Current Status of Watermelon Chlorotic Stunt Virus (WmCSV) on Some Cucurbit Plants *(Cucurbitaceae)* in Alahsa Region of Saudi Arabia

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ABSTRACT
Cucurbit crops are widely grown and consumed in large quantities in Saudi Arabia and Middle East countries. Viral diseases are among the most limiting factors that affect the production of cucurbit crops around the world. The infection percentage of watermelon chlorotic stunt disease and molecular characterization of Saudi isolate of WmCSV are described in this study. Symptomatic leaf samples of zucchini (*Cucurbita pepo*), Cucumber (*Cucumis sativus*) and melon (*Cucumis melo L*) plants were tested for WmCSV and squash leaf curl virus (SLCV) infection serologically using the double antibody sandwich- enzyme-linked immunosorbent assay (DAS-ELISA) test and polymerase chain reaction (PCR) using degenerate and specific overlapping primers for WmCSV to amplify 2108 nucleotides of DNA-A. The data of DAS-ELISA test showed that 69% of all tested zucchini samples were infected with WmCSV, the percentage of infection increased to 72.7% when using PCR test. All tested samples of other cucumber and melon tested negative for WmCSV while SLCV was not detected in all tested samples. The obtained sequence analysis data demonstrated that the isolated WmCSV shared high nucleotide identity 98% with other isolates of Oman. The nucleotide sequences of WmCSV obtained in this study was submitted to the GenBank (International Nucleotide Sequence Database Collaboration) under accession number KC876038.

Key Words: Begomovirus, Geminiviruses, whitefly, WmCSV, Zucchini

INTRODUCTION
Viruses are the most common cause of diseases affecting cucurbits around the world. These diseases result in losses through reduction in growth, yield, and sometimes malformation of fruits. Cucurbit crops are widely grown the Middle East (total 226,492 ha) and are consumed in large quantities in traditional diet. In Saudi Arabia, cucurbit crops are grown all year around. The total cultivated area was 37,694 ha with an average annual production of 718,124 tons (Anonyms, 2013). Viral diseases are among the most limiting factors affecting the production of cucurbit crops in the Middle East. This is due to the widespread distribution of the whitefly *Bemisia tabaci type B* (*Gennaduis*) (*Aleyrodidae: Hemiptera*) vector in all vegetable producing areas of the Middle East countries (Gill and Brown, 2010). Based on genome organization, host range, and insect vector, the family of Geminiviridae has been divided into nine genera (Zerbini *et al.*, 2017). Begomoviruses have become a major limiting factor in the production of various fiber crops such as cassava and cotton, and food crops such as cucurbits, tomato, beans, and pepper (Varma and Malathi 2003). WmCSV causes severe diseases to cucurbits across Eastern Mediterranean countries and recently reported to be associated with cucurbits disease in the western hemisphere (Dominguez-Duran *et al.*, 2017). *Begomovirus* genus consists of viruses that are transmitted by whiteflies (*Bemisia tabaci*) that infect a wide range of plants that growon in gradually worm temperature, sub-tropical, and tropical regions (Moffat 1999; Zerbini *et al.*, 2017). WmCSV is a bipartite and has two single strand DNA (ssDNA) components known as DNA-A and DNA-B (Khan *et al.*, 2012). DNA-A component encoded six genes; four of them are in the complementary-sense (AC1, AC2, AC3 and AC4) and two are in the virion-sense

