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African Basil (*Ocimum gratissimum*) Is a Reservoir of Divergent Begomoviruses in Uganda

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Abstract

Begomoviruses are plant viruses that cause major losses to many economically important crops. Although they are poorly understood, begomoviruses infecting wild plants may have an important role as reservoirs in the epidemiology of viral diseases. This study reports the discovery and genomic characterization of three novel bipartite begomoviruses from wild and cultivated African basil (*Ocimum gratissimum*) plants collected in Uganda, East Africa. Based on the symptoms shown by the infected plants, the names proposed for these viruses are Ocimum yellow vein virus (OcYVV), Ocimum mosaic virus (OcMV), and Ocimum golden mosaic virus (OcGMV). Genome and phylogenetic analyses suggest that DNA-A of OcGMV is mostly related to begomoviruses infecting tomato in Africa, whereas those of OcYVV and OcMV are closely related to one another and highly divergent within the Old World begomoviruses. The DNA-A of all characterized begomovirus isolates are of a recombinant nature, revealing the role of recombination in the evolution of these begomoviruses. The viruses characterized here are the first identified in *O. gratissimum* and the first in *Ocimum* spp. in the African continent and could have important epidemiological consequences for cultivated basils and other important crops.

Keywords: African basil, begomoviruses, *Geminiviridae*, *Ocimum gratissimum*, phylogenetic analysis, recombination

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are plant viruses with circular single-stranded DNA genomes encapsidated in twin (geminate) quasi-icosahedral virions. A high number of begomoviruses cause severe damage to many vegetable and fiber crops worldwide. They are transmitted by whiteflies (Hemiptera: Aleyrodidae) of the *Bemisia tabaci* complex (Navas-Castillo et al. 2011). Begomovirus genomes are monopartite or bipartite (Zerbini et al. 2017). The genomes of bipartite begomoviruses consist of DNA-A and DNA-B components, each of 2.5 to 2.6 kb. DNA-A encodes the coat protein, a putative movement protein (MP) (present only in Old World begomoviruses), replication associated protein (Rep), a transcriptional activator, a replication enhancer, and the C4 protein. DNA-B encodes a nuclear shuttling protein and MP. Both components share a common region of approximately 200 nt within an intergenic region (IR) that includes the replication origin. Rep initiates viral DNA replication by binding to iterative sequences (iterons) within the IR and introducing a nick into the conserved TAATATT↓AC sequence. The genomes of monopartite begomoviruses resemble the DNA-A component of bipartite begomoviruses. In some cases, begomoviruses are associated with DNA satellites—namely, betasatellites, alphasatellites, and deltasatellites (Lozano et al. 2016; Zhou 2013).

In East Africa, begomoviruses causing cassava mosaic disease have been extensively studied (reviewed by Rey and Vanderschuren 2017). However, little attention has been devoted to viruses infecting minor crops, weeds, and other wild plants. In Uganda, for example, isolates of only two begomovirus species infecting wild plants have been fully characterized: *Vernonia crinkle virus* (associated with a betasatellite) from *Vernonia amygdalina* (Compositae) and *Desmodium mottle virus* (the first legumovirus from East Africa) from *Desmodium* sp. (Leguminosae) (Mollel et al. 2017a, b).

Ocimum spp., generically named as basils, are aromatic plants belonging to the family Lamiaceae (Labiatae). Members of the genus include annual herbs, suffrutices, and shrubs native to the tropical and warm temperate regions, with the greatest number of species in Africa (Paton 1992; Paton et al. 1999). *O. gratissimum*, African basil, is widespread in the Paleotropics, from India to West Africa, and naturalized in tropical America. In Africa, where *O. gratissimum* forms a variable polymorphic complex, it is a common culinary herb also used in traditional medicine against a range of illnesses such as cough, fever, ear infection, and abdominal pain (Kokwaro 2009). *O. gratissimum* also has major potential economic importance, owing to the production of essential oil that has a large antimicrobial spectrum (Lawrence 1992). The objective of this study was to search and characterize the putative begomoviruses infecting wild and cultivated *O. gratissimum* plants in Uganda. The full-length genome of DNA-A and DNA-B was cloned and sequenced from six field-collected plants expressing typical begomovirus symptoms. The genome and phylogenetic analyses showed the presence of three novel begomoviruses, two of them highly divergent. To our knowledge, there have been no previous reports of viruses infecting *Ocimum* spp. in Africa nor *O. gratissimum* anywhere in the world.

Materials and Methods

Sample collection and plant identification. As part of a survey for begomoviruses in Uganda, leaf samples from six *O. gratissimum* plants were collected from different locations in the Central and Western regions of the country in March 2015 (Fig. 1; Table 1). The survey

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was conducted along main roads, where wild-plant specimens were collected, and in some backyard gardens, where cultivated plants were sampled. All plants exhibited symptoms suspicious of begomovirus infection (Fig. 2). Morphological identification of the plant species was confirmed molecularly by DNA barcoding using the chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit and maturase K genes (Hollingsworth et al. 2009).

