Iranian J Biotech. 2017 October;15(4):e1541 **DOI:10.15171/ijb.1541**

Brief Report

Presence of Recombinant Strain of *Cucurbit Aphid Borne Yellows Virus* in Iran

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Received: 14 April 2016; **Revised**: 14 Oct. 2016; **Accepted**: 20 June 2017 ; **Published online:** 29 December 2017

Background: *Cucurbit aphid-borne yellows virus* (CABYV) is among the most important yellowing viruses on cucurbits in Iran. The presence of CABYV has been previously reported from the major cucurbit growing areas in Iran, however, there are few studies concerning the detection of the different strains of this virus in the grower's fields, and especially, there is no report of the weed host plants near crop fields.
Objectives: This study was done in order to detect the new strains of the CABYV polerovirus in cucurbits and the weed plants in the Lorestan province, Iran, as an introductory investigation for initiating a program of the breeding for resistance.
Material and Methods: During a survey carried out in 2013-2014 in Lorestan province; Iran, 189 cucurbit and 261 weed samples were investigated for the presence of CABYV using RT-PCR. In addition, the phylogeny and nucleotide similarities were discussed on the basis of the partial nucleotide sequence of RNA dependent RNA polymerase (RdRP) gene.
Results: The RT-PCR carried out on leaf samples revealed the infection with the CABYV in 43 cucumber and 12 weed samples. RT-PCR using strain specific primers detected the presence of the both common (C) and recombinant (R) strains of CABYV in the tested samples. On the basis of the phylogenetic analyses, the CABYV-C isolates from Iran were clustered into two distinct sub-populations (CI and CII), such that all the weed samples with two sequenced cucumber isolates were clustered in the CI sub-population. Meanwhile, a distinct sub-population of the isolates was clustered in the CABYV-R group showed a shared sequence identity of 97% to a Taiwanese isolate (JQ700306).
Conclusions: This study has indicated the incidence of CABYV-R in the Southwest Asia; Iran for the first time. We were also able to show CABYV occurrence in *Sysimbrium irio* and *Citrullus colocynthis* from this area of the world. Identification of cucurbit infecting viruses and studying their distribution and potential reservoir hosts are important for developing successful control programs for virus disease management.

Keywords: CABYV-R; *Cucurbit aphid-borne yellows virus*; Iran; Weeds.

1. Background

In Iran, cucurbit cultivation was found to be severely affected by the various viruses that are transmitted by aphids in a non-persistent manner, among which *Cucumber mosaic virus* (CMV, genus *Cucumovirus,* family *Bromoviridae*), *Watermelon mosaic virus* (WMV, genus *Potyvirus*, family *Potyviridae*) and *Zucchini yellow mosaic virus* (ZYMV, genus *Potyvirus,* family *Potyviridae*) are the most frequent viruses in the regions. Furthermore, *Cucurbit aphid borne yellows virus* (CABYV, genus *Polerovirus*, family *Luteoviridae*) and *Cucumber vein yellow virus* (CVYV, genus *Ipomovirus*, family *Potyviridae*) have been detected in cucurbit plantations in Iran, as well (1-5).

CABYV is among the most important yellowing viruses, which over the past two decades has been reported from various cucurbit crops in temperate, Mediterranean, and subtropical climatic regions of the world (6). The main symptoms caused by CABYV include yellowing and thickening, which are more obvious in older leaves (7). Up to 100% of plants show yellowing symptoms by the end of the growing season (8, 9). CABYV induces the high percentage flower abortion in cucumber and melon, which reduces the number of fruits per plant and yield (51 and 40 %, respectively), however, CABYV infection does not affect the fruit

shape or quality (7). CABYV is transmitted persistently by specific aphid vectors (10). *Myzus persicae* and *Aphis gossypii* are the most prevalent aphid vectors of CABYV. As well, the mechanical transmission has never been reported for this viral infection (7). CABYV infects more crop plants other than cucurbits, like *Lactuca sativa* and *Beta vulgaris*, as well as common weeds of the species *Capsella bursa-pastoris*, *Crambre abyssinica*, *Bryonia dioica*, *Ecballium elaterium*, *Lamium amplexicaule*, *Montia perfoliata*, *Papaver rhoeas*, and *Senecio vulgaris* which seems to serve as the virus reservoirs (7, 11, 12).

