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Research Article

FIRST REPORT OF Cucumis melo endornavirus WITH A NEW HOST, THE GHERKIN (*Cucumis anguria* Linn.), IN TURKEY

Ali KARANFİL*, Savaş KORKMAZ

Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, 17100, Çanakkale, TURKEY

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Yıldız Aydın

Corresponding Author:
Ali Karanfil
ali.karanfil@hotmail.com

ORCID ID:
orcid.org/0000-0002-4503-6344

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Abstract: More than 50 viruses causing infection in members of the family Cucurbitaceae have been identified in the world so far. Because of the development of virus detection methods, new viruses are added to the known infectious cases list every day. One of the viruses recently identified is the Cucumis melo endornavirus (CmEV) which has been reported from different countries all over the world. However, no study for determination of CmEV has been done so far in Turkey. For the purpose of this study, 59 cucurbit plants showing virus and virus-like symptoms were collected from Manisa and İzmir provinces in Turkey. The samples were tested, for presence of CmEV, by reverse-transcriptase polymerase chain reaction and CmEV infections were detected in 47 samples, of which 44 were melon (*Cucumis melo* Linn.), and the remaining were gherkins (*C. anguria* Linn.). Among the infected samples, five samples (three were melon, and two were gherkin) were chosen for sequence analysis. After evaluating the sequence analysis results, it was shown that the Turkish isolates presented 93%-99% and 93%-98% identities at the nucleotide level and 94%-99% and 91%-98% identities at the amino acid level among each other and worldwide isolates, respectively. To the best of our knowledge, this is the first report of CmEV in gherkin as a new host both in Turkey and worldwide.

Özet: Dünyada gerçekleştirilen çalışmalar sonucunda kabakgil familyasındaki bitkileri enfekte eden 50'den fazla virüs varlığı tanımlanmıştır. Virüs tespit metotlarındaki gelişmelere bağlı olarak bu sayıya her geçen gün yenileri eklenmektedir. Son yıllarda tanılanan bu virüs hastalıklarından bir tanesi de Cucumis melo endornavirus (CmEV)'dır. CmEV'nin enfeksiyonu dünyanın farklı ülkelerinden bildirilmiştir. Ancak, ülkemizde şimdiye kadar CmEV varlığının belirlenmesine yönelik bir çalışma gerçekleştirilmemiştir. Bu amaçla, Manisa ve İzmir illerinden virüs ve virüs-benzeri belirti gösteren 59 kabakgil bitkisinden örnekler alınmıştır. Toplanan örnekler ters transkriptaz-polimeraz zincir reaksiyonu ile test edilmiştir. Gerçekleştirilen bu testler sonucunda 47 örnekte CmEV enfeksiyonu tespit edilmiştir. Enfekteli 47 örneğin 44 tanesi kavun (*Cucumis melo* Linn.), kalan 3 örnek ise acurdur (*C. anguria* Linn.). Enfekteli örnekler arasından, beş tanesi (üç tanesi kavun, iki tanesi acur) sekans analizleri için seçilmiştir. Gerçekleştirilen sekans analizleri sonucunda CmEV izolatları kendi aralarında nükleotit düzeyinde %93-99, amino asit düzeyinde ise %94-99 benzerlikler gösterdiği belirlenmiştir. Dünya izolatları ile yapılan benzerlik analizleri sonucunda ise nükleotit düzeyinde %93-98, amino asit düzeyinde ise %91-98 benzerlikler tespit edilmiştir. Gerçekleştirilen bu çalışma ile ülkemizde ilk kez CmEV enfeksiyonu kavun ve dünya için yeni bir konukçu kaydı olan acur bitkisinde tespit edilmiştir.

Introduction

Turkey is characterized with a wide range of ecological diversity which allows cultivation of different plant taxa one of which includes the cucurbit plants in the family Cucurbitacea (Çat *et al.* 2016). The currently known 825 species of this family are placed in 118 genera (Jeffrey 1980). The most important species of this family include melon (*Cucumis melo* Linn.), watermelon (*Citrullus lanatus* Thumb.), gherkin (*Cucumis anguria* Linn.), and zucchini/squash/pumpkin (*Cucurbita* spp.) all which can be cultivated in almost every region of the world and provide important economic inputs to their producers.

