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Development and application of sugarcane yellow leaf virus vectors in sugarcane

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Abstract

The aphid-transmitted polerovirus, sugarcane yellow leaf virus (SCYLV), is one of the most widespread sugarcane viruses and causes huge economic losses to the global sugarcane production. Molecular studies and disease resistance breeding against SCYLV are impeded both by the lack of infectious clones and comprehensive knowledge of this virus. In this study, we present the development of the first infectious cDNA clone of SCYLV, which could efficiently infect *Nicotiana benthamiana, Arabidopsis thaliana* and sugarcane plants. SCYLV-based recombinant heterologous EGFP protein vector was also developed. Using this system, we identified the green peach aphid, *Myzus persicae*, as a new compatible transmission vector and recognized LC05-136 and GT10-2118 as more resistant cultivars of SCYLV than Badila. This system provides an indispensable resource for both SCYLV biological research and sugarcane breeding efforts.

Keywords Sugarcane yellow leaf virus, Sugarcane, Aphid, Infectious clone, Expression vector

Background

As a pivotal tropical cash crop, sugarcane (*Saccharum* spp.) is a broadly cultivated C4 plant for sugar and bioenergy production in Brazil, China, Australian India, Africa, Southeast Asia, etc. (Lu et al. 2024). Although the traditional breeding has greatly improved yield and some disease resistance persisted over decades, challenges such as climate change, limited cultivation resources, and the difficulty in segregating complex genes reveal its limitations (Budeguer et al. 2021; Lu et al. 2024). Sugarcane cultivation is generally limited by a rapid increase of inter-regional spread of sugarcane viral diseases like sugarcane yellow leaf disease (YLD) and sugarcane mosaic disease (SMD) (Lu et al. 2021; Yin et al. 2024). YLD has a significant impact on sugarcane production in India and America, with severe cases leading to a reduction in crop yield by up to 50% (Saritha et al. 2023). YLD, which caused by mixed-infections by both sugarcane yellows phytoplasma (SCYP) and sugarcane yellow leaf virus (SCYLV, *Polerovirus SCYLV*), globally leads to degeneration of the elite sugarcane varieties in the field (Parmessur et al. 2002; Viswanathan 2021). To date, there is yet no efficient method to evaluate SCYLV resistance without contamination of other pathogens or aphids.

As one of YLD-causing pathogens, SCYLV belongs to the family *Solemoviridae* with a single-stranded positive-sense RNA genome of about 6 kb in size (Lin et al. 2015). SCYLV infection has significantly affected sugarcane development, chlorophyll content, sugar transport, and sucrose accumulation in susceptible sugarcane varieties. The virus is transmitted over long distances through sugarcane tubers trade and locally by insect vector aphids,

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e.g. *Melanaphis sacchari* (Bertasello et al. 2021). Poleroviruses, including potato leafroll virus (PLRV), Brassica yellows virus (BrYV), and turnip yellows virus (TuYV), are pathogens that cause significant damage to major crops (Zhang et al. 2018; Li et al. 2019). These viruses infect phloem-associated tissues of plants mostly and are transmitted to these tissues by aphids through feeding (Zhang et al. 2015). Virion of SCYLV is 24–29 nm in diameter and comprises of 180 coat protein subunits (Holkar et al. 2020). SCYLV genome encodes seven proteins, and three of them are expressed by the genomic RNA and four by subgenomic RNAs. The three open reading frames (ORFs) associating virus replication are translated directly from the genomic RNA including ORF0 (encoding a 30 kD RNA-silencing suppressor of the virus), ORF1 (encoding the 72 kD multifunctional protein viral protease), and ORF2, which is translated via a ribosomal frameshift within ORF1 to yield the 120 kD RNA-dependent RNA polymerase (RdRp). Four other ORFs associating virus movement are expressed by subgenomic RNAs including ORF3a (encoding the 4.8 kD viral system movement protein), ORF3 (encoding the 21 kD viral capsid protein), ORF4 (encoding a 17 kD putative movement protein), and ORF5 (expressed by translational read-through as a fusion protein with the capsid protein) (Smirnova et al. 2015). SCYLV is restricted in sugarcane phloem and changes the physiological indicators in sugarcane leaves, especially higher starch concentration (Lehrer et al. 2010). SCYLV may enhance aphid feeding and increase transmission efficiency by the change of conditions in sugarcane plants. The different physiological indicators are key mechanism to attracting the aphid vector to stimulate the speed of spread (Zhao et al. 2019; Ye et al. 2021).