The genome of CABYV consists of a single stranded positive-sense RNA molecule containing at least six open reading frames (ORFs). Open reading frames 0, 1, and 2 are expressed from the genomic RNA, whereas the other three (ORFs 3, 4, and 5) are expressed from a subgenomic RNA (sgRNA1). The two additionally predicted ORFs (ORFs 6 and 7) might be expressed from a second sgRNA (sgRNA2). The protein product of ORF 0 (P0) is an active silencing suppressor and an enhancer of pathogenicity. The proteins, putatively encoded by the ORF 1 (P1) and ORF 2 (P2 domain in P1-2), have regions of the amino acid sequence similarity with the serine proteases and the genome-linked viral proteins (VPgs) of the other poleroviruses, as well as amino acid motifs typical for the RNA-dependent RNA polymerases (RdRPs), respectively. The P1-2 fusion protein is generated as a result of a ribosomal frameshift in the C-terminal portion of ORF1 that occurs during translation of ORF1. CABYV coat protein (P3 or CP) is encoded by the ORF 3, which takes part in virus transmission, virion assembly, and virus multiplication titer in the plant. ORFs 4 and 5 encode proteins P4 (a movement protein-MP), and P3-5 (a read-through protein needed for the aphid transmission of the virus) (13). The CABYV population has been previously classified into two strains named as common (CABYV-C) and recombinant (CABYV-R) by using a set of universal polerovirus primers (14). The recombinant CABYV-R strain most likely has emerged through recombination in the intergenic rejoin (IR), a recombination hot-spot of the poleroviruses, between ancestors of *Melon aphid-borne yellows virus* (MABYV) and CABYV-C strain (11, 14).

2. Objectives

Studying the distribution and sequence data of the viruses and information on their potential reservoir hosts in the main cucurbit production regions of Iran is critical in the planning and employing the successful strategies for the viral disease management. CABYV has been previously reported from melon, cucumber, squash, and watermelon in the main cucurbit cultivation areas in Iran (1, 15). Despite the importance and a wide spread of the CABYV in Iran, few studies were done for identifying the different strains of this virus in the fields, and especially, there is no report of this virus in the weed plants near the crop fields. Identification of the native hosts may provide clues with respect to the vectors of the virus, and studies of the isolates from weeds may indicate the relationships between the existing virus isolates from weeds and those from the other agronomic plants such as cucurbits. The study described here was performed in order to extend our knowledge about the presence and distribution of the cucurbit-infecting CABYV-C and CABYV-R strains in Iran as an introduction for initiating a program of breeding for attaining viral resistance. The conventional virus resistance to the CABYV has been described in cucurbit genetic resources (16). However, resistance specificity should be determined at the first steps of resistance characterization, when encountering the resistant cucurbit plants to a collection of CABYV isolates representative of its variability.

3. Materials and Methods

3.1. Sample Collection

To determine the frequency and the potential weed reservoir hosts for CABYV, a survey within and adjacent cucumber fields was performed during 2013-2014 in five counties of Lorestan province in the west of Iran. Only symptomatic samples were randomly collected from each county. In total, 175 cucumber (*Cucumis sativus* L.), 14 snake melon (*Cucumis melo* var. *flexuosus*), and 261 weed plants belonging to fifteen families including *Amaranthaceae* (n=16), *Asteraceae* (n=41), *Brassicaceae* (n=31), *Caryophyllaceae* (n=13), *Chenopodiaceae* (n=8), *Convolvulacae* (n=11), *Cucurbitaceae* (n=14), *Euphorbiaceae* (n=8), *Fabaceae* (n=25), *Labiatea* (n=3), *Malvaceae* (n=21), *Papaveraceae* (n=17), *Plantaginaceae* (n=8), *Solanaceae* (n=26), and *Ranunculaceae* (n=19) were collected. Leaf samples from symptomatic plants showing chlorosis, yellowing, and thickening of the older lower leaves, were collected, labeled and transported to the laboratory on ice and kept at 4 °C.

3.2. RNA Isolation and RT-PCR

The collected samples were evaluated for CABYV infection by using RT-PCR. Total RNA was extracted from leaves using Tri-Reagent (Sigma, St. Louis, MO,

USA) and RNX plus™ (CinaGen Inc, Tehran, Iran) according to the manufacturers' instructions. RNA was finally precipitated by isopropanol, washed with 75% ethanol, resuspended in diethyl pyrocarbonate (DEPC)-treated water, and stored at -70 °C until use. Symptomatic leaf samples were tested for the presence of CABYV, using CAB.cp.f and CAB.cp.r primers designed for the conserved nucleotide region of the coat protein (CP) gene (supplementary Table 1) (15). Furthermore, the RT-PCR protocols based on the previously described specific primers sets including, CA-C-2891-F and CA-3372-R with an expected amplified product length of 532-bp, and CA-R-3050-F and CA-3372-R which specifically amplify a 372-bp product were used to distinguish between the common and recombinant strains, respectively (supplementary Table 1) (14).

