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The hypervariable N-terminal of soybean mosaic virus P1 protein influences its pathogenicity and host defense responses

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Abstract

Soybean mosaic virus (SMV; *Potyvirus*, *Potyviridae*) is one of the most prevalent and destructive viral pathogens in the world. The P1 protein is the first N-terminal product in the potyvirus genome and shows a high sequence variability that may be related to virus adaptation to hosts. In this work, we focused on the different functions of P1 proteins in two SMV isolates SMV^{GZL} and SMV^{NB} during their infection of plants. Isolate SMV^{GZL} induced weaker symptoms than SMV^{NB} in mechanical inoculation assays, and the accumulation level of SMV CP in SMV^{GZL}-infected leaves was lower than that in leaves infected with SMV^{NB}, especially at the late stage of infection. The isolates SMV^{GZL} and SMV^{NB} had a high similarity in genome sequence except for the P1 region. $P1^{GZL}$ induced a higher salicylic acid (SA) response than $P1^{NB}$ in *Nicotiana benthamiana*, which may explain the lower virus titers in plants infected with SMV^{GZL}. Our results suggest that the divergence in the P1 proteins of these SMV isolates influenced their virulence via differentially regulating SA signaling.

Keywords: Soybean, Soybean mosaic virus, P1, Pathogenicity difference, Host defense

Background

Soybean, *Glycine max* (L), is one of the world's most important economic crops, providing high-quality vegetable oil and protein for human and animal diets (Wilson 2008; Gao et al. 2015). Soybean is a natural host to many plant viruses, and some of them can cause significant losses in soybean yield and quality. Soybean mosaic virus (SMV) is the most economically damaging of these and is a long-standing problem in all soybean-growing areas in the world. It typically causes 8–35% yield losses (Hill and Whitham 2014), but losses of 50–100% have been reported during severe outbreaks (Song et al. 2016).

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SMV is a member of the genus *Potyvirus* in the family *Potyviridae* (Hajimorad et al. 2018; Gao et al. 2020). It is transmitted in the field by aphids in a nonpersistent manner and is also spread through infected seeds (Widyasari et al. 2020). Plants infected by SMV show leaf mosaic, mottling, wrinkling, stem necrosis and plant dwarfing, which greatly reduces yield. Different strains of SMV have been recognized based on the different phenotypic responses to the virus by a set of soybean cultivars. Based on the different identification systems, SMV isolates in the United States have been classified into seven strains (G1–G7) (Cho and Goodman 1979), while 22 strains (SC1–SC22) have been recognized in China (Li et al. 2010; Wang et al. 2013). Like other potyviruses, the SMV genome consists of a single-stranded RNA that is approximately 10 kb in length and has a single open reading frame (ORF) (Hajimorad et al. 2018). The polyprotein encoded is processed by self-proteolysis into a series of

multifunctional proteins known as P1, helper component proteinase (HC-Pro), P3, PIPO (a product of slippage in the P3 coding sequence), 6K1, cylindrical inclusion (CI) protein, 6K2, nuclear inclusion a (NIa-Pro) protein, nuclear inclusion b (NIb) protein and capsid protein (CP) (Urcuqui-Inchima et al. 2001; Gao et al. 2020; Widyasari et al. 2020).

The P1 protein is at the N-terminal of the viral polyprotein and has serine protease activity (Tena Fernandez et al. 2013) which enables it to cleave itself from the adjacent HC-Pro, a protein that is critical for inhibiting host RNA silencing defenses (Verchot et al. 1991; Adams et al. 2005). Earlier studies suggested that P1 enhanced the RNA silencing suppressor activity of HC-Pro. The potato virus Y (PVY) HC-Pro expressed from a P1-HC-Pro sequence increased the accumulation of a reporter gene, whereas protein expressed from an HC-Pro sequence alone did not (Anandalakshmi et al. 1998; Tena Fernandez et al. 2013). The P1 proteins of potyviruses are highly variable both in length and in amino acid sequence, especially at their N-termini (Valli et al. 2007; Shan et al. 2018). These highly disordered N-termini modulate potyviral replication and host defense responses by negatively regulating the self-cleavage activity of P1, which contributes to the long-term replication of virus (Pasin et al. 2014). Removal of the N-terminus of P1 enhances early amplification of virus, and induces salicylate-dependent defense responses (Pasin et al. 2014). Thus, although P1 is not directly involved in suppressing RNA silencing, it can modulate the function of HC-Pro by its self-cleavage activity, and therefore modulates the level of virus amplification and alleviates the host antiviral responses.

Pathogenesis-related (PR) proteins are components of the innate immunity system in plants induced by various viral infections and environmental stresses (Li et al. 2021), and are recognized as markers for systemic acquired resistance (SAR) (van Loon and van Strien 1999; Nürnberger and Brunner 2002). Salicylic acid (SA) plays an important role in SAR. Following pathogen infection, SA accumulation induces the expression of multiple PR protein genes by binding to the key regulator NPR1 (Non-expressor of pathogenesis-related gene 1) (Gaffney et al. 1993; van Loon et al. 2006). PR proteins have been classified into 17 families (PR-1 to PR-17) based on their sequence similarity and molecular biological properties (van Loon et al. 1994; Christensen et al. 2002) and some of these PR protein genes have been characterized in many plants (van Loon et al. 2006; Shin et al. 2014; Almeida-Silva and Venancio 2022). For example, overexpressing the *PR-1* gene of rice increases resistance to bacterial infection (Shin et al. 2014). Exogenous SA treatment activates the resistance responses associated with TMV-induced hypersensitivity in tobacco plants by inducing *PR-2* and *PR-3* transcription (Yalpani et al. 1991; Heitz et al. 1994). Thus, the accumulation of PR proteins is closely associated with the severity of symptoms and the host defense response to pathogen infection (Wang et al. 2020).

In a previous study of SMV isolates from different soybean-growing areas in China, we identified isolate SMV_JL_GZL (SMV^{GZL}) that causes leaf crinkling of soybean and clusters separately from other Chinese SMV isolates in phylogenetic analyses (Wei et al. 2021). We now report another SMV isolate, SMV_ZJ_NB (SMV^{NB}), which causes severer symptoms and accumulates virions in plants to a greater extent than SMV^{GZL}. The P1 protein sequences of these two isolates are divergent, especially at the N-terminus. Both $P1^{GZL}$ and $P1^{NB}$ localized in chloroplasts where $P1^{GZL}$-GFP gave a stronger GFP signal. In mechanical inoculation assays, SMV^{NB} and its $P1^{NB}$ protein induced weaker expression of salicylate-dependent pathogenesis-related genes, and caused more severe symptoms. Thus, our results show that the divergence in P1 sequence is associated with the severity of SMV symptoms.

Results

SMV^{NB} and SMV^{GZL} isolates caused different symptoms on soybean

SMV_ZJ_NB (SMV^{NB}), an isolate of SMV newly obtained from soybean in Ningbo, China, was compared with isolate SMV_JL_GZL (SMV^{GZL}, GenBank Access no. MW354946.1) that had been obtained from northeast China through high-throughput sequencing (HTS) in a previous study (Wei et al. 2021). Both isolates were inoculated to soybean cv. Dongnong 51 in a mechanical transmission assay. The plants inoculated with SMV^{GZL} developed leaf crinkling, while SMV^{NB} induced severe leaf curling and crinkling (Fig. 1a). Reverse transcription-quantitative PCR (RT-qPCR) (Fig. 1b) and western-blotting analyses (Fig. 1c) confirmed the presence of SMV RNA and coat protein (CP), respectively, with greater accumulation of both in SMV^{NB}-infected leaves than in those inoculated with SMV^{GZL}.