There are many viral diseases known to affect members of this important plant family (Zitter *et al.* 1996) by causing economic losses. New viruses are increasingly being added to the list of disease agents and they threaten the production of cucurbit plants. Some of these viruses are routinely studied, while others are not. Therefore, there is no reliable available information about the importance and prevalence of these viruses.

A number of viruses infecting cucurbit plants in Turkey were identified (Köklü & Yilmaz 2006, Ozaslan *et al*. 2006, Kamberoglu *et al*. 2016), among which the most prevalent viruses are the Watermelon mosaic virus, Cucumber mosaic virus, and the Zucchini yellow mosaic virus (Kaya & Erkan 2011, Keçe & Kamberoğlu 2016, Topkaya *et al*. 2019).

Cucumis melo endornavirus (CmEV) is one of the recently identified viral disease agent infecting the cucurbit plants. CmEV is a member of the endornavirus family that infects plants and fungi. The causal agent has (+)ssRNA genome containing nucleotides of around 15 kb (Valverde *et al*. 2019).

The presence of CMeV has so far been reported only from Ecuador, USA and Brazil (Quito-Avila *et al*. 2014, Sabanadzovic *et al*. 2016, da Costa *et al*. 2019). Based on a Japanese study, it was shown that dsRNA bands close to the CmEV genome were obtained from melon via dsRNA analysis (Fukuhara *et al*. 2006). CmEV sequences from South Korea are available in the GenBank. However, there has been no attempt in Turkey so far to detect the presence of CmEV infection. In the present study, field studies were performed on cucurbit plants grown in Manisa and İzmir provinces on Turkey and the presence of the CmEV as the causal agent was investigated.

Materials and Methods

Sampling and virus detection

Samples were collected in randomly selected fields in İzmir and Manisa provinces among cucurbit plants only showing the virus and virus-like symptoms (Fig. 1). The field studies were carried out from June to September in 2019. When similar symptoms were observed in the same field, only three samples were collected.

The presence of CmEV in the collected samples was determined by reverse-transcriptase polymerase chain reaction (RT-PCR) using a virus-specific primer pair. Before performing RT-PCR, total nucleic acid (TNA) isolation was performed by the cetyl trimethylammonium bromide (CTAB) method (Li *et al*. 2008). The resulting TNAs were checked by agarose gel electrophoresis and stored at -80 ºC until used.

To determine the presence of CmEV in the resulting TNAs, complementary DNAs (cDNAs) were initially synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific ™, USA). The presence of CmEV was then screened by PCR with the CmEV-primer pair (Table 1) specific to the partial polyprotein gene. PCR tests were performed according to conditions as indicated by Quito-Avila *et al*. (2014) using 2X EmeraldAmp® Max PCR Master Mix (Takara, Japan).

Fig. 1. Map showing the provincial borders of the two provinces (shaded in green) in the western part of Turkey where the field samplings were performed. The numbers in parentheses correspond to the sampling numbers in each province.

Sequence analysis

Five samples were chosen among the samples which were accepted to be infected with CmEV based on host species and the geographic origin, where they were obtained from. The resulting RT-PCR products were purified by EZ-10 Spin Column PCR Products (BioBasic, Canada) and sequenced bilaterally.

The assembled sequences were used to match with other world CmEV isolates from the GenBank (Table 2). Sequence identities of the isolates were determined with the sequence Demarcation Tool V. 1.2 (Muhire *et al*. 2014). The phylogenetic relationship was determined in CLC Main Work Bench V. 20 packet program (Qiagen, Canada) using the neighbor-joining method by applying Kimura 2-parameter with 1000 bootstrap replications.

Table 1. Primer pair used in RT-PCR for determination of CmEV infection in the collected samples.

Primer code	Primer sequence (5'-3')	Sense	Fragment size	Reference
CmEVF	GGTGGAATATGGGTTGATGCTAG	Forward	413 bp	Quito-Avila *et al*. (2014)
CmEVR	CGTCGTGATGGACATCAACTCTAC	Reverse		

Table 2. CmEV isolates used in molecular characterization studies

Isolate Code	Accession Number	Host	Origin	Reference
SJ1	KX641269	Korean melon	South Korea	Baek *et al.* (2016)
CL-01	NC_029064	Melon	USA	Sabanadzovic *et al.* (2016)
BRA/TO-74/2010	MH365459	Not known*	Brazil	da Costa *et al.* (2019)
BRA/TO-23/2014	MH365458	Not known*	Brazil	da Costa *et al.* (2019)
MAN2	MN985120	Melon	Turkey	This study
MAN22	MN985121	Gherkin	Turkey	This study
MAN25	MN985122	Melon	Turkey	This study
IZM7	MN985123	Gherkin	Turkey	This study
IZM36	MN985124	Melon	Turkey	This study

*found in human stool samples

Results

A total of 59 cucurbit plants with viruses and virus-like symptoms were collected. Forty-nine of these were melon, seven were pumpkins, and three were gherkins. CmEV was detected in 47 of the samples (Table 3).

