Australasian Plant Disease Notes (2024) 19:27
https://doi.org/10.1007/s13314-024-00551-4

First Report of cucumber mosaic virus in *Zinnia elegans* in Indonesia

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Received: 24 July 2024 / Accepted: 30 September 2024 / Published online: 10 October 2024

Abstract

Zinnia elegans of Family Asteraceae is a flowering plant grown widely in gardens in Indonesia. The plant is also often integrated into pest management of different agricultural commodities as its colourful flowers may attract beneficial insects including natural enemies. A total of eight viral symptomatic and four non-symptomatic *Z. elegans* samples were collected from four districts in Kulon Progo and Sleman Regencies of Special Region of Yogyakarta. They were molecularly tested using two universal primer pairs for begomoviruses, and two specific primer pairs for cucumber mosaic virus (CMV, *Cucumovirus*) detections. The eight symptomatic samples were all positive for CMV but negative for begomoviruses infections. The four non-symptomatic samples were tested negative to begomoviruses and CMV. Partial RNA2 and RNA3 segments of four CMV isolates were sequenced to demonstrate that they are members of subgroup IB. 4Ze-Ngaglik isolate was shown to be mechanically transmitted to healthy *Z. elegans* and cucumber cv. Baresta (*Cucumis sativus*). To the best of our knowledge, this report confirmed the first CMV occurrence in *Z. elegans* in Indonesia.

Keywords Artificial inoculation · Identification · PCR · Phylogeny · Recombination

Introduction

Zinnia elegans (Family Asteraceae) is among favourite ornamentals grown in gardens in Indonesia due to its beautiful flowers and easy-care nature. However, the plant is a subject for viral infections such as by begomoviruses (Li et al. 2013; Snehi et al. 2022) and cucumber mosaic virus (CMV, genus *Cucumovirus*) (Min et al. 2020). Members of the genus *Begomovirus* have circular single stranded DNA as either mono- or bipartite genome, and mainly vectored by *Bemisia tabaci* (Brown et al. 2015). On the other hand, CMV single stranded RNA genome is three segmented into RNA1, RNA2, and RNA3 (Palukaitis and García-Arenal 2003). The virus global distribution can be attributed to mechanical and aphid transmissions as well as its capability to infect wide range of crops, ornamentals, and weeds (Rossinck et al. 1999; Shahmohammadi et al. 2015; Santosa et al. 2021).

Two *Z. elegans* plants showing conspicuous leaf mosaic and deformation, and one non-symptomatic plant were collected in Nanggulan District, Kulon Progo Regency in 2023. Two *Z. elegans* with similar mosaic on leaf and one non-symptomatic plants were further collected in each of Mlati, Ngaglik, and Gamping Districts, Sleman Regency in 2024 (Fig. 1). Both Kulon Progo and Sleman Regencies are in Special Region of Yogyakarta. In total, eight symptomatic and four non-symptomatic samples were brought to Plant Pathology Laboratory in Universitas Gadjah Mada to be molecularly tested against begomoviruses and CMV.

Total DNA and RNA were obtained from the samples following standard procedures of the respective 'Genomic DNA Mini Kit (Plant)' and 'Total RNA Mini Kit (Plant)' kits provided by their manufacturer (Geneaid Biotech Ltd., Taiwan). The cDNAs were synthesized using ReverTra Ace kit (Toyobo, Japan) then applied as templates in the subsequent PCRs against CMV using two CMV specific primer pairs: RNA2F 5`- GTTTATTTACAAGAGCGTACGG-3`/RNA2R 5`- GGTTCGAA(AG)(AG)(AT)ATAACCGGG-3` to amplify 650 bp of RNA2 (Finetti Sialer et al. 1999), and RNA3F 5`-GTAGACATCTGTGACGCGA-3`/RNA3R

