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Molecular characterization of a novel *Conyza canadensis*-infecting begomovirus in China

Pengbai Li¹, Ke Li¹, Chenchen Jing³, Rui Wu¹, Gentu Wu¹, Mingjun Li¹ and Ling Qing^{1,2*} 

Abstract

A novel monopartite begomovirus was found in naturally infected *Conyza canadensis* plants exhibiting typical begomovirus-induced yellow vein symptoms in Yunnan Province of China. Analysis of two obtained full-length viral genome sequences showed that they shared 99.8% nucleotide (nt) sequence similarity, and both consisted of 2733 nts (GenBank accession no. OK120264 and OK120266, respectively). Further analysis showed that these two sequences shared the highest nt sequence similarity (78.9%) with tomato yellow leaf curl Vietnam virus (TYLCVV) (EU189150). In the assayed *C. canadensis* plants, a betasatellite of 1,341 nts (Accession no. OK120265 and OK120267, respectively) was also found, and it shared 98.1% sequence similarity with malvastrum yellow vein betasatellite (MaYVB) (JX679254). Based on the current classification criteria for begomoviruses, we consider that the two obtained viral isolates are a novel begomovirus, and named it as conyza yellow vein virus (CoYVV). Our further analysis result showed that CoYVV is likely originated from a recombination event between tomato yellow leaf curl Yunnan virus (TYLCYNV) and tomato yellow leaf curl China virus (TYLCCNV). To investigate the effect of CoYVV infection in plant, we constructed two infectious clones (i.e., pCoYVV and pMaYVB), and inoculated them individually or together to *Nicotiana benthamiana* plants through agro-infiltration. The result showed that the plants co-inoculated with CoYVV and MaYVB developed yellow vein and downward leaf-curling symptoms, whereas the plants inoculated with CoYVV alone showed no clear virus-like symptoms. Virus infection in the inoculated *N. benthamiana* plants was confirmed through polymerase chain reaction (PCR). The result of quantitative PCR (qPCR) showed that in the presence of MaYVB, the accumulation level of CoYVV DNA was significantly increased compared to that in the plants infected with CoYVV alone.

Keywords: *Conyza canadensis*, *Begomovirus*, Conyza yellow vein virus, Malvastrum yellow vein betasatellite, Virus recombination

Background

Geminiviruses are plant-infecting viruses with circular single-stranded DNA (ssDNA) genomes that are encapsidated in twined icosahedral particles. The size of geminivirus particle is about 18 nm × 30 nm (Fauquet et al. 2008). Based on genome structures, insect vectors, and host ranges, members in the family *Geminiviridae* are

divided into fourteen genera (*Becurtovirus*, *Begomovirus*, *Grablovirus*, *Capulavirus*, *Citodlavirus*, *Eragrovirus*, *Mastrevirus*, *Maldovirus*, *Mulcrilevirus*, *Turncurtovirus*, *Opunvirus*, *Curtovirus*, *Topilevirus*, and *Topocuvirus*) (Varsani et al. 2014, 2017; Roumagnac et al. 2022). According to the International Committee on Taxonomy of Viruses, genus *Begomovirus* contains over 400 species, which cause severe economic losses to many crop plants (Varma et al. 2003; Zerbini et al. 2017). Based on the number of genomic components, begomoviruses are further divided into the monopartite and bipartite begomoviruses (Fauquet et al. 2008; Briddon et al. 2010). Bipartite

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begomoviruses contain two genomic components known as DNA-A and DNA-B, and each genomic component has approximately 2.5–2.7 kilo-bases (kb). The monopartite begomoviruses contain one genomic component (DNA-A) of about 2.7 kb in size. In general, DNA-A contains six open reading frames (ORFs) and encode the coat protein (AV1/V1) and the movement protein (AV2/V2) from the virion sense DNA strand, and the replication-associated protein (AC1/C1), transcriptional-activator protein (AC2/C2), replication-enhancer protein (AC3/C3), and a multifunctional protein (AC4/C4) from the complementary sense DNA strand (Fondong et al. 2013). Some begomoviruses also encode a AC5/C5 protein from the complementary sense strand, and a V3 protein from the virion sense strand (Li et al. 2015, 2021; Gong et al. 2021, 2022). Many recent reports have also shown that most monopartite begomoviruses are associated with satellite DNA molecules known as the alphasatellites or betasatellites (Briddon et al. 2018), and these satellite DNA molecules share no significant sequence similarities with the DNA-A components (Zhou et al. 2013; Yang et al. 2019).