Traditional breeding program for disease resistance against SCYLV exclusively necessitates aphid for virus inoculation, making it difficult to distinguish sugarcane resistance between aphid and SCYLV or other aphid-transmitted viruses since the sugarcane aphid could transmit multiple viruses (Lehrer et al. 2007; Bertasello et al. 2023). Although tissue culture techniques could be effective in the management of YLD, virus resistance breeding is still the most effective way for disease control. Infectious clones have been widely utilized for researches including functional genomics, replication and expression of viral proteins, genome editing for host-virus interactions and breeding for virus resistance (Zhang et al. 2024). Virus infectious clones allow for precise evaluation of plant resistance to single virus without the interference of other pathogens or insect vectors. However, the lack of infectious clones of SCYLV hinders research on the detailed mechanisms and biology of SCYLV, besides SCYLV-resistant sugarcane breeding.

In this study, we constructed an infectious cDNA clone of SCYLV and validated its infectivity in both *Nicotiana benthamiana* (dicot), *Arabidopsis thaliana* (dicot) and *Saccharum* spp. (monocot). The major *Saccharum* hybrids phenotypes were also tested, and the cultivars LC05-136 and GT10-2118 were identified as more resistant to SCYLV than Badila. Our agroinfection method would be a sufficient SCYLV resistance sources screening system for both the conventional resistance breeding program and biotechnological breeding by transgenic sugarcane or genome editing strategies.

Results

Characterization of SCYLV from sugarcane in China

In February 2023, a sugarcane yellow leaf disease (YLD) associated with yellowish midrib of leaves was found in plantations in Nanning city, Guangxi province, China (Fig. 1a). It was speculated that SCYLV was the possible causing agent of the disease since the symptoms resembled those caused by SCYLV and the aphid vector—*M. sacchari* was present. An amplification of partial SCYLV coat protein gene was obtained in 3/9 sugarcane samples (Fig. 1b). These partially cloned SCYLV sequences are identical to the reference genome sequence of SCYLV (NC_000874.1) in GenBank, which was isolated from host *Saccharum* spp. cultivar CP65-77 from Florida, USA, and confirmed by a phylogenetic tree constructed based on the CP gene of SCYLV (Fig. 1c). We failed to acquire the complete genome of SCYLV from these samples even with several pairs of specific primers designed according to all known SCYLV accessions in the GenBank.

Establishment of an infectious clone of SCYLV in *N. benthamiana*

To establish an infectious clone, we synthesized the full-length SCYLV genomic cDNA based on the reference sequence of SYCLV in GenBank (NC_000874.1) in pCB301 vector. An infectious cDNA clone of SCYLV was constructed by combining four essential element components. These components include cauliflower mosaic virus (CaMV) 35S promoter, SCYLV cDNA, hepatitis D virus ribozyme (HDVRz) and the nopaline synthase gene (*NOS*) terminator. The final infectious vector construct was named pCB301-SCYLV (Fig. 2a). The plasmid pCB301-SCYLV with sequencing confirmation was then transformed into the *A. tumefaciens* strain EHA105. *Agrobacterium* containing pCB301-SCYLV was infiltrated onto leaves of *N. benthamiana* seedlings at the stage of 4–6 true leaves to evaluate its infectivity. The presence of SYCLV could be detected in *N. benthamiana* stems by RT-PCR and there is no positive band of SCYLV from plants inoculated with

