Phytopathology Research

RESEARCH

Open Access

Construction and characterization of the infectious cDNA clone of the prevalent Chinese strain SC3 of soybean mosaic virus

Hui Liu[1†], Huanfang Zheng[1†], Wenyang Xiang[1†], Yingpei Song[1], Bowen Li[1], Jinlong Yin[2], Mengzhuo Liu[1], Xiaoxiang Liu[1], Liqun Wang[1], Shouping Yang[1*], Kai Xu[2*] and Haijian Zhi[1*]

Abstract

The resistance of different soybean varieties to the Chinese isolate SC3 of soybean mosaic virus (SMV) was systematically studied. However, the resistance of different germplasm sources is still poorly understood. We constructed an infectious DNA clone of SMV-SC3 (pSC3) and modified it to allow the expression of yellow fluorescence protein (YFP)/red fluorescence protein (RFP) during viral infection. By using the infection of pSC3-RFP, we can classify the resistance of different soybean cultivars to SMV-SC3 to the extreme resistance that restricts initial viral replication and the resistance that allows viral replication in the initially inoculated cells but restricts further movement. Also, we tracked the SMV-SC3 infection in susceptible cultivar Nannong 1138-2 (NN1138-2) and found that the seed transmission of SMV to the offspring plants can be tracked and recorded by imaging the virus-driven expression of YFP. Overall, we reveal new aspects of soybean resistance to SMV-SC3 and provide an essential tool to study the infection and transmission of SMV-SC3, which will help decipher the genes involved in SMV pathogenesis and host resistance.

Keywords Soybean mosaic virus, *Glycine max*, Extreme resistance, Viral spread

Background

Soybean mosaic virus (SMV) is an economically important pathogen affecting soybean production worldwide (Hill and Whitham 2014). Its host range is relatively narrow, generally limited to legumes (Zhou 1990; Zheng et al. 2000). SMV is a positive-sense single-stranded RNA virus that belongs to the *Potyvirus* genus of the *Potyviridae* family. Its RNA genome is approximately 10,000 nt in length (Hajimorad et al. 2018). SMV infection of soybean can cause visible symptoms such as mosaic leaves, deformed leaves, dwarfing, and in severe cases, top necrosis, leading to yield loss (Widyasari et al. 2020). Moreover, SMV is transmitted by aphids to nearby soybean plants in a non-persistent manner or through seeds to offspring (Hajimorad et al. 2018). Since SMV is one of the pests in plant quarantine practices, different countries established different pathosystems to study soybean interaction with domestic SMV isolates (Usovsky et al. 2022). For example, SMV was grouped into strains G1–G7 in the United States (Cho and Goodman 1979, 1982), strains A–E were reported in Japan (Takahashi et al.

†Hui Liu, Huanfang Zheng and Wenyang Xiang contributed equally to this work

*Correspondence:
Shouping Yang
spyang@njau.edu.cn
Kai Xu
xukai@njnu.edu.cn
Haijian Zhi
zhj@njau.edu.cn
[1] National Center for Soybean Improvement, National Key Laboratory for Crop Genetics and Germplasm Enhancement, Key Laboratory of Biology and Genetic Improvement of Soybean-Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, China
[2] Jiangsu Key Laboratory for Microbes and Functional Genomics, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China

1980), while the Chinese SMV isolates were classified into 22 strains (SC1–SC22) (Wang et al. 2014).

Many resistance loci on the soybean genomes have been identified (Widyasari et al. 2020). For example, the *Rsv1* allele in cultivar L78-378 and the *Rsv3* allele in cultivar L29 mediate extreme resistance (ER), which does not allow SMV-G2 or G7 strain to replicate in the primarily inoculated cells (Hajimorad et al. 2018). In contrast to the extreme resistance, the *Rsv4* allele in cultivar V94-5152 only restricts the cell-to-cell and systemic movement of strains SMV-G2 and SMV-G7 (Wang et al. 2015). Many efforts were made to identify the genetics of the resistance to different Chinese SMV isolates (Fu et al. 2006; Li et al. 2006; Li et al. 2010; Ma et al. 2011; Wang et al. 2011; Yan et al. 2015; Li et al. 2016; Liu et al. 2016; Karthikeyan et al. 2017, 2018; Li et al. 2017; Yuan et al. 2020b). Among them, the *Rsc7* locus in soybean cultivar Kefeng No. 1 mediates resistance to SMV strain SC7 (Fu et al. 2006; Yan et al. 2015), the *Rsc3* and *Rsc3Q* loci in soybean cultivar Qihuang No. 1 are involved in resistance to SMV strain SC3 (Li et al. 2017), and the *Rsc4* locus in cultivar Dabaima confers resistance against aggressive SMV strains including SC3, SC4, and SC7. Recently, the *Rsc4-3* from the *Rsc4* locus encoding a coiled-coil (CC) domain-containing nucleotide-binding site (NBS)-leucine-rich repeat (LRR) (CC-NBS-LRR) protein was identified as the resistance gene (Yin et al. 2021). However, whether resistances from different soybean cultivars act similarly or differently on the locally isolated SMV strains has not yet been assessed.

After establishing the first infectious clone of the brome mosaic virus (Ahlquist et al. 1984), various plant viruses could be recovered from infectious DNA clones (Bao et al. 2020; Wang et al. 2021). The stable infectious clones are essential for the analysis of viral replication, movement, symptom development, host range, and virus-host interactions (Tuo et al. 2017). In this work, we constructed an infectious clone of Chinese SMV isolate SC3 and engineered it to express the yellow fluorescence protein (YFP)/ red fluorescence protein (RFP) for tracking viral infection and host resistance. Using these clones, we have observed the viral infection on six soybean cultivars grown in soybean production areas in China. We discovered that, in contrast to the susceptible cv. NN1138-2, the soybean cvs. Dabaima, Kefeng No. 1, and Qihuang No. 1 display extreme resistance to SMV SC3 infection, while Soybean cvs. Zaoshu No.18 and Davis allow viral replication at the inoculation sites but restrict viral movement to adjacent cells and distal leaves. In addition, we showed that the susceptible cv. NN1138-2 allows SMV SC3 to infect the seeds in a small percentage, and those virus-infected seeds could passage the virus to the offspring. Overall, our study provided new insight into the compatible and incompatible interactions between various soybean cultivars and SMV-SC3.

Results

Construction of the SMV-SC3 infectious clone

The full-length SMV-SC3 cDNA was PCR amplified and inserted into the pBR322 vector under the control of the Cauliflower mosaic virus 35S promoter (Fig. 1a). A modified version with multi-cloning sites (MCS) was constructed, in which a foreign gene can be inserted into the MCS between the coding region of viral protein P1 and HC-Pro, a commonly used strategy to express foreign genes for many potyviral infectious clones (Beauchemin et al. 2005; Olspert et al. 2015; Seo et al. 2016). The translated polyprotein can be cleaved by viral encoded proteases P1 and NIa-pro at the protease cleavage site during viral infection (Fig. 1b). Next, we inserted a yellow fluorescence protein coding sequence into the MCS, resulting in the pSC3-YFP clone for tracking the viral replication and infection within host plants (Fig. 1c). To evaluate the infectivity, the plasmids of pSC3, pSC3-MCS, or pSC3-YFP was rub-inoculated onto the leaves of susceptible cultivar NN1138-2. After 7 days, all the inoculated SMV clones induced mosaic leaves (Fig. 2a). We observed that the symptomatic leaf areas in pSC3-YFP-infected leaves are accompanied by successful SMV infection, as shown by the yellow fluorescence under a stereoscopic fluorescence microscope (Fig. 2b). The accumulation of YFP mRNA and protein could be detected by RT-PCR and Western blotting analysis (Fig. 2c). Besides the phenotypic observation, we demonstrated qualitatively that the SMV viral protein was successfully expressed in the plant through DAS-ELISA assay (Fig. 2d). Furthermore, we detected the viral accumulation by Western blotting analysis (Fig. 2e). We have shown that the SMV recovered from SMV-SC3 infectious clones can replicate to a similar or higher level than the wild type (WT) SMV-SC3. Overall, the constructed SMV infectious clones can effectively cause infection and can be used for tracking the virus in soybean plants.