Weeds and many other wild plant species are known as important host plants of many plant-infecting viruses, and play vital roles in virus disease epidemiology (Li et al. 2018; Liu et al. 2021; Wang et al. 2021). For example, horseweed (*Conyza canadensis*, family Convolvulaceae) is one of the most common weeds in fields, and can be infected with many viruses, including tomato spotted wilt virus (TSWV) and tomato chlorosis virus (ToCV) (Shah et al. 2014; Kil et al. 2015). To date, whether begomoviruses can infect *C. canadensis* in field is unknown. In this study, we analyzed *C. canadensis* plants showing virus-like yellow vein symptoms, and identified a novel begomovirus. Based on sequence analysis result, we named this virus as conyza yellow vein virus (CoYVV). A betasatellite molecule, malvastrum yellow vein betasatellite (MaYVB), was found in the samples. Inoculation of *N. benthamiana* plants with CoYVV yielded an asymptomatic infection, whereas inoculation of *N. benthamiana* plants with both CoYVV and MaYVB caused yellow vein and downward leaf-curling symptoms.

Results

Identification of a novel begomovirus and a betasatellite in *Conyza canadensis*

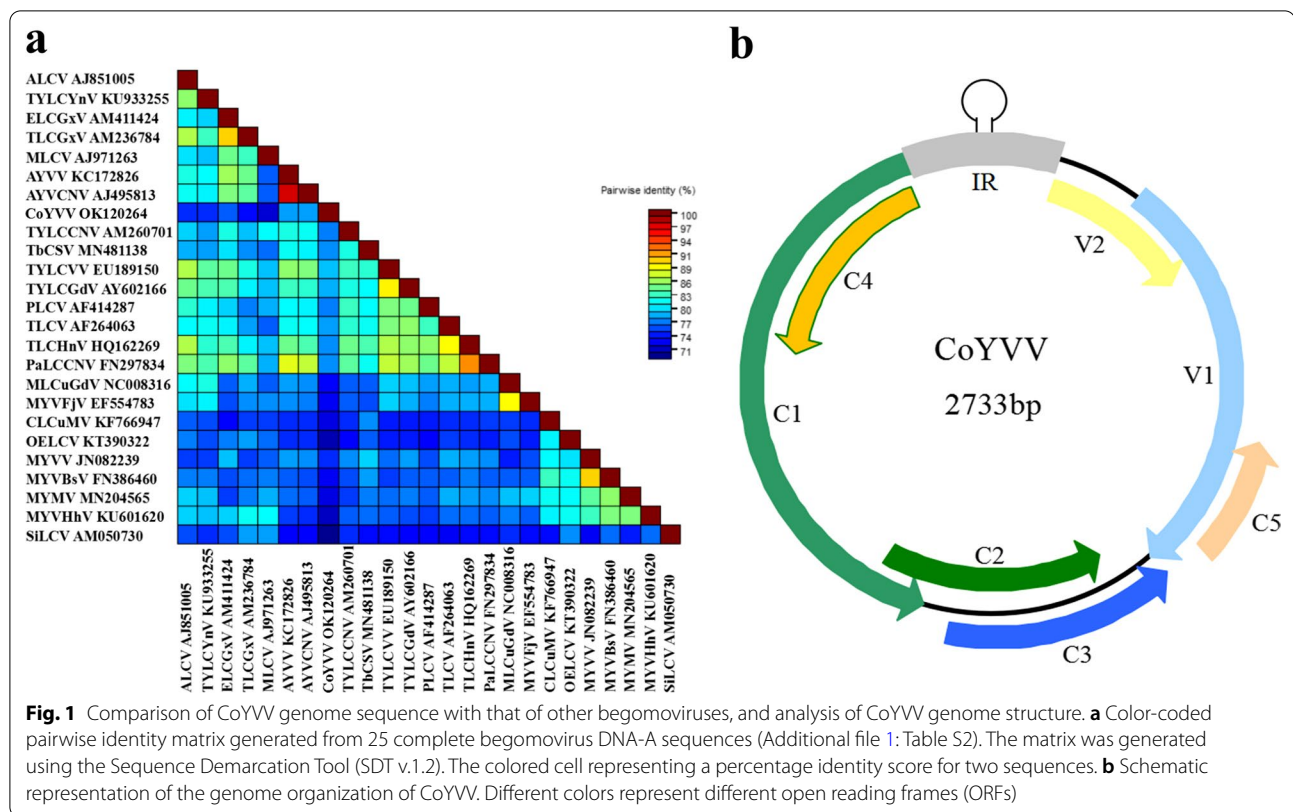
Total DNA was extracted from *C. canadensis* plants with typical begomovirus-induced yellow vein symptoms, and analyzed through PCR using primer PA and PB, reported to be universal to all members in the genus *Begomovirus* (Khan et al. 2000). Fragments of ~500 bp were PCR-amplified and cloned for sequencing. Analysis of five clones from each of the two assayed samples revealed

