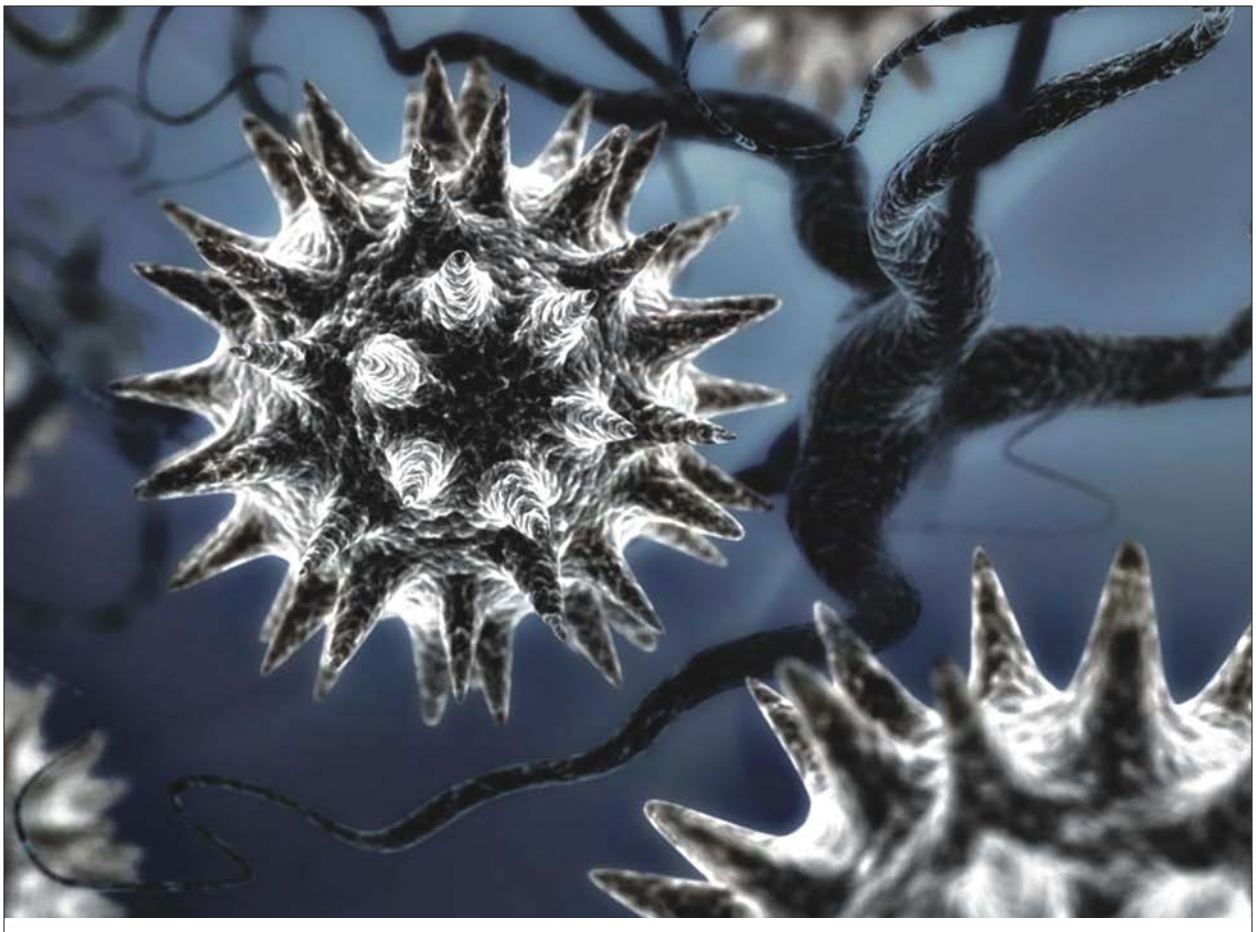

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 11 (1): 1-11, 2015
ISSN 1816-4900 / DOI: 10.3923/ijv.2015.1.11

Serological and Molecular Characterization of *Cucurbit chlorotic yellows virus* Affecting Cucumber Plants in Egypt