Dynamics of viral local and systemic movements

Next, we aim to observe the SMV spread within the susceptible cultivar NN1138-2. When the unifoliolate leaf was fully expanded, we inoculated the pSC3-YFP clone onto the leaf (inoculated leaf). We observed that when the cell-to-cell movement of SMV-YFP reached the veins at 7 days post-inoculation (dpi), the upper leaf (systemically infected leaf) started to show scattered fluorescence representing successful infection of SMV. Albeit the local infection of SMV was not widely dispersed even till 9 dpi, the viral infection on the upper leaves is dominated by SMV-YFP at most of

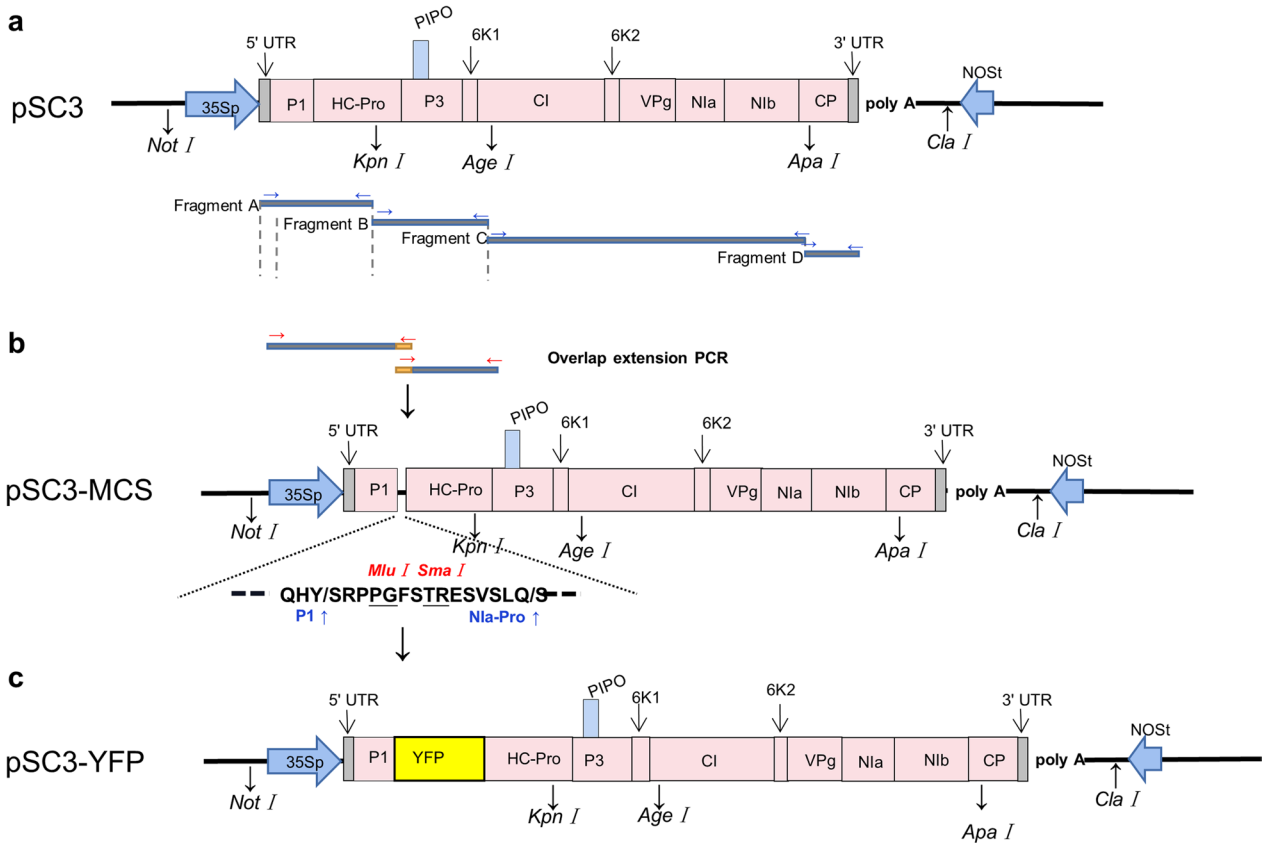

Fig. 1 Schematic description of the infectious clones of Soybean mosaic virus Chinese isolate SC3. **a** Scheme of the cloning strategy for SMV infectious clone pSC3. The genomic sequence of SMV-SC3 was amplified as four overlapping segments, namely Fragment A–D. Four fragments are recombined and inserted into pBR322G7 carrier plasmid via Gibson Assembly. The blue arrows represent different primer pairs. **b** The sequence of an MCS was inserted between the P1 and HC-Pro coding regions. The red-colored arrows represent different primer pairs. The Peptide cleavage sites identified by P1 or NIa-Pro viral proteases are indicated by arrows. **c** Map of the infectious clone pSC3-YFP. DNA fragment containing the yellow fluorescence protein (YFP) coding sequence was inserted into the *SmaI*/*MluI* digested pSC3-MCS, resulting in pSC3-YFP

the leaf areas (Fig. 3a). Moreover, the yellow fluorescence can be detected on a specific upper leaf even at 90 dpi, indicating that pSC3-YFP clones could be continuously expressed in soybean, and could be used in subsequent experimental studies of SMV propagation tracking (Fig. 3b).

To understand whether SMV-YFP could reach the whole plant or seeds, we inoculated the pSC3-YFP to the third trifoliolate leaf of the susceptible soybean cultivar NN1138-2 when the third trifoliolate leaf was unfolded (V3 stage). When the plant reached the V6 stage, we observed that all the trifoliolate leaves were infected by SMV-YFP, as shown by the presence of yellow fluorescence (Fig. 4a). We also observed the presence of SMV-YFP in various tissues of the plant, including roots, flowers, and seeds, when the infected plants were grown for an extended period (Fig. 4b).

SMV-SC3 can infect the seeds and be transmitted to the offspring plants

We have harvested the seeds from SMV-YFP-infected susceptible cultivar NN1138-2. Compared with seeds from healthy plants, seeds infected with SMV-YFP had brown spots on their epidermis, and the brown spot rate was about 44.98% (Fig. 5a). The germination rate of brown spotted seeds (69.64%, n = 56) was lower than that of healthy seeds (89.47%, n = 57). Furthermore, in the seeds harvested in the previous generation, we peeled the seed coat and found that SMV-SC3 not only replicated in the seed coat but also infected the hypocotyl and cotyledon (Fig. 5b), where yellow fluorescence was observed in about 1.67% (n = 539) of the seeds in the embryo. When these seeds with fluorescence in the seed embryos were planted in pots kept in moisturized condition, the leaves of young

