METHODOLOGY

Open Access

A highly efficient Agrobacterium-mediated infectious system for *Sweet potato leaf curl virus* and a deltasatellite-based VIGS vector

Yi Zhang[1†], Xuangang Yang[1,2†], Lifei Huang[3] and Shulin Deng[1,4*]

Abstract

Plant virus disease is one of the major threats to the yield and quality of sweetpotato (*Ipomoea batatas* [L.] Lam.), the seventh most important food crop in the world in terms of production. Sweepoviruses are a group of monopartite geminiviruses (genus *Begomovirus*, family *Geminiviridae*) that infect *Ipomoea* plants and cause significant damage. However, investigations on the mechanisms of sweepovirus infection are scarce due to the low efficiency of the current infectious system. In this work, we used the genomic sequence of *Sweet potato leaf curl virus* (SPLCV) to construct an effective infectious clone, SPLCV-1.01. We optimized the inoculation procedure in *Nicotiana benthamiana* by improving the *Agrobacterium* virulence using an induction medium. Finally, we established a highly efficient agroinoculation protocol for SPLCV on sweetpotato. Furthermore, a simple and reliable "Agro-soaking" method was developed for SPLCV on sweetpotato and *Ipomoea aquatica*. The "Agro-soaking" method also worked well with *Tomato yellow leaf curl China virus* (TYLCCNV) and *Beet severe curly top virus* (BSCTV) on tomato. Based on the "Agro-soaking" method and a deltasatellite, the SBG51 VIGS vector worked with the SPLCV-1.01 infectious clone and silenced two endogenous genes *IbCHL* (*magnesium-chelatase subunit*) and *IbPDS* (*phytoene desaturase*) in two weeks. Moreover, the VIGS system identified *IbADK1* (*adenosine kinase 1*) as a positive regulator to dampen the SPLCV accumulation in sweetpotato. Our work provides a practical method for studying the interaction between sweetpotato and SPLCV and the functional studies of genes in sweetpotato.

Keywords Sweetpotato, SPLCV, Infection system, VIGS, Deltasatellite

†Yi Zhang and Xuangang Yang contributed equally to this work.

*Correspondence:
Shulin Deng
sldeng@scbg.ac.cn
[1] Key Laboratory of National Forestry and Grassland Administration on Plant Conservation and Utilization in Southern China & Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China
[2] University of Chinese Academy of Sciences, Beijing 101408, China
[3] Guangdong Key Laboratory of Crops Genetics and Improvement, Crops Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[4] National Engineering Research Center of Navel Orange, Gannan Normal University, Ganzhou 341000, China

Background

Sweetpotato (*Ipomoea batatas* [L] Lam.) belongs to the Convolvulaceae family, and the storage root of sweetpotato is rich in starch and other nutrients. Sweetpotato has been cultivated worldwide with excellent tolerance to abiotic stresses, such as drought, salt, and heat stress. Nevertheless, as a vegetatively propagated plant, the yield and quality of sweetpotato are threatened by viral diseases. Sweetpotato is usually coinfected by two or more viruses. The most severe disease of sweetpotato is caused by coinfection of two RNA viruses, *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*) and *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*), which is collectively called sweet potato virus disease (SPVD)

Zhang *et al. Phytopathology Research*   (2024) 6:23

Page 2 of 14

(Loebenstein 2015). Besides SPFMV and SPCSV, the other main viruses infecting sweetpotato include two RNA viruses, *Sweet potato mild mottle virus* (SPMMV, genus *Ipomovirus*), *Sweet potato latent virus* (SPLV, genus *Potyvirus*), and one DNA virus, *Sweet potato leaf curl virus* (SPLCV, genus *Begomovirus*) (Loebenstein 2015). SPLCV belongs to a group of single-strand DNA viruses called sweepoviruses (genus *Begomovirus*, family *Geminiviridae*) transmitted by the whitefly *Bemisia tabaci*. Sweepoviruses are distinct from the Old and New World begomovirus groups (Fauquet and Stanley 2003) and infect *Ipomoea* plants in many countries worldwide. SPLCV is known as a monopartite geminivirus without DNA-B and satellite DNA (Bi and Zhang 2012). However, a noncoding satellite DNA, which was named a deltasatellite associated with SPLCV, was recently found in Spain and Venezuela (Hassan et al. 2016; Lozano et al. 2016).

Although SPLCV is the most severe DNA virus disease of sweetpotato and has been even worse in recent decades, studies on the interaction between SPLCV and sweetpotato are restricted to virus detection, cloning, and identification due to lacking a highly efficient infectious system in laboratories. Grafting or whitefly transmission is used for SPLCV detection and studying the host range (Barkley et al. 2011; Ling et al. 2011). However, it is obvious that graft and insect transmission are uncontrollable approaches. Agroinoculation is currently the most efficient method for geminivirus infection, but previous reports have shown that it is difficult to infect sweetpotato by agroinoculation. The efficiency of agroinoculation on sweetpotato is poor, and the latent period is long (4–6 months) in laboratories (Trenado et al. 2011). The improved agroinoculation method by vacuum infiltration of sweetpotato leaves shortened the latent period to 35 day post inoculation (dpi), but the infection rate was only about 25% (Bi and Zhang 2014). Therefore, a highly efficient infectious system for SPLCV on sweetpotato is urgently needed.

Although the transformation methods for sweetpotato were developed recently, it is still challenging to knock out the endogenous genes in sweetpotato, because sweetpotato is an allohexaploid species with a $B_1B_1B_2B_2B_2B_2$ composition genome (Yang et al. 2017a). In addition, sweetpotato is self-incompatible, which makes it almost impossible to generate a homologous mutagenesis on a target gene by selfing. Virus-induced gene silencing (VIGS) would still be a powerful technology for functional studies of genes in sweetpotato. Geminiviruses genomes have been developed into viral vectors for biotechnological applications. Geminivirus-based vectors are used as gene expression vectors and VIGS tools to study gene function in many important crops (Yang et al. 2017b). The geminivirus protein AC1 (Rep) can recognize

the geminivirus intergenic region (IR), therefore any sequence flanked by IR would form an episomal replicon with AC1 protein (Lozano-Duran 2016). The SPLCV genome was developed into an expression vector, which highly expressed the target gene and improved the efficiency of CRISPR-Cas-mediated RNA targeting and gene editing in *N. benthamiana* (Yu et al. 2020). In the SPLCV replicon, the *AV1* and *AV2* genes were replaced by the target expression cassette. In addition, the *AC2* and *AC4* genes were mutated, and only *AC1* and *AC3* genes were retained in the SPLCV replicon vector. However, it was reported that the coat protein (CP) encoded by the *AV1* gene in monopartite geminivirus was essential for virus movement (Sharma and Ikegami 2009). The *AV1*-deleted SPLCV replicon unsurprisingly only expressed the target gene in situ (Yu et al. 2020). Therefore, monopartite geminiviruses are usually developed into viral vectors associated with satellite DNAs (Yang et al. 2017b). The betasatellite-based VIGS vector successfully silenced *N. benthamiana*, tomato, cotton, and Arabidopsis genes with *Tomato yellow leaf curl China virus* (TYLCCNV) and *Cotton leaf curl multan virus* (CLCuMuV) as the helper virus (Kumar et al. 2014; Tao and Zhou 2004). In addition, the *N. benthamiana* endogenous genes *NbTOM1* and *NbTOM3* were silenced by the alphasatellite-based vector 2mDNA1 associated with *Tobacco curly shoot virus* (TbCSV), which resulted in a resistant phenotype to *Tobacco mosaic virus* (TMV) (Huang et al. 2009).

In this study, 1.01 copies of the SPLCV genome were inserted into the pCAMBIA1300 binary vector to generate an infectious clone, SPLCV-1.01. We optimized the agroinoculation method and established a simple, reliable, and highly efficient infectious system for SPLCV. Moreover, the simple and efficient method was also used for tomato infection by TYLCCNV and BSCTV. Based on the infectious system, a deltasatellite-based VIGS vector was developed, which dramatically silenced the endogenous gene in a short period. By this system, we identified that *IbADK1* is a positive regulator to dampen the SPLCV accumulation in sweetpotato, which convinced us it is a powerful tool for functional study in sweetpotato.