Virus component cloning. Total DNA was extracted from leaf tissue using a modified cetyl trimethyl ammonium bromide method (Permingeat et al. 1998) and was used as a template for rolling-circle amplification (RCA) using φ29 DNA polymerase (illustra TempliPhi 100 Amplification Kit; GE Healthcare, Buckinghamshire, U.K.). Amplified RCA products were digested with *Hpa*II, a 4-nt restriction enzyme commonly used for preliminary restriction fragment length polymorphism (RFLP) analysis of RCA products obtained from samples infected by ssDNA viruses, and restriction products analyzed on a 1% agarose gel. Then RCA products were digested with a set of 6-nt restriction enzymes (*Bam*HI, *Eco*RI, *Hind*III, *Nco*I, *Nhe*I, and *Sal*I). Selected fragments (~2.7 kb) of RCA products putatively corresponding to full-length begomoviral components were cloned into pBlueScript II SK (+) (Stratagene, La Jolla, CA) or closed pGEM-T Easy Vector (Promega, Madison, WI). Recombinant plasmid DNAs were transformed into *Escherichia coli* DH5α by electroporation and selected clones were sequenced at Macrogen Inc. (Seoul, South Korea).

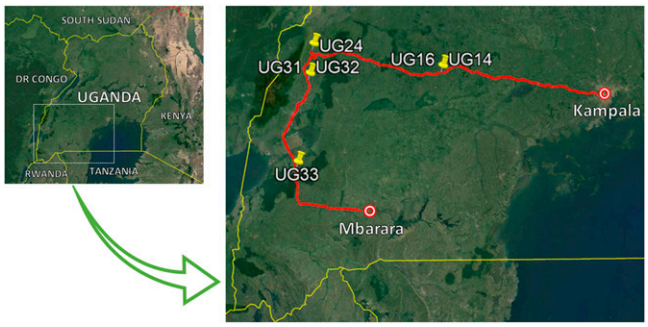

Fig. 1. Map showing the survey route in southwestern Uganda and the locations of the six *Ocimum gratissimum* plants sampled and analyzed in this work.

Sequence analysis. The initial nucleotide sequence similarity comparison was performed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned with Multiple Sequence Comparison by Log-Expectation (MUSCLE) (Edgar 2004) and pairwise identity scores were calculated using the SDT sequence demarcation tool (Muhire et al. 2014). In silico digestion analysis of cloned begomovirus sequences was performed with Restriction Analyzer (http://www.molbiotools.com/restrictionanalyzer.html).

Phylogenetic analysis. Molecular Evolutionary Genetics Analysis 7 (MEGA 7) was used for phylogenetic analysis using the maximum-likelihood method (Kumar et al. 2016). The best-fit model

Table 1. Samples of *Ocimum gratissimum* collected in southwestern Uganda

Sample code	GPS coordinates	Field type	Symptoms
UG14	0°29.817′N 31°16.003′E	Backyard garden (cultivated)	Yellow vein and mosaic
UG16	0°29.828′N 31°15.993′E	Backyard garden (cultivated)	Yellow vein and mosaic
UG24	0°41.002′N 30°14.063′E	Roadside (wild)	Mosaic
UG31	0°26.706′N 30°12.203′E	Roadside (wild)	Golden mosaic
UG32	0°26.726′N 30°12.206′E	Roadside (wild)	Yellow vein and mosaic
UG33	0°15.669′S 30°06.138′E	Roadside (wild)	Golden mosaic