(AV1 and AV2). The DNA-B component of WmCSV and all other bipartite Begomoviruses includes one virion-sense gene (BV1) encoding the nuclear shuttle protein (NSP) and one complementary-sense gene (BC1) that encodes movement protein (MP) (Gutierrez *et al*., 2002). DNA-A and DNA-B components are sharing small sequence identity for a common region (CR) of 200–400 bp. This common region contains a predicted stem-loop structure with the Nona-nucleotide motif TAATATTAC in the loop that is the origin responsible for replication (Ori) for all geminiviruses groups (Laufs *et al*., 1995; Harrison and Robinson 2002). WmCSV was first identified in Yemen in 1982 (Jones *et al*., 1988) and reported across the Middle East and North Africa afterward in the following years in Sudan (Kheyr-Pour *et al*., 2000), Jordan (Al-Musa *et al*., 2011), Oman (Khan *et al*., 2012), and Palestine (Ali-Shtayeh *et al*., 2014). The symptoms caused by WmCSV are chlorotic mottling, stunting, vein yellowing, and severe decrease of yield, particularly in watermelon crops (*Citrullus spp.*). The host range of this virus covers most of cucurbits including zucchini, cucumber, and melons, in addition to pumpkin. Some investigatied plants like *Nicotiana glutinosa N. benthamiana* have been found to be hosts for WmCSV (Bananej *et al*., 2002). This research aims to identify the isolate of WmCSV from zucchini plants grown in Alahsa region, located in Eastern Saudi Arabia. The identification of isolated virus was carried out using polymerase chain reaction (PCR) and nucleotide sequences analysis.

MATERIALS AND METHODS

Sample collection

In the springs of 2012 and 2013, viral disease symptoms including yellowing, chlorosis, curling, stunting, and mottling (fig. 1) were observed on cucurbits in fields highly infected with whitefly (*Bemisia tabaci*) in Alahsa governorate, Saudi Arabia.

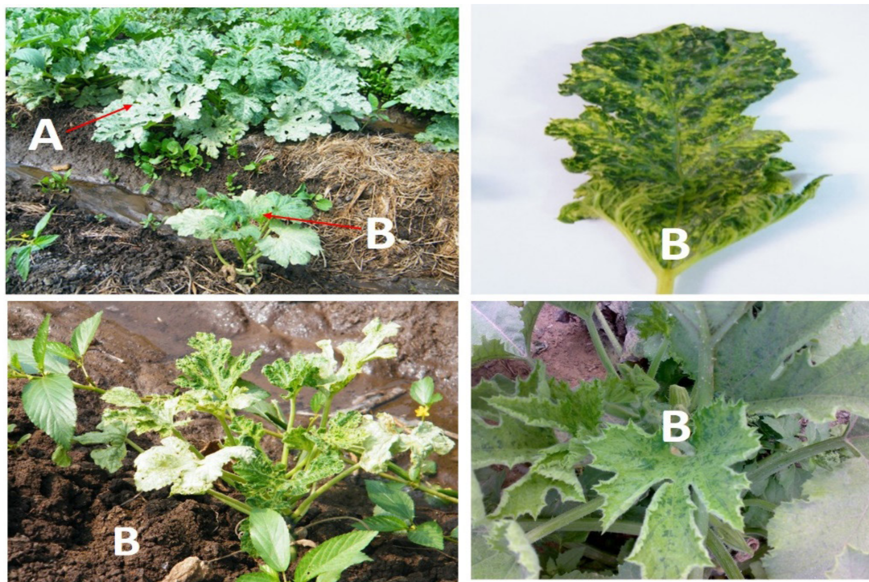

Fig. (1): Natural WmCSV-like symptoms (yellowing, chlorosis, stunting, Curling, and mottling) observed on zucchini plants (B) comparing with healthy looking zucchini plants (A) in open fields in Alahsa, Saudi Arabia.

To study the etiology of the disease, two hundreds ninety six symptomatic and non-symptomatic leaf samples of cucumber (*Cucumis sativus*) (72 samples), zucchini (*Cucurbita pepo*) (214 samples), and melon (*Cucumis melo L*) (10 samples) were collected from different fields at diverse locations of Alahsa governorate, Saudi Arabia. Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) test was used for the detection of WmCSV and SLCV in the collected samples using BIOREBA kit and

according to its manufacturer (BIOREBA, AG, Reinach, Switzerland; SEDIAG S.A.S. Longvich, France). In brief: the extracts of the collected plants were added to ELISA plates previously coated with specific antisera for each virus and incubated at 4 ºC overnight. The enzyme (alkaline phosphatase)-conjugate were diluted 1:1,000 in conjugation buffer and added after washing. The palates were incubated for 5 hours at 30 ºC. Substrate (p-nitrophenyl phosphate, 1mg/ml in substrate buffer) was added to each well after one more washing and the plates were incubated in the dark for one to two h at room temperature. Absorbance values were measured at 405 nm using an ELISA reader (MEDISPEC ESR-200).