Results
Construction of the SPLCV-1.01 infectious clone and infectivity on *N. benthamiana*
Most geminivirus infectious clones were usually generated by tandem repeats and contained 1.3 ~ 2.0 units of the virus genome. It was reported that a 41 nt conserved stem loop region is essential and sufficient to generate an infectious clone of *Tomato yellow leaf curl virus* (TYLCV) (Urbino et al. 2008). We used the SPLCV isolate GZ02 (2829 nt, GenBank: JX286654.1) genomic sequence as a

reference. We repeated the 41 nt stem loop and synthesized a dsDNA fragment of 2870 bp (Additional file 1: Figure S1). The 1.01 copies of the SPLCV genome were inserted into the pCAMBIA1300 binary vector to generate an infectious clone, SPLCV-1.01 (Fig. 1a, b). *N. benthamiana* is a sensitive host for many geminiviruses, thus we first tested the infectious clone of SPLCV-1.01 on *N. benthamiana*. The 4-week-old *N. benthamiana* stems were injected with *Agrobacterium tumefaciens* strain GV3101 containing SPLCV-1.01 infectious clone or empty vector, pCAMBIA1300, as a mock treatment. The mottle leaves were observed at 15 dpi, and the top leaves became curly at 20 dpi with SPLCV-1.01 (Fig. 1c). The growth of top leaves and buds was strongly inhibited after the symptoms appeared. At the later inoculation stage (35 dpi), the infected *N. benthamiana* showed dwarfing phenotypes compared with the mock-inoculated plants that already flowered (Fig. 1c). We randomly collected the top symptomatic leaves from three plants for Southern blotting. The 1095 bp coding sequence (CDS) of the *AC1* gene was used as a probe to detect the viral DNA. As shown in Fig. 1d, single-stranded (ss) SPLCV was the primary form of SPLCV in host cells. In addition, the supercoiled (sc), linear (lin), and open-circular (oc) virus DNA forms were also detected (Fig. 1d). We also attempted to construct an SPLCV-1.25 clone containing a repeated *AV1* and *AV2* region and an SPLCV-1.75 clone containing a repeated *AC1–AC4* region, but we accidentally introduced a "GGG to GAG" mutation in the stem loop region. Compared with the SPLCV-1.01 infectious clone, these two mutated clones had a much lower infection rate, longer latent period, and milder symptoms (Additional file 1: Figure S2).

The induction medium significantly improved the agroinoculation efficiency

The SPLCV-1.01 infectious clone was further tested on the sweetpotato cultivar Guangshu 87 (G87), but the plants were still asymptomatic until 35 dpi. Four samples of top leaves were randomly collected to detect viral DNA by PCR, and only one sample was weakly positive (Additional file 1: Figure S3). This infection rate was
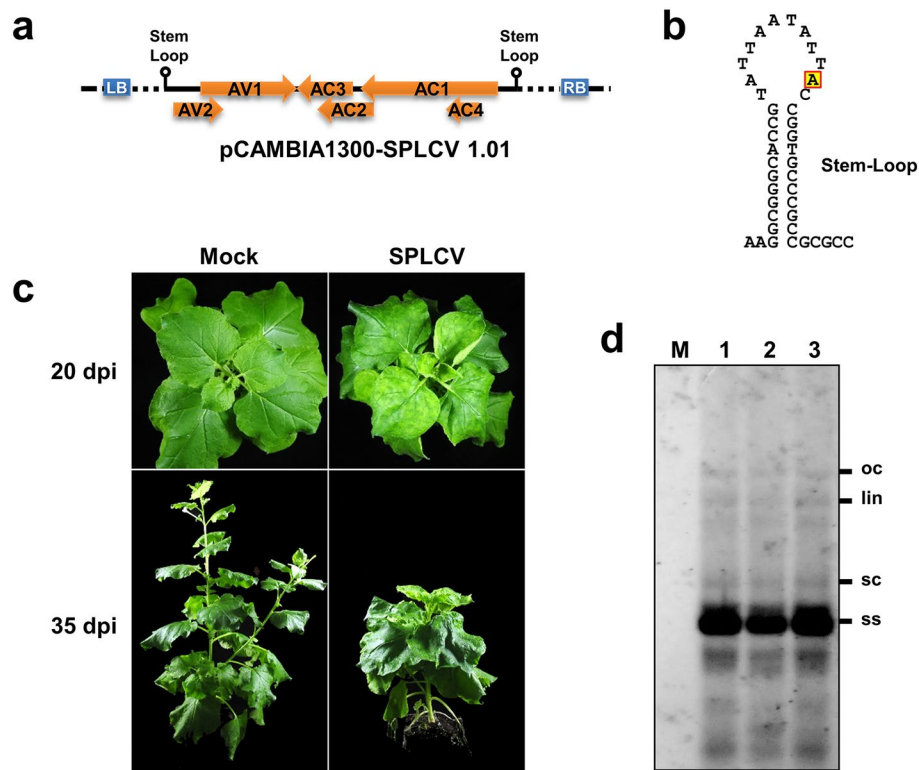

Fig. 1 Schematic of the SPLCV-1.01 infectious clone and *N. benthamiana* infected with SPLCV-1.01. **a** Schematic of the pCAMBIA1300-SPLCV-1.01 infectious clone. The orange arrows represent viral genes as indicated. The 41 nt stem loop is represented by a bar with a circle on the top. The blue boxes represent the left border (LB) and right border (RB) region of T-DNA. The dashed line represents the other sequence in the T-DNA region. **b** Schematic of the stem loop sequence. The highlighted "A" base is the start site of replication. **c** Symptoms of SPLCV-1.01 infected *N. benthamiana*. **d** Southern blot analysis of SPLCV DNA in *N. benthamiana* at 35 dpi. ss, single-stranded DNA; sc, supercoiled DNA; lin, linear DNA; oc, open-circular. The empty vector pCAMBIA1300 was inoculated as a mock (M)

Zhang *et al. Phytopathology Research*   (2024) 6:23

Page 4 of 14

consistent with previously reported data (Bi and Zhang 2014; Trenado et al. 2011). In order to increase the poor infectivity of agroinoculation on sweetpotato, we firstly tried to improve the agroinoculation efficiency by optimizing the *Agrobacterium* culture and inoculation procedure. It was reported that an induction medium with AB salt and glucose was used to induce the *vir* genes of *Agrobacterium* (Gelvin 2006). The finished induction medium and inoculation buffer were introduced into the SPLCV inoculation procedure (Additional file 1: Figure S4). As shown in Fig. 2a, three inoculation methods (method 2 ~ 4) were evaluated, and the GV3101 strain containing an empty vector was used as a mock-inoculation control (method 1). Compared with the conventional agroinoculation method (method 2), the finished induction medium (methods 3 and 4) significantly increased the infection rate to 100% and shortened the latent period for symptom appearance (Fig. 2b). In addition, methods 3 and 4 resulted in more severe symptoms, including shorter plant heights (Fig. 2c, d). The SPLCV DNA level was quantified by qPCR, which showed that methods 3 and 4 resulted in more viral DNA accumulation in host plants (Fig. 2e). Comparing method 3 with 4, the MES $MgCl_2$ buffer had a slightly better effect than the ¼ MS infiltration buffer, but the difference was not significant (Fig. 2).

An efficient "Agro-injection" method for sweetpotato

The improved "Agro-injection" method was tested for sweetpotato inoculation. *Agrobacterium* strain GV3101 or AGL1 containing the SPLCV-1.01 clone was cultured in LB medium and finished induction medium in sequence (Fig. 3a). The cultured *Agrobacterium* cells were resuspended in the MES $MgCl_2$ buffer to inject the sweetpotato stems (Fig. 3a). It was reported that the AGL1 strain showed the optimum transformation efficiency for sweetpotato compared with the other common strains, GV3101 and EHA105 (Mei et al. 2024). At 30 dpi, the accumulation of SPLCV virus DNA in AGL1-inoculated samples was also much higher than that in GV3101-inoculated samples (Fig. 3b). AGL1 was further used to evaluate the efficiency of the "Agro-injection" method on two cultivars, Guangshu 87 (G87) and Longshu 28 (L28). At 10 dpi, viral DNA was detected in eight out of ten G87 and six out of ten L28 plants (Fig. 3c). However, there was wide variation in the viral DNA level of each plant (Fig. 3c). Three out of ten G87 initiated symptoms at 25 dpi. The top buds and palmate leaves were wrinkled, brown, and tiny at 35 dpi (Fig. 3d, e). The cordate leaves of L28 were obviously curly at 18 dpi (Fig. 3f, g). The mild symptom at 35 dpi was curly, brown, and tiny leaves (Fig. 3h), and the severe symptom was dead top buds and leaves

(Fig. 3i). In summary, the "Agro-injection" was an efficient inoculation method for SPLCV on sweetpotato. However, the injection step cannot control the consistent inoculation dosage, which results in variable symptoms. Moreover, the "Agro-injection" is a laborious method and does not apply to large-scale screening, so a simple and reliable method is still needed.

