https://doi.org/10.17221/191/2020-PPS

Molecular characterisation of *Zucchini yellow mosaic virus* infecting *Cucurbita pepo* in Egypt

Engy E. Abdel Aleem, Mona Rabie, Faiza A. Fattouh*

Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt

**Corresponding author: eengy24@yahoo.com, engy.essam@alexu.edu.eg*

Citation: Abdel Aleem E.E., Raibe M., Fattouh F.A. (2021): Molecular characterisation of *Zucchini yellow mosaic virus* infecting *Cucurbita pepo* in Egypt. Plant Protect. Sci., 57: 263–270.

Abstract: The complete nucleotide sequence of *Zucchini yellow mosaic virus* isolate from Egypt (ZYMV-Egz_MT383108) was determined. The sequence comparisons suggested that the isolate belongs to Group A. The sequence analysis of the Egyptian isolate showed the highest similarity (~96–97%) with the isolates leaf1 (KJ923767.1) and PA_2006 (JQ716413.1) from the USA and the lowest similarity (84%) with an isolate (AF014811.2) from Singapore. The phylogenetic analysis revealed that ZYMV-Egz occupied a distinct clade together with the USA isolates in Group A, known to be the most widespread throughout the world. This is a first record of the complete nucleotide sequence of an Egyptian isolate of ZYMV.

Keywords: cucurbits; genome sequence; phylogenetic analysis; RT-PCR; virus identification

Cucurbit crops are infected by several viruses such as *Papaya ringspot virus* (PRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV), belonging to the *Potyvirus* genus. Virus infections result in economic damage in many parts in the world (Simmons et al. 2013). ZYMV is one of the most abundant potyviruses and was reported to cause yield losses of up to 100% (Moradi et al. 2019). ZYMV was first reported in Italy in 1973 (Lisa et al. 1981) and in France (Lecoq et al. 1981). The virus is currently reported to cause disease in Africa, America, Asia, Europe, the Middle East and Oceania (Spadotti et al. 2015; Bubici et al. 2020). In Egypt, ZYMV is associated with significant yield losses in a wide range of cucurbit crops including cucumbers, melons, pumpkins, squashes, vegetable marrows, watermelons, and especially zucchinis.

A ZYMV infection could result in symptoms consisting of mosaic, yellowing and eventually "shoestring" in the leaves. The fruits are stunted, twisted and deformed, resulting in a reduced yield making the produce unmarketable; especially zucchini squash (Massumi et al. 2011). ZYMV has an experimental host range that includes species in eleven families of dicotyledons although it mostly infects cucurbits in natural conditions (Romay et al. 2014).

ZYMV has a genome of a single positive-sense RNA molecule of about 10 kb and encodes a polyprotein that is proteolytically processed into ten mature proteins; *P1* (protease), *HC* (helper component/protease), *P3*, *6K1*, *CI* (cylindrical inclusions), *6K2*, *NIa* (nuclear inclusion a), *VPg* (viral protein linked genome), *NIb* (nuclear inclusion b) and *CP* (Lin et al. 2001; Moradi et al. 2019). The virus can be transmitted not only by 26 species of aphids such as *Myzus persicae*, in a non-persistent manner (Gal-On 2007), but also mechanically (Coutts et al. 2013; Tymchyshyn et al. 2017) and through the seeds (Simmons et al. 2011).

Several studies have indicated the ZYMV infections of cucurbits in Egypt (Nasr-Eldin et al. 2016; Kheder et al. 2017). Reverse transcription polymerase chain reaction (RT-PCR) has been used to detect the virus coat protein (*CP*) gene or other genome segments. Phylogeny studies of such genes have indicated their relatedness to other isolates limited to this genome segment. However, there is no information on the whole viral genome sequence or the virus genotyping. Such

https://doi.org/10.17221/191/2020-PPS

information is needed and may be critical to understand the virus genetic diversity and phylogenetic and evolutionary patterns. It could provide insights into the disease epidemiology and aid in breeding resistant varieties and hybrids. Thus, here, we attempt the full-length sequencing of a ZYMV local isolate obtained from the zucchini for further molecular characterisation and genotyping of the virus in Egypt.

MATERIAL AND METHODS

Samples collection and RNA extraction. Zucchini (*Cucurbita pepo* Linnaeus) leaf samples showing virus-induced symptoms such as mosaic, yellowing and leaf deformation were collected in June 2018 in the locations Kom Hamada and Markaz Badr in the El-Beheira governorate in Egypt. The collected samples were kept frozen at –20 °C till testing. The total RNA was isolated from 50 symptomatic squash plant samples using a GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, USA) following the manufacturer's instructions.