that these cloned sequences were 99.9–100% identical. We then designed a pair of primer, Y-full-F and Y-full-R, and used them to amplify the full-length viral genome sequences. Analysis of two full-length viral genome sequences (Y250 and Y251, GenBank accession no. OK120264 and OK120266) showed that both sequences had 2733 nucleotides (nts) and were 99.8% similar to each other. To confirm the above result, we performed a rolling-circle amplification (RCA) assay and found that these two sequences represented the same begomovirus. Our result also showed that these two viral sequences shared the highest nucleotide sequence similarity (78.9%) with that of tomato yellow leaf curl Vietnam virus (TYLCVV) (Fig. 1a). Based on the current classification criteria for begomoviruses (a threshold value of 91% for species demarcation), we considered that the newly identified virus is a novel begomovirus and named it as conyza yellow vein virus (CoYVV).

To determine whether CoYVV is a monopartite or a bipartite begomovirus, we conducted PCR amplification using primer CR01 and CR02, designed according to the published B component sequences of begomoviruses (Fondong et al. 2000). The result showed that the two analyzed tissue samples had no B components, indicating that the causal virus in these samples is a monopartite begomovirus. Further analysis of the two samples using universal abutting primers for betasatellites reported previously (Briddon et al. 2002), we obtained a 1.3 kb sequence from both samples. Sequence analysis of five clones from each of the two samples showed that both samples had a betasatellite molecule of 1341 bp in size (GenBank accession no. OK120265 and OK120267, respectively), and the two obtained betasatellite sequences shared 98.1% sequence identity with that of MaYVB (JX679254). To investigate whether these two samples also had alphasatellite, we conducted PCR assays using a pair of alphasatellite-specific primer (UN101 and UN102) reported previously (Bull et al. 2003). The result revealed that these two samples had no potential alphasatellite.

Characterization of CoYVV genome

Analysis of genome organization of CoYVV DNA showed that it is a typical Old World begomovirus and contains six open reading frames (ORFs). The ORF V1 (nt position 293–1063) and ORF V2 (nt position 133–480) are on the virus-sense strand, and encode a coat protein (CP) of 257 amino acids (aa) and a movement protein (MP) of 116 aa, respectively. Three ORFs were found on its complementary-sense strand. The ORF C1 (nt position 1512–2600) encodes a replication-associated protein (Rep) of 363 aa, the ORF C2 (nt position 1205–1612) encodes a transcriptional-activator protein (TrAP) of 136 aa, and the



ORF C3 (nt position 1060–1464) and ORF C4 (nt position 2150–2572) encode a replication-enhancer protein (Ren) of 135 aa and a multifunctional protein of 141 aa, respectively. To investigate whether CoYVV also encodes a AC5/C5 protein as previously reported for several other begomoviruses (Li et al. 2015, 2021), we analyzed the nucleotide sequence of CoYVV using the ORF Finder software (<https://www.ncbi.nlm.nih.gov/orffinder/>). The result indicated that CoYVV can encode a C5 protein (nt position 707–997) of 97 aa (Fig. 1b). In addition, the intergenic region (IR) contained a putative stem-loop structure with a conserved nonanucleotide sequence TAATATT/AC (nt position 2727–2).

