

First Report of a Complete Genome Sequence for a Begomovirus Infecting *Jatropha gossypifolia* in the Americas.

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Abstract

Jatropha gossypifolia is a weed that is commonly found with yellow mosaic symptoms growing along the roadside and in close proximity to cultivated crops in many farming communities in Jamaica. For the first time, the complete genome sequence of a new begomovirus designated Jatropha mosaic virus-[Jamaica:Spanish Town:2004] (JMV-[JM:ST:04]) was determined from field-infected *J. gossypifolia* in the Western Hemisphere. DNA-A nucleotide sequence comparisons showed closest identity (84%) to two tobacco-infecting viruses from Cuba, (Tobacco mottle leaf curl virus-[Cuba:Sancti Spiritus:03] (TbMoLCV-[CU:SS:03]), Tobacco leaf curl Cuba virus-[Cuba:Taguasco:2005] (TbLCuCUV-[CU:Tag:05])); and two weed-infecting viruses from Cuba and Jamaica, Rhynchosia rugose golden mosaic virus-[Cuba:Camaguey:171:2009] (RhRGMV-[CU:Cam:171:09]) and Wissadula golden mosaic St. Thomas virus-[Jamaica:Albion:2005] (WGMSTV-[JM:Alb:05]). Phylogenetic analysis revealed that JMV-[JM:ST:04] was most closely related to tobacco and tomato viruses from Cuba and, WGMSTV-[JM:Alb:05], a common malvaceous weed-infecting virus from Eastern Jamaica, and is distinct from begomoviruses infecting *Jatropha* species in India and Nigeria.

Keywords: begomovirus, *Jatropha gossypifolia*, Jamaica

Jatropha gossypifolia is a wild or uncultivated shrub that grows ubiquitously in Jamaica, oftentimes displaying yellow mosaic symptoms typical of begomovirus infection (Fig 1). *J. gossypifolia* belongs to the *Euphorbiaceae*



Fig 1 (A) Healthy and (B) field infected *Jatropha gossypifolia* with yellow mosaic symptoms.

family which includes important crop species such as *Ricinus communis* (castor bean), *Hevea brasiliensis* (rubber tree) and, *Manihot esculenta* Crantz (cassava) which is widely cultivated in many African countries and around the world. *J. gossypifolia* can be found growing wild in Africa, North America, India and the Caribbean [16]. It contains active antimicrobial components [11] and has many medicinal uses including the treatment of dysentery [16, 18] even though it is known to be potentially toxic when ingested [4]. *Jatropha* species have been harnessed for their seed oil that is used in the making of glue, dye and soap but more importantly as feedstock for biodiesel production. The seed cake, which is the byproduct of the seed oil extraction process, is high in nitrogen and phosphorus, thus making it ideal as a natural fertilizer [10].