A simple, reliable, and highly efficient "Agro-soaking" method for sweetpotato

Sweetpotato is a vegetatively propagated plant with excellent regeneration capacity via cuttings, and *Agrobacterium* can easily infect the wounded tissue. Therefore, we wondered whether *Agrobacterium* containing the SPLCV-1.01 infectious clone could infect sweetpotato from cuttings. The "Agro-soaking" method is shown in Fig. 4a. The AGL1 strain containing the SPLCV-1.01 clone was cultured in LB medium and finished induction medium in sequence. The cultured *Agrobacterium* cells were resuspended in a ¼ MS inoculation buffer, which was a better condition for sweetpotato cuttings. As shown in Additional file 1: Figure S5, the cuttings with 6–8 leaves were soaked in the freshly prepared *Agrobacterium* suspension for 3–18 h in the dark, and the soaking time depended on the different cultivars and growth status (Additional file 1: Figure S5a, b). After inoculation, the cuttings were recovered in water for another 12 h. Additional leaves were cut off, and only 3–4 leaves were kept before the cuttings were transferred into the pots with soil (Additional file 1: Figure S5c). The typical symptoms were observed at 10–30 dpi, depending on the different cultivars. Three cultivars were inoculated by the "Agro-soaking" method. At 10 dpi, the infection rate of G87 was 90%, but that of Pushu 32 (P32) and L28 was 100% (Fig. 4b, c). The infection rates of all the three cultivars were 100% at 20 dpi (Fig. 4b, c). L28 was a sensitive cultivar in the field, showing the shortest latent period (10 dpi) and the most SPLCV DNA accumulation (Fig. 4c, d). We also checked the viral DNA in L28 leaves at 30 dpi by Southern blotting. Single-stranded (ss) SPLCV was the main form of SPLCV in host cells (Fig. 4e). In addition, the supercoiled (sc), linear (lin), and open-circular (oc) virus DNA forms were also detected (Fig. 4e). P32 was moderate, and G87 had a long (23 dpi) and least viral DNA accumulation, which indicated that G87 is a relatively resistant cultivar (Fig. 4c, d). In summary, the "Agro-soaking" method has less workload and is more stable, making it a good choice for large-scale screening work. Details of the protocol and timeline of "Agro-injection" and "Agro-soaking" are shown in Additional file 1: Figure S6.
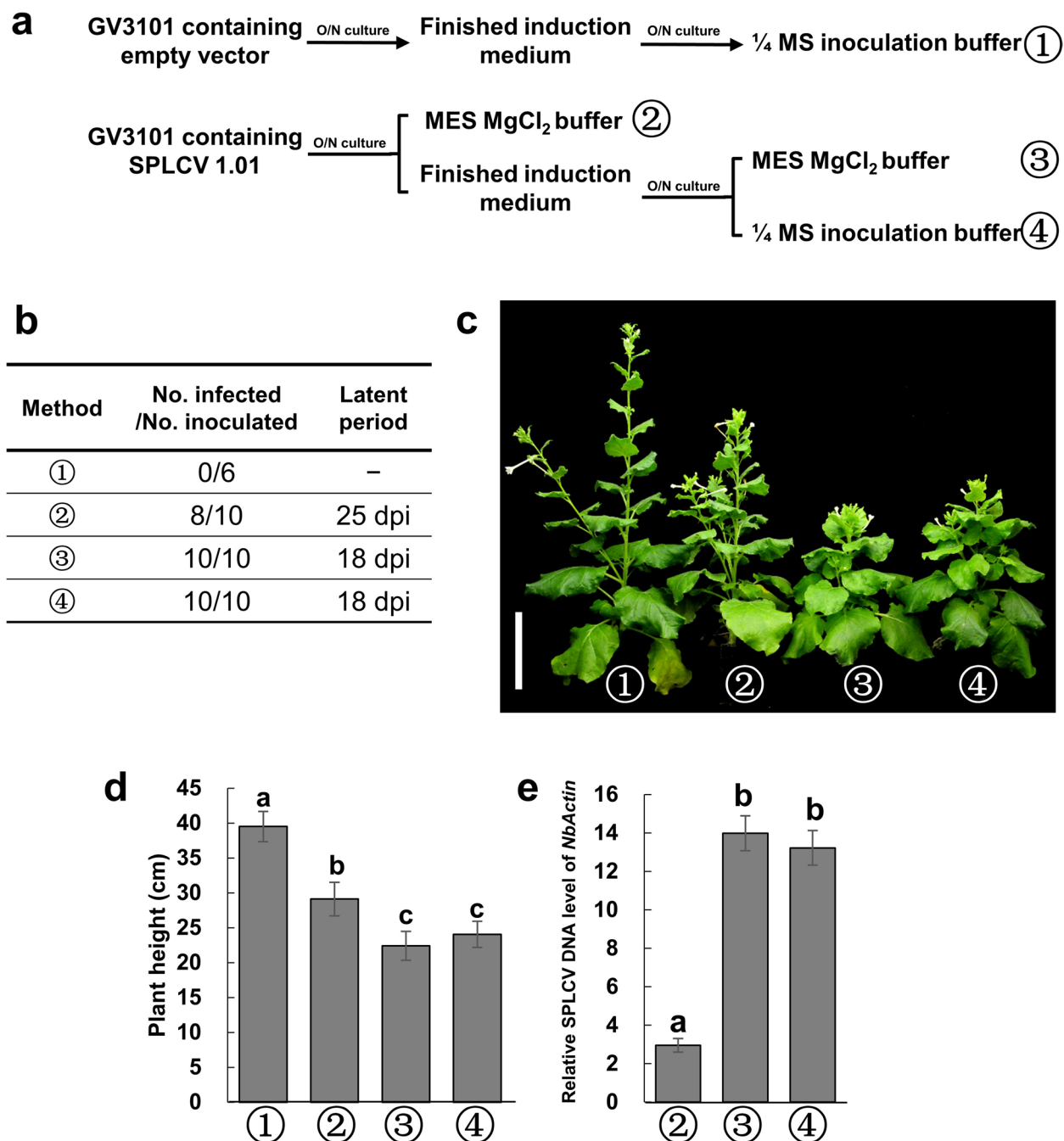
Fig. 2 The induction medium increases the infection effects of SPLCV-1.01. **a** Schematic of different culture conditions for agroinoculation. **b** The different infection rates and latent periods in different culture conditions. **c** The symptoms of *N. benthamiana* are inoculated by different cultural conditions at 30 dpi. Scale bars: 10 cm. **d** Plant heights of *N. benthamiana* inoculated by different culture conditions at 30 dpi. **e** Quantification of SPLCV DNA in *N. benthamiana* at 30 dpi. Different letters indicate significant differences at $p < 0.05$ by the One-way ANOVA test

The "Agro-soaking" method was applied to *Ipomoea aquatica* and tomato

It was reported that SPLCV successfully infected *Ipomoea nil* and *I. setosa* (Hassan et al. 2016). Another important *Ipomoea* vegetable plant, *I. aquatica*, was

also tested here. The mild curly leaves and bright yellow mottled veins inhibited apical buds, and more lateral bud growth symptoms were observed at 20 dpi (Fig. 5a), which was consistent with the reported symptoms infected by grafting (Lotrakul et al. 1998). The infection
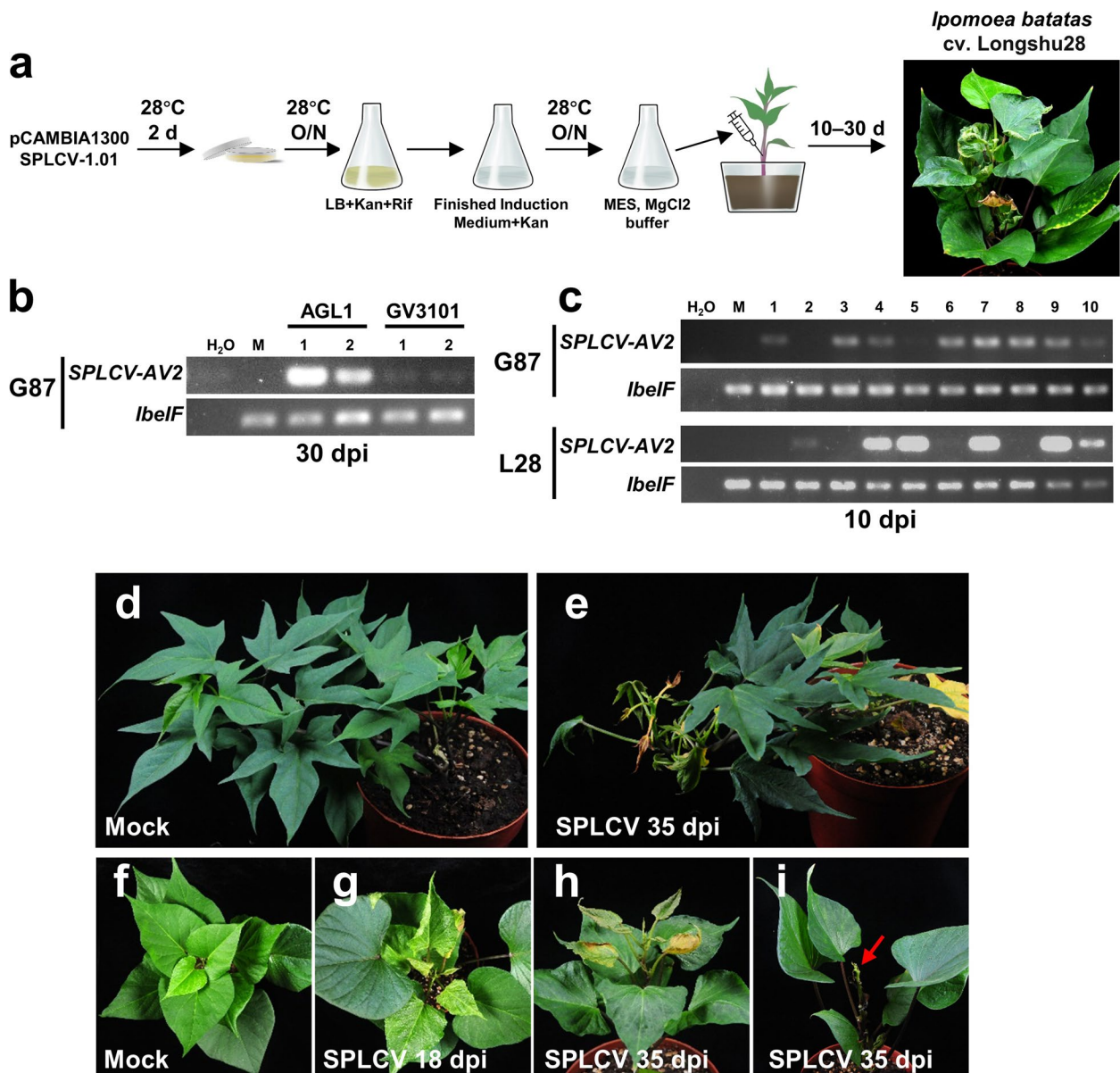
Fig. 3 Sweetpotato infected by the "Agro-injection" method. **a** Schematic of the "Agro-injection" method and the symptoms of sweetpotato Longshu 28 inoculated with SPLCV-1.01 by the "Agro-injection" method. **b** PCR detection of SPLCV DNA in sweetpotato leaves inoculated with *Agrobacterium* strain AGL1 or GV3101 at 30 dpi. **c** PCR detection of SPLCV DNA in sweetpotato Guangshu 87 (G87) and Longshu 28 (L28) leaves inoculated with *Agrobacterium* strain AGL1 at 10 dpi. **d** and **e** Symptoms of sweetpotato G87 inoculated by the "Agroinjection" method. **f** and **i** Symptoms of sweetpotato L28 inoculated by "Agro-injection" method. **g** Curly leaves of L28 at 18 dpi. **h** The mild symptom of L28 at 35 dpi. **i** The severe symptom of L28 at 35 dpi. The red arrow indicates the dead top bud and leaves. The empty vector pCAMBIA1300 was inoculated as a mock (M)

