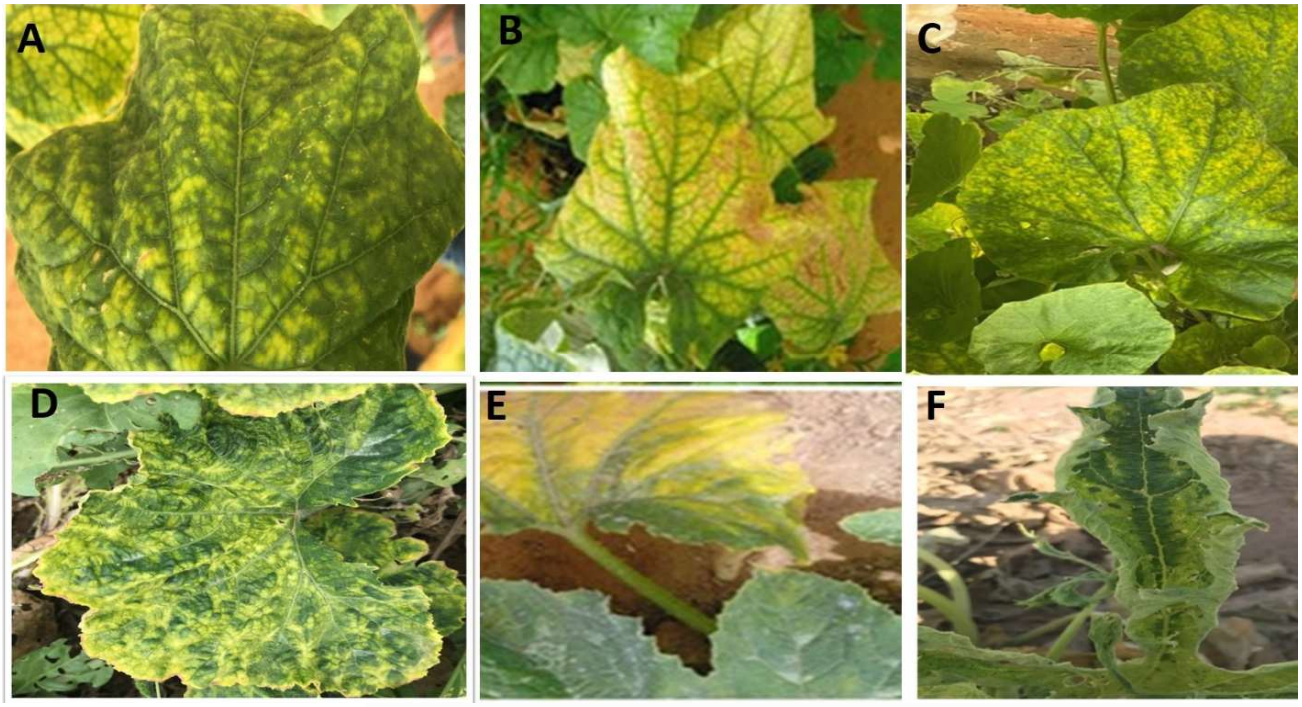

Figure 1. Natural symptoms in cucumber (A, B, C) and squash (D, E, F) plants infected with suspected CABYV show growth reduction, interveinal chlorosis, and yellowing of the basal leaves. (D) General yellowing symptoms in an open field at the end of the growing season (b).