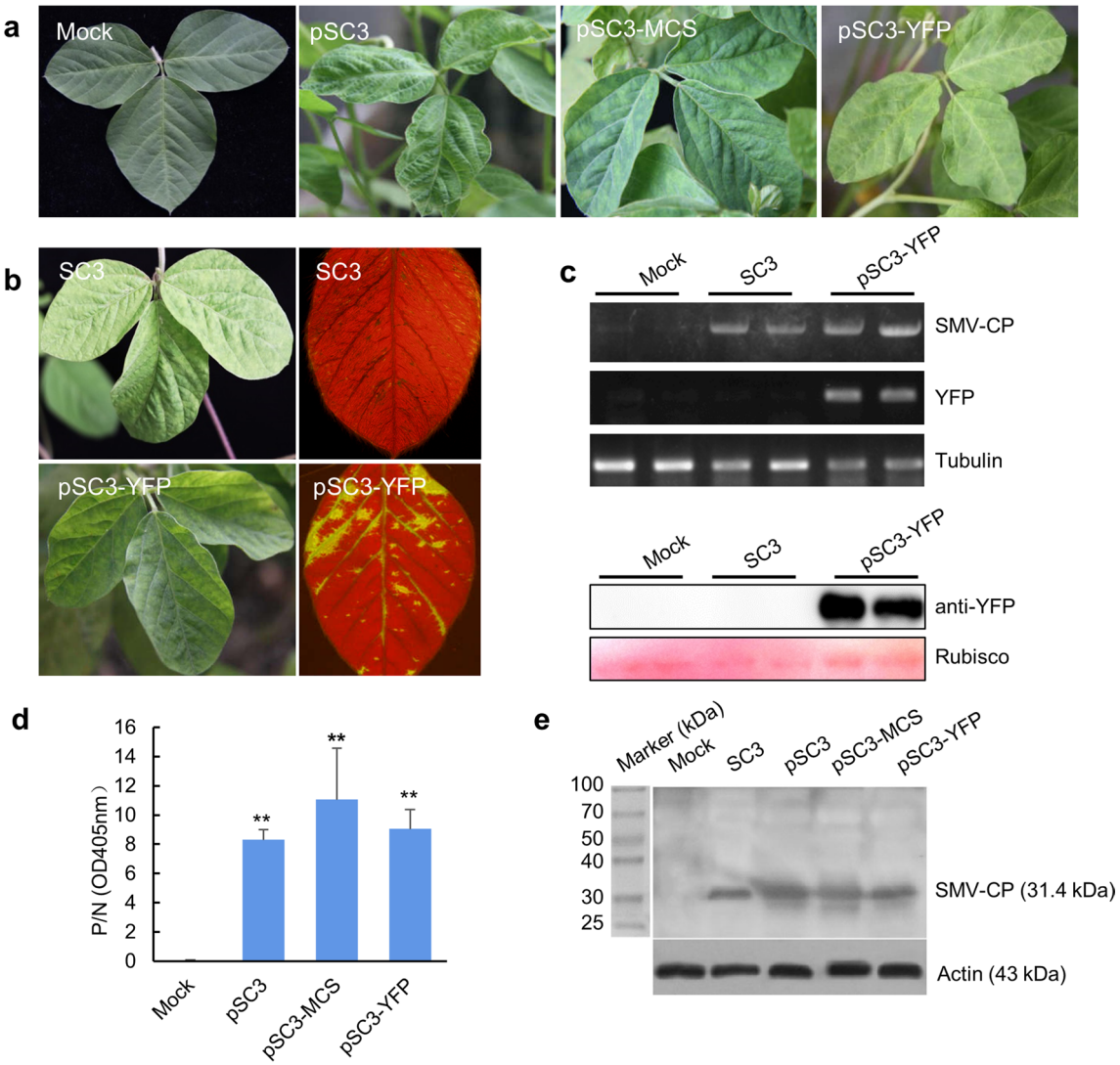

Fig. 2 Infectivity of SMV recovered from the pSC3, pSC3-MCS, or pSC3-YFP clones in soybean plants. **a** Symptoms of pSC3, pSC3-MCS, and pSC3-YFP-infected soybean leaves at 7 days post-inoculation (dpi). **b** Symptoms of pSC3-YFP-infected soybean leaves were observed under normal light (left) or the laser light that excites the yellow fluorescence of YFP at 7 dpi. The wild type SC3 strains were inoculated as control. **c** Detection of viral RNAs coding for CP and YFP by RT-PCR (above) and Western blotting analysis (below). The plants infected with SC3, pSC3-YFP were tested, and healthy plants were used as a negative control. Gm-β -tubulin was tested as a reference gene. **d** Detection of SMV accumulation by DAS-ELISA assay. The plants infected with pSC3, pSC3-MCS, pSC3-YFP were tested, and 9 samples were taken from each type. Healthy plants were used as a negative control. The value of P/N (OD405nm) represents OD value of sample/OD value of negative control and the asterisk indicates *P*-value between groups. $^{**}P \leq 0.05$; $^{***}P \leq 0.01$. **e** Western blotting detects the accumulation of SMV coat protein in soybean plants inoculated with pSC3, pSC3-MCS, pSC3-YFP, and wild type (WT) SMV-SC3. Actin (NCBI, Accession number: AW350943.1) is used as a loading control. The expected size of SMV CP is 31.4 kDa, and the size of actin is 43 kDa

seedlings exhibited typical SMV symptoms (Fig. 5c). These results demonstrated that SMV-YFP could be transmitted by seed during the infection of the cv. NN1138-2 with similar infectivity as the natural virus strain SC3 (Song et al. 2015), and also provided a convenient method in tracking the seed transmission.

SMV-SC3 can be used to detect the resistance level in different soybean cultivars

Previous studies showed that many soybean cultivars are resistant to the SMV-SC3 strain (Li et al. 2010; Wang et al. 2018; Yuan et al. 2020a). YFP fluorescence was observed by simulating rubbing inoculated (with buffer

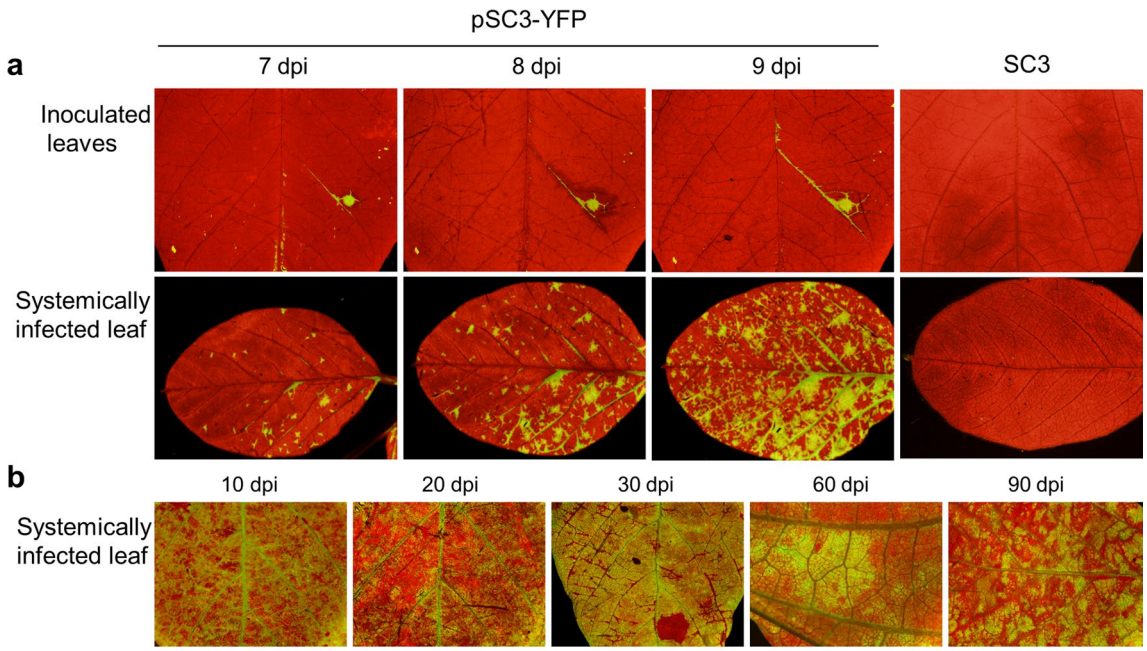

Fig. 3 Local and systemic infection of SMV-YFP in susceptible soybean cultivar NN1138-2. **a** At 7 days post-inoculation (dpi), upper leaves of inoculated soybean cultivar NN1138-2 start to accumulate SMV-YFP, while the veins of the inoculated leaves start to show signs of viral movement. At 8 or 9 dpi, the upper leaves accumulated more virus, while the inoculated leaf shows a clear path of viral movement through the vein that near the inoculation site. The same leaves were continuously imaged at 7, 8, and 9 dpi. The wild type SC3 strains were inoculated as control. **b** Extended observation of SMV-YFP infected upper leaves at 10, 20, 30, 60, and 90 dpi under a stereoscopic fluorescence microscope