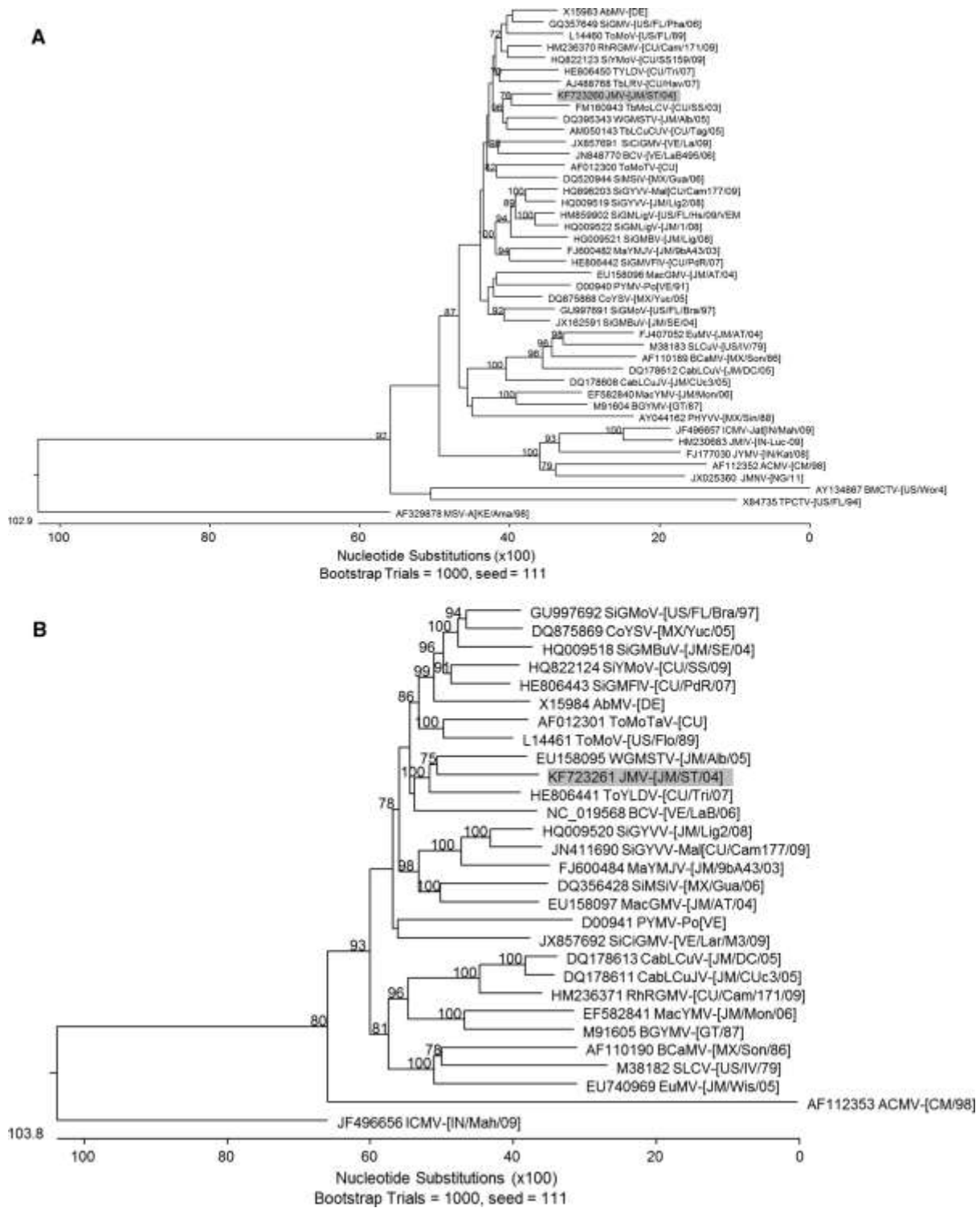
J. gossypifolia susceptibility to begomovirus infection was reported in 1930s in Puerto Rico [3], in 1951, symptoms were observed in Jamaica, Martinique and Guadeloupe [2]. Viruses associated with *Jatropha* mosaic disease have also been reported in Cuba [8], India [15, 16], Kenya [12], Singapore [6] and Nigeria [7]. *J. gossypifolia* has also shown susceptibility to begomoviruses from another euphorbiaceous species, *Croton* sp. [15].

The *Geminiviridae* family of viruses cause significant yield loss in crops. Members of the *Begomovirus* genus are whitefly-transmitted viruses that infect dicotyledons [19]. Wild plants can serve as alternate hosts and reservoirs for some crop infecting geminiviruses [1, 9, 14]. In Jamaica, begomoviruses have been characterized from vegetable crops such as tomato, pepper and cabbage and numerous uncultivated plants with yellow mosaic and leaf curl symptoms [17]. Partial DNA-A and DNA-B genomic sequences have been reported for a virus isolated from *J. gossypifolia* in Jamaica, tentatively named *Jatropha mosaic virus* (JMV, [13]). Complete DNA-A sequences are a minimum for establishing virus species [5]. Complete genome sequences also provide thorough insights into the relationship between the circulating begomoviruses and are useful for understanding virus evolution and emergence of new outbreaks. Here we report the first complete genome sequences of a bipartite begomovirus that infects *Jatropha* spp. in the Western Hemisphere.

Total DNA was extracted from symptomatic leaves of *J. gossypifolia* collected from Spanish Town, Jamaica in 2004. The previously reported nucleotide sequences for JMV (accession numbers AF324410, AF324410) were used to design overlapping primers to amplify complete DNA components. The DNA-A primers pJAC1v2123 (5'-TGGAATTCTCCCCATTCCAGT GTATCTCC) and pJAC1c2132 (5'-GAGAATTCCAGATCGACGGACGAAGTGC) overlapped at an *EcoRI* site, whilst the DNA-B primers