In studies of plants at different time points after inoculation, both SMV^{NB} and SMV^{GZL} induced mild leaf mosaic at 5 days post-inoculation (dpi) but subsequently showed differences in symptoms. The isolate SMV^{NB} caused leaf crinkling at 10 dpi, while SMV^{GZL} caused leaf crinkling till 15 dpi (Fig. 2a). Further results from western blotting assay confirmed that accumulation levels of SMV CP were greater in SMV^{NB}-infected leaves than in those inoculated with SMV^{GZL} (Fig. 2b).

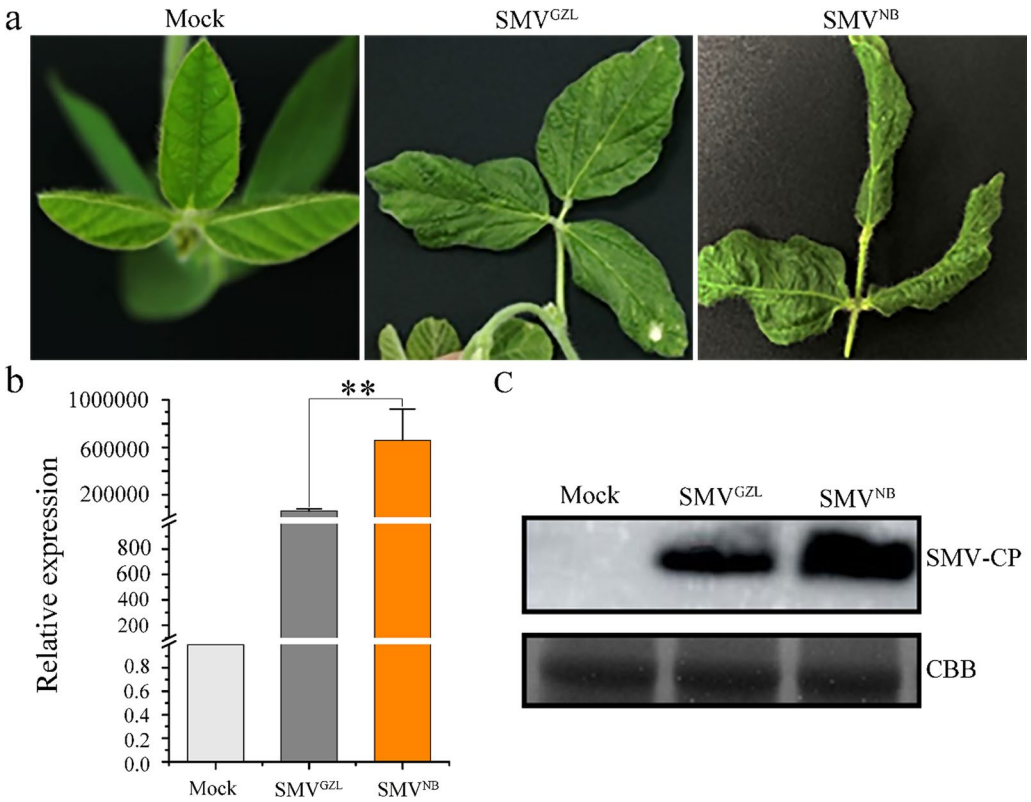

Fig. 1 Symptoms of SMV isolates SMV^{GZL} and SMV^{NB} infection on soybean. **a** Photographs of systemic (non-inoculated) leaves of Dongnong 51 plants at 30 dpi. **b** RNA levels of SMV^{NB} and SMV^{GZL} in infected plants determined by RT-qPCR. Data are shown as mean ± SD of three biological replicates. $^{**}P < 0.01$, Student's *t* tests. **c** Immunoblots showing levels of SMV coat protein (CP) in plants mock-inoculated or inoculated with SMV^{NB}/SMV^{GZL}. Coomassie brilliant blue (CBB) staining of the same extracts shows equal loading

Phylogenetic analysis of SMV^{NB} and SMV^{GZL} isolates

To explore the basis underlying different symptoms caused by these two SMV isolates, we first obtained the near-complete genome sequence of isolate SMV^{NB} via transcriptome sequencing (GenBank Access no. OK625818). The phylogenetic relationship between the two isolates was then examined by constructing a Maximum-Likelihood (ML) tree based on their genome sequences and those of other representative SMV genomes retrieved from GenBank (G1–G7, United States; SC3, SC7, SC15, SMV_AH_SZ, SMV_N1, SMV_N3, SMV_NE-N1 and SMV_4469-4, China). The 17 SMV isolates segregated into two major clades with clade I containing three subgroups (Fig. 3). Isolate SMV^{NB} clustered with the Chinese SC7 strain in clade Ib, while isolate SMV^{GZL} grouped with the SC3 strain within clade Ic. The isolates G1–G7 from USA clustered either in clade Ia or in clade II. This result suggested that the isolates SMV^{NB} and SMV^{GZL} are members of different SMV strains.

The P1 proteins of SMV^{NB} and SMV^{GZL} are very different

The ORF of isolate SMV^{NB} is 9606 nt in length (encoding 3203 amino acids), while that of isolate SMV^{GZL} is shorter (9204 nt; 3,068 aa). Most of the mature viral proteins encoded by these two isolates are similar to each other (95.4–100% nt identity in the coding regions), but the similarity was much lower in the P1 cistron (42.8% nt identity) (Fig. 4a). The P1 of SMV^{NB} isolate ($P1^{NB}$) has 444 amino acids, while that of SMV^{GZL} isolate ($P1^{GZL}$) has only 309 amino acids for the differences in the N-terminal region of the protein (Fig. 4b). The sequence difference between the two isolates is therefore mainly due to these differences in the N-terminus of the P1 protein.

To determine whether the variability of P1 protein is common in SMV strains, we performed a phylogenetic analysis based on the nucleotide sequence of the P1 protein using ML methods. It was revealed that the P1 proteins from different SMV strains were clustered in two different genotypes (Fig. 5). Type I was represented by the $P1^{NB}$, which encodes longer P1 proteins (1332 nt). While,