RT-PCR was carried out by synthesizing the first strand of cDNA using 5 µL of template RNA (1.5 µg), 1µL of the reverse primer (20 pMol.mL^{-1}), one µL of the dNTPmix (10 mM each) (CinnaGen, Iran), and 1 µL of RevertAid™ M-MuLV reverse transcriptase (200 unit.mL^{-1}) (Fermentas, Lithuania) in a final reaction volume of 20 µL according to the manufacturer's instructions. The reaction was incubated for 60 min at 42°C and then terminated by heating the mixture for 10 min at 72°C. PCR amplification was conducted in a final volume of 50 µL consisting 5 µL of 10X PCR buffer, 0.2 mM of each dNTP, two units of *Pfu* DNA polymerase (CinnaGen, Iran), 5 µL of cDNA (as a template), and specific primers for common and recombinant CABYV strains (20 µM each primer). The PCR program consisted of the initial denaturation step for 2 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at the 55 °C for 1 min, and extension at 72 °C for 1 min. The final extension step was performed at 72 °C for 10 min.

3.3. Phylogenetic Analysis

The amplified DNA (corresponding to the CABYV RdRP gene) was isolated from agarose gel slices using the Wizard SV Gel and PCR Clean-Up system (Promega, USA) following the manufacturer's recommendations. DNA amplicons were sequenced in both directions with dideoxy Sanger method (PGGene; www.pggene.ir).

The nucleotide sequences of the seven cucumbers and eleven weeds Iranian CABYV isolates were identified in this study and forty-one CABYV data available from the GenBank (National Center of Biotechnology Information-NCBI) were aligned using CLUSTAL X 2 program (supplementary Table 2) (6, 17) and the phylogenetic tree was constructed using the Neighbor-Joining (NJ) method implemented in MEGA 5 (18). The sequence of the Suakwa aphid-borne yellows virus (SABYV) isolate (ac. no. GU324111) was used as an out-group as it was previously applied for CABYV phylogenetic analysis (19). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the relationships of the sequences.

4. Results

A DNA fragment of about 479 bp was amplified using CP specific primers in the forty-three cucumber samples (24.5%), three snake melon samples (21.4%), and twelve weed samples (4.6 %) belonging to the seven genera. The highest virus infection among the weeds was found in *Bryonia dioca* (n= 4 infected samples), followed by *Sysimbrium irio* (n=3 infected samples), and the 2 infected samples of *Capsella bursa-pastoris*. In addition, for some weeds including *Eruca sativa*, *Citrullus colocynthis*, and Papaver rhoeas just one sample was found to be infected with CABYV (Table 1).

Using RT-PCR through the application of CA-R-3050-F and CA-3372-R primers specific for CABYV-R, a DNA amplicon of about 372 bp was amplified in seven yellowing cucumber samples (16.2%) out of the forty-three positive CABYV samples, which indicated the presence of recombinant CABYV strain in Lorestan province plantation area.

However, the amplification of a DNA band about 532 bp in 12 weed in addition to other cucumber samples indicated the prevalence of CABYV-C (i.e., the common strain) in the weeds. No DNA fragment was amplified in the weed samples relating to the recombinant strain of CABYV. CABYV isolates were further divided into two groups, representing the CABYV-C and CABYV-R. The same topology has been obtained using Minimum Evolution (ME) and Maximum Likelihood (ML) trees, (data not shown). Interestingly, a distinct sub-population of the Iranian cucumber isolates was clustered for the recombinant strain of CABYV. These isolates of the cucumber from Lorestan plantation area in Iran shared a sequence identity of 97% to a Taiwanese isolate (ac. no. JQ700306). The CABYV isolates from different weeds were clustered with those previously reported for Iranian isolates (15) in sub-population C-I. Although, three CABYV isolates from North-East Iran (Khorasan-e-Razvi province) (KF425566, KF425568, and KF425569) fell into sub-population C-II (Fig. 1).

