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Short Notes

Characterization of two *Cucumber mosaic virus* isolates infecting *Allium cepa* in Turkey

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Summary. *Cucumber mosaic virus* (CMV) is polyphagous, infecting plants in several families. CMV has occurred as a minor pathogen in *Allium* crops in several Mediterranean countries, but little was known of the virus naturally infecting *Allium* spp. This study completed molecular and biological characterization of CMV-14.3Po and CMV-15.5Po, two newly identified CMV isolates infecting onion (*Allium cepa* L.) in Turkey. Phylogenetic, and nucleotide and amino acid sequence identity analyses of partial RNA2 and RNA3 of the two isolates showed that they were very similar to other CMV isolates from Mediterranean, European, and East Asian countries. Phylogenetic analysis of the partial sequence of RNA3 also showed that the onion isolates belong to subgroup IA. Onion isolates were mechanically transmissible, and caused mild leaf malformation on onion, severe leaf malformation and stunting on garlic (*Allium sativus* L.), and mosaic and mottle on cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.).

Keywords. Onion, phylogenetic analysis, host indexing.

INTRODUCTION

Allium crops, including onion (*Allium cepa* L.), garlic (*Allium sativus* L.), and leek (*Allium ampeloprasum* L.), have been reported to be infected by viruses belonging to *Potyvirus*, *Carlavirus*, *Tospovirus*, and *Allexivirus* (Shahraeen *et al.*, 2008; Tomassoli *et al.*, 2009; Parrano *et al.*, 2012). Several of these viruses, including *Leek yellow stripe virus* (LYSV), *Onion yellow dwarf virus* (OYDV), *Iris yellow spot virus* (IYSV), *Shallot latent virus* (SLV), and *Garlic common latent virus* (GarCLV), are frequently found in *Allium* crops (Dovas and Volvas, 2003; Ward *et al.*, 2009; Sevik and Akcura, 2013; Bag *et al.*, 2015; Abraham *et al.*, 2019). Other viruses, including *Turnip mosaic virus* (TuMV), *Shallot yellow stripe virus* (SYSV), and *Groundnut bud necrosis virus* (GBNV), have been considered as minor pathogens that occasionally infect *Allium* spp. only in particular regions (Gera *et al.*, 1997; Chen *et al.*, 2005; Sujitha *et al.*, 2012).

Cucumber mosaic virus (CMV) (*Cucumovirus*, *Bromoviridae*) has a very broad host range. It usually causes diseases on plant in *Cucurbitaceae*, *Sola-*

naceae, *Brassicaceae*, and *Fabaceae*, and typically induces mild to severe systemic mosaic symptoms on these plants (Brunt *et al.*, 1996). More than 80 aphid species have been recorded to non-persistently non-circulatively transmit CMV (Palukaitis and Garcia-Arenal, 2003). CMV is a tripartite virus, with the genome in three single-stranded plus-sense RNAs (RNA1-3). RNA1 encodes 1a protein and RNA2 encodes 2a protein, which are both involved in the replicase complex (Hayes and Buck, 1990). A small 2b protein involved in cell-to-cell movement, suppression of post-transcriptional gene silencing, and symptom induction, is also expressed in RNA2 (Bujarski *et al.*, 2019). RNA3 encodes 3a protein (movement protein (MP)) and coat protein (CP), which are important in viral movement processes (Boccard and Baulcombe, 1993).

CMV was probably first recorded infecting *Allium* when it was serologically detected in a single garlic sample in Zagreb, Croatia (formerly Yugoslavia) (Stefanac, 1980). A large survey on *Allium* viruses in the East Mediterranean region of Turkey identified CMV as a minor virus infecting garlic in the area (Fidan, 2010). The CMV-ALC1 isolate was detected in leek in Alicante, Spain, and its nucleotide sequence was registered in NCBI GenBank with accession no. JN806091 (Alfaro-Fernandez *et al.*, unpublished). These findings showed presence of CMV isolates naturally infecting *Allium* crops in different Mediterranean regions.