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ABSTRACT

This study was aimed to identify the causal agent inducing virus-like symptoms on cucumber plants for the first report of *Cucurbit chlorotic yellows virus* (CCYV) in Egypt. A total of 45 symptomatic and asymptomatic cucumber samples were collected from five locations in El-Behira, government, Egypt during June, 2014. Out of 45 samples analyzed by DAS-ELISA, twenty two samples were found infected by CCYV only, two and four samples showed mixed infection with *Cucurbit yellow stunting disorder virus*+CCYV and *Cucurbit aphid-borne yellows virus*+CCYV, respectively, whereas the rest of the samples were negative to all three viruses. No RT-PCR products were obtained with all tested samples when the specific primer for *Beet pseudo-yellows virus* was used. Total RNA was extracted from ELISA positive samples for CCYV and a 353-bp DNA fragment of the HSP70 gene was amplified by RT-PCR using specific primer for CCYV. The synthesized cDNA probe for CCYV was used to confirm the detection of CCYV in singly and mixed infections using dot blot hybridization. The highest similarity (100%) was found with 22 isolates of CCYV isolated from melon, watermelon, cucumber and muskmelon in Japan, China, Taiwan, Lebanon and Sudan was found but the lowest similarity (94.3%) was found with two isolates from cucumber and melon in Iran. To our knowledge, this is the first report of CCYV on cucumber in Egypt.

Key words: CCYV, DNA sequencing, ELISA, HSP70 gene, RT-PCR

INTRODUCTION

Cucurbits are of great economic importance among the major vegetable crops in Egypt, where total production of cucurbits reached 3972500 t from a cultivated area of 528588 feddan (1 feddan = 0.42 ha) in 2012 (Ministry of Agriculture and FAO reports). In the Mediterranean region, at least 28 viruses have been reported to infect cucurbit crops (Lecoq, 2003; Lecoq and Desbiez, 2012; Abrahamian *et al*., 2013) and several are discovered each year (Hassan and Duffus, 1990; Brown *et al*., 2011; Lecoq *et al*., 2011; Dong *et al*., 2012; Omar and Bagdady, 2012; El-Rahmany *et al*., 2014). *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucurbit chlorotic yellows virus* (CCYV) are two emergent viruses that belong to the genus *Crinivirus* in the family Closteroviridae. The CCYV is a novel cucurbit-infecting crinivirus which was first identified in melon crops in Kumamoto, Japan, in 2004 (Gyoutoku *et al*., 2008) and is now widespread throughout the country (Furuta *et al*., 2008; Okuda *et al*., 2010). Since its first report in 2004 in Japan (Gyoutoku *et al*., 2008) CCYV has spread to Taiwan (Huang *et al*., 2010), China

(Gu *et al*., 2011; Zeng *et al*., 2011), Sudan (Hamed *et al*., 2011), Saudi Arabia (Al-Saleh *et al*., 2014) and Lebanon (Abrahamian *et al*., 2012). CCYV remains a tentative member of the genus *Crinivirus* since it was first fully sequenced in 2010 (Okuda *et al*., 2010). CCYV has a typical bipartite crinivirus genome, encoding four proteins in RNA1 and eight in RNA2 (Okuda *et al*., 2010). Recently, mixed infections by CYSDV and CCYV were reported in commercially cultivated cucurbits (Abrahamian *et al*., 2012). Both viruses cause considerable damage mainly to the Cucurbitaceae family and can infect field crops or weeds (Wintermantel *et al*., 2009; Okuda *et al*., 2010). In addition to CCYV and CYSDV, other known whitefly-transmitted yellowing viruses are *Lettuce infectious yellows virus* (LIYV), *Tomato infectious chlorosis virus* (TICV), *Tomato chlorosis virus* (ToCV), *Sweet potato chlorotic stunt virus* (SPCSV), *Potato yellow vein virus* (PYVV) and *Lettuce chlorosis virus* (LCV) (Hartono *et al*., 2003; Boubourakas *et al*., 2006; Tzanetakis *et al*., 2013). CCYV and CYSDV are transmitted by *Bemisia tabaci* Mediterranean (MED) (previously biotype Q) and MEAM1 complexes in a semi-persistent manner (Gyoutoku *et al*., 2008; De Barro *et al*., 2011). Symptoms of *Criniviruses* develop on older leaves and progress toward newer growth inducing nutrition deficiency-like symptoms, interveinal chlorosis, brittleness and thickening of leaves. Symptoms induced by CYSDV, CCYV and other *Criniviruses* are very similar and their differentiation relies on the application of laboratory-based detection techniques (Wintermantel and Wisler, 2006). CCYV causes chlorotic leaf spots and complete yellowing of leaves of cucumber (*Cucumis sativus*), melon (*C. melo*) and watermelon (*Citrullus lanatus*) plants, resulting in severe economic losses. For efficient control of CCYV, simple and accurate diagnosis is urgently required. Several detection methods for CCYV using Enzyme-linked Immunosorbent Assay (ELISA), Tissue-Blot Immuno Assay (TBIA), Dot-Blot Immuno Assay (DBIA), dot-blot hybridization and Reverse Transcription (RT), Polymerase Chain Reaction (PCR) (Hourani and Abou-Jawdah, 2003; Ruiz *et al*., 2002; Gyoutoku *et al*., 2008). During 2014, yellowing symptoms were frequently observed in cultivated cucumber fields. The present study aim to investigate the incidence of the CCYV was the causal agent of yellowing disease of cucumber, also, conducted a preliminary analysis to genetically characterize Egyptian CCYV isolates. To achieve this, serological and molecular methods such as DAS-ELISA, RT-PCR, dot blot hybridization and sequencing analysis were applied for identification of the CCYV.