Table 1. Detection of the *Cucurbit aphid borne yellows virus* in weeds collected within or adjacent to the cucurbit fields during 2013-2014

Family	Species	Common name	No. Sample collected	No. Positive samples with CABYV
Amaranthaceae	*Amaranthus retroflexus*	Slim amaranth	10	-
	Atriplex hortensis	Orach	6	-
Asteraceae	*Sonchus oleraceous*	Prickly sow-thistle	5	-
	Carthamus tinctorius	Safflower	3	-
	Achillea sp.		1	-
	Senecio vulgaris	Common groundsel	14	-
	Matricaria discoidea	Mayweed	3	-
	Silybum sp.	milk thistle	7	-
	Centurea sp.	Knapweed	11	-
Bracicaceae	*Cardaria deraba*	Hoary cress	4	-
	Capsella bursa-pastoris	Shepherd's-purse	9	2
	Sysimbrium irio	London rocket	10	3
	Carthamus oxyacantha	Safflower	5	-
	Eruca sativa	Rocket	3	1
Caryophyllaceae	*Stellaria media*	Chickweed	13	-
Chenopodiaceae	*Chenopodium sp*	Lamb squarters	2	-
	Chenopodium murale	Nettle-leaved goosefoot	6	-
Convolvulaceae	*Convolvolus arvensis*	Field bindweed	11	-
Cucurbitaceae	*Bryonia dioca*	red bryony	9	4
	Citrullus colocynthis	Colocynth	5	1
Euphorbiaceae	*Euphorbia sp.*	Milkwort	8	-
Fabaceae	*Melilotus officinalis*	Yellow sweet clover	7	-
	Lathyrus sp.	Pea vine	4	-
	Trifolium pretense	Red clover	7	-
	Trifolium sp.	Huban clover	3	-
	Trifolium repens	White clover	4	-
Labiatea	*Lamium amplexicaule*	Henbit	3	-
Malvaceae	*Hibiscus esculentus*	Gombo	2	-
	Malva sp.	Common malva	19	-
Papaveraceae	*Papaver rhoeas*	Common poppy	17	1
Plantaginaceae	*Plantago psyllium*	Musa, Flax-seed	4	-
	Veronica anagallis	Speedwell	4	-
Solanaceae	*Solanum nigrum*	Black nightshade	15	-
	Datura stramonium	Jimson weed	8	-
	Hyoscyamus niger	Black hinbane	3	-
Ranunculaceae	*Ranunculus sardous*	Crowfoot	6	-
	Adonis aestivalis L.	Pheasant's-eye flower	13	-
Total number of weed samples			261	12