Total nucleic acid extraction

Total nucleic acids were extracted from the collected samples using modified Dellaporta methods as described by (Anfoka *et al*., 2008; Rezk and Alhudaib 2017). In brief, 50 mg of plant tissue were grinded in 500-µl extraction buffer (mM EDTA, 100 mM Tris-HCl, 500 mM NaCl, 10 mM ß-mercaptoethanol) and incubated at 65°C for 10 min after adding 33 µl of 20% of SDS. The mixture was centrifuged for 10 min at 10,000 rpm after adding 1/5 volume of potassium acetate (5 M, pH 8.0). The supernatant was carefully transferred to a clean tube and an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added. The mixture was vortexed for 5 min and clarified by centrifugation at 10,000 rpm for 5 min and the upper phase were transferred to a new clean tube. The total DNA was precipitated by adding an equal volume of cold isopropanol was added to the mixture followed by a 15 min of centrifugation at 14,000 rpm. The DNA pellet was then washed with 70% ethanol and suspended in 100 µl sterile deionized water.

Polymerase Chain Reaction (PCR) Amplification

The extracted DNA was used as a templet PCR using set of primers as shown in table 1. Set of primers AVcore and ACcore were used as a degenerate primer for begomoviruses group to amplify about 580 bp. Other sets of primer WmF1/WmR1, WmF2/WmR2 and WmF3/WmR3 were designed to use as a specific primers for component A (DNA-A) of WmCSV to amplify 650 bp, 500 bp and 970 bp respectively. PCR reactions were optimized as following “for 50µl reaction and the final concentration of the components were 25 µM of each deoxynucleotide triphsphate (dNTPs), 1 x PCR buffer, 2.5 µM $MgCl_2$, 3 units *Taq* DNA polymerase, 0,2 µM of each complementary and viral sense primer and five µl of DNA used as template”. PCR cycle parameters for all reactions were as follows; two minutes at 94°C for initial start followed by 35 cycles at 94°C for 1 min, 55 °C for 2 min, and 72 °C for 2 min, and final additional cycle for extension at 72 °C for 10 min.

Table1: Oligo primers used to amplify partial fragments of DNA-A of WmCSV using polymerase chain reaction

Primer name	Nucleotide sequence 5--------------------------------3	Fragment size/ bp	Reference
WmF1	GCCCATGTACAGGAAGCCGAGG	650	This study
WmR1	ATTGGAAAATGTCCTTGTTGGG		This study
WmF2	CGGATATATTTTATGACTCA	500	This study
WmR2	CCCCTCTTCTCAGCCGAGC		This study
WmF3	TTGATCCGCAGTAATGTATTCCCC	970	This study
WmR3	AGCTACTTTCTTTTTTAGTCGGCC		This study
AVcore	GCCHATRTAYAGRAAGCCMAGRAT	580	Brown *et al*., 2001
ACcore	GAATTCATGRTNRGGTGYATHGANAAYGG		Brown *et al*., 2001

DNA Cloning and Sequencing

PCR product obtained using primers are designed to amplify overlapping fragments of WmCSV-DNA-A were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). The purified DNAs were cloned into the cloning vector pGEM-T Easy (Promega, USA) according to its manufacturer's instructions then transformed into high library-efficiency competent *Eschrichia coli* DH5α strain (Invitrogen Corporation, USA).

DNA sequences were determined by the didexynucleotide method in Macrogen Company, South Korea, using Thermo Sequenase Dye terminator cycle sequencing kit, on ABI 377 DNA sequencer (Perkin-Elmer, Applied Biosystems, USA).

Nucleotide sequence analysis:

Alignment analysis was done for partial nucleotide sequences Saudi Arabian isolate of WmCSV using the online BLAST service of the National Centre for Biotechnology Information (NCBI) (URL: www.ncbi.nih.gov BLAST/) and DNAMAN software version 8. Homology tree was constructed from the multiple alignments with WmCSV isolates sequences available in GenBank (International Nucleotide Sequence Database Collaboration) database (table 2) using the DNAMAN software (Lynnon,Canada) with the Jukes-Cantor distance-correction method (Jukes and cantor, 1969) and the neighbor-joining method (Saitou and Nei,1987).