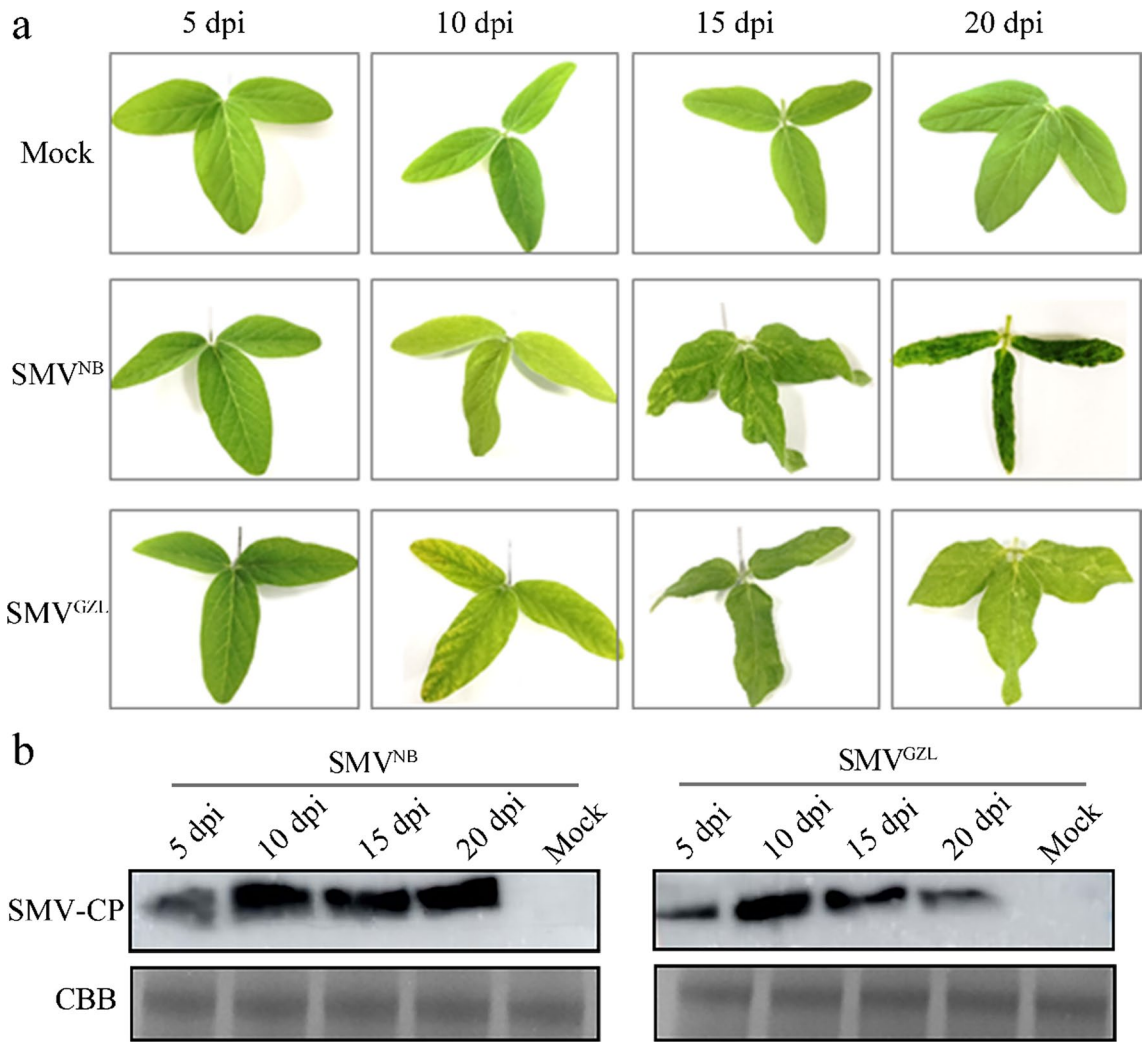

Fig. 2 Symptom development on soybean plants at different time points after inoculation with SMV^{GZL} or SMV^{NB}. **a** Symptoms on systemic (non-inoculated) leaves of Dongnong 51 plants at 5, 10, 15 and 20 dpi. **b** Immunoblots showing levels of SMV coat protein (CP) in plants mock-inoculated or inoculated with SMV^{NB}/SMV^{GZL} at different time points after inoculation. Coomassie brilliant blue (CBB) staining of the same extracts shows equal loading

the other genotype was similar to $P1^{GZL}$, which lacks residues at the N-terminus and encodes shorter P1 proteins (924–927 nt). These results suggested that P1 is a highly divergent protein with variable sequences in SMV, and $P1^{GZL}$ and $P1^{NB}$ belong to two distinct genotypes.

Subcellular localization of $P1^{GZL}$ and $P1^{NB}$ in *N. benthamiana*

To determine the subcellular localization of different P1 proteins, GFP fusions driven by the CaMV35S promoter were constructed and transiently expressed in *N. benthamiana* leaves. Free GFP, used as a control, was observed in the cytoplasm and nucleus (Fig. 6c), whereas the fusion proteins were observed in structures similar to chloroplasts but with a weaker GFP signal for $P1^{NB}$-GFP (Fig. 6a) than for $P1^{GZL}$-GFP (Fig. 6b). Further co-localization showed that the GFP signals of $P1^{GZL}$-GFP and $P1^{NB}$-GFP merged with the autofluorescence of chloroplasts, confirming that they were indeed localized in the chloroplast (Fig. 6).

Differential regulation of SA-dependent immune response by SMV $P1^{GZL}$ and $P1^{NB}$

SA plays an important role in plant immune responses against viruses including SMV (Zhao et al. 2018). The SA receptor NPR1 and the SAR marker gene PR1 can be induced by pathogen infection and have previously been used to evaluate the immune response of plants

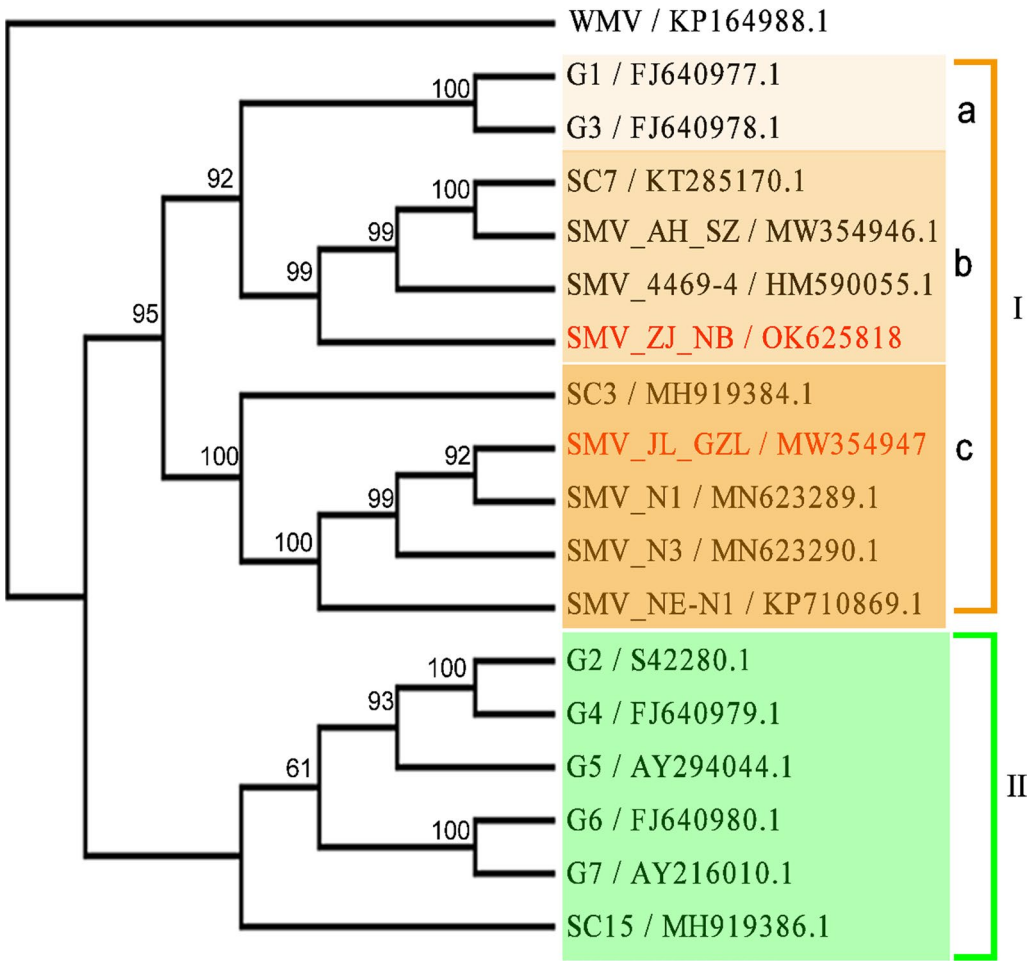

Fig. 3 Phylogenetic relationships among SMV isolates from different countries. The Maximum Likelihood phylogenic tree was constructed using MEGAX. The complete genome sequences of SMV isolates were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The colored bars (orange and green) indicate the different clades observed in the phylogenies. The letters a, b and c indicate different subgroups in Clade I

to viruses (van Loon and van Strien 1999; Nürnberger and Brunner 2002). We therefore compared the accumulation of *GmNPR1* and *GmNPR2* transcripts in soybean plants infected with SMV^{GZL} or SMV^{NB}. *GmNPR1* and *GmNPR2* accumulation was significantly higher in plants infected with SMV^{GZL} despite the fact that SMV CP level was lower than those in plants infected with SMV^{NB} (Fig. 7a–d). Quantification of hormone contents further revealed that the production of SA was higher in SMV^{GZL}-infected plants than that in SMV^{NB}-infected plants (Fig. 7e).