MATERIALS AND METHODS

Sample collection: Seven cucumber open fields were surveyed in different locations 48 symptomatic and two asymptomatic cucumber samples from seven open fields distributed in a geographical area in El-Behira government, Egypt during June, 2014 were collected for virus identification. Viral symptoms included chlorotic spots followed by yellowing of whole leaves and thickening of older leaves were observed. A maximum of ten samples per field from symptomatic plants were collected and processed in Plant Virology Lab, Plant Protection Department, College of Food and Agricultural Sciences, King Saud University.

Serological detection: The presence of CCYV and other common cucurbit viruses (*Cucurbit chlorotic yellows virus* (CCYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucurbit aphid-borne yellows virus* (CABYV) in samples was ascertained by using Double-Antibody Sandwich (DAS) ELISA as described by Clark and Adams (1977) with polyclonal

antibodies obtained from LOEWE CO, Germany) and polyclonal antibodies of CABYV was kindly provided by Dr. H. Lecoq (INRA Montfavet, France were used to serologically identify the viruses infecting cucumber). Leaf samples were ground in a pre-cooled mortar and pestle with an extraction buffer (PBST: 0.13 M NaCl, 0.003 M KCl, 0.008 M Na_2HPO_4, 0.001 M KH_2PO_4, pH 7.4) containing 0.05% Tween 20 and 0.1% nonfat dry milk and were placed in wells that had been pre-coated with specific polyclonal antisera diluted in a carbonate buffer (pH 9.6). Plates were incubated at 4°C overnight and washed three times with PBST-Tween 20 buffer. Plates were then coated with alkaline phosphatase conjugated antibody diluted in extraction buffer and incubated for 2 h at 37°C. After washing, p-nitrophenyl phosphate in diethanolamine substrate buffer (0.5 mg mL^{-1}, pH 9.8) was added to each well and incubated at room temperature for 30-120 min. The reaction was detected colorimetrically at A405 nm using an ELISA reader (BioTek, Instruments, Inc., USA). Two wells were used per sample. Virus-free cucurbit species grown in insect- proof cages were used as negative controls. Positive and healthy controls were included in all tests. Samples were considered to be positive if the A405 nm values were more than three times those of the healthy control.

Reverse transcription-polymerase chain reaction (RT-PCR) detection: The original field samples were used to RNA extraction using Isolate II RNA Kit (Bioline) according to the manufacturer's protocol. RT-PCR was carried out using One-step RT-PCR reagent (PrimeScript One-step RT-PCR kit ver 2; Takara BIO. Inc. Japan). Amplifications were carried out in a Mastercycler gradient (Eppendorf, Germany). A pair of primers, BPYV I: 5'-tcg aaa gtc caa caa gac gt-3' and BPYV II: 5'-ctg atg gtg cgc gag tg-3'), were used to detect genera *Beet pseudo-yellows virus* (BPYV) according to Boubourakas *et al*. (2006). The HSP70h gene region of CCYV was amplified using Crini-s2 (5'-cat tcc tac ctg ttt agc ca-3') and Crini-as2 (5'-tgc act tat aat ctg ctg gta c-3') primer designed according to Hamed *et al*. (2011) from CCYV sequences available at GenBank to confirm virus infection under the following reaction conditions: RT, 42°C for 30 min and 94°C for 2 min; PCR, 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, followed by 72°C for 5 min. The PCR amplification products were then examined by agarose gel electrophoresis (1.5% agarose, TAE), followed by ethidium bromide staining according to Sambrook and Russell (2001).

