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Strawberry vein banding virus-based vector for transient overexpression in strawberry plants

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Abstract

Strawberry vein banding virus (SVBV) is a double-stranded DNA virus. In our previous studies, we generated an infectious clone of SVBV, pSVBV, which causes light-green vein banding symptoms along the leaf veins in strawberry plants (*Fragaria vesca*). In this study, we constructed pSVBV-P1-MCS and pSVBV-P4-MCS, two recombinant virus vectors containing a multiple cloning site (MCS) downstream of the SVBV-encoded movement protein gene (*P1*) and coat protein gene (*P4*), respectively. At 35 days post-inoculation, the two SVBV-based vectors could produce light-green vein banding symptoms on the systemic leaves of strawberry plants, indicating that they could successfully cause infection. Furthermore, the infectivity rates of the recombinant virus vectors pSVBV-P1-MCS and pSVBV-P4-MCS were similar to that of the wild-type infectious clone pSVBV, indicating that the insertion of MCS did not affect the infectivity of SVBV-based vectors. Additionally, we engineered SVBV as a transient overexpression vector, which could be used for the overexpression of exogenous green fluorescent protein in strawberry plants. Collectively, these SVBV-based vectors provide a new approach for the analysis of gene functions in strawberry plants.

Keywords: Strawberry vein banding virus (SVBV), Infectious clone, Virus-mediated overexpression (VOX)

Background

Gene overexpression systems have been widely used to study the functions of genes in plants (Ayre et al. 2020). Techniques for the overexpression of genes include stable genetic transformation and transient overexpression. Although the stable genetic transformation method is widely used for gene function analysis, it has several notable disadvantages including long cycles, heavy workloads, cumbersome processes, and complete dependence on the efficiency of the plant transformation system (Amara et al. 2013). In contrast, virus-mediated overexpression (VOX) has more advantages, particularly when time, cost, and effort are considered. Consequently, it has become an effective method of studying gene functions in plants (Marillonnet et al. 2004; Lee et al. 2012).

As plant virus-based vectors can cause systemic infections in their host plants, foreign genes are highly expressed in infected plants (Gleba et al. 2007; Vasques et al. 2019). Cultivated strawberry (*Fragaria ananassa*) is one of the most economically important small fruits, and the area of strawberry cultivation and yield have been increasing annually owing to increased consumption and its relatively high economic value in China (Yuan et al. 2021). However, to date, few viral vectors have been applied in the gene function analysis of strawberry plants. Although the tobacco rattle virus (TRV)-based vector has been used in *F. ananassa* (Tian et al. 2014), the infectivity rate of *F. ananassa* inoculated with TRV is very low because it is not a natural host for TRV. Thus, it is difficult to perform strawberry gene function analysis using

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a TRV-based vector. Apple latent spherical virus (ALSV)-based vectors have a wide host range. pALSV can infect *F. ananassa* tissue culture seedlings by gene-gun inoculation. ALSV-based vectors have been successfully used to silence the *F. ananassa* phytoene desaturase (PDS)-coding gene (*FaPDS*) and to induce early flowering by overexpressing the *Arabidopsis thaliana* flowering locus T gene in *F. ananassa* (Li et al. 2019). However, the gene-gun inoculation method is expensive. Additionally, it is difficult to obtain *F. ananassa* tissue culture seedlings, and the inoculation materials are too limited to be widely used in strawberry gene function analysis studies.

Strawberry vein banding virus (SVBV), which belongs to the genus *Caulimovirus* of the *Caulimoviridae* family, can infect both *F. vesca* and *F. ananassa* (Ratti et al. 2009). The SVBV genome is composed of double-stranded DNA with approximately 7876 nucleotides (Pattanaik et al. 2004). The genomic DNA of SVBV contains seven open reading frames encoding seven proteins: movement protein (P1), aphid transmission protein (P2), DNA-binding protein (P3), coat protein (P4), reverse transcriptase (P5), multifunctional protein (P6), and an unknown-function protein (P7) (Ryabova et al. 2002). In our previous studies, we constructed an infectious clone of SVBV (Feng et al. 2016) and identified P6 as an RNA silencing suppressor, symptom determinant, and trans-activating factor (Feng et al. 2018; Li et al. 2018). Additionally, we observed that SVBV-P1 could form inclusion bodies associated with microtubules and the endoplasmic reticulum and interact with P4 to promote SVBV cell-to-cell movement (Rui et al. 2021).

In this study, we engineered pSVBV as an effective virus-based overexpression vector by inserting a multiple cloning site (MCS) into the SVBV genome. The pSVBV-based vector could effectively infect strawberry plants and successfully overexpress the green fluorescent protein (GFP) in strawberry plants. We anticipate that the developed SVBV-based vector system could be a useful tool to the research community for functional studies of strawberry genes.

