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Detection and complete sequence of a divergent isolate of cucumber fruit mottle mosaic virus on *Coccinia grandis* in Sudan

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Abstract

Cucurbit-infecting tobamoviruses known so far belong to six acknowledged or tentative species. Except cucumber green mottle mosaic virus (CGMMV) present worldwide, they are geographically restricted, mostly to Asia, and have not been observed in Africa so far. A tobamovirus isolate infecting a wild coccinia grandis plant was collected in central Sudan in 2012. Its host range appeared mostly limited to cucurbits. A full-length sequence was established and it presented 85% nt identity with isolates of cucumber fruit mottle mosaic virus (CFMMV) described in Israel and Korea, whereas aa identity with CFMMV isolates was 92 to 95% depending on the protein. Based on its biological and molecular properties, we suggest to consider the Sudanese isolate as a divergent isolate of CFMMV. This constitutes the first description of CFMMV in Africa. Its high divergence with isolates from Israel and Korea suggests a lack of recent exchanges between CFMMV from Sudan and the other known populations.

A sample of the wild cucurbit *Coccinia grandis* was collected during a survey performed in the Singa region in central Sudan in December 2012. After mechanical inoculation on *Cucurbita pepo*, severe mosaic symptoms were observed, and electron microscope observations revealed the presence of rod-shaped particles suggestive of the presence of a tobamovirus, as well as flexuous particles and pinwheels that were found to correspond to the ipomovirus coccinia mottle virus (CocMoV) [4]. CocMoV was eliminated after mechanical inoculation of *Nicotiana benthamiana*, as confirmed by RT-PCR with specific CocMoV primers [4], but the tobamovirus-like particles were still present and the inoculated *N. benthamiana* plants displayed systemic mosaic symptoms. Mosaic and deformations were observed after mechanical inoculation from *N. benthamiana* to melon and cucumber. Two primer pairs were designed through alignments of full-length sequences of known cucurbit-infecting tomaboviruses: cucumber green mottle mosaic virus (CGMMV) present worldwide [6], cucumber fruit mottle mosaic virus (CFMMV) detected so far only in Israel and Korea [2, 7], kyuri green mottle mosaic virus (KGMMV) found in Korea, Japan, Indonesia and China, cucumber mottle virus (CMoV) described in Japan and zucchini green mottle mosaic virus (ZGMMV) described in Korea and China. Primer pair 1 (TobCuc-1150-5': ACGTTYTCTGTGTGGTTTCC and TobCuc-2700-3': CCACADCCVGGMACNCCATC targeted a 1550nt fragment in the replicase region, and pair 2 (TobCuc-4980-5': TMGAYGATGCWATCCACGA and TobCuc-6240-3': GCRGCNGTAGACGCRTCGTC targeted a 1300nt fragment encompassing the complete movement protein and part of the coat protein regions. RT-PCR fragments of the expected size were obtained for the coccinia isolate as well as an isolate from *Luffa acutangula* collected in the same area. Blast analyses on partial sequences revealed 82% and 88% nt identity in the first and second regions respectively between the Sudan isolates and CFMMV isolates from Israel and Korea (GenBank accessions AF321057 and JN226146 respectively), and more than 99% nt identity between the Sudanese coccinia and loofah isolates. Specific primers (Supporting information S1) were designed using the coccinia partial sequences and complete CFMMV sequences of Israeli and Korean isolates (AF321057 and JN226146, 98.2% identity between the two sequences). After RT-PCR amplification, Sanger sequencing and manual assembly, a full-length sequence of 6574 nt was obtained (GenBank accession MT989352). The sequenced virus had a typical tobamovirus genetic organization [1] with a 131 kDa nonstructural protein (1165 amino-acids) terminated by an amber (UAG) stop codon, a 188.6 kDa (1669 aa) protein expressed by readthrough of the amber stop codon, a putative 27.7 kDa (258 aa) movement protein and a 17.4 kDa (163 aa) coat protein. It showed circa 85% overall nt identity (92 to 95% aa identity depending on the protein) with CFMMV isolates from Korea and Israel (6571 and 6562 nt respectively) (Table 1). Nt identity was 72-75% with KGMMV and ZGMMV, 62-63% with CGMMV, CMoV and the tentative tobamovirus watermelon green mottle mosaic virus (WGMMV) described recently in Taiwan and the USA [3], and less than 55% with tobamoviruses outside the “cucurbit” group (Table 1). Multiple sequence alignments were performed with ClustalW included in MEGA6 [8]. The best nt and aa substitution models were determined with MEGA6 and used for building maximum likelihood trees (Figure 1) with 500 bootstrap replicates, confirming the clustering of the Sudanese isolate with CFMMV within the “cucurbit tobamovirus” group, and its closer relation with KGMMV and ZGMMV than with the other cucurbit-infecting tobamoviruses. The species demarcation threshold for tobamoviruses is 90% overall nt identity, but numerous pairs of tobamovirus isolates showing 85 to 90% identity are considered as belonging to the same species (https://www.ncbi.nlm.nih.gov/sutils/pasc/). Also, for all proteins, the Sudanese isolate presented more than 92% aa identity with Israeli and Korean CFMMV isolates (Table 1). After mechanical inoculation, *Chenopodium amaranticolor*, *C. quinoa* and tobacco cv. Xanthi showed no symptoms and the virus was not detected by RT-PCR at the apex of the plants (data not shown), whereas CFMMV isolates from Israel and Korea did not infect tobacco but were reported to induce local lesions on *C. amaranticolor* and symptomless systemic infection of *C. quinoa*. Indeed, the biological and molecular differences between the Sudanese isolate and isolates of CFMMV are similar to those between the Y and C isolates of KGMMV [9]. Too few isolates are characterized to determine if there is a molecular continuum between CFMMV-Su and the other isolates, and if differences in biological properties are frequent for this virus. Based on the current knowledge on its biological and molecular properties, we propose to consider the Sudanese tobamovirus as a divergent isolate of CFMMV rather than a member of a new species