RT-PCR amplification of the *CP* gene for the detection of ZYMV infection. The first-strand cDNA was synthesised using a Thermo Scientific™ RevertAid™ first strand cDNA Kit (Thermo Scientific, USA) according to the manufacturer's instructions, and the amplification was performed using a Gene Amp 9700 thermocycler (Applied Biosystem ABI, USA). 2.5 µL of each cDNA sample was added to a 25 µL PCR reaction mixture. The mixture consisted of 12.5 µL of a Dream Taq Green PCR Master Mix (2X) and 0.5 µL (20 pmol) of each primer (Zy1F 5'-CGCGTGAAAGGAAATGTGATAC-3' and Zy1R 5'-GGCCAAACAACCTTGAAGAAA-3') corresponding to the *CP* gene and 9 µL of nuclease-free water. The PCR profile consisted of 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 62 °C

Table 1. Primers used for the amplification of the total genomic RNA of *Zucchini yellow mosaic virus*

Primer name	Primer sequence (5'–3')	Region amplified	Expected product size (bp)	Reference
ZY-1 F	CTATAATTCGCACAGCATCAAGAA	1–377	377	this study
ZY-1 R	CTCGTCAGTGCTGAAGAAATTG			
ZY-2 F	CAGTGTGCAAACACTCAAGTAAG	140–734	595	
ZY-2 R	GCCAATCATCTCAACAGGGATA			
ZY-3 F	GCGAGACATACACTCACCTTC	712–1 069	358	
ZY-3 R	GTCTCTACCTCGAATCACCAAAT			
ZY-4 F	AAGCACTCACATGAAGCAAATAC	1 009–2 437	1 429	
ZY-4 R	GGATCACCAGAAGCTCCTATAAC			
ZY-5 F	GGCATGATTTCACCCACTATTC	2 380–3 475	1 096	
ZY-5 R	TTGCCATCGCTCTCTCTTT			
ZY-6 F	GGAGCAGTAGGTTCTGGAAAG	3 430–4 647	1 218	
ZY-6 R	GTTCCATGCGTTTCAATGGTAG			
ZY-7 F	ACAGAAGCAGCAGCCTTATC	4 597–5 533	937	
ZY-7 R	CTGAGCTGATAGTGTCGAAGTG			
ZY-8 F	CTCGGACTTCGCGGTAAAT	5 487–6 262	776	
ZY-8 R	CTCAGGCTCCACACCATATAAA			
ZY-9 F	TGTCGAGTTGGAGAGCAAATC	6 207–6 991	785	
ZY-9 R	GCTCAAGTGCGTGGCTATAA			
ZY-10 F	CACAGAGCAAGCGAGAAAGA	6 927–7 827	901	
ZY-10 R	CAACCCACCAATCCTCCATATAA			
ZY-11 F	AACTGCGAGGAGATTGAGAATAG	7 767–8 542	776	
ZY-11 R	GTCTTGATGGAGGGCTTGTAG			
ZY-12 F	CAGATGCTGGAGCTACAAAGA	8 487–9 382	896	
ZY-12 R	GGTTTCGAAGCAAACCATACC			
ZY-13 F	GATGTTAATAGAAACATGCACACCT	9 327–9 593	267	
ZY-13 R	AGGCTCACACTAAAGCTTCC			

Primer combinations produced thirteen overlapping segments covering the whole genome

https://doi.org/10.17221/191/2020-PPS

for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 5 minutes. The RT-PCR reaction mixes were analysed by agarose gel electrophoresis.

Amplification of total genomic RNA of ZYMV-Egz isolate. Viral cDNAs measuring 2.5 µL were amplified by PCR in a 25 µL reaction mixture. Thirteen overlapping primer pairs covering the whole genome were designed from the complete genome sequences of the ZYMV isolates available in the GenBank and used for the amplification of the whole ZYMV genome, as shown in Table 1.

Sequencing and phylogenetic analyses. To sequence the amplified fragments, the amplicons of the expected size were purified from the agarose gel using a Wizard® SV Gel PCR Clean-Up System kit (Promega, USA). The identities of the amplicons were verified by sequencing at GATC Biotech (Germany) and the sequences obtained were submitted to the GenBank database. Amino acid sequences were obtained using an online translation tool (www.expasy.ch/tool/translate).

