First report of Capsicum chlorosis virus naturally infecting *Ageratum conyzoides* in China

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Capsicum chlorosis virus (CaCV; family *Tospoviridae*, genus *Orthotospovirus*) was first reported to infect capsicum (*Capsicum annuum)* and tomato (*Solanum lycopersicum*) in Australia in 2002 (McMichael et al., 2002). Subsequently, its infection was detected in different plants including waxflower (*Hoya calycina* Schlecter) in the United States (Melzer et al. 2014), peanut (*Arachis hypogaea*) in India (Vijayalakshmi et al. 2016), and spider lily (*Hymenocallis americana*) (Huang et al. 2017), Chilli pepper (*Capsicum annuum*) (Zheng et al. 2020), and Feiji cao (*Chromolaena odorata*) (Chen et al. 2022) in China. *Ageratum conyzoides* L. (commonly known as goat weed, family *Asteraceae*) is a natural weed in crop fields distributed in subtropical and tropical areas and a reservoir host of numerous plant pathogens (She et al. 2013). In April 2022, we observed that 90% of plants of *A. conyzoides* in maize fields in Sanya, Hainan province, China, exhibited typical virus-like symptoms of vein yellowing, leaf chlorosis, and distortion (Fig. S1 A-C). Total RNA was extracted from one symptomatic leaf of

A. conyzoides. Small RNA libraries were constructed using the small RNA Sample Pre Kit (Illumina, San Diego, USA) for sequencing with an Illumina Novaseq 6000 platform (Biomarker Technologies Corporation, Beijing, China). A total 15,848,189 clean reads were obtained after removing low-quality reads. Quality-controlled qualified reads were assembled into contigs using Velvet 1.0.5 software with a k-mer value of 17. One hundred contigs shared nucleotide identity ranging from 85.7% to 100% with the CaCV using BLASTn searches online (https://blast.ncbi.nlm.nih.gov/Blast.cgi?). Numerous contigs (45, 34, and 21) obtained in this study were mapped to the L, M, and S RNA segments of the CaCV-Hainan isolate (GenBank accession no. KX078565- KX078567) from spider lily (*Hymenocallis americana*) in Hainan province, China, respectively. The full-length of L, M, and S RNA segments of CaCV-AC were determined to be 8,913, 4,841, and 3,629 bp, respectively (GenBank accession no. OQ597167- OQ597169). Furthermore, five symptomatic leaf samples were tested to be positive for CaCV using a CaCV enzyme-linked immunosorbent assay (ELISA) kit (MEIMIAN, Jiangsu, China) (Fig. S1-D). Total RNA from these leaves was amplified by RT-PCR with two sets of primer pairs. Primers CaCV-F (5′-ACTTTCCATCAACCTCTGT-3′) and CaCV-R (5′-GTTATGGCCATATTTCCCT-3′) were used for the amplification of 828 bp fragment from nucleocapsid protein (NP) on CaCV S RNA. While another, primers gL3637 (5′-CCTTTAACAGTDGAAACAT-3′) and gL4435c (5′-CATDGCRCAAGARTGRTARACAGA-3′) were used for the amplification of 816 bp fragment from RNA-dependent RNA polymerase (RdRP) on CaCV L RNA (Fig. S1-E and -F) (Basavaraj et al. 2020). These amplicons were cloned into the pCE2 TA/Blunt-Zero vector (Vazyme, Nanjing, China) and three independent positive colonies of *Escherichia coli* DH5α carrying each viral amplicon were sequenced. These sequences were deposited in the GenBank database under accession nos. OP616700-OP616709. Pairwise sequence comparison revealed that nucleotide sequences of NP and RdRP genes of the five CaCV isolates shared 99.5% (812 bp out of 828 bp) and 99.4% (799 bp out of 816 bp) nucleotide identities, respectively. They showed 86.2-99.2% and 86.5-99.1% nucleotide identities with corresponding nucleotide sequences of other

CaCV isolates derived from GenBank database, respectively. The highest nucleotide sequence identity (99%) of the CaCV isolates obtained in the study was observed with the CaCV-Hainan isolate. Phylogenetic analysis based on NP amino acid demonstrated that six CaCV isolates (this study = 5 and NCBI database = 1) clustered into one distinct clade (Fig. S2). Our data confirmed for the first time the presence of CaCV naturally infecting *A. conyzoides* plant in China, which enriches information on the host range and will be helpful for disease management.

