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ANNOTATED SEQUENCE RECORD

Identification and genomic characterization of a novel tobamovirus from prickly pear cactus

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Abstract

In this work, we describe the complete sequence and genome organization of a novel tobamovirus detected in a prickly pear plant (*Opuntia* sp.) by high-throughput sequencing, tentatively named "opuntia virus 2". The full genome of opuntia virus 2 is 6,453 nucleotides in length and contains four open reading frames (ORFs) coding for the two subunits of the RNA polymerase, the movement protein, and the coat protein, respectively. Phylogenetic analysis using the complete nucleotide sequence revealed that the virus belongs to the genus *Tobamovirus* (family *Virgaviridae*), showing the highest nucleotide sequence identity (49.8%) with cactus mild mottle virus (CMMoV), being indicating that it belongs in the Cactaceae subgroup of tobamoviruses.

Prickly pear (*Opuntia* sp.) is an important crop in Mexican culture and gastronomy [1]. The main viruses affecting this crop are the potexviruses opuntia virus X and schlumbergera virus X and the tobamovirus rattail cactus necrosis-associated virus, which are associated with chlorotic ring spots [2–4]. In cacti, two tobamoviruses have been identified to date: cactus mild mottle virus (CMMoV), isolated from *Gymnocalycium mihanovichii* [5], and rattail cactus necrosis-associated virus (RCNaV), isolated from *Aporocactus flagelliformis* [6]. Tobamoviruses are economically important in agriculture, affecting plants belonging to the families Solanaceae and Cucurbitaceae [7, 8]. In 2017, prickly pear fruit and vegetable crops (*O. albicarpa* and *O. ficus-indica*) showing chlorotic annular spots (Fig. 1a) and located on farms in the municipality of Otumba, state of Mexico, were subjected to high-throughput sequencing. To do that, total messenger RNA extracted from a mixed sample of symptomatic cladodes of the two species (*O. albicarpa* and *O. ficus-indica*) were sequenced, yielding approximately 30 million sequences of 75 bp each. All sequences were filtered and trimmed using Geneious 9.0 software (Biomatters Ltd, Auckland, New Zealand), and *de novo* assembly was performed using Trinity software, which uses Bruijn graphs [9]. Contigs were analyzed using BLASTx [10], and the identified ORFs were aligned using Geneious 9.0. A contig of 6456 nt from the mixed sample of prickly pear plants was identified, and it showed the highest percentage of sequence identity to CMMoV and RCNaV (49.8%) (Supplementary Table 1). The 5′ and 3′ termini of the new virus were determined by RACE analysis (Roche Diagnostics. Mannheim Germany) using specific primers (VP3374AsTobamo-5' and VP3373sTobamo-3'; Supplementary Table 2) designed from the combined RNA-seq sequence and total RNA extracted from a symptomatic *O. ficus-indica* plant collected from a farm in Otumba, state of Mexico. In the next step, the full genome of the new virus was amplified by RT-PCR using SuperScript III One-Step High Fidelity (Thermo Fisher Scientific, Carlsbad, USA) system, total RNA extracted from the same sample used in RACE analysis, and the primers VP3379sTobamo_genome and VP3380sTobamo_genome (Supplementary Table 2), targeting the 5' and 3' end, respectively. An amplicon of 6.5 kb was obtained and cloned using a CloneJET PCR Cloning Kit (Thermo