A computer-based comparison of nucleotide sequences of the Egyptian isolates of ZYMV to those present in the GenBank was carried out by using the CLUSTALW program in MEGA 7 software (version 7) (Kumar et al. 2016). Phylogenetic and molecular evolutionary analyses were conducted using the Maximum likelihood (ML) method and Bootstrap test of phylogeny with 1 000 replicates in the MEGA 7 software. The ZYMV sequences downloaded from the GenBank database and used in phylogenetic analysis are described in Tables 2 and 3.

RESULTS

Detection of ZYMV in *Cucurbita pepo* L. ZYMV related symptoms were found in the *Zucchini* survey fields in the El Beheira governorate. Samples showed mild to severe virus disease symptoms (Figure 1A–D). A total of 50 symptomatic samples were screened by RT-PCR for the virus detection.

Amplification of the *CP* gene. ZYMV was detected in only four of the collected samples by the use of the Zy1F/Zy1R primer, which amplified a region of the ZYMV-*CP* gene (840 nts). Sequence comparisons using BLAST confirmed the identity of the sequences as being that of ZYMV. The nucleotide sequences obtained were submitted to the National Center for Biotechnology Information (NCBI) GenBank, (accession numbers MT383104, MT383105, MT383106, and MT383107).

Table 2. List of 65 *Zucchini yellow mosaic virus* isolates used to construct the phylogenetic analysis based on the *coat protein* gene (sequence reference: NCBI)

Accession number	Isolate	Geographical origin
MK457355.1	Sohag-Eg-2	Egypt
MK457354.1	Sohag-Eg-1	Egypt
MG021246.1	Qalyubia-EG	Egypt
KJ923768.1	leaf17	USA
KC665629.1	FG1	USA
KU743336.1	13-YY-X7-22	China
KU743350.1	15-LD-ZMQ-368	China
KU743351.1	15-LD-ZMQ-369	China
JN192427.1	2nd	USA
JN192426.1	3nd	USA
AJ429071.1	isolate A	South Korea
EF062583.1	AG	Israel
GQ251520.1	Aligarh	India
AJ420015.1	Austria 10	Austria
FJ705252.1	Aza.Mah.W	Iran
FJ705272.1	Azr.Mak.W	Iran
AY188994.1	B*	Israel
AM422386.1	begonia	Taiwan
AY074809.1	Beijing	China
AJ420019.1	Berlin 1	Germany
MH042025.1	BR2	South Korea
MH042026.1	BR3	South Korea
L31350.1	California	USA
HM768180.1	CF732	USA
HM768185.1	CG714	USA
AY611021.1	CH99/116	China
AY611023.1	CH99/193	China
KJ848744.1	Changji-1	China
KU366270.1	Cha-Zuc	Iran
D00692.1	Connecticut	USA
AF062518.1	cu	Korea
AJ307036.2	CU	China
EU561043.1	cu	Poland
JF792448.1	Cvn-1	Australia
AF486822.1	dongyang	China
JN861005.1	E15	France
JN192420.1	E72	USA
FJ705258.1	Far.Mar.M	Iran
AJ459954.1	H266-2	Hungary
AF486823.1	hainan	China
FJ705260.1	Ham.Aas.C	Iran
AY611026.1	HN-01	China
AJ420020.1	Italy 1	Italy
JF792363.1	Knx-1	Australia
AY278998.1	KR-PA	Korea

https://doi.org/10.17221/191/2020-PPS

Table 2. to be continued

Accession number	Isolate	Geographical origin
AB063251.1	M39	Japan
JN861007.1	MT92-7	USA
JF792440.1	Nt-1	Australia
AJ316227.1	p	China
AB127936.1	pak	Pakistan
JX028593.1	RSHS	USA
AJ316228.2	SG	China
AB458595.1	SYZY-1	Syria
AB458596.1	SYZY-3	Syria
KJ848773.1	Tuokexun-3	China
MG214089.1	Y21	Turkey
KP872579.1	Y23	Turkey
KM405803.1	Z106-2	China
KM405795.1	Z11-11	China
KU244514.1	DE_2014	Germany
JQ716413.1	PA_2006	USA
JX502667.1	ZYMV-DF	Brazil
AB004640.1	169	Japan
KY225543.1	694K	Australia
AF014811.2	Singapore	Singapore