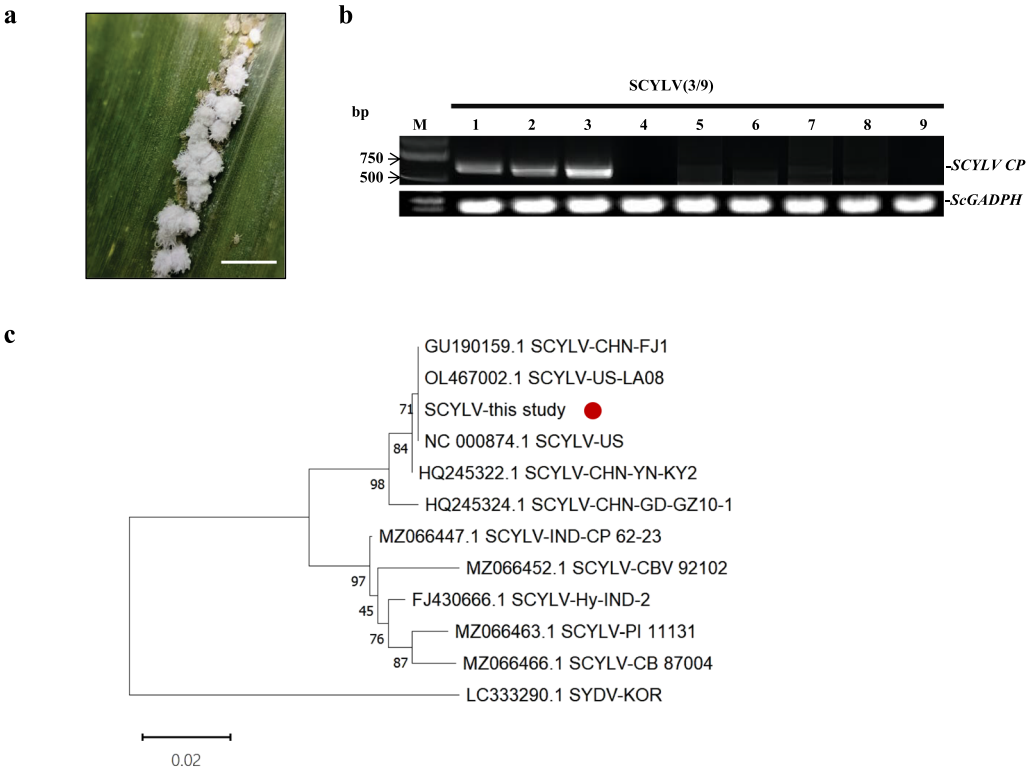

Fig. 1 The Identification and detection of sugarcane yellow leaf virus (SCYLV) in sugarcane. **a** Sugarcane leaves collected from field in Nanning, Guangxi Province, China, in 2023. Bar = 1 cm. **b** SCYLV detection was performed using RT-PCR with SCYLV-specific primers (SCYLV-TF and SCYLV-TR) that amplify a 591 bp fragment of the *CP* gene. Sugarcane internal control *ScGADPH* gene. **c** Neighbor-joining tree calculated from the *CP* gene of SCYLV in Guangxi (circle symbol), other 10 SCYLV isolates and Setaria yellow dwarf virus (SYDV). The SYDV was used as an outgroup. The terminal node consists of the accession number, isolate location and name

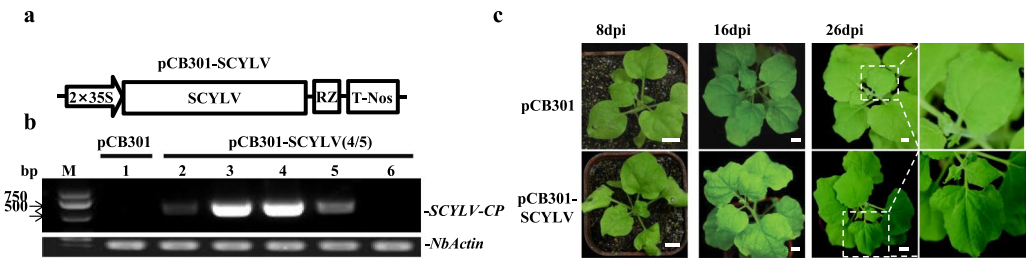

Fig. 2 Construction and validation of infectious clone of *SCYLV* in *N. benthamiana*. **a** Schematic representation of the constructing strategy for the full-length cDNA clone of SCYLV. **b** RT-PCR detection of stalks of *N. benthamiana* infected by *Agroinoculation* with the infectious clone of pCB301-SCYLV at 8dpi. SCYLV detection was performed using RT-PCR with SCYLV-specific primers (SCYLV-TF and SCYLV-TR). *NbACTIN* as a *N. benthamiana* reference gene. **c** Symptoms of *N. benthamiana* at 8, 16, and 26 days after inoculation with pCB301 or pCB301-SCYLV by *Agroinoculation*. Bars = 1 cm

empty vector (Fig. 2b) in the newly emerged leaves of *N. benthamiana* plants inoculated with SCYLV showed mild curl of top leaves at 8dpi (Fig. 2c). In a later stage, mild crinkling leaf symptoms could be observed in all SCYLV-positive *N. benthamiana* plants (Fig. 2c).

Heterologous expression of EGFP by recombinant SCYLV in *N. benthamiana*

To test whether this vector could be further developed to express heterologous protein in plants, an *EGFP* (the gene of Enhanced Green Fluorescent Protein) and

a second subgenomic promoter of the SCYLV movement protein gene were inserted into the upstream of 3′-UTR of SCYLV genome, named pCB301-SCYLV-EGFP (Fig. 3a). RT-PCR confirmed the presence of both viral and exogenous EGFP RNAs of SCYLV-EGFP in *N. benthamiana* stems, supported by the anticipated size of amplified cDNA fragments for both *EGFP* and 3′-UTR (Fig. 3b). Western blot analysis also confirmed the presence of the EGFP in *N. benthamiana* stems, suggesting successful SCYLV-EGFP infection in *N. benthamiana* (Fig. 3c). We further used epifluorescence microscopy to examine the systemic movement of SCYLV-EGFP in *N. benthamiana.* At 8 dpi, EGFP fluorescence could be observed in these vascular cells in stems of *N. benthamiana*, but no positive fluorescence was observed in the control empty vector (pCB301)-inoculated plants (Fig. 3d).