Recently, two onion samples from Ankara province in Turkey tested positive for CMV and negative for LYSV, OYDV, GarCLV, and SLV infections. The two CMV isolates were subsequently named CMV-14.3Po and CMV-15.5Po, and the partial nucleotide (nt) sequence of their RNA2 were deposited in NCBI GenBank (accession no. MN070136-37). Initial phylogenetic analysis showed that the isolates were similar to isolates from Iran, Serbia, Hungary, Poland, Germany, Japan, China, and South Korea, which indicated very wide distribution of CMV isolates genetically similar to onion isolates (Santosa and Ertunc, 2020).

The relationship between CMV and *Allium* spp. still requires investigation, because CMV is prevalent and many *Allium* spp. are economically important crops. Wide phylogenetic analyses of partial nt sequences of RNA2 and RNA3, and host indexing of CMV-14.3Po and CMV-15.5Po, are presented in this study, to provide further knowledge about CMV infection in onion and other *Allium* spp.

MATERIALS AND METHODS

Nucleotide sequences of RNA2 of onion isolates

The previously reported partial nt sequences of RNA2 were 521 bp long (Santosa and Ertunc, 2020), covering 27 bp of 5' UTR and 494 bp of partial 2a protein gene (1-494 position), for reference isolate accession no. D10538. These are the only nt sequences of CMV isolates naturally infecting onion that are currently available in NCBI GenBank. Therefore, a portion of their RNA3 was also sequenced in the present study to provide more information about their genetic makeup.

RT-PCR and sequencing of partial RNA3

Total nucleic acid was extracted from onion samples using tris-EDTA buffer (100 mM tris, 50 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol, pH 8.0) (Presting *et al.*, 1995). RT-PCR was performed in two stages (Santosa and Ertunc, 2020). A primer pair F-GTAGACATCTGTGACGCGA and R-GCGC-GAAACAAGCTTCTTATC was used in the PCR to amplify 540 bp of RNA3 (De Blas *et al.*, 1994).

PCR cycles were carried out using a thermocycler (Biometra, Germany). Each cycle consisted of initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 60 s, annealing at 54°C for 60 s, elongation at 72°C for 60 s, and a final extension at 72°C for 10 min. Products were visualized on a 1% (w/v) tris-acetate agarose gel stained with ethidium bromide. Successfully amplified RT-PCR products were sent to BM Lab. Ltd. (Ankara, Turkey) for purification and Sanger sequencing. The obtained nt sequences were then submitted to NCBI GenBank.

Neighbor-joining tree construction, and nucleotide and amino acid sequences identity percentage calculation

Genes that were homologous to Turkish onion isolates were determined using the BLAST program from the the NCBI website. The Turkish onion isolates were then compared with reference CMV isolates from GenBank. The selected CMV isolates were mostly those which each had both RNA2 and RNA3 sequences available in GenBank. All sequences were aligned using ClustalW version 1.6, with default parameters in MEGA7 software (megasoftware.net). MEGA7 then applied a Neighbor-joining algorithm with both transition and transversion substitutions, and uniform rates, to construct two phylogenetic trees for RNA2 and RNA3

comparisons (Hall, 2013). Statistical significance of isolate clusters were tested using 1000 bootstrap replicates in the Tamura 3 parameter model (Tamura, 1992). Nucleotide (nt) and amino acid (aa) similarity percentages among tested isolates were estimated using Sequence Demarcation Tool (SDT) v1.2 software (Muhire *et al.*, 2014).

Host indexing

Each onion isolate of CMV was mechanically inoculated to four onion, garlic, cucumber (*Cucumis sativus* L.), and melon (*Cucumis melo* L.) plants, to determine host responses. Prior to inoculation, plants were tested to be CMV-free by RT-PCR. Onion and garlic plants were also tested to be free from LYSV, SLV, GarCLV, and OYDV infections using RT-PCR and specific primers for each virus (Fajardo *et al.*, 2001; Majumder *et al.*, 2008; Parrano *et al.*, 2012; Nam *et al.*, 2015).