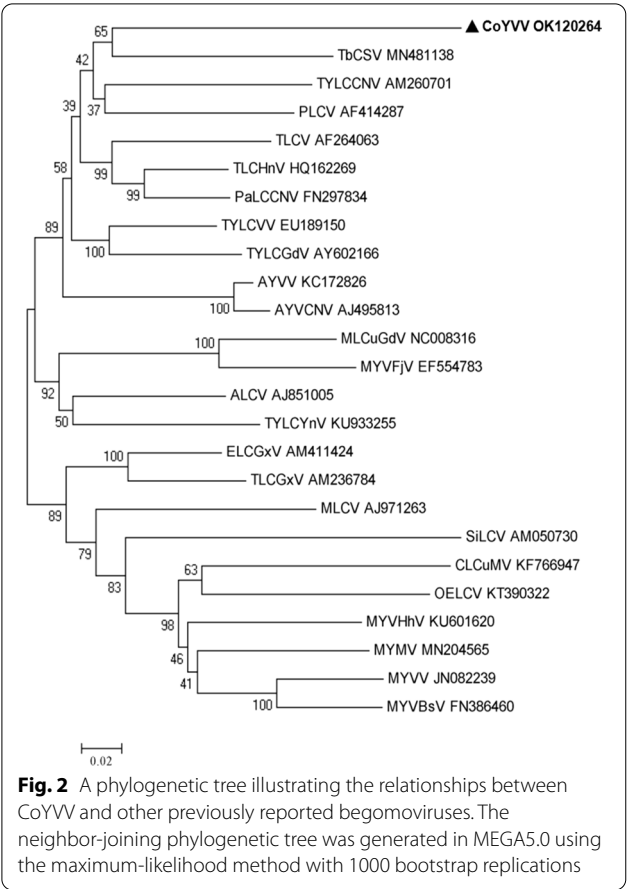
Phylogenetic relationship between CoYVV and other geminiviruses

To investigate the phylogenetic relationship between CoYVV and other begomoviruses, we constructed a phylogenetic tree using full-length viral genome sequences. According to the phylogenetic tree, CoYVV was clustered with tobacco curly shoot virus (TbCSV, MN481138) (Fig. 2). The recombination event analysis indicated that there was at least one significant recombination event in the CoYVV genome. The recombination breakpoint was 589 nucleotide long (nt position 2089–2677), covering part of the IR, the C1 ORF, and the entire C4 ORF.

The major parent was predicted to be tomato yellow leaf curl Yunnan virus (TYLCYnV) and the minor parent was tomato yellow leaf curl China virus (TYLCCNV) (Table 1).

Infectivity of CoYVV and MaYVB

To investigate the pathogenic effect of CoYVV and MaYVB in infected plants, we produced infectious clone pCoYVV and pMaYVB, and inoculated *N. benthamiana* plants with pCoYVV or pCoYVV and pMaYVB through agro-infiltration. Fourteen days post inoculation (dpi), the *N. benthamiana* plants inoculated with CoYVV alone showed no virus like symptoms (Fig. 3a). However, the plants co-inoculated with CoYVV and MaYVB showed yellow vein in systemic leaves and downward leaf-curling (Fig. 3a, b). The result of PCR showed that CoYVV did accumulate in the *N. benthamiana* plants inoculated with CoYVV alone or co-inoculated with CoYVV and MaYVB. MaYVB accumulation was detected in the plants co-inoculated with CoYVV and MaYVB. When the inoculated plants were analyzed through qPCR, we found that the accumulation level of CoYVV was significantly increased in the *N. benthamiana* plants co-inoculated with CoYVV and MaYVB compared to that in the plants inoculated with CoYVV alone (Fig. 3c).



Discussion

In China, geminivirus-caused diseases occur in many regions and are continuously spreading (Jing et al. 2016; Rojas et al. 2018). *Bemisia tabaci* is an important transmission vector of many begomoviruses (Mansoor et al. 2003; Rojas et al. 2018). With rapid increase of international food trade and global warming, populations of *B. tabaci* in many regions of China have greatly increased in recent years. Weeds are intermediate hosts of many viruses and play important roles in virus disease epidemics (Xie et al. 2013). For example, *B. tabaci* transmitted geminiviruses can spread effectively between food crops

and weeds and cause severe yield and economic losses in crop productions (Mansoor et al. 2003; Varma et al. 2003).