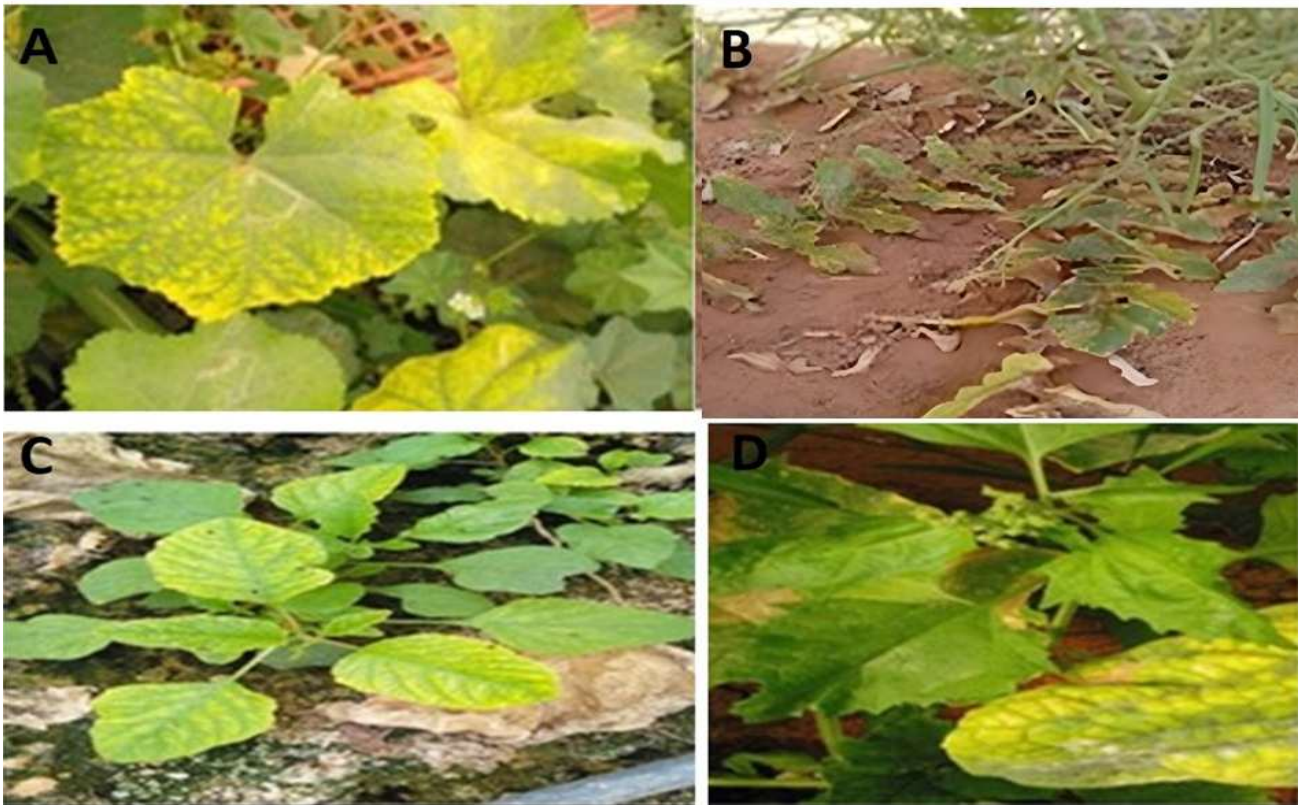

Figure 2. Naturally, symptoms are present in *M. parviflora* (A), *S. oleraceous* (B), *S. nigrum* (C), and *Ch. murale* (D) plants. *M. parviflora* (A), *S. oleraceous* (B), *S. nigrum* (C), and *Ch. murale* (D) plants infected with suspected CABYV showing yellowing symptoms.

RESULTS

Serological detection: Samples that were collected from asymptomatic and symptomatic cucumber, squash (Figure 1) and different weeds (Figure 2) in greenhouses and open fields distributed in Al-Kharj location, region of Saudi Arabia exhibited interveinal chlorosis patches, thickening of older leaves, and yellowing symptoms. Samples were tested against CYSDV, CCYV, PRSV, MNSV, CGMMV, WmCSV, ZYMV, CMV, WMV, SqMV and CABYV using the specific antibodies by ELISA. The obtained results showed that 30/50 and 29/38 of cucumber and squash samples tested positive for CABYV, but negative for other tested cucurbit viruses (ZYMV, CYSDV, WmCsV, CGMMV, CMV, CVYV, MNSV, WMV, SqMV and PRSV). Additionally, CABYV was found in all four weed plant species that were growing nearby cucurbit plants, including *M. parviflora* (4/8), *Ch. murale* (5/12), *S. oleraceous* (2/5), and *S. nigrum* (1/2).

RT-PCR: The RT-PCR results yielded fragments of the same size as the CABYV CP gene (598 bp) (Figure 3) when a specific primer for CABYV was used. CABYV was detected in four *M. parviflora, Ch. murale, S. oleraceous*, and *S. nigrum* (Figure 4). This amplified product contains the CP gene using specific primers for CABYV. No fragments were amplified from the RNA isolated from a healthy cucumber plant.