A new compatible aphid vector *M. persicae* carrying SCYLV

To date, three aphid species have been reported as efficient transmission vectors for SCYLV. To find new insect vectors, we tested the ability of the green peach aphid *Myzus persicae* in transmitting SCYLV-EGFP. After feeding *M. persicae* on SCYLV-EGFP-infected *N. benthamiana* or pCB301- inoculated *N. benthamiana* for 28 days (Fig. 4a), total RNA of *M. persicae* were extracted and was detected by this virus-specific RT-PCR. RT-PCR analysis verified the presence of SCYLV-EGFP in *M. persicae*, with expected fragment size (Fig. 4b). We also conducted transmission of SCYLV-EGFP from *N. benthamiana* to *A. thaliana* with *M. persicae* as the transmission vector. Western blot analysis confirmed the presence of EGFP in SCYLV-EGFP-infected *A. thaliana* leaves (Fig. 4c). Furthermore, there are green fluorescence signals in the vascular bundles of *A. thaliana* inoculated with aphids carrying SCYLV-EGFP, demonstrating systemic infection of SCYLV-EGFP in *A. thaliana* (Fig. 4d). SCYLV-EGFP caused mild symptoms including yellowish leaves in *A. thaliana* (Fig. 4e).

Infectivity of the infectious clone of SCYLV-EGFP in sugarcane

To test the infectivity of SCYLV on its natural host, we inoculated SCYLV infectious clone to sugarcane (Badila cultivar) seedlings. At 40 dpi, sugarcane inoculated with the SCYLV infectious clone did not exhibit any apparent symptoms. RT-PCR result (12/17) confirmed high infectivity of SCYLV infectious clone in Badila sugarcane (Fig. 5a), but significantly lower (2/8) for the infectious clones of a recombinant SCYLV-EGFP vector (Fig. 5b, c). Western blot analysis also confirmed the presence of the EGFP protein in sugarcane, suggesting successful infection of SCYLV-EGFP in sugarcane (Fig. 5d). This evidence indicate the efficient infection of SCYLV and SCYLV-EGFP infectious clone in sugarcane.

Identification of sugarcane varietal resistance to SCYLV

To find resistant cultivars with our infectious SCYLV clones, two hybrid sugarcane cultivars GT10-2118 and LC05-136 were used. LC05-136 is one of the most cultivated cultivars and has been cultivated more than 1.67 million hectares in China in the past 10 years (Wu et al. 2024b). Both GT10-2110 and LC05-136 were derived from the same crossing between the maternal parent CP81-1254 and the paternal parent ROC22. These two sugarcane cultivars are notable for their high yield, sugar

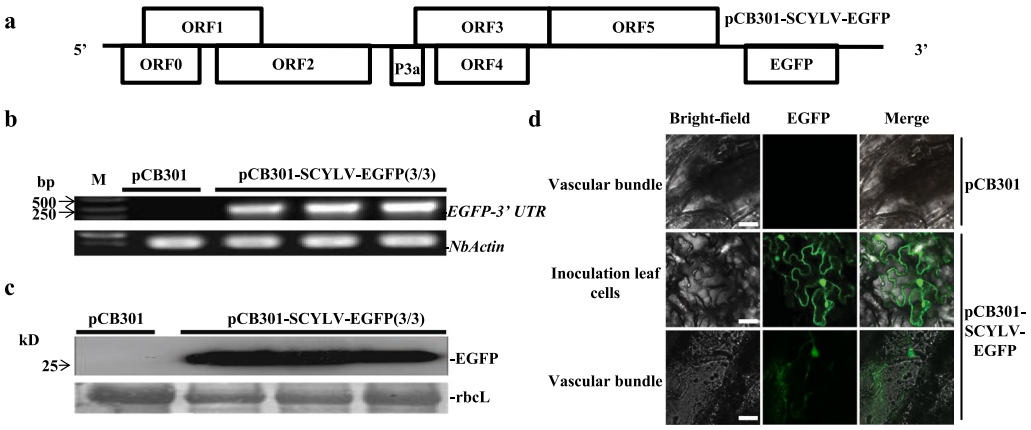

Fig. 3 Recombinant *SCYLV* vector expressing exogenous EGFP in *N. benthamiana*. **a** Schematic diagram of SCYLV-EGFP construct. **b** SCYLV-EGFP detection was performed by RT-PCR with SCYLV-EGFP specific primers (EGFP-TF and SCYLV-2R) to amplify a 302 bp region from partial EGFP to the 3' UTR of SCYLV-EGFP in *N. benthamiana* plants. *NbACTIN* as a *N. benthamiana* internal control gene. **c** Detection of the EGFP by western blotting. **d** Cellular localization of EGFP fluorescence in the inoculated leaves or systemic of infected *N. benthamiana* plants after aphid delivery of SCYLV or SCYLV-EGFP. Bars = 20 μm