In this study, we identified a novel begomovirus in *C. canadensis* plants, a common weed in many regions of China. Based on our phylogenetic analysis result, we named this virus as conyza yellow vein virus (CoYVV). Our sequence analysis result indicated that CoYVV is a novel virus with typical monopartite begomovirus genome organization. Recombination event analysis result showed that CoYVV is likely originated from a recombination between TYLCYnV and TYLCCNV. To date, there is no report on TYLCYnV and/or TYLCCNV infection in *C. canadensis* plants, and these two begomoviruses were not detected in the *C. canadensis* samples analyzed in this study. Therefore, we speculate that the recombination event occurred in an unidentified host plant co-infected with both TYLCYnV and TYLCCNV, and the recombined virus was then transmitted to other plant species, including *C. canadensis*, via *B. tabaci*.

Previous reports have indicated that spread and replication of betasatellites depend on their helper viruses (Zhou et al. 2013). On the other hand, the presence of most betasatellites can enhance the accumulation levels of their helper viruses and induce more severe disease symptoms (Briddon et al. 2003). In this study, we found a betasatellite in the CoYVV-infected *C. canadensis* plants, and the betasatellite was identified as MaYVB. Furthermore, the *N. benthamiana* plants inoculated with CoYVV alone showed no clear virus-like disease symptoms. Whereas the *N. benthamiana* plants co-inoculated with CoYVV and MaYVB developed yellow vein and downward leaf-curling symptoms. In addition, the presence of MaYVB significantly increased the accumulation level of CoYVV. This finding supports a recent report showing that co-inoculation of *N. benthamiana* plants with tomato yellow leaf curl China betasatellite (TYLCCNB) and ageratum leaf curl Sichuan virus (ALCScV) caused more severe disease symptoms, and an approximately tenfold increase of ALCScV DNA compared to that in the plants inoculated with ALCScV alone (Li et al. 2020).

Due to their wide geographic distribution and rapid propagation, weeds are known as shelters for many plant

Table 1 The region in the CoYVV genome corresponding to the recombination breakpoint detected by at least six different methods in the RDP4 package

Recombination breakpoint	Potential parent sequence [#]		P-value	Methods used to detect recombination [*]
	Major	Minor		
2089–2677	TYLCVnV (KU933255)	TYLCCNV (AM260701)	3.121 × 10 ^{−25}	R, G, B, M, C, S

R, RDP; G, GeneConv; B, Bootscan; M, MaxChi; S, CHIMAERA; C, SiScan; 3S, 3SEQ; TYLCVnV, tomato yellow leaf curl Yunnan virus (GenBank Accession No. KU933255); TYLCCNV, tomato yellow leaf curl China virus (GenBank accession no. AM260701)

[#] Major parent refers to the parental isolate that contributes a larger fraction of the recombined sequence as compared to the minor parent