To our knowledge, this is the first observation of CFMMV in Africa and its first detection on *Coccinia grandis* and *Luffa acutangula*. These plants could constitute reservoirs and contribute to virus maintenance and dissemination, whereas CFMMV had been observed previously only on cultivated cucurbits in natural conditions [2, 7]. As observed for the ipomovirus cucumber vein yellowing virus [5], the high sequence divergence between the Sudanese CFMMV isolate and the other isolates indicates that the presence of CFMMV in distant parts of the world is not related to recent exchanges of contaminated material between Sudan and the other places.

Figure legends

Fig. 1: Maximum-likelihood tree obtained for full-length nucleotide sequences of tobamoviruses. Bootstrap values above 70% (500 bootstraps) are indicated for each node. The scale bar represents a genetic distance of 0.2. Subgroups of tobamoviruses infecting members of different plant families are indicated. The Sudanese isolate analyzed here is indicated in bold

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Declarations

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Ethical statement: This research did not involve any human participants and/or animals.

Conflict of interest: The authors declare that they have no conflict of interest.

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Table 1 : sequence identity (%) between the Sudanese coccinia tobamovirus isolate and other tobamoviruses, for the full-length nucleotide sequence and for deduced amino-acid sequences of the polymerase (RdRp), movement protein (MP) and coat protein (CP)