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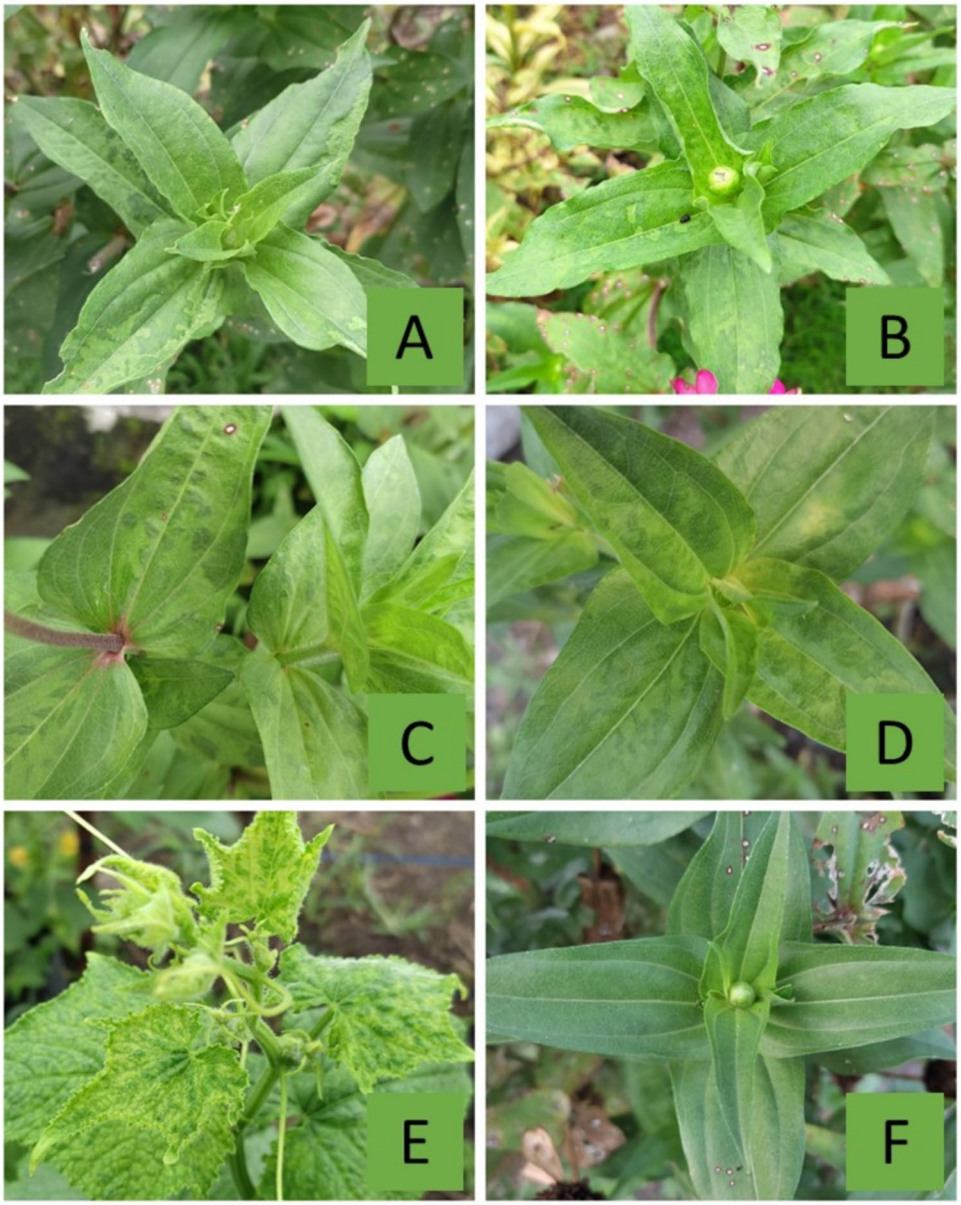

Fig. 1 Symptoms of CMV infection on naturally infected and inoculated *Zinnia elegans* and cucumber (*Cucumis sativus*). (**A**) Mosaic on *Z. elegans* from Kulon Progo regency infected by 8Ze-Nanggulan isolate, (**B**) Mosaic on *Z. elegans* from Sleman regency infected by 2Ze-Mlati isolate, (**C**) Mosaic on *Z. elegans* from Sleman regency infected by 6Ze-Gamping isolate, (**D**) Mosaic on *Z. elegans* inoculated with 4Ze-Ngaglik isolate, (**E**) Mosaic and leaf deformation on cucumber inoculated with 4Ze-Ngaglik isolate, (**F**) Healthy *Z. elegans* from Sleman regency