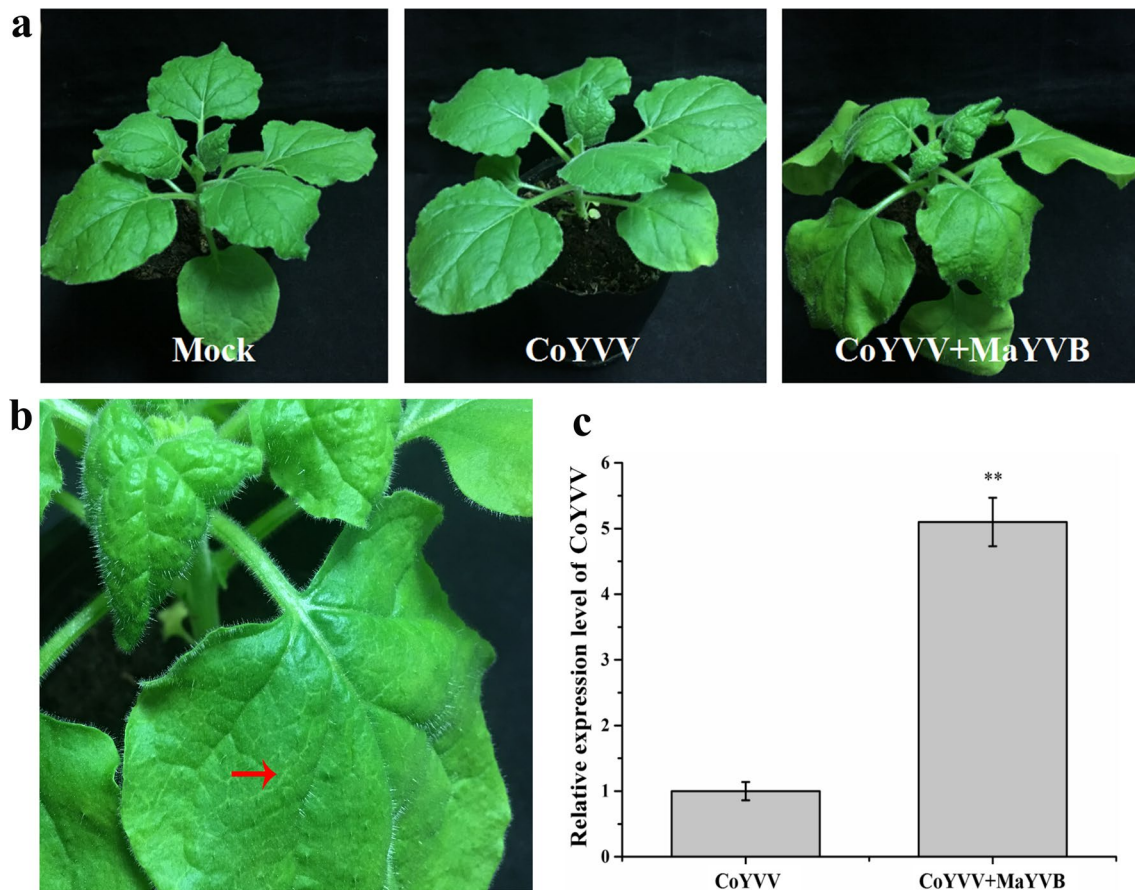


Fig. 3 Symptoms induced by CoYVV infection or CoYVV and MaYVB co-infection in *N. benthamiana*. **a** Symptoms induced by CoYVV infection or by CoYVV and MaYVB co-infection in *N. benthamiana*. **b** A close up image showing the yellow vein (red arrow) and downward-leaf rolling symptoms caused by CoYVV and MaYVB co-infection. The plants were photographed at 14 dpi. **c** qPCR analysis of CoYVV accumulation in *N. benthamiana* plants infected with CoYVV or co-infected with CoYVV and MaYVB. ** indicates a significant difference ($P < 0.01$) between the treatments, determined using the Student's *t*-test

viruses and insect vectors, and play important roles in virus epidemics and evolution (Rubio et al. 2020). Therefore, further studies are needed to determine the geographical distribution and host range of CoYVV, and the species of betasatellites that can co-infect plants with CoYVV.

Conclusions

In this study, we identified a novel begomovirus in *Conyza canadensis* in China, and named it as CoYVV. In the analyzed *C. canadensis* plants, CoYVV was found to co-exist with MaYVB. Our recombination event analysis result indicated that CoYVV is likely originated from a recombination between TYLCYnV and TYLCCNV. Pathogenicity study showed that inoculation of plants with both CoYVV and MaYVB caused typical begomovirus-induced yellow vein and downward leaf-curling symptoms. Geographic distribution of CoYVV

and its potential threat to agriculture require further investigations.

Methods

Plant materials

Field-grown *C. canadensis* plants displaying typical geminivirus-induced yellow vein symptoms were collected in 2014 in Yunnan Province of China.

Determination of full-length viral genomic sequences

Total DNA was extracted from the collected plant tissue samples using the CTAB method (Zhou et al. 2001), followed by PCR application using degenerate primers (PA and PB) that are conserved for all members of the genus *Begomovirus* (Khan et al. 2000). The resulting PCR products were separated in 1% agarose gels through electrophoresis, and fragments of ~500 bp were purified and cloned into the pGEM-T Easy Vector (Promega,

Madison, WI, USA) for sequencing. Based on the sequencing results, a primer pair (YN-full-F and YN-full-R, Additional file 1: Table S1) was designed for the amplification of the full-length virus genome sequence.

To confirm that the collected *C. canadensis* samples were indeed infected with a single begomovirus, we amplified the viral DNAs from individual samples using the rolling-circle amplification (RCA) technology. The RCA products were digested with *Cla*I restriction enzyme and then separated in 1% agarose gels. The resulting 2.7-kb products were purified and ligated into the pGEM-3Zf vector (Promega Corporation, Madison, WI, USA) for sequencing.