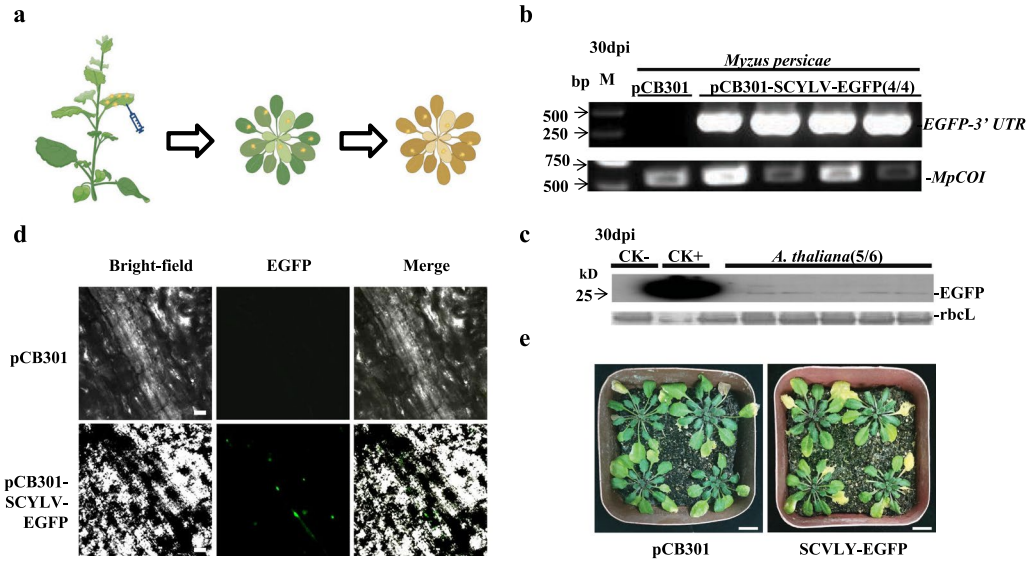

Fig. 4 The aphid-mediated transmission of SCYLV-EGFP in *Arabidopsis thaliana*. **a** Schematic diagram of aphid-mediated transmission of SCYLV-EGFP in *A. thaliana*. After feeding of SCYLV-EGFP recombinant virus on *N. benthamiana* leaves at 30 days post-infestation (dpi), viruliferous *Myzus persicae* was transferred onto *Arabidopsis thaliana*. The picture was drawn by Figdraw. **b** Detection of SCYLV-EGFP in *Myzus persicae* by RT-PCR in 30 dpi and aphid gene *MpCOI* was served as an internal control. **c** Detection of the EGFP in virus infected *Arabidopsis* plants by Western blotting. Empty vector control (pCB301)-*N. benthamiana* infested *Myzus persicae* was used as negative control (CK–). GFP-transgene *N. benthamiana* line 16c was served as positive control (CK+). *A. thaliana* refers to *A. thaliana* plants inoculated with SCYLV-EGFP-carrying *Myzus persicae*. **d** Cellular localization of EGFP fluorescence in the leaves of SCYLV-EGFP infected *A. thaliana*. Bars = 20 μm. **e** Symptoms of SCYLV-EGFP infection transmitted by *Myzus persicae* in *A. thaliana* plants. Bars = 1 cm. Manifestations of SCYLV-EGFP infection transmitted by the green peach aphid in A. thaliana plants

