



# Complete Genome Sequence of a *Zucchini Yellow Mosaic Virus* Isolated from Pumpkin in Oklahoma

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**ABSTRACT** *Zucchini yellow mosaic virus* (ZYMV) (genus *Potyvirus*, family *Potyviridae*) was first isolated in Europe during the 1970s and in the United States in 1981. Here, we report the complete genome sequence of ZYMV isolate BL-67, isolated from pumpkin in Oklahoma during the 2016 growing season.

Cucurbits are commonly grown as cash crops in the United States. The six major cucurbits (cantaloupe, cucumber, honeydews, pumpkin, squash, and watermelon) in the United States generated \$1,732 million in 2017, which is 12.5% of the total value of production from the fresh vegetable market (1). *Zucchini yellow mosaic virus* (ZYMV) is a member of the family *Potyviridae* and the genus *Potyvirus*. (2). During the 1970s, ZYMV was simultaneously described in Italy from zucchini (3) and in France from muskmelon (4). ZYMV has worldwide distribution, mostly in tropical, subtropical, and temperate regions, and members of the family Cucurbitaceae are its primary hosts (5). ZYMV is transmitted in a nonpersistent manner by more than 26 aphid vectors (3, 4, 6) and also by seeds (7, 8).

ZYMV was first described in the United States from squash in 1981 (9). Simmons et al. (10) sequenced the complete genome of a single field isolate of ZYMV from wild gourd (*Cucurbita pepo* subsp. *texana*). Subsequently, the same field isolate of ZYMV was transmitted mechanically and by aphid vectors to obtain 24 subisolates of ZYMV, which were also completely sequenced. There are no other complete genome sequences of ZYMV available from any other regions of the United States, despite ZYMV being an emerging viral disease in the country, particularly in the southern states (11–13).

During a survey in the 2016 growing season, leaf samples from plants showing virus-like symptoms were collected from pumpkin fields in Blaine County, Oklahoma. Leaf samples were serologically screened using a dot-immunobinding assay (DIBA) (14) against antisera of ZYMV (AC Diagnostics). Among the several DIBA-positive samples, an isolate named BL-67 was used for molecular characterization of ZYMV. Infected tissues of isolate BL-67 were mechanically inoculated into pumpkin seedlings (15). Two weeks postinoculation, total RNA was extracted from leaves according to the TRI Reagent method (Molecular Research, USA) (15).

Nine pairs of overlapping primers were designed from the complete genome sequences of ZYMV isolates available in GenBank and synthesized commercially (Integrated DNA Technologies, USA). All nine fragments were successfully amplified by reverse transcription-PCR (RT-PCR), confirmed on 1% agarose gels, and cleaned with ExoSap-IT (Affymetrix, USA) (16). Purified PCR products were directly sequenced in both directions using an Applied Biosystems 3130 analyzer at the Department of Biological Science at The University of Tulsa. Nucleotide sequences were visualized using FinchTV version 1.4 (Geospiza Inc., USA) and were aligned using ClustalW (17).

The complete genome of ZYMV was 9,553 nucleotides long, encoding a polyprotein of 3,080 amino acid residues comprising 10 different genes. Nucleotide BLAST searches showed that ZYMV isolate BL-67 showed 83% to 96% nucleotide sequence identity and

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86% to 98% amino acid sequence identity with the available ZYMV complete genome sequences in the GenBank database. The highest nucleotide (96%) and amino acid (98%) sequence similarities were observed with a South Korean isolate (GenBank accession no. [MH042025](#)). Interestingly, isolate BL-67 showed only 92% nucleotide and 95% amino acid identities with the isolate of ZYMV from the United States (10).

**Data availability.** The complete genome sequence of ZYMV isolate BL-67 was deposited in GenBank under the accession no. [MK124612](#).