Dot blot hybridization assay: The obtained RT-PCR product (353 bp) of partial Hsp70h gene of these CCYV was purified using Matrix Gel Extraction System, Life Technologies and directly labeled with digoxigenin (DIG) labeling system according to Feinberg and Vogelstein (1983) and Holtke and Kessler (1990). Sap extractions were prepared from all collected samples from cucumber (45 samples) by grinding according to Laulhere and Rozier (1976) and Podleckis *et al*. (1993). The DNA was fixed on the membranes by Ultraviolet cross-linked for 30 sec. Prehybridization, hybridization and immunological detection were carried out using the DIG DNA labeling and Detection kit (Roche Diagnostics) according to the manufacturer's recommendation.

Partial nucleotide sequence cloning and analysis of CCYV HSP70h gene: Amplified RT-PCR products subsequently were ligated into pGEM-T Easy vector (Promega, Madison, WI) according to the manufacturer's instructions and transformed into DH5a *Escherichia coli* cells. CCYV DNA products from RT-PCR were used directly for sequence analysis. Ethidium bromide strained DNA bands of interest were purified (Matrix Gel Extraction System, Life Technologies).

Table 1: Sequence identities between Egyptian isolates and thirty other *Cucurbit chlorotic yellows virus* (CCYV) sequences retrieved from GenBank databases

Accession No.	Isolate	Organic host	Country	KP204871 EG-B1	KP204872 EG-B2	KP204873 EG-B3
AB457591	-	Melon	Japan: Kumamoto	100	100	100
AB523789	-	Melon	Japan: Kumamoto	100	100	100
KP204871	EG-B1	Cucumber	Egypt	100	100	100
KP204872	EG-B2	Cucumber	Egypt	100	100	100
KP204873	EG-B3	Cucumber	Egypt	100	100	100
GU721106	Sgw	Watermelon	China: Shouguang	99.5	99.5	99.5
GU721107	Sgm	Melon	China: Shouguang	99.5	99.5	99.5
GU721108	Nbw	Watermelon	China: Ningbo	100	100	100
GU721109	Nbm	Melon"	China: Ningbo	100	100	100
GU721110	Nbc	Cucumber	China: Ningbo	100	100	100
GU721111	Btc	*Bemisia tabaci*	China: Shouguang	100	100	100
HG939521	CC1-10HSP	Cucumber	Greece: Tympaki, Crete	99.5	99.5	99.5
HM581659	Nbc	Cucumber	China: Ningbo	100	100	100
HQ148667	Shjd	Melon	China	100	100	100
JF502222	TW	Melon	Taiwan: Yunlin	100	100	100
JN126045	Erlun	Muskmelon	Taiwan: Yunlin, Erlun	100	100	100
JN126046	Yilan	Muskmelon	Taiwan: Yilan"	100	100	100
JQ904629	Beijing	Cucumis	China: Beijing	100	100	100
KC559449	Sem6	Cucumber	Iran: Semnan	94.3	94.3	94.3
KC559450	Sem26	Melon	Iran: Semnan	94.3	94.3	94.3
KC633821	Jb2	Cucumber	Lebanon	99.5	99.5	99.5
KC633822	CC2a	Cucumber	Lebanon	100	100	100
KC633823	Jb8	Cucumber	Lebanon	100	100	100
KC633824	M2	Cucumber	Lebanon	99.5	99.5	99.5
KC633825	JC7	Cucumber	Lebanon	100	100	100
KC633826	M1	Cucumber	Lebanon	100	100	100
KC633827	TS14	Cucumber	Lebanon	100	100	100
KC677623	Sud.C.35	Pumpkin	Sudan	100	100	100
KC677624	Su.C.37	Pumpkin	Sudan	100	100	100
KC990509	TS16	Cucumber	Lebanon	100	100	100
KC990510	JC5	Cucumber	Lebanon	100	100	100
KC990511	JC8	Cucumber	Lebanon	100	100	100
LM653107	CC-HSP-5	Cucumber	Saudi Arabia	95.5	95.5	95.5