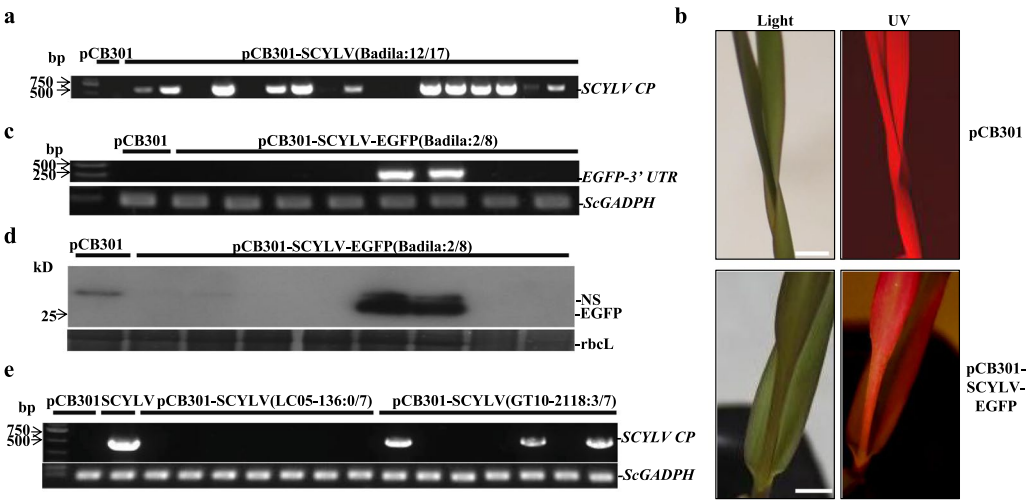

Fig. 5 Infectivity of SCYLV and SCYLV-EGFP infectious clone in sugarcane and assessment of sugarcane resistance of SCYLV. **a** SCYLV detection was performed using RT-PCR with SCYLV-specific primers (SCYLV-TF and SCYLV-TR). **b** Infectivity of SCYLV-EGFP in Badila observed under white light (left panel) and UV light (right panel) at 40 dpi. Bars = 1 cm. **c** RT-PCR detection of SCYLV in Badila systemic leaves by EGFP-TF and SCYLV-2R. **d** Detection of SCYLV-EGFP by Western blot using antibody against EGFP at 40 dpi in sugarcane plants. Badila plants inoculated with empty vector or SCYLV-EGFP, respectively. NS represents non-specific binding. **e** Detection of the SCYLV infected LC05136 cultivar or GT10-2118 cultivar by RT-PCR. Sc*GADPH* as a sugarcane reference gene

content, drought and cold tolerance, strong regrowth, and broad adaptability (Wu et al. 2024b). The infection of SCYLV was only successful in two out of seven GT10-2118 plants but failed in all 7 LC05136 plants (Fig. 5e). It seems that both LC05136 and GT10-2118 are more resistant to SCYLV than Badila. In addition,

inoculation of SCYLV-EGFP infectious clone was unsuccessful in both LC05136 and GT10-2118 (Table 1).

Discussion

The SCYLV has greatly affected sugarcane yield, reducing it by up to 50% (Saritha et al. 2023). Efforts in breeding SCYLV-resistant sugarcane cultivars and understanding the detailed interaction mechanism between the virus and its host have been hindered by the absence of infectious virus clones. We established SCYLV or SCYLV-EGFP reporter infectious cDNA clones based on an *Agroinfection* system in the sugarcane cultivars Badila and two hybrid cultivars, LC05-136 and GT10-2118 (*Saccharum officinarum L.*). Only Badila was successfully infected with SCYLV-EGFP (Fig. 5c, d). Our results indicate that Badila was the most sensitive cultivars among the tested germplasms, the cultivars LC05-136 and GT10-2118 possess the resistance to SCYLV to a certain extent (Fig. 5b, e). The cultivar LC05-136 is known for several significant advantages, including high yield and high sugar content among others, and has been cultivated across more than 1.67 million hectares in China up to 2023 (Wu et al. 2024b). Our research added an additional advantage to LC05-136, providing resistance to SCYLV (Fig. 5d). GT10-2118 is one of the most promising sixth-generation sugarcane varieties, boasting high yield, high sugar content, and resistance to another important fungal disease, sugarcane smut. Even though it does possess weaker resistance to SCYLV infection compared with LC05-136, GT10-2118 represents a more resistant cultivar to SCYLV than Badila in this research. Future work is needed to verify its virus resistance under field conditions.

SCYLV is known to be transmitted by three aphid species, including *M. sacchari, Rhopalosiphum maidis* and *R. rufiabdominalis* (Schenck and Lehrer. 2000; Behary Paray et al. 2011). Here we showed that *M. persicae* is another compatible aphid species. Traditional methods involve crossing resistant varieties with high-yielding but susceptible ones, followed by selection and back-crossing to combine desirable traits (Wu et al. 2024a). In recent years, some progress has been made in screening germplasm for SCYLV resistance. Techniques such as RNAi or CRISPR/CASs technology could be other alternative methods to enhance virus resistance in sugarcane together with our infectious clones ready for use (Khalil et al. 2018). Our system can readily test the resistance to SCYLV of new sugarcane cultivars to promote sugarcane breeding.