With the exception of five collected melon samples, nearly all were found to be infected with CmEV. Three CmEV infections were detected in gherkin plants. CmEV infection was detected in none of the pumpkin plants.

As a result of the sequence analysis performed, it was determined that Turkish CmEV isolates showed 93%-99% and 94%-99% identities with each other at the nucleotide and amino acid levels, respectively. In multiple sequence alignments with world CmEV isolates, it was observed that Turkish CmEV isolates showed 93%-98% and 91%-98% identities at the nucleotide and amino acids levels, respectively. The highest nucleotide identity rate between the Turkish and world CmEV isolates was found to be 98% between MAN25 with BRA/TO-74/2010 and BRA/TO-23/2014 isolates, while the least nucleotide identity was found to be 93% between MAN22 with CL01 and SJ1 isolates (Fig. 2).

Based on the phylogenetic analysis, all of the Turkish CmEV isolates, except MAN25 and IZM36, were closely related and formed a separate clade. While the world isolates form a clade with MAN25, IZM36 was found as a separate clade that consists of only itself (Fig. 3).

Table 3. The numerical distribution of the sampled cucurbits in each province. The numbers of infected samples were also given for each different plant.

Cucurbit Species	Province		Total Number of Infected and Collected Samples
	Manisa	İzmir	
	Number of Infected/Collected Samples	Number of Infected/Collected Samples	
Melon	24/24	20/25	44/49
Pumpkin	0/1	0/6	0/7
Gherkin	2/2	1/1	3/3
Total Number of Infected/Collected Samples	26/27	21/32	47/59

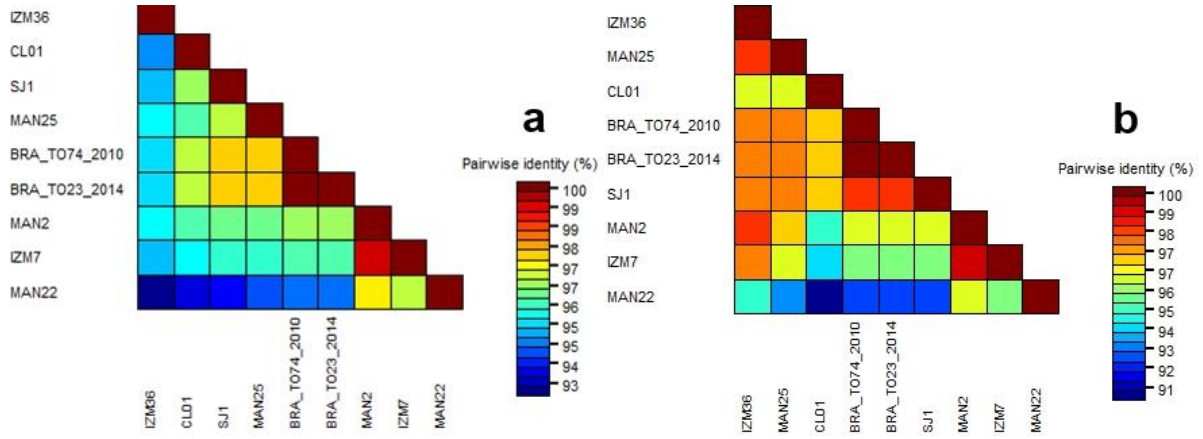

Fig. 2. Identity matrix of Turkish and world CmEV. The colored identity matrix was generated by using partial polyprotein gene region of CmEV based on nucleotide (a) and amino acid (b) sequences.