only) control leaves. We found that autofluorescence was induced in the wounded area. Therefore, we constructed the clone of pSC3-RFP (replacing the YFP sequence of the pSC3-YFP cloning vector with the RFP sequence) to distinguish between viral replication at the site of SMV infection and autofluorescence caused by injury (Fig. 6a). To test the infectivity of SMV-SC3 on different soybean cultivars, we inoculated the pSC3-RFP clone onto a small area each of the leaves from different cultivars and observed the viral accumulation and spread. In a previously reported susceptible cultivar NN1138-2, the SMV-RFP infection was successfully established at 5 dpi as the red fluorescence can be observed at both the initially inoculated site and the veins that are close to the inoculation site. The virus continuously moved to the adjacent leaf areas through the veins (Fig. 6b).

Similarly, we inoculated pSC3-RFP onto the leaves of different resistant soybean cultivars, including Dabaima (Wang et al. 2017), Qihuang No. 1, Zaoshu No.18, Davis, and Kefeng No. 1 (Li et al. 2010). The SMV-RFP can replicate at the initial inoculation sites in cultivars Zaoshu No.18 and Davis without further movement, as indicated by the restricted viral clone-driven expression of RFP at the inoculation sites (Fig. 6c). In contrast, in the cultivars Dabaima, Qihuang No. 1, and Kefeng No. 1, SMV-RFP infection was not established even at the inoculation sites (Fig. 6d). These data suggest that resistances to SMV in various soybean cultivars are different.

Discussion

Similar to the studies of the United States/South Korea soybean-SMV pathosystem, the decades-long systematic genetic studies on Chinese soybean cultivars identified a similar but larger set of soybean accessions that mediate resistance to various SMV strains isolated in China (Wang et al. 2018; Yuan et al. 2020a). However, whether these cases of resistance fall into the category of extreme resistance (ER) or the resistance that restricts viral movements were previously unknown.

The infectious clone containing complete genomic information of the plant virus is a powerful tool for studying plant-virus interactions (Brewer et al. 2018). Although the infectious clones were made for SMV strains isolated from the United States (Usovsky et al. 2022), it is rarely reported for Chinese SMV isolates. This situation hampered the progress in understanding the SMV resistance (compatible interaction) of various local Chinese soybean cultivars and limited understanding of the viral replication and transmission of many Chinese SMV isolates (incompatible interaction).

Here, we have chosen one of the prevalent SMV strains SC3, that causing diseases in Huang-Huai-Hai

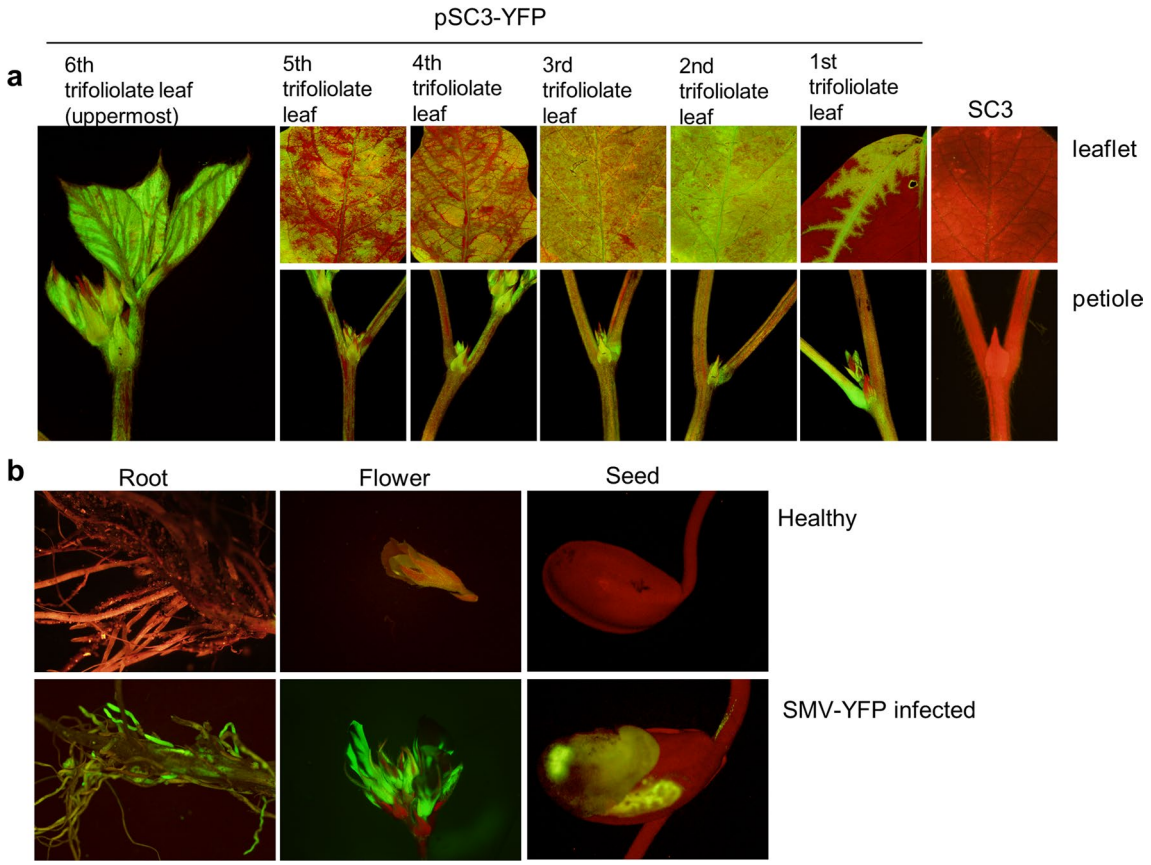

Fig. 4 Presence of SMV-YFP in different parts of soybean plants. **a** Plasmids of pSC3-YFP were rub-inoculated onto the third trifoliolate leaf of the susceptible cultivar NN1138-2 at the V3 growth stage. Stereoscope images show that SMV-YFP spread to all six trifoliolate leaves on the V6 growth stage of the soybean. The wild type SC3 strains were inoculated as control. **b** SMV-driven YFP expression can be observed in roots, flowers, and seeds

and Yangtze River basins in China, and successfully constructed its infectious cDNA clone. The SC3 strain was used as a reference virus to evaluate the resistance to SMV in many Chinese soybean cultivars (Ma et al. 2011; Li et al. 2017). Instead of using viral-driven expression of β-glucuronidase followed by staining of the infected leaves (Yu et al. 2004; Wang et al. 2015; Hajimorad et al. 2018), we constructed SMV-SC3 clones expressing YFP/RFP that can be tracked under a stereoscopic fluorescence microscope. We found that the soybean resistance to SMV-SC3 also includes both extreme resistance (Kefeng No. 1, Qihuang No. 1, Dabaima) and movement-restricting resistance (Zaoshu No.18, Davis). The resistance gene *Rsc4-3* in Dabaima and its allele *NBS_C* in soybean cv. L29 were recently reported (Yin et al. 2021). The *Rsc4-3/NBS_C* genes encode a CC-NBS-LRR protein localizing to the cell wall. Interestingly, both Dabaima in this study and L29 in the previous study (Hajimorad et al. 2018) were reported to mediate extreme resistance against SMV-SC3 or SMV-G7 infection, confirming the same resistance mechanism of *Rsc4-3/NBS_C* genes on different soybean cultivars. Furthermore, it will be interesting to investigate which gene mediates the observed movement-restricting resistance in soybean cvs. Zaoshu No.18, and Davis to SMV-SC3.

