Phytoparasitica (2012) 40:177–184
DOI 10.1007/s12600-011-0212-2

Cucurbit aphid-borne yellows virus in Egypt

Ayman F. Omar • Naser A. Bagdady

Received: 6 June 2011 / Accepted: 13 December 2011

Abstract During 2010, yellowing symptoms were fre- quently observed in cultivated squash fields in Egypt. A total of 717 symptomatic squash leaf samples were collected from four regions where squash cultivation is of economic importance for the country: Kafrelsheikh, El-Behira, El-Sharkia and El-Ismailia. Serological anal- ysis showed that 95.6% of the symptomatic squash samples were infected by *Cucurbit aphid-borne yellows virus* (CABYV), and visual estimation of the incidence of yellowing symptoms suggested a very high incidence of CABYV in the fields. Twelve CABYV isolates were characterized by sequencing two regions of the viral genome, open reading frame (ORF) 3 and ORFs 4/5. Overall, Egyptian isolates were very similar among them, and had higher similarity values with a French than with a Chinese isolate. The average nucleotide diversity for ORF 3 was significantly higher than for the other two regions, indicating that variability is not evenly distributed along the viral genome. The ratios between nucleotide diversity values in non-synonymous (d_N) and synonymous (d_S) positions (d_N/d_S) for each ORF showed that the three ORFs are evolving under different pressures, although predominantly under purifying selection. Phylogenetic analyses revealed that these Egyptian isolates, with only one exception, shared the same clade with a French isolate. Moreover, these analyses suggested that Egyptian isolates belong to the Mediterranean group described previously.

Keywords CABYV · Cucurbit diseases · *Luteoviridae* · *Polerovirus*

Introduction

Cucurbits include economically important vegetable crops in many countries of the world. In recent decades, approximately 39 different virus species have been reported to infect cucurbits naturally (Lecoq 2003). One of these is *Cucurbit aphid-borne yellows virus* (CABYV), which is a member of the genus *Polerovirus*, family *Luteoviridae* (Mayo and D'Arcy 1999). Its viral particles are isometric, roughly 25 nm in diameter, and encapsidate the CABYV genome, which consists of a single-stranded positive sense RNA molecule of 5.7 Kb containing six major open reading frames (ORFs) which are separated into two regions by an internal non-coding sequence stretch. The 5′ proximal ORFs are expressed from the genomic RNA and yield P1, P2 and the translational frameshift protein P2-3. The 3′ proximal ORFs (ORFs 4, 5 and 6) are translated from a subgenomic RNA and yield

A. F. Omar (✉)
Department of Plant Pathology, Faculty of Agriculture,
Kafrelsheikh University,
33516 Kafrelsheikh, Egypt
e-mail: omar12744@gmail.com

N. A. Bagdady
Department of Genetics, Faculty of Agriculture,
Kafrelsheikh University,
33516 Kafrelsheikh, Egypt

Published online: 04 January 2012

proteins P4 (the coat protein, CP), which is involved in viral transmission, particle packaging and viral accumulation within the plant; P5 (the movement protein, MP); and P6. which is expressed only as a read-through protein (P4-P6), and is needed for aphid transmission of the virus (Ziegler-Graff *et al*. 1996).

CABYV is transmitted in a persistent manner by aphids of the species *Aphis gossypii* and *Myzus persicae* (Lecoq 1999; Lecoq *et al*. 1992), while mechanical transmission has never been reported. It has been detected in many countries, including France, Italy, Lebanon, Spain, Iran, Turkey, Tunisia, Slovakia, USA and China (Bananej *et al*. 2009; Tomassoli and Meneghini 2007; Xiang *et al*. 2008; Yardımcı and Özgönen 2007).

CABYV disease symptoms were first described by Lecoq *et al*. (1992) as chlorotic spots on lower leaves with interveinal chlorotic areas, with leaves becoming sometimes thickened and brittle. These symptoms were often attributed to nutritional disorders (mainly to Mg^{2+} and Mn^{2+} deficiencies), or even to pesticide phytotoxicity. Up to 100% of plants may show yellowing symptoms by the end of the growing season (Lemaire *et al*. 1993).

In Egypt, squash is one of the most important commercial cucurbit crops (FAO 2010). During 2010, yellowing symptoms were frequently observed in cultivated squash fields. In the present study, a large-scale survey was performed in open field squash crops to test whether CABYV was the causal agent of the yellowing disease of squash. We also conducted a preliminary analysis to genetically characterize Egyptian CABYV isolates.

