J. Trop. Plant Pests Dis.
Vol. 24, No. 1, Maret 2024
Pages: 1–9

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.1241-9

RESEARCH PAPER

Begomoviruses on two chili types in Southeast Sulawesi Indonesia: variation of symptom severity assessment and DNA-betasatellite identification

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Manuscript received: 21 February 2023. Revision accepted: 19 April 2023. Available online: 10 November 2023.

ABSTRACT

The association of viral satellite DNA with Begomoviruses influences symptom expression in infected plants. Normalized Difference Vegetation Index (NDVI) is an image processing method used to assess plant health based on the plant's ability to absorb sunlight for photosynthesis. Therefore, this study aims to assess symptom severity based on symptom variation and NDVI, as well as to detect and identify the presence of beta-satellite DNA associated with chili plants. The study was conducted in North Kolaka Regency, Southeast Sulawesi Province, Indonesia. It involved observations and image recording of symptom variations in the sampled cayenne and big red chili plants, followed by the detection of beta-satellite DNA in the samples using the polymerase chain reaction (PCR). The results confirmed the presence of non-coding satellite DNA associated with the *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) in all severe, mild, or asymptomatic plant samples. In the phylogenetic tree, the non-coding satellite DNA isolates from Southeast Sulawesi are included in the betasatellite group and exhibit different genetic characteristics from other isolates available in the GenBank database. The cayenne chili with severe symptoms had lower NDVI values than the mild and asymptomatic categories, indicating that this type of chili with severe symptoms had a lower ability to absorb light. Further studies are needed to characterize the alpha-satellite type in the Southeast Sulawesi region for potential use in cross-protection mechanisms against plant virus infections.

Key words: beta-satellite, Normalized Difference Vegetation Index (NDVI), phylogeny tree, the severity of viral symptoms

INTRODUCTION

Curly yellow symptoms on the leaves of chili plants are generally caused by a viral infection (Shingote et al., 2022). Chili yellow curl diseases can be caused by several species of Begomovirus, namely, *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), *Ageratum yellow vein virus* (AYVV), and *Tomato leaf curl Java virus* (ToLCJaV) (Kenyon et al., 2014; Wilisiani et al., 2014).

In Indonesia, several Begomovirus species have also been reported, including ToLCJaV, AYVV, TYLCKaV, PepYLCIV, *Mungbean yellow mosaic India virus* (MYMIV) *Tomato leaf curl New Delhi virus* (ToLCNDV), and *Squash leaf curl China virus* (SLCCV) (Kon et al., 2007; Nurulita et al., 2015; Paradisa et al., 2022; Selangga & Listihani, 2021; Subiastuti et al., 2019; Sutrawati et al., 2020).

Initially, Begomovirus infection-related illnesses were limited to the lowlands. However, the disease has recently also been discovered in the highlands region (Kandito et al., 2021).

Begomovirus is generally transmitted by whitefly (*Bemisia tabaci*) biotype B, which is known to be invasive and polyphagous on many hosts, including chili peppers (Subiastuti et al., 2019). Whiteflies transmit Begomovirus persistently in plants (Subiastuti et al., 2019), can acquire and transmit viruses from many host plant species (Gilbertson et al., 2015), and a single whitefly can associate with more than one Begomovirus species (Purwoko et al., 2015). This virus has a wide host range of about 600 plant species, including chili, eggplant, cucumber, some weeds, string beans, tomatoes, and Ageratum (Annisaa et al., 2021; Gaswanto et al., 2016). Begomovirus can also be transmitted through seeds (Fadhila et al., 2020). These biological properties make the Begomovirus very difficult to control.

Viruses in the genus Begomovirus are often accompanied by symptoms of vein clearing, leaf

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cupping, malformations, and shrinkage. It has been reported that plants exhibiting curly yellow symptoms are infected with viruses from the Begomovirus group (Lavanya & Arun, 2021). The presence of DNA satellites influences the severity of Begomovirus symptoms. Alpha, beta, and delta are types of satellite DNA that are often detected in association with Begomovirus (Kandito et al., 2021).