Phylogenetic analysis. The phylogenetic trees generated from alignment of the nucleotide sequence of the *CP* gene of the four isolates and 65 ZYMV isolates from different geographical areas available in the GenBank showed three distinct groups (A, B and C) [(Figure S1A in electronic supplementary material (ESM) – For ESM see the electronic version]. All four Egyptian isolates (ZYMV-Egz1-4) were in group A. All four isolates appeared to be closely related to each other (identity ~99–98.6 %) and showed high identity (~99–98%) against the isolates CF732 (HM768180.1) and leaf17 (KJ923768.1) from the USA. The highest phylogenetic distance was detected against isolate (AF014811.2) from Singapore and Knx-1 isolate (JF792363.1) from Australia in group C (identity 86–85%). The sequence identity (90–92%) was also detected against the isolates of group B, the isolate begonia (AM422386.1) from Taiwan and isolate A (AJ429071.1) from South Korea. Interestingly, the ZYMV isolates from Egypt reported in previous studies showed less similarity (93%) to the Egz1-4 isolates, indicating a possible genetic diversity in ZYMV in Egypt. Collectively, the four Egyptian isolates showed 85 to 99% nucleotide sequence identity with the other reported isolates. Similar results were obtained comparing the amino acid sequences of the *CP* for the new Egyptian isolates which occupied group A and showed a closer relatedness to the isolates from USA [CF732 (HM768180.1) and leaf17 (KJ923768.1)] (Figure S1B in ESM). The four isolates showed 88 to 96.6% amino acid sequence identity with the available ZYMV *CP* sequences in the NCBI GenBank database.

Table 3. List of the 23 *Zucchini yellow mosaic virus* isolates used for the phylogenetic analysis based on the ZYMV complete genome (sequence reference: NCBI)

Accession Number	Isolate	Geographical origin
AB369279.1	RDA	South Korea
AF014811.2	Singapore	Singapore
AJ307036.2	CU	China
AJ316228.2	SG	China
AM422386.1	begonia	Taiwan
AY278998.1	KR-PA	Korea
AY278999.1	KR-PE	Korea
JQ716413.1	PA_2006	USA
KJ923767.1	leaf1	USA
KJ875864.1	ZYMV	USA
MN598576.1	Nt-5	Australia
MK956829.1	Z-104	Italy
MF072714.1	Trini3	Trinidad and Tobago
AY188994.1	B*	Israel
EF062583.1	AG	Israel
KF976712.1	H	Czech Republic
MN598566.1	Cvn-19	Australia
KY225553.1	14Br	Australia
MG967620.1	SB02	India
AB188116.1	2002	Japan
AF127929.2	TW-TN3	Taiwan
KX421104.1	zz	China
MK033874.1	CN:Lc:17	China

Amplification of ZYMV-Egz total genome. The genome of isolate ZYMV-Egz (accession number MT383108), is 9593 bp long, containing a 5' untranslated region (UTR) of 139 nt, a 3' UTR of 211'nt, and a single open reading frame (ORF), spanning from position 140 to 9 379, encoding a polyprotein of 3 080 amino acids comprising ten different genes.

For the amino acid sequences of the Egz isolate, nine cleavage sites in the polyprotein were identified which could give rise to ten mature proteins: The *P1*/*Hc-Pro* cleavage site (Y/S) identified in motif VDHY/SS. The *Hc-Pro*/*P3* cleavage site (G/G) identified in motif RVG/GS. The *P3*/*6K1* cleavage

https://doi.org/10.17221/191/2020-PPS

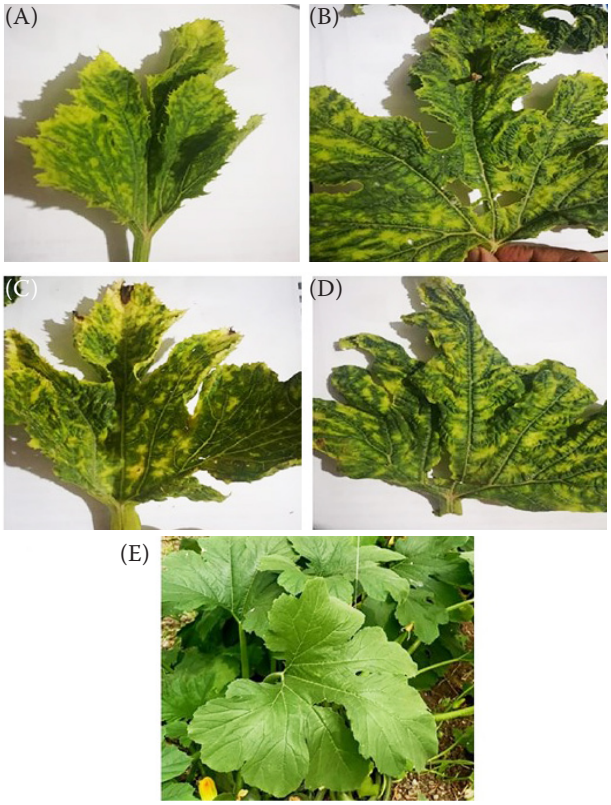

Figure 1. ZYMV-related symptoms in the Squash (*Cucurbita pepo* L.) surveyed fields