Table 2: GenBank accession numbers of selected WmCSV sequences used in this study for analysis of the isolated WmCSV (KC876038)

Accession Number	Country	Reference
KC876038	Saudi Arabia – Alhasa	This study
AJ245652	France	Kheyr-Pour,A. *et al*. (2000)
AJ245650	France	Kheyr-Pour,A. *et al*. (2000)
JN618984	Oman - Muscat	Khan,A.J. *et al*. (2012)
JN618981	Oman - Muscat	Khan,A.J. *et al*. (2012)
KJ854919	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
KJ854918	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
KJ854912	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
JN618982	Oman - Muscat	Khan,A.J. *et al*. (2012)
KJ854917	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
JN624386	Oman - Muscat	Khan,A.J. *et al*. (2012)
KJ854915	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
KJ854913	Palestine – Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
JX131283	Jordan - Al-Balqa'	Anfoka,G.H. *et al*. (2012)
KJ854916	Palestine Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
KJ854914	Palestine - Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
JN618983	Oman - Muscat	Khan,A.J. *et al*. (2012)
KM066100	Saudi Arabia - Jeddah	Al-Saleh,M.A. *et al*. (2014)
KC462552	Palestine - Nablus	Ali-Shtayeh,M.S. *et al*. (2014)
HM368371	Lebanon - Beirut	Samsatly,J. *et al*. (2012)
EU561237	Jordan - Amman	Al-Musa,A.M. *et al*. (2011)

RESULTS

Sampling and PCR amplification:

Through springs of 2012 and 2013, plant samples showing disease symptoms were collected from 296 cucumber, zucchini and melon plants in several fields in Alhasa

governorate (table 3). All tested samples of cucumber zucchini and melon were negative for SLCV. WmCSV was found only in zucchini and none in cucumber and melon. The incidence of WmCSV disease as shown DAS-ELISA test was 69%. This percentage increased up to 75.7% when PCR was used for diagnoses (table 3). The analysis of the collected samples by PCR using AVcore and ACcore set of primers as degenerate primers for the entire begomoviruses group revealed that positive samples gave bands in expected size, which is about 580 bp (fig. 2). Other primers that is specifically designed for WmCSV were used with samples that gave positive reactions with degenerate primers (table 1). The designed specific primers successfully amplified the expected size of DNA and gave strong band as showed in fig. 3. Those primers were designed to amplify overlap regions for DNA-A component of WmCSV.

Table 3: ELISA test and PCR amplification to detect watermelon chlorotic stunt virus (WmCSV) and squash leaf curl virus (SLCV)

Location	No. of Samples	Type of samples	(+ve with ELISA test)		+ve PCR (deg. Primers*)(%)
			WmCSV(%)	SLCV	
Alahsa	72	Cucumber	0	0	0
	214	Zucchini	148 (69%)	0	162(75.7%)
	10	Melon	0	0	0

*degenerate primer used to detect *Begomoviruse* members.

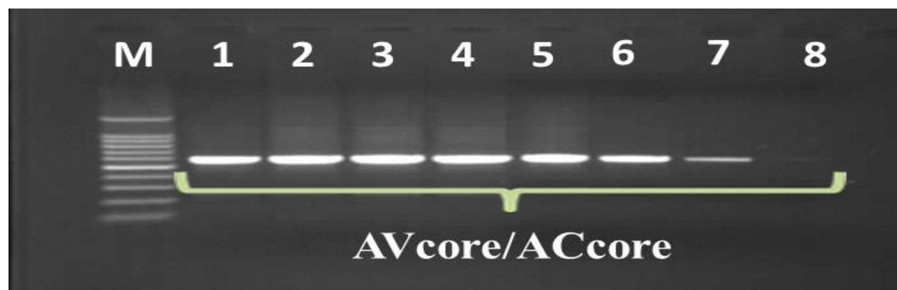

Fig 2: Gel electrophoresis of PCR products using the degenerate primers AVcore and ACcore; M= 100 bp marker (Promiga), Lanes from 1 to 8 zucchini samples collected from Alahsa.