The presence of alpha-satellite in plants can interfere with the replication process and reduce the accumulation of viruses, resulting in milder symptoms (Idris et al., 2011). On the other hand, beta-satellite suppresses plant defense mechanisms, such as transcription of silencing genes and jasmonic acid synthesis. This mechanism plays a significant role in suppressing viral replication, resulting in the emergence of more severe symptoms in plants (Kandito et al., 2019).

The role of delta-satellite is unknown, but in some cases, its DNA has been reported to increase or decrease the accumulation of other helper viruses. It also influences symptom severity (Fiallo-Olivé et al., 2021). Satellite DNA has been reported in chili plants infected by Begomovirus in several areas, including Magelang and Yogyakarta (Kandito et al., 2019; Kandito et al., 2020).

Visual observation and digital image processing technology are two methods of assessing disease severity based on symptoms in the field. Visual observation is a conventional technique, while digital image processing is a new technology for evaluating the severity of viral symptoms. This approach is expected to provide precise information and reduce the bias. This bias is mainly caused by the subjectivity factor and variations in the evaluator's skill level, which often occur during visual observations. A previous study has reported that digital image processing can assess the severity of viral symptoms in the field (Hasan et al., 2022) and in experimental gardens (Hasan et al., 2021a).

Furthermore, the NDVI (Normalized Difference Vegetation Index) image processing method can detect differences between virus-infected and virus-free plants (Hasan et al., 2021b). It is even easier to detect chili plants with Begomovirus symptoms on a large scale in the field by processing plant images recorded using a UAV (Unmanned Aerial Vehicle) camera (Solahudin et al., 2015).

The presence of Begomovirus satellite DNA has not been reported in mainland Southeast Sulawesi. The discovery of cayenne and big red chili with varying Begomovirus symptoms in Southeast Sulawesi may be attributed to the presence of beta-satellite types. Therefore, it is important to confirm the presence of beta-satellite types in plants infected with Begomovirus, exhibiting varying symptom severities. This study aims to detect the presence of beta-satellite types in cayenne and big red chili infected with Begomovirus at various levels of symptom severity. The assessments were based on visual observations and digital image processing using NDVI images.

MATERIALS AND METHODS

Research Site. The survey was conducted in North Kolaka Regency, Southeast Sulawesi Province Indonesia, from August to September 2022 to collect sample cultivars infected with Begomovirus, such as the cayenne and big red chili. The survey included observation and image recording of symptom variations. The presence of satellite DNA in sampled plants was determined using polymerase chain reaction (PCR) at the Laboratory of Plant Virology, Gadjah Mada University (UGM).

Observation of Symptom Variation and Visual Assessment of Severity. Symptom variation was described and then categorized as mild or severe. A greater variety of viral symptoms in a sample indicated a higher level of severity. Typical variations of Begomovirus symptoms include curly leaves, yellowing, vein clearing, leaf cupping, malformations, or leaf shrinking. Asymptomatic plants were also observed for comparison. Two samples were observed for each category of symptom severity, namely asymptomatic, mild, and severe, for both cayenne and big red chili. In total, 12 plants (two sample plants in each of the three symptom categories for both chili cultivars) were described, recorded, and used as test samples in the laboratory.

Recording and Processing of NDVI-Image. The sample plant images were captured and processed at 10:00 am UTC+8, under clear sky conditions with relatively high sun intensity. Based on a visual assessment, images of the cayenne and big red chili samples were recorded for each symptom severity category. The time required to capture the image of each sample in the same planting area was approximately 2 min. The camera specifications (Canon EOS 750D, Japan) and settings used were referenced from a previous study (Hasan et al., 2021b) but were modified to a maximum focal length of 29 mm for the lens. The camera was positioned perpendicular to the surface of

the plant crown, with a distance of approximately 60 cm (using a tripod) during image capture.

NDVI image processing is carried out using the Fiji-ImageJ application (Schindelin et al., 2012), which has the Photo Monitoring plugin installed (Horning, 2012), as in a prior study (Hasan et al., 2021b). The processing stage commences with the preparation of a 6000×4000-pixel digital image of the plant samples in TIFF format. Determination of the leaf area to be assessed (region of interest, ROI) is performed on each sample plant image using the rectangle tool. The size of all ROI images is standardized to 512×512 pixels using the resize tool, and they are subsequently converted to NDVI. Following this, the digital numbers (NDVI values) are extracted using the measurement tool.