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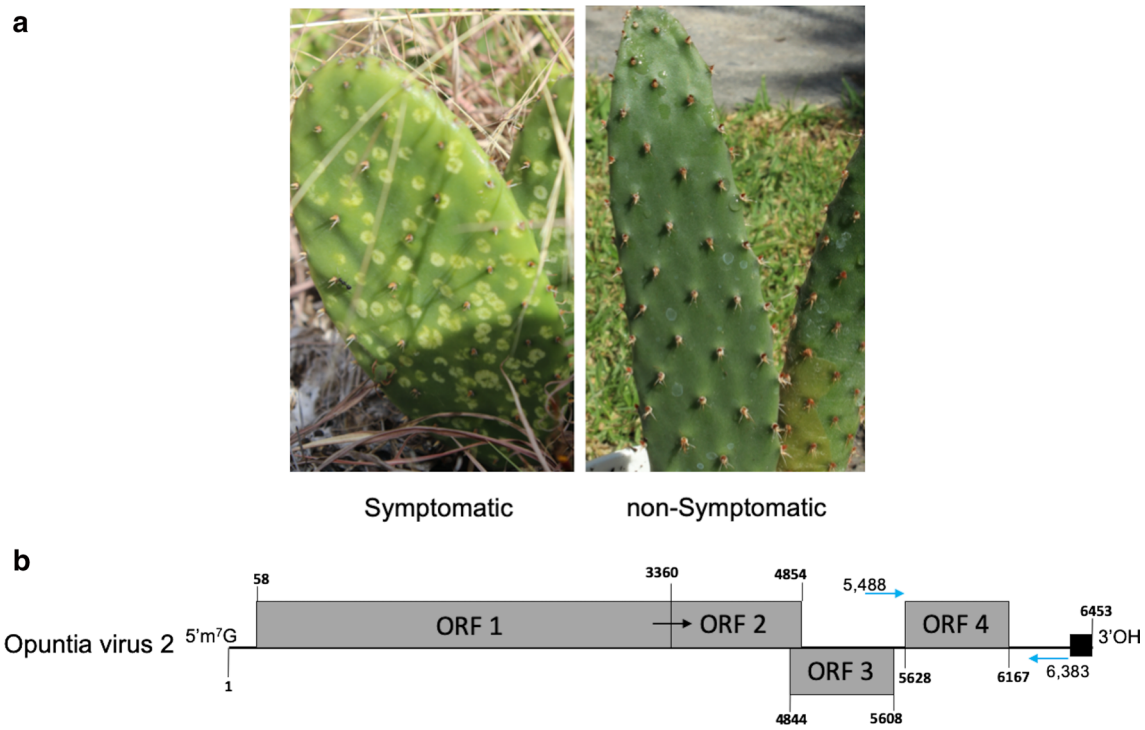

Fig. 1 **a**. *O. albicarpa* plants showing chlorotic annular spots (left) and plants showing no symptoms (right), **b**. Genomic organization of opuntia virus 2 isolated from nopal verdura. Numbers indicate the initial and last nucleotide of each ORF and the complete genome. The tRNA structure at 3' end is represented by a dark square. The black arrow indicates the site of the readthrough leaky termination codon of the 128-kDa protein. The blue arrows indicate the location of the binding sites for the Tobamo_2nopF and Tobamo_2nopR primers used in the diagnostic RT-PCR assay

Fisher Scientific, Carlsbad, USA), and the sequence of an individual colony was determined using specific primers (Supplementary Table 2). The complete genome sequence of the selected individual clone has 6543 nt long (accession number MF434821) and showed 98.4% nucleotide sequence identity to the combined RNA-seq sequence. The complete genome of the new virus, which we tentatively named 'opuntia virus 2' (OV2), has the typical genomic organization of other members of the genus *Tobamovirus*: the 5' and 3' non-coding regions of 57 nt and 286 nt, respectively; two 5'-proximal ORFs (ORF 1 and ORF 2) of 3303 nt and 4797 nt coding for the two subunits of the RNA polymerase of 128 kDa and 187 kDa, respectively; an ORF 3 of 765 nt coding for the movement protein (MP) of 27.8 kDa; and, finally, the 3' proximal ORF 4 of 540 nt coding for the capsid protein (CP) of 19.2 kDa (Fig. 1b). Phylogenetic analysis of the complete genome sequence was performed using MEGA 7 [11], and a neighbor-joining tree was constructed using the Jukes-Cantor substitution model and 1500 bootstrap pseudoreplicates. A dendrogram was obtained by aligning the complete genome sequence of OV2 and tobamoviruses of 34 species (39 sequences) available in the GenBank database. Members of other genera of the family *Virgaviridae* were included as an outgroup. The genus *Tobamovirus* has been historically divided into three subgroups based on natural host range, genomic organization and phylogenetic clustering. Based on the families of plants that are infected by tobamoviruses, these viruses have been divided into five (Brassicaceae, Cactaceae, Cucurbitaceae, Malvaceae, and Solanaceae/Orchidaceae) [5], or six subgroups (the previous five plus members infecting members of the family Passifloraceae) [12]. The phylogenetic tree included OV2 in the Cactaceae subgroup, and it was found to be closely related to other viruses isolated from cacti (CMMoV and RCNaV), with a bootstrap value of 100% (Fig. 2). Recently, Gibbs et al. [13] showed that, with the exception of three of the 29 species, all tobamoviruses fall into three clusters that have either asteroid or rosid or caryophyllid hosts (i.e., the major subdivisions of eudicotyledonous plants). OV2 clustered in the caryophyllid group, as expected (Fig. 2), reinforcing the hypothesis that tobamoviruses and their hosts have probably co-diverged [13].