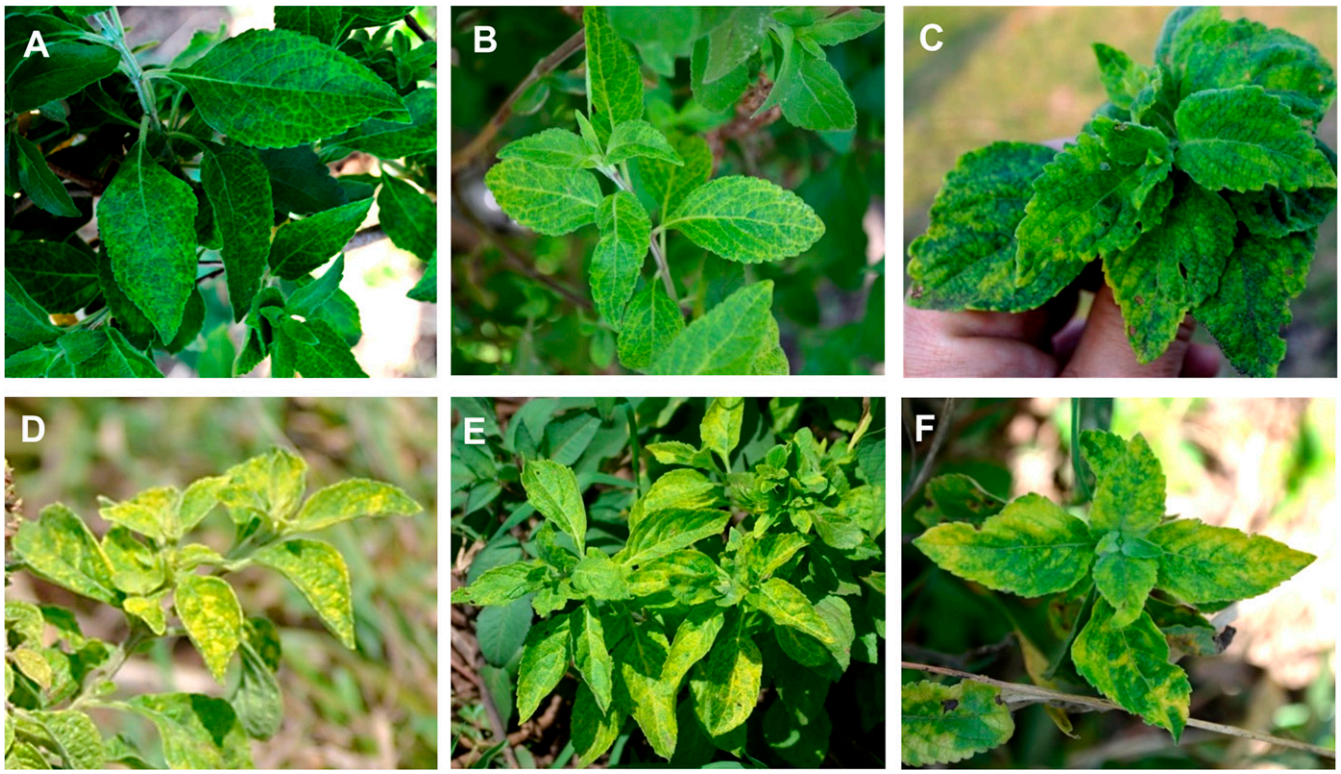

Fig. 2. Photographs of the collected *Ocimum gratissimum* plants, showing typical begomovirus symptoms: **A,** UG14, **B,** UG16, **C,** UG24, **D,** UG31, **E,** UG32, and **F,** UG33.

of nucleotide substitution was selected based on the corrected Akaike information criterion and the Bayesian information criterion as implemented in MEGA 7 (Kumar et al. 2016).

Recombination detection analysis. Detection of potential recombinant sequences, identification of likely parental sequences, and localization of recombination breakpoints were carried out with the RDP4 recombination detection program (Martin et al. 2015). The analysis was performed with default settings for the different detection methods and a Bonferroni-corrected *P*-value cutoff of 0.05. Only recombination events detected with five or more methods with *P* values lower than $<10^{-2}$ were considered. Sequences used for recombination analysis were selected using SWeBLAST (Sliding Window Web-based BLAST) with a window size of 200 and a step size of 200 (Fourment et al. 2008) from an alignment generated with MUSCLE (Edgar 2004).

Results

Genome characterization of novel begomovirus species. Digestion with *Hpa*II of RCA-amplified products from the six *O. gratissimum* leaf samples yielded restriction patterns supporting the suspected begomovirus infections, based on the symptomatology observed in the field (Fig. 3A). Symptoms included yellow vein and/or (golden) mosaic. Sequencing of cloned DNA fragments following digestion with 6-nt enzymes confirmed the presence of DNA-A and DNA-B genomic components in the six samples (Table 2). The genome organization of the six begomoviruses was typical of Old World bipartite begomoviruses, with six and two proteins encoded by DNA-A and DNA-B, respectively (GenBank accession numbers MN313658 to MN313669).

The initial nucleotide sequence similarity comparison for DNA-A and DNA-B performed using BLASTN and/or BLASTX showed