Plant sap was prepared by grinding 1 g of naturally infected onion leaf samples in 5 mL of 0.01 M potassium phosphate buffer (pH 7) using a mortar and pestle. Leaves of treated plants were dusted with abrasive-celite, then plant sap was manually rubbed on the leaves. Four onion, garlic, cucumber, and melon plants were each dusted with abrasive-celite then rubbed with potassium phosphate buffer as controls. Ten minutes after treatment, inoculated and control plants were rinsed with distilled water. The plants were then kept in a greenhouse at 24–28°C, and any symptoms were recorded during the following 2 to 5 weeks (Hill, 1984; Ohno *et al.*, 1997; Hull, 2009). At 5 weeks after inoculation, a composite sample of leaves of the inoculated plants of the each species were collected for RT-PCR. This allowed onion isolates infection on each plant species to be confirmed by a small number of RT-PCRs, but infection rates were unknown. A primer pair of F-GTTTATTTACAAGAGCGTACGG and R-GGTTTCGAA(AG)(AG)(AT)ATAACCGGG, to amplify 650 bp of RNA2, was used in the PCR (Finetti Sialer *et al.*, 1999).

RESULTS

Sequencing of RNA3

The obtained partial nt sequences of RNA3 were 540 bp long, covering 216 bp of 5' UTR and 324 bp of the partial CP gene (1–324 position) for reference isolate accession no. D10538. NCBI GenBank accession no. MN864792-93 were acquired for them.

Phylogenetic analysis and nucleotide and amino acid sequences identity percentage

The phylogenetic tree constructed based on the partial RNA2 comparison was divided into four groups (1–4). The two onion isolates clustered with 21 other isolates in Group 1. The tree constructed based on the partial RNA3 comparison was also divided into four groups (1–4). However, the onion isolates only clustered in Group 1 with 15 other CMV isolates, which showed that not all isolates used in this study had high identities to the onion isolates in their RNA2 and RNA3 sequences (Figure 1).

CMV-14.3Po and CMV-15.5Po shared very high nt and aa sequence similarities in both RNA2 and RNA3. All the compared CMV isolates showed high nt (91.2–99.4%) and aa (89–100%) in partial RNA2 to onion isolates. The compared CMV isolates showed relatively lower nt (86.7–99.8%) and aa (81.8–100%) sequences to the onion isolates in comparison of partial RNA3 (Table 1).

TUR84, TUR86, I17F, PV0187, Ns, Rs, Gd, Ri-8, CMV21, NND454J and Can isolates had very high nt and aa similarities in both RNA2 and RNA3 sequences to the onion isolates, and also were clustered in the same group with onion isolates in both phylogenetic trees. The outcome showed that these isolates were genetically more similar to CMV Turkish onion isolates than the other virus isolates compared in this study.

Host indexing

Both of the onion isolates caused mild leaf malformations on all the inoculated onion plants, which was difficult to differentiate from the controls. Severe leaf malformations and stunting were observed on all inoculated garlic plants. Mottle symptoms appeared on leaves of most of the cucumber and melon plants inoculated with both onion isolates. Only one cucumber and one melon plant produced clear mosaic symptoms after inoculation with isolate CMV-14.3Po (Table 2; Figure 2). Infections on all the tested plant species were confirmed by RT-PCR (Figure 3).

DISCUSSION

Based on partial RNA2 and RNA3 sequence comparisons, the two onion isolates (CMV-14.3Po and CMV-15.5Po) had high similarities to at least 11 other CMV isolates. Some of these other isolates were also originally from Turkey, while some were from geographically close countries (Iran, Hungary, Germany, Austria,