Plant Sampling and Beta-satellite Type Satellite DNA Detection. Some symptomatic and asymptomatic leaves of the cayenne and big red chili plants were wrapped in dry tissue and placed in a plastic sample container containing calcium chloride ($CaCl_2$). This container was then stored in a styrofoam box and sent to the Plant Virology Laboratory at Gadjah Mada University for a PCR test. The satellite DNA analysis followed the procedures from a previous study (Kandito et al., 2020).

DNA extraction was carried out using a total DNA extraction kit for plants (Geneaid, Germany), following the manufacturer's instructions. The total extracted DNA was used as a template for amplification. The PCR reaction included MyTaq Redmix Polymerase (Bioline, Germany), ddH_2O, forward primer, reverse primer, and DNA templates, with total volume of 50 µL. The PCR program for the primer pair PAR1C715/PALIV1978 (Rojas, 1993), which amplified the Begomovirus DNA-A region, involved pre-denaturation at 95 ºC for 3 min, followed by 40 cycles of denaturation at 95 ºC for 1 min, annealing of the primer at 55 ºC for 30 s, and extension for 1 min 30 s. The final extension was carried out at 70 ºC for 10 min.

The PCR program for the β01/β02 primer (Briddon et al., 2002) which amplified the complete genome of the beta satellite, involved pre-denaturation at 95 ºC for 3 min, followed by 40 cycles of denaturation at 95 ºC for 1 min, annealing at 65 ºC for 30 s, and an extension for 1 min 45 s. The final extension was at 72 ºC for 10 min. PCR results were visualized on 1% (w/v) agarose gel, running at 50 V for 50 min.

Sequencing and Construction of the Phylogenetic Tree. The PCR product from two primers, namely β01/β02, were sequenced using general sequencing (Sanger sequencing method) conducted by Integrated Research and Testing Laboratory, Gadjah Mada University (LPPT-UGM). The T7 plasmid was employed to clone the betasatellite sequence. The MEGA 7 program, BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), ORF Finder program (https://www.ncbi.nlm.nih.gov/orffinder/), and Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) were used to examine the nucleotide sequence data. Data analysis included alignment and homology percentages using BLAST, ORF detection, and phylogenetic tree construction. The phylogenetic tree was constructed with 1000 bootstraps using the Neighbor-Joining method.

NDVI Image Value Based Symptom Severity Assessment. The average digital number of each NDVI image of the sample plants is used to assess symptom severity. The digital number considered in the assessment is > 0.00 (Beisel et al., 2018). Furthermore, the symptoms of the virus in the plant are categorized as more severe when the digital number is lower (close to 0.00), and vice versa.

Data Analysis. The data were tabulated and analyzed descriptively using the Microsoft Excel application.

RESULTS AND DISCUSSION

A severe case was characterized by a variety of symptoms such as stripes, curly leaves, yellowing, vein clearing, leaf cupping, and shrinking (Figure 1). Dwarf chili plants were found in this category. In contrast, the mild symptom category was only characterized by yellowish stripes, as shown in Table 1.

Table 1 illustrates that the average NDVI value for the severe symptom category was lower than for mild and asymptomatic symptoms. In cayenne chili, the average NDVI value for the severe symptom category only reached 0.462. In contrast, the mild symptom category could reach 0.490, slightly lower than the asymptomatic category of 0.492. This demonstrates that a greater variety of virus symptoms results in a lower NDVI value.

In big red chili plants, the average NDVI value for the severe symptom category reached 0.493, while the mild and asymptomatic categories reached 0.498. This indicates that a plant appearing asymptomatic does not necessarily mean that the plant is healthy, as viruses can infect without causing visible symptoms (latent symptoms). Even if typical symptoms are not visible, plant tissues infected by the virus can be

damaged, especially the chloroplasts, which impacts the destruction of chlorophyll pigments formed within the chloroplasts (Bhattacharyya & Chakraborty, 2018). Damaged chlorophyll results in suboptimal photosynthesis. Moreover, environmental factors such as low air temperature can trigger invisible symptoms (Arsi et al., 2023; Zhao et al., 2016).