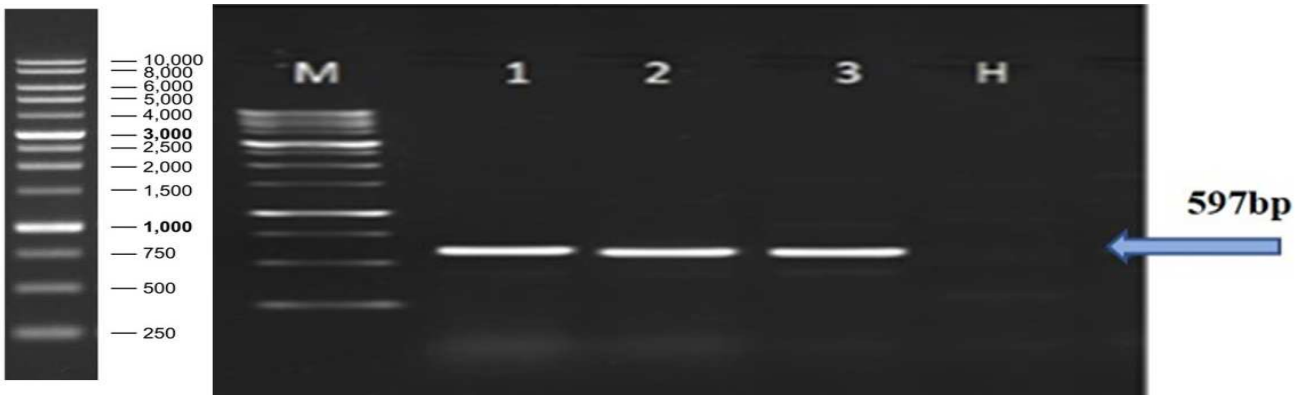

Figure 3: Agarose gel electrophoresis 1% of RT-PCR amplification of a 597 bp fragment using the specific primer for CABYV-CP gene. Lane M represents 1 KB DNA Ladder Gene Direx. The amplified product is 597 bp. Lanes 1, 2 show fragments from infected cucumber and lane 3 shows fragments from infected squash plants collected from the Al-Kharj region. No RT-PCR amplification was observed in uninfected sample cucumber tissue (lane H) as a negative control.

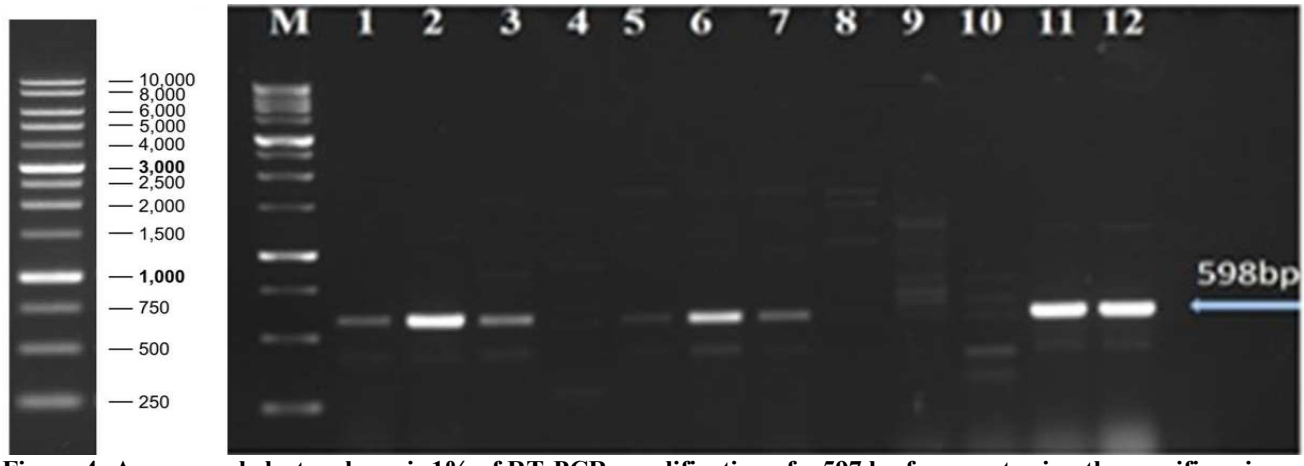

Figure 4: Agarose gel electrophoresis 1% of RT-PCR amplification of a 597 bp fragment using the specific primer for CABYV-CP gene. Amplified products from symptomatic and asymptomatic arable weeds including *M. parviflora* (Lane 1,2), *Ch. murale* (Lane 3,4,5), and *S. nigrum* (Lane 6,7,8,9,10), *S. oleraceous* (Lane 11,12), No PCR product was amplified from asymptomatic *M. parviflora, Ch. murale, S. oleraceus, and S. nigrum* collected from Al-Kharj region (lane 4,8,9,10), Lane M represents 1 KB DNA Ladder Gene Direx.