Accession	Tobamovirus	Genome (nt)	RdRp (aa)	MP (aa)	CP (aa)
NC_002633.1	Cucumber fruit mottle mosaic virus (CFMMV)	85.5	92.7	95.3	93.8
JN226146	CFMMV-Cm	85.9	94.9	95.3	93.8
NC_003610.1	Kyuri green mottle mosaic virus (KGMMV)	74.6	84.2	78.7	83.2
AB015145	KGMMV-Yodo	73.9	83.3	79.1	82.0
AB162006	KGMMV-YM	72.4	78.7	78.3	80.7
NC_003878.1	Zucchini green mottle mosaic virus (ZGMMV)	73.5	83.5	77.9	77.6
NC_001801.1	Cucumber green mottle mosaic virus (CGMMV)	63.3	66.1	62.8	45.3
NC_008614.1	Cucumber mottle virus (CMoV)	62.1	64.1	59.3	41.4
MH837097	Watermelon green mottle mosaic virus (WGMMV)	61.7	64.0	58.4	42.6
NC_016519.1	Clitoria yellow mottle virus (ClYMV)	49.4	44.8	24.6	41.7
NC_025381.1	Hibiscus latent Fort Pierce virus (HLFPV)	53.5	51.5	27.2	39.9
NC_008310.2	Hibiscus latent Singapore virus (HLSV)	53.3	51.0	28.4	38.7
NC_015552.1	Passion fruit mosaic virus (PafMV)	50.2	47.7	22.2	33.3
NC_008716.1	Maracuja mosaic virus (MarMV)	50.7	47.7	24.9	33.3
NC_014546.1	Frangipani mosaic virus (FrMV)	51.2	45.6	36.4	36.2
NC_026816.1	Plumeria mosaic virus (PluMV)	50.6	45.4	33.9	36.8
NC_001873.1	Turnip vein-clearing virus (TVCV)	50.8	48.1	31.0	34.4
NC_002792.2	Ribgrass mosaic virus (RMV)	51.0	47.8	31.5	33.8
NC_003355.1	Wasabi mottle virus (WMoV)	50.7	47.9	31.9	31.2
NC_004422.1	Youcai mosaic virus (YoMV)	50.7	47.9	32.8	31.8
NC_008365.1	Streptocarpus flower break virus (SFBV)	51.0	46.2	31.9	34.3
NC_010944.1	Brugmansia mild mottle virus (BrMMV)	51.5	47.4	32.9	33.3
NC_003852.1	Obuda pepper virus (ObPV)	50.8	46.0	32.5	32.7
NC_004106.1	Paprika mild mottle virus (PaMMV)	51.7	46.6	31.5	34.0
NC_001556.1	Tobacco mild green mosaic virus (TMGMV)	50.0	46.0	31.9	33.3
NC_030229.1	Tropical soda apple mosaic virus (TSAMV)	51.1	46.4	34.5	36.9
NC_003630.1	Pepper mild mottle virus (PMMoV)	50.8	46.3	33.9	36.3
NC_001728.1	Odontoglossum ringspot virus(ORSV)	50.8	45.4	32.6	35.3
NC_009642.1	Bell pepper mottle virus (BPeMV)	51.1	47.1	31.3	33.8
NC_002692.1	Tomato mosaic virus (ToMV)	51.2	47.5	30.5	32.1
NC_022230.1	Tomato mottle mosaic virus (ToMMV)	50.7	47.8	29.7	30.8

NC_028478.1	Tomato brown rugose fruit virus (ToBRFV)	51.0	47.7	31.1	32.7
NC_022801.1	Yellow tailflower mild mottle virus (YTMMV)	51.4	46.8	34.9	34.8
NC_001367.1	Tobacco mosaic virus (TMV)	51.0	47.3	31.8	35.2
NC_009041.1	Rehmannia mosaic virus (ReMV)	51.2	47.8	30.5	34.0
NC_016442.1	Rattail cactus necrosis associated virus (RCNaV)	49.1	44.3	22.9	27.0
NC_011803.1	Cactus mild mottle virus (CMMoV)	48.7	44.4	20.5	25.2