Reverse genetics system has been a pivotal tool in studying the process of viral infection. In a past study, a fusion EGFP protein and CP-RTD protein method was applied to trace the process of poleroviral infection (Boissinot et al. 2017). Due to the influence of RTD function, a significant reduction in the viral load carried by aphids after remodeling of viruses by the strategy (Bortolamiol-Becet et al. 2018). By subgenomic promoter expression of EGFP and repeat P3a expression strategy applying in SCYLV, the problem seems to be solved. In addition, it is well known that poleroviruses are phloem-restricted

Table 1 Primer sequences used in this study

The primer name	Primer sequences	Purpose of primers
SCYLV-TF/ SCYLV-TR	CAGAAGACGCGCTAACCGTC/ CTATTTGGGATTCTGGAAAAGGC	Detection of SCYLV
ScGAPDH-qPCRF/ ScGAPDH-qPCRR	CACGGCCACTGGAAGCA/ TCCTCAGGGTTCCTGATGCC	GADPH control detection using RT-PCR
SCYLV-1F	GTTCATTTCATTTGGAGAGGCCTACAAAATATATCGGGAGGGAAACCCC	Amplified F1 of SCYLV
SCYLV-ORF5R-2	CCGTATTCAACCAAACAAGGAGGAGG	Amplified F1 of SCYLV
SCYLV-2U-2-F	GTTTGGTTGAATACGGAGGGGCATCCC	Amplified F2 of SCYLV
SCYLV-2UTB-R	TCCTTCGTGAGCGGTTAGCGCCCGTATTCGTTCACTATCGCTCGGATGTGTGC	Amplified F2 of SCYLV
EGFP-F-TYCZ-SCYLV	ACCGCTCACGAAGGAATGGTGAGCAAGGGCGAGG	Amplified F3 of SCYLV
EGFP-R-TYCZ2-SCYLV	CTCGACACTTACTTGTACAGCTCGTCCATGC	Amplified F3 of SCYLV
SCYLV-3UTR-2-F	GTACAAGTAAGTGTCGAGCCACTATAACGCTCC	Amplified F4 of SCYLV
SCYLV-2R	GATGCCATGCCGACCCATTTCGGTGACTAGGATATACGGGAGG	Amplified F4 of SCYLV and detection of SCYLV-EGFP
EGFP-TF	GCGATCACATGGTCCTGCTG	Detection of SCYLV-EGFP
LEP-F/LEP-R	ATTCAACCAATCATAAAGATATTGG/ AAACTTCTGGATGTCCAAAAAATCA	Detection of Aphid

possibly due to their weak movement proteins (Taliansky et al. 2003). Additionally, recent findings revealed that tombusvirus-like associated RNAs (tlaRNAs) or umbravirus-like RNA viruses (ULVs) enhancing poleroviral RNA levels and facilitating the accumulation of poleroviruses, thereby exacerbating symptom severity (Sanger et al. 1994; Ryabov et al. 2001; Yoshida. 2020; Zhao et al. 2021a; Simon et al. 2024). Currently, sugarcane umbra-like viruses had been reported in sugarcane (Lehrer et al. 2007; Tahir et al. 2021). It would be possible to enhance SCYLV infection efficiency and protein expression using our system in the future by co-infection.

Conclusions

In conclusion, we constructed an infectious cDNA clone of SCYLV and confirmed its infectivity in *N. benthamiana*, *A. thaliana* and sugarcane. Despite the modest expression of EGFP protein in *N. benthamiana*, the capacity of aphids to translocate SCYLV-EGFP to other plants is not impeded by the viral reconfiguration process. This suggests that the aphid-mediated transmission of the virus remains efficacious despite the relatively subdued fluorescence exhibited by the viral vector. Additionally, LC05-136 and GT10-2118 are more resistant cultivars to SCYLV than Badila in our system. The tool will serve as a resource and furnish a critical foundation for researchers of the viruses in the *Polerovirus* genus, thereby facilitating future research in this field.

Materials and methods

Plant and insect materials and growth conditions

A. thaliana in this study represents *A. thaliana* (Col-0). *N. benthamiana* and *A. thaliana* plants were meticulously cultivated in controlled-environment growth chambers, maintained at a constant temperature of 25 °C, under a photoperiod of 12 h of light (80 μmoL m^{-2} s^{-1}) alternating with 12 h of darkness (Wu et al. 2023). Green peach aphids were carefully tended within our laboratory at a constant temperature of 25 °C in an insect-rearing cage. *M. persicae* was confirmed by PCR and Sanger sequencing with universal primers LEP-F and LEP-R. During the process of *M. persicae* transmitting SCYLV, *M. persicae* and plants were enclosed in plastic bottles capped with 250-mesh screens to prevent aphid escape. A brush was used to remove all aphids gently from *N. benthamiana A. thaliana plants* to prevent virus contamination from aphids.