Table 1. A list of the CABYV Saudi Arabian and other isolates originating from different countries was used in this study.

Accession No.	Isolate	Host	Country
MH192385	CaBYV-SA-cucumber	Cucumber	Saudi Arabia
e	CaBYV-SA-Squash-1	Squash	Saudi Arabia
MH192387	CaBYV-SA-Squash-2	Squash	Saudi Arabia
MH192388	CaBYV-SA-Chenopodiummurale	*Chenopodiastrum murale*	Saudi Arabia
MH192389	CaBYV-SA-Solanumnigrum	*Solanum nigrum*	Saudi Arabia
MH192390	CaBYV-SA-Sonchusoleraceus	*Sonchus oleraceus*	Saudi Arabia
MH192391	CaBYV-SA-Malvaparviflora	*Malva parviflora*	Saudi Arabia
MH414922	GRB-Bjt2	Squash	Morocco
MK059479	B1	Squash	Poland
MN145451	D332/17	Squash	Slovenia
MN746322	44-7/10/19	Cucumber	Germany
MT027103	EM160093	Melon	France
MT384365	CABYV-2018/1	Squash	Poland
MW051363	LP63	Watermelon	Spain
OM948845	22.1Z/M	Zucchini	Spain
OM948849	59.1M/A	Melon	Spain
OP909798	Cuc-cp-541-5	*Cucumis* sp.	Brazil
OP909799	Mac-cp-543-1	*Macroptilium* sp.	Brazil
OP909801	Sty-cp-757-3	Stylosanthes sp.	Brazil
OP909806	PF-799	*Squirting cucumber*	Brazil
OQ703996	LE	Cucumber	Iran
OQ865059	Iraq	Squash	Iraq
AY529653	36-2	Squash	Spain
EF029112	S89	*Squirting cucumber*	Italy
EF029113	S23	Melon	Italy
EF187338	9FB	Melon	Tunisia
EF187339	14-S	Cucumber	Tunisia
e	ESH-25	Zucchini	Tunisia
EF187341	AZ	Watermelon	Tunisia
EF187344	LR	Lettuce	Tunisia
EU244316	Xinjiang	Cantaloupe	China
EU244323	Tianjin	Squash	China
EU244327	Guangxi	Cucumber	China
FR717145	Kafrelsheikh 1	*Cucurbita* sp.	Egypt
FR717147	Badr1	Squash	Egypt
FR822383	Elsalheia	*Cucurbita* sp.	Egypt
FR822384	Elkoreen-1	*Cucurbita* sp.	Egypt
GQ221224	CABYV-JAN	Cucumber	Japan
GU324099	CABYV-R-TW36	Squash	Taiwan
GU324102	CABYV-R-TW82	Luffa	Taiwan
HM771271	Rak-1	Squash	Czech Republic
JX262071	66-09	Squash	Serbia
KF791037	CABYV-R-TH1	Bitter gourd	Thailand
KF815683	CABYV-C-TH235	Cucumber	Thailand
KR231962	SW25	Melon	South Korea
KX398664	200/15	Squash	Montenegro
MF281974	BG1	Bitter gourd	India
OR060954	3AP	Melon	Iran

Nucleotide sequence: The CABYV CP gene (598 bp) that was isolated from cucumber, squash, *M. parviflora*, *S. oleraceous*, *S. nigrum*, and *Ch. murale* were sequenced and posted to the GenBank with the following accession no's: MH192385, MH192386, MH192387, MH192388, MH192389, MH192390 and MH192391 for CaBYV-SA-Cucumber, CaBYV-SA-Squash-1, CaBYV-SA-Squash-2, CaBYV-SA-*Ch. murale*, CaBYV-SA-*S. nigrum*, CaBYV-SA-*S. oleraceus* and CaBYV-SA-*M. parviflora* isolates, respectively. The analysis of CABYV sequences

has shown that CABYV isolates from Al-Kharj region possess 98-100% identity among themselves. A comparison of all seven Saudi isolates of CABYV in this study with other isolates showed nucleotide identity range from 93.7 to 100%.

The Saudi isolates (MH192385, MH192386, MH192387, MH192388, MH192389, MH192390 and MH192391) shared the highest nucleotide identity (98-100%) with one melon isolate from Egypt (FR822383) squash. The CABYV-Saudi Arabian isolates share the lowest similarity (93.7-94.4%) with squash CABYV isolate obtained from Taiwan (GU324099).