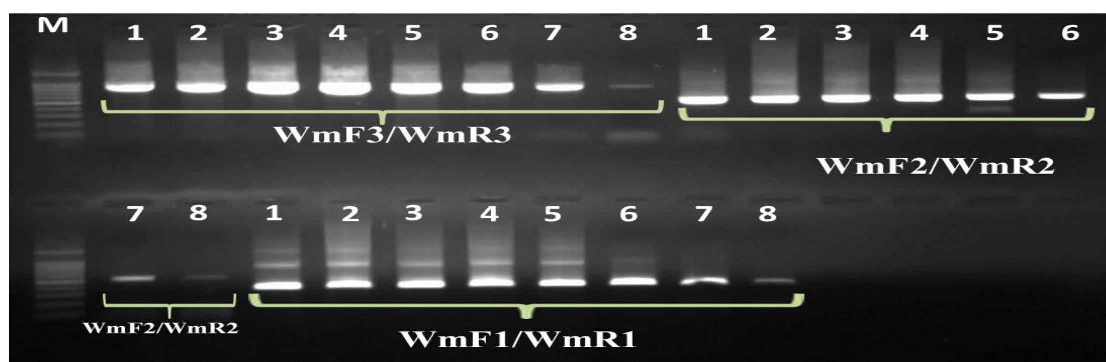

Fig 3: Gel electrophoresis of PCR products using the specific primers WmF1/WmR1, WmF2/WmR2 and WmF3/WmR3 ;M= 100 bp marker (Promiga), Lanes from 1 to 8 zucchini samples collected from Alahsa.

Nucleotide Sequence analysis:

The obtained PCR products from the tested zucchini samples using previous generate and specific primers were cloned into pGEM T-Easy vector (Promiga) and sequenced to identify virus strain. The core region of the

virus, that amplified using primers AVcore/ACcore, was sequenced. Phylogenetic analysis was carried out as a primary indicator for the partial sequences this begomovirus common region. The obtained sequence referred to the isolated virus is WmCSV. From this primary data, specific primers of WmCSV were designed to amplify about 2,108 bp of WmCSV component-A, using the sequence data of WmCSV isolate from Oman (JN618984) isolated by Khan *et al.* (2012) as a template for designing the specific primers. The amplified fragments using designed specific primers WmF1/WmR1, WmF2/WmR2, and WmF3/WmR3 were cloned and sequenced. The obtained sequence were analyzed using (URL: www.ncbi.nih.gov BLAST/) and DNAMAN software version 8. The obtained sequence was submitted in GenBank under accession number KC876038 compared and aligned with the representative sequences of WmCSV isolates from different countries available in GenBank as in table 2.

The homology tree (Fig. 4) showed the aligned sequences separated in three clusters; the first one includes isolates from Oman and France. The second includes isolates from Palestine and Jordan while the third one includes isolates from Jeddah, Saudi Arabia.