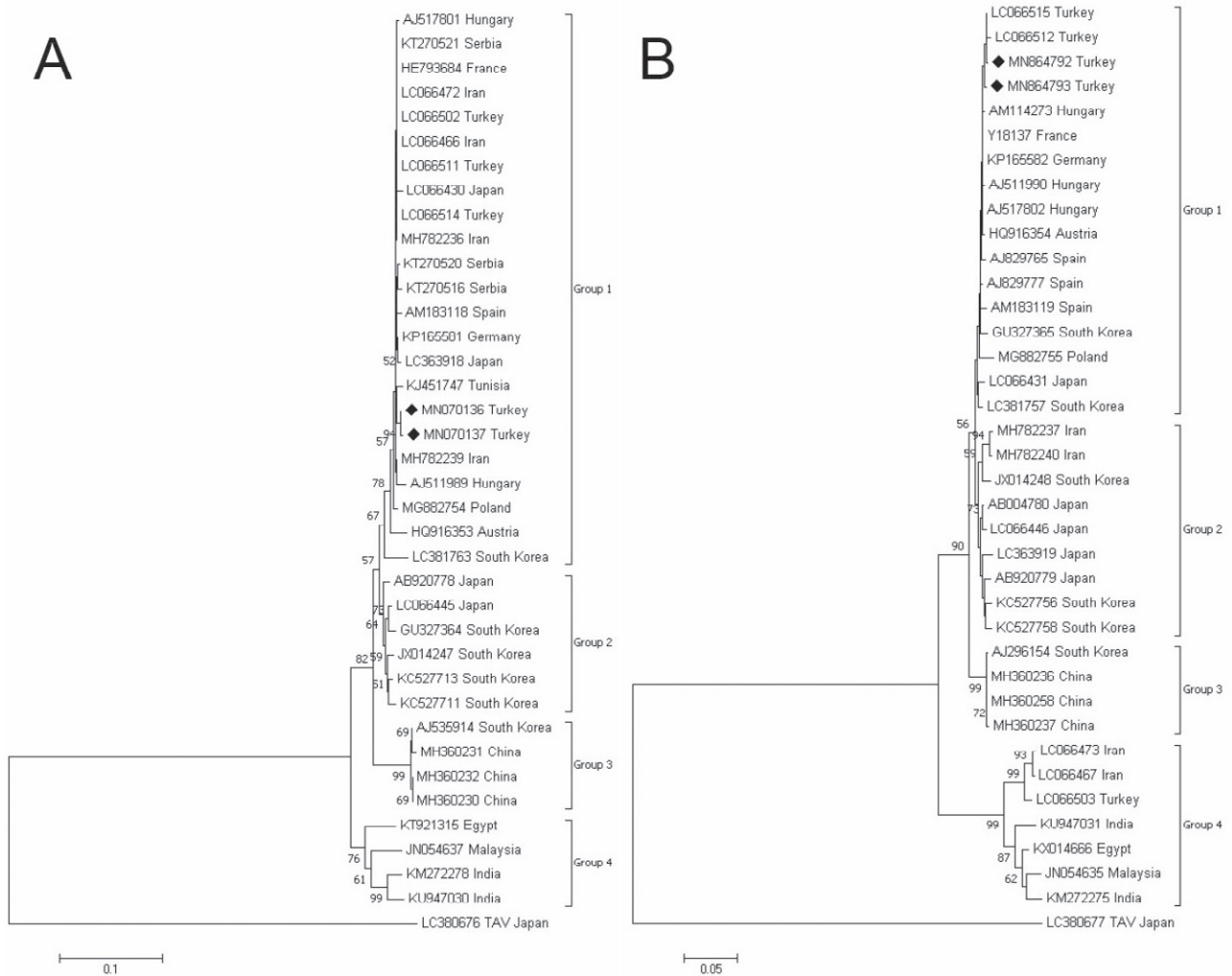


Figure 1. A. Phylogenetic tree based on nucleotide sequence comparisons of partial RNA2 of two onion isolates (MN070136-37) and 35 other CMV isolates. B. Phylogenetic tree based on nucleotide sequence comparisons of partial RNA3 of two onion isolates (MN864792-93) and 35 other CMV isolates. Bootstrap values on each branch were supported by 1000 replicates, and only values greater than 50% are shown. An isolate of *Tomato aspermy virus* (TAV Japan) was used as out-group.

France, Spain, and Poland), and some others were from East Asian countries (South Korea and Japan). The two onion isolates were also similar to three Serbian isolates (650-07, 581-11, and 473-12) and one Tunisian isolate (89-2012) only in the RNA2 sequence comparisons, since these isolates have no information on their RNA3 sequences available in NCBI GenBank. In only the RNA3 sequence comparisons, the two onion isolates were highly homologous to one Hungarian isolate (Le02) and two Spanish isolates (MAD01/2 and BAR96/1).