The CABYV isolated from Cucumber (MH192385) shared the highest nucleotide identity (99.1-100%) with three isolates including MT027103 (France), EF029112 (Italy), MT384365 (Poland), isolated from melon, squirting cucumber, squash, and four isolates FR717145, FR717147, FR822383 and FR822384 isolated from *Cucurbita* sp. in Egypt. The CABYV isolated from squash (MH192386 and MH192387) shared the highest nucleotide identity (98%), with two isolates isolated from Cucurbita spp. (MT384365 and FR7171450 from Poland and Egypt.

For CABYV isolated from CaBYV-SA-*Ch. murale* (MH192388), CaBYV-SA-*S. nigrum* (MH192389) and CaBYV-SA-*S. oleraceus* (MH192390) the highest nucleotide sequence identity (97.5-98%) with MT027103 (melon), MW051363 (watermelon), OM948845 (Zucchini), OM948849 (Melon), EF029112 (squirting cucumber), OQ703996 (Cucumber), EU244327 (Cucumber), FR822384 (*Cucurbita* sp), FR717147 (squash), FR717145 (*Cucurbita* sp.), FR822383 (*Cucurbita* sp.), MT384365 (*Cucurbita pepo*) from France, Spain, Italy, Iran, China, Egypt and Poland. Whereas the CABYV isolate (CaBYV-SA-*Malva parviflora*) isolated from *M. parviflora* (MH192391) shared the highest nucleotide sequence identity (98.6-100%) with six isolates from Serbia, Czech Republic, Montenegro, France, Italy, Poland and four isolates from Egypt isolated from squash, melon, squirting cucumber and *Cucurbita* sp. Phylogenetic tree, SDT and the pairwise nucleotide identity percentage of CABYV Saudi isolates as compared with other retrieved isolates from GenBank globally (Figure 5, 6; Table 2).