In light of the divergence between the SMV P1 proteins and also the fact that the N-terminal region of P1 of another potyvirus (plum pox virus, PPV) negatively regulates viral replication and alleviates the host antiviral responses (Pasin et al. 2014), we therefore tested whether the differences between $P1^{GZL}$ and $P1^{NB}$ are related to the expression of SA marker genes. When $P1^{GZL}$ and $P1^{NB}$ were transiently expressed in *N. benthamiana* leaves, SA marker genes *NbPR1, NbNPR1* and *NbSABP2* (Jiang et al. 2021) accumulated to a significantly greater extent in leaves expressing $P1^{GZL}$ than in those expressing $P1^{NB}$ (Fig. 7f and Additional file 1: Figure S1), and similar results were obtained in soybean inoculated with the respective viruses. $P1^{GZL}$ and $P1^{NB}$ were then transiently expressed in transgenic *N. benthamiana* NahG plants (Fig. 7f and Additional file 1: Figure S1), which have a defect in SA-dependent defense response signaling (Govrin and Levine 2002). The accumulation of SA marker genes was markedly reduced in NahG plants, compared to that in wild-type plants (Fig. 7f). Notably, the fold change for the differential expression of SA marker genes induced by $P1^{GZL}$ and $P1^{NB}$ was much weaker in NahG plants than that in wild-type plants (Fig. 7f), suggesting that SA-mediated

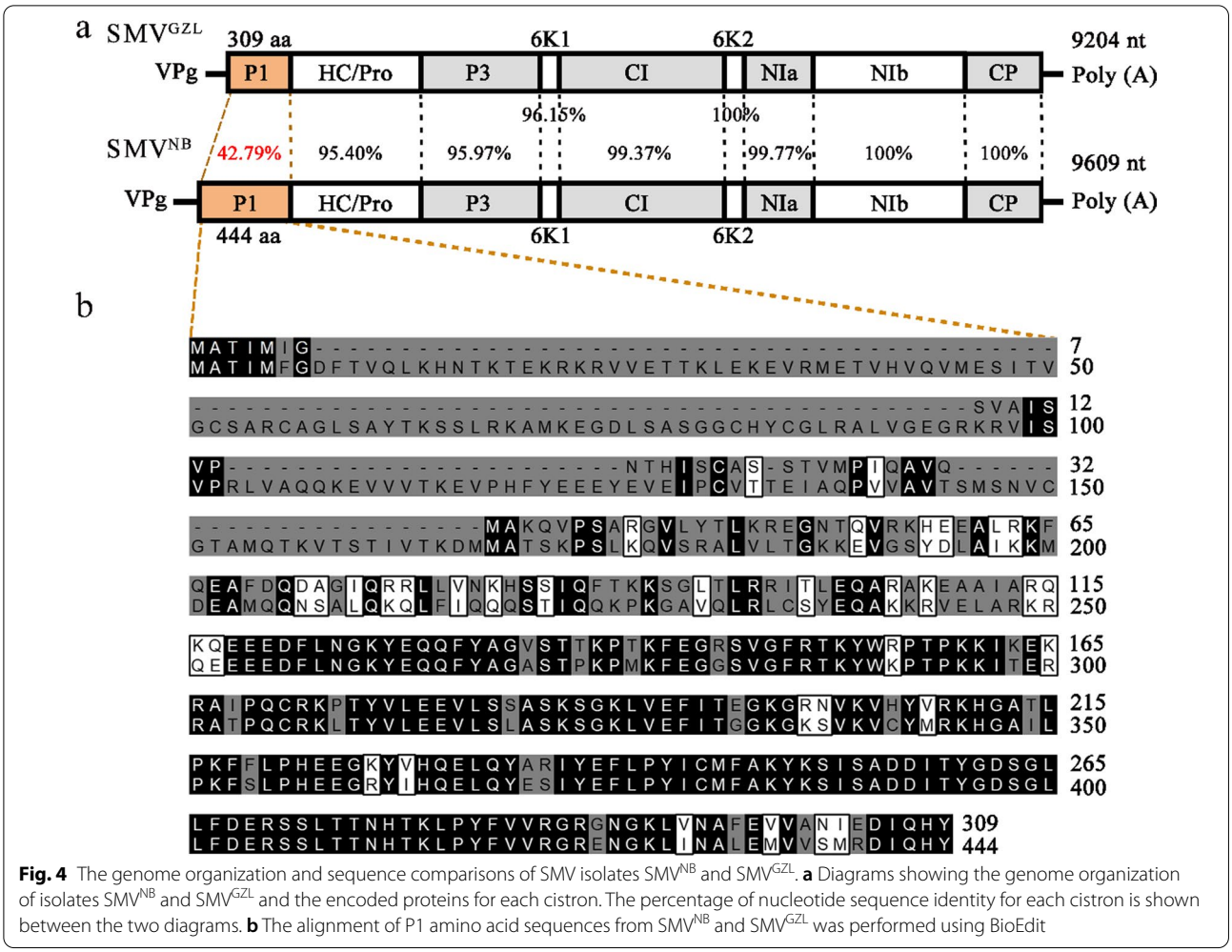

Fig. 4 The genome organization and sequence comparisons of SMV isolates SMV^{NB} and SMV^{GZL}. **a** Diagrams showing the genome organization of isolates SMV^{NB} and SMV^{GZL} and the encoded proteins for each cistron. The percentage of nucleotide sequence identity for each cistron is shown between the two diagrams. **b** The alignment of P1 amino acid sequences from SMV^{NB} and SMV^{GZL} was performed using BioEdit

antiviral pathways are involved in the SMV P1^{GZL} and P1^{NB} immune responses.

Discussion

Previous studies have suggested that several of the SMV-encoded proteins influence symptom development through interacting with the host plant. For example, SMV P1 plays a role in symptom development and host adaptation via its interaction with the Rieske Fe/S protein of the host (Shi et al. 2007), while CI acts as a virulence determinant and is also involved in inducing severe symptoms on soybean (Zhang et al. 2009). The two Chinese SMV isolates compared here were similar in most parts of their genomes (96.7% nt identity) but differed greatly in the size and sequence of their P1 proteins, prompting us to further investigate the role of SMV P1 in symptom development and host defense response.