There is no sequence of CMV isolates from Croatia available in NCBI GenBank to be compared to onion isolates examined in the present study, but the onion isolates were very similar to isolates from Serbia, Hungary,

and Austria, which are direct neighbours of Croatia. Thus, there was possibility that the CMV garlic isolate (CMV-G) that was reported in Croatia (Stefanac, 1980) also had high nt sequence similarities to CMV-14.3Po and CMV-15.5Po.

Two Iranian CMV isolates (IRN-REY4 and IRN-REY10) and one Turkish isolate (TUR54) all from radish (*Raphanus sativus* L.) were very similar in RNA2 sequences, but were divergent in RNA3 sequences, in comparison to the two onion isolates. These isolates clustered together with onion isolates in Group 1 of the phylogenetic tree of RNA2, but clustered in Group 4 of the tree of RNA3, which was very distant to the onion isolates. The RNA3 of CMV was known to be little conserved, as was also shown by the sequences iden-

Table 1. CMV isolates used in this study. The partial RNA2 and RNA3 nucleotide and amino acid sequence percentages to onion isolates are indicated.

No.	Isolate name ^a	Origin	Host	RNA2 comparison				RNA3 comparison			
				Accession No.	Phylo group	nt identity (%)	aa identity (%)	Accession No.	Phylo group	nt identity (%)	aa identity (%)
1.	14.3Po^b	Turkey	<i>Allium cepa</i>	MN070136	1	100	100	MN864792	1	100	100
2.	15.5Po^b	Turkey	<i>Allium cepa</i>	MN070137	1	100	100	MN864793	1	100	100
3.	Rs	Hungary	<i>Raphanus sativus</i>	AJ517801	1	99.0-99.2	99.4	AJ517802	1	99.4-99.6	100
4.	IRN-REY4	Iran	<i>Raphanus sativus</i>	LC066466	1	99.2-99.4	100	LC066467	4	87.8-88.3	83.5
5.	TUR86	Turkey	<i>Rapistrum rugosum</i>	LC066514	1	99.2-99.4	100	LC066515	1	99.6-99.8	100
6.	TUR84	Turkey	<i>Rapistrum rugosum</i>	LC066511	1	99.2-99.4	100	LC066512	1	99.3-99.4	99.4
7.	TUR54	Turkey	<i>Raphanus sativus</i>	LC066502	1	99.2-99.4	100	LC066503	4	87.8-88	81.8
8.	MeEs	Iran	<i>Cucumis melo</i>	MH782236	1	99.2-99.4	100	MH782237	2	97.0-97.2	98.9
9.	IRN-REY10	Iran	<i>Raphanus sativus</i>	LC066472	1	99.2-99.4	100	LC066473	4	87.6-88.1	83
10.	NND454J	Japan	<i>Raphanus sativus</i>	LC066430	1	98.7-98.8	100	LC066431	1	98.5-98.7	98.3
11.	I17F	France	<i>Solanum lycopersicum</i>	HE793684	1	99.2-99.4	100	Y18137	1	99.4-99.6	100
12.	Ri-8	Spain	<i>Solanum lycopersicum</i>	AM183118	1	98.7-98.8	100	AM183119	1	98.7-98.9	98.9
13.	PV0187	Germany	-	KP165581	1	99.0-99.2	100	KP165582	1	99.4-99.6	100
14.	Co-46	Japan	<i>Corchorus olitorius</i>	LC363918	1	98.7-98.8	100	LC363919	2	97.0-97.2	98.3