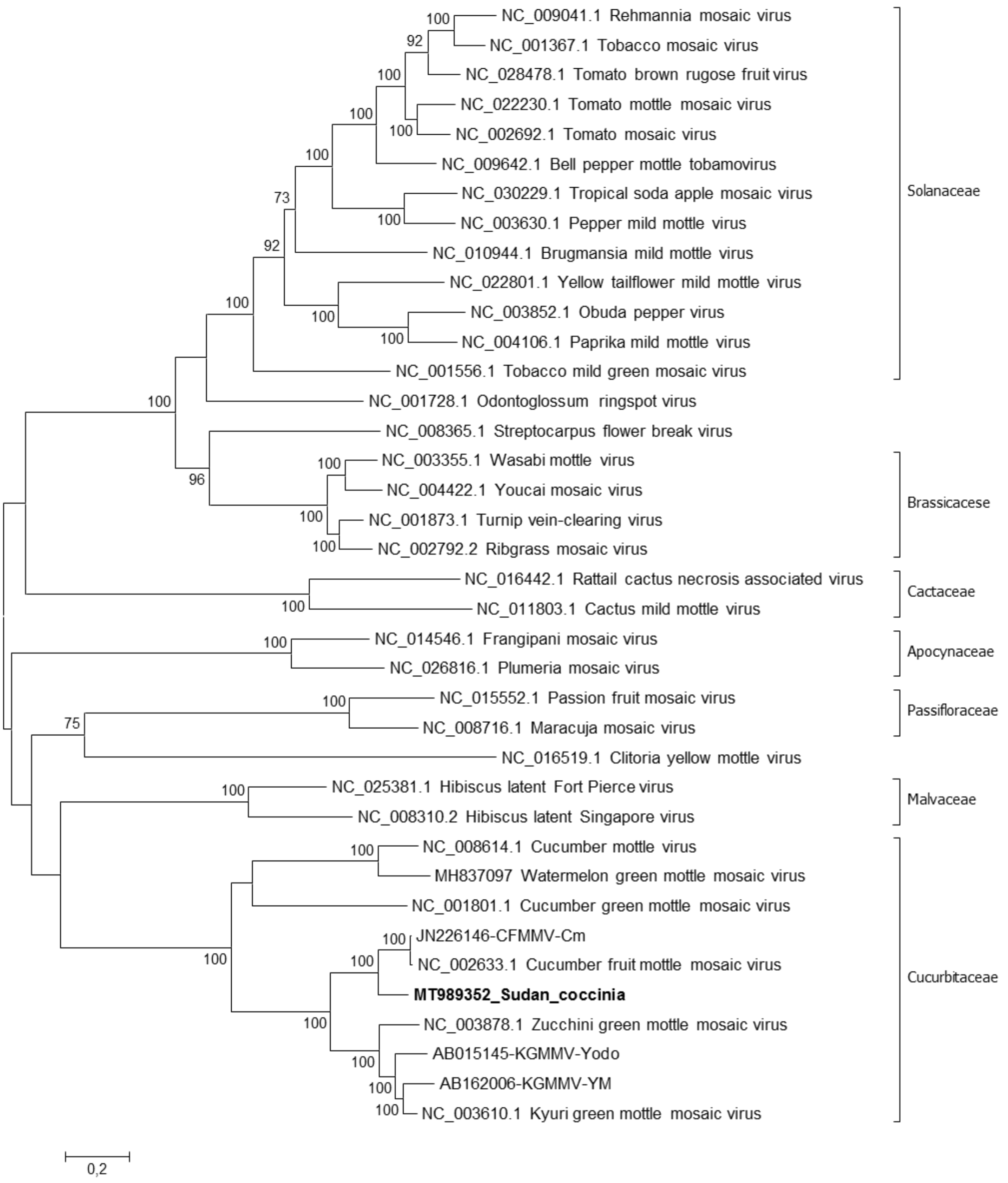
100
NC_009041.1 Rehmannia mosaic virus
92
NC_001367.1 Tobacco mosaic virus
100
NC_028478.1 Tomato brown rugose fruit virus
100
NC_022230.1 Tomato mottle mosaic virus
100
NC_002692.1 Tomato mosaic virus
100
NC_009642.1 Bell pepper mottle tobamovirus
73
NC_030229.1 Tropical soda apple mosaic virus
100
NC_003630.1 Pepper mild mottle virus
92
NC_010944.1 Brugmansia mild mottle virus
NC_022801.1 Yellow tailflower mild mottle virus
100
NC_003852.1 Obuda pepper virus
100
100
NC_004106.1 Paprika mild mottle virus
NC_001556.1 Tobacco mild green mosaic virus
100
NC_001728.1 Odontoglossum ringspot virus
NC_008365.1 Streptocarpus flower break virus
100
NC_003355.1 Wasabi mottle virus
96
NC_004422.1 Youcai mosaic virus
100
NC_001873.1 Turnip vein-clearing virus
100
NC_002792.2 Ribgrass mosaic virus
NC_016442.1 Rattail cactus necrosis associated virus
100
NC_011803.1 Cactus mild mottle virus
100
NC_014546.1 Frangipani mosaic virus
NC_026816.1 Plumeria mosaic virus
100
NC_015552.1 Passion fruit mosaic virus
75
NC_008716.1 Maracuja mosaic virus
NC_016519.1 Clitoria yellow mottle virus
100
NC_025381.1 Hibiscus latent Fort Pierce virus
NC_008310.2 Hibiscus latent Singapore virus
100
NC_008614.1 Cucumber mottle virus
MH837097 Watermelon green mottle mosaic virus
NC_001801.1 Cucumber green mottle mosaic virus
100
JN226146-CFMMV-Cm
100
100
NC_002633.1 Cucumber fruit mottle mosaic virus
MT989352_Sudan_coccinia
100
NC_003878.1 Zucchini green mottle mosaic virus
100
AB015145-KGMMV-Yodo
100
AB162006-KGMMV-YM
100
NC_003610.1 Kyuri green mottle mosaic virus
Solanaceae
Brassicacese
Cactaceae
Apocynaceae
Passifloraceae
Malvaceae
Cucurbitaceae
0,2

Figure 1