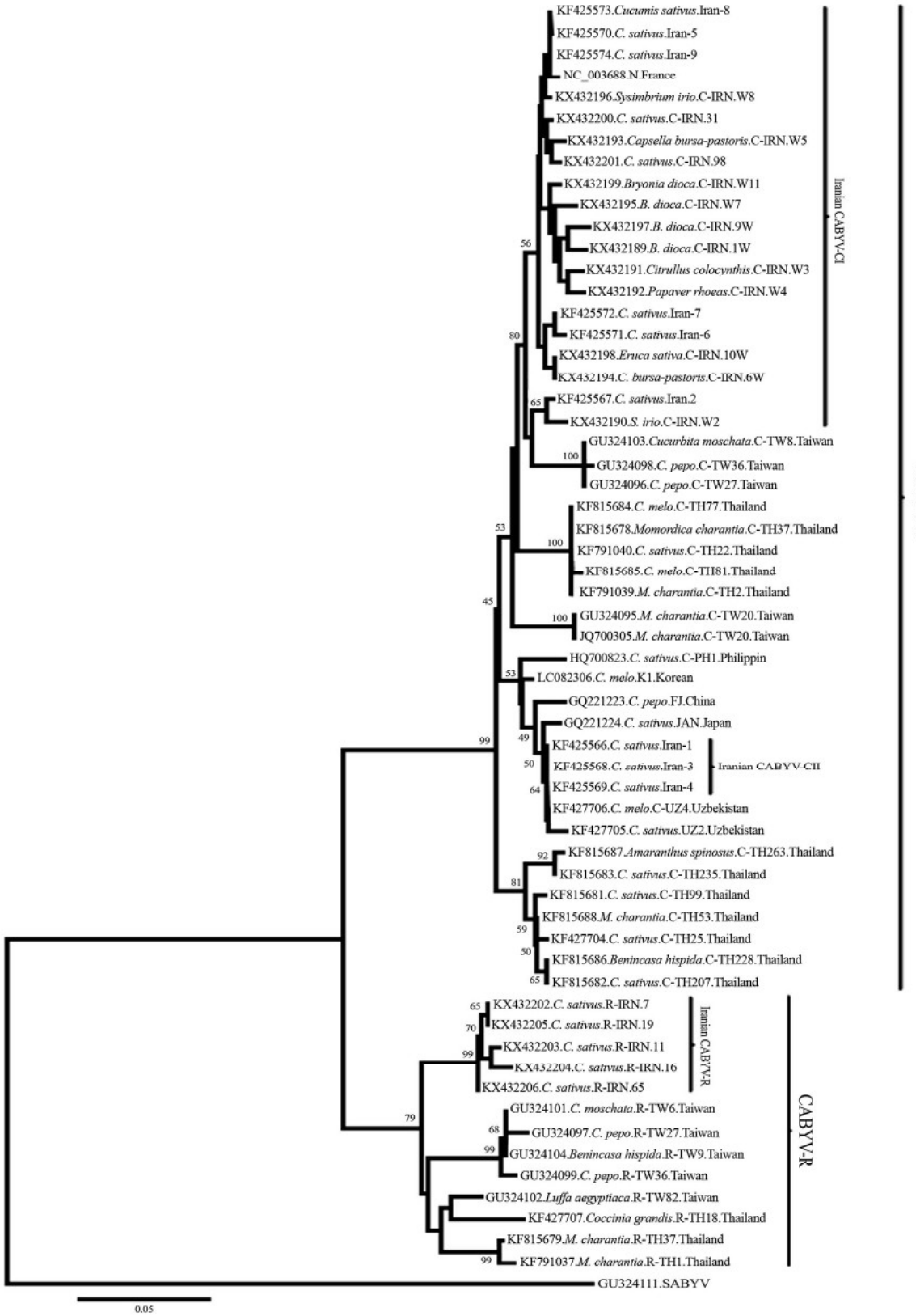

Figure 1. Neighbor-Joining phylogenetic tree showing the relationship between Iranian *Cucurbit aphid-borne yellows virus* isolates using the partial sequences of RNA dependent RNA polymerase-RdRp. Numbers at each node indicate the percentage of supporting puzzling steps (or bootstrap samples) in NJ method. Iranian CABYV isolates from weed are shown in boxes. The sequence of the Suakwa aphid-borne yellows virus (ac. no. GU324111) is used as out-group.

5. Discussion

CABYV is one of the most important yellowing viral diseases of the cucurbit crops (11, 20) as it can cause yield losses up to 40-50% in the cucumber and melon, if the infection occurs at an early stage (7). In Iran, also CABYV is one of the most prevalent viruses in the major cucurbit crops and has been detected in the main cucurbit producing provinces; both in the greenhouses as well as open field plantation areas (15). Our results showed for the first time the high incidence of CABYV in different regions of Lorestan province (West of Iran). Also, this is the first report regarding CABYV infection in the snake melon (*Cucumis melo* var. *flexuosus*) in Iran. In addition, our study reveals the important role that weed species may play as an alternative host and the potential virus reservoir of the CABYV in the areas that were subjected to the investigation in the present study (supplementary Table 2). Our results also showed the infection of S. *irio* and *C. colocynthis* as the reservoir hosts for CABYV in planting area of this country, as well. Although Kassem *et al*. (11) have suggested the CABYV infection for *Senecio vulgaris* and *Lamium amplexicaule*, no infection was found among the collected samples from these species in this investigation.

Our data suggest that weeds could be a critical factor in the maintenance and dissemination of the CABYV infections in cucurbit crops. They probably serve as primary source of the CABYV and their presence would greatly increase the chance that a vector will feed on the infected weeds which could result in an increased CABYV-viruliferous aphids. Therefore, a rigorous broadleaf weed control is the primary component of the integrated open fields and greenhouses management programs. The control of polerovirus diseases through the application of insecticides against aphid vectors is both costly and unreliable, and potentially dangerous to the environment. Resistant cucurbit cultivars provide a safer and more sustainable alternative for viral disease management (16).