A previous study deploying the DAS-ELISA method found that the seed-transmission incidence of SMV-SC3 in various domestic soybean cultivars is between 0 and 13.68%, with an overall ratio of 1.47% (Song et al. 2015), although the test efficiency of that method is low. And the SMV-positive seeds can not germinate into offspring seedlings due to the usage for molecular detection. The utilization of viral-clone driven-YFP/RFP expression shown in this study provided an easy way to track seed transmission. Moreover, by using these SMV clones, we have provided direct and visible evidence that SMV could infect the embryo within the seeds.

Conclusions

In summary, we constructed an infectious DNA clone of the SMV type-strain SC3. SMV-SC3 is a prevalent and virulent strain that is epidemic in the soybean

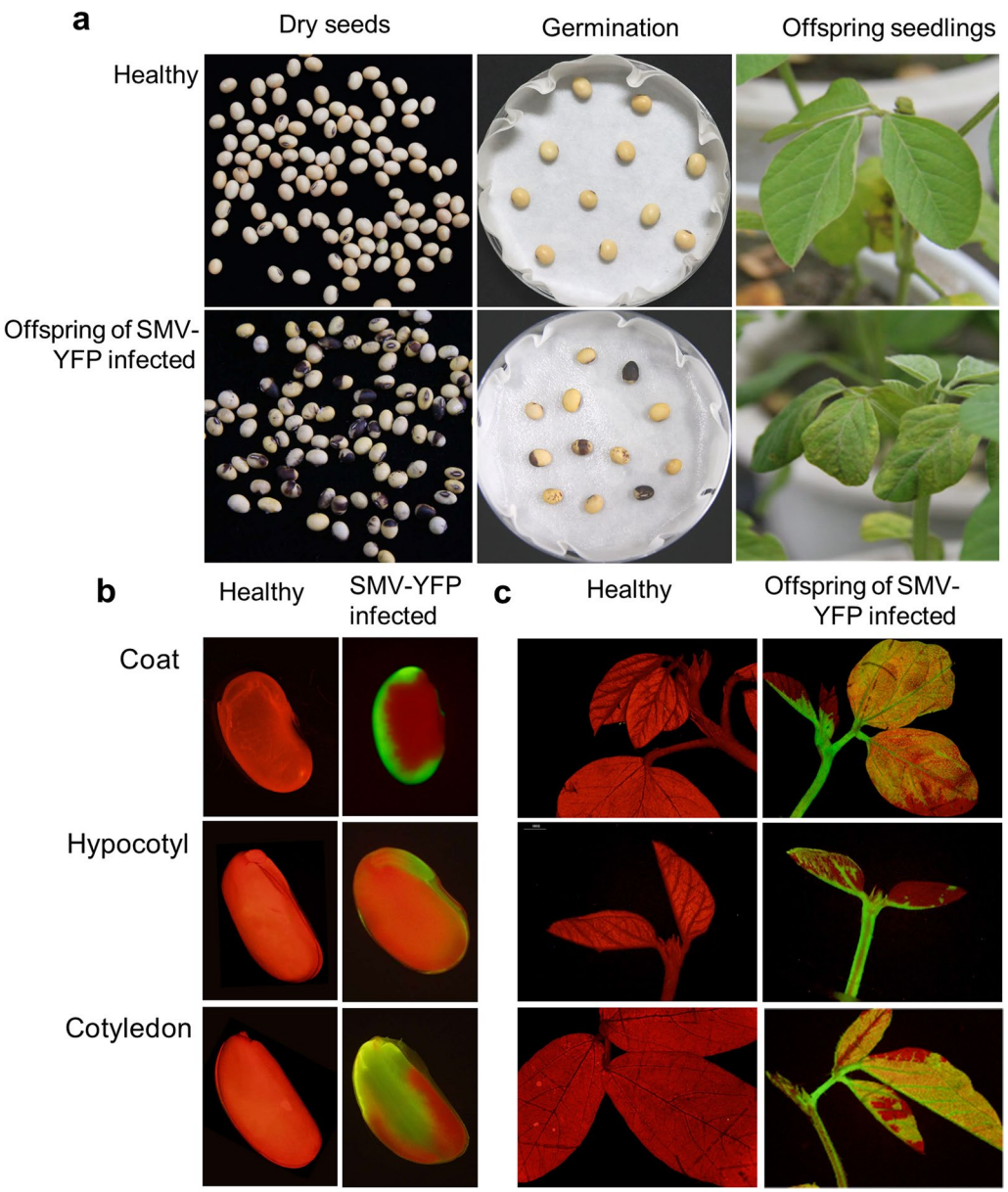

Fig. 5 Phenotypes of offspring plants infected with SMV. **a** Phenotypes of dry seeds, germinated seeds, and offspring seedlings derived from healthy soybean plants and SMV-YFP infected soybean plants. **b** The presence of the yellow fluorescence in the seed coat, the hypocotyl, and the cotyledon of the seeds collected from SMV-YFP infected NN1138-2 soybean plants. **c** SMV-YFP infection could be detected in the offspring plants grown from the SMV-YFP positive seeds (right), but not from virus-free seeds from the healthy plants (left)

(See figure on next page.)

Fig. 6 SMV-RFP accumulation as observed via the presence of the red fluorescence on different soybean cultivars inoculated with pSC3-RFP. **a** The injured area was observed by YFP fluorescence and RFP fluorescence by simulating (buffer only) rubbing of inoculated control leaves. Scale bars, 1 mm. **b** The pSC3-RFP plasmids were directly rub-inoculated onto one spot of each leaf of the soybean cultivar NN1138-2 using a glass rod. Rescued SMV-RFP can accumulate at the inoculation site. The progression of the viral spread through the leaf vein was captured at 5, 6, 7, 8, 9, and 10 days post-inoculation (dpi) via a fluorescent stereoscope. **c** Soybean cultivars Zaoshu No.18 and Davis could restrict SMV-RFP spread within the leaf areas but supported SMV-RFP replication at the inoculation sites. **d** SMV-RFP could not replicate in the inoculated leaves from the soybean cultivars Qihuang No. 1, Dabaima, and Kefeng No. 1 as indicated by the lack of red fluorescence at the inoculation site. The images shown are representative ones from at least five repeated experiments