The nucleotide sequence of the partial Hsp70h gene of these CCYV isolates detected in cucumber (EG-B1, EG-B2 and EG-B3) were determined with the same primers as those used for amplification using an ABI 310 DNA Analyzer (Perkin Elmer Applied Biosystems, HITACHI). Alignment analysis for calculation of percentage identity was carried out using the DNASTAR program. Analysis of the obtained sequences was carried out using the DNASTAR software trial. To achieve a valid comparison, 30 CCYV isolate sequences belonging to different countries obtained from GenBank database (Table 1) were reduced to the longitude of the isolated sequences in this study. Sequences used for comparisons were retrieved from GenBank (www.ncbi.nlm.nih.gov). The accession numbers of international CCYV isolates, along with the country of origin, are shown in Table 1. The partial of Hsp70h gene sequence of three Egyptian CCYV isolates; EG-B1, EG-B2 and EG-B3 have been deposited in the EMBL/GenBank/DDBJ databases.

RESULTS

Detection and identification of CCYV in infected field samples by DAS-ELISA: Symptoms on infected cucumber plants included yellowing and thickening of the basal and older leaves which occasionally also showed slight epinasty, or resulted in flower abortion and a reduction in the number of fruits per plant. Frequently, the chlorotic basal leaves of older plants developed large interveinal necrotic areas (Fig. 1). Forty eight samples of symptomatic plants taken from seven cucumber fields in El-Behira, government, Egypt, were analyzed. Twenty samples were found infected by CCYV only, two samples showed mixed infection with CYSDV and CCYV, four samples showed mixed infection with CABYV and CCYV whereas the rest of the samples were negative to all three viruses when DAS-ELISA against CYSDV, CABYV and CCYV using specific polyclonal antiserum.

RT-PCR: The obtained results reveled that no RT-PCR products (251 bp) were obtained with all tested samples when the specific primers for BPYV (BPYV-CP-5' and BPYV-CP-3') was used. Six out of twenty six samples that were positive using DAS-ELISA for CCYV infection were checked by RT-PCR to confirm CCYV identification. DNA amplification product of approximately 353 bp was observed in samples that were positive to CCYV in DAS-ELISA. No DNA product was amplified in healthy plant extracts (Fig. 2).

Dot blot hybridization: A cDNA probe for CCYV was synthesized using the Crini-s2 and Crini-as2 primer. Figure 3 illustrate dot blot hybridization of total RNA extraction from all naturally symptomatic and asymptomatic cucumber plant samples collected from El-behira government. Positive hybridization reaction indicated by formation of purple color on the nitrocellulose membrane was observed in RNA extracts of 26 out of 45 cucumber samples but no hybridization was observed between the cDNA probe and nucleic acid extraction from the rest collected samples (Fig. 3). These results were confirming the obtained results from DAS- ELISA and RT-PCR analysis.

Fig. 1(a-d): Symptoms in cucumber plants infected with *Cucurbit chlorotic yellows virus* (CCYV): (a) Interveinal chlorosis, (b) Yellowing and thickening of the basal leaves and (c) Reduction in growth in an open field and (d) No symptoms were observed on healthy plant

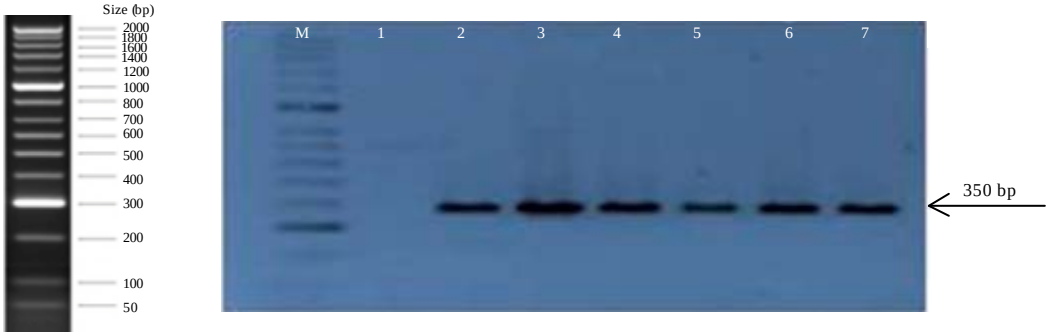

Fig. 2: Gel electrophoresis of RT-PCR amplification of a 353 bp fragment from the CCYV- heat shock protein 70 kDa homologous gene (HSP70h) gene using the Crini-s2 and Crini-as2 primer pair. Lane M: 50 bp DNA ladder (Bioline). Lane 1: Leaf samples from healthy cucumber as a negative control, Lanes 2, 3, 4, 5: Selected samples from infected cucumber plants collected from El-behira government