P1 is the most divergent of the potyvirus proteins both in sequence and length (Adams et al. 2005), and it is thought that P1 diversification has contributed to host adaptation (Valli et al. 2007). Based on their functional diversity and host factor requirements, P1 proteins of members of the family *Potyviridae* have been categorized into two types, A and B (Rodamilans et al. 2013, 2021). Type A proteins are highly variable at their N terminus. They do not have RNA silencing suppression (RSS) activity, and require the assistance of a host factor for self-cleavage (e.g. PVY P1protein, TuMV P1 protein and CVYV P1a protein) (Rodamilans et al. 2013). Type B P1 proteins have RSS activity and contain a conserved zinc-finger motif at the N-terminal (e.g. WSMV P1protein and CVYV P1b protein) (Rodamilans et al. 2013, 2021). Although the proteins P1^{GZL} and P1^{NB} are divergent at their N-termini, nevertheless, they both cluster together with the P1 proteins of all the other members of the genus *Potyvirus* in clade type A (Additional file 1: Figure S2). The highly variable N-terminus of P1 may play an important role in host adaptation (Shi et al. 2007). Although many functions have been attributed to the P1, the location of the SMV P1 protein in plants is still

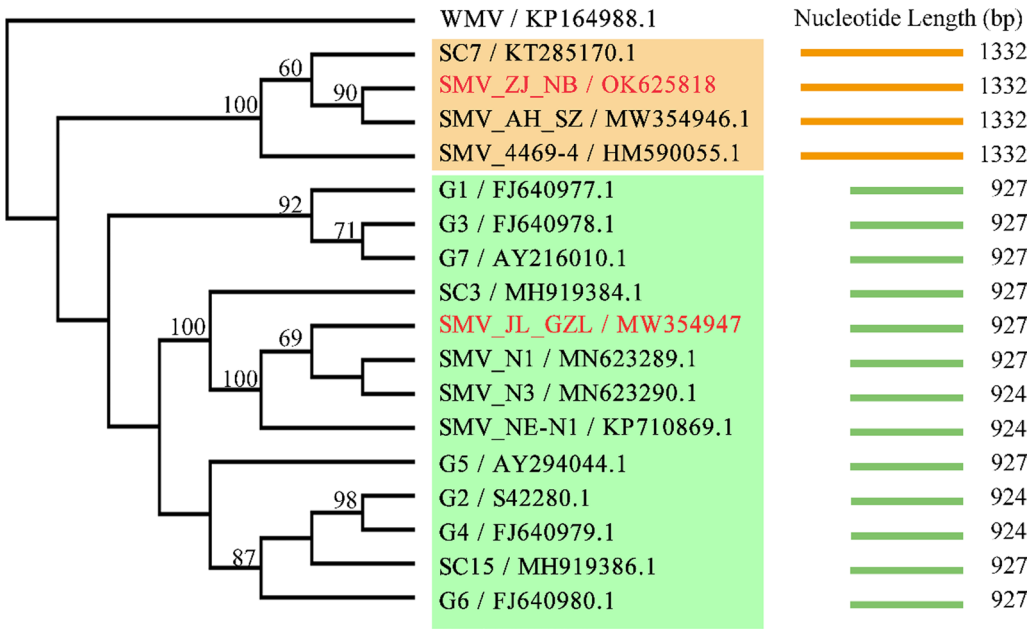

Fig. 5 Phylogenetic relationship among the P1 proteins of soybean mosaic virus (SMV) isolates from different countries. The phylogenic tree was constructed using MEGAX. The nucleotide sequence of P1 protein were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The colored bars (orange and green) indicate the different clades observed in the phylogenies. The sequence length of P1 protein is shown in the right panel

unclear. Our transient expression assay showed that both $P1^{GZL}$ and $P1^{NB}$ were localized to the chloroplasts of *N. benthamiana* (Fig. 6), and $P1^{GZL}$-GFP gave a stronger GFP signal (Fig. 6c). Therefore, it does not appear that the N-terminal of P1 has any significant effect on the localization of the protein but is more likely to affect protein stability or degradation.

The C-terminal region of P1 is relatively well conserved within and even between species and is essential for self-cleavage from the viral polyprotein (Verchot et al. 1991). Recent work has shown that the N-terminal region of PPV P1 protein acts as a negative regulator for its self-cleavage, modulating viral replication and host defense response during virus infection (Pasin et al. 2014; Shan et al. 2018). In PPV infection, the first 164N-terminal residues of P1 have an antagonistic effect on its self-processing, and thus alleviate the host defense responses and regulate the level of virus amplification, contributing to long-term infection with higher replicative capacity. In the later stage of PPV infection, removal of the P1 N-terminus induced the accumulation of PR proteins and reduced the viral loads (Pasin et al. 2014). PR proteins have been found to be inducible by infection with various types of pathogens in many plants, and are recognized as markers for SAR (van Loon and van Strien 1999). Most PRs and related proteins are induced by the signaling compounds SA, jasmonic acid, or ethylene, and further modulated by abscisic acid (van Loon et al. 2006). In our case, transcript accumulations of *GmNPR1* and *GmNPR2* were significantly higher in SMV^{GZL}-infected soybean plants than those in leaves infected by SMV^{NB}, and the levels of viral CP were lower in the later stages of infection. Similar patterns of PR accumulation and viral loads were induced by these P1 proteins in transient expression assays in *N. benthamiana*. These results may indicate that the P1 N-terminus modulates the viral amplification and host defense response.

Conclusions

In this work, we identified two SMV isolates, SMV^{GZL} and SMV^{NB}, which showed differences in symptoms and virus accumulation during their infection of host plants. These two isolates shared a very high similarity in genome sequence except for the P1 region (especially on the P1 N-terminus), and $P1^{GZL}$ and $P1^{NB}$ belong to two typical genotypes that are widely present in SMV isolates. In addition, our results suggested that these two typical P1 proteins influenced the pathogenesis of SMV isolates by differentially regulating SA signaling.

Methods

Mechanical transmission assay

Inoculation of soybean plants was performed as previously described (Wei et al. 2021). Sap from symptomatic plants was used to inoculate a susceptible soybean variety (Dongnong 51). Each inoculum was prepared from 1 g of