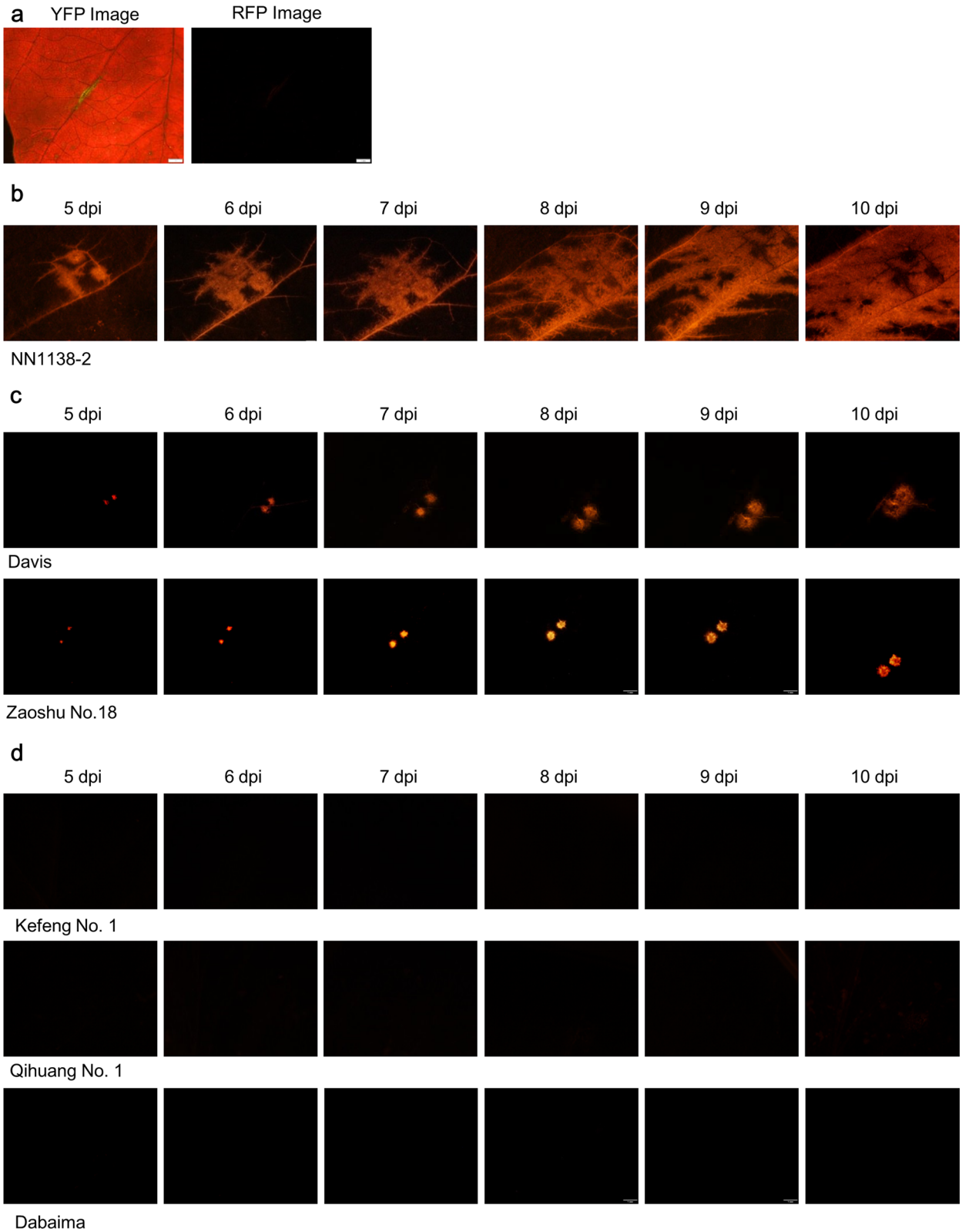

Fig. 6 (See legend on previous page.)

production areas of the Huang-Huai-Hai basin region in China. This clone has application value in screening for resistant germplasm and the study of resistance mechanisms. We showed that the resistance of different soybean cultivars to SMV-SC3 includes two types: the extreme resistance that restricts initial viral replication, and the resistance that allows viral replication in inoculated cells but restricts viral movement within the plants. Moreover, the transmission of SMV seeds to the offspring plants can be tracked and recorded by imaging the virus-driven expression of YFP. Identifying different resistant types and the easy method of tracking seed transmission will be conducive to understanding the interaction between soybean and local SMV strains.

Methods

Plant materials and SC3 strain

The soybean leaves infected with SMV strain SC3 were kept at −80°C and were used for virus propagation in the susceptible cv. NN1138-2. All soybean (*Glycine max* (L.) Merr.) accessions and SMV strains were provided by the National Center for Soybean Improvement (Nanjing Agricultural University, Nanjing, China). Soybean plants were grown in a growth chamber set at 25°C under long-day conditions (16 h light/8 h dark).

Construction and inoculation of the full-length infectious cDNA clones of pSC3, pSC3-MCS, pSC3-YFP, and pSC3-RFP

Total RNAs were isolated from soybean seedlings infected with the SMV-SC3 strain and reverse-transcribed according to the user's manual (HiScript III 1st Strand cDNA Synthesis Kit, Vazyme, Nanjing, China). The genomic sequences of the SMV were obtained from the GenBank of the National Center for Biotechnology Information (NCBI) (Accession number: JF833013.1) for the design of SMV-specific primer pairs (Table 1). The SC3 sequence was amplified via PCR (PrimerSTAR Max DNA Polymerase, TAKARA) as four overlapping segments, namely Fragment A–D (Fig. 1a). Subsequently, the PCR products were purified and assembled by overlapping PCR technology and Gibson Assembly Cloning® Kit (New England Biolabs, Beijing, China), following the manufacturer's instructions. The assembled full-genomic sequence of SMV-SC3 was doubly-digested by *Not*I and *Cla*I and inserted into pBR322G7 (a modification vector based on pBR322) digested with the same restriction enzymes. The full-length cDNA clone of SMV-SC3 was named pSC3 (Fig. 1a). The restriction enzyme sites of *Mlu*I and *Sma*I were introduced between the coding regions of P1 and HC-Pro by overlapping PCR, resulting in pSC3-MCS (Fig. 1b). Subsequently, the DNA fragment of YFP was amplified by PCR from plasmid pA7YFP (BioVectorNTCC, Beijing, China), digested with *Sma*I and *Mlu*I, and inserted into pSC3-MCS digested with *Sma*I and *Mlu*I. The resulting vector was named pSC3-YFP

Table 1 Primer sequences used for amplifying SMV-SC3 genome sequences

Scheme	Forward primer (5′→ 3′)	Reverse primer (5′→ 3′)
35S	TCGAGCGGCCGCCTACTCCAAGAATATCAA	TTTATGAGTAGTTTTAATTTCCTCTCCAAA TGAAATGAAC
A	GTTCATTTCATTTGGAGAGGAAATTAAAACTACTCATAAA	GGTACCCAACTGTTAAAGATCCAAA
B	TTTTGGATCTTTAACAGTTGGGTAC	CCGGTTATCTGTATCAAGAGTAGCC
C	GGCTACTCTTGATACAGATAACCGG	GGCCCATGCCCAGAAGAGTGTGCAT
D	ATGCACACTCTTCTGGGCATGGGCC	GCTATCGATTTTTTTTTTTTTTTTTTTTTT
YFP	TCCCCCGGGGGTGAGCAAGGGCGAGGAGCTGT	CGACGCGTCTTGTACAGCTCGTCCATGCC

The primers were designed according to the full-length reference genome sequence of SMV-SC3. The adjacent PCR fragments overlap to each other with a 25 bp long homologous arm

Table 2 The primers of pSC3-MCS vector

Sequence name	Forward primer (5′→ 3′)	Reverse primer (5′→ 3′)
NotI -MluI	TCGAGCGGCCGCCTACTCCAAGAATATCAAAGATACAGTCTCAGAAGACC	CCCGGGGGAAAAACG CGTTTTCCTCTCCAAATG AAATGAACTTCCTT
SmaI-KpnI	CCCGGGGGAAAATTAAAACTACTCATAAAGACAAC	CATGGTACCCAACTGTCA AGGATCCAAAAGAGTCAA TCACATGCATGGTT

(Fig. 1c). Primers used were provided (Table 2). Plasmids in this study were extracted by Plasmid Miniprep Kit (Axygen, New York, USA), then directly rub-inoculated on fully-expanded primary leaves. Each leaf received approximately 20 μg plasmids dissolved in 20 μl of phosphate buffer (0.1 M, pH 7.0).