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References:

Basavaraj, B. Y., et al. 2020. Plant Dis. 104:3086.

Chen, L., et al. 2022. Plant Dis. 106:1077.

Huang, Y., et al. 2017. Arch. Virol. 162:3229-3232.

Melzer, M. J., et al. 2014. Plant Dis. 98:571.

McMichael, L. A., et al. 2002. Aust. Plant Pathol. 31:231-239.

She, X. M., et al. 2013. Plant Dis. 97: 418.

Vijayalakshmi, G. et al. 2016. Plant Dis. 100:2339.

Zheng, K. Y., et al. 2020. Plant Dis. 104:1175-1182.

Supplementary data

Supplementary Figure S1. Symptoms, DAS-ELISA, and RT-PCR detection for

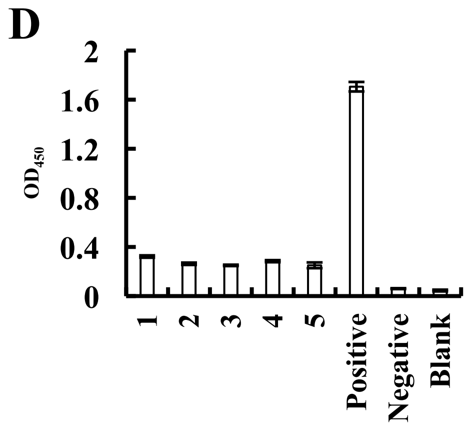
Capsicum chlorosis virus (CaCV) infecting *Ageratum conyzoides* leaves. A-C, symptoms of vein yellowing, leaf chlorosis, and distortion in leaves; D, DAS-ELISA assay on *A. conyzoides* samples by CaCV ELISA kit (MEIMIAN, Jiangsu, China). 1-5, five symptomatic leaf samples, positive control, recombinant protein; negative control, protein from CaCV-free tissue; Blank control, H_2O. E-F, RT-PCR detection of CaCV in *A. conyzoides* samples using primer pairs CaCV-F/CaCV-R and gL3637/gL4435c, respectively. Lanes 1-5, five symptomatic leaf samples; M, DL 2000 DNA marker; PC, sample from small RNA sequencing.

Supplementary Figure S2. A neighbor-joining phylogenetic tree was constructed based on NP amino acid sequences by MEGA6.0 software with 1,000 bootstrap replicates. The nucleotide sequences from CaCV isolates obtained in this study with red letters.