5`-GCGCGAAACAAGCTTCTTATC-3` for amplification of 540 bp of RNA3 (De Blas et al. 1994). The obtained DNA were applied in PCRs with begomovirus primer pairs: Krusty 5`-CCNMRDGGHTGTGARGGNCC-3`/Homer 5`-SVDGCRTGVGTRCANGCCAT-3` for amplification of 580 bp of partial AV1 region (Revill et al. 2003), and SPG1 5`-CCCCKGTGCGWRAATCCAT-3`/SPG2 5`-ATCCVAAYWTYCAGGGAGCT-3` to amplify 900 bp of partial AC2 and AC1 regions (Li et al. 2004).

All eight symptomatic samples were tested positive for CMV infection by forming the targeted bands on agarose gel after electrophoresis, and negative for begomoviruses. Four non-symptomatic samples were negative for CMV and begomoviruses according to PCR results. RT-PCR products of one positive sample from each sampling location were submitted to LPPT Universitas Gadjah Mada for bidirectional sequencing of partial RNA2 and RNA3 regions using Sanger methods. The obtained sequences of 2Ze-Mlati, 4Ze-Ngaglik, 6Ze-Gamping, and 8Ze-Nanggulan isolates were deposited in NCBI GenBank with acc. no. PP970548 – PP970551 (RNA2) and PP970552 – PP970555 (RNA3).

As for 3 July 2024, there are five CMV *Z. elegans* isolates available in GenBank but only sequences of 'Ze' from South Korea can be aligned with those obtained in this study. Twenty-two Indonesian isolates in GenBank also cannot be aligned since the sequences cover different regions than those of obtained in this study. Sequences of the four Indonesian *Z. elegans* isolates were then aligned with 19 isolates in