Table 1 also indicates that the variation in NDVI values was lower in the cayenne chili (standard deviation= 0.273) and big red chili (standard deviation = 0.280) in the category of severe symptoms compared to the mild and no symptoms category. This indicated that damage to chlorophyll in the severe symptom category was more evenly distributed across all leaf areas, unlike to the mild symptom and no symptom categories.

Damage to chlorophyll due to virus infection results in the absorption of visible light for photosynthesis, such as blue light, becoming less effective, causing visible light to be reflected more. Healthy plant leaves generally reflect less visible light (red and blue) and more near-infrared light than diseased plant leaves (Kitchen & Goulding, 2001; Lei et al., 2016; Ozyavuz et al., 2015).

Figure 1. RGB images of the severe symptom of Begomovirus infection. A. Curly, leaf cuping, vein banding, and malformation; B. Yellowing, striped, curly leaves, vein banding, leaf cuping and shrinking.

Table 1. Variation of visual symptoms and results of health assessments on the cayenne and big red chili

Chili types	Visual symptoms variation	Symptom severity assesment	
		Visual observations	NDVI image processing (The average of NDVI[1)] Value ± SD[2)])
Cayenne chili	Visual symptoms are not visible	Asymptomatic	0.492 ± 0.281
	Yellow stripes	Mild	0.490 ± 0.280
	Striped, curly leaves, yellowing, vein banding, leaf cupping, leaf shrinking, or malformation	Severe	0.462 ± 0.273
Big red chili	Visual symptoms are not visible	Asymptomatic	0.498 ± 0.283
	Yellow stripes.	Mild	0.498 ± 0.283
	Striped, leaf curl, yellowing, vein clearing, leaf cupping, leaf malformation, or stunting	Severe	0.493 ± 0.280

[1)]Normalized Difference Vegetation Index; [2)]Standard of deviation.

A possible cause of the various symptoms (Table 1) is infection with a mixture of other virus types in the sample chili plants, aside from Begomovirus. This can lead to the occurrence of recombination between viruses within host plants (Wilisiani et al., 2019) and has the potential to generate new virus strains (Annisaa et al., 2021). In other research, it has been reported that beta-satellite can be associated with MYMIV infection in long bean plants. This beta-satellite belongs to the same group as the non-coding satellites from PepYLCIV and TYLCKaV (Pertiwi et al., 2021). The beta-satellite linked to MYMIV is also referred to as a non-coding DNA satellite because, in the open reading frame (ORF) section, amino acid insertions and premature stop codons lead to incomplete translation of amino acids during the coding of the βC1 protein.

In Indonesia, Begomovirus infections associated with beta-satellites have been previously reported. These include DNA-β associated with ToLJaV in Ageratum conyzoides weed, DNA-β01 associated with ToLCJaV, DNA-β associated with PepYLCIV in chili plants, DNA-β02 associated with AYVV [Java] in tomato plants, as well as non-coding satellites associated with TYLCKaV and PepYLCIV from eggplant and chili plants (Jamsari & Pedri, 2013; Kandito et al., 2019; Kandito et al., 2020; Kandito et al., 2021; Kon et al., 2007).

Ilyas et al. (2010) reported that in Pakistan, Begomovirus associated with long bean plants causes yellow mosaic symptoms. Based on the results of laboratory tests, it was found that recombination had occurred between DNA-B of PepYLCIV and a beta-satellite. It is suspected that a mixed infection or co-infection of PepYLCIV and MYMIV in long bean plants is the cause. PepYLCIV is a virus in the Begomovirus group that causes yellow curly leaf disease in chili plants in Indonesia and has an extensive host range, including the Leguminosae family.

The laboratory detection results using universal DNA primers did not successfully amplify complete satellite DNA. However, they confirmed the presence of non-coding or incomplete satellite DNA associated with PepYLCIV in all sample categories, as shown in Figure 2. This confirms that the cayenne and big red chili cultivated by farmers at the sampling locations in the Southeast Sulawesi region were indeed infected with Begomovirus.