Materials and methods

Field surveys Forty squash (*Cucurbita pepo*) open fields were surveyed in different areas in Egypt, including Kaferlsheikh, El-Behira, El-Sharkia and El-Ismailia. In each field, incidence of yellowing symptoms was estimated visually by examining approximately 200 to 300 plants during the 2010 growing season. Sampling periods varied between regions: in Kaferlsheikh between March and May, in El-Behira between May and August, and in the last two regions between August and October. Fields were visited twice, in the middle and at the end of the season. At each visit, leaf samples from symptomatic squash plants were collected, placed in plastic bags and maintained at −70°C until either ELISA or RT-PCR analyses were performed. Virus incidence (%) in each region was estimated by the proportion of positive samples (%) multiplied by the proportion (%) of symptomatic plants according to the visual estimates.

Virus detection and RNA extractions Sample testing for CABYV was carried out by double antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (1977). Antibodies, conjugated antibodies, positive and negative control samples were provided by Dr. H. Lecoq (INRA, Montfavet Cedex, France). Optical densities (OD) were recorded at 405 nm with a microplate reader (Stat Fax® 4200, USA) and samples showing OD values higher than twice the average of the negative control were considered positive (Mnari-Hattab *et al*. 2009).

Depending on ELISA results, fresh squash tissues were taken from CABYV positive samples. Total RNA from each sample was extracted using RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols.

PCR amplification and sequencing OneStep RT-PCR Kit (Qiagen) was used for reverse transcription (RT) and PCR amplification. The RT-PCR reaction was set up in 50 μl as follows: 1 μl RNA sample, 20 μl RNase-free water, 10 μl 5× Qiagen OneStep RT-PCR buffer (containing 12.5 mM MgCl2), 10 μl 5× Q-Solution, 2 μl dNTP mix (10 mM each), 2 μl Qiagen OneStepRT-PCR Enzyme Mix and 2.5 μl of each primer, CE9 (forward primer: (5′-GAATACGGTCGCGGCTAGAAATC-3′, 10 pmol), CE10 (reverse primer (5′-CTATTTCGGG TTCTGGACCTGGC-3′, 10 pmol) (Juarez *et al*. 2004) to amplify fragments from nucleotide 3507 to 4104 of ORFs 4/5, and the primers pairs CE-124 (5′-CTCCT TCCGATATTGGCTCG-3′) and CE-123 (5′-CCCAT TCTGCGCCGCAGTGG-3′) (Juarez *et al*. 2006), which were designed based on the nucleotide sequence of isolate CABYV-N (GenBank accession no. X76931) to amplify fragments from nucleotide 2316 to 2979 of ORF 3. The reaction was performed at 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and a final extension period of 72°C for 10 min. The PCR product was run on a 1.5% agarose gel with 1-Kb plus DNA

Ladder (Invitrogen, Carlsbad, CA, USA) as molecular weight marker.

Nucleotide sequencing analysis Amplified PCR products from infected squash samples were purified from agarose gels after electrophoresis using GENECLEAN turbo columns (Q-Biogene) and then sequenced (Secugen, Madrid, Spain) with the same primers as those used for amplification. Raw sequence chromatograms were assembled and edited using GAP4 (Bonfield *et al.* 1995). Homologies between known sequences were detected using the BLASTX algorithms against the non-redundant GenBank database at http://www.ncbi.nlm.nih.gov/blast. Multiple alignments were performed using ClustalW (Thompson *et al.* 1994) and the phylogenetic analyses were conducted with MEGA4 (Tamura *et al.* 2007) using the minimum evolution method (Rzhetsky and Nei 1992). The MEGA4 package was used also to compute the total nucleotide diversity π (Nei 1987), using the Kimura 2-parameters method (Kimura 1980) and to estimate nucleotide diversity values in non-synonymous (d_N) and synonymous positions (d_S) using the Pamilo-Bianchi-Li method (PBL) (Li 1993). Recombination events were evaluated using the RDP3 package (Martin *et al.*, 2005). The partial ORF 4 (coat protein, P4) gene sequence of 12 Egyptian CABYV isolates, Kafrelsheikh 1, Kafrelsheikh 2, Badr 1, Badr 2, Badr 3, Badr 4, Badr 5, Elkoreen1, Elkoreen 2, Elkoreen 3, Elkoreen 4 and Elsalheia have been deposited in the EMBL/GenBank/DDBJ databases under accession numbers FR717145, FR717146, FR717147, FR717148, FR717149, FR717150, FR717151, FR822384, FR822385, FR822386, FR822387 and FR822383, respectively, and the partial sequences of ORFs 4/5 of these isolates were deposited under accession numbers FR838009, FR838010, FR838004, FR838005, FR838006, FR838007, FR838008, FR838014, FR838012, FR838013, FR838011 and FR838015, respectively. Sequences used for comparisons were retrieved from GenBank (www.ncbi.nlm.nih.gov). The accession numbers of international CABYV isolates, along with the country of origin, are shown below in Figs. 3 and 4.