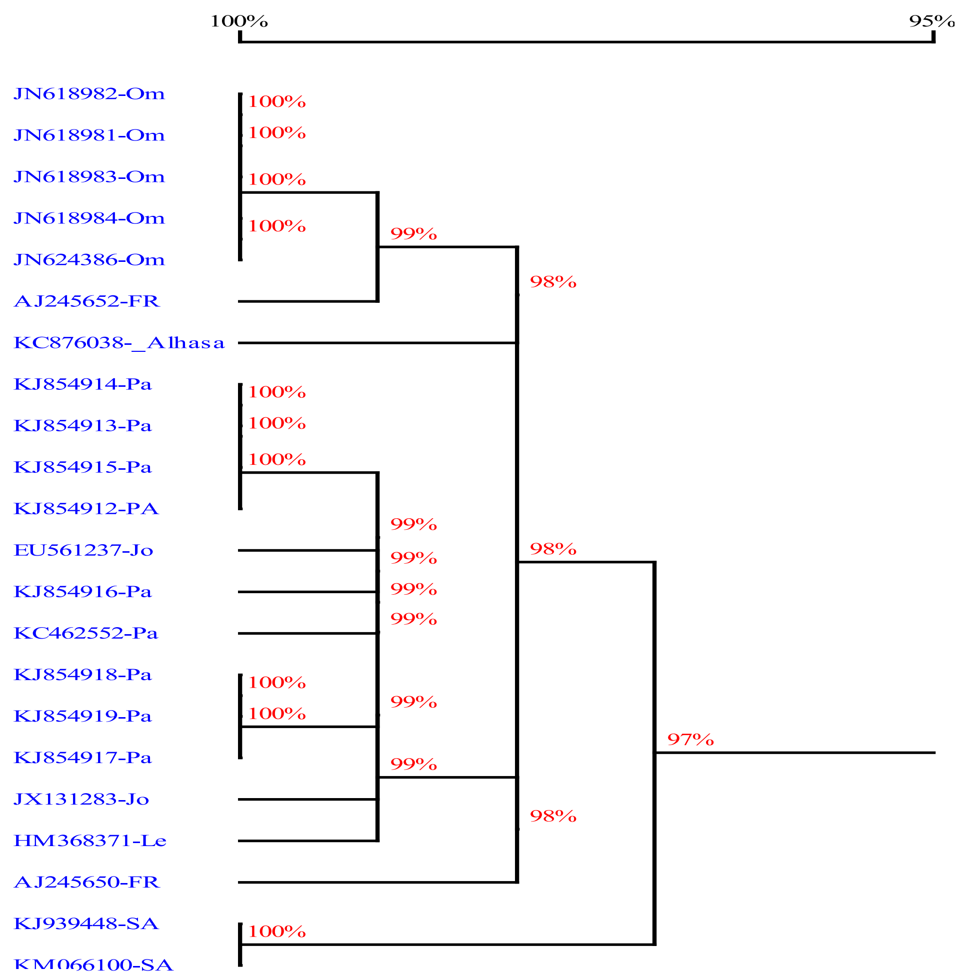

Fig 4: Neighbor joining tree showing the genetic homology among the WmCSV-DNA component for the genome sequence obtained in this study (KC876038) and other sequences selected from GenBank.

DISCUSSION

During field visits in spring seasons of 2012 and 2013 to some fields in Alahsa governorate, many complains were received from farmers of sever diseases symptoms on cucurbit plants. These symptoms represented in

yellowing plants, stunting and drastically reduced the yield production. These symptoms was associated with the presence of high population of whiteflies (*B. tabaci*). The observed disease symptoms were similar to those described previously by (Ali-Shtayeh ., 2014) that was associated with WmCSV in Palestine. In addition, these symptoms were similar to most diseases caused by *Begomoviruses* members with high numbers of whiteflies in these fields. Disease incidence of WmCSV reached 75.7 % of tested zucchini plants. Other virus such as cucurbit yellow stunt disorder virus (CYSDV) (Rezk *et al*., 2015) was recorded in same area and both viruses are transmitted by whiteflies. This may partially explain the decline of zucchini cultivation area in Saudi Arabia from 225 ha in 2009 to 189 ha in 2015(Agricultural statistic book, Ministry of Agriculture, Saudi Arabia, 2016). The percentage of virus detection in examined zucchini plants increased from 69% to 75.7 % when PCR using degenerate primers was used. This is due to the high sensitivity of PCR compared to ELISA test. The samples from one to seven gave very strong bands while the sample 8 gave faint band. This was also observed for the same samples with the degenerate primer and all used primers in this study. This may be due to the concentration of the template DNA in sample 8. The alignment and homology tree analysis demonstrated that the isolated virus from Alahsa in this study was located same distance between the cluster of the Omani isolates (Khan *et al*., 2012) and other Palestinian isolates (Ali-Shtayeh, *et al*, 2014), and Jordan (Almusa *et al*, 2011) with 98% similarity. However, the identity with the other isolates of Saudi Arabia that isolated from Jeddah by Al-Saleh *et al*. (2014) was 97%. The sequence segregate was mostly close to the sequences of WmCSV isolate from Oman in addition the short distance between both countries suggesting the probability that the virus entered Alahsa from Oman and especially transmitted by whitefly. This study shows that case is a viral disease transmitted by Whitefly. Due to the speed of transmission of the insect between crops, the infection spread among most plants since this virus infects most of *Cucurbitaceae*. The early identification of the disease causal and mode of transmission is beneficial for the control process. It could be concluded that there is a new isolate of WmCSV in Saudi Arabia that is closely related to that found in Oman. However, due to the expanded area and diversity of climate of Saudi Arabia, there might be different strains of this virus. Thus, research will be of interest in next studies on sequencing a complete genome of WmCSV DNA-A and DNA-B in addition to studying the genetic diversity of this virus throughout the Kingdom of Saudi Arabia as a future goal.