A

B

C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0.4
0
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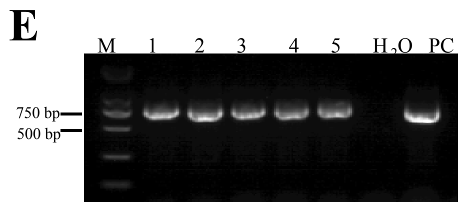
2
3
4
5
Positive
Negative
Blank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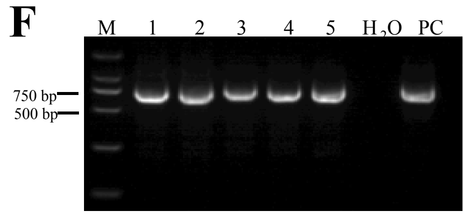
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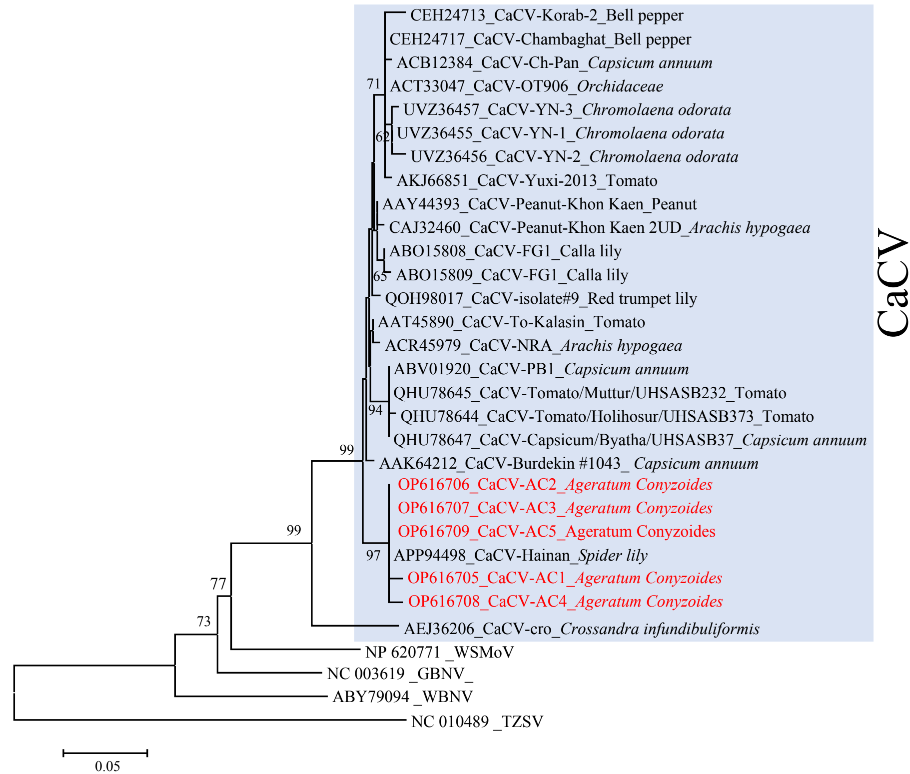
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CEH24717_CaCV-Chambaghat_Bell pepper
ACB12384_CaCV-Ch-Pan_Capsicum annuum
71
ACT33047_CaCV-OT906_Orchidaceae
UVZ36457_CaCV-YN-3_Chromolaena odorata
62
UVZ36455_CaCV-YN-1_Chromolaena odorata
UVZ36456_CaCV-YN-2_Chromolaena odorata
AKJ66851_CaCV-Yuxi-2013_Tomato
AAY44393_CaCV-Peanut-Khon Kaen_Peanut
CAJ32460_CaCV-Peanut-Khon Kaen 2UD_Arachis hypogaea
ABO15808_CaCV-FG1_Calla lily
65
ABO15809_CaCV-FG1_Calla lily
QOH98017_CaCV-isolate#9_Red trumpet lily
AAT45890_CaCV-To-Kalasin_Tomato
ACR45979_CaCV-NRA_Arachis hypogaea
ABV01920_CaCV-PB1_Capsicum annuum
QHU78645_CaCV-Tomato/Muttur/UHSASB232_Tomato
94
QHU78644_CaCV-Tomato/Holihosur/UHSASB373_Tomato
QHU78647_CaCV-Capsicum/Byatha/UHSASB37_Capsicum annuum
99
AAK64212_CaCV-Burdekin #1043_ Capsicum annuum
OP616706_CaCV-AC2_Ageratum Conyzoides
OP616707_CaCV-AC3_Ageratum Conyzoides
99
OP616709_CaCV-AC5_Ageratum Conyzoides
97
APP94498_CaCV-Hainan_Spider lily
77
OP616705_CaCV-AC1_Ageratum Conyzoides
OP616708_CaCV-AC4_Ageratum Conyzoides
73
AEJ36206_CaCV-cro_Crossandra infundibuliformis
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NC 003619 _GBNV_
ABY79094 _WBNV
NC 010489 _TZSV
0.05
CaCV