The prevalence of OV2 in cultivars of prickly pear plants and other wild species of *Opuntia*, collected from Puebla and Mexico City, was analyzed by RT-PCR (Superscript III One-step, Thermo Fisher Scientific, Carlsbad, USA) using specific primers (Tobamo_2nopF and Tobamo_2nopR) targeting a conserved region of the capsid protein gene. Total

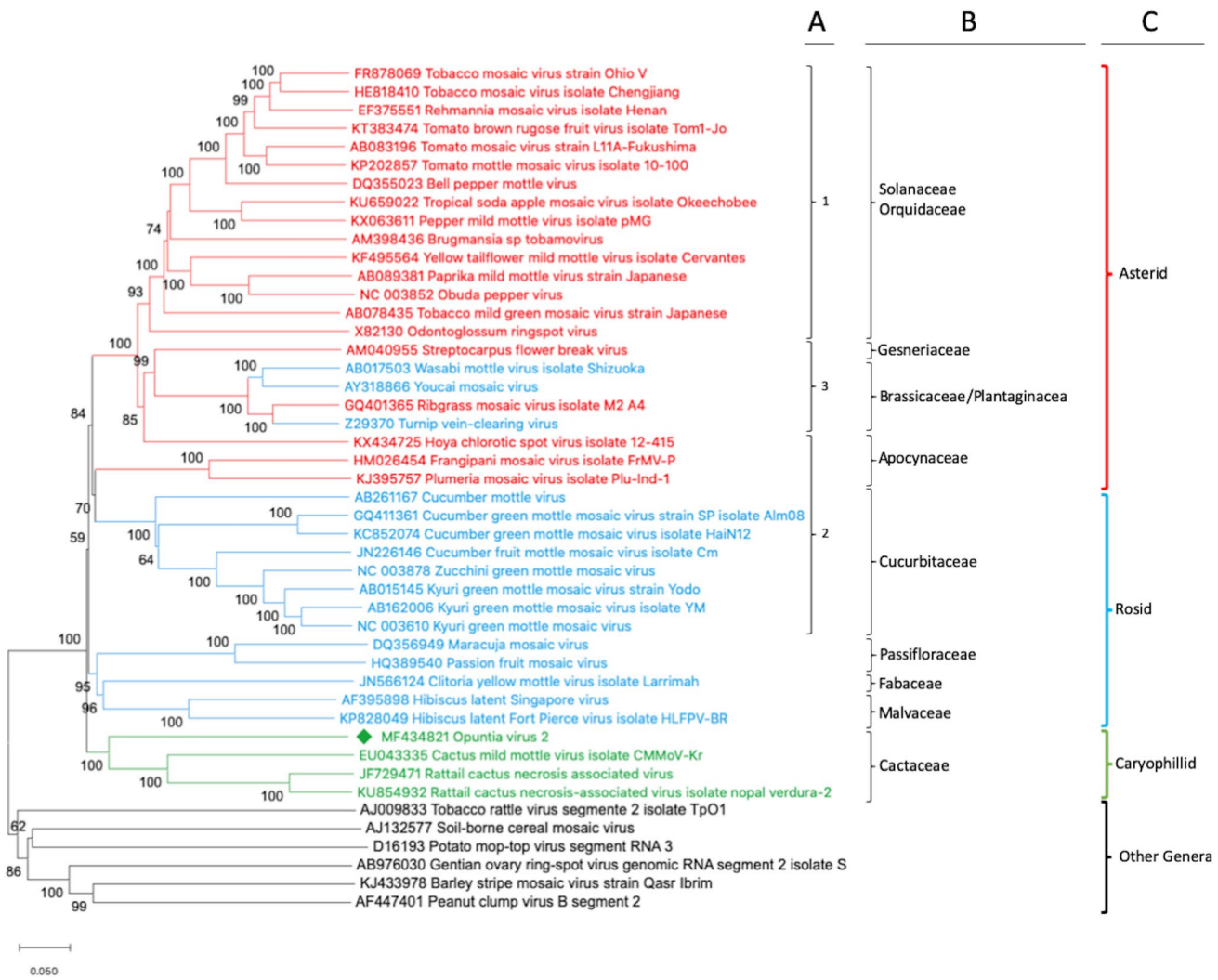

Fig. 2 Phylogenetic analysis of members of the genus *Tobamovirus* using the complete nucleotide sequences of members of 34 species available in the GenBank database plus the new virus, opuntia virus 2, identified in prickly pear cactus (MF434821), which is indicated by a diamond (♦). Tobamovirus clustering based on the phylogenetic relationships of the original host is conserved with respect to different classification approaches: A. Historical classification based on natural host range, genomic organization, and site of assembly origin. B. Classification in host families according to Song et al. 2006 [12]. C. Division on three groups of eudicotyledonous host plant lineages – asterids, rosids, and caryophyllids – according to Gibbs et al. 2015 [13]. Numbers indicate the bootstrap percentage value (1500 pseudoreplicates) for each node. Branches with bootstrap values less than 50% were collapsed. 'Other genera' refers to members of other genera in the family *Virgaviridae*. The scale bar shows the number of substitutions per site