15.	SqSh	Iran	<i>Cucurbita pepo</i>	MH782239	1	99.0-99.2	100	MH782240	2	97.2-97.4	98.9
16.	Ns	Hungary	<i>Nicotiana glutinosa</i>	AJ511989	1	98.5-98.7	99.4	AJ511990	1	99.3-99.4	100
17.	CMV21	Poland	<i>Cucurbita pepo</i>	MG882754	1	98.5-98.7	100	MG882755	1	97.8-98	96.6
18.	Gd	Austria	<i>Cucurbita pepo</i>	HQ916353	1	97.1-97.3	97.1	HQ916354	1	99.3-99.4	100
19.	Can	S. Korea	<i>Canna generalis</i>	LC381763	1	96.5-96.7	97.7	LC381757	1	98.5-98.7	98.9
20.	650-07	Serbia	<i>Nicotiana tabacum</i>	KT270521	1	99.2-99.4	100	-	-	-	-
21.	581-11	Serbia	<i>Capsicum annuum</i>	KT270520	1	98.9-99	99.4	-	-	-	-
22.	473-12	Serbia	<i>Citrullus lanatus</i>	KT270516	1	98.7-98.8	99.4	-	-	-	-
23.	89-2012	Tunisia	<i>Cynara scolymus</i>	KJ451747	1	98.9-99	98.8	-	-	-	-
24.	Le02	Hungary	<i>Lycopersicon esculentum</i>	-	-	-	-	AM114273	1	99.4-99.6	100
25.	MAD01/2	Spain	<i>Diplotaxis eruroides</i>	-	-	-	-	AJ829765	1	99.1-99.3	99.4
26.	BAR96/1	Spain	<i>Cucumis melo</i>	-	-	-	-	AJ829777	1	99.1-99.3	99.4
27.	KM	Japan	<i>Cucumis melo</i>	-	-	-	-	AB004780	2	98.0-98.5	98.3
28.	m1	Japan	<i>Nicotiana tabacum</i>	AB920778	2	96.5-96.7	97.7	AB920779	2	97.2-97.4	98.3
29.	TKD766J	Japan	<i>Raphanus sativus</i>	LC066445	2	96.4-96.5	97.1	LC066446	2	97.6-98.1	98.3
30.	Rb	S. Korea	<i>Rudbeckia hirta</i>	GU327364	2	96.4-96.5	95.4	GU327365	1	98.5-98.7	99.4
31.	Va	S. Korea	<i>Vigna angularis</i>	JX014247	2	96.5-96.7	96.5	JX014248	2	97.2-97.8	98.3
32.	RP30	S. Korea	<i>Capsicum annuum</i>	KC527713	2	96.5-96.7	97.1	KC527758	2	97.0-97.2	97.2
33.	RP28	S. Korea	<i>Capsicum annuum</i>	KC527711	2	96.4-96.5	95.9	KC527756	2	97.0-97.2	97.7
34.	Ly2	S. Korea	<i>Lilium longiflorum</i>	AJ535914	3	93.7-93.9	94.2	AJ296154	3	96.5-96.7	94.3
35.	C4	China	<i>Lilium</i> spp.	MH360231	3	93.3-93.5	93.6	MH360236	3	96.7-96.9	94.9
36.	C6	China	<i>Lilium</i> spp.	MH360232	3	93.7-93.9	93.6	MH360237	3	96.5-96.7	94.3
37.	C2	China	<i>Lilium longiflorum</i>	MH360230	3	93.7-93.9	93.6	MH360258	3	96.7-96.9	94.9
38.	HM3	Egypt	<i>Solanum lycopersicum</i>	KT921315	4	93.1-93.2	91.3	KX014666	4	87.8-88.3	83
39.	CLW2	Malaysia	<i>Cucumis sativus</i>	JN054637	4	92.7-92.9	89.6	JN054635	4	87.0-87.6	83
40.	KO	India	<i>Capsicum annuum</i>	KM272278	4	91.7-91.9	88.4	KM272275	4	86.7-87.2	81.8
41.	ICAR-IISR PN25	India	<i>Piper nigrum</i>	KU947030	4	91.2-91.3	89	KU947031	4	87.2-87.4	83

^a Isolates that name typed in bold are those that both RNA2 and RNA3 sequences are available in NCBI GenBank.^b CMV onion isolates that were identified in this study.

Table 2. Symptoms caused from inoculations with two CMV isolates on different inoculated test plants.