Results and discussion

CABYV symptoms and incidence During 2010 a high incidence of yellowing symptoms in squash growing in Egyptian fields was observed mainly at the end of the growing season (Fig. 1a and b). In some fields, yellowing was noted in 100% of the plants. In general, symptoms consisted of interveinal chlorosis of the basal and older leaves with brittle and thick aspect. Similar symptoms have been described previously in

Fig. 1 Symptoms in squash plants infected with Cucurbit aphid-borne yellows virus: Interveinal chlorosis, yellowing of the basal leaves and reduction in growth (a). General yellowing symptoms in an open field at the end of the growing season (b)

cucurbit crops and found to be caused by CABYV (Kassem *et al*. 2007; Lecoq *et al*. 1992; Mnari-Hattab *et al*. 2009). In addition, we noticed in symptomatic plants a reduction of the fruit number, but no effect on fruit quality and shape was detected (not illustrated). This observation is incongruent with results obtained by Lecoq *et al*. (1992) with plants of zucchini squash F1 hybrid ('Diamant'), where no effect on fruit yield was detected in CABYV-infected plants. However, in the same study a CABYV infection in melon and cucumber crops caused a significant reduction in fruit production without alteration in fruit shape or quality (Lecoq *et al*. 1992).

A total of 717 symptomatic squash leaf samples were collected from four regions where squash cultivation is of economic importance for the country: Kafrelsheikh, El-Behira, El-Sharkia and El-Ismailia. Serological analysis showed that 95.6% of the symptomatic squash samples were infected by CABYV. This important percentage reflected the high incidence of CABYV in the fields (Table 1), and demonstrated the prevalence of CABYV in squash-growing fields of Egypt. Such a high incidence of CABYV was reported also in other countries like Lebanon, Spain, Tunisia and China (Abou-Jawdah *et al*. 2000; Kassem *et al*. 2007; Mnari-Hattab *et al*. 2009; Shang *et al*. 2009). In accord with our observations, all these regions had an abundance of aphids in open fields. Noticeably, our results showed that this incidence increased at the end of the growing season, reaching 100% of analyzed samples in some cases, except for in the Kafrelsheikh region, where it remained relatively low (around 25%). This could be explained by the earlier growing season of squash (March–May) in this region, which also had relatively lower temperatures and lower aphid abundance (data not shown).

Partial sequencing of Egyptian CABYV isolates and phylogenetic analysis cDNAs for two regions of the CABYV genome from 12 CABYV 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 without previous cloning. Thus, we sequenced fragments of 663 and 597 pb, corresponding to the partial sequences of ORF 3 (*P3* gene) and ORFs 4/5 (complete *P4* and partial *P5* genes), respectively.

Nucleotides and amino acids alignments of these sequences were performed separately in order to analyze the phylogenetic relationship between isolates. Multiple alignments of amino acid of ORF 4 (Fig. 2a) and ORF 3 (Fig. 2b) encoding partial coat protein P4 and P3 showed that four and five amino acid positions, respectively, are variable between Egyptian isolates. A phenylalanine amino acid of the ORF 4 region at position 88 in the partial coat protein (P4) appeared as a signature for Kafrelsheikh isolates.