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REFERENCES

Al-Musa, A., Anfoka, G., Al-Abdulat, A., Misbeh, S., Haj Ahmed, F., and Otri, I., 2011. Watermelon chlorotic stunt virus (WmCSV): A serious disease threatening watermelon production in Jordan. Virus Genes 43: 79-89.

Al-Saleh, M. A., Ahmad, M. H., Al-Shahwan, I. M., Brown, J. K., and Idris, A. M. 2014. First report of watermelon chlorotic stunt virus Infecting watermelon in Saudi Arabia. Plant Dis. 98 (10):1451.

Ali-Shtayeh, M. S., Jamous, R. M., Mallah, O. B. and Abu-Zeitoun, S.Y. 2014. Molecular characterization of watermelon chlorotic stunt virus (WmCSV) from Palestine. Viruses 6(6): 2444-2462.

Anfoka G., Abhary, M., and Haj Ahmad, F. 2008. Survey of tomato yellow leaf curl disease-associated viruses in the Eastern Mediterranean basin. Journal of Plant Pathology. 90: 311–20.

Anonyms 2013. Agricultural Statistics, Ministry of Agriculture, Saudi Arabia.

Bananej, K., Dafalla, G. A., Ahoonmanesh, A., and Kheyr-Pour, A. 2002. Host range of an Iranian isolate of watermelon chlorotic stunt virus as determined by whitefly-mediated inoculation and agroinfection, and its geographical distribution. J. Phytopath. 150: 423–430.

Brown J. K., Idris, A. M., Torres-Jerez, I., Banks, G. K., and Wyatt, S. D. 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of Begomoviruses. Arch Virol. 146: 1581–1598.

Domínguez-Durána, G, Rodríguez-Negreteb, E. A., Morales-Aguilara, J., Camacho-Beltrána, E., Romero-Romeroa, J. L., Rivera-Acostaa, M. A., and Leyva-Lópeza,Molecular, N. 2017. Biological characterization of watermelon chlorotic stunt virus (WmCSV): An Eastern Hemisphere begomovirus introduced in the Western Hemisphere. Crop Protection. 103: 51–55. doi.org/10.1016/j.cropro.2017.09.006.

Gill, R., and Brown, J. K. 2010. Systematics of *Bemisia* and *Bemisia* Relatives: Can molecular techniques solve the *Bemisia tabaci* complex conundrum, a Taxonomist's viewpoint. pp. 5–29. *In*: Stansly, P. A., and Naranjo, S. E. (Eds.) Bionomics and Management of a Global Pest. Springer, Amsterdam, Netherlands.

Gutierrez, C. 2002. Strategies for geminivirus DNA replication and cell cycle interference. Physiol. Mol. Plant Pathol. 60: 219–230.

Harrison, B.D., and Robinson, D.J. 2002. Green shoots of geminivirology. Physiol. Mol. Plant Pathol. 60: 215–218.

Jones, P., Sattar, M. H. A., and Al Kaff, N. 1988. The incidence of virus disease in watermelon and sweet melon crops in the People Democratic Republic of Yemen and its impact on cropping policy. Asp. Appl. Biol. 17:203-207.

Jukes, T.H., and Cantor, C.R. 1969. Evolution of Protein Molecules. Academic Press, New York. pp. 21–132.

Khan, A.J., Akhtar, S., Briddon, R.W., Um Ammara, Al-Matrooshi A.M., and Mansoor, S. 2012. Complete nucleotide sequence of watermelon chlorotic stunt virus originating from Oman. Viruses 4: 1169-1181.

Kheyr-Pour, A., Bananej, K., Dafalla, G. A., Caciagli, P., Noris, E,. Ahoonmanesh, A., Lecoq, H., and Gronenborn, B. 2000. Watermelon chlorotic stunt virus from the Sudan and Iran: Sequence comparisons and identification of a whitefly transmission. Phytopathology. 90(6): 629-635.