A, B – mosaic pattern and mottles; C – leaf with distorted and serrated edges; D – leaf curled downwards from leaf edges and vein banding; E – healthy control

site (Q/A) identified in motif STQ/AK. The *6K1*/*CI* cleavage site (Q/G) identified in motif RLQ/GL. The *6K2*/*VPg* and *VPg*/*NIa* cleavage sites (E/S) identified in motif RVE/SK and ELE/SK, respectively. The *CI*/*6K2, NIa*/*NIb* and *Nib*/*CP* cleavage sites (Q/S) identified in motif VLQ/SK, ETQ/SK and MLQ/SG, respectively. All the conserved potyvirus motifs were identified in the polyprotein.

Phylogenetic analysis illustrating evolutionary relationships. The phylogenetic tree based on the complete genome showed three distinct groups (A, B and C) (Figure 2). ZYMV-Egz clustered with members of group A similar to the *CP* gene. The ZYMV-Egz isolate (MT383108) showed a nucleotide sequence identity (~82–97%) with the available ZYMV complete genome sequences in the GenBank, suggesting a genetic diversity between the Egz isolate and the other reported isolates. ZYMV-Egz showed a high identity (~96–97%) against the isolates leaf1 (KJ923767.1) and PA_2006 (JQ716413.1) from the USA. A sequence identity (90–91%) was also detected against the isolates of group B such as RDA (AB369279.1) from South Korea and zz (AJ429071) from China. The highest phylogenetic distance was detected against the Singapore isolate (AF014811.2) of group C (identity 84%).

DISCUSSION

Cucurbitaceous crops, belonging to the family *Cucurbitaceae*, are important food sources and valued for their nutritional and medicinal worldwide (Kwon et al. 2005). *C. pepo* L. is an economically important crop for local consumption that may be

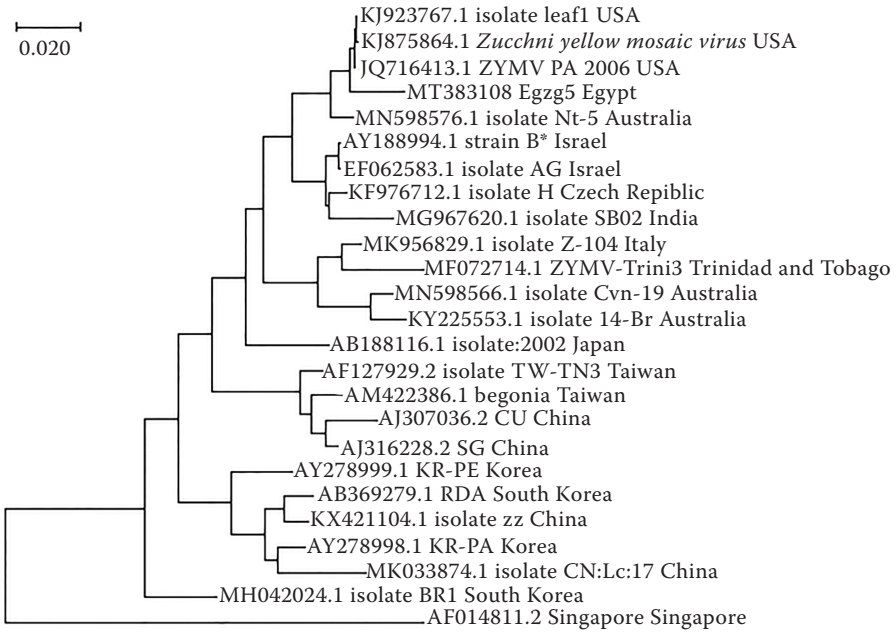

Figure 2. Phylogenetic analysis of the full-length genome of the ZYMV-Egz isolate (MT383108) and its comparison to other *Zucchini yellow mosaic virus* isolates based on the nucleotide sequences

https://doi.org/10.17221/191/2020-PPS

produced almost year-round (Mohamed et al. 2003). Significant yield losses due to ZYMV infections have been reported ranging from 20 to 78% in Egypt (Kheder et al. 2017); 50 to 94% or up to 80% in Western Australia (Coutts et al. 2011) and in Germany (Müller et al. 2006), respectively; and 19.7 to 84.9% in the Ivory Coast (Kone et al. 2017).