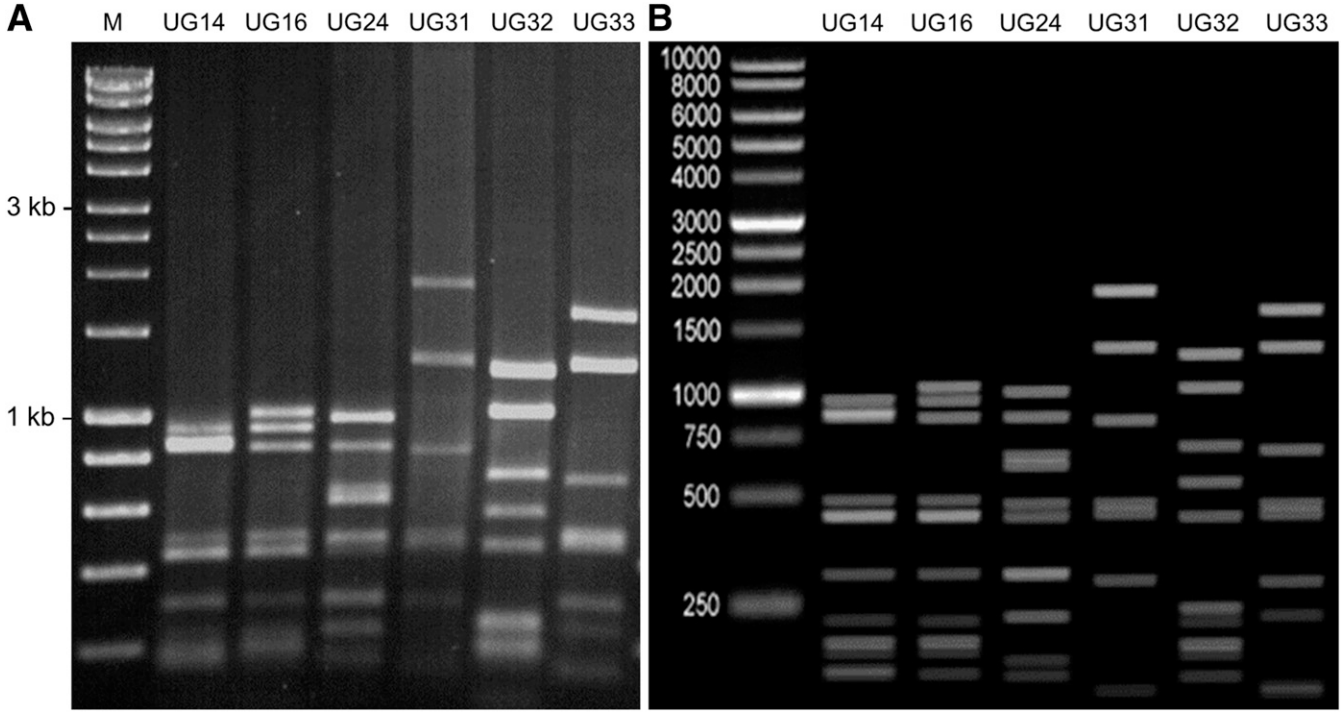

Fig. 3. Restriction fragment length polymorphism (RFLP) analysis. **A,** RFLP performed by digestion of rolling circle amplification products obtained from *Ocimum gratissimum* plant DNA extracts with the restriction enzyme *Hpa*II revealed on a 1% agarose gel. M = molecular weight marker (HyperLadder 1 kb, Bioline). **B,** In silico RFLP analysis of the DNA-A plus DNA-B sequences obtained from each sample was performed with Restriction Analyzer (http://www.molbiotools.com/restrictionanalyzer.html).

Table 2. Nucleotide percentage identity of the full-length DNA-A and DNA-B components of the begomoviruses isolated from *Ocimum gratissimum* plants, with the most closely related begomovirus genomes available in GenBank

O. gratissimum begomoviruses						Begomoviruses with the highest nucleotide identity			
Virus[a]	Sample	DNA component	GenBank accession	Enzyme used for cloning	Size (nt)	Virus[b]	Country	GenBank accession	Identity (%)
OcYVV	UG14	DNA-A	MN313667	*Eco*RI	2,804	ClGMJsV	China	FN396966	72.4
		DNA-B	MN313666	*Eco*RI	2,752	ToLCNDV	Pakistan	KT948073	64.5
	UG16	DNA-A	MN313665	*Sal*I	2,804	ClGMJsV	China	FN396966	72.6
		DNA-B	MN313664	*Eco*RI	2,753	ToLCNDV	Pakistan	KT948073	64.6
	UG32	DNA-A	MN313663	*Eco*RI	2,805	ClGMJsV	China	FN396966	72.3
		DNA-B	MN313662	*Eco*RI	2,755	ToLCNDV	Pakistan	KT948073	63.8
OcMV	UG24	DNA-A	MN313669	*Nco*I	2,794	ClGMJsV	China	FN396966	71.8
		DNA-B	MN313668	*Nco*I	2,760	ToLCNDV	India	KC874496	62.8
OcGMV	UG31	DNA-A	MN313661	*Nco*I	2,752	ToLCUV	Uganda	DQ127170	82.7
		DNA-B	MN313660	*Nco*I	2,678	ToLCGUV	India	KP235538	62.2
	UG33	DNA-A	MN313659	*Eco*RI	2,750	ToLCUV	Uganda	DQ127170	83.3
		DNA-B	MN313658	*Eco*RI	2,678	ToLCGUV	India	KP235538	63.4

[a] OcYVV = Ocimum yellow vein virus, OcMV = Ocimum mosaic virus, and OcGMV = Ocimum golden mosaic virus.
[b] ClGMJsV = *Clerodendrum golden mosaic Jiangsu virus*, ToLCNDV = *Tomato leaf curl New Delhi virus*, ToLCUV = *Tomato leaf curl Uganda virus*, and ToLCGUV = *Tomato leaf curl Gujarat virus*.