Based on the phylogenetic tree (Figure 3), it is evident that the non-coding satellite DNA (PepYLCIV satDNA) isolates (samples A1-B3) from Southeast Sulawesi belong to a different group than the betasatellite isolates available in the GenBank database. This indicates that the non-coding satellite DNA isolate from Southeast Sulawesi obtained in this study exhibits distinct genetic characteristics from other isolates, making it a new isolate within the betasatellite group.

The results of this study provide intriguing information: sample plants that showed no visual symptoms actually contained beta-satellite. Therefore, it is possible that chili plants infected with Begomovirus, even without symptoms, may also contain alpha-satellite and beta-satellite. The presence of alpha-satellite enhances plant resistance by suppressing virus replication within plants. According to a study, the presence of alpha-satellite can reduce the accumulation of beta-satellite in plants (Idris et al., 2011). Both of these satellites are believed to contribute to the mild or even asymptomatic symptoms of Begomovirus infection.

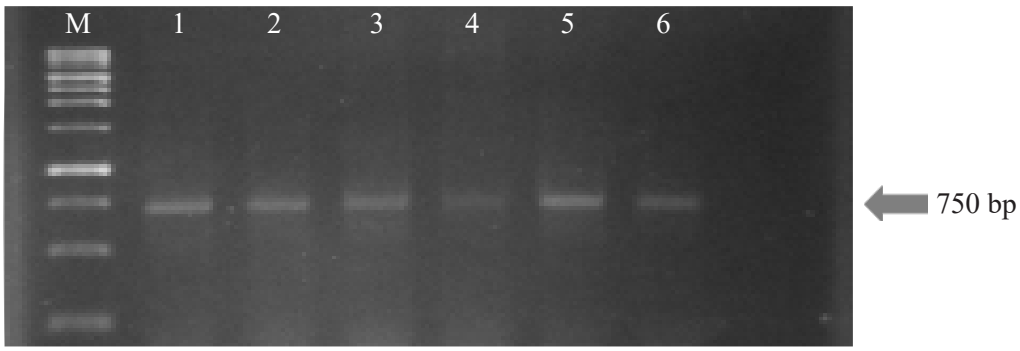

Figure 2. The results of detecting the presence of non-coding satellite DNA in the cayenne chili (1= severe, 2= mild, 3= no symptoms) and the big red chili (4= severe, 5= mild, 6= no symptoms) using the PCR technique.