The nucleotide sequences of French (X76931) and Chinese isolates (EU636992 and EU000535), available in the databases, were included in the alignment. Overall, the Egyptian isolates shared 95.3% to 96.6% nucleotide identity with the French isolate and 92.9% to 93.9% with the Chinese isolate. For ORFs 4/5, a close relationship was observed among isolates, with pairwise nucleotide identities ranging between 99% and 100%, including the French isolate (more than 98.9% nucleotide identity), placing all together in the same phylogenetic group, clearly separated from the Chinese isolate (94.4-95.0% nucleotide identity) (Fig. 3a). For ORF 3, isolates were genetically more separated, with nucleotide identities ranging from 96.6% to 100%. Egyptian isolates shared the same clade with the French isolate, except for Badr-3

Table 1 Incidence of *Cucurbit aphid-borne yellows virus* in squash detected by ELISA of samples collected from open fields during 2010

Region of sampling	First survey			Second survey		
	Visual inspection Symptomatic/total	ELISA Positive/ tested plants	Virus incidence (%)	Visual inspection Symptomatic/total	ELISA Positive/ tested plants	Virus incidence (%)
Kafrelsheikh	60/1000	12/15	4.80	384/1200	77/97	25.40
El-Behira	1050/3000	67/70	33.50	3000/3000	96/100	96
El-Sharkia	1800/4000	114/120	42.75	4000/4000	100/100	100
El-Ismailia	575/1200	102/115	42.25	2400/2400	100/100	100

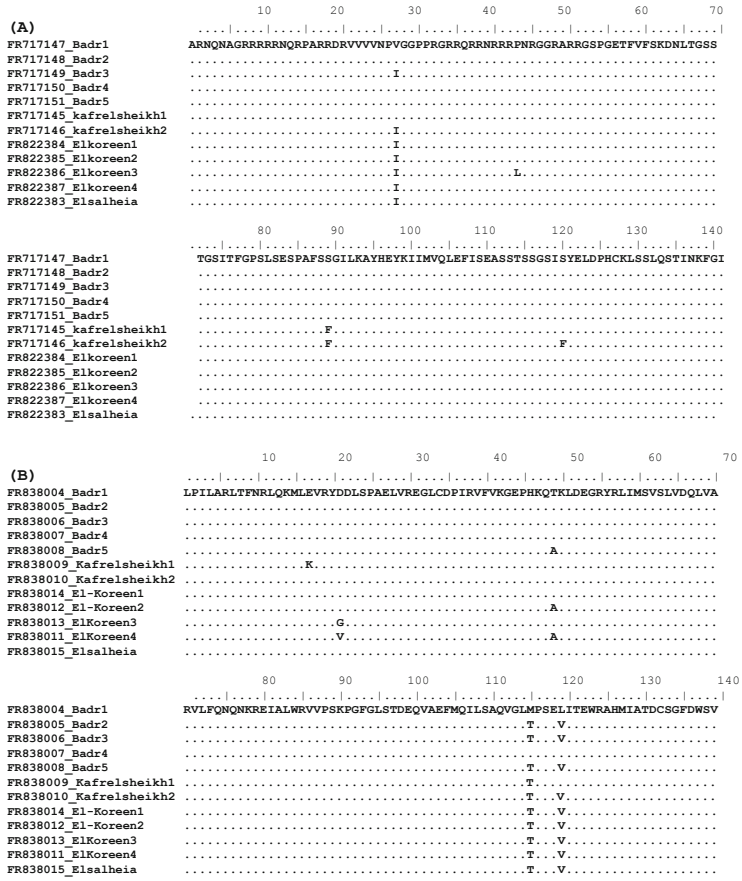

Fig. 2 Amino acid sequences alignments of ORF4 (A) and ORF3 (B) regions of 12 Egyptian *Cucurbit aphid-borne yellows virus* isolates

(FR838006), which seemed to be genetically different with bootstrap support equal to 90%. This allows speculation that an analysis of more Egyptian isolates could differentiate in the field at least two distinctly related groups for this region of the genome (Fig. 3b): one dominant (with high frequencies) and another minor in the population. Moreover, these trees suggested that Egyptian isolates belong to the