similarities with other Old World begomoviruses. Pairwise nucleotide identity analysis revealed that most DNA-A and DNA-B from the six samples showed the highest nucleotide identities with begomoviruses from Asia (i.e., China, Pakistan, and India) (Table 2). DNA-A of samples UG14, UG16, UG24, and UG32 showed the highest identity (72.4, 72.6, 71.8, and 72.3%, respectively) with the monopartite begomovirus *Clerodendrum golden mosaic Jiangsu virus*, isolated from the weed *Clerodendrum cyrtophyllum* (Lamiaceae) in China (Li and Zhou 2010). DNA-B from the same samples showed the highest identities (64.5, 64.6, 62.8, and 63.8%, respectively) with isolates of *Tomato leaf curl New Delhi virus* from pumpkin collected in Pakistan and potato in India. DNA-A of samples UG31 and UG33 showed the highest identities (82.7 and 83.3%, respectively) with the monopartite begomovirus *Tomato leaf curl Uganda virus* (ToLCUV), isolated from tomato in Uganda (Shih et al. 2006a), whereas DNA-B from the same samples showed the highest identities (62.2 and 63.4%, respectively) with *Tomato leaf curl Gujarat virus*, isolated from tomato in India.

Based on their sequence identities, the DNA-As of the begomoviruses described in this work are clustered in three groups (Fig. 4). In accordance with the begomovirus species demarcation criterion (<91% nucleotide identity for DNA-A) (Brown et al. 2015), the begomoviruses described here should be assigned to three new species. The following names are proposed for them: Ocimum yellow vein virus (OcYVV; isolates Uganda-UG14-2015, Uganda-UG16-2015, and Uganda-UG32-2015 for samples UG14, UG16, and UG32, respectively), Ocimum mosaic virus (OcMV; isolate Uganda-UG24-2015 for sample UG24), and Ocimum golden mosaic virus (OcGMV; isolates Uganda-UG31-2015 and Uganda-UG33-2015 for samples UG31 and UG33, respectively).

DNA-A and DNA-B genomic components isolated from each sample showed common regions with identities of 83.9 (UG14), 83.3 (UG16), 91.4 (UG24), 94.6 (UG31), 89.5 (UG32), and 93.3% (UG33). Furthermore, iterons were essentially identical in sequence and position in both components from the same sample, demonstrating that both DNA-A and DNA-B from each sample constitute a cognate pair (Fig. 5).

In silico digestion analysis of cloned begomovirus sequences produced an RCA-RFLP pattern from each sample (Fig. 3B) identical to those experimentally obtained (Fig. 3A). This analysis unequivocally confirms that the only begomovirus present in an amplifiable amount in each sample is the one cloned and sequenced in this work.

Phylogenetic and recombination analysis of OcYVV, OcMV, and OcGMV. Phylogenetic analysis of DNA-A and DNA-B of the begomoviruses described in this work showed that they are related to Old World begomoviruses from Africa and Asia (Fig. 6). OcGMV DNA-A (from samples UG31 and UG33) grouped with ToLCUV as the sister species. OcYVV DNA-A (from samples UG14, UG16, and UG32) and OcMV DNA-A (from sample UG24) were highly divergent and grouped in a single and differentiated cluster supported by a high bootstrap value (Fig. 6A). However, DNA-B of all begomoviruses described in this work clustered together (Fig. 6B). RDP analysis showed a recombinant origin for all DNA-A molecules sequenced in this work (Table 3). Begomoviruses involved in recombination events of DNA-A infect cassava, tomato, and soybean in Asia; tomato in the Indian Ocean Islands; and cassava, tomato, and *Desmodium* sp. in Africa (Lefeuvre et al. 2007; Mollel et al. 2017b; Shih et al. 2006b). Minor parents of the three recombination events detected in both isolates of OcGMV are unknown, but in one of them (nucleotides 2,322/2,320 to 2,626/2,641) the most closely related sequence was, surprisingly, from a New World begomovirus, *Sida micrantha mosaic virus* (FN436005; Paprotka et al. 2010). With respect to the DNA-Bs, only the one of OcMV was a recombinant. The minor parent of the recombination event detected in OcMV DNA-B, interestingly, is an isolate of OcYVV and the major parent, although unknown, would be related to OcGMV.

Discussion

Begomoviruses are a major constraint to production of economically important crops around the world, including Sub-Saharan Africa, where great effort has been devoted to characterizing viruses infecting major crops, exemplified by cassava (Rey and Vanderschuren 2017). Nevertheless, begomoviruses infecting minor crops, weeds, and other wild plants are poorly studied in this continent. However, understanding the viral diversity in minor crops and wild plants is important because it has epidemiological implications. Thus, these plants can act as reservoirs of viruses infecting important crops (García-Arenal and Zerbini 2019; Navas-Castillo et al. 2011) and even as niches for the development of new strains or species by recombination (García-Andrés et al. 2006).