Results

Infection analysis of the recombinant virus vector pSVBV-P1-MCS

As shown in the construction diagram of the recombinant virus vector pSVBV-P1-MCS, MCS was inserted downstream of the *P1* gene (Fig. 1a). To verify whether the recombinant virus vector pSVBV-P1-MCS could infect the host, strawberry plants were inoculated with pSVBV-P1-MCS and pSVBV by *Agrobacterium*-mediated vacuum infiltration. At 35 days post-inoculation (dpi), systemic leaves of strawberry plants inoculated with pSVBV-P1-MCS or pSVBV exhibited light green vein banding symptoms (Fig. 1b). In contrast, no symptoms were observed on the systemic leaves of strawberry plants inoculated with pCB301, which served as negative controls (Fig. 1b). To further confirm this, Southern blot assay was performed to analyze the accumulation of SVBV in the systemic leaf tissues shown in Fig. 1b. The results showed clear accumulation of SVBV DNA in the strawberry plants inoculated with pSVBV-P1-MCS or pSVBV, whereas SVBV DNA could not be detected in the strawberry plants inoculated with pCB301 (Fig. 1c). These results indicate that pSVBV-P1-MCS could systemically infect strawberry plants.

Expression of GFP in strawberry plants inoculated with pSVBV-P1-GFP

To investigate whether the recombinant virus vector pSVBV-P1-MCS could express foreign genes in strawberry plants, the *GFP* gene was inserted into the MCS of pSVBV-P1-MCS to generate the overexpression vector pSVBV-P1-GFP (Fig. 2a). Then, pSVBV-P1-GFP was transformed into *Agrobacterium tumefaciens* with which the strawberry plants were inoculated via *Agrobacterium*-mediated vacuum infiltration. At 15 dpi, the infiltrated plants were illuminated with UV-B light. It was shown that green fluorescence was observed on the veins and petioles of strawberry plants inoculated with pSVBV-P1-GFP. In contrast, no green fluorescence was observed in the systemic leaves of the plants inoculated with pSVBV-P1-MCS (Fig. 2b). Western blotting analysis confirmed the accumulation of SVBV P4 in strawberry plants inoculated with pSVBV-P1-MCS or pSVBV-P1-GFP, and also the expression of GFP protein in the pSVBV-P1-GFP infected plants (Fig. 2c). It should be noted that no symptoms were induced by pSVBV at 15 dpi (Fig. 2b); however, at 35 dpi, the symptoms could be observed (Additional file 1: Figure S1a). Therefore, these data suggest that pSVBV-P1-GFP was infectious and could express GFP in strawberry plants.

Infection analysis of the recombinant virus vector pSVBV-P4-MCS

To further explore alternative strategies for the construction of SVBV-based vectors, we designed and tested another way. As shown in the construction diagram of the recombinant virus vector pSVBV-P4-MCS, MCS was inserted downstream of the *P4* gene (Fig. 3a). Strawberry plants were then inoculated with pSVBV-P4-MCS or pSVBV by *Agrobacterium*-mediated vacuum infiltration. Interestingly, at 35 dpi, the systemic leaves of strawberry plants inoculated with pSVBV-P4-MCS or pSVBV exhibited light-green vein banding symptoms (Fig. 3b). No symptoms were observed