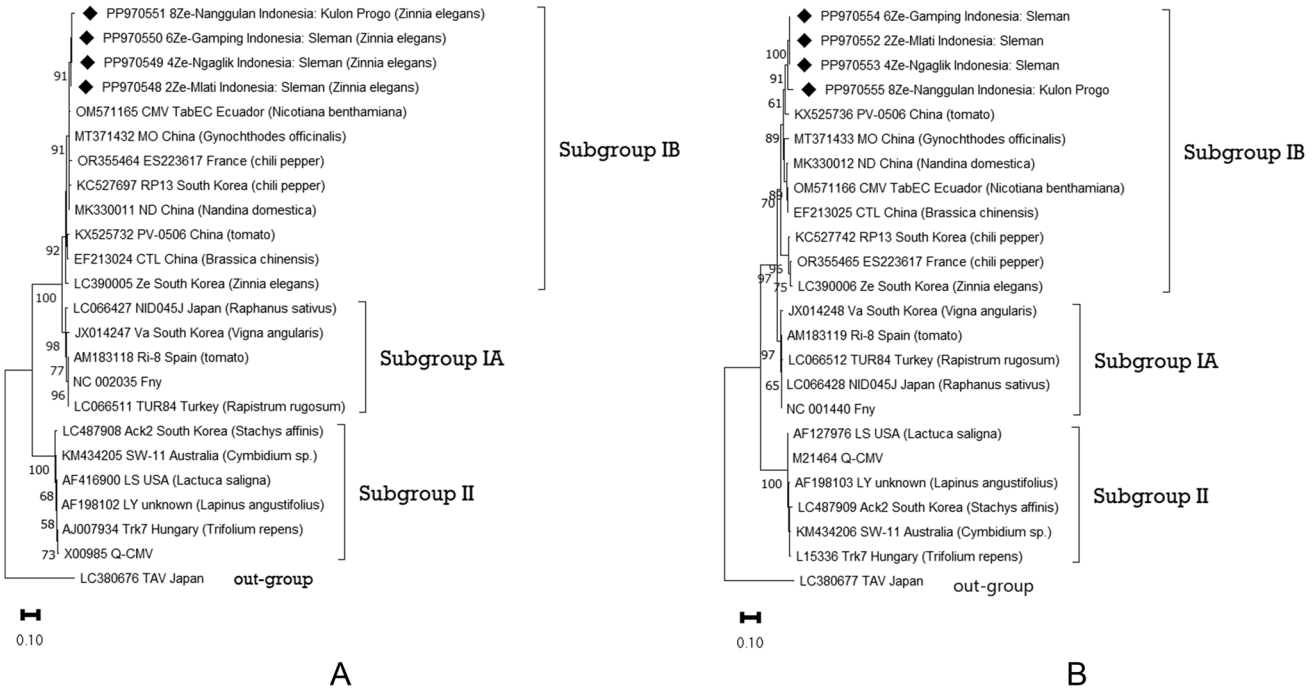

Fig. 2 Phylogenetic trees based on nucleotide sequences of (**A**) partial RNA2 (±600 bp) and (**B**) partial RNA3 (±500 bp) of cucumber mosaic virus genome. The trees were constructed using MEGA11 software with 1000 bootstrap replicates (only values > 50% were shown). Indonesian *Zinnia elegans* isolates reported here were noted with black rhombuses. An isolate of tomato aspermy virus (TAV) was included as out-group

GenBank, including 'Ze', using ClustalW algorithm in MEGA11 software (Tamura et al. 2021). Recombination Detection Program v5.30 (Martin et al. 2021) did not find recombination signal on the sequences of tested isolates. Both the RNA2 and RNA3 trees built by MEGA11 indicated that all four Indonesian *Z. elegans* isolates belong to subgroup IB thus no reassortment was observed (Fig. 2). 'Ze' was reported to be also a IB isolate (Min et al. 2020). The four Indonesian *Z. elegans* isolates shared 70–98% and 62–97% identities at nucleotide (nt) and amino acid (aa) levels, respectively, at partial RNA2, and 73–95% nt and 66–99% aa identities at partial RNA3 with the other 19 analysed isolates, as calculated by Sequence Demarcation Tool v1.2 (Muhire et al. 2014).

Sap of 4Ze-Ngaglik isolate was prepared with phosphate buffer then used in the mechanical inoculation to healthy *Z. elegans* and cucumber (*Cucumis sativus*) cultivar Baresta at two true leaf stage (Santosa et al. 2018). Mosaic and deformation symptoms were observed on leaves of *Z. elegans* and cucumber three weeks after inoculation (Fig. 1). CMV infection on both inoculated species were verified by RT-PCR using the same primer pairs mentioned before. *Z. elegans* is often integrated in the management of several pests in Indonesia due to its colourful flowers attract natural enemies. Therefore, the possible transmission of CMV from infected *Z. elegans* to other susceptible hosts could emphasize the necessity to remove symptomatic individual plants from agricultural fields.

CMV had been reported infecting *Z. elegans* in Iran (Shahmohammadi et al. 2015) and South Korea (Min et al. 2020). This was the first confirmation of the virus infection in *Z. elegans* in Indonesia to the best of our knowledge. Additional samples are needed in the future to determine whether isolates from other subgroups are able to infect *Z. elegans* in nature or not.

Acknowledgements Not Applicable.

Author contributions Conceptualization: H.A.Z., N.K.D., and A.I.S., Methodology: A.I.S. and F.R-Z., Investigation: H.A.Z. and N.K.D., Validation: A.I.S. and W.P., Formal analysis: A.I.S., W.P., and F.R-Z., Writing—original draft preparation: A.I.S., Writing—review and editing: W.P. and F.R-Z., Visualization: A.I.S., Supervision: A.I.S., Project administration: A.I.S. All authors have read and agreed to the published version of the manuscript.

Funding This research received no external funding.

Data Availability Nucleotide sequences of partial RNA2 and partial RNA3 segments of four CMV isolates reported in this study have been made available in NCBI GenBank, reference numbers PP970548-PP970555.

Declarations

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

Competing interests The authors declare that they have no competing interests.

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