In the present study, symptomatic cultivated and wild *O. gratissimum* plants from Uganda were found to be commonly infected with begomoviruses, being the first report of a viral infection in this species. This is also the first report of a virus infecting *Ocimum* spp. in Africa. Outside Africa, basil (*O. basilicum*) has been reported to be infected by *Alfalfa mosaic virus* (*Alfamovirus*) and *Impatiens necrotic spot virus* (*Orthotospovirus*) in the United States (Poojari and Naidu 2013; Wintermantel and Natwick 2012) and *Pepino mosaic virus* (*Potexvirus*) in Italy (Davino et al. 2009). On the other hand, holy basil [*O. tenuiflorum* (syn. *O. sanctum*)] has been reported

A

	UG14	UG16	UG32	UG24	UG31	UG33
UG14	100					
UG16	99.4	100				
UG32	97.3	97.1	100			
UG24	90	90	90.1	100		
UG31	70.1	70.3	70	69.2	100	
UG33	70.1	70.2	70.5	69.6	96.3	100

B

	UG14	UG16	UG32	UG24	UG31	UG33
UG14	100					
UG16	99.3	100				
UG32	97.2	97	100			
UG24	83.1	83.1	82.7	100		
UG31	81	81.2	81.1	77.3	100	
UG33	81.8	81.7	81.1	77.8	93.4	100

Fig. 4. Identities between the begomovirus isolates characterized in this work. Identities for **A,** DNA-A and **B,** DNA-B were obtained using the SDT sequence demarcation tool (Muhire et al. 2014). Each group, which corresponds to a single begomovirus species (<91% DNA-A identity between species), is highlighted with a different color.

Fig. 5. Alignment of the DNA-A and DNA-B common region of Ocimum yellow vein virus from samples UG14, UG16, and UG32; Ocimum mosaic virus from sample UG24; and Ocimum golden mosaic virus from samples UG31 and UG33. The TATA box is boxed. Arrows indicate the position and orientation of the iterons.

to be infected by *Cucumber mosaic virus* (*Cucumovirus*) in India (Khan et al. 2011). With regard to begomovirus infections, *O. basilicum* has been found to be infected by *Chilli leaf curl virus* and *Tomato yellow leaf curl virus* in Oman (Ammara et al. 2015) and *O. tenuiflorum* by *Tomato leaf curl Gujarat virus* in India (Gaur 2012; Nehra et al. 2019).

The three novel begomovirus species, reported here for the first time, belonged to the Old World phylogenetic group of begomoviruses, but the DNA-A of two of them were highly divergent. In contrast, all of the DNA-B components were closely related to one another and grouped in a single cluster. This example demonstrates that the two genome components, DNA-A and DNA-B, have distinct