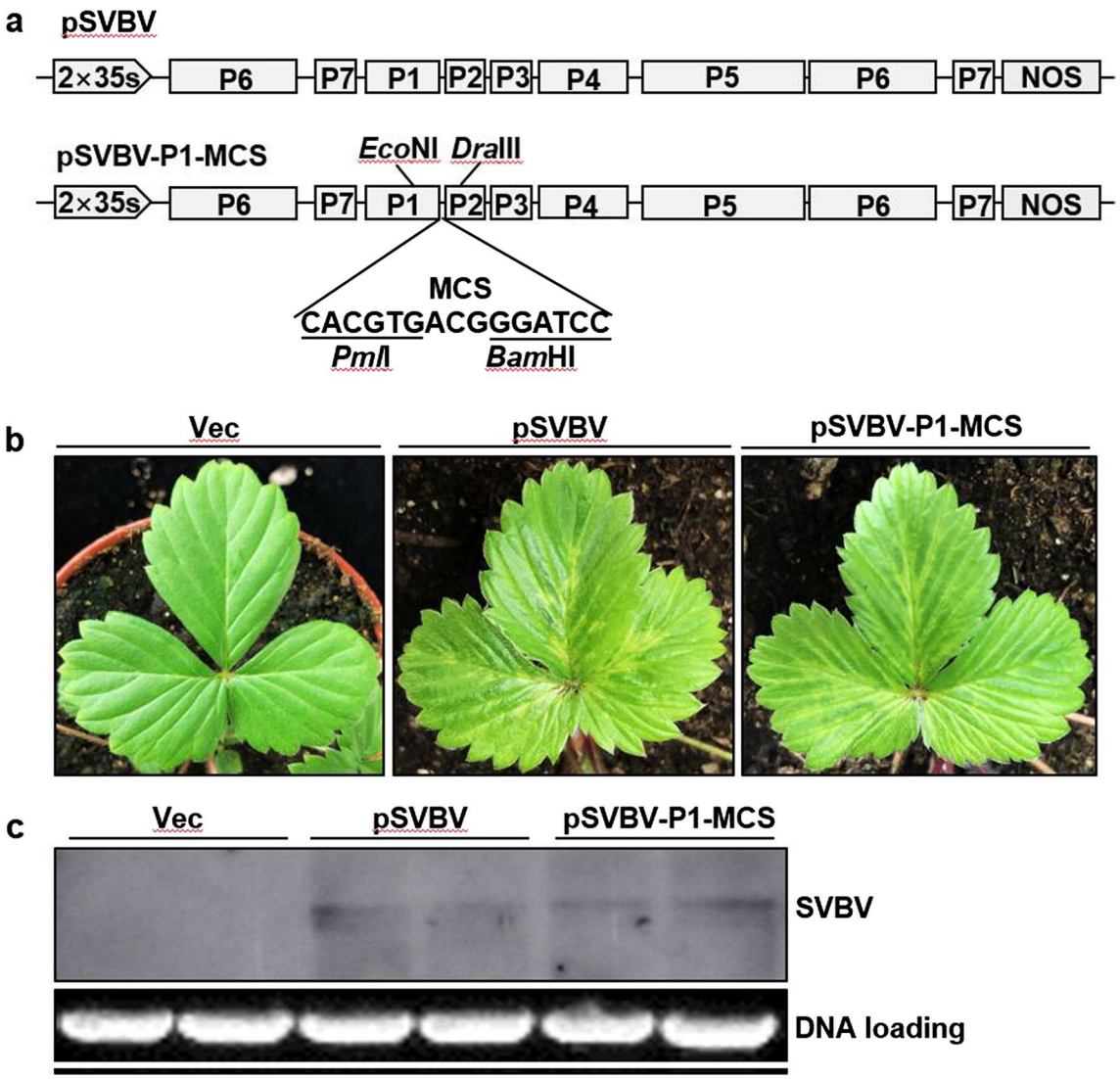

Fig. 1 The recombinant virus vector pSVBV-P1-MCS could induce systemic infection in strawberry plants. **a** Schematic representation of the construction of pSVBV and pSVBV-P1-MCS vector. A total of 1.25 copies of the full-length SVBV genome were inserted into pCB301 to construct the infectious clone pSVBV. The MCS sequence was CACGTGACGGGATCC, with the underlined part representing the recognition sequence of restriction endonucleases *Pml*I and *Bam*HI. Fragment P1-MCS was cloned into the plasmid pSVBV via the *Eco*NI/*Dra*III to generate pSVBV-P1-MCS, which has a MCS downstream of the *P1* gene; **b** Light-green vein banding symptoms were observed in systemic leaves of strawberry plants agroinfiltrated with pSVBV or pSVBV-P1-MCS. Strawberry plants agroinfiltrated with pCB301 were used as negative controls; **c** Accumulation of SVBV in pCB301-, pSVBV-, or pSVBV-P1-MCS-agroinfiltrated strawberry plants determined by Southern blot analysis. Blot was probed with the *CP* gene sequence of SVBV. The lower panel represents an ethidium bromide-stained gel of DNA samples as a loading control

on the systemic leaves of strawberry plants inoculated with pCB301, which served as negative controls (Fig. 3b). Southern blot results indicated clear accumulation of SVBV DNA in strawberry plants inoculated with pSVBV-P4-MCS or pSVBV; however, no SVBV DNA accumulation was observed in the strawberry plants inoculated with pCB301 (Fig. 3c). These results indicate that engineering MCS into the viral genome did not affect the replication competence and infectivity of the virus in strawberry plants.