In Iran, the latest official reports show that approximately 7,000 hectares are dedicated to cucurbit crops (including cucumber, melon, squash, and watermelon) in Lorestan province, West Iran. Among these, cucumber is a very important cash crop (grown on approximately 6,000 hectares) (21). Recently, three cucurbit viruses, CMV, WMV, and ZYMV have been detected with the prevalence of 14.7, 24.2, and 10 %, respectively, in Lorestan province. Furthermore, viral infection was found among different weed samples including *Lathyrus sp.*, *Trifolium repens*, *Sonchus oleraceous*, *Chenopodium sp* and *Solanum nigrum* with the CMV, *Sonchus oleraceous* and *Malva sp.* with the WMV virus, and *B. dioca* with the ZYMV viral infection, respectively (5).

Based on partial RdRP gene sequence analysis, the phylogenetic assessment of the CABYV-C isolates from Iranian cucumber plantation area suggested the occurrence of at least two CABYV-C sub-populations in this region. It is interesting from the phylogenetic standpoint that previous study on Iranian CABYV-C isolates using partial coat protein (CP) gene sequences showed that the sequences of CP gene differed slightly (1). From the seven Iranian cucumber isolates of the CABYV obtained in this study, five were grouped in CABYV-R cluster and two placed in the CABYV-C group, but none of the Iranian weed isolates of CABYV were grouped in CABYV-R cluster. Other studies on non-Iranian CABYV isolates have shown that the structure of CABYV population does not seem to be correlated with the date and locality of the sample collection or crop species. As well, ecological elements including aphid vector efficiency in transmission of virus and adaptation to the alternative hosts could be considered in the genetic structure of CABYV (11). Our studies were done on a limited number of Iranian CABYV isolates. Further statistical analysis using longer parts of the genome is required for a better understanding of the CABYV population structure in Iran. CABYV was first identified and described in 1992 in France (7) and reported from cucurbit plantings of the Iran recently (15). Thus, the presence of the two different strains of the CABYV and its widespread distribution in Iran does not agree well with a recent introduction in the region, rather, it matches with an older presence of the CABYV or with multiple introduction events. Additional research, increasing the spatial and temporal ranges of sampling are needed to resolve this issue.

The recombinant CABYV strain was reported from a number of the Southeast Asian countries including China (22), Philippine (23), Taiwan (14), and Thailand (2). This analysis revealed the high diversity of CABYV isolates in Iran and to the best of our knowledge, this is the first report on the detection of the recombinant strain of the CABYV beyond Southeast Asia, in Iran; mid-Eurasia region. However, more research on the genome diversification, biological properties and interactions between the different species and strains are required, especially for breeding cucurbits harboring multiple resistance to the various polerovirus species (14). In addition, identification of the other cause or causes of the yellowing symptoms in samples that cannot be ascribed to the polerovirus infection is also required.

6. Conclusions

This investigation indicated for the first time, the incidence of the recombinant strain of CABYV in Southwest Asia; Iran. Also, the infection of S. *irio* and *C. colocynthis* as the hosts of the CABYV is reported for the first time. The definite and detailed status of the phylogenetic relationships and comparisons among distantly related virus genomes provide an important insight into the basic evolutionary mechanisms, and studies on these variations are required for achieving progress in the management programs of the viral diseases prevention and their expansion.

Acknowledgements

This work was supported by the Khorramabad Branch of the Islamic Azad University [Grant number 51485921021002].

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Supplementary Table 1. Primer sets used for RT-PCR detection of CABYV.

Name	Sequence (5' to 3')	Tm (°C)	Target region [a]	Ampl. size	Strain [b]	Ref
CAB.cp.f	CGCGTGGTTGTGGTCAACCC	57.9	CP	479 bp	C+R	15
CAB.cp.r	CCYGCAACCGAGGAAGATCC	57.9				
CA.C.2891.F	GAYGGAACATTATTAGCGCAGAGA	54.0	RdRP	532 bp	C	14
CA.3372.R	AATCTATTGTKTGGACTCTTDGTAACGA	54.1				
CA.R.3050.F	ACCTAGCGAAATACGCTGAGCTA	55.3	RdRP	372 bp	R	14
CA.3372.R	AATCTATTGTKTGGACTCTTDGTAACGA	54.1				

a. CP: coat preotein gene, RdRP: RNA dependent RNA polymerase gene;

b. C: common strain, R: recombinant strain

Supplementary Table 2. The isolates of the Cucurbit aphid borne yellows virus obtained from the local plantation area in the present study and those other isolates reported from elsewhere in the world that were used for analysis.