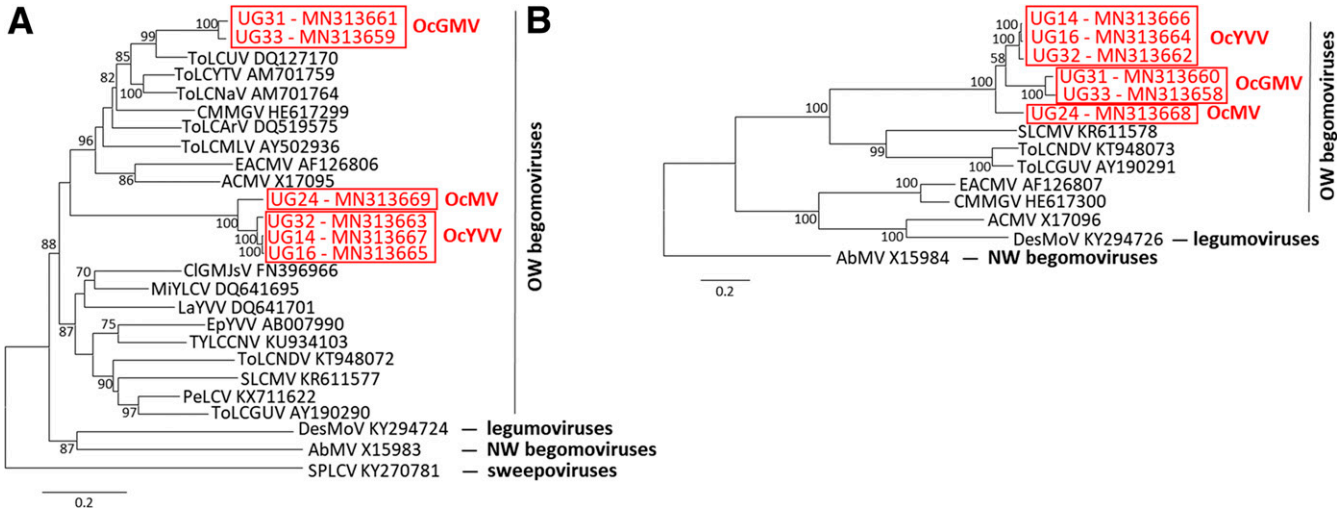

Fig. 6. Phylogenetic trees illustrating the relationship of Ocimum yellow vein virus (OcYVV), Ocimum mosaic virus (OcMV), and Ocimum golden mosaic virus (OcGMV) **A,** DNA-A and **B,** DNA-B to other Old World begomoviruses: *Tomato leaf curl Uganda virus* (ToLCUV), *Tomato leaf curl Mayotte virus* (ToLCYTV), *Tomato leaf curl Namakely virus* (ToLCNaV), *Cassava mosaic Madagascar virus* (CMMGV), *Tomato leaf curl Arusha virus* (ToLCArV), *Tomato leaf curl Mali virus* (ToLCMLV), *East African cassava mosaic virus* (EACMV), *African cassava mosaic virus* (ACMV), *Clerodendrum golden mosaic Jiangsu virus* (ClGMJsV), *Mimosa yellow leaf curl virus* (MiYLCV), *Lindernia anagallis yellow vein virus* (LaYVV), *Eupatorium yellow vein virus* (EpYVV), *Tomato yellow leaf curl China virus* (TYLCCNV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Sri Lankan cassava mosaic virus* (SLCMV), *Pedilanthus leaf curl virus* (PeLCV), and *Tomato leaf curl Gujarat virus* (ToLCGUV). The legumovirus *Desmodium mottle virus* (DesMoV), the New World (NW) begomovirus *Abutilon mosaic virus* (AbMV), and the sweepovirus *Sweet potato leaf curl virus* (SPLCV) were included as outgroups. The trees were constructed by the maximum-likelihood method (1,000 replicates) using the Molecular Evolutionary Genetics Analysis MEGA 7 program with the best-fit model, TN93+G+I for DNA-A, and HKY+G+I for DNA-B. The bar below each tree indicates the number of nucleotide substitutions per site. Only bootstrap values >50% are shown.

Table 3. Recombination events within the DNA-A sequence of *Ocimum gratissimum* begomoviruses detected by at least five methods included in the RDP4 package

Recombinant genome (GenBank accession)[a]	Recombination breakpoints	Parent-like sequence (GenBank accession)[b]		*P* value	Methods that detected recombination[c]
		Major	Minor		
OcYVV DNA-A (MN313667)	1,183–1,378	SLCMV (KR611577)	TYLCCNV (KU934103)	3.289×10^{-04}	R, B, M, C, S, 3S
	2,131–2,279	ToLCYTV (AM701759)	DesMoV (KY294724)	1.830×10^{-06}	R, B, M, C, 3S
OcYVV DNA-A (MN313665)	1,183–1,378	SLCMV (KR611577)	TYLCCNV (KU934103)	3.289×10^{-04}	R, B, M, C, S, 3S
	2,131–2,279	ToLCYTV (AM701759)	DesMoV (KY294724)	1.830×10^{-06}	R, B, M, C, 3S
OcYVV DNA-A (MN313663)	986–1,410	SLCMV (KR611577)	TYLCCNV (KU934103)	3.289×10^{-04}	R, B, M, C, S, 3S
	2,120–2,278	ToLCYTV (AM701759)	DesMoV (KY294724)	1.830×10^{-06}	R, B, M, C, 3S
OcMV DNA-A (MN313669)	989–1,369	SLCMV (KR611577)	TYLCCNV (KU934103)	3.289×10^{-04}	R, B, M, C, S, 3S
	2,132–2,272	ToLCYTV (AM701759)	DesMoV (KY294724)	1.830×10^{-06}	R, B, M, C, 3S
OcMV DNA-B (MN313668)	2,608–582	Unknown	OcYVV (MN313666)	1.403×10^{-25}	R, G, B, M, C, S, 3S
OcGMV DNA-A (MN313661)	361–481	EACMV (HG530114)	Unknown	1.185×10^{-04}	R, G, B, M, C, 3S
	1,971–2,109	ToLCArV (DQ519575)	Unknown	2.143×10^{-03}	R, G, B, M, C
	2,322–2,626	ToLCNaV (AM701764)	Unknown	4.931×10^{-11}	R, G, B, M, C, S, 3S
OcGMV DNA-A (MN313659)	359–488	EACMV (HG530114)	Unknown	1.185×10^{-04}	R, G, B, M, C, 3S
	1,969–2,107	ToLCArV (DQ519575)	Unknown	2.143×10^{-03}	R, G, B, M, C
	2,320–2,641	ToLCNaV (AM701764)	Unknown	4.931×10^{-11}	R, G, B, M, C, S, 3S

[a] OcYVV = Ocimum yellow vein virus, OcMV = Ocimum mosaic virus, and OcGMV = Ocimum golden mosaic virus.

[b] SLCMV = *Sri Lankan cassava mosaic virus*, TYLCCNV = *Tomato yellow leaf curl China virus*, ToLCYTV = *Tomato leaf curl Mayotte virus*, DesMoV = *Desmodium mottle virus*, EACMV = *East African cassava mosaic virus*, ToLCArV = *Tomato leaf curl Arusha virus*, and ToLCNaV = *Tomato leaf curl Namakely virus*.