Expression of GFP in strawberry plants inoculated with pSVBV-P4-GFP

To verify the expression of exogenous proteins using the pSVBV-P4-MCS vector in strawberry plants, the *GFP*

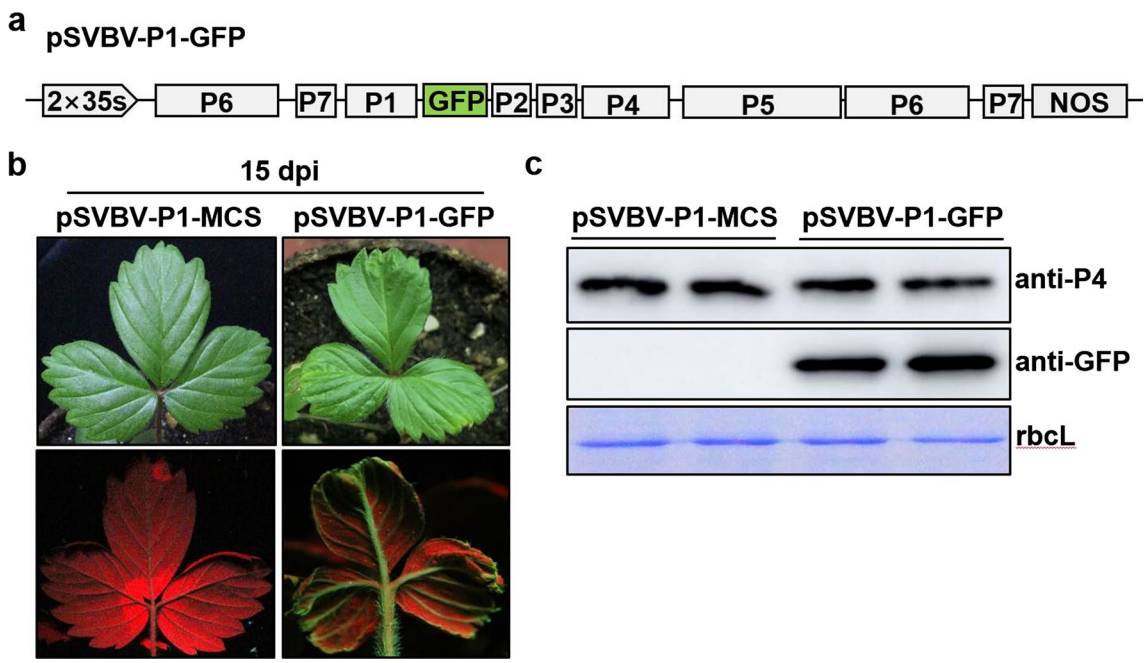

Fig. 2 Expression of GFP in strawberry plants inoculated with the pSVBV-P1-GFP vector. **a** Schematic representation of the overexpression vector pSVBV-P1-GFP. The *GFP* gene was cloned into pSVBV-P1-MCS via the *Pml*I-*Bam*HI sites. **b** Detection of fluorescence signals in the leaves of strawberry plants inoculated with pSVBV-P1-MCS or pSVBV-P1-GFP. The back of the strawberry leaves was illuminated under UV-B light. **c** Western blot analysis of the leaf samples shown in (**b**) using antibodies for SVBV P4 and GFP. Coomassie brilliant blue (CBB)-staining of the RuBisCO large subunit (rbcL) was used as a loading control

gene was cloned into pSVBV-P4-MCS via the *Pml*I and *Bam*HI sites, yielding a construct designated as pSVBV-P4-GFP (Fig. 4a). Strawberry plants were infiltrated with *A. tumefaciens* containing pSVBV-P4-GFP or pSVBV-P4-MCS by *Agrobacterium*-mediated vacuum infiltration. At 15 dpi, the infiltrated plants were illuminated under UV-B light and strong GFP expression was detected in the veins and petioles of strawberry plants inoculated with pSVBV-P4-GFP. By contrast, no expression of GFP was detected in the systemic leaves of the plants inoculated with pSVBV-P4-MCS (Fig. 4b). To validate these results, we tested strawberry plant samples by western blotting. The results revealed that SVBV was accumulated in the strawberry plants inoculated with pSVBV-P4-MCS or pSVBV-P4-GFP (Fig. 4c). Strawberry plants exhibited no symptoms when inoculated with pSVBV-P4-MCS or pSVBV-P4-GFP at 15 dpi (Fig. 4b); however, at 35 dpi, the symptoms induced by pSVBV were observed (Additional file 1: Figure S1b), which was similar to that of the pSVBV-P1-MCS and pSVBV-P1-GFP vectors. These results suggest that pSVBV-P4-GFP could infect and express GFP in strawberry plants.

Comparison of infectivity rates between pSVBV and SVBV-based vectors in strawberry plants

To determine the efficiency of SVBV-based vectors, the infectivity rates of pSVBV and SVBV-based vectors were statistically analyzed. As shown in Table 1, the infectivity rates of both pSVBV-P4-GFP (74.28%) and pSVBV-P4-MCS (75.76%) were very similar to that of pSVBV (77.14%), while the incidences of pSVBV-P1-GFP (60.60%) and pSVBV-P1-MCS (62.16%) were slightly lower. This indicates that the insertion of MCS and foreign genes did not affect the infectivity of SVBV-based vectors. Therefore, SVBV-based vectors could be used to study the functions of genes in strawberry plants.