(See figure on next page.)
Fig. 4 Sweetpotato infected by the "Agro-soaking" method. **a** Schematic of the "Agro-soaking" method and the symptoms of sweetpotato Longshu 28 inoculated with SPLCV-1.01 by the "Agro-soaking" method. **b** PCR detection of SPLCV DNA in sweetpotato G87, Pushu 32 (P32), and L28 leaves inoculated with the "Agro-soaking" method at 10 dpi. **c** Infectivity analysis of the "Agro-soaking" method on different sweetpotato cultivars. **d** Quantification of SPLCV DNA in sweetpotato leaves at 10 dpi by qPCR. The *IbeIF* gene was used as an internal reference. Error bars show the mean values (± SD) of three independent samples. **e** Southern blot analysis of SPLCV DNA in L28 at 30 dpi. The empty vector pCAMBIA1300 was inoculated as a mock (M)
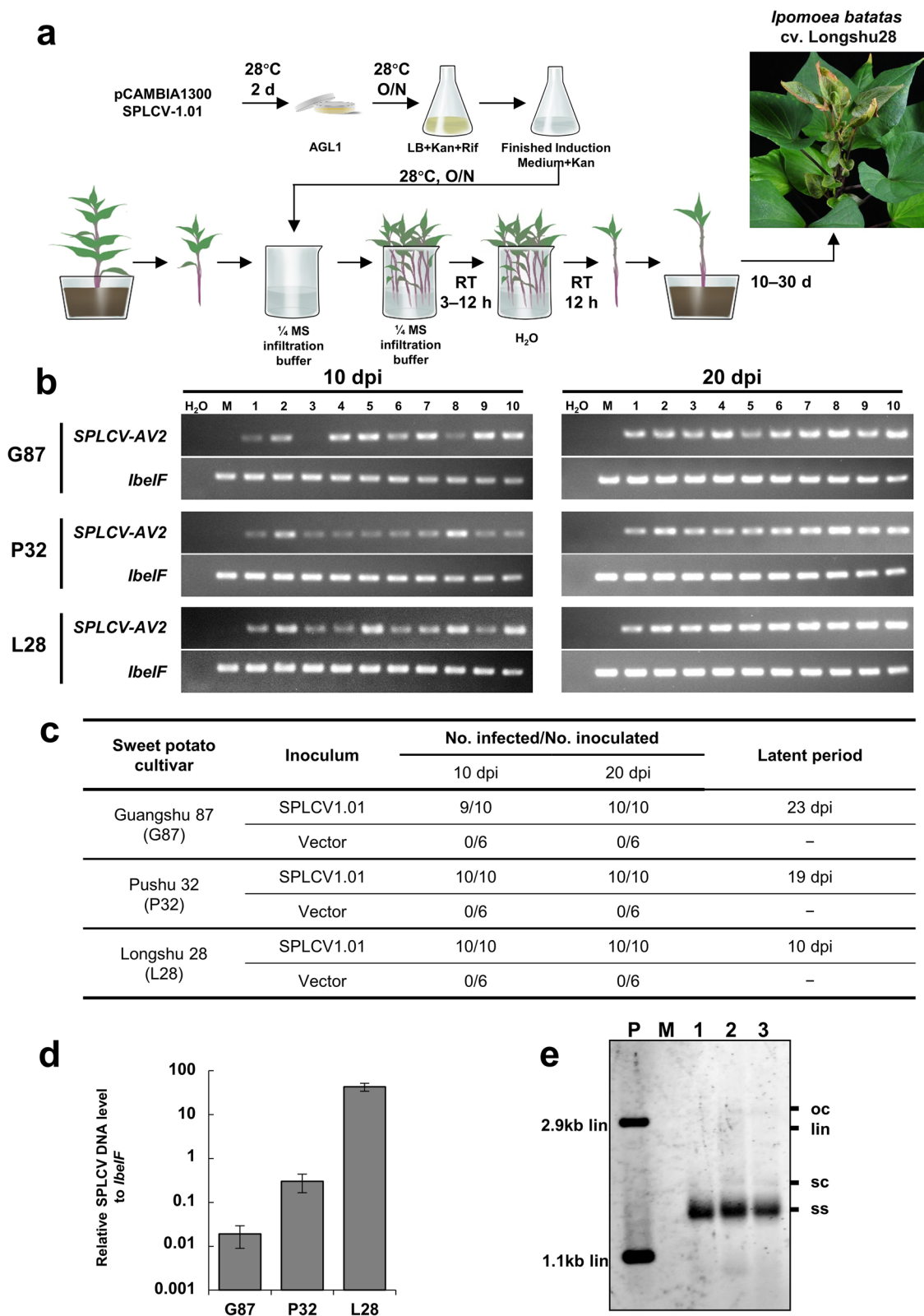
Fig. 4 (See legend on previous page.)

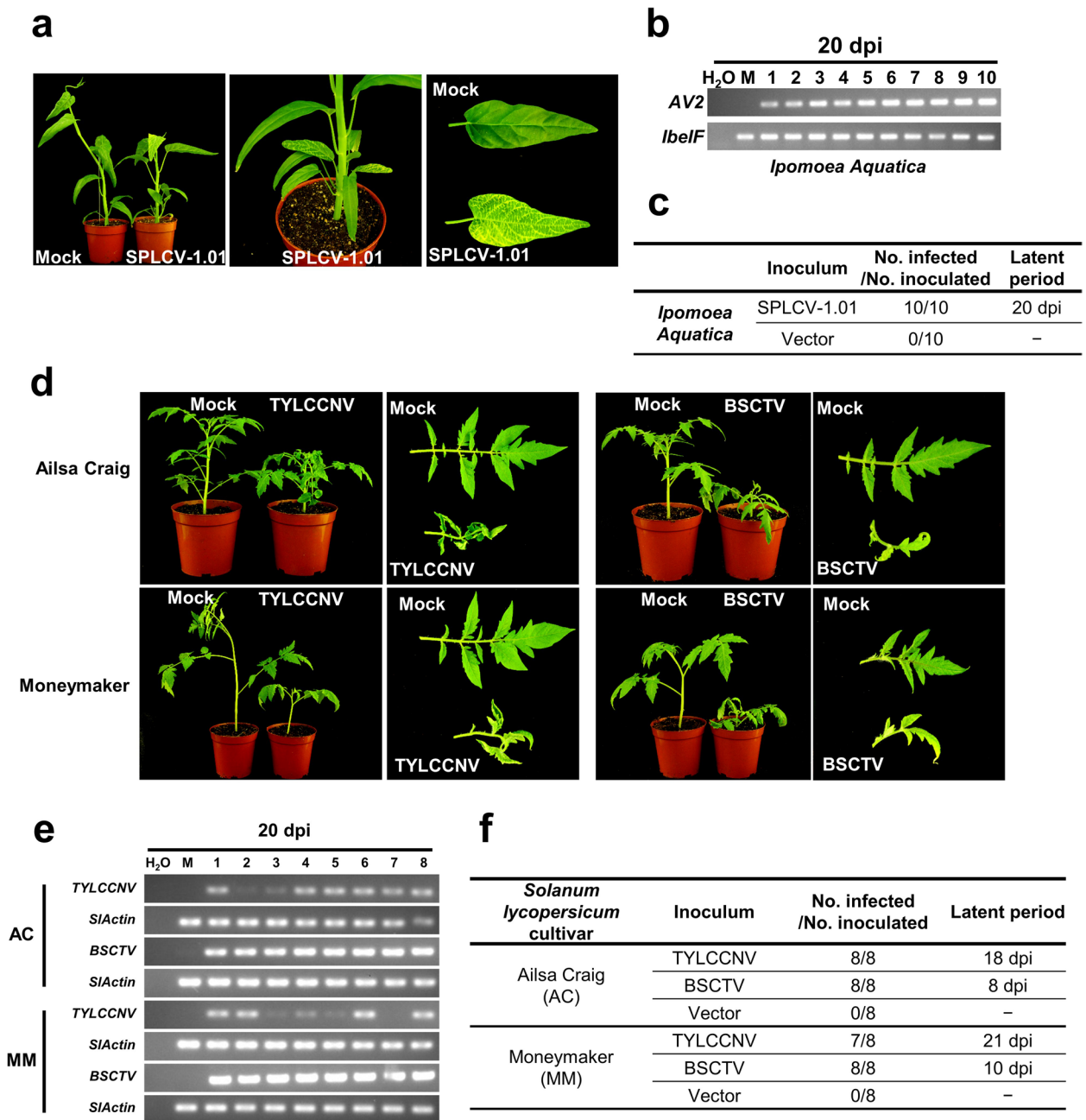Zhang *et al. Phytopathology Research*   (2024) 6:23