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## REFERENCES

- National Agricultural Statistics Service. 2018. Vegetables: 2017 summary. United States Department of Agriculture, National Agricultural Statistics Service (USDA-NASS), Washington, DC. <https://usda.library.cornell.edu/concern/publications/02870v86p>.
- King A, Adams M, Carstens E, Lefkowitz E. 2012. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses. Academic Press, London, UK.
- Lisa V, Boccardo G, D'Agostino G, Dellavalle G, D'Aquilio M. 1981. Characterization of a potyvirus that causes zucchini yellow mosaic. *Phytopathology* 71:667–672. <https://doi.org/10.1094/Phyto-71-667>.
- Lecoq H, Pitrat M, Clément M. 1981. Identification et caractérisation d'un potyvirus provoquant la maladie du rabougrissement jaune du melon. *Agronomie* 1:827–834. <https://doi.org/10.1051/agro:19811001>.
- Desbiez C, Lecoq H. 1997. *Zucchini yellow mosaic virus*. *Plant Pathol* 46:809–829. <https://doi.org/10.1046/j.1365-3059.1997.d01-87.x>.
- Katis NI, Tsitsipis JA, Lykouressis DP, Papapanayotou A, Margaritopoulos JT, Kokinis GM, Perdiki DC, Manoussopoulos IN. 2006. Transmission of *Zucchini yellow mosaic virus* by colonising and noncolonising aphids in Greece and new aphid species vectors of the virus. *J Phytopathol* 154:293–302. <https://doi.org/10.1111/j.1439-0434.2006.01096.x>.
- Al-Musa AM. 1989. Overwintering hosts for some cucurbit viruses in the Jordan Valley. *J Phytopathol* 127:49–54. <https://doi.org/10.1111/j.1439-0434.1989.tb04502.x>.
- Simmons HE, Holmes EC, Gildow FE, Bothe-Goralczyk MA, Stephenson AG. 2011. Experimental verification of seed transmission of *Zucchini yellow mosaic virus*. *Plant Dis* 95:751–754. <https://doi.org/10.1094/PDIS-11-10-0843>.
- Purcifull DE, Adlerz WC, Simone GW, Hiebert E, Christie SR. 1984. Serological relationships and partial characterization of zucchini yellow mosaic virus isolated from squash in Florida. *Plant Dis* 68:230–233. <https://doi.org/10.1094/PD-68-230>.
- Simmons HE, Dunham JP, Stack JC, Dickens BJA, Pagan I, Holmes EC, Stephenson AG. 2012. Deep sequencing reveals persistence of intra- and inter-host genetic diversity in natural and greenhouse populations of zucchini yellow mosaic virus. *J Gen Virol* 93:1831–1840. <https://doi.org/10.1099/vir.0.042622-0>.
- Ali A, Abdalla O, Bruton B, Fish W, Sikora E, Zhang S, Taylor M. 2012. Occurrence of viruses infecting watermelon, other cucurbits and weeds in the southern United States. *Plant Health Prog* 4:e00299-16. <https://doi.org/10.1094/PHP-2012-0824-01-RS>.
- Provvidenti R. 1991. Inheritance of resistance to the Florida strain of zucchini yellow mosaic virus in watermelon. *HortScience* 26:407–408.
- Provvidenti R, Gonsalves D, Humaydan HS. 1984. Occurrence of zucchini yellow mosaic virus in cucurbits from Connecticut, New York, Florida, and California. *Plant Dis* 68:443–446. [https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1984Abstracts/PD\\_68\\_443.htm](https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1984Abstracts/PD_68_443.htm).
- Ali A, Randles JW. 1997. Early season survey for pea viruses in Pakistan and the detection of two new pathotypes of pea seed-borne mosaic potyvirus. *Plant Dis* 81:343–347. <https://doi.org/10.1094/PDIS.1997.81.4.343>.
- Ali A, Mohammad O, Khattab A. 2012. Distribution of viruses infecting cucurbit crops and isolation of potential new virus-like sequences from weeds in Oklahoma. *Plant Dis* 96:243–248. <https://doi.org/10.1094/PDIS-05-11-0419>.
- Khanal V, Ali A. 2018. First Report of *Cucurbit aphid-borne yellows virus* infecting *Cucurbita pepo* in Oklahoma. *Plant Dis* 102:1046. <https://doi.org/10.1094/PDIS-10-17-1675-PDN>.
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. 2014. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539. <https://doi.org/10.1038/msb.2011.75>.