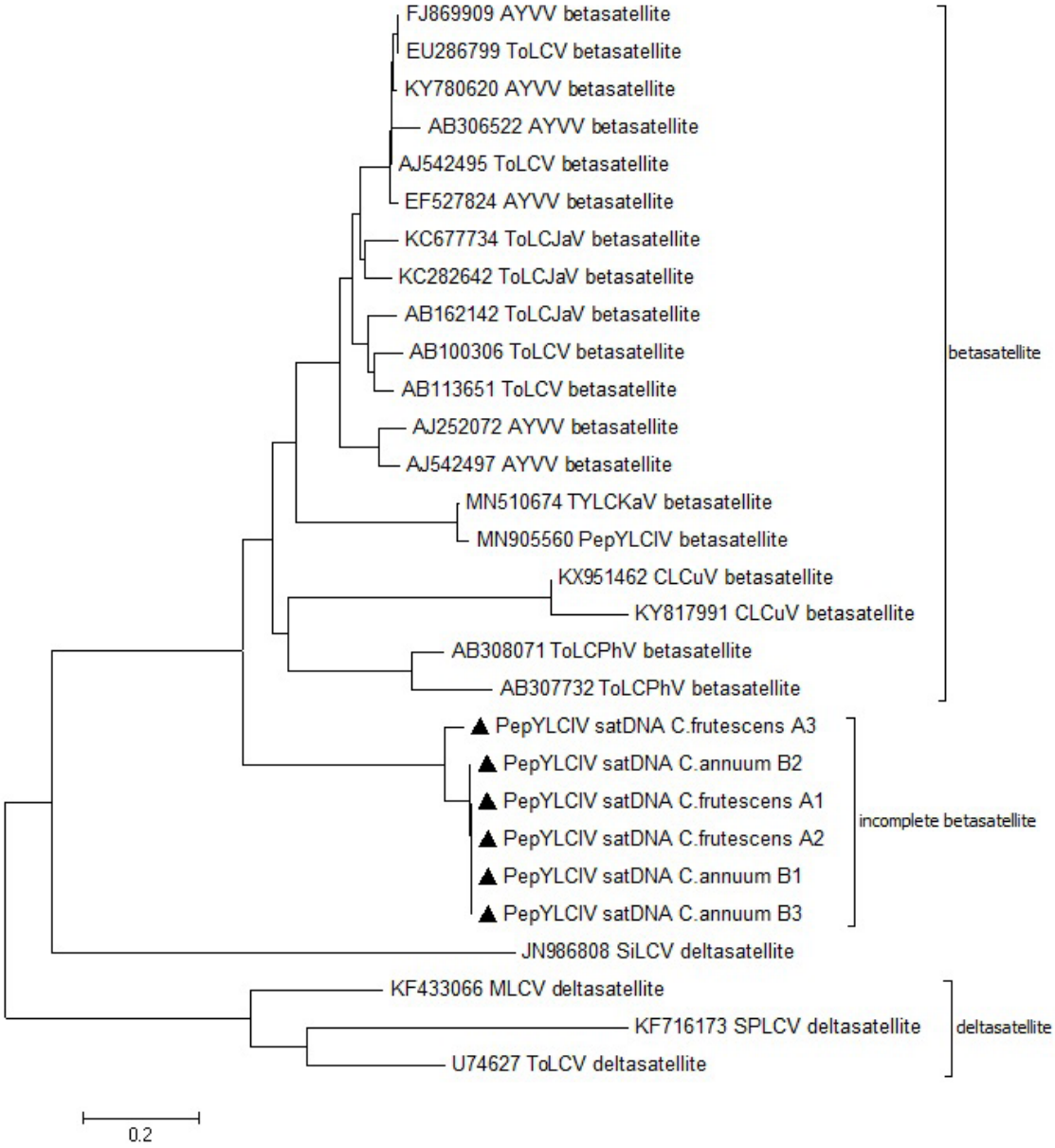

Figure 3. Phylogeny tree of non-coding satellites DNA (betasatellites group) detected from samples of cayenne (A1-A3) and big red chili (B1-B3) from Southeast Sulawesi, constructed using Neighbor-Joining method with 1000 bootstraps (used Mega 7 program).

CONCLUSION

Based on the search results of previous publications, this study is, without a doubt, the first report on the presence of non-coding satellite DNA associated with PepYLCIV in two chili plants, namely cayenne and big red chili in Southeast Sulawesi. These DNA satellites belong to the group of beta-satellite types. NDVI imaging could differentiate mild/asymptomatic symptoms from severe symptoms in cayenne chili. Subsequent studies will focus on detecting the presence of alpha-satellite, which is associated with chili plants infected with Begomovirus, even when they do not exhibit symptoms. This investigation is essential due to the potential of alpha-satellite in cross-protection mechanisms to suppress Begomovirus infection.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia 2021 for funding this study, according to contract Number 50/UN29.20/PG/2022, on May 12, 2022. The authors also express gratitude to the LPPM Halu Oleo University for administrative support, chili farmers who have helped carry out the

study, and all parties.

FUNDING

The Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia 2021 (contract number 50/UN29.20/PG/2022, on May 12, 2022).

AUTHORS' CONTRIBUTIONS

MT considered and planned the experiment; performed PCR-based Begomovirus and DNA-betasatellite detection; wrote and reviewed the manuscript. MZF, GHS, and VIV reviewed the manuscript. SY and MB conducted surveys and took plant samples for virus detection. AH considered and planned the experiment; recorded and processed plant images; analyze data; wrote and reviewed manuscripts.

COMPETING INTEREST

No competing interest.

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