Page 8 of 14

Fig. 5 *I. aquatica* and tomato infected by the "Agro-soaking" method. **a** Symptoms of SPLCV-1.01 infected *I. aquatica* at 20 dpi. **b** PCR detection of SPLCV DNA in *I. aquatica* leaves at 20 dpi. **c** Infectivity analysis of the "Agro-soaking" method on *I. aquatica*. **d** Symptoms of TYLCCNV and BSCTV infected tomato using the "Agro-soaking" method at 30 dpi. **e** PCR detection of TYLCCNV and BSCTV DNA in tomato leaves at 20 dpi. **f** Infectivity analysis of the Agro-soaking method on tomato. The empty vector pCAMBIA1300 was inoculated as a mock (M)

rate of *I. aquatica* was also 100% (Fig. 5b, c). These data suggest that "Agro-soaking" is applied to SPLCV infection of *Ipomoea* plants.

Furthermore, we tested the "Agro-soaking" method on the interaction between two tomato (*Solanum lycopersicum*) cultivars Ailsa Craig and Moneymaker and two geminiviruses, TYLCCNV and BSCTV. The typical dwarf plants with yellow and curly leaves were observed (Fig. 5d). In general, the infection rate of tomato was almost 100% for all experiments, and BSCTV caused more severe symptoms and a shorter latent period (Fig. 5e, f). These data suggest that the "Agro-soaking"

method can be extended to the other cutting propagation plants.

A deltasatellite-based VIGS vector silenced the sweetpotato genes

As a monopartite geminivirus, the movement of SPLCV would be broken by the deletion of the *coat protein* (*CP*, also called *AV1*) gene. The DNA β satellite from TYL-CCNV was modified to a VIGS vector (Tao and Zhou 2004), and a noncoding DNA satellite, deltasatellite, was also identified in some SPLCV-infected samples in Spain (Hassan et al. 2016; Lozano et al. 2016). The deltasatellite could be transmitted with SPLCV and reduced symptom severity (Hassan et al. 2016), which would be the advantage of a VIGS vector. The reported SBG51 deltasatellite (GenBank: FJ914390.1) was used as a template (Lozano et al. 2016). The conserved stem loop was repeated once, and an MCS (multiple cloning site) was inserted before the A-rich region to generate an SBG51 deltasatellite-based VIGS vector (Fig. 6a and Additional file 1: Figure S7). The 513 bp fragment of *IbCHL* (*magnesium-chelatase subunit*) CDS and 376 bp fragment of *IbPDS* (*phytoene desaturase*) were inserted into the MCS of the VIGS vector, which were used as targets to test the VIGS vector. The AGL strain containing the SPLCV-1.01 infectious clone was mixed with the strain containing the SBG51-based VIGS vector at a 1:1 ratio. Sweetpotato plants were inoculated with the mixed AGL1 strain by the "Agro-soaking" method. As shown in Fig. 6b, silencing of the endogenous *IbCHL* gene caused bright yellow leaves, and silencing of the *IbPDS* gene caused white-mottled veins on newly emerged leaves at 14 dpi (Fig. 6b). In comparison, inoculation with the SPLCV VIGS-empty vector did not show any phenotype or virus symptoms at 14 dpi (Fig. 6c). The qPCR results showed that the endogenous target genes were significantly silenced in the plants inoculated with SPLCV VIGS-*IbCHL* or SPLCV VIGS-*IbPDS* at 14 dpi (Fig. 6c, d). We tested the VIGS vector on five sweetpotato cultivars, G87, P32, L28, Mianfen1 (M1), and Annou-Imo (AI). In general, about 60% of plants showed varying degrees of gene silencing phenotype at about 10 dpi (Fig. 6e). G87 is a relatively resistant cultivar, while G87 did not show any gene silencing phenotype. The sensitive cultivar, L28, showed 100% gene-silencing

phenotype. Our results indicated that the SBG51 deltasatellite-based VIGS vector with the highly efficient infection method could silence an endogenous gene in two weeks.

It was reported that ADK1 (adenosine kinase 1) was an anti-virus regulator, and the Arabidopsis *adk1* mutant was hypersusceptible to the geminivirus *Cabbage leaf curl virus* (CaLCuV) (Raja et al. 2008). We tested the function of *IbADK1* in the interaction between sweetpotato and SPLCV using our VIGS system. As shown in Fig. 6f, the expression level of *IbADK1* was reduced to about 40% in the VIGS-*IbADK1* plants compared to the empty vector control plants at 14 dpi (Fig. 6f). At the same inoculation time, the VIGS-*IbADK1* plants were more susceptible than the empty vector control plants (Fig. 6g). The SPLCV DNA in the empty vector control plants was very low at 14 dpi, but the VIGS-*IbADK1* plants accumulated significantly higher virus DNA (Fig. 6g). The results indicated that the VIGS system could efficiently silence sweetpotato endogenous genes and would be a practical tool in functional studies of sweetpotato genes.

Discussion

To date, sweepovirus disease has been one of the significant threats to sweetpotato. Generating a highly efficient infection method is essential to study the interaction between sweetpotato and sweepovirus. Compared with other methods, such as particle bombardment, grafting, or infection by insect vectors, agroinoculation is a simple and reproducible method used for most geminiviruses in laboratories. Our work started with constructing the SPLCV infectious clone and optimized the inoculation procedure. Finally, we established a simple, reliable, and highly efficient infectious system for SPLCV. It was reported that most geminivirus infectious clones, including SPLCV, were generated by the rolling circle amplification (RCA) method (Bang et al. 2014; Choi et al. 2012; Wu et al. 2008). The reported SPLCV infectious clones contained a 1.3- or 2.0-unit virus genome (Bi and Zhang 2012; Choi et al. 2012). Here, we repeated the 41 nt stem loop sequence and obtained a simple infectious clone, SPLCV-1.01 (Fig. 1a, b). When we tried to generate an SPLCV-1.25 clone containing a repeated *AV1* and *AV2* region and an SPLCV-1.75 clone containing a repeated

(See figure on next page.)