In the same way as the above construction method of pSC3-YFP, the YFP sequence of the pSC3-YFP cloning vector was replaced with the RFP sequence to generate the pSC3-RFP clone.

Total RNA extraction, cDNA synthesis, and RT-PCR

Total RNAs were extracted from fresh soybean leaves using TRIzol reagent (Art. No. 15596-026, Invitrogen, USA), respectively. For RT-PCR, synthesis of the first-strand cDNA template was performed using HiScript II qRT SuperMix for qPCR (+gDNAwiper) (R223-01, Vazyma, Nanjing, China), followed by gDNA eraser enzyme (Vazyme, Nanjing, China) to eliminate genomic DNA. All RT-PCR reactions were performed using three biological replicates of each sample. Gm-β-tubulin (GenBank: NM_001252709.2) was used as an internal control.

Detection of SMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

The accumulation of SMV after inoculation was analyzed by DAS-ELISA kits (AC Diagnostics Inc., Fayetteville, AR, USA). Three leaf discs (from different soybean plants) were randomly collected from soybean leaves with typical SMV symptoms and combined as one sample for the test, and then nine samples each were taken for detection. Soybean cv. NN1138-2 inoculated with the viral strain SC3 or mock-inoculated (buffer only) were used as positive or negative controls, respectively. The DAS-ELISA analysis was carried out according to the manufacturer's protocols. The optical density (OD) was measured at the wavelength of 405 nm, and readings were presented as the fold-increase to the negative control (P/N) as previously described (Zhang et al. 2011). Samples with fold-increase equal to or higher than 2.0 were considered positive.

Western blotting analysis

The accumulation level of the SMV in soybean plants infected with these cloning vectors or wild-type SMV-SC3 was determined using Western blotting. Total proteins were extracted from new leaves of soybean plants, separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred to a PVDF membrane. The PVDF membrane was blocked with 5% defatted milk powder in pH 7.6 Tris-buffered saline containing 0.05% Tween-20 (TBST) for 2 h at room temperature, incubated in antiserum against SMV CP (Zhu et al. 2016) and YFP diluted at 1:10,000 (V/V) for 2 h, followed by 3–5 h incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG diluted in 1:10,000 and visualized by chemiluminescence as instructed by the manufacturer.

Fluorescence microscope observation

Soybean tissue inoculated with pSC3-YFP/pSC3-RFP clones was placed under a stereoscopic fluorescence microscope (Olympus MVX10) with an MVPLAPO 0.63X objective lens (1× magnification) and an Olympus DP80 camera (ISO: 1600; exposure time: 90 ms) for observation. For YFP imaging, a BP490-500HQ excitation filter and a DM505HQ long-pass emission filter were used. To image the RFP fluorescence, a BP535-555HQ excitation filter and a BA570-625HQ band-pass emission filter were used.

Statistical analysis

Data were analyzed by the one-factor variance method using SPSS software (version 18). All experiments were repeated three times, and the average of three separate experiments was presented.

Abbreviations

DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
dpi	Days post-inoculation
ER	Extreme resistance
MCS	Multi-cloning sites
OD	Optical density
RFP	Red fluorescence protein
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SMV	Soybean mosaic virus
YFP	Yellow fluorescence protein

Acknowledgements

The authors thank Prof. Xiaorong Tao (Nanjing Agricultural University) for providing SMV antiserum.

Authors' contributions

HZ (Haijian Zhi) and KX conceptualized the study. HL, HZ (Huanfang Zheng), WX, YS, BL, ML, XL, JY, and LW performed experiments, HL, HZ (Huanfang Zheng), WX, and KX analyzed the results and wrote the manuscript. HL, SY, and KX interpreted data and read and approved the final manuscript. All authors read and approved the final manuscript.

Funding

This research was funded by the National Key R&D Program of China (2022YFF1001500), the Core Technology Development for Breeding Program of Jiangsu Province (JBGS-2021–014), the Jiangsu Collaborative Innovation Center for Modern Crop Production (JCIC-MCP), the National Soybean Industrial Technology System of China (CARS-004), the National Natural Science Foundation of China (31770164), and the Jiangsu Province's Innovation Program (JSSCTD202142).

Availability of data and materials

Not applicable.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 19 October 2022 Accepted: 24 January 2023
Published online: 14 March 2023

References

Ahlquist P, French R, Janda M, Loesch-Fries LS. Multicomponent RNA plant virus infection derived from cloned viral cDNA. Proc Natl Acad Sci. 1984;81:7066–70. https://doi.org/10.1073/pnas.81.22.7066.

Bao WH, Yan T, Deng XY, Wuriyanghan H. Synthesis of full-length cDNA infectious clones of soybean mosaic virus and functional identification of a key amino acid in the silencing suppressor Hc-Pro. Viruses. 2020;12:886. https://doi.org/10.3390/v12080886.

Beauchemin C, Bougie V, Laliberté J. Simultaneous production of two foreign proteins from a polyvirus-based vector. Virus Res. 2005;112(1–2):1–8. https://doi.org/10.1016/j.virusres.2005.03.001.

Brewer HC, Hird DL, Bailey AM, Seal SE, Foster GD. A guide to the contained use of plant virus infectious clones. Plant Biotechnol J. 2018;16:832–43. https://doi.org/10.1111/pbi.12876.

Cho EK, Goodman RM. Strains of soybean mosaic virus: classification based on virulence in resistant soybean cultivars. Phytopathology. 1979;5:467–70. https://doi.org/10.1094/phyto-69-467.

Cho EK, Goodman RM. Evaluation of resistance in soybeans to soybean mosaic virus strains. Crop Sci. 1982;69:1133–6. https://doi.org/10.2135/cropsci1982.0011183X002200060012x.

Fu SX, Zhan Y, Zhi HJ, Gai JY, Yu DY. Mapping of SMV resistance gene Rsc-7 by SSR markers in soybean. Genetica. 2006;128:63–9. https://doi.org/10.1007/s10709-005-5535-9.

Hajimorad MR, Domier LL, Tolin SA, Whitham SA, Saghai Maroof MA. Soybean mosaic virus: a successful potyvirus with a wide distribution but restricted natural host range. Mol Plant Pathol. 2018;19:1563–79. https://doi.org/10.1111/mpp.12644.

Hill JH, Whitham SA. Control of virus diseases in soybeans. Adv Virus Res. 2014;90:355–90. https://doi.org/10.1016/B978-0-12-801246-8.00007-X.

Karthikeyan A, Li K, Jiang H, Ren R, Li C, Zhi HJ, et al. Inheritance, fine-mapping, and candidate gene analyses of resistance to soybean mosaic virus strain SC5 in soybean. Mol Genet Genomics. 2017;292:811–22. https://doi.org/10.1007/s00438-017-1310-8.

Karthikeyan A, Li K, Li C, Yin JL, Li N, Yang YH, et al. Fine-mapping and identifying candidate genes conferring resistance to soybean mosaic virus strain SC20 in soybean. Theor Appl Genet. 2018;131:461–76. https://doi.org/10.1007/s00122-017-3014-1.