Sugarcane germplasm

Badila, LC05136, and GT10-2118 were all propagated and cultivated using virus-free tubers. The growth conditions for the sugarcane were similar to *N. benthamiana* and *A. thaliana* plants, except that it was maintained at the constant temperature of 30 °C.

Construction of the infectious cDNA clones of SCYLV and SCYLV-EGFP

The binary vector pCB301 served as the backbone for constructing the infectious clone of SCYLV. The vector was doubly digested with *Stu*I and *Sma*I in rCutSmart Buffer™ (NEB, USA) to prepare for the desired fragment insertions. The construction of SCYLV-EGFP involves the following steps: amplification of fragment1 using SCYLV-1F and SCYLV-ORF5R-2, amplification of fragment2 using SCYLV-2U-2-F and SCYLV-2UTB-R, amplification of fragment 3 using EGFP-F-TYCZ-SCYLV and EGFP-R-TYCZ2-SCYLV, and amplification of fragment 4 using SCYLV-3UTR-2-F and SCYLV-2R. Fragments 1, 2 and 4 were amplified using SCYLV as the template, while fragment 3 used the *EGFP* gene as template. These four fragments (1, 2, 3, 4) were then ligated into the double-digested pCB301 vector using homologous recombination.

Agrobacteria and *Agroinfiltration*

Constructs of pCB301-SCYLV and pCB301-SCYLV-EGFP were independently introduced into the *A. tumefaciens* strain EHA105 through electroporation following standard laboratory protocols (Li et al. 2014). *A. tumefaciens* cells harboring the corresponding clones were cultured for 48 h in Luria–Bertani medium supplemented with kanamycin (50 mg/L) and rifampicin (50 mg/L). The bacterial cells were harvested and suspended with MMA buffer (10 mM MES, 10 mM $MgCl_2$, 200 μM acetosyringone) for 3 h at optical density 600 (OD_{600}) = 1.5. *N. benthamiana* plants aged 3–4 weeks were agroinfiltrated using a disposable sterile syringe without needle. Plants infiltrated with *Agrobacterium* transformed with pCB301 empty vector were served as vector control. Immersed sugarcane seedlings with primary roots and tuber in an *Agrobacterium* solution at an optical density of OD_{600} = 0.5 for 10 h. Subsequently, rinse the seedlings with water and transfer them to soil to facilitate further growth.

RT-PCR detection

Total RNA was extracted from the plant samples using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription of the RNA was conducted with the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) (Wang et al. 2022). Polymerase chain reaction (PCR) was performed using 2×Phanta Flash Master Mix (Vazyme #P520). SCYLV detection was performed using RT-PCR with SCYLV-specific primers (SCYLV-TF and

SCYLV-TR) to amplify a fragment of the *CP* gene. Sugarcane *ScGADPH* gene and *N. benthamiana NbACTIN* gene served as internal controls. SCYLV-EGFP detection was performed by RT-PCR with SCYLV–EGFP specific primers (EGFP-TF and SCYLV-2R) to amplify a 302-bp region from partial *EGFP* to the 3'-UTR of SCYLV-EGFP in plants and *M. persicae*.

Serological assays

Western blot analysis was conducted according to our previously published methods (Zhao et al. 2021b). Total soluble protein was extracted from the systemically infected leaves of sugarcane and *N. benthamiana* plants, then analyzed by 10% SDS-PAGE, and blotted onto a Merck Millipore PVDF membrane (IPVH00010, Germany). Finally, EGFP was detected using anti-EGFP polyclonal antibodies with a dilution ratio of 1:2000.

Abbreviations

YLD	Yellow leaf disease
SMD	Sugarcane mosaic disease
SCYP	Sugarcane yellows phytoplasma
SCYLV	Sugarcane yellow leaf virus
PCR	Polymerase chain reaction
EGFP	Enhanced green fluorescent protein
N. benthamiana	*Nicotiana benthamiana*
A. thaliana	*Arabidopsis thaliana*

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00295-0.

Additional file 1. Full uncropped images.

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Author contributions

YY conducted the experiments, conceived, and wrote the manuscript. PZ revised the manuscript. YS, MY, JW, and HZ provided experimental materials. TH and MW conducted the experiments. JY conceived and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

JY and YY are inventors on a patent related to this work, filed by the Chinese Academy of Sciences (No. 202411414324X, filed Oct 11th, 2024). The authors declare that they have no competing interests.

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