Fig. 6 Schematic of the SBG51 deltasatellite-based VIGS vector and gene silencing in sweetpotato. **a** Schematic of the SBG51 deltasatellite-base VIGS vector and the stem loop sequence. **b** Phenotypes of VIGS-*IbCHL* and VIGS-*IbPDS* sweetpotato at 14 dpi. **c** and **d** Quantification of the gene expression levels of *IbCHL* and *IbPDS* in SPLCV, SPLCV-VIGS-*IbCHL*, and SPLCV-VIGS-*IbCHL* infected sweetpotato by qPCR. **e** Summary of VIGS phenotype on different sweetpotato cultivars. **f** Quantification of the gene expression levels of *IbADK1*. **g** Quantification of SPLCV DNA in sweetpotato leaves at 14 dpi by qPCR. The *IbeIF* gene was used as an internal reference. Error bars show the mean values (± SD) of three independent samples. ** $p < 0.01$ (Student's *t*-test)
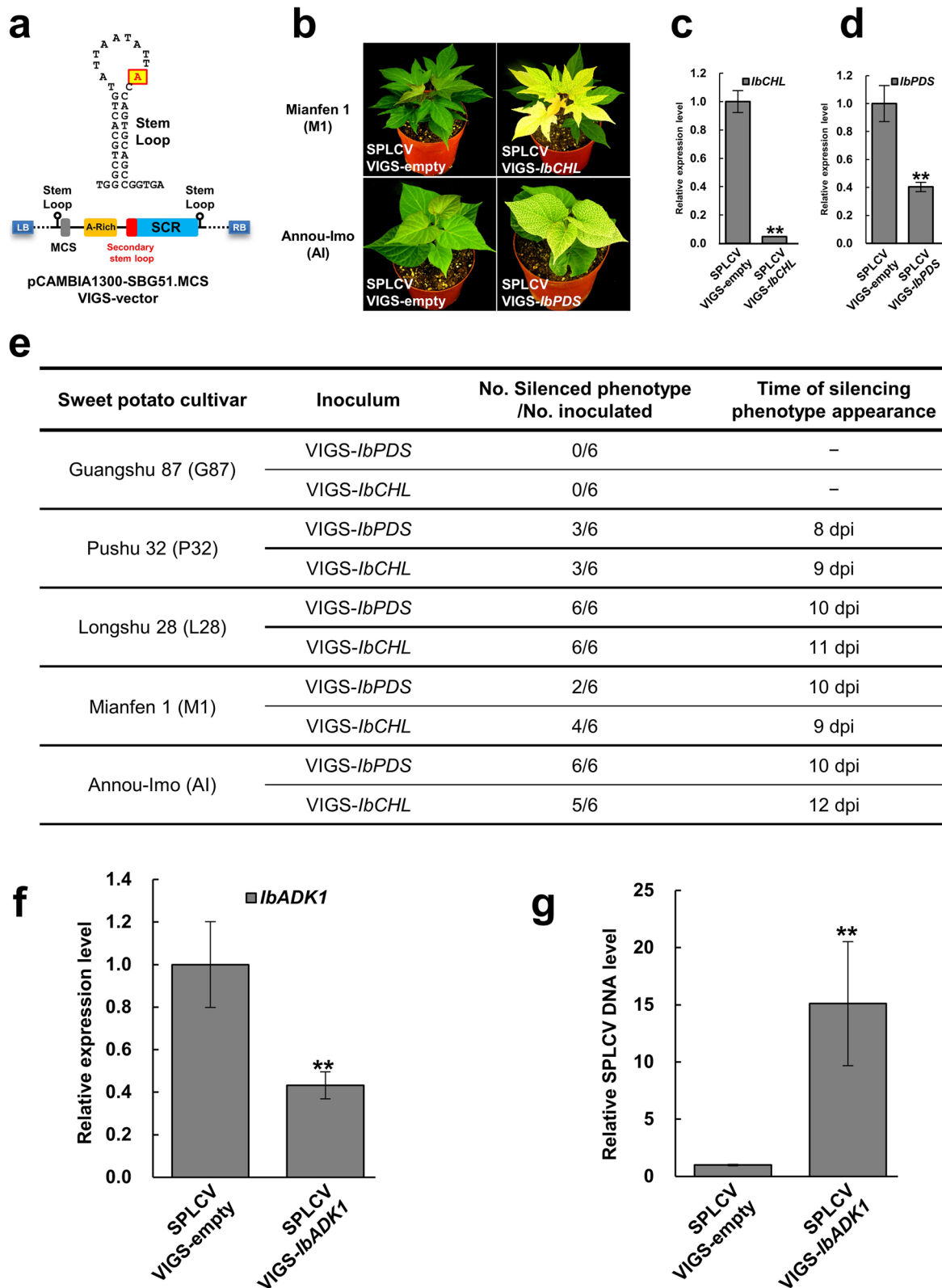
Sweet potato cultivar	Inoculum	No. Silenced phenotype /No. inoculated	Time of silencing phenotype appearance
Guangshu 87 (G87)	VIGS-*IbPDS*	0/6	–
	VIGS-*IbCHL*	0/6	–
Pushu 32 (P32)	VIGS-*IbPDS*	3/6	8 dpi
	VIGS-*IbCHL*	3/6	9 dpi
Longshu 28 (L28)	VIGS-*IbPDS*	6/6	10 dpi
	VIGS-*IbCHL*	6/6	11 dpi
Mianfen 1 (M1)	VIGS-*IbPDS*	2/6	10 dpi
	VIGS-*IbCHL*	4/6	9 dpi
Annou-Imo (AI)	VIGS-*IbPDS*	6/6	10 dpi
	VIGS-*IbCHL*	5/6	12 dpi

Fig. 6 (See legend on previous page.)

AC1–4 region, we only obtained constructs containing a "GGG to GAG" or "GGG to GGA" mutation in the stem loop region. It is possible that the SPLCV-1.25 and SPLCV-1.75 constructs with the correct sequence are unstable in *Escherichia coli.* The 100% infection rate of the SPLCV-1.01 clone and lower infection rates of SPLCV-1.25m and SPLCV-1.75m indicates that the repeated stem loop is essential and sufficient for an effective infectious clone.

The agroinoculation method is a process of DNA transfer from *Agrobacterium* to the host. Therefore, increasing *Agrobacterium* virulence could enhance the infection rate of SPLCV by agroinoculation. An acidic pH (5.2–6.0), sugars such as glucose, and acetosyringone (3,5-methoxy-4-hydroxyacetophenone [AS]) were reported to induce *Agrobacterium* virulence. We optimized the *Agrobacterium* culture and inoculation procedure based on a previously reported protocol (Gelvin 2006). The finished induction buffer significantly enhanced the infection rate and virus symptom severity (Fig. 2). The MES MgCl$_2$ buffer was used for the "Agro-injection" method because it had a slightly better effect on *N. benthamiana* compared with the ¼ MS infiltration buffer (Fig. 2 and Additional file 1: Figures S4 and S6). In contrast, the ¼ MS inoculation buffer was used for the "Agro-soaking" method (Additional file 1: Figures S4 and S6).

Both "Agro-injection" and "Agro-soaking" are effective inoculation methods for SPLCV on sweetpotato, but each has advantages and disadvantages. Cutting off the shoots from the plant is unnecessary for "Agro-injection", thus, any shoot of sweetpotato can be inoculated by "Agro-injection" at any growth stage. It is suitable for studying the interaction of viruses and hosts in a particular tissue or a special growth and development period. In addition, when SPLCV is used as a gene silencing vector to study functional genes for growth and development, the "Agro-injection" method is also a good choice. However, the disadvantages of "Agro-injection", wide variation and more workload, are also apparent. The "Agro-injection" method also needs well-trained individuals to get a higher infection rate. The "Agro-soaking" method was developed for large-scale screening work, with less workload and consistent inoculation concentration and time for each cutting. It is also a simple method, which does not require highly trained researchers to perform. In addition, the "Agro-soaking" method does not need any special tools and equipment, such as syringes, a vacuum pump, and a vacuum container. "Agro-soaking" also had a higher infection rate than "Agro-injection" and was applied to the other cutting propagation plants, such as *I. aquatica* and tomato (Fig. 5). Although tomatoes are reported to be easily infected by the conventional agroinjection method with acceptable efficiency, it still remains

a challenge to inject a large number of plants one by one for screening work. We expect that the "Agro-soaking" method could be used to screen the geminivirus-resistant sweetpotato or tomato cultivars.

Due to the excellent regeneration capacity, several transformation methods for sweetpotato have been developed recently (Cao et al. 2023; Mei et al. 2024; Zhang et al. 2023). However, VIGS is still a powerful tool for studying gene function, especially those for virus resistance. The process of VIGS is also that of the infection of plant viruses. Therefore, geminivirus-based VIGS vectors can be used to study the interaction between geminiviruses and host plants. For example, the ACMV (*African cassava mosaic virus*)-based vector silenced the cassava endogenous gene *MePOLD1* to study its function in cassava mosaic disease (CMD) caused by ACMV (Lentz et al. 2018; Lim et al. 2022).

The previously reported VIGS method for sweetpotato was *Tobacco rattle virus* (TRV) based vector and sprout vacuum infiltration, which required several weeks to generate a gene-silenced grown plant seedling (Xue et al. 2022). Because of the native host-virus relationship between sweetpotato and SPLCV, developing a VIGS vector and system based on our highly efficient infection method is expected. SPLCV is a monopartite geminivirus, and the six genes are essential for its infection and movement. Therefore, satellite DNA is required to generate a VIGS vector. Deltasatellites were first discovered in association with New World bipartite begomovirus, *Sida golden yellow vein virus* (SiGYVV-Ma), infecting the *malvaceous* species *Malvastrum coromandelianum* (Fiallo-Olive et al. 2012). Interestingly, deltasatellites can be replicated by a New World monopartite begomovirus, *Tomato leaf deformation virus* (ToLDeV), but not by Old World begomoviruses such as TYLCV or the curtovirus *Beet curly top virus* (BCTV) (Fiallo-Olive et al. 2016). Deltasatellites may affect disease symptoms and reduce the accumulation of helper viruses (Fiallo-Olive et al. 2012, 2016). Several deltasatellites have been associated with sweepovirus in Spain and Venezuela. Moreover, chimeric sweepovirus-DNA satellite molecules containing the *AV2* and *AV1/CP* genes have also been found (Lozano et al. 2016). These results imply that deltasatellites are potential vectors carrying large DNA fragments that can replicate with sweepovirus. Our SBG51 deltasatellite-based VIGS vector silenced an endogenous gene in two weeks. VIGS plants and control plants did not show obvious disease symptoms, which is an advantage compared with TRV-based VIGS vectors. However, deltasatellite is not essential for SPLCV replication, thus it could be lost during virus replication. This limitation of deltasatellite-based VIGS can be improved by combining a deficient SPLCV clone with a complementary satellite DNA.

ADK1 is a key component in dampening the geminiviruses accumulation. In Arabidopsis, geminivirus proteins AC2/AL2/L2 interact with and inactivates AtADK1 (Wang et al. 2003). The *adk1* mutant is more susceptible to CaLCuV (Raja et al. 2008). Our results showed that at 14 dpi with SPLCV VIGS-*IbADK1*, the sweetpotato plants accumulated much higher virus DNA than those inoculated SPLCV empty vector (Fig. 6 g). The hypersusceptible phenotype of VIGS-*IbADK1* was due to the silenced *IbADK1* gene expression (Fig. 6f). It indicates that IbADK1 is also an anti-virus protein in sweetpotato as AtADK1 in Arabidopsis. Our data suggests that the VIGS system has great potential in the functional investigation of sweetpotato genes.