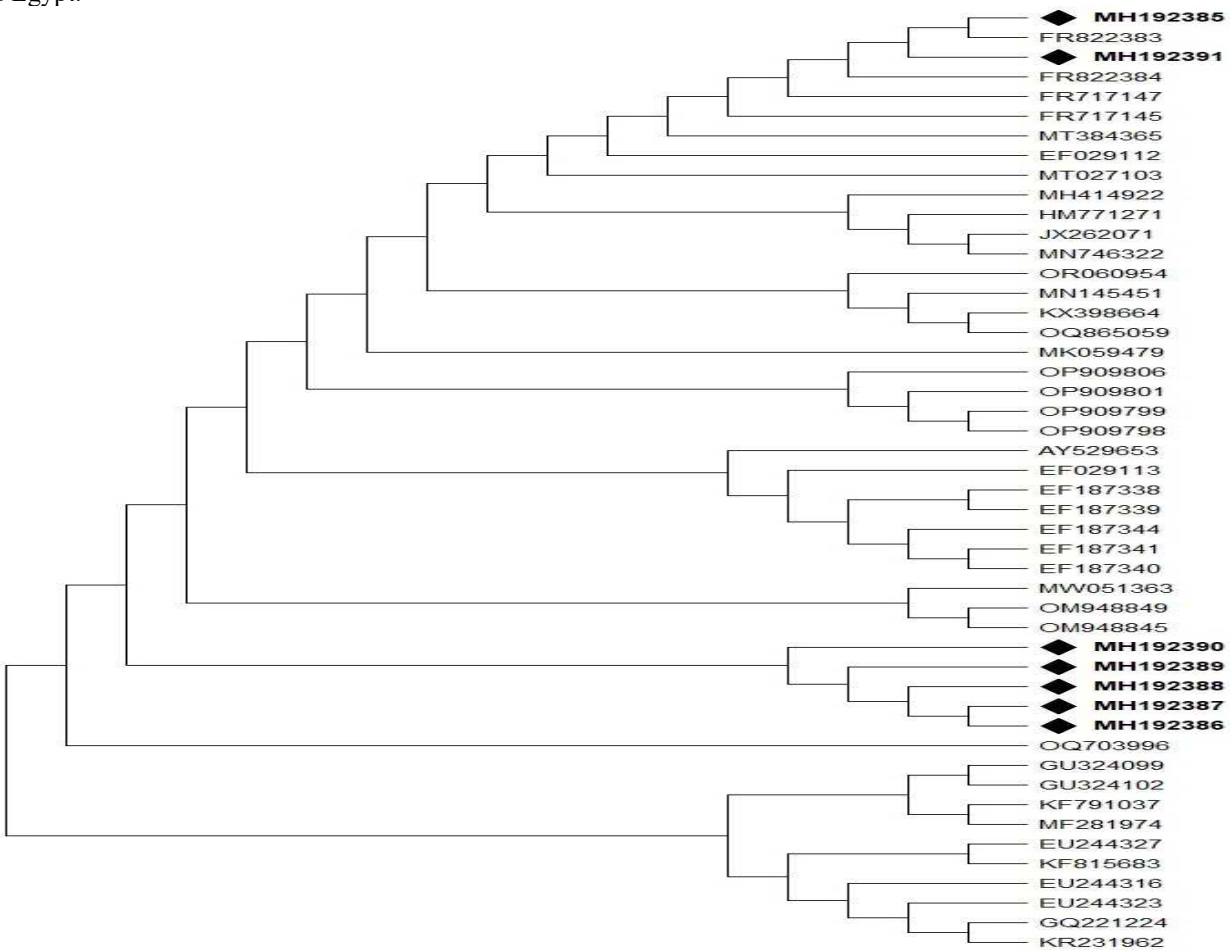

Figure 5. Phylogenetic relationships tree among the multiple sequence alignments of three Saudi Arabian isolates of CABYV, other selected 30 CABYV different isolates from other countries referenced in GenBank