Discussion

Multiple cloning sites (MCS) are generally inserted into the suitable sites of viral infectious clones to generate plant virus vectors. The position where MCS is inserted in the infectious virus clones has differential effects on virus infection and replication. There are three common strategies to develop plant virus vectors. First, a specific gene encoded by the virus can be replaced with MCS. In a previous study, the coat protein (*CP*) gene encoded by tomato mosaic virus (ToMV) was replaced by MCS to obtain the recombinant virus vector pToMV. It was observed that pToMV could infect *Nicotiana*

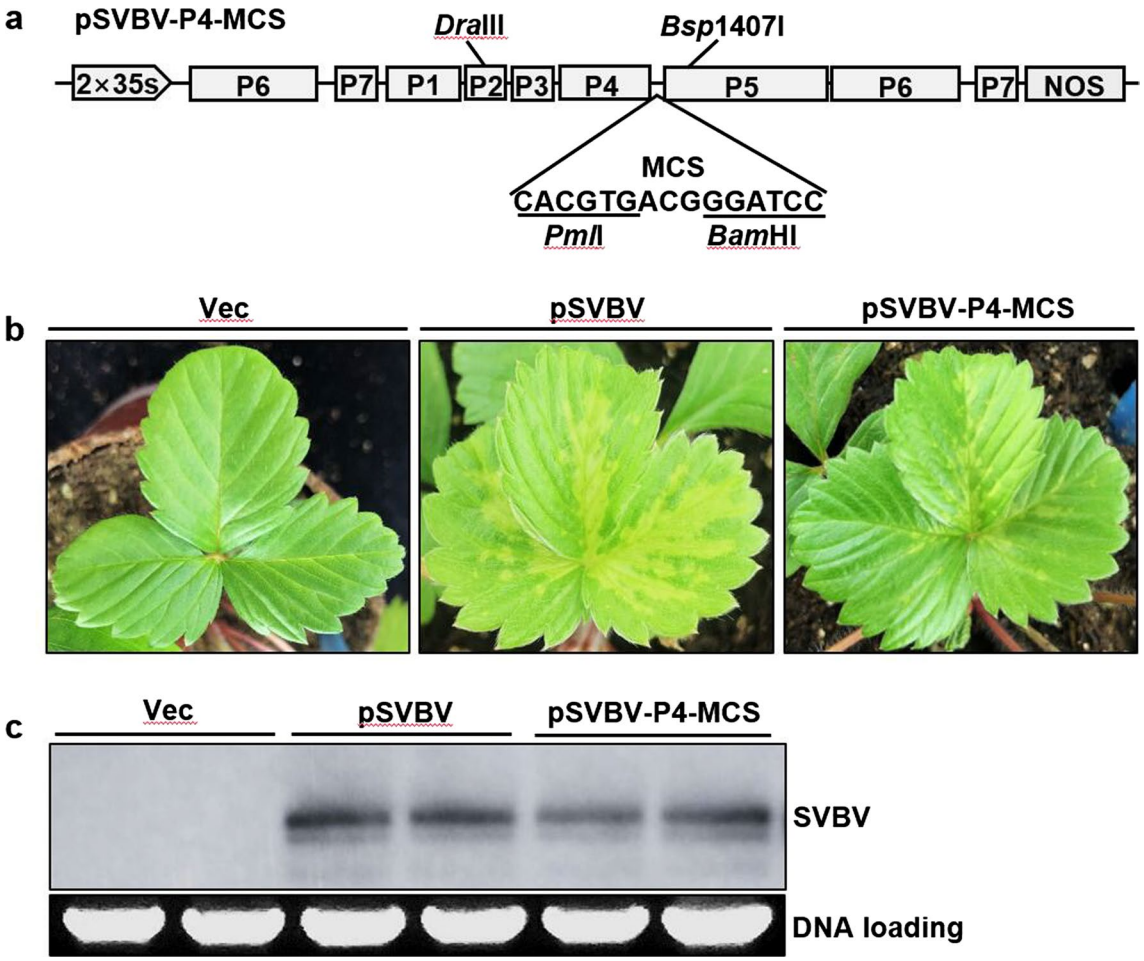

Fig. 3 The recombinant virus vector pSVBV-P4-MCS could induce systemic infection in strawberry plants. **a** Schematic representation of the pSVBV-P4-MCS vector. The MCS sequence was CACGTGACGGGATCC, with the underlined part representing the recognition sequence of restriction endonucleases *Pml*I and *Bam*HI. The fragment P4-MCS was cloned into the plasmid pSVBV via the *Dra*III/*Bsp*1407I sites to generate pSVBV-P4-MCS, which has a MCS downstream of the *P4* gene; **b** Light-green vein banding symptoms in strawberry plants agroinfiltrated with pSVBV or pSVBV-P4-MCS. Strawberry plants agroinfiltrated with pCB301 were used as negative controls; **c** Accumulation of SVBV in pCB301-, pSVBV-, or pSVBV-P4-MCS-agroinfiltrated strawberry plants by Southern blot analysis. SVBV DNA was detected using a digoxigenin-labeled probe specific for the SVBV *CP* gene. The lower panel represents an ethidium bromide-stained gel of DNA samples as a loading control