Laufs, J., Jupin, I., David, C., and Schumacher, S. 1995. Heyraud-Nitschke, F.B.G. geminivirus replication: Genetic and biochemical characterization of Rep protein function, a review. Biochimie. 77: 765–773.

Moffat, A.S. 1999. Geminiviruses emerge as serious crop threat. Science. 286, 1835.

Rezk A., and Alhudaib, K. 2017. Molecular biodiversity of tomato yellow leaf curl- like viruses in Saudi Arabia. Journal of Plant Science. Doi:10.3923/jps.2017.

Rezk, A., Alhudaib, K. and Soliman, A. 2015. Characterization of cucurbit yellow stunt disorder virus (CYSDV) and development monoclonal antibodies using recombinant coat protein. Egyptian Journal of Virology. (12): 93-104.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4): 406-425.

Samsatly, J., Sobh, H., Jawhari, M., Najjar, C., Haidar, A., and Abou-Jawdah, Y. 2012. First report of watermelon chlorotic stunt virus in cucurbits in Lebanon. Plant Dis. 96(11): 1703.

Varma, A., and Malathi, V. G. 2003. Emerging geminivirus problems: A serious threat to crop production. Ann. Appl. Biol. 142: 145–164.

Zerbini F. M., Briddon, R. W., Idris, A., Martin, D. P., Moriones, E., Navas-Castillo, J., Rivera-Bustamante, R., Roumagnac, P., and Varsani, A. 2017. ICTV virus taxonomy profile: Geminiviridae. J. Gen. Virol. 98: 131–133.

الوضع الحالي لفيروس اصفرار وتقزم البطيخ على بعض النباتات القرعية بمنطقة الأحساء بالمملكة العربية السعودية

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الملخص

تزرع محاصيل القرعيات على نطاق واسع، وتستهلك بكميات كبيرة في المملكة العربية السعودية ودول الشرق الأوسط. الأمراض الفيروسية هي من بين العوامل المحددة الرئيسة التي تؤثر على إنتاج محاصيل القرعيات في جميع أنحاء العالم. وخلال هذه الدراسة تم تحديد نسبة الإصابة بفيروس اصفرار وتقزم البطيخ وكذلك التعريف الجزيئي للعزلة السعودية للفيروس. أجريت الاختبارات السيرولوجية (الأليزا) وكذلك تفاعل البلمرة المتسلسل باستخدام بادئات عامة لمجموعة الفيروسات التوأمية للكشف عن فيروس اصفرار وتقزم البطيخ، وفيروس تجعد أوراق الكوسة على مجموعة من العينات الورقية التي جمعت من نباتات كوسة وخيار وشمام تحمل أعراضًا مرضية. أجري خلال الدراسة تحليل ودراسة التتابع النيوكليوتيدي لعدد 2108 نيوكليوتيدة من DNA-A للفيروس. أظهرت نتائج الأليزا أن نسبة 69 % من نباتات الكوسة المختبرة كانت مصابة بفيروس اصفرار وتقزم البطيخ، وهذه النسبة زادت إلى 72.7 % عند استخدام تفاعل البلمرة المتسلسل. العينات النباتية للخيار والشمام المختبرة كانت سالبة لفيروس اصفرار وتقزم البطيخ بالإضافة إلى عدم تواجد فيروس تجعد أوراق الكوسة في كل العينات المختبرة؛ سواء باستخدام تفاعل البلمرة المتسلسل أو باستخدام الأليزا. وأظهرت بيانات تحليل دراسة التتابع النيوكليوتيدي أن الفيروس المعزول من منطقة الأحساء كان متشابهًا جينيًّا مع عزلات أخرى من سلطنة عُمَان، وبنسبة تشابه وصلت إلى 98 %. تم إدراج التسلسل النيوكليوتيدي للفيروس المعزول خلال هذه الدراسة في بنك الجينات تحت رقم KC876038.

الكلمات المفتاحية: الذبابة البيضاء، فيروس اصفرار وتقزم البطيخ، الفيروسات التوأمية، الكوسة.