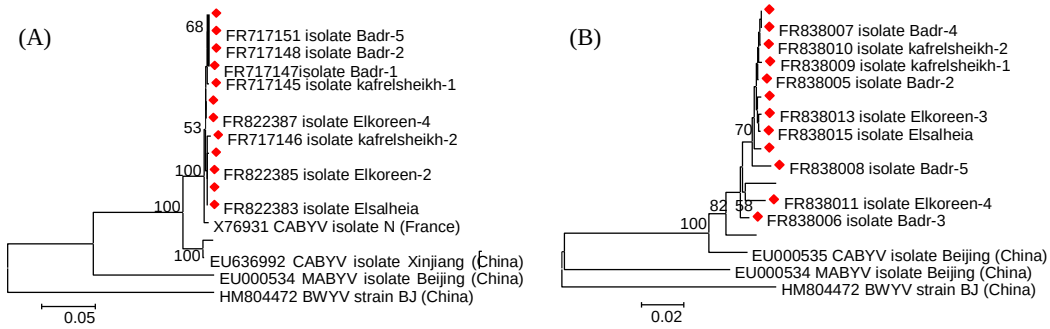

Fig. 3 Phylogenetic tree of (A) ORFs 4/5 and (B) ORF 3 of *Cucurbit aphid-borne yellows virus* Egyptian isolates ( ) sequenced in this study and of additional isolates available in Genebank. The evolutionary history was inferred using the Minimum Evolution method. Bootstrap percentages above 50% are shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted using MEGA4

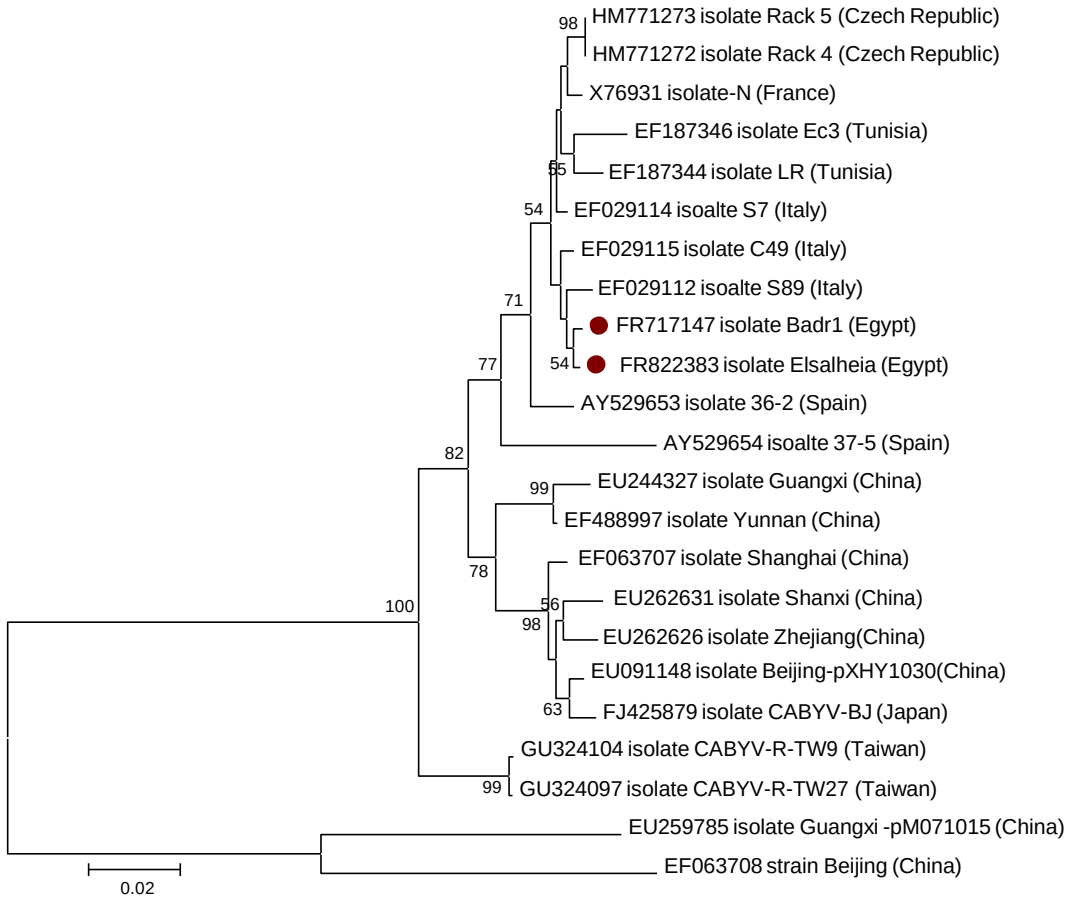

Fig. 4 Phylogenetic tree of ORFs 4/5 of 23 worldwide *Cucurbit aphid-borne yellows virus* isolates (including the two Egyptian isolates – FR717147 and FR822383 – sequenced in this study). The evolutionary history was inferred using the Minimum Evolution method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset. In the final dataset there were 472 positions. Phylogenetic analyses were conducted with MEGA4