Sequence and phylogenetic analyses

The obtained full-length sequences of CoYVV were analyzed using the DNASTar and DNAMAN Version 4.0 software followed by sequence alignment using the MUSCLE software. Twenty-four begomoviruses with high nucleotide similarity to CoYVV were selected for pairwise alignment and evolutionary analysis. Pairwise identity scores were calculated using the Sequence Demarcation Tool (SDT 1.2) (Muhire et al. 2014). A neighbor-joining phylogenetic tree was constructed using the maximum-likelihood method in MEGA5.0, with 1000 bootstrap replications (Tamure et al. 2011). The recombination event analysis was conducted using the Recombination Detection Program version 4 (RDP v4.69) with seven recombination analysis methods (i.e., RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, and 3Seq). The recombination events detected by more than six methods (P -value $> 1 \times 10^{-6}$) were considered as the 'clear' recombination as described previously (Martin et al. 2010). GenBank accession numbers of the sequences analyzed in the study are listed in the Additional file 1: Table S2.

Construction of infectious clones

To investigate the infectivity of the identified CoYVV and MaYVB, we constructed a pCoYVV infectious clone through the seamless cloning method. Briefly, the full-length CoYVV sequence was PCR-amplified using primer Y250-InFu-F1 and Y250-InFu-R1 (Additional file 1: Table S1), and named it as 1.0A. The second PCR was performed using primer Y250-InFu-F2 and Y250-InFu-R2 (Additional file 1: Table S1) to amplify a 0.9-mer CoYVV fragment and named it as 0.9A. Both 1.0A and 0.9A fragments were inserted into the pBinPLUS vector through the seamless cloning method to generate a recombinant pBinPLUS-CoYVV vector. Meanwhile, an infectious clone of MaYVB was also constructed using the double-digestion method reported previously (Guo et al. 2008).

Agro-infiltration assays

The produced virus expression vectors were transformed individually into *Agrobacterium tumefaciens* strain GV3101 cells, which were grown at 28 °C for 48 h. The *Agrobacterium* cells were pelleted through centrifugation, resuspended with an inoculation buffer to $OD_{600} = 1.0$, and then infiltrated into leaves of *N. benthamiana* plants with 4–6 leaves.

Quantitative PCR analysis

Quantitative detection of CoYVV accumulation in the inoculated plants was done through qPCR using primer CoYVV-qPCR-F and CoYVV-qPCR-R (Additional file 1: Table S1). Each qPCR reaction was 20 μ L [10 μ L 2 \times Novostart SYBR qPCR SuperMix Plus Kit (Novoprotein), 0.5 μ L forward primer (10 mM), 0.5 μ L reverse primer (10 mM), 1 μ L total DNA (100 ng/ μ L), and 8 μ L RNase-free ddH₂O] and was performed on a real-time C1000 TouchTM Thermal Cycler (Bio-rad, Hercules, CA). The conditions for qPCR were as follows: 95 °C for 1 min followed by 40 cycles of 95 °C, 10 s; 60 °C, 20 s; and 72 °C, 30 s. The expression of *N. benthamiana* 25S rRNA gene was used as an internal control. The resulting data was analyzed using the $2^{-\Delta\Delta CT}$ method (Livak et al. 2001).

Abbreviations

aa: Amino acids; ALCScV: Ageratum leaf curl Sichuan virus; CoYVV: Conyza yellow vein virus; CP: Coat protein; dpi: Days post inoculation; IR: Intergenic region; MaYVB: Malvastrum yellow vein betasatellite; MP: Movement protein; ORF: Open reading frame; qPCR: Quantitative polymerase chain reaction; RCA: Rolling-circle amplification; Ren: Replication-enhancer protein; Rep: Replication-associated protein; ssDNA: Single-stranded DNA; ToCV: Tomato chlorosis virus; TrAP: Transcriptional-activator protein; TSWV: Tomato spotted wilt virus; TYLCCNV: Tomato yellow leaf curl China virus; TYLCCNB: Tomato yellow leaf curl China betasatellite; TYLCVV: Tomato yellow leaf curl Vietnam virus; TYLCYNV: Tomato yellow leaf curl Yunnan virus.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-022-00118-0>.

Additional file 1. Table S1: Primers used in this study. **Table S2:** Names, abbreviations, and GenBank accession numbers of begomoviruses used in pairwise identity comparisons and phylogenetic analysis.

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Author contributions

LQ conceived and designed the experiments. PL, KL, CJ and RW conducted the experiments. GW, ML and PL analyzed the experimental data. All authors read and approved the final manuscript.

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Availability of data and materials

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Declarations**Ethics approval and consent to participate**

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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