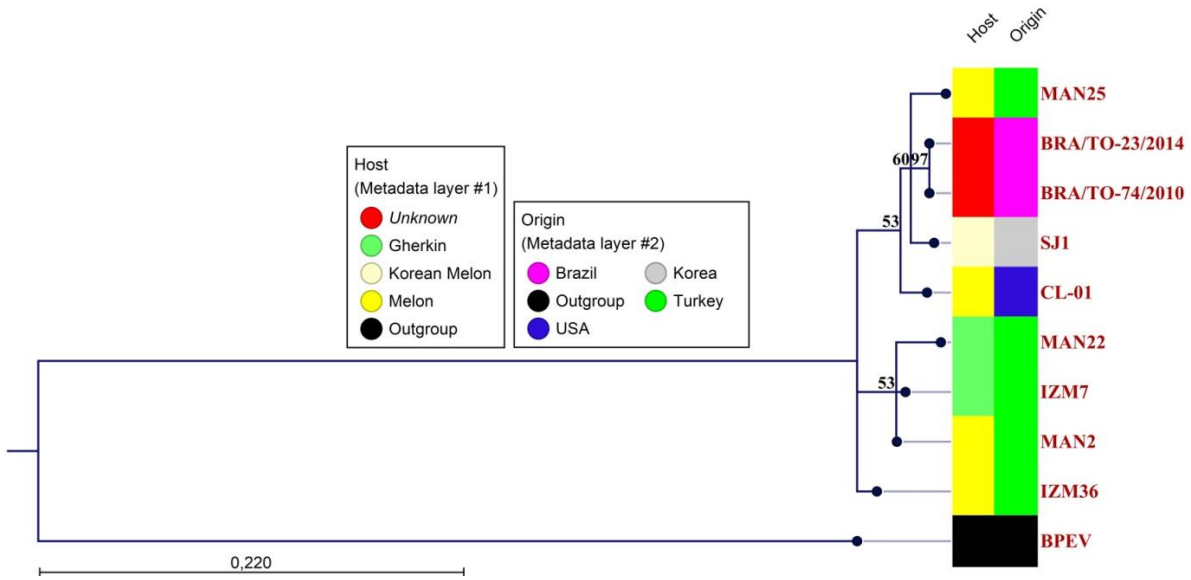

Fig. 3. Phylogenetic relationship of Turkish CmEV isolates based on the partial polyprotein gene region. The phylogenetic tree was generated by neighbor-joining method applying Kimura 80 parameters with 1000 bootstrap replications. The bootstrap threshold was implemented at 50%. Bell pepper endornavirus (BPEV) was used as the outgroup.

Discussion

Although the presence of CmEV from different countries has been reported, only one study (Quito-Avila *et al*. 2014) about the prevalence of the causal agent comes to the forefront. As a result of this study conducted in cucurbit production fields in Ecuador, it was reported that 95% of melon plants with and without virus-like symptoms were infected with CmEV. No CmEV infection was detected in watermelon and cucumber plants (Quito-Avila *et al*. 2014). In this context, the results obtained in the present study were found to be in parallel with the results of Quito-Avila *et al*. (2014).

It is known that endornaviruses infect plants and fungi. There are many economically important crops that are infected with these viruses. Some of these crops are rice, barley, pepper, and common bean (Wakarchuk & Hamilton 1985, Zabalgogeazcoa & Gildow 1992, Fukuhara *et al*. 1993, Okada *et al*. 2011). In a recent study from the United States (US), endornaviruses were detected in non-cultivated plant species (Herschlag *et al*. 2019). In addition to the known host range of endornaviruses, gherkin was found to be a new host for CmEV as the result of this study.

In a study carried out in the US, it was reported that CmEV populations had 10% and 6% genetic variation with each other at the nucleotide and amino acid levels, respectively (Sabanadzovic *et al*. 2016). In this context, the identity rates obtained in this study showed great parallelism with that study.

It was determined that the isolates did not show phylogenetic distribution according to their geographic origin and host (Fig. 3). However, it is considered necessary to perform analyses using a much larger number of isolates with larger sequences for more reliable results.

As a result of the recent worldwide studies, the host range of endornaviruses has been expanded. However, the impact of these viruses on crop yields or growth parameters is still unclear (Escalante *et al*. 2016, Fukuhara 2019), especially in cases in which the CmEV infected plant is co-infected with severe viruses. To the best of our knowledge, this is the first report of CmEV with gherkin (*C. anguria* Linn.) as a new host both in Turkey and worldwide. Moreover, it is thought that the virus may present a more extensive worldwide distribution than previously reported.

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