Isolate	Plant species	Symptoms on inoculated plant number ^a			
		1	2	3	4
CMV-14.3Po	Onion	Mild leaf malformation	Mild leaf malformation	Mild leaf malformation	Mild leaf malformation
	Garlic	Severe leaf malformation and stunting	Severe leaf malformation and stunting	Severe leaf malformation and stunting	Severe leaf malformation and stunting
	Cucumber	Mottle	Mottle	Mosaic	Mottle
	Melon	Mottle	Mottle	Mottle	Mosaic and mottle
CMV-15.5Po	Onion	Mild leaf malformation	Mild leaf malformation	Mild leaf malformation	Mild leaf malformation
	Garlic	Severe leaf malformation and stunting	Severe leaf malformation and stunting	Severe leaf malformation and stunting	Severe leaf malformation and stunting
	Cucumber	Mottle	Mottle	Mottle	Mottle
	Melon	Mottle	Mottle	Mottle	Mottle

^a Mottle was considered mild, while mosaic was considered as a more severe symptom.

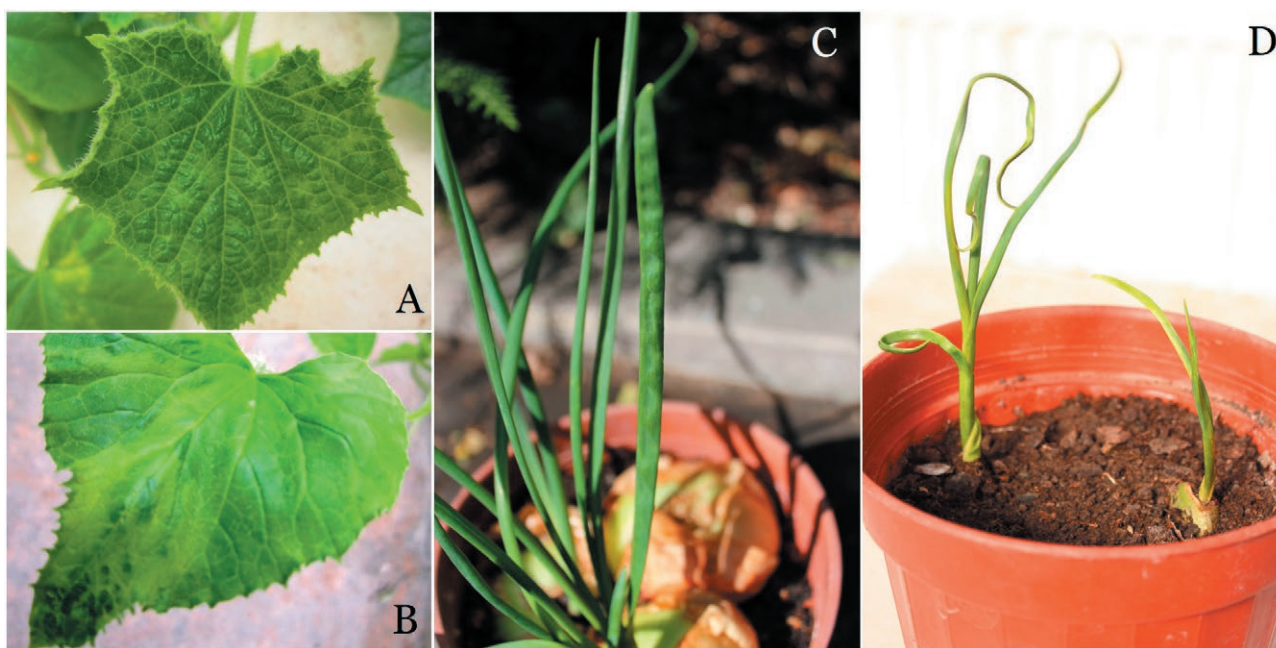


Figure 2. Symptoms on plants mechanically inoculated with two CMV isolates from onion. A. Mosaic on cucumber inoculated with CMV-14.3Po, B. Mosaic and mottle on melon inoculated with CMV-14.3Po, C. Mild leaf malformation on onion inoculated with CMV-15.5Po, D. Severe leaf malformation and stunting on garlic inoculated with CMV-15.5Po.

tity analyses of this study. Phylogenetic analyses on this region were useful for classification of CMV isolates into subgroups IA, IB, and II (Roossinck *et al.*, 1999). The two onion isolates belonged to subgroups IA according to phylogenetic analysis in the present study.