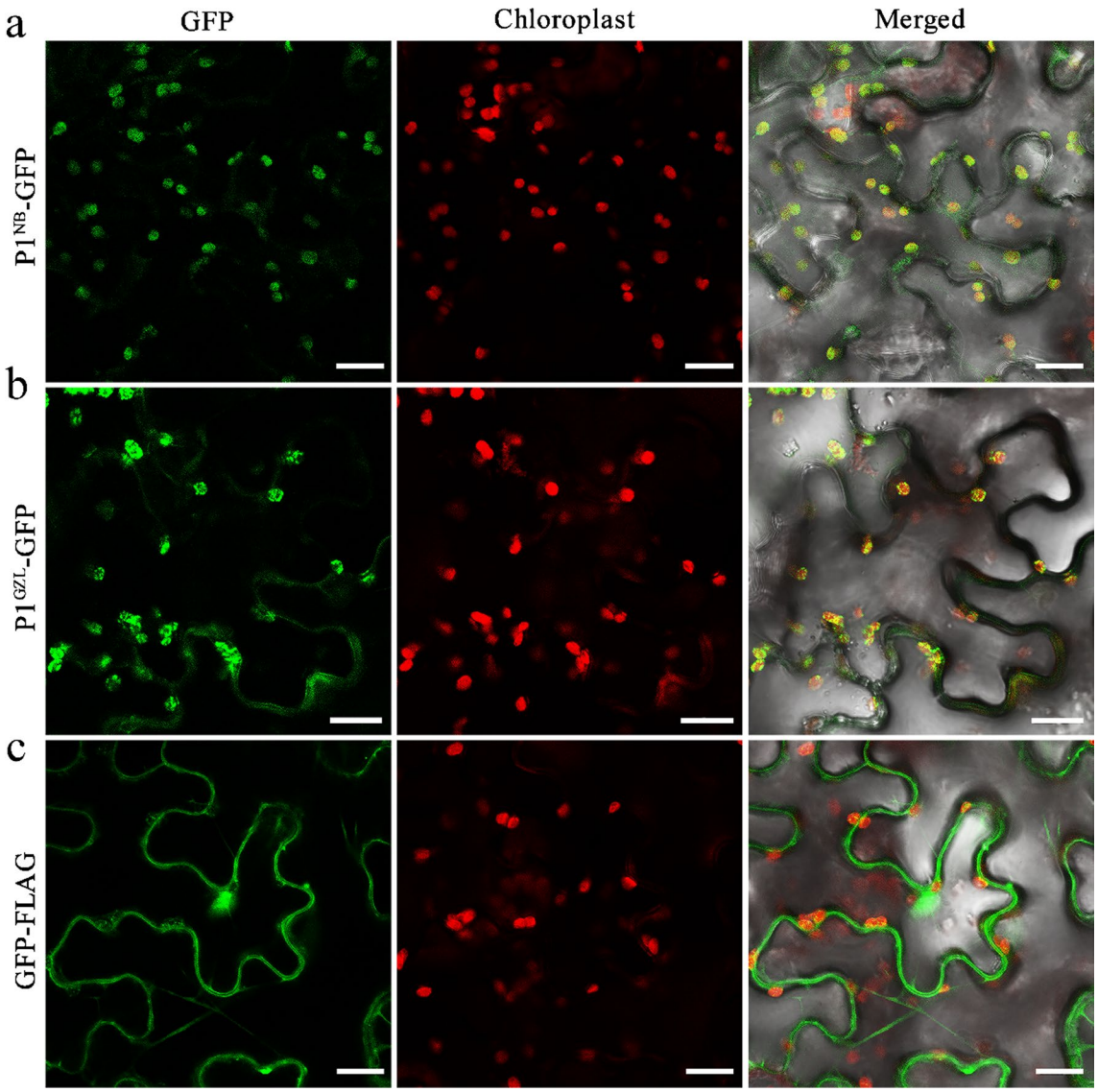

Fig. 6 Subcellular localization of P1^{NB}-GFP and P1^{GZL}-GFP in *N. benthamiana*. Confocal images of *N. benthamiana* cells showing the subcellular localization patterns of **a** P1^{NB}-GFP, **b** P1^{GZL}-GFP and **c** GFP-FLAG. Images were taken at 48 h post-infiltration. Scale bars: 20 μm

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Fig. 7 Viral accumulation and induction of immune responses by SMV^{NB} and SMV^{GZL} in soybean and *N. benthamiana*. **a** Symptoms on systemic (non-inoculated) leaves of Dongnong 51 plants at 15 dpi. **b** RNA levels of SMV^{NB} and SMV^{GZL} in infected plants as determined by RT-qPCR. Data are shown as mean ± SD of three biological replicates. $^{**}P < 0.01$, Student's *t* tests. **c** Immunoblots showing levels of SMV coat protein (CP) levels in plants mock-inoculated or inoculated with SMV^{NB}/SMV^{GZL}. Coomassie brilliant blue (CBB) staining of the same extracts shows equal loading. **d** Transcript levels of *GmNPR1* and *GmNPR2* in soybean plants infected with SMV^{NB} or SMV^{GZL} as determined by RT-qPCR. Average SMV^{GZL} value equal to 100. **e** Levels of endogenous salicylic acid (SA) in mock and SMV-infected soybean plants at 15 dpi. Data are shown as mean ± SD of three biological replicates. $^{**}P < 0.01$, Student's *t* tests. **f** Transcript levels of *NbPR1*, *NbNPR1* and *NbSABP2* in *N. benthamiana* plants transiently expressing P1^{NB} or P1^{GZL} as determined by RT-qPCR. Average P1^{GZL} value equal to 100. The numbers above the error bars indicate the fold change of gene expression compared with P1^{GZL} in *N. benthamiana* (WT) and transgenic *N. benthamiana* NahG plants. Statistically significant differences indicated by different lowercase letters are analyzed by Student's *t* tests ($P < 0.05$). Data are shown as mean ± SD of three biological replicates

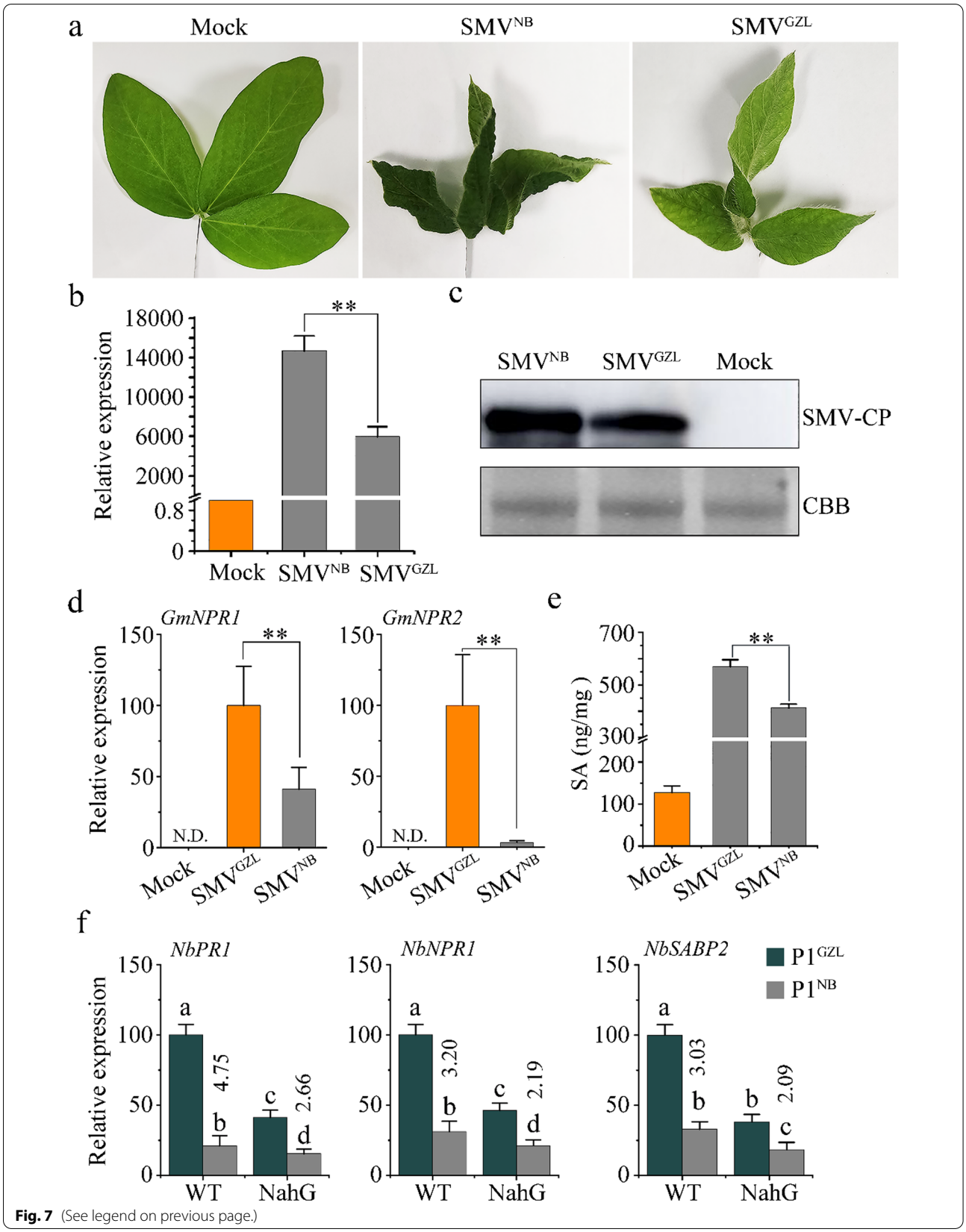

Fig. 7 (See legend on previous page.)

infected leaf tissue, which was homogenized in 10 mL of 0.1 M sodium phosphate buffer, pH 7.0, using a mortar and pestle. Inoculation was performed before the trifoliate leaves emerged. Unifoliate soybean leaves were dusted with carborundum before inoculation, then rubbed softly with a cotton puff to distribute the inoculum, and finally rinsed with tap water. Plants inoculated with phosphate-buffered saline were used as controls. Inoculated plants were grown at 25–28 °C (16-h light/8-h dark). Three independent experiments were conducted to provide data for statistical analysis, Values are means ± standard deviation (SD).