Isolates	Original host	Country	Accession no.
Iran-1	*Cucumis sativus*	Iran	KF425566
Iran-2	*C. sativus*	Iran	KF425567
Iran-3	*C. sativus*	Iran	KF425568
Iran-4	*C. sativus*	Iran	KF425569
Iran-5	*C. sativus*	Iran	KF425570
Iran-6	*C. sativus*	Iran	KF425571
Iran-7	*C. sativus*	Iran	KF425572
Iran-8	*C. sativus*	Iran	KF425573
Iran-9	*C. sativus*	Iran	KF425574
CABYV-C-IRN.W1	*Bryonia dioca*	Iran	KX432189
CABYV-C-IRN.W2	*Sysimbrium irio*	Iran	KX432190
CABYV-C-IRN.W3	*Citrullus colocynthis*	Iran	KX432191
CABYV-C-IRN.W4	*Papaver rhoeas*	Iran	KX432192
CABYV-C-IRN.W5	*Capsella bursa-pastoris*	Iran	KX432193
CABYV-C-IRN.W6	*C. bursa-pastoris*	Iran	KX432194
CABYV-C-IRN.W7	*B. dioca*	Iran	KX432195
CABYV-C-IRN.W8	*S. irio*	Iran	KX432196
CABYV-C-IRN.W9	*B. dioca*	Iran	KX432197
CABYV-C-IRN.W10	*Eruca sativa*	Iran	KX432198
CABYV-C-IRN.W11	*B. dioca*	Iran	KX432199
CABYV-C-IRN.31	*C. sativus*	Iran	KX432200
CABYV-C-IRN.98	*C. sativus*	Iran	KX432201
CABYV-R-IRN.7	*C. sativus*	Iran	KX432202
CABYV-R-IRN.11	*C. sativus*	Iran	KX432203
CABYV-R-IRN.16	*C. sativus*	Iran	KX432204
CABYV-R-IRN.19	*C. sativus*	Iran	KX432205
CABYV-R-IRN.65	*C. sativus*	Iran	KX432206
N	-	France	NC_003688
CABYV-FJ	*Cucurbita pepo*	China	GQ221223
CABYV-JAN	*C. sativus*	Japan	GQ221224
CABYV-C-TW20	*Momordica charantia*	Taiwan	JQ700305
CABYV-C-TW20	*M. charantia*	Taiwan	GU324095
CABYV-C-TW27	*C. pepo*	Taiwan	GU324096
CABYV-R-TW27	*C. pepo*	Taiwan	GU324097
CABYV-C-TW36	*C. pepo*	Taiwan	GU324098
CABYV-R-TW36	*C. pepo*	Taiwan	GU324099
CABYV-R-TW6	*C. moschata*	Taiwan	GU324101
CABYV-R-TW82	*Luffa aegyptiaca*	Taiwan	GU324102
CABYV-C-TW8	*Cucurbita moschata*	Taiwan	GU324103
CABYV-R-TW9	*Benincasa hispida*	Taiwan	GU324104
K1	*C. melo*	Korean	LC082306
R-TH18	*Coccinia grandis*	Thailand	KF427707
C-UZ4	*C. melo*	Uzbekistan	KF427706
UZ2	*C. sativus*	Uzbekistan	KF427705
C-TH25	*C. sativus*	Thailand	KF427704
CABYV-C-TH53	*M. charantia*	Thailand	KF815688
CABYV-C-TH263	*Amaranthus spinosus*	Thailand	KF815687
CABYV-C-TH228	*Benincasa hispida*	Thailand	KF815686
CABYV-C-TH81	*C. melo*	Thailand	KF815685
CABYV-C-TH77	*C. melo*	Thailand	KF815684

Table 2. continued.

CABYV-C-TH235	*C. sativus*	Thailand	KF815683
CABYV-C-TH207	*C. sativus*	Thailand	KF815682
CABYV-C-TH99	*C. sativus*	Thailand	KF815681
CABYV-R-TH37	*M. charantia*	Thailand	KF815679
CABYV-C-TH37	*M. charantia*	Thailand	KF815678
CABYV-C-TH22	*C. sativus*	Thailand	KF791040
CABYV-C-TH2	*M. charantia*	Thailand	KF791039
CABYV-R-TH1	*M. charantia*	Thailand	KF791037
CABYV-C-PH1	*C. sativus*	Philippine	HQ700823