Li HC, Zhi HJ, Gai JY, Guo DQ, Wang YW, Li K, et al. Inheritance and gene mapping of resistance to soybean mosaic virus strain SC14 in soybean. J Integr Plant Biol. 2006;2021:C14. https://doi.org/10.1111/j.1744-7909.2006.00365.x.

Li K, Yang QH, Zhi HJ, Gai JY. Identification and distribution of soybean mosaic virus strains in southern China. Plant Dis. 2010;94:351–7. https://doi.org/10.1094/PDIS-94-3-0351.

Li N, Yin JL, Li C, Wang DG, Yang YQ, Karthikeyan A, et al. NB-LRR gene family required for Rsc4-mediated resistance to soybean mosaic virus. Crop Pasture Sci. 2016;67:541–52. https://doi.org/10.1071/CP15165.

Li C, Adhimoolam K, Yuan Y, Yin JL, Ren R, Yang YH, et al. Identification of candidate genes for resistance to soybean mosaic virus strain SC3 by using fine mapping and transcriptome analyses. Crop Pasture Sci. 2017;68:156–66. https://doi.org/10.1071/CP16353.

Liu JZ, Fang Y, Pang HX. The current status of the soybean-soybean mosaic virus (SMV) pathosystem. Front Microbiol. 2016;7:1906. https://doi.org/10.3389/fmicb.2016.01906.

Ma Y, Wang DG, Li HC, Zheng GJ, Yang YQ. Fine mapping of the Rsc14Q locus for resistance to soybean mosaic virus in soybean. Euphytica. 2011;181:127–35. https://doi.org/10.1007/s10681-011-0457-3.

Olspert A, Chung BY, Atkins JF, Carr JP, Firth AE. Transcriptional slippage in the positive-sense RNA virus family Potyviridae. Embo Rep. 2015;16:995–1004. https://doi.org/10.15252/embr.201540509.

Seo JK, Choi HS, Kim KH. Engineering of soybean mosaic virus as a versatile tool for studying protein-protein interactions in soybean. Sci Rep. 2016;6:22436. https://doi.org/10.1038/srep22436.

Song YP, Gao L, Liu ZT, Li K, Shen YC, Zhong YK, et al. Evaluation of adult resistance and soybean seed resistance to soybean mosaic virus. Soybean Sci. 2015;34:1015–9.

Takahashi K, Tanaka T, Iida W, Tsuda Y. Studies on virus diseases and causal viruses of soybean in Japan. Bull Tohoku Natl Agric Exp Station. 1980;62:1–130.

Tuo D, Fu LL, Shen WT, Li XY, Zhou P, Yan P. Generation of stable infectious clones of plant viruses by using Rhizobium radiobacter for both cloning and inoculation. Virology. 2017;510:99–103. https://doi.org/10.1016/j.virol.2017.07.012.

Usovsky M, Chen P, Li D, Wang A, Shi A, Zheng C, et al. Decades of genetic research on Soybean mosaic virus resistance in Soybean. Viruses. 2022;14(6):1122. https://doi.org/10.3390/v14061122.

Wang DG, Ma Y, Yang YQ, Liu N, Li C, Song YP, et al. Fine mapping and analyses of R (SC8) resistance candidate genes to soybean mosaic virus in soybean. Theor Appl Genet. 2011;122:555–65. https://doi.org/10.1007/s00122-010-1469-4.

Wang DG, Wei LH, Zhi HJ, Tian Z, Chen HU, Hu GY, et al. Identification of strains and screening of resistance resources to soybean mosaic virus in Anhui Province. Chin J Oil Crop Sci. 2014;36:374–9. https://doi.org/10.7505/j.issn.1007-9084.2014.03.013.

Wang Y, Khatabi B, Hajimorad MR. Amino acid substitution in P3 of soybean mosaic virus to convert avirulence to virulence on Rsv4-genotype soybean is influenced by the genetic composition of P3. Mol Plant Pathol. 2015;16:301–7. https://doi.org/10.1111/mpp.12175.

Wang DG, Zhao L, Li K, Ma Y, Wang LQ, Yang YQ, et al. Marker-assisted pyramiding of soybean resistance genes Rsc4, Rsc8, and Rsc14Q to soybean mosaic virus. J Integr Agric. 2017;16:2413–20. https://doi.org/10.1016/S2095-3119(17)61682-4.

Wang DG, Li K, Zhi HJ. Progresses of resistance on soybean mosaic virus in soybean. Sci Agric Sin. 2018;51:3040–59. https://doi.org/10.3864/j.issn.0578-1752.2018.16.002.

Wang DG, Cui LY, Zhang L, Ma ZN, Niu YB. Complete genome sequencing and infectious cdna clone construction of soybean mosaic virus isolated from Shanxi. Plant Pathol J. 2021;37:162–72. https://doi.org/10.5423/PPJ.OA.11.2020.0209.

Widyasari K, Alazem M, Kim KH. Soybean resistance to soybean mosaic virus. Plants (Basel, Switzerland). 2020;9:219. https://doi.org/10.3390/plants9020219.

Yan HL, Wang H, Cheng H, Hu ZB, Chu SS, Zhang GZ, et al. Detection and fine-mapping of SC7 resistance genes via linkage and association analysis in soybean. J Integr Plant Biol. 2015;57:722–9. https://doi.org/10.1111/jipb.12323.

Yin JL, Wang LQ, Jin TT, Nie Y, Liu H, Qiu YL, et al. A cell wall-localized NLR confers resistance to soybean mosaic virus by recognizing viral-encoded cylindrical inclusion protein. Mol Plant. 2021;14:1881–900. https://doi.org/10.1016/j.molp.2021.07.013.

Yu JH, Hamari Z, Han K, Seo JA, Reyes-Domínguez YR, Scazzocchio C. Double-joint pcr: a PCR-based molecular tool for gene manipulations in filamentous fungi. Fungal Genet Biol. 2004;41:973–81. https://doi.org/10.1016/j.fgb.2004.08.001.

Yuan Y, Yang YQ, Shen YC, Yu KS, Wang LQ, Ren R, et al. Mapping and functional analysis of candidate genes involved in resistance to soybean (Glycine max) mosaic virus strain SC3. Plant Breed. 2020a;139:618–25. https://doi.org/10.1111/pbr.12799.

Yuan Y, Yang YQ, Yin JL, Shen YC, Zhi HJ. Transcriptome-based discovery of genes and networks related to Rsc3Q-mediated resistance to soybean mosaic virus in soybean. Crop Pasture Sci. 2020b;71(12):987–95. https://doi.org/10.1071/CP20253.

Zhang XC, Sato S, Ye XH, Dorrance AE, Morris TJ, Clemente TE, et al. Robust RNAi-based resistance to mixed infection of three viruses in soybean plants expressing separate short hairpins from a single transgene. Phytopathology. 2011;101:1264–9. https://doi.org/10.1094/PHYTO-02-11-0056.
Zheng CM, Chang RZ, Qiu LJ. Progress on the disease of soybean mosaic virus. Acta Phytopathol Sin. 2000;2:97–105. https://doi.org/10.13926/j.cnki.apps.2000.02.001.
Zhou XP. Natural infection of Pisum sativum by soybean mosaic virus in Nanjing. J Nanjing Agric Uni. 1990;01:53–6 (in Chinese).
Zhu M, Zhang WN, Tian JY, Zhao WY, Chen ZQ, Sun LH, et al. Development of a lateral-flow assay (LFA) for rapid detection of soybean mosaic virus. J Virol Methods. 2016;235:51–7. https://doi.org/10.1016/j.jviromet.2016.05.011.