Symptoms characteristic of a ZYMV infection were clearly detected in the surveyed fields (Figure 1). Similar symptoms have been previously reported worldwide by Sharma and Gaur (2015), Wang and Li (2017). Confirmation of a ZYMV infection using the Zy1F and Zy1R primer pairs, designed specifically for the *CP* gene, produced 840 bp amplicons as reported earlier (Simmons et al. 2011). The molecular characterisation of the ZYMV isolates is an important aspect in the development of tools to detect this virus. The information about the sequence diversity among the isolates may help design primers for the reliable detection of all the known virus isolates. Earlier studies on the virus in Egypt (El-Hoseny et al. 2010; Kheder et al. 2017) were based on the RT-PCR detection and the genetic diversity of the virus in Egypt was lacking. Based on the *CP* phylogenetic analyses from previous worldwide studies, the ZYMV isolates have been classified into two or three major groups (Coutts et al. 2011; Massumi et al. 2011; Maina et al. 2017). The phylogenetic trees generated from the alignments of the nucleotide sequences of the Egz1-4 *CP* gene revealed three distinct groups (A, B and C), all the ZYMV Egz1-4 isolates were located in group A. Nasr-Eldin et al. (2016) reported ZYMV isolates that clustered in group A, but in different subgroups. The Egz1-4 isolate showed a closer relatedness (98–99%) to the isolates from the USA, but not others previously reported from Egypt. The isolates of group A are the most widely spread (Romay et al. 2014; Spadotti et al. 2015).

There is little evidence of clustering of the isolates according to their geographical origin. This is in agreement with the study of Bubici et al. (2020), but not in agreement with Glasa and Pittnerová (2006) and Maghamnia et al. (2018). The phylogenetic tree was constructed with the complete genome sequence of the Egz isolate and the other 23 reported ZYMV isolates (Figure 2), the ZYMV isolates were clustered into three groups, as reported by Maghamnia et al. (2018). The Egz isolate clustered in clade A, in close relationship to some USA isolates (KJ923767.1 and JQ716413.1). The other isolates from South Korea, China, and Korea were placed in group B. A single isolate from Singapore was in group C. This study and earlier reports indicated that all the isolates from the USA, Iran (Maghamnia et al. 2018), South Korea, Australia, Israel, Italy (Bubici et al. 2020), Taiwan (Lin et al. 2000), and China are more closely related to each other than the Singapore isolate.

The polyprotein of Egz had nine cleavage sites that could give rise to ten mature proteins as reported for the other ZYMV isolates (Choi et al. 2007; Maghamnia et al. 2018 and Bubici et al. 2020). However, the *Hc-Pro/P3* cleavage site of the Egz isolate was G/G in motif RVGGS which was not in agreement with the *Hc-Pro /P3* cleavage site G/G that was found in motif RVGGT that was conserved in all the potyviruses (Choi et al. 2007).

For the amino acid sequences of the Egz isolate, all the conserved potyvirus motifs were identified in the polyprotein. In the N terminal region of the *CP*, the diacylglycerol (DAG) motif that plays an important role in the aphid-transmission was identified. In the *HC-Pro* protein of the Egz isolates, PTK (Phe-Thr-Lys) and KLSC (Lys-Leu-Ser-Cys) motifs were found which play an important role in the aphid-transmission (Glasa & Pittnerová 2006; Maghamnia et al. 2018). However, in the other ZYMV isolates, the KITC (Lys-Ile-Thr-Cys) motif was identified instead of KLSC in ZYMV-Kuchyna (Choi et al. 2007). It was reported that KLSC was permissive for the transmission and efficient aphid transmissibility to zucchinis and cucumbers (Glasa & Pittnerová 2006).

HC-Pro protein has two motifs in its sequence related to the virus aggressiveness: the FRNK (Phe-Arg-Asn-Lys) motif and the CDNQLD motif, both motifs are found in Egz. It is suggested that this isolate may represent an aggressive strain. Previous studies reported that mutations in either the FRNK (Gal-On 2000; Shiboleth et al. 2007) or CDNQLD motifs (Desbiez et al. 2010) cause attenuation of the virus aggressiveness.

Prendeville et al. (2012) and Romay et al. (2014) explained the epidemiology in tropical and subtropical regions, because cucurbits can be cultivated all year and several aphid vectors can transmit ZYMV from one crop to the next one and alternatively, thus wild cucurbits can be efficient virus reservoirs.