Mediterranean group described previously by Shang *et al*. (2009). To test this hypothesis, we introduced available CABYV sequences originating from Italy, Spain, Tunisia, Czech Republic, China, Japan and

Table 2 Nucleotide diversities* ± standard errors for different regions of the *Cucurbit aphid-borne yellows virus* genome

d_N/d_S	d_S	d_N	π	
0.071	0.042±0.010	0.003±0.002	0.013±0.003	ORF 3
1	0.003±0.003	0.003±0.002	0.003±0.002	ORF 4
0.4	0.005±0.004	0.002±0.002	0.002±0.002	ORF 5

*Nucleotide diversity is defined here as the mean number of nucleotide substitutions per site (π). Nucleotide diversities were computed separately for synonymous ($\boldsymbol{d_S}$) and non-synonymous ($\boldsymbol{d_{NS}}$) positions using the PBL method. Standard errors were calculated by using the bootstrap method implemented in the MEGA4 program

ORF, open reading frame

Taiwan to the ORFs 4/5 sequence alignment. From this alignment we deduced a new phylogenetic tree (Fig. 4). Three main branches were detected in the Mediterranean group: one including the Egyptian isolates with French and two Italian isolates; a second including Czech, Tunisian, Italian and Spanish isolates; and a third group including only Spanish isolates (37-5).

Values of nucleotide diversity were estimated for each of the ORFs analyzed (Table 2). The average nucleotide diversity for ORF 3 was significantly higher than for the other two regions, indicating that variability is not evenly distributed along the viral genome. We also estimated nucleotide diversity values in non-synonymous (d_N) and synonymous positions (d_S) for each ORF in order to estimate the d_N/d_S ratio, our results showing that the three genes are evolving under different pressures. Similar to results obtained for other poleroviruses (Pagán and Holmes 2010; Wu *et al*. 2011), the d_N/d_S ratio was significantly smaller for ORF 3 than for the two other ORFs, suggesting that purifying selection has been stronger for this ORF than for the other two. ORF 5 appeared to be also under negative selection, whereas the d_N/d_S value was significantly higher for ORF4 (equal to 1, i.e., evolving neutrally).

Due to the important role of recombination in luteovirus diversification, an analysis of potential recombination events within the sequenced regions was carried out. However, our results did not reveal any potential recombination event within these genes, in agreement with other studies (Pagán and Holmes 2010; M. Kassem, M. Juarez, P. Gomez, S. F. Elena, & M. A. Aranda, unpublished).

In conclusion, our analyses showed that CABYV is widespread in the most important squash-growing fields of Egypt. CABYV infection was associated with the typical yellowing symptoms of older leaves and yield reductions. The CABYV population has a low diversity and seems to be related genetically to the French isolate, especially in ORFs 4/5 of its genome. Our results also demonstrate that Egyptian CABYV isolates belong genetically to the Mediterranean group.

Acknowledgments Part of this work was supported by grant 11934/PI/09 (Fundación Séneca de la Región de Murcia, Spain). We thank Miguel A. Aranda and Verónica Truniger for critically reading the manuscript, and Mari Carmen Montesinos and Blanca Gosalvez for their excellent technical assistance.

References

Abou-Jawdah, Y., Sobh, H., El-Zammar, S., Fayad, A., & Lecoq, H. (2000). Incidence and management of virus diseases of cucurbits in Lebanon. *Crop Protection, 19*, 217–224.

Bananej, K., Vahdat, A., Predajna, L., & Glasa, M. (2009). Molecular characterization of geographically different cucurbit aphid-borne yellows virus isolates. *Acta Virologica, 53*, 61–64.

Bonfield, J. K., Smith, K. F., & Staden, R. (1995). A new DNA sequence assembly program. *Nucleic Acids Research, 24*, 4992–4999.

Clark, M. F., & Adams, A. N. (1977). Characteristics of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology, 34*, 475–483.

FAO (2010). Statistical agriculture databases. http://faostat.fao.org/

Juarez, M., Kassem, M., Sempere, R., & Aranda, M. (2006). *Cucurbit aphid-borne yellows virus* in Spain. In G. P. Rao, P. Lavakumar, & R. J. Holguín-Peña (Eds.), *Characterization, diagnosis and management of plant viruses, Vol. 3* (pp. 303-315). *Vegetable and pulse crops.* Houston, TX, USA: Studium Press.