benthamiana leaves; however, it could not induce systematic infection in *N. benthamiana* plants (Liu et al. 2014). The second strategy is the insertion of MCS downstream of the virus-encoded movement protein gene (*MP*). MCS was inserted downstream of the *MP* gene encoded by broad bean wilt virus (BBWV) to produce the recombinant virus vector pBBWV2-R2-OE, which could express recombinant proteins in plants (Choi et al. 2019). The third strategy is to insert the MCS downstream of the virus-encoded *CP* gene. MCS was inserted downstream of the *CP* gene encoded by tobacco rattle virus (TRV) RNA2 to obtain the recombinant virus vector pTRV2, which has been widely used to induce endogenous gene silencing in plants such as *N. benthamiana* (Li et al. 2019), *A. thaliana* (Bilichak and Kovalchuk 2017), *Capsicum annuum* L. (Chung et al. 2004), and *Mirabilis jalapa* (Singh et al. 2012). In this study, we successfully constructed recombinant virus vectors pSVBV-P1-MCS and pSVBV-P4-MCS by inserting MCS downstream of the movement protein gene *P1* and coat protein gene *P4*, respectively. The two SVBV-based vectors could infect strawberry plants and their infectivity rates were similar to that of pSVBV. Therefore, the findings of this study lay a foundation for the subsequent use of SVBV-based vectors to induce transient overexpression of foreign genes in strawberry plants.

At present, gene function analysis studies in strawberry plants mainly depend on genetic transformation. For

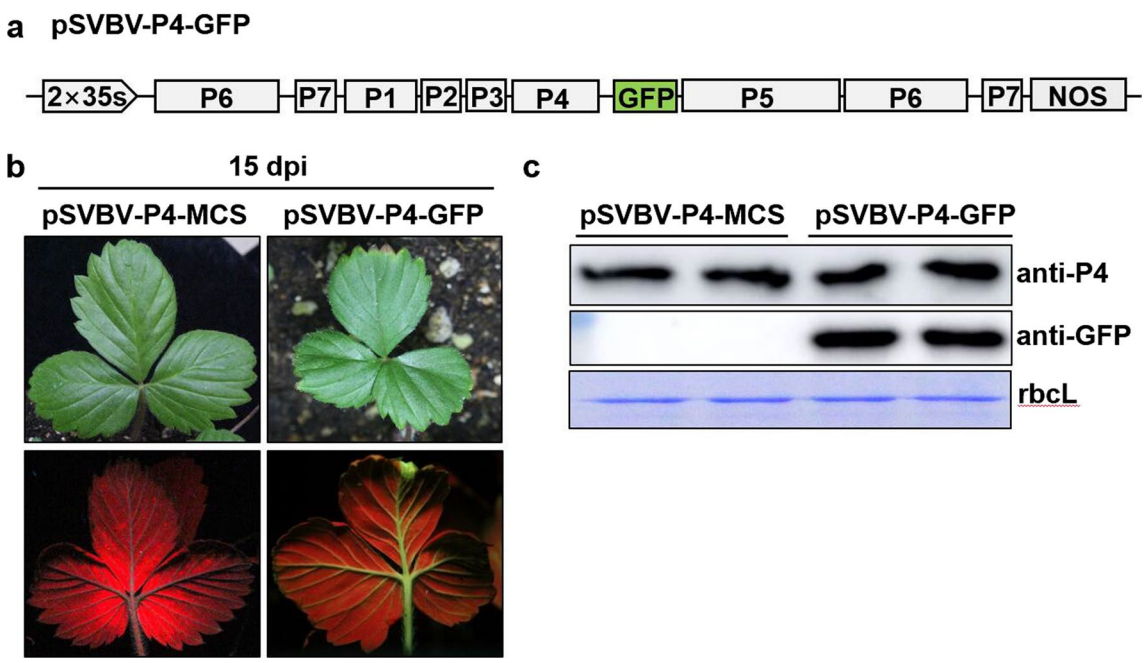

Fig. 4 Expression of GFP in strawberry plants using the pSVBV-P4-GFP vector. **a** Schematic representation of the overexpression vector pSVBV-P4-GFP. The *GFP* gene was cloned into pSVBV-P4-MCS via the *Pml*I-*Bam*HI sites. **b** Detection of fluorescence signals in the leaves of strawberry plants inoculated with pSVBV-P4-MCS or pSVBV-P4-GFP. Backs of the strawberry leaves were illuminated under UV-B light. **c** Western blot analysis of leaf samples shown in (**b**) using antibodies for SVBV P4 and GFP. Coomassie brilliant blue (CBB)-staining of the RuBisCO large subunit (rbcL) was used as a loading control