This study is a first record of the complete genome nucleotide sequence (9 593 bp) of an Egyptian ZYMV isolate. However, it is not the first record of the presence or detection of the virus by a molecular technique. In previous studies, ZYMV was

https://doi.org/10.17221/191/2020-PPS

detected by RT-PCR amplification and detection of either the *CP* (840 bp) or other genome segments followed by the sequencing and phylogeny of such genes. We compared the *CP* sequence of our isolates with other previous Egyptian isolates (*CP* gene) and they were found to be 93% accurate. The sequence and phylogeny of the whole viral genome of our isolate Egz (9 593) was compared to other full genome nucleotide sequences from other geographical areas of the world. There is no record of previous full genome Egyptian isolate sequences in the GenBank to compare to our isolate.

The control of virus diseases is best achieved through limiting the sources of infection and the identification of possible genetic biodiversity in the local viral population. Minimising the introduction of viruses to the country through regulation of seed exchange policies over a wide range of geographically distant areas and routine identification of introduced plant material can reduce the occurrence of viral infections, thus preventing new disease outbreaks.

REFERENCES

Bubici G., Navarro B., Carluccio A.V., Ciuffo M., Serio F.D., Fabrizio Cillo F. (2020): Genomic sequence variability of an Italian *Zucchini yellow mosaic virus* isolate. European Journal of Plant Pathology, 156: 325–332.

Choi S.K., Yoon J.Y., Sohn S.H. (2007): Analysis of the complete genome sequence of *Zucchini yellow mosaic virus* strain A isolated from Hollyhock. Plant Pathology Journal, 23: 245–250.

Coutts B.A., Kehoe M.A.A., Jones R.A. (2013): *Zucchini yellow mosaic virus:* Contact transmission, stability on surfaces, and inactivation with disinfectants. Plant Disease, 97: 765–771.

Coutts B.A., Kehoe M.A., Jones R.A.C. (2011): Minimising losses caused by *Zucchini yellow mosaic virus* in vegetable cucurbit crops in tropical, subtropical and Mediterranean environments through cultural methods and host resistance. Virus Research, 159: 141–161.

Desbiez C., Girard M., Lecoq H. (2010): A novel natural mutation in HC-Pro responsible for mild symptomatology of *Zucchini yellow mosaic virus* (ZYMV, Potyvirus) in cucurbits. Archives of Virology, 155: 397–401.

El-Hoseny M.E., El-Fallal A., Amira A.K., El-Sayed A.K., Diab A., Sadik A.S. (2010): Biology, cytopathology and molecular identification of an Egyptian isolate of *Zucchini yellow mosaic potyvirus* (ZYMV-Eg). Pakistan Journal of Biotechnology, 7: 75–80.

Gal-On A. (2000): A point mutation in the FRNK motif of the Potyvirus helper component-protease gene alters symptom expression in cucurbits and elicits protection against the severe homologous virus. Phytopathology, 90: 467–473.

Gal-On A. (2007): *Zucchini yellow mosaic virus*: Insect transmission and pathogenicity – The tails of two proteins. Molecular Plant Pathology, 8: 139–150.

Glasa M., Pittnerová S. (2006): Complete genome sequence of a Slovak isolate of *Zucchini yellow mosaic virus* (ZYMV) provides further evidence of a close molecular relationship among central European ZYMV isolates. Journal of Phytopathology, 154: 436–440.

Kheder A.A., Taqwa M., Sulaiman T.M., Ghanem G.A.M., Tohamy M.R. (2017): Biological, serological and molecular characterization of Egyptian *Zucchini yellow mosaic virus* isolate infecting squash plants in Fayoum governorate. Egyptian Journal of Phytopathology, 45: 1–14.

Kone N., Asare-Bediako E., Silue S., Kone D., Koita O., Wulf M., Stephan W. (2017): Influence of planting date on incidence and severity of viral disease on cucurbits under field condition. Annals of Agricultural Sciences, 62: 99–104.

Kumar S., Stecher G., Tamura K. (2016): MEGA7: Molecular evolutionary genetics analysis, version 7.0. for bigger datasets. Molecular Biology and Evolution, 33: 1870–1974.

Kwon S.W., Kim M.S., Choi H.S., Kim K.H. (2005): Biological characteristics and nucleotide sequences of three Korean isolates of *Zucchini yellow mosaic virus*. Journal of General Plant Pathology, 71: 80–85.

Lecoq H., Pitrat M., Clément M. (1981): Identification et caractérisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. Agronomie, 1: 827–834.

Lin S.S., Hou R.F., Yeh S.D. (2000): Heteroduplex mobility and sequence analyses for assessment of variability of *Zucchini yellow mosaic virus*. Phytopathology, 90: 228–235.

Lin S.S., Hou R.F., Yeh S.D. (2001): Complete genome sequence and genetic organization of a Taiwan isolate of *Zucchini yellow mosaic virus*. Botanical Bulletin of Academia Sinica, 42: 243–250.