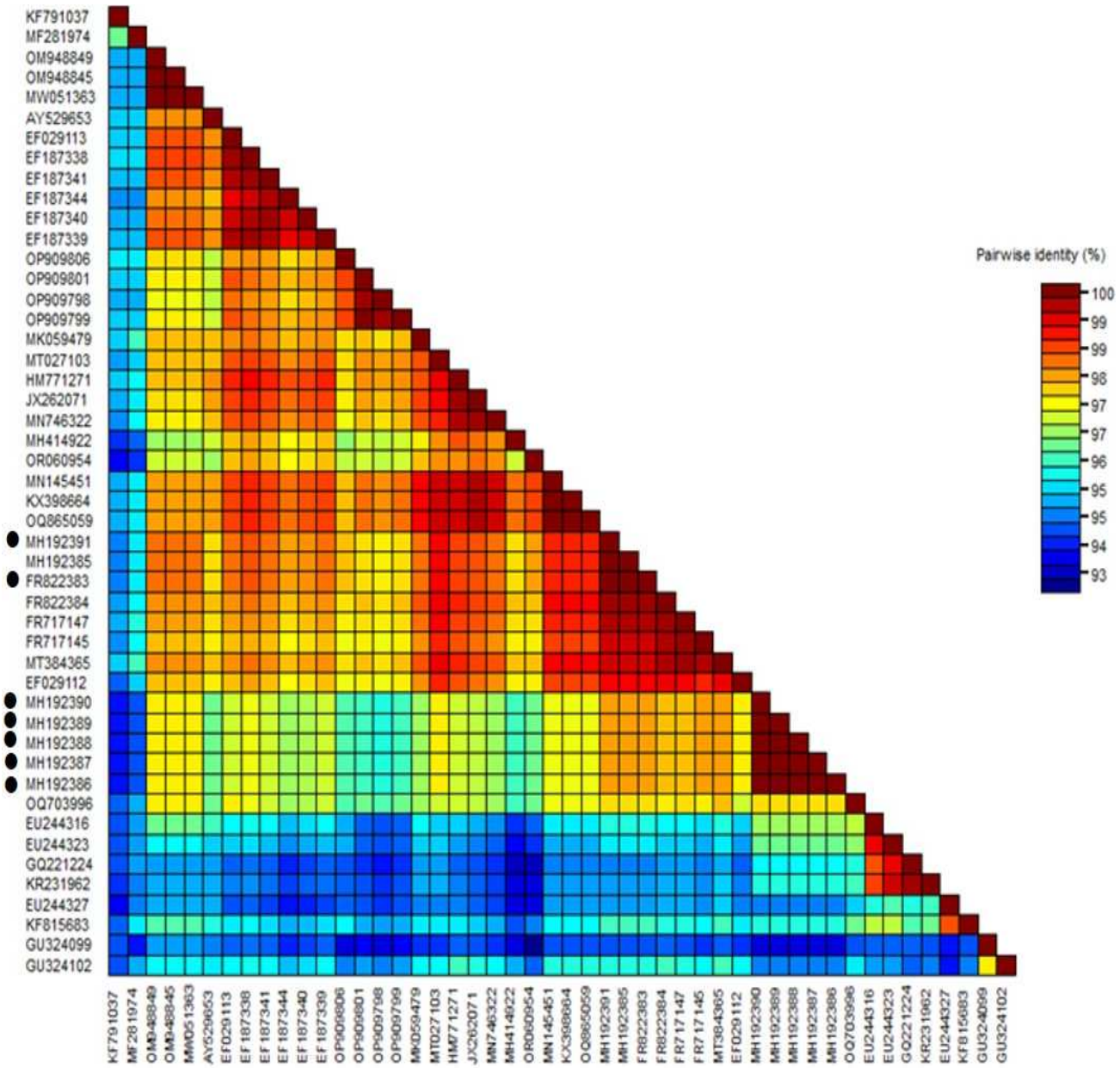

Figure 6. Distance matrix illustrating, the pairwise nucleotide identity percentage of CABYV Saudi isolates as compared with other retrieved isolates from GenBank globally.

Table 2. Percentage identity of the nucleotide sequence of the Coat protein gene CABYV Saudi Arabian isolates compared with other accession numbers isolates from GenBank originating from different countries.

Accession No.	MH192385	MH192386	MH192387	MH192388	MH192389	MH192390	MH192391
MH192385	100	98	98	98	98	98	100
MH192386		100	100	100	100	100	98
MH192387			100	100	100	100	98
MH192388				100	100	100	98
MH192389					100	100	98
MH192390						100	98
MH192391							100
MH414922	97.7	96	96	96	96	96	97.7

MK059479	98.4	96.8	96.8	96.8	96.8	96.8	98.4
MN145451	98.9	97.3	97.3	97.3	97.3	97.3	98.9
MN746322	98.4	96.7	96.7	96.7	96.7	96.7	98.4
MT027103	99.1	97.5	97.5	97.5	97.5	97.5	99.1
MT384365	99.5	98	98	98	98	98	99.5
MW051363	98.4	97.5	97.5	97.5	97.5	97.5	98.4
OM948845	98.4	97.5	97.5	97.5	97.5	97.5	98.4
OM948849	98.4	97.5	97.5	97.5	97.5	97.5	98.4
OP909798	97.5	95.9	95.9	95.9	95.9	95.9	97.5
OP909799	97.7	96	96	96	96	96	97.7
OP909801	97.7	96	96	96	96	96	97.7
OP909806	97.8	96.2	96.2	96.2	96.2	96.2	97.8
OQ703996	97.7	97.7	97.7	97.7	97.7	97.7	97.7
OQ865059	98.9	97.3	97.3	97.3	97.3	97.3	98.9
AY529653	97.7	96.4	96.4	96.4	96.4	96.4	97.7
EF029112	99.1	97.5	97.5	97.5	97.5	97.5	99.1
EF029113	98.4	97.1	97.1	97.1	97.1	97.1	98.4
EU244316	95.9	96.8	96.8	96.8	96.8	96.8	95.9
EU244323	95.5	96.4	96.4	96.4	96.4	96.4	95.5
EU244327	95	94.8	94.8	94.8	94.8	94.8	95
FR717145	99.5	98	98	98	98	98	99.5
FR717147	99.6	97.8	97.8	97.8	97.8	97.8	99.6
FR822383	100	98	98	98	98	98	100
FR822384	99.8	97.8	97.8	97.8	97.8	97.8	99.8
GQ221224	94.8	95.7	95.7	95.7	95.7	95.7	94.8
GU324099	94.4	93.7	93.7	93.7	93.7	93.7	94.4
GU324102	95.9	94.8	94.8	94.8	94.8	94.8	95.9
HM771271	98.7	97.1	97.1	97.1	97.1	97.1	98.7
JX262071	98.6	96.9	96.9	96.9	96.9	96.9	98.6
KF791037	94.8	94.1	94.1	94.1	94.1	94.1	94.8
KF815683	96	95.9	95.9	95.9	95.9	95.9	96
KR231962	95	95.9	95.9	95.9	95.9	95.9	95
KX398664	98.9	97.3	97.3	97.3	97.3	97.3	98.9
MF281974	95.5	94.4	94.4	94.4	94.4	94.4	95.5
OR060954	98	96.4	96.4	96.4	96.4	96.4	98
EF187338	97.3	97.5	97.5	97.3	97.3	97.3	97.3
EF187339	97.1	97.3	97.3	97.1	97.1	97.1	97.1
EF187340	96.9	97	97	96.9	96.9	96.9	96.9
EF187341	97.1	97.3	97.3	97.1	97.1	97.1	97.1
EF187344	96.8	96.9	96.9	96.8	96.8	96.8	96.8