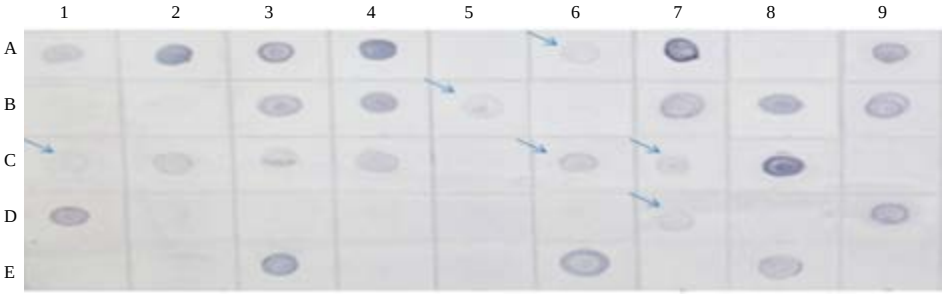

Fig. 3: Detection of isolates of CCYV by dot blot hybridization. The total nucleic acid was extracted from all collected cucumber samples and spotted on a nylon membrane and hybridized with a CCYV cDNA probe containing digoxigenin labeled preparations from plasmid clones representing both partial Hsp70h gene of the virus. Spot in row A: 6 and B: 5 shows positive reaction from mixed (CCYV+CYSDV) infections samples and spot in row C: 1, 6, 7 and D: 7 shows positive reaction from mixed (CCYV+CABYV) infections samples. Row A: 1, 2, 3, 4, 7, 9; Row B: 3, 4, 7, 8, 9; Row C: 2, 3, 4, 8; Row D: 1, 9, and Row E: 3, 6, 8. No hybridization reaction was observed with the rest of the samples (Row D: 3, 4, and 5)

Partial nucleotide sequence of CCYV HSP70h gene: The RT-PCR products obtained with the leaf samples were cloned and sequenced to identify the suspected CCYV isolates. The HSP70h which was amplified with the primers Crini-s2 and Crini-as2 primer (353 bp) were sequenced and submitted in GenBank under accession numbers (KP204871, KP204872 and KP204873). Phylogenetic analysis was carried out for the partial sequences of the HSP70h of the obtained sequences for Egyption isolates (EG-B1, EG-B2 and EG-B3) and representative sequences of CCYV isolates from Japan, China, Taiwan, Iran, Lebanon, Sudan and Saudi Arabia as shown in Table 1. The phylogenetic tree showed that all Egyptian CCYV isolates in this study are in one clusters with them. The analysis also showed that the highest nucleotide identities were 100%