Juarez, M., Truniger, V., & Aranda, M. A. (2004). First report of cucurbit aphid-borne yellows virus in Spain. *Plant Disease, 88*, 907.

Kassem, M. A., Sempere, R. N., Juárez, M., Aranda, M. A., & Truniger, V. (2007). Cucurbit aphid-borne yellows virus is prevalent in field-grown cucurbit crops of southeastern Spain. *Plant Disease, 91*, 232–238.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*, 111–120.

Lecoq, H. (1999). Epidemiology of cucurbit aphid-borne yellows virus. In H. G. Smith & H. Baker (Eds.), The *Luteoviridae* (pp. 243–248). Wallingford, UK: CABI Publishing.

Lecoq, H. (2003). Cucurbits. In G. Loebenstein & G. Thottapilly (Eds.), *Virus and virus-like diseases of major crops in developing countries* (pp. 665–687). Dordrecht, the Netherlands: KluweAcademic Publishers.

Lecoq, H., Bourdin, D., Wipf-Scheibel, C., Bon, M., Lot, H., Lemaire, O., *et al*. (1992). A new yellowing disease of cucurbits caused by a luteovirus, Cucurbit aphid-borne yellows virus. *Plant Pathology, 41*, 749–761.

Lemaire, O., Gubler, W. D., Valencia, J., Lecoq, H., & Falk, B. W. (1993). First report of cucurbit aphid-borne yellows luteovirus in the Unites States. *Plant Disease, 77*, 1169.

Li, W.-H. (1993). Unbiased estimation of the rates of synonymous and non-synonymous substitution. *Journal of Molecular Evolution, 36*, 96–99.

Martin, D., Williamson, C., & Posada, D. (2005). RDP2: recombination detection and analysis from sequence alignments. Bioinformatics, 21, 260–262.

Mayo, M. A., & D'Arcy, C. J. (1999). Family Luteoviridae: A reclassification of luteoviruses. In H. G. Smith & H. Barker (Eds.), The Luteoviridae (pp. 15–22). Wallingford, UK: CABI Publishing.

Mnari-Hattab, M., Gauthier, N., & Zouba, A. (2009). Biological and molecular characterization of the *Cucurbit aphid-borne yellows virus* affecting cucurbits in Tunisia. *Plant Disease, 93,* 1065–1072.

Nei, M. (1987*). Molecular evolutionary genetics*. New York, NY: Columbia University Press.

Pagán, I., & Holmes, E. C. (2010). Long-term evolution of the Luteoviridae: time-scale and mode of virus speciation. *Journal of Virology, 84,* 6177–6187.

Rzhetsky, A., & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Molecular Biology and Evolution, 9,* 945–967.

Shang, Q. X., Xiang, H. Y., Han, C. G., Li, D. W., & Yu, J. L. (2009). Distribution and molecular diversity of three cucurbit-infecting poleroviruses in China. *Virus Research, 145,* 341–346.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24,* 1596–1599.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research, 22,* 4673–4680.

Tomassoli, L., & Meneghini, M. (2007). First report of Cucurbit aphid-borne yellows virus in Italy. *Plant Pathology, 56,* 720.

Wu, B., Blanchard-Letort, A., Liu, Y., Zhou, G., Wang, X., & Elena, S. F. (2011). Dynamics of molecular evolution and phylogeography of *Barley yellow dwarf virus-PAV.* PLoS ONE, 6(2), e16896.

Xiang, H. Y., Shang, Q. X., Han, C. G., Li, D. W., & Yu, J. L. (2008). First report on the occurrence of *Cucurbit aphid-borne yellows virus* on nine cucurbitaceous species in China. *Plant Pathology, 57,* 390.

Yardımcı, N., & Özgönen, H. (2007). First report of *Cucurbit aphid-borne yellows virus* in Turkey. *Australian Plant Disease, 2,* 59.

Ziegler-Graff, V., Brault, V., Mutterer, J. D., Simonis, M.-T., Herrbach, E., Guilley, H., et al. (1996). The coat protein of beet western yellows luteovirus is essential for systemic infection but the viral gene products P29 and P19 are dispensable for systemic infection and aphid transmission. *Molecular Plant-Microbe Interactions, 9,* 501–510.