Lisa V., Boccardo G., D' Agostino G., D' Aquilio M. (1981): Characterization of a potyvirus that causes *Zucchini yellow mosaic*. Phytopathology, 71: 667–672.

Maghamnia H.R., Hajizadeh M., Azizi A. (2018): Complete genome sequence of *Zucchini yellow mosaic virus* strain Kurdistan, Iran. 3 Biotech, 8: 147. doi: 10.1007/s13205-018-1177-3

Maina S., Coutts B.A., Edwards O.R., Almeida L., Kehoe M.A., Ximenes A., Jones R.A.C. (2017): *Zucchini yellow mosaic virus* populations from East Timorese and Northern Australian cucurbit crops: Molecular properties, genetic connectivity, and biosecurity implications. Plant Disease, 101: 1236–1245.

Massumi H., Shaabanian M., Heydarnejad J., Hosseini Pour A.H., Rahimian H. (2011): Host range and phyloge-

https://doi.org/10.17221/191/2020-PPS

netic analysis of Iranian isolates of *Zucchini yellow mosaic virus*. Journal of Plant Pathology, 93: 187–193.

Mohamed M. F., Refaei E.F.S., Shalaby G.I. (2003): Growth and yield of inbred zucchini squash (*Cucurbita pepo* L.) lines developed under adverse climatic conditions. Assiut University Bulletin for Environmental Researches, 6: 109–114.

Moradi Z., Mehrvar M., Nazifi E. (2019): Population genetic analysis of *Zucchini yellow mosaic virus* based on the *C* gene sequence. Journal of Cell and Molecular Research, 10: 76–89.

Müller C., Bröther H., Von Bargen S., Büttner C. (2006): *Zucchini yellow mosaic virus* incidence and sources of virus infection in field-grown cucumbers and pumpkins in the Spreewald, Germany. Journal of Plant Diseases and Protection, 113: 252–258.

Nasr-Eldin M. A., Hayam S., Abdelkader H.S., Abo-Senna A.S., Othman B.A. (2016): Characterization and phylogenetic analysis of *Zucchini yellow mosaic virus* infecting *Cucurbita pepo* in Egypt. Journal of American Science, 12: 93–104.

Prendeville H.R., Ye X., Morris J., Pilson D. (2012): Virus infections in wild plants are both frequent and often unapparent. American Journal of Botany, 99: 1033–1042.

Romay G., Lecoq H., Geraud-Pouey F., Chirinos D.T., Desbiez C. (2014): Current status of cucurbit viruses in Venezuela and characterization of Venezuelan isolates of *Zucchini yellow mosaic virus*. Plant Pathology, 63: 78–87.

Sharma A., Gaur D. (2015): First report of *Zucchini yellow mosaic virus* on Muskmelon in India. Journal of Plant Pathology, 97: 541–551.

Shiboleth Y.M., Haronsky E., Leibman D., Arazi T., Wassenegger M., Whitham S.A., Gaba V., Gal-On A. (2007): The conserved FRNK box in HC-Pro, a plant viral suppressor of gene silencing, is required for small RNA binding and mediates symptom development. Journal of Virology, 81: 13135–13148.

Simmons H.E., Dunham J.P., Zinn K.E., Munkvold G.P., Holmes E.C., Stephenson, A.G. (2013): *Zucchini yellow mosaic virus* (ZYMV, *Potyvirus*): Vertical transmission, seed infection and cryptic infections. Virus Research, 176: 259–264.

Simmons H.E., Holmes E.C., Gildow F.E., Bothe-Goralczyk M.A., Stephenson A.G. (2011): Experimental verification of seed transmission of *Zucchini yellow mosaic virus*. Plant Disease, 95: 751–754.

Spadotti D.M.A., Wassano D.T., Rezende J.A.M., Camargo L.E.A., Inoue-Nagata A.K. (2015): Biological and molecular characterization of Brazilian isolates of *Zucchini yellow mosaic virus*. Scientia Agricola, 72: 187–191.

Tymchyshyn O., Shevchenko T., Shevchenko O., Budzanivska I. (2017): Phylogenetic analysis of seed-transmitted isolate of *Zucchini yellow mosaic virus*. Bulletin of Taras Schevchenko National University of Kyiv, 2: 47–50.

Wang D., Li G. (2017): Biological and molecular characterization of *Zucchini yellow mosaic virus* isolates infecting melon in Xinjiang, China. Canadian Journal of Plant Pathology, 39: 48–59.

Received: December 31, 2020
Accepted: June 16, 2021
Published online: September 7, 2021