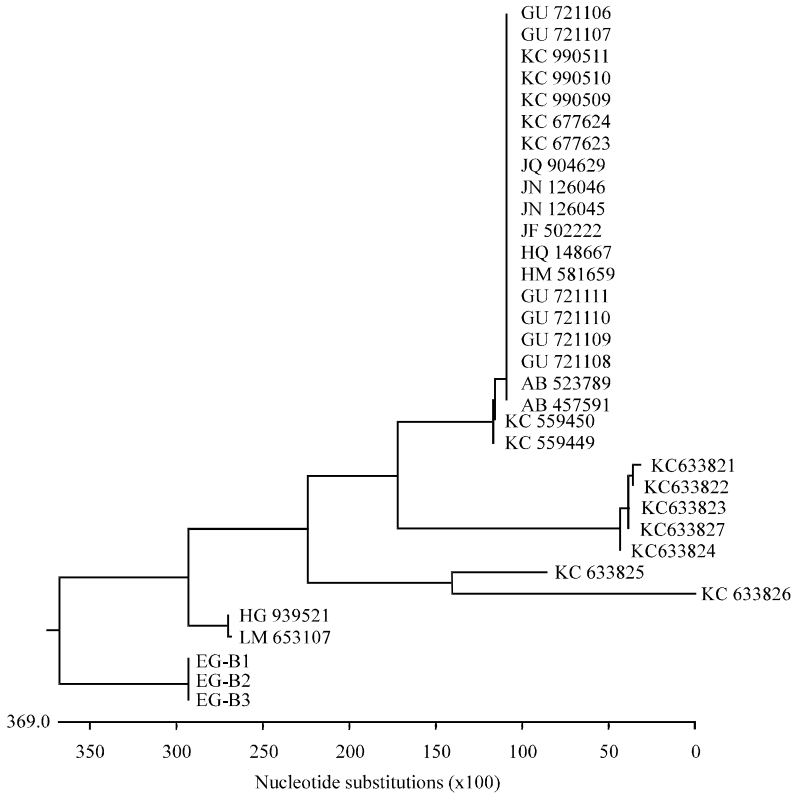

Fig. 4: Phylogenetic relationships tree among the multiple sequence alignments of Egyptian isolates of CCYV and 32 different CCYV isolates referenced in GenBank

obtained with two isolates (AB457591 and AB523789) of CCYV from Japan isolated from melon, seven isolates (GU721108, GU721109, GU721110, GU721111, HM581659, HQ148667 and JQ904629) isolated from melon, watermelon, cucumber and whitefly from China, three isolates (JF502222, JN126045 and JN126046) isolated from melon and muskmelon from Taiwan, eight isolates (KC633822, KC633823, KC633825, KC633826, KC633827, KC990509, KC990510 and KC990511) isolated from cucumber from Lebanon and two isolates isolated from pumpkin from Sudan (KC677623 and KC677624). While it was 95.5% with CCYV isolate from watermelon and melon (GU721106 and GU721107) from China and 4 isolates isolated from cucumber from Greece, Lebanon and Saudi Arabia (HG939521, KC633821, KC633824 and LM653107). The lowest similarity (94.3%) also was found with the Sem6 (KC559449) and Sem26 (KC559450) isolates from Iran isolated from cucumber and melon respectively when compared with the five isolates of CCYV from Egypt (Fig. 4 and Table 1).

DISCUSSION

Cucurbit viruses have always caused major losses in the quantity and quality of cucurbit crops in many countries of the world and they represent one of the most important limiting factors for growers (Provvidenti, 1996). In recent decades, approximately 39 different virus species have been reported to infect cucurbits naturally (Lecoq, 2003). Yellowing virus diseases (BPYV, CYSDV, CCYV and CABYV) are emerging as a major agricultural threat causing significant yield losses in cucumber and cucurbits. Several cucumber-growing regions of cucumber plants in open-field crops

in El-Behira, government, Egypt were collected with symptoms similar to those induced by CCYV (interveinal chlorosis of middle leaves and yellowing of older leaves). Similar symptoms were observed in 2012 in open field watermelon (*Citrullus lanatus*) plants in Rhodes and in November 2013 in a cucumber greenhouse in Tympaki, Crete (Orfanidou *et al*., 2014). The yellowing symptoms differed considerably from three endemic viral diseases of cucurbits, *Watermelon mosaic virus*, *Zucchini yellow mosaic virus* and *Cucumber mosaic virus* (Avgelis, 1983; Boubourakas *et al*., 2006). Symptoms of affected cucurbit plants appeared to be more similar to those caused by whitefly transmitted yellowing criniviruses, *Cucurbit yellow stunting disorder virus* and *Beet pseudo-yellows virus*, as well as *Cucurbit chlorotic yellows virus* (Okuda *et al*., 2010), or even similar to the aphid transmitted polerovirus, CABYV which also induces yellowing symptoms in cucurbits (Omar and Bagdady, 2012). Mixed infection was found in approximately 12.5% of infected cucumber samples, whereas out of 45 samples, two samples showed mixed infection with CYSDV+ CCYV and four samples showed mixed infection with CABYV+CCYV when DAS-ELISA was used. In Lebanon, some 13% of melon and 30% of squash plants showed mixed infections of ZYMV+CABYV (Abou-Jawdah *et al*., 2000). The highest incidence (15%) was found in cucurbits of Turkey infected with ZYMV+WMV (Sevik and Arli-Sokmen, 2003). Mixed infections are associated with enhanced symptom expression and synergistic effects are reported for viruses in the family Luteovirridae (Savenkov and Valkonen, 2001). The high incidence of CCYV found in this study, together with the less number of mixed infections, suggests that CCYV is an important threat for cucurbit crops in Egypt. CCYV has been reported only in Japan, China, Taiwan, Lebanon and Sudan (Gu *et al*., 2011; Zeng *et al*., 2011; Hamed *et al*., 2011; Huang *et al*., 2010; Abrahamian *et al*., 2013; Bananej *et al*., 2013; Orfanidou *et al*., 2014). Symptoms and the presence of CCYV were confirmed by RT-PCR (Hamed *et al*., 2011). The cDNAs for the Hsp70h gene of CCYV genome from three CCYV isolates chosen randomly from the previous survey were produced by RT-PCR. For all samples, the expected RT-PCR products were obtained and were directly sequenced with previous cloning. Thus, fragments of 353 pb, corresponding to the partial sequences of Hsp70h gene was sequenced. The alignment of partial Hsp70h gene nucleotide sequences of three CCYV Egyptian isolates showed 100% identity, when compared with other CCYV isolates from Japan, China, Taiwan, Lebanon and Sudan while the lowest similarity (94.3%) was found with the Sem6 and Sem26 isolates from Iran isolated from cucumber and melon, respectively when compared with the five isolates of CCYV from Egypt. These isolates did not only show high similarity among themselves but they also showed significant similarity with isolates of CCYV in some neigh-boring countries, as well as in distantly located Asia and European countries too, suggesting probable transmission of this virus between these countries through transmission methods such as seeds and whiteflies.