Table 1 Infectivity rates of strawberry plants inoculated with pSVBV or SVBV-based vector

Infectious clones	No. of plants inoculated	No. of plants infected	GFP-positive	Infectivity rates (%)
pSVBV	35	27	–	77.14
pSVBV-P1-GFP	33	20	20	60.60
pSVBV-P1-MCS	37	23	–	62.16
pSVBV-P4-GFP	35	26	26	74.28
pSVBV-P4-MCS	33	25	–	75.76

example, strigolactone inhibits the formation of branches and regulate plant growth and development. Overexpression of the key gene *DWARF27 (FveD27)* for strigolactone biosynthesis inhibits the formation of new strawberry stem branches and increases the number of inflorescences and strawberry yield (Sun et al. 2021). Similarly, overexpressing the transcription factors *TEOSINTE BRANCHED 1, CYCLOIDEA,* and *PROLIFERATING4 CELL FACTORS* (*FvTCP9*) improves fruit maturation by promoting the biosynthesis of abscisic acid and anthocyanins (Xie et al. 2020). Additionally, it may be possible to improve the cell membrane permeability of mature strawberry fruit by overexpressing *NOD26-like intrinsic protein* (*FaNIP1;1*) genes to allow water accumulation in strawberry fruits (Molina-Hidalgo et al. 2015). However, the genetic transformation of strawberries is time-consuming, expensive and has low transformation rate. The highest transformation rate was reported to be no more than 20%, and it required 25 weeks for the transformation process to be completed (Gruchała et al. 2004). By contrast, the SVBV-based overexpression vector could express exogenous genes in strawberry plants at 15 dpi, and our data (Table 1) indicated that the lowest transformation rate of SVBV-based vector was more than 60%. Therefore, this modified vector has the potential to be used as a simple and rapid tool for functional gene research in strawberry plants.

Plant virus-based vectors have been used as gene expression tools for studying insect and plant functional genes (Gao et al. 2019). In this study, although the SVBV-based vectors have not been demonstrated to be effective for the expression of endogenous genes of strawberry, the results have showed that the pSVBV-P1-GFP and pSVBV-P4-GFP could infect strawberry plants and successfully express exogenous gene. At 15 dpi, GFP was detected in the systemic leaves of strawberry plants inoculated with the two SVBV-based vectors, while no symptoms were observed on other parts of the plants. More importantly, SVBV-induced symptoms emerged

until 35 dpi (Additional file 1: Figure S1). As the expression of foreign proteins was observed before the appearance of virus-induced symptoms, the symptoms will not significantly affect the phenotype of strawberry plants overexpressing foreign genes. Therefore, SVBV-based vectors are suitable for systemic gene function analysis in strawberries.

Conclusions

Our results suggest that SVBV-based vectors constructed in this study could effectively infect strawberry plants and successfully overexpress exogenous genes in strawberry plants. Furthermore, the developed SVBV-based vectors will provide important tools for exploring gene functions of strawberries.

Methods

Plant material, agrobacterium transformation, and vacuum-infiltration

Strawberry (*F. vesca*) plants were grown in the greenhouse of Anhui Agricultural University (31°83′N, 117°25′E). The culture conditions were as follows: temperature 23 ± 2 °C, humidity 65 ± 5%, and light intensity 3000 Lx with a 16 h light/8 h dark cycle (Tian et al. 2015).

Agrobacterium transformation was conducted as previously described (Wieczorek et al. 2020). The binary vector pCB301, SVBV, and its derivatives were transformed into *A. tumefaciens* strain AGL1. A single colony of *A. tumefaciens* strain AGL1 containing pCB301 or SVBV-related constructs was grown in YEP liquid medium (supplemented with 50 mg/L rifampicin and 100 mg/L kanamycin) at 28 °C for 12 h with shaking. The *A. tumefaciens* cells were then harvested and resuspended to an OD600 = 1.0 using infiltration buffer (1 M $MgCl_2$, 0.1 M 2-(4-morpholino)-ethane sulfonic acid, and 2 mM acetosyringone) and incubated in dark for 3 h before inoculation.

Vacuum infiltration was performed as previously described (Tian et al. 2015). Strawberry plants were removed from the nutrient soil and rinsed with tap water. Then, 0.01% Silwet® L-77 was added to the inoculation solution before the immersion of whole plant. Vacuum-infiltration was carried out for 20–25 s, and the plants were gently rinsed with water and cultured in nutrient soil.