Conclusions

In summary, our work offers a practical infectious system to study the interaction between sweetpotato and SPLCV, even in a wide range of plant species and geminiviruses. Moreover, the SPLCV and deltasatellite based VIGS system is a powerful tool for studying gene function in sweetpotato, especially in the interaction between sweetpotato and SPLCV.

Methods

Plant materials and growth conditions

Nicotiana benthamiana and tomato (*Solanum lycopersicum*) were germinated from seeds and grown in pots made of peat moss substrates and perlite at a ratio of 3:1. The virus-free sweetpotato (*Ipomoea batatas* (L.) Lam.) were grown in the field in Zhanjiang, Guangdong province or Longyan, Fujian province, and the cuttings from virus-free sweetpotato were grown in pots made of peat moss substrates, perlites, and sands at a ratio of 3:1:1 for 2–3 weeks. The consistent cuttings in pots were used for agroinoculation. *I. aquatica* were geminated from seeds and grown in pots made of peat moss substrates, perlites, and sands at a ratio of 3:1:1. All plants in pots were cultivated in a growth room with 16 h light and 8 h dark at 26°C.

The constructs of SPLCV-1.01 infectious clone and SBG51-MCS vector

The SPLCV isolate GZ02 (2829 nt, GenBank: JX286654.1) genomic sequence was used as a template, and the 41 nt conserved stem loop was repeated (Additional file 1: Figure S1). The 2870 bp of the SPLCV-1.01 dsDNA sequence was synthesized by GENEWIZ from Azenta Life Sciences (Additional file 1: Figure S1). SPLCV-1.01 was inserted into the pCAMBIA1300 binary vector at the *Kpn*I and *Xba*I sites. The SBG51 deltasatellite (GenBank: FJ914390.1) was used as a template, and the 39 nt conserved stem loop was repeated (Additional file 1: Figure

S7). The 740 bp SBG51-MCS dsDNA sequence was synthesized by GENEWIZ from Azenta Life Sciences (Additional file 1: Figure S7). SBG51-MCS was inserted into the pCAMBIA1300 binary vector by *Eco*RI and *Hin*dIII sites.

The constructs of VIGS-vectors

The genome and transcripts database of *Ipomoea trifida* (NSP306) was used as a sweetpotato gene sequence reference, available from Sweetpotato Genomics Resource (http://sweetpotato.uga.edu/). The 513 bp fragment of *IbCHL* (itf01g05440.t1) was amplified by the primer IbCHL.KpnI.F (5'-ATA<u>GGTACC</u>TGAAATTATGCC TTTTGCTCA-3') and IbCHL.XbaI.R (5'-AGA<u>TCTAGA</u> GGTTTCCAGAGCCAATAAG-3'). The 376 bp fragment of *IbPDS* (itf11g08190.t2) was amplified by the primer IbPDS.KpnI.F (5'- ATA<u>GGTACC</u>TTGGAGTTCCAG TAATAAATGTTC-3') and IbPDS.XbaI.R (5'- AGA<u>TCT AGA</u>TCTATGGGGGATTTTTGCAA-3'). The 372 bp fragment of *IbADK1* (itf04g06000.t1) was amplified by the primer IbADK1.KpnI.F (5'- ATA<u>GGTACC</u>AAGATC CCAAGGCTAAAAC-3') and IbADK1.XbaI.R (5'- AGA <u>TCTAGA</u>CTAATACACTTTCTGGGAGC-3'). The VIGS fragments were inserted into SBG51-MCD by the *Kpn*I and *Xba*I sites.

Agroinoculation

The infectious clone or VIGS vector was heat-shock transformed into *Agrobacterium* strain GV3101 for *N. benthamiana* inoculation or AGL1 for sweetpotato inoculation. *Agrobacterium* cells were plated on Luria-Bertani (LB) agar plates with appropriate antibiotics and incubated at 28°C for 2 days. *Agrobacterium* colonies were picked into the LB liquid medium with appropriate antibiotics and cultured at 28°C and 200 rpm for 24 h. *Agrobacterium* cells were centrifuged at $2500 \times g$ for 10 min, and the pellet was resuspended in a finished induction medium (Additional file 1: Figure S4). *Agrobacterium* cells were cultured at 28°C and 200 rpm overnight. *Agrobacterium* cells were centrifuged at $2500 \times g$ for 10 min and the pellet was resuspended either in a MES $MgCl_2$ buffer for the "Agro-injection" method or in a ¼ MS infiltration buffer for the "Agro-soaking" method (Additional file 1: Figure S4). The OD_{600} of the *Agrobacterium* culture was adjusted to 1. For the "Agro-injection" method on *N. benthamiana*, 4-week-old *N. benthamiana* stems were injected using a 1 mL syringe. For the "Agro-injection" method on sweetpotato, fresh and young sweetpotato stems were injected using a 1 mL syringe. For the "Agro-soaking" method, the cuttings were soaked in the freshly prepared *Agrobacterium* suspension for 3–18 h in the dark at room temperature, and the soaking time depended on the growth status of the cuttings. As shown

in Additional file 1: Figure S5, the cuttings of about 15 cm should be soaked for 8–10 h. If the cuttings are thinner and younger, the soaking time should be reduced. The cuttings from strong and old plants in fields need longer soaking time. If the leaves on cuttings become wilted, it is time to stop soaking. Cuttings were transferred into H_2O for another 12 h. Cuttings were transferred into the soil for further culture.

Virus detection in infected plants by PCR

The total DNA of the third newly emerged leaves was extracted by the standard CATB method. The concentration of gDNA was measured by Nanodrop and diluted to 10 ng/µL for PCR or qPCR. PCR was performed in a 20 µL reaction using Green Taq Mix (P131-01, Vazyme). qPCR was performed using gene-specific primers and ChamQ SYBR qPCR Master Mix (Q311–02, Vazyme). The *IbeIF* gene was used as an internal control for sweetpotato and *I. aquatica*. *NbActin* and *SlActin* were used as internal controls for *N. benthamiana* and tomato, respectively. The relative SPLCV DNA level was calculated according to the formula $2^{-\Delta Ct}$. The relative gene expression was calculated according to the formula $2^{-\Delta\Delta Ct}$. All primers used in qRT-PCR are shown in Additional file 2: Table S1.

Southern blotting

The amplified full-length SPLCV-AC1 PCR product was labeled as a probe using the DIG High PrimeDNA Labeling and Detection Starter Kit (11,745,832,910, Roche). For Southern blotting, 5 µg of total DNA extracted from the inoculated sweetpotato leaves or 500 ng of total DNA extracted from the inoculated *N. benthamiana* leaves were electrophoresed on 0.8% agarose gels and then transferred onto Amersham™ Hybond™ -N + Membranes (GE Healthcare). 50 ng of 1095-bp AC1 and 50 ng of 2870-bp SPLCV-1.01 were used as positive controls. The hybridization and detection of the probe were performed following the manufacturer's protocol (11745832910, Roche).

Total RNA extraction, cDNA synthesis, and gene expression analysis

Total RNA was extracted from 100 mg of sweetpotato leaves using AG RNAex Pro Reagent (AG21102, Accurate Biotechnology) following the manufacturer's protocol. First-strand cDNA synthesis was performed using a HiScript II 1st Strand cDNA Synthesis Kit (R212–02, Vazyme). qRT-PCR was performed to check the expression of *IbCHL, IbPDS,* and *IbADK1* using ChamQ SYBR qPCR Master Mix (Q311–02, Vazyme). The *IbeIF* gene was used as an internal reference. All primers used in qRT-PCR are shown in Additional file 2: Table S1.

Abbreviations

ADK1	*Adenosine kinase 1*
AI	Annou-Imo
BSCTV	*Beet severe curly top virus*
CDS	Coding sequence
CHL	*Magnesium-chelatase subunit*
CP	Coat protein (CP)
dpi	Day postinoculation
G87	Guangshu 87
IR	Intergenic region
L28	Longshu 28
M1	Mianfen 1
MCS	Multiple cloning site
P32	Pushu 32
PDS	*Phytoene desaturase*
SPLCV	*Sweet potato leaf curl virus*
SPVD	Sweet potato virus disease
TYLCCNV	*Tomato yellow leaf curl China virus*
VIGS	Virus-induced gene silencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00244-x.

Additional file 1: Figure S1. DNA sequence of SPLCV-1.01. **Figure S2.** Infection rate and symptoms of *N. benthamiana* infected with different SPLCV infectious clones. **Figure S3.** PCR detection of SPLCV DNA in sweetpotato leaves inoculated by agroinoculation. **Figure S4.** Preparation and recipe of buffers and media. **Figure S5.** The sweetpotato cuttings used for "Agro-soaking" methods. **Figure S6.** Schematic timeline of the "Agro-injection" and "Agro-soaking" methods. **Figure S7.** DNA sequence of SBG51-MCS.

Additional file 2: Table S1. Primers used in this paper.

