Diseases Caused by Viruses

Occurrence of *Chenopodium quinoa* Mitovirus 1 in *Chenopodium quinoa* in China

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Funding: The authors thank the funding support from the National Key Research and Development Program of China (2017YFF0210203, 2018YFC0809200) and Science and Technology Program of Hangzhou (20190101A01). Plant Dis. 105:715, 2021; published online as <https://doi.org/10.1094/PDIS-02-20-0428-PDN>. Accepted for publication 2 October 2020.

Chenopodium quinoa mitovirus 1 (CqMV1), a member of *Mitovirus* in the family *Mitoviridae*, is the first identified plant mitovirus (Nerva et al. 2019), which has been reported to be capable of infecting different cultivars of *Chenopodium quinoa* including Cherry Vanilla quinoa, GQU-7356 campesino quinoa, and wild quinoa (Nerva et al. 2019). Cultivation of *C. quinoa* has increased notably in China, with good agricultural and industrial results due to its nutritional value (Vega-Gálvez et al. 2010). In September 2019, leaf mottling and plant stunting were observed on *C. quinoa* (cv. Longli 1) plants in a field of about 0.9 acre in Qingyuan County, Zhejiang Province, China. About 33.3% (401/1,200) of *C. quinoa* showed leaf mottling and plant stunting symptoms. To identify viral agents potentially associated with this disease, an sRNA library from a symptomatic leaf sample was generated and sequenced. Total RNA was extracted using RNAiso Plus (TaKaRa, Tokyo, Japan), and the library was constructed using the TruSeq Small RNA Library preparation kit (Illumina, U.S.A.). Approximately 14 million raw reads were obtained from the Illumina MiSeq platform. The clean reads were obtained and assembled using the VirusDetect pipeline version 1.6 (Zheng et al. 2017) for virus identification. A total of 22 assembled contigs, with sizes ranging from 42 to 306 nt, could be aligned to the genome of CqMV1 isolate Che1 (accession no. MF375475) with nucleotide identities of 96.3 to 99.1% and a cumulative alignment coverage of the CqMV1 genome of 84.0%. Except for CqMV1,

no other viruses or viroids were found in the sample. Based on the assembled contigs and the reference CqMV1 genome, we designed two primer pairs (PIF: 5'-TCCGAATCTCATTTCGGAGTGGGTAGA-3' and PIR: 5'-CAGACTTTAGATCAAATGAATACACATGT-3'; P2F: 5'-TCCAGTATACCTGTGGATAGTACTTTCA-3' and P2R: 5'-CGATCTCTGCTACCAAATACTCGTGAGCC-3') to obtain the genome sequence of CqMV1 isolate Zhejiang (CqMV1-ZJ). Total RNA from the CqMV1-infected *C. quinoa* plant was subject to reverse transcription (RT) using AMV reverse transcription (TaKaRa, Japan) with random primers N6 (TaKaRa). The cDNA was then used as the template to amplify two regions in the genome, which together covered the entire genome of CqMV1-ZJ, using high-fidelity DNA polymerase KOD-Plus-Neo (Toyobo, Japan). The PCR products were cloned into the pLB vector (Tiagen, China) and Sanger sequenced (YouKang Co., China). The obtained sequences were assembled into a 2,730-nt contig, representing the complete genome of CqMV1-ZJ (GenBank accession no. MT089917). Pairwise sequence comparison using the Sequence Demarcation Tool version 1.2 (Muhire et al. 2014) revealed that CqMV1-ZJ shared a sequence identity of 96.9% with the sole CqMV1 sequence available in GenBank (MF375475), thus confirming the identity of the virus as CqMV1. Furthermore, we performed RT-PCR detection on 10 collected samples using the primer pair PIF and PIR. All seven symptomatic plants tested positive for CqMV1 infection, whereas three asymptomatic plants were CqMV1-free, suggesting a possible association between the virus and the symptoms observed. However, in the study by Nerva et al., two CqMV1-infected accessions (cvs. Regalona and IPSP1) were found to be asymptomatic (Nerva et al. 2019); we therefore speculated that the symptom caused by CqMV1 varies between different *C. quinoa* varieties or its growth environment. To the best of our knowledge, this is the first report of CqMV1 infecting *C. quinoa* in China. Its ability to be transmitted through seeds (Nerva et al. 2019) and the possible pathogenicity in *C. quinoa* raise serious concerns for the local *C. quinoa* industry. The findings reported here will assist further investigations on the epidemiology and biological characteristics of CqMV1 in Zhejiang, China.

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The author(s) declare no conflict of interest.

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Keywords: virus, quinoa, pathogen detection

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