RNA isolation and quantitative real-time PCR (qPCR) assays

Total RNA was extracted from soybean leaves or *N. benthamiana* using TRIzol reagent (Invitrogen, United States) according to the manufacturer's instructions. About 1.5 μg of total RNA was reverse transcribed to cDNA using a reverse transcription kit (Tiangen Company, Beijing, China). qPCR was conducted using ChamQTM SYBR qPCR Master Mix (Low ROX Premixed) by an ABI7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA, United States). The qPCR conditions were as follows: 95 °C for 4 min; 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 15 s. The relative expression levels of genes were determined using the $2^{-\Delta\Delta C\ (t)}$ method. The *Actin11* (soybean) and *NbActin* (*N. benthamiana*) genes were used as internal controls. Three biological and two technical replicates were conducted to determine gene expression. The qPCR primer sequences used in this study are listed in Additional file 2: Table S1.

Western blot analysis

The SMV-infected soybean leaves were extracted by sodium dodecylsulfate (SDS) lysis buffer (100 mm Tris–HCl, pH 6.8, 10% SDS, and 2.0% β-mercaptoethanol). The crude extracts were centrifuged at 12,000×*g* for 10 min at room temperature, and the resulting supernatant (8 μL per sample plus 2 μL 5 × SDS loading buffer) was electrophoresed in 10–12% SDS-PAGE gels. Western blot analysis was done as previously reported (Zhang et al. 2019). Proteins were transferred to polyvinylidene difluoride (PVDF, Millipore, United States) membranes using the Trans-Blot Turbo transfer system (Bio-Rad, United States). Polyclonal antibody anti-SMV-CP (at 1:3000 dilution, synthesized by Huabio, China) was used to detect the SMV infection. Anti-Myc (at 1:5000 dilution, TransGen Biotech) was used to test the P1 protein.

Phylogenetic analysis

The nearly complete genome sequences or amino acid sequences of virus isolates were aligned using Clustal X. Phylogenetic trees were constructed using the Maximum-Likelihood (ML) method with the best-fitting model: GTR + G + I (General time reversible + Gama distributed with invariant sites). Numbers on the nodes indicate percentage of bootstrap support from 1000 replicates. For the construction of SMV phylogenetic tree, Watermelon mosaic virus (WMV; genus *Potyvirus*, family *Potyviridae*) was used as the outgroup.

Agrobacterium tumefaciens-mediated transient expression in *N. benthamiana*

The recombinant binary constructs of P1 were introduced into *A. tumefaciens* strain GV3101 by electroporation. Transformed bacteria were grown overnight at 28 °C in Luria–Bertani (LB) medium supplemented with the appropriate antibiotic mixture. The cultures were collected and resuspended using an agroinfiltration buffer (10 mM $MgCl_2$, 10 mM morpholineethanesulfonic acid, pH 5.6, and 150 μM acetosyringone). The suspensions were adjusted to an optical density of 0.5 at 600 nm (OD_{600}) before leaf infiltration. The cell suspensions were infiltrated into 4-to 6-week-old *N. benthamiana* leaves using a 2.5 mL sterile syringe. The plants were kept in a growth chamber for 36–44 h. Fluorescence signals for subcellular localization were observed using a confocal laser scanning microscope (Nikon Microsystems) (Sun et al. 2013).

SA measurement

Leaves of mock-treated and SMV-infected soybean plants were collected at 15 dpi, ground in liquid nitrogen and used for hormone extraction and analysis as previously described (He et al. 2017). Three biological replicates were conducted to provide data for statistical analysis.

Abbreviations

SMV: Soybean mosaic virus; SMV^{GZL}: SMV_JL_GZL; SMV^{NB}: SMV_ZJ_NB; $P1^{GZL}$: SMV_JL_GZL P1; $P1^{NB}$: SMV_ZJ_NB P1; ORF: Open reading frame; HC-Pro: Helper component proteinase; CI: Cylindrical inclusion; NIa-Pro: Nuclear inclusion a; NIb: Nuclear inclusion b; CP: Capsid protein; SA: Salicylic acid; SAR: Systemic acquired resistance; PR: Pathogenesis-related; NPR1: Non-expressor of pathogenesis-related gene 1; SABP2: Salicylic acid-binding protein 2; RSS: RNA silencing suppression; PPV: Plum pox virus; PVY: Potato potyvirus Y; TuMV: Turnip mosaic virus; CVYV: Cucumber vein yellowing virus; WSMV: Wheat streak mosaic virus.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-022-00115-3.

Additional file 1. **Figure S1**. Expression of P1 proteins in *N. benthamiana* by Western blot. **Figure S2**. Phylogenetic analysis of amino acid sequences of the conserved serine protease domain of P1 proteins of 36 representative viruses in the family *Potyviridae*.

Additional file 2. **Table S1**. List of primer sequences used in this study.

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Authors' contributions
CM, ZW and ZS designed the research; CM, SS, YH, HZ, CJ and YL performed the experiments; CM drafted the manuscript. ZW and ZS revised the manuscript; ZW, ZS and JC supervised the project. All authors read and approved the final manuscript.

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Availability of data and materials
The sequences of SMV^{GZL} and SMV^{NB} isolates were deposited in the GenBank database, with accession numbers MW354946.1 and OK625818.

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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References

Adams MJ, Antoniw JF, Beaudoin F. Overview and analysis of the polyprotein cleavage sites in the family *Potyviridae*. Mol Plant Pathol. 2005;6(4):471–87. https://doi.org/10.1111/j.1364-3703.2005.00296.x.

Almeida-Silva F, Venancio TM. Pathogenesis-related protein 1 (PR-1) genes in soybean: genome-wide identification, structural analysis and expression profiling under multiple biotic and abiotic stresses. Gene. 2022;809:146013. https://doi.org/10.1016/j.gene.2021.146013.

Anandalakshmi R, Pruss GJ, Ge X, Marathe R, Mallory AC, Smith TH, et al. A viral suppressor of gene silencing in plants. Proc Natl Acad Sci U S A. 1998;95(22):13079–84. https://doi.org/10.1073/pnas.95.22.13079.

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

Christensen AB, Cho BH, Naesby M, Gregersen PL, Brandt J, Madriz-Ordenana K, et al. The molecular characterization of two barley proteins establishes the novel PR-17 family of pathogenesis-related proteins. Mol Plant Pathol. 2002;3(3):135–44. https://doi.org/10.1046/j.1364-3703.2002.00105.x.

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, et al. Requirement of salicylic acid for the induction of systemic acquired resistance. Science. 1993;261(5122):754–6. https://doi.org/10.1126/science.261.5122.754.

Gao L, Zhai R, Zhong Y, Karthikeyan A, Ren R, Zhang K, et al. Screening isolates of soybean mosaic virus for infectivity in a model plant, *Nicotiana Benthamiana*. Plant Dis. 2015;99(4):442–6. https://doi.org/10.1094/PDIS-04-14-0405-RE.

Gao L, Luo J, Ding X, Wang T, Hu T, Song P, et al. Soybean RNA interference lines silenced for eIF4E show broad potyvirus resistance. Mol Plant Pathol. 2020;21(3):303–17. https://doi.org/10.1111/mpp.12897.

Govrin EM, Levine A. Infection of Arabidopsis with a necrotrophic pathogen, *Botrytis cinerea*, elicits various defense responses but does not induce systemic acquired resistance (SAR). Plant Mol Biol. 2002;48(3):267–76. https://doi.org/10.1023/a:1013323222095.