Plasmid construction

SVBV infectious clone was constructed as previously described (Feng et al. 2016). A total of 1.25 copies of the full-length SVBV Shenyang isolate genome were inserted into the pCB301 vector to construct the infectious clone pSVBV. To construct the recombinant virus vector pSVBV-P1-MCS, the pSVBV plasmid was amplified with the primer pair P1L-SY-F/P1L-SY-R or P1R-SY-F/P1R-SY-R by polymerase chain reaction (PCR) to generate P1L-MCS and P1R-MCS fragments, P1L-MCS and P1R-MCS was amplified with the primer pair P1L-SYF/P1R-SY-R by PCR to generate a 1347 bp P1-MCS fragment. The fragment P1-MCS was ligated into the plasmid pSVBV to generate pSVBV-P1-MCS (Fig. 1a). The GFP fragment was amplified using the primer pair GFP-F/GFP-R and cloned into pSVBV-P1-MCS via the *Pml* I-*Bam*H I sites to generate pSVBV-P1-GFP (Fig. 2a). The primers used for plasmid construction are listed in Additional file 2: Table S1.

The overexpression vector pSVBV-P4-GFP was constructed following a similar method. First, the fragment P4-MCS was PCR-amplified using the primer pairs P4L-SY-F/P4L-SYR and P4R-SY-F/P4R-SY-R. Then, the fragment P4-MCS was ligated into the plasmid pSVBV to generate pSVBV-P4-MCS (Fig. 3a). The GFP fragment was amplified using the primer pair GFP-F/GFP-R and cloned into pSVBV-P4-MCS via the *Pml* I-*Bam*H I sites by enzyme digestion followed by ligation to generate pSVBV-P4-GFP (Fig. 4a). The primers used for plasmid construction are also listed in Additional file 2: Table S1.

Southern blot analysis

Southern blot analysis was conducted as described previously (Feng et al. 2016). Systemic leaves in pSVBV-, pSVBV-P1-MCS-, and pSVBV-P4-MCS-inoculated strawberry plants were harvested at 35 dpi. The total DNA was extracted using the Plant gDNA Isolatin Kit (Biomiga, Shanghai, China), fractionated on a 0.8% agarose gel for 4–6 h, and then transferred onto Hybond-N^+ membranes (Amersham Pharmacia, Little Chalfont, Buckinghamshire, England). Detection was performed with digoxigenin-labeled probes specific for the SVBV *CP* (primers SbCP-F/SbCP-R) gene using a DIG High Prime DNA Labeling and Detection Starter Kit II (Roche, Mannheim, Germany).

GFP imaging and western blot

GFP imaging was conducted as described previously (Tian et al. 2015). The fluorescence of strawberry leaves was monitored by illuminating them with a 100 W hand-held long-wave ultraviolet lamp (UV products, Upland, CA 91,786, Black Ray model B 100AP/R). The leaves were then photographed using a Nikon 4500 digital camera with Kodak Wratten Filter 15 in the dark.

Western blotting was also conducted as described previously (Feng et al. 2018). Briefly, the total protein was extracted from systemic leaves inoculated with pSVBV-P1-MCS and pSVBV-P1-GFP, pSVBV-P4-MCS, or pSVBV-P1-GFP using RIPA lysis buffer (Sangon Biotech,

Shanghai, China); the crude proteins were then fractionated on an SDS-PAGE gel and transferred onto a PVDF membrane; anti-GFP or anti-P4 antibody was then used to probe the blot. The immunoblot signals were then detected using an EasySee Western Blot Kit (TransGen Biotech, Beijing, China).

Abbreviations

ALSV: Apple latent spherical virus; BBWV: Broad bean wilt virus; CBB: Coomassie brilliant blue; CP: Coat protein; dpi: Days post-inoculation; GFP: Fluorescent protein; MCS: Multiple cloning site; MP: Movement protein; PCR: Polymerase chain reaction; PDS: Phytoene desaturase; rbcL: RuBisCO large subunit; SVBV: Strawberry vein banding virus; TRV: Tobacco rattle virus; ToMV: Tomato mosaic virus; VOX: Virus-mediated overexpression.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00113-5.

Additional file 1: Figure S1. Symptoms in strawberry plants agroinfiltrated with SVBV-based vectors. Light-green vein banding symptoms in strawberry plants agroinfiltrated with pSVBV-P1-MCS or pSVBV-P1-GFP (**a**) and pSVBV-P4-MCS or pSVBV-P4-GFP (**b**) at 35 dpi.

Additional file 2: Table S1. Primers used in the study.

Acknowledgements

Not applicable.

Authors' contributions

TJ and LJ designed the study. XY, QZ, and XJ performed the experiments and drafted the manuscript. ZW provided suggestions for revision of the manuscript and advised on the experiments. JL, LJ, and TJ finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

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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