Isolates that have high genomic similarities to CMV-14.3Po and CMV-15.5Po were identified from different plant species from several families. As comparison, four isolates in Group 3 (Ly2-CMV, C2, C4, and C6) were

all identified from *Lilium* spp. Results from phylogenetic and similarity analyses also showed that CMV isolates with genome signature similar to CMV-14.3Po and CMV-15.5Po naturally had wide host ranges, and had been found in a broad geographic area, comprising Mediterranean, European, and East Asian countries. CMV should therefore be included in future virus surveys of *Allium* conducted in these regions, especially where CMV was known to be prevalent.

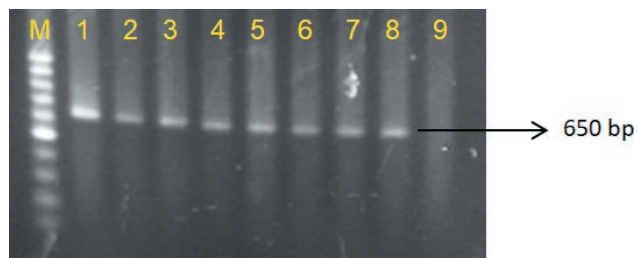


Figure 3. Gel from extracts of onion, garlic, cucumber, and melon plants mechanically inoculated with two onion isolates of CMV in the host indexing study gave positive results in RT-PCR to amplify partial RNA2 of CMV (650 bp). Isolate CMV-14.3Po in lane 1. onion, lane 2. garlic, lane 3. cucumber, and lane 4. Melon. Isolate CMV-15.5Po in lane 5. onion, lane 6. garlic, lane 7. cucumber, lane 8. Melon. Lane 9. negative control (uninoculated onion). Lane M = 100 bp DNA ladder marker (Thermo Fisher Scientific).

Partial RNA3 sequences of CMV-14.3Po and CMV-15.5Po shared high similarities to two other Turkish CMV isolates (TUR84 and TUR86) identified from *Rapistrum rugosum* (Oshima *et al.*, 2016). This provided more evidence to the previous suggestion based on phylogenetic analyses of partial RNA2, that onion isolates were probably transmitted from *R. rugosum*, or other weed species, in onion fields. During the survey that was conducted in Ankara province, only two of 210 onion samples tested positive for CMV infections. This low infection rate may have been due to absence of aphid vectors (Santosa and Ertunc, 2020). The two onion isolates were found in two different fields close to each other during a survey that covered a wide area. The isolates were therefore likely to only spread locally, because natural mechanical transmission in onion was difficult.

CMV-14.3Po and CMV-15.5Po were shown to be mechanically transmissible to onion, garlic, cucumber, and melon, based on host indexing results. CMV-G was also reported to be transmitted to onion and cucumber by mechanical inoculation. Similar to CMV-14.3Po and CMV-15.5Po, CMV-G produced severe symptoms on garlic (Stefanac, 1980). CMV-G caused severe symptom (necrotic streaks) on onion, contrary to CMV-14.3Po and CMV-15.5Po that only produced mild leaf malformation on onion. These results probably showed response of different onion varieties to CMV isolates. The mild leaf malformation on inoculated onions was consistent with symptoms on inoculum sources (samples that were naturally infected by CMV-14.3Po and CMV-15.5Po) (Santosa and Ertunc, 2020). Onion isolates mostly only caused mild symptom (mottle) on inoculated cucumber and melon, with only one of each cucumber and melon plants developing severe symptoms (mosaic).

Based on low infection rate and mild symptom severity, it can be shown that CMV is a minor virus that does not pose serious threats to onion production. CMV could be a threat for garlic cultivation, since it caused severe damage to garlic plants, and garlic vegetative propagation would easily transmit viruses to next generations. However, CMV infection on garlic was determined to be rare in a survey (Fidan, 2010). Severe symptoms on garlic which could eliminate the possibility for infected plants to become planting material, and the difficulty to transmit CMV-G by aphids, were thought to be the reasons for rare CMV infection on garlic (Stefanac and Milicic, 1992).

Phylogenetic and similarity analyses and host indexing that were performed on CMV-14.3Po and CMV-15.5Po revealed knowledge of CMV isolates that naturally infect onion, which was lacking prior to this study. However, information on distribution, transmission, economic importance and genomic diversity of CMV in onion and other *Allium* spp. is still needed to be further investigated.

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