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(7):1563–79. https://doi.org/10.1111/mpp.12644.

He Y, Zhang H, Sun Z, Li J, Hong G, Zhu Q, et al. Jasmonic acid-mediated defense suppresses brassinosteroid-mediated susceptibility to rice black streaked dwarf virus infection in rice. New Phytol. 2017;214(1):388. https://doi.org/10.1111/nph.14376.

Heitz T, Fritig B, Legrand M. Local and systemic accumulation of pathogenesis-related proteins in tobacco plants infected with tobacco mosaic virus. Mol Plant Microbe Interact. 1994;7(6):776–9. https://doi.org/10.1094/MPMI-7-0776.

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.

Jiang Y, Zheng W, Li J, Liu P, Zhong K, Jin P, et al. NbWRKY40 positively regulates the response of *Nicotiana benthamiana* to tomato mosaic virus via salicylic acid signaling. Front Plant Sci. 2021;11: 603518. https://doi.org/10.3389/fpls.2020.603518.

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

Li J, Kolbasov VG, Pang Z, Duan S, Lee D, Huang Y, et al. Evaluation of the control effect of SAR inducers against citrus Huanglongbing applied by foliar spray, soil drench or trunk injection. Phytopathol Res. 2021;3:2. https://doi.org/10.1186/s42483-020-00079-2.

Nürnberger T, Brunner F. Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecular patterns. Curr Opin Plant Biol. 2002;5(4):318–24. https://doi.org/10.1016/s1369-5266(02)00265-0.

Pasin F, Simon-Mateo C, Garcia JA. The hypervariable amino-terminus of P1 protease modulates potyviral replication and host defense responses. PLoS Pathog. 2014;10(3): e1003985. https://doi.org/10.1371/journal.ppat.1003985.

Rodamilans B, Valli A, Garcia JA. Mechanistic divergence between P1 proteases of the family Potyviridae. J Gen Virol. 2013;94(Pt_6):1407–14. https://doi.org/10.1099/vir.0.050781-0.

Rodamilans B, Casillas A, Garcia JA. P1 of sweet potato feathery mottle virus shows strong adaptation capacity, replacing P1-HCPro in a chimeric plum pox virus. J Virol. 2021;95(14):e00150-e221. https://doi.org/10.1128/JVI.00150-21.

Shan H, Pasin F, Tzanetakis IE, Simon-Mateo C, Garcia JA, Rodamilans B. Truncation of a P1 leader proteinase facilitates potyvirus replication in a non-permissive host. Mol Plant Pathol. 2018;19(6):1504–10. https://doi.org/10.1111/mpp.12640.

Shi Y, Chen J, Hong X, Chen J, Adams MJ. A potyvirus P1 protein interacts with the Rieske Fe/S protein of its host. Mol Plant Pathol. 2007;8(6):785–90. https://doi.org/10.1111/j.1364-3703.2007.00426.x.

Shin SH, Pak JH, Kim MJ, Kim HJ, Oh JS, Choi HK, et al. An acidic *pathogenesis-related1* gene of *Oryza grandiglumis* is involved in disease resistance response against bacterial infection. Plant Pathol J. 2014;30(2):208–14. https://doi.org/10.5423/PPJ.NT.11.2013.0112.

Song Y, Li C, Zhao L, Karthikeyan A, Li N, Li K, et al. Disease spread of a popular soybean mosaic virus strain (SC7) in southern China and effects on two susceptible soybean cultivars. Philipp Agric Sci. 2016;99(4):355–64.

Sun Z, Yang D, Xie L, Sun L, Zhang S, Zhu Q, et al. Rice black-streaked dwarf virus P10 induces membranous structures at the ER and elicits the unfolded protein response in *Nicotiana benthamiana*. Virology. 2013;447(1–2):131–9. https://doi.org/10.1016/j.virol.2013.09.001.

Tena Fernandez F, Gonzalez I, Doblas P, Rodriguez C, Sahana N, Kaur H, et al. The influence of cis-acting P1 protein and translational elements on the expression of potato virus Y helper-component proteinase (HCPro) in heterologous systems and its suppression of silencing activity. Mol Plant Pathol. 2013;14(5):530–41. https://doi.org/10.1111/mpp.12025.

Urcuqui-Inchima S, Haenni AL, Bernardi F. Potyvirus proteins: a wealth of functions. Virus Res. 2001;74(1–2):157–75. https://doi.org/10.1016/s0168-1702(01)00220-9.

Valli A, Lopez-Moya JJ, Garcia JA. Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. J Gen Virol. 2007;88(Pt 3):1016–28. https://doi.org/10.1099/vir.0.82402-0.

van Loon LC, Van Strien EA. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol. 1999;55(2):85–97. https://doi.org/10.1006/pmpp.1999.0213.
van Loon LCPW, Boller T, Conejero V. Recommendations for naming plant pathogenesis-related proteins. Plant Mol Biol. 1994;12(3):245–64. https://doi.org/10.1007/BF02668748.
van Loon LC, Rep M, Pieterse CM. Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol. 2006;44(1):135–62. https://doi.org/10.1146/annurev.phyto.44.070505.143425.
Verchot J, Koonin EV, Carrington JC. The 35-kDa protein from the N-terminus of the potyviral polyprotein functions as a third virus-encoded proteinase. Virology. 1991;185(2):527–35. https://doi.org/10.1016/0042-6822(91)90522-d.
Wang D, Tian Z, Li K, Li H, Huang Z, Hu G. Identification and variation analysis of soybean mosaic virus strains in Shandong, Henan and Anhui provinces of China. Soybean Sci. 2013;32:806–9 (**(in Chinese)**).
Wang L, Dong M, Zhang Q, Wu Y, Hu L, Parson JF, et al. Silicon modulates multi-layered defense against powdery mildew in Arabidopsis. Phytopathol Res. 2020;7:2. https://doi.org/10.1186/s42483-020-00048-9.
Wei Z, Mao C, Jiang C, Zhang H, Chen J, Sun Z. Identification of a new genetic clade of cowpea mild mottle virus and characterization of its interaction with soybean mosaic virus in co-infected soybean. Front Microbiol. 2021;12: 650773. https://doi.org/10.3389/fmicb.2021.650773.
Widyasari K, Alazem M, Kim KH. Soybean resistance to soybean mosaic virus. Plants (basel). 2020;9(2):219. https://doi.org/10.3390/plants9020219.
Wilson RF. Soybean: market driven research needs. New York: Springer; 2008. https://doi.org/10.1007/978-0-387-72299-3_1.
Yalpani N, Silverman P, Wilson T, Kleier DA, Raskin I. Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. Plant Cell. 1991;3(8):809–18. https://doi.org/10.1105/tpc.3.8.809.
Zhang C, Hajimorad MR, Eggenberger AL, Tsang S, Whitham SA, Hill JH. Cytoplasmic inclusion cistron of soybean mosaic virus serves as a virulence determinant on Rsv3-genotype soybean and a symptom determinant. Virology. 2009;391(2):240–8. https://doi.org/10.1016/j.virol.2009.06.020.
Zhang H, Tan X, He Y, Xie K, Li L, Wang R, et al. Rice black-streaked dwarf virus P10 acts as either a synergistic or antagonistic determinant during superinfection with related or unrelated virus. Mol Plant Pathol. 2019;20(5):641–55. https://doi.org/10.1111/mpp.12782.
Zhao Q, Li H, Sun H, Li A, Liu S, Yu R, et al. Salicylic acid and broad spectrum of NBS-LRR family genes are involved in SMV-soybean interactions. Plant Physiol Biochem. 2018;123:132–40. https://doi.org/10.1016/j.plaphy.2017.12.011.

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