pJBV1v556 (5'- CATGCATGAGAATCAGTATGGGCCTGA) and pJBV1c563 (5'- CATGCATGCGTTGACATGACATCTTGG) overlapped at an *NsiI* site, and amplified the expected 2.7 kb fragment. Amplicons were cloned using the *TOPO* TA cloning kit (Invitrogen) and sequenced by MacroGen, South Korea. The Clustal V algorithm in MegAlign (DNASar, Lasergene 7.0) was used to construct the multiple alignments, phylogenetic trees and compute sequence distances. Nucleotide sequences were obtained for three DNA-A clones (JCA1, 2 and 3; KF723258, KF723259, KF723260) which were 2610 nts and were between 98.9-100% identical, as well as two DNA-B clone JCB1 and 2, (KF723261, KF723262) which were 2588 nts and were 100% identical. The JCA clones possessed the begomovirus coat protein, replication initiator protein, transcriptional activator protein and replication enhancer protein genes in their canonical positions. The nuclear shuttle protein and movement protein genes were observed in the JCB clones. A comparison of the JCA and JCB clones revealed a 165 bp common region (CR) sequence of 99.4-100% identity. The CR contained the invariant geminivirus nonanucleotide (TAATATTAC), the TATA box and a virus specific iterated sequence GGGGGAAC, which occurred three times in the CR, twice as direct adjacent repeats and once as an inverted repeat (GTTCCCCC). The CR identity and architecture suggest that these sequences represent DNA-A and DNA-B isolates that could form an infectious begomovirus unit.

The sequence of the JCA and JCB clones were used in a BLASTn search of the NCBI database. Sequences sharing at least 85% similarity to JCA with a minimum of 90% sequence coverage were selected based on the BLAST local alignment for inclusion in a DNA-A multiple sequence alignment that also contained all reported begomoviruses that infect *Jatropha* spp., begomoviruses from Jamaica and representatives from the Western Hemisphere begomovirus clades. The JCA clones showed highest sequence identity (84%) to four begomoviruses from the Caribbean region, Rhynchosia rugose golden mosaic virus-[Cuba:Camaguey:171:2009] (RhRGMV-[CU:Cam:171:09]), Tobacco mottle leaf curl virus-[Cuba:Sancti Spiritus:2003] (TbMoLCV-[CU:SS:03]), Tobacco leaf curl Cuba virus-[Cuba:Taguasco:2005] (TbLCuCUV-[CU:Tag:05]) and Wissadula golden mosaic St. Thomas virus-[Jamaica:Albion:2005] (WGMSTV-[JM:Alb:05]). In accordance with the 89% sequence similarity rule for demarcating begomovirus species (Fauquet et al 2008), the JCA sequences represent the complete DNA-A of a distinct begomovirus species. The JCB sequences shared highest nucleotide identity (70%) to WGMSTV-[JM:Alb:05], a weed-infecting virus from Jamaica.



The previously reported partial JMV DNA-A isolated from *J. gossypifolia* in Jamaica was 93% identical to nucleotides 1772-547 of sequence the JCA clones, whilst the JCB clones shared 85% identity to partial JMV DNA-B clones. The JCA clones are likely the complete DNA-A isolates of JMV species based on DNA-A similarity and association with the yellow mosaic of *J. gossypifolia* in Jamaica. The JCA and JCB clones therefore denote the first complete DNA-A and DNA-B for a begomovirus infecting *J. gossypifolia* in the Americas and are designated isolates 1-3 of Jatropha mosaic virus-[Jamaica:Spanish Town:2004] (JMV-[JM:ST:04]).

Phylogenies inferred from the multiple alignments show the DNA-A of JMV-[JM:ST:04] clustering with TbLCuCUV-[CU:Tag:05], WGMSTV-[JM:Alb:05] and TbMoLCV-[CU:SS:03], whilst the DNA-B clustered with WGMSTV-[JM:Alb:05] and Tomato yellow distortion leaf virus-[Cuba:Trinidad:2007] (ToYDLV-[CU:Tri:07]) (Fig 2). Both phylogenies were well supported with bootstrap values over 95% and show that JMV-[JM:ST:04] was more closely related to begomoviruses in the Americas than to monopartite begomoviruses infecting *Jatropha* spp. in the Eastern Hemisphere [7, 16] which were 52-54% identical to JMV-[JM:ST:04]. A close evolutionary relationship between JMV-[JM:ST:04] and begomoviruses infecting tobacco and tomato in Cuba has been observed.

Acknowledgements

Funding was provided by the School of Graduate Studies and Research and the Principal's New Initiative Fund, University of the West Indies, Mona.

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