Authors' contributions

YZ and SD designed the research, YZ and XY performed the experiments, YZ and XY analyzed the data, LH provided the materials, YZ wrote the manuscript and revised the manuscript by SD; all the authors have read and approved the manuscript.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Zhang *et al. Phytopathology Research*   (2024) 6:23

Page 14 of 14

References

Bang B, Lee J, Kim S, Park J, Nguyen TT, Seo YS. A rapid and efficient method for construction of an infectious clone of *tomato yellow leaf curl virus*. Plant Pathol J. 2014;30:310–5. https://doi.org/10.5423/PPJ.NT.03.2014.0025.

Barkley NA, Pinnow DL, Wang ML, Ling KS, Jarret RL. Detection and classification of SPLCV isolates in the US sweetpotato germplasm collection via a real-time PCR assay and phylogenetic analysis. Plant Dis. 2011;95:1385–91. https://doi.org/10.1094/PDIS-01-11-0012.

Bi H, Zhang P. Molecular characterization of two sweepoviruses from China and evaluation of the infectivity of cloned SPLCV-JS in *Nicotiana benthamiana*. Arch Virol. 2012;157:441–54. https://doi.org/10.1007/s00705-011-1194-6.

Bi H, Zhang P. Agroinfection of sweet potato by vacuum infiltration of an infectious sweepovirus. Virol Sin. 2014;29:148–54. https://doi.org/10.1007/s12250-014-3430-1.

Cao X, Xie H, Song M, Lu J, Ma P, Huang B, et al. Cut–dip–budding delivery system enables genetic modifications in plants without tissue culture. Innovation. 2023;4:100345. https://doi.org/10.1016/j.xinn.2022.100345.

Choi E, Lee G, Park J, Lee TK, Choi HS, Lee S. Molecular characterization and an infectious clone construction of sweet potato leaf curl virus (SPLCV) isolated from Korea. Acta Virol. 2012;56:187–98. https://doi.org/10.4149/av_2012_03_187.

Fauquet CM, Stanley J. Geminivirus classification and nomenclature: progress and problems. Ann Appl Biol. 2003;142:165–89. https://doi.org/10.1111/j.1744-7348.2003.tb00241.x.

Fiallo-Olive E, Martinez-Zubiaur Y, Moriones E, Navas-Castillo J. A novel class of DNA satellites associated with New World begomoviruses. Virology. 2012;426:1–6. https://doi.org/10.1016/j.virol.2012.01.024.

Fiallo-Olive E, Tovar R, Navas-Castillo J. Deciphering the biology of deltasatellites from the New World: maintenance by New World begomoviruses and whitefly transmission. New Phytol. 2016;212:680–92. https://doi.org/10.1111/nph.14071.

Gelvin SB. Agrobacterium virulence gene induction. Methods Mol Biol. 2006;343:77–84. https://doi.org/10.1385/1-59745-130-4:77.

Hassan I, Orilio AF, Fiallo-Olive E, Briddon RW, Navas-Castillo J. Infectivity, effects on helper viruses and whitefly transmission of the deltasatellites associated with sweepoviruses (genus *Begomovirus*, family *Geminiviridae*). Sci Rep. 2016;6:30204. https://doi.org/10.1038/srep30204.

Huang C, Xie Y, Zhou X. Efficient virus-induced gene silencing in plants using a modified geminivirus DNA1 component. Plant Biotechnol J. 2009;7:254–65. https://doi.org/10.1111/j.1467-7652.2008.00395.x.

Kumar J, Gunapati S, Kumar J, Kumari A, Kumar A, Tuli R, et al. Virus-induced gene silencing using a modified betasatellite: a potential candidate for functional genomics of crops. Arch Virol. 2014;159:2109–13. https://doi.org/10.1007/s00705-014-2039-x.

Lentz EM, Kuon JE, Alder A, Mangel N, Zainuddin IM, McCallum EJ, et al. Cassava geminivirus agroclones for virus-induced gene silencing in cassava leaves and roots. Plant Methods. 2018;14:73. https://doi.org/10.1186/s13007-018-0340-5.

Lim YW, Mansfeld BN, Schlapfer P, Gilbert KB, Narayanan NN, Qi W, et al. Mutations in DNA polymerase delta subunit 1 co-segregate with CMD2-type resistance to Cassava Mosaic Geminiviruses. Nat Commun. 2022;13:3933. https://doi.org/10.1038/s41467-022-31414-0.

Ling KS, Harrison HF, Simmons AM, Zhang SC, Jackson DM. Experimental host range and natural reservoir of *sweet potato leaf curl virus* in the United States. Crop Prot. 2011;30:1055–62. https://doi.org/10.1016/j.cropro.2011.03.009.

Loebenstein G. Control of sweet potato virus diseases. Adv Virus Res. 2015;91:33–45. https://doi.org/10.1016/bs.aivir.2014.10.005.

Lotrakul P, Valverde RA, Clark CA, Sim J, De La Torre R. Detection of a geminivirus infecting sweet potato in the United States. Plant Dis. 1998;82:1253–7. https://doi.org/10.1094/PDIS.1998.82.11.1253.

Lozano G, Trenado HP, Fiallo-Olive E, Chirinos D, Geraud-Pouey F, Briddon RW, et al. Characterization of non-coding DNA satellites associated with sweepoviruses (genus *Begomovirus*, *Geminiviridae*) - definition of a distinct class of begomovirus-associated satellites. Front Microbiol. 2016;7:162. https://doi.org/10.3389/fmicb.2016.00162.

Lozano-Duran R. Geminiviruses for biotechnology: the art of parasite taming. New Phytol. 2016;210:58–64. https://doi.org/10.1111/nph.13564.

Mei G, Chen A, Wang Y, Li S, Wu M, Hu Y, et al. A simple and efficient in planta transformation method based on the active regeneration capacity of plants. Plant Commun. 2024:100822. https://doi.org/10.1016/j.xplc.2024.100822.

Raja P, Sanville BC, Buchmann RC, Bisaro DM. Viral genome methylation as an epigenetic defense against geminiviruses. J Virol. 2008;82:8997–9007. https://doi.org/10.1128/Jvi.00719-08.

Sharma P, Ikegami M. Characterization of signals that dictate nuclear/nucleolar and cytoplasmic shuttling of the capsid protein of Tomato leaf curl Java virus associated with DNA beta satellite. Virus Res. 2009;144:145–53. https://doi.org/10.1016/j.virusres.2009.04.019.

Tao X, Zhou X. A modified viral satellite DNA that suppresses gene expression in plants. Plant J. 2004;38:850–60. https://doi.org/10.1111/j.1365-313X.2004.02087.x.

Trenado HP, Orilio AF, Marquez-Martin B, Moriones E, Navas-Castillo J. Sweepoviruses cause disease in sweet potato and related Ipomoea spp.: fulfilling Koch's postulates for a divergent group in the genus *begomovirus*. PLoS ONE. 2011;6:e27329. https://doi.org/10.1371/journal.pone.0027329.

Urbino C, Thebaud G, Granier M, Blanc S, Peterschmitt M. A novel cloning strategy for isolating, genotyping and phenotyping genetic variants of geminiviruses. Virol J. 2008;5:135. https://doi.org/10.1186/1743-422X-5-135.

Wang H, Hao L, Shung CY, Sunter G, Bisaro DM. Adenosine kinase is inactivated by geminivirus AL2 and L2 proteins. Plant Cell. 2003;15:3020–32. https://doi.org/10.1105/tpc.015180.

Wu CY, Lai YC, Lin NS, Hsu YH, Tsai HT, Liao JY, et al. A simplified method of constructing infectious clones of begomovirus employing limited restriction enzyme digestion of products of rolling circle amplification. J Virol Methods. 2008;147:355–9. https://doi.org/10.1016/j.jviromet.2007.10.002.

Xue L, Wei Z, Zhai H, Xing S, Wang Y, He S, et al. The IbPYL8-IbbHLH66-IbbHLH118 complex mediates the abscisic acid-dependent drought response in sweet potato. New Phytol. 2022;236:2151–71. https://doi.org/10.1111/nph.18502.

Yang J, Moeinzadeh MH, Kuhl H, Helmuth J, Xiao P, Haas S, et al. Haplotype-resolved sweet potato genome traces back its hexaploidization history. Nat Plants. 2017;3:696–703. https://doi.org/10.1038/s41477-017-0002-z.

Yang QY, Ding B, Zhou XP. Geminiviruses and their application in biotechnology. J Integr Agric. 2017b;16:2761–71. https://doi.org/10.1016/S2095-3119(17)61702-7.

Yu Y, Wang X, Sun H, Liang Q, Wang W, Zhang C, et al. Improving CRISPR-Cas-mediated RNA targeting and gene editing using SPLCV replicon-based expression vectors in *Nicotiana benthamiana*. Plant Biotechnol J. 2020;18:1993–5. https://doi.org/10.1111/pbi.13384.

Zhang W, Zuo Z, Zhu Y, Feng Y, Wang Y, Zhao H, et al. Fast track to obtain heritable transgenic sweet potato inspired by its evolutionary history as a naturally transgenic plant. Plant Biotechnol J. 2023;21:671–3. https://doi.org/10.1111/pbi.13986.