RNA extracted from germinated *O. albicarpa* plants was used as a negative control. For the analysis, we selected 20 symptomatic wild (*O. pilifera*: 3 plants; *O. depressa:* 2 plants; *O. tomentosa*: 2 plants; *O. robusta*: 2 plants; *O. streptacantha*: 1 plant) and commercial (*O. albicarpa*: 7 plants; *O. ficus-indica*: 3 plants) plants. The expected amplicon of 890 nt (extending from the CP gene to the 3' end) was obtained in all of the samples analyzed, indicating an association of symptoms with OV2 infection. No amplification was observed in the negative control. To confirm the presence of the new virus, the amplicons obtained from *O. streptacantha, O. pilifera*, *O. depressa, O. robusta* and *O. ficus-indica* samples were extracted from a gel, using a Wizard DNA Clean-up System (Promega, Madison, USA) and sequenced directly. All amplicons corresponded to OV2, with identity values ranging from 94. 2% to 96.6%.

Based on the results presented here, we propose that opuntia virus 2 should be placed in the genus *Tobamovirus*, family *Virgaviridae*. Based on species demarcation criteria proposed by the International Committee of Taxonomy of Virus [14], opuntia virus 2 should be considered a member of a new species in the genus *Tobamovirus* due to the low level of sequence identity (less than 90%) of the complete genome to those of other tobamoviruses. Further work is

needed to evaluate more precisely the frequency of OV2 infection in other prickly pear plants grown in commercial plots or wild *Opuntia* species throughout the country, where the specific primers described here could be a useful tool.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies performed on human participants or animals by any of the authors.

References

1. Sánchez LDL, López GC, Ávalos HI (2013) Nomenclatura vernácula, uso y manejo de Opuntia spp. en Santiago Bayacora, Durango, México. Revista Chapingo Serie Horticultura 19:367–380
2. Alonso BB, Mora AG, Valdovinos PG, Ochoa MDL, Rodríguez LE, De La Torre AR (2015) Asociación de un Potexvirus como agente causal de manchas cloróticas en Opuntia ficus-indica. Rev Mex Fitopatol 33:75–86
3. De La Torre AR, Salgado OH, Salazar SM, Pallás V, Sánchez NJA, Valverde RA (2016) First report of Schlumbergera virus X in prickly pear (*Opuntia ficus-indica*) in Mexico. Plant Dis 100(8):1799
4. De La Torre AR, Salgado OH, Salazar SM, Pallás V, Sánchez NJA, Valverde RA (2016) First report of Rattail cactus necrosis-associated virus in prickly pear fruit (Opuntia albicarpa Scheinvar) in Mexico. Plant Dis 100(11):2339
5. Min BE, Chung BN, Kim MJ, Ha JH, Lee BY, Ryu KH (2006) Cactus mild mottle virus is a new cactus-infecting tobamovirus. Arch Virol 151(1):13–21
6. Kim NR, Hong JS, Song YS, Chung BN, Park JW, Ryu KH (2012) The complete genome sequence of a member of a new species of tobamovirus (rattail cactus necrosis-associated virus) isolated from Aporocactus flagelliformis. Arch Virol 157(1):185–187
7. Adams MJ, Antoniw JF, Kreuze J (2009) Virgaviridae: a new family of rod-shaped plant viruses. Arch Virol 154(12):1967–1972. https://doi.org/10.1007/s00705-009-0506-6
8. Lecoq H, Desbiez C (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. Adv Virus Res 84:67–126
9. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Regev A (2013) De novo transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat Protoc 8(8):1–43
10. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402
11. Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874
12. Song YS, Min BE, Hong JS, Rhie MJ, Kim MJ, Ryu KH (2006) Molecular evidence supporting the confirmation of Maracuja mosaic virus as a species of the genus Tobamovirus and production of an infectious cDNA transcript. Arch Virol 151:2337–2348
13. Gibbs AJ, Wood J, Garcia-Arenal F, Ohshima K, Armstrong JS (2015) Tobamoviruses have probably co-diverged with their eudicotyledonous hosts for at least 110 million years. Virus Evol 1(1):vev019
14. Adams MJ, Adkins S, Blagard C et al (2018) International Committee on Taxonomy of Viruses (ICTV) Report on the taxonomy of the Virgaviridae. http://www.ictv.global/report/virgaviridae. Accessed 19 Oct 2018