[c] The method with the lowest *P* value obtained for each region is underlined. R = RDP recombination detection program, B = BootScan, M = MaxChi, C = Chimaera, S = SiScan, 3S = 3Seq, and G = GENCONV.

molecular evolutionary histories. This phenomenon reflects component exchange between different viruses, which has been shown to be much more frequent in Old World begomoviruses than in New World begomoviruses (Briddon et al. 2010). This has been shown previously, for example, for the New World begomoviruses *Rhynchosia rugose golden mosaic virus* (Fiallo-Olivé et al. 2010a) and *Pepper golden mosaic virus* (Brown et al. 2005) and the Old World begomoviruses *Desmodium mottle virus* (Mollel et al. 2017b) and those belonging to the cassava mosaic virus complex (De Bruyn et al. 2016). In the case of *O. gratissimum*, it can be hypothesized that the DNA-B components of the three begomoviruses have evolved to adapt to this host. It would be interesting to test whether they are also well adapted to other species of the genus *Ocimum*.

Begomoviruses have been mostly described from annual crops but, particularly in the last few years, the availability of RCA and next-generation sequencing techniques has revealed a higher diversity in this genus of plant viruses, including those present in wild plants, many of them perennial. The finding of novel begomovirus species in *O. gratissimum* is a clear example of the hidden viral diversity present in understudied hosts and geographic areas. Our survey was carried out in southwestern Uganda following a transect along the main local roads with some stops to visit backyard gardens. Only six *O. gratissimum* plants were observed with symptoms but all of them were infected by begomoviruses belonging to three new species. Africa is largely underrepresented in relation to other tropical regions such as Latin America or the Indian subcontinent, with respect to plant-virus identification. Increased efforts, therefore, should be implemented to survey new areas and wild host-plants, which will surely be rewarded by the discovery of novel viruses.

The results of phylogenetic and recombination analyses obtained in this work emphasize the interconnection between cultivated and wild plants in relation to the exchange of viruses between them (Cooper and Jones 2006; García-Arenal and Zerbini 2019). There are a number of examples of begomoviruses initially identified in wild plants and later in crops, as is the case of *Euphorbia mosaic virus* in the New World, which was first identified in pioneering research in the 1950s, infecting several species of the genus *Euphorbia* (Bird et al. 1975; Costa and Bennett 1950; Debrot and Centeno 1986) and later found causing emergent diseases in sweet pepper and tobacco crops (Fiallo-Olivé et al. 2010b; Gregorio-Jorge et al. 2010). A similar example from the Old World is *Ageratum yellow vein virus*, first isolated from the common weed *Ageratum conyzoides* (Tan et al. 1995) and later from soybean (Samretwanich et al. 2001) and tomato (Andou et al. 2010).

Additional work is needed to determine the geographical distribution of the begomoviruses described in this work across Africa and their potential harmfulness to crops including other cultivated species of the genus *Ocimum* and economically important crops. OcGMV, one of the begomovirus species characterized here, is closely related with a begomovirus isolated from tomato also in Uganda (Fig. 6; Table 2), so OcGMV could potentially infect this crop too. It is also important to consider that *O. gratissimum*, being a perennial plant, could be a reservoir acting as a melting pot for accumulation of different begomoviruses prone to recombine and driving the generation of new recombinant viruses, as has been shown for other wild plants (García-Andrés et al. 2006).

Although the begomoviruses discovered in *O. gratissimum* are the suspected causal agent of the symptoms shown by the hosts, Koch's postulates, as well as the possibility that these viruses infect certain crops, still need to be proven. This is important because their abilities to infect crops may result in future plant-virus epidemics in East Africa.

On the other hand, it would be interesting to determine which species of the *B. tabaci* complex present in East Africa are responsible for the transmission of the begomoviruses characterized in this work. Low populations of *B. tabaci* were found infesting some of the *O. gratissimum* plants sampled in this work but they were not genetically characterized. In a study carried out in 2003/2004 to identify the host-plant distribution of *B. tabaci* in Uganda, Sseruwagi et al. (2005) reported the occurrence of the genotype Uganda 3, currently named Sub-Saharan Africa 6 species (SSA6; Mugerwa et al. 2018), only on wild *O. gratissimum* plants. However, SSA6 was found to occur on a wider host range in Uganda, including several wild plant species (Mugerwa et al. 2018).

The importance of *O. gratissimum* as a host and reservoir of begomoviruses and whiteflies is highlighted by the results shown here and this knowledge also provides new insights into the epidemiological situation of plant viruses in Africa. These findings also have the potential to contribute to the design of more effective and specific control strategies against the panoply of viruses that have been and are being described in this continent, which otherwise constitute probably only the tip of the iceberg of virus threats to economically and socially important crops.

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