DISCUSSION

During the growing season of 2014-2015, a significant incidence of symptoms of yellowing in cucumber and squash growing in the Al-Kharj region of Saudi Arabia fields was observed mainly, which reached up to 100% in some cases at the end of the season. It has previously been reported that CABYV causes interveinal chlorosis of the basal leaves in cucurbit crops, which is accompanied by brittle and thicker older leaves (Mnari-Hattab *et al*., 2009; Shang *et al*., 2009) and to Criniviruses CCYV and CYSDV (Al-Saleh *et al*., 2015b; Shakeel *et al*., 2018). Based on serology, RT-PCR and aphid transmission, it was confirmed that CABYV was involved in causing the yellowing disease observed in cucumber and squash crops. The present study also showed that CABYV was detected in a high proportion (67%) of cucumber and squash samples collected from open field and glasshouse cucurbit crops and the weeds collected (44.4%). Choi *et al*. (2015) investigated that about 5% of the melon samples that were collected and had yellowing signs had CABYV infection, as opposed to 30–50% in other studies (Kassem *et al*., 2007; Xiang *et al*., 2008, Shang *et al*., 2009). This difference may result from the presence of other species that produce similar signs of yellowing. Abou-Jawdah *et al*., 2000; Mnari-Hattab *et al*., 2005; Kassem *et al*., 2007 have demonstrated the effectiveness and reliability of DAS-ELISA for detecting CABYV. Figueira *et al*., 1997; Van den Heuvel *et al*., 1989 have indicated that its sensitivity

is insufficient to identify CABYV and other luteoviruses in plant tissue. In this study, we also demonstrated that DAS-ELISA and RT-PCR are reliable for detecting CABYV. Besides these viruses, cucurbits are frequently reported to be infected with other viruses (Ahsan *et al*., 2023). Phylogenetic tree analysis, sequence alignment and SDT make it implausible that CABYV isolates are not subdivided into subgroups. The results were determined using many CABYV isolates from various geographic locations and survey years.

The high frequency of CABYV (30/50 and 29/38) in squash and cucumber crops in this survey indicates that CABYV may pose a significant danger to cucurbit crops in Saudi Arabia, indicating the need for immediate management measures. Additionally, CABYV has been found in several weed species, including non-cucurbits like *Laminium amplexicaule*, *Capsella bursa-pastoris*, *Ch. murale*, *Bryonia dioica*, *Citrullus colocynthis*, *Coccinia grandis*, and *Ecballium elaterium* (Kassem *et al*., 2013; Vafaei and Mahmoodi, 2017; Shates *et al*., 2019).

Cucurbit yellows disease has been reported to be caused by viruses like CCYV, CYSDV, and CABYV (Desbiez *et al*., 2003; Wintermantel *et al*., 2009). An alternative detection method for these viruses' prevalence in cucurbit crops was investigated using RT-PCR. The presence of these viruses in weed and cucurbit plant species significantly impacts their spread in squash and cucumber and squash, especially in the presence of aphid species identified in the surveyed fields. These aphid species acquire the virus from the weeds and transmit it to cultivated plants.

Moreover, the phylogenetic tree and distance matrix indicated that Saudi isolates are members of the Mediterranean group, as previously described by Shang *et al*. (2009) and Omar and Bagdady (2012). To investigate this hypothesis, CABYV sequences originating from Poland, Slovenia, Germany, France, Spain, Brazil, Iran, Iraq, Italy, Tunisia, Morocco, China, Egypt, Japan, Taiwan, Czech Republic, Serbia, Thailand, South Korea, India and Montenegro were added to the sequence alignment with the Saudi isolates. This study may help better understand the frequency and distribution of CABYV, the genetic variability of CABYV isolates, and the phylogenetic relationship between Saudi isolates and globally reported isolates.

Conclusion: The findings revealed that the cucurbit aphid-borne yellows virus is highly distributed in squash and cucumber-growing fields in the Al-Kharj region of Saudi Arabia. The characteristic yellowing symptoms observed in older leaves and yield losses have been associated with this virus infection. The present isolates of this virus show low diversity. They are genetically related to the France, Italy, Poland, Spain, Iran, China, Serbia, Czech Republic, Montenegro, and Egyptian isolates, especially in the CP gene, and they belong genetically to the Mediterranean group isolates.

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