A non-radioactive dot-blot hybridization technique have been used to detect several plant viruses and have been shown to be more sensitive and more specific than serology (Eiras *et al*., 2001; Sanchez-Navarro *et al*., 1996, 1998; James *et al*., 1999; Kataya *et al*., 2008). In dot-blot hybridization experiments, the virus could be detected using a DIG-labelled probe. Non-isotopic dot-blot hybridization becomes more accessible to non-specialized or less well equipped laboratories, since leaf samples can be homogenized even in the field and sap extracts applied immediately to nylon membranes. The necessity to fix the RNA onto the membrane using a UV light source is a disadvantage but the technique has several advantages: speed, the probe can be re-used up to three times and hundreds of samples can be processed simultaneously and samples can be stored until required. This becomes essential when early detection is required, particularly with the known phloem tropism and low virus concentration of criniviruses in plants (Kataya *et al*., 2008).

Phylogenetic tree constructed by DNASTAR (Fig. 4) divided CCYV isolates into five clusters: Group I include isolates from Japan, Sudan, China and two isolates isolated from muskmelon from Taiwan. Group II included the two isolates isolated from cucumber from Iran. Group III included the five isolates isolated from cucumber from Lebanon. Group VI included the two isolates isolated from cucumber from Lebanon. Group VII included the two isolates isolated from cucumber from Greece and Saudi Arabia, whereas the Group VIII include the three Egyptian isolates of CCYV from Egypt. The data presented here show that it was one of the most prevalent and wide-spread viruses in open field crops of the El-behira region. In agreement with the present findings, field-grown cucurbit crops (cucumber, melon, squash and watermelon) from Iran, China, Greece, Lebanon, also showed high CCYV incidence (Gu *et al*., 2011; Zeng *et al*., 2011; Abrahamian *et al*., 2012; Bananej *et al*., 2013; Orfanidou *et al*., 2014). In conclusion, symptoms, RT-PCR and sequencing results confirm the presence and establishment of CCYV in cucumber crops in Egypt. It is remarkable that the sequences obtained from the Egyptian samples show only negligible sequence differences from some Iran isolates. Because of the large whitefly vector populations, the spread of CCYV to neighboring countries in Africa and potentially southern Europe, or wherever cucurbits are grown, can be expected. To our knowledge, this is the first report of CCYV in Egypt.

ACKNOWLEDGMENTS

The author gratefully acknowledges the financial support by the Agricultural Research Center, Deanship of Scientific Research of, King Saud University, Riyadh, King Saud University. A special thank also to Dr. M.A. Al-Saleh at Plant Protection Department, College of Food and Agricultural Sciences, King Saud University, for useful advice, suggestion and provision of the necessary facilities valuable for this research.

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