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Insights into geminiviral pathogenesis: interaction between β C1 protein and *GLABROUS1* enhancer binding protein (GeBP) in Solanaceae

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Abstract

Transcription factors (TFs) play crucial roles in plant development and pathogen defense. However, plant viruses can exploit TFs to facilitate their infection or transmission. In this study, we confirmed the β C1 proteins, encoded by tobacco curly shoot virus (TbCSV)- and tomato yellow leaf curl China virus (TYLCCNV)-associated betasatellites, interacted with *GLABROUS1* enhancer binding protein (GeBP) TFs from solanaceous plants including *Nicotiana benthamiana*, *Solanum lycopersicum*, *S. tuberosum*, and *Capsicum annuum*. Further analysis verified the nuclear localization, homodimerization, and DNA-binding ability of the GeBP TFs, along with its interaction with β C1 in the nucleus. PVX-mediated overexpression of *NbGeBP* showed no effect on the accumulation of viral and betasatellite DNAs in *N. benthamiana* plants after infection with TbCSV and its heterologous betasatellite, malvastrum yellow vein virus associated betasatellite (MaYVB), or its homologous betasatellite, TbCSB. However, both TbCSV and MaYVB caused a decrease in *NbGeBP* expression during the early stages of infection, regardless of the presence of homologous or heterologous betasatellites, implying that *NbGeBP* might play a role in virus infection. TbCSV/TbCSB and TYLCCNV/TYLCCNB infect many solanaceous plants, and solanaceous GeBP proteins interact with β C1 proteins from TbCSB and TYLCCNB. The yeast two-hybrid and bimolecular fluorescence complementation assays showed that AtGeBP from *Arabidopsis thaliana* could not interact with TbCSB β C1, revealing that the GeBP- β C1 interactions might only exist in GeBP proteins from solanaceous plants. Importantly, the β C1 protein from MaYVB, which was almost not reported on natural infection in solanaceous plants, could not interact with GeBP, suggesting the potential roles of GeBP in monopartite begomovirus infection of solanaceous plants.

Keywords Geminivirus, β C1 protein, Transcription factor, *GLABROUS1* enhancer binding protein (GeBP), Nuclear localization, Solanaceous plants, Pathogen-host interaction

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Background

Plant transcription factors (TFs) play essential roles in regulating gene expression during developmental processes and stress responses by binding to the *cis*-regulatory elements of genes. TFs can regulate defense-related gene expression to defend against virus infection in plants. For example, the expression of *NbWRKY1* TF gene is induced by tomato yellow leaf curl China virus/tomato yellow leaf curl China betasatellite (TYLCCNV/TYLCCNB) infection, and *NbWRKY1* TF binds to the promoter of the *NbWHIRLY1* TF gene to repress the expression of *NbWHIRLY1*, a negative regulator of plant antiviral RNAi defense (Sun et al. 2023). Conversely, some plant viruses can either manipulate the gene expression of TFs or directly hijack TF proteins to facilitate virus infection or transmission. Rice stripe virus and Southern rice black-streaked dwarf virus (SRBSDV) upregulate the expression of the *OsNF-YA* TF family genes, and *OsNF-YAs* interact with JA signaling TFs *OsMYC2/3* and inhibit plant antiviral JA signaling defense (Tan et al. 2022). Additionally, proteins encoded by SRBSDV interact with *OsMYC3* and *OseIL2* TFs to interfere with plant hormone signaling defense responses, promoting viral infection and transmission (Li et al. 2021; Zhao et al. 2022). The *GLABROUS1* enhancer-binding protein (GeBP), a plant-specific DNA-binding protein first identified in *Arabidopsis thaliana*, and its homologs contain two conserved domains: a central DNA-binding domain and a C-terminal putative leucine zipper domain, which are crucial for activating downstream gene expression (Curaba et al. 2003; Ma et al. 2021). Previous research has underscored the function of the *GeBP* gene family in the growth and developmental aspects of plants. *GeBP* modulates trichome development by regulating the expression of *GLABROUS1*, a key gene involved in trichome initiation (Curaba et al. 2003). It also affects trichome elongation by influencing gibberellins and cytokinins (Yanai et al. 2005). Furthermore, *GeBPs* act in modulating the cytokinin hormone pathway (Chevalier et al. 2008). The *Arabidopsis Constitutive Expressor of Pathogenesis-Related Gene-5* (*CPR5*) exhibits diverse functions such as cell expansion, and *GeBPs* can regulate cell expansion in a *CPR5*-dependent manner by manipulating a group of genes within the *CPR5* pathway (Perazza et al. 2011). In addition to their roles in plant growth and development, *GeBPs* have been observed to respond to abiotic stresses. For instance, *GeBP* is upregulated in rice panicle tissue under water-deficit stress (Ray et al. 2011), and the ectopic expression of *MdGeBP3* decreases resistance against drought in *Arabidopsis* (Liu et al. 2023). Besides, *GeBP-LIKE4* (*GPL4*) has been identified to inhibit root growth under cadmium stress by regulating the levels of reactive oxygen species, and it responds to

excess copper and zinc in the same manner (Khare et al. 2017). *GeBPs* also have been found to respond to biotic stresses; loss-of-function mutations in *GeBP* cause differential expression of numerous biotic stress-response genes in *Arabidopsis* (Garcia-Cano et al. 2018), and transgenic *Arabidopsis* plants overexpressing *GPL2* exhibit enhanced resistance against the *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (Perazza et al. 2011). Collectively, *GeBP* family genes manipulate the developmental process and mediate plant responses to abiotic and biotic stresses, while the specific roles of *GeBP* genes in virus infection are still largely unknown.

Geminiviruses cause devastating diseases in many crops worldwide (Boulton 2003; Rojas et al. 2005; Mansoor et al. 2006; Sattar et al. 2013; Yang et al. 2019). The family *Geminiviridae* contains 14 genera, classified based on genome structure, host range, and insect vector (Roumagnac et al. 2022). *Begomovirus* is the largest genus, with 445 species, and have either one (monopartite) or two (bipartite) genomic components (Harrison and Robinson 1999; Hanley-Bowdoin et al. 2013). Monopartite begomoviruses consist of a DNA component which encodes at least six proteins (C1, C2, C3, C4, V1, and V2). Some monopartite begomoviruses are associated with betasatellites and the complex causes more severe diseases (Zhou et al. 2003; Cui et al. 2004; Saeed et al. 2005; Zhou 2013). Tobacco curly shoot virus (TbCSV), a member of monopartite begomoviruses first reported in China (Xie et al. 2002), can cause symptoms such as leaf curling, shoot bending, stunting, and vein darkening in various plants, including oriental tobacco and tomato (Li et al. 2004, 2005; Wu and Zhou 2005). Some isolates of TbCSV (such as the isolate Y35) are associated with a betasatellites (TbCSB) that can exacerbate symptoms in certain hosts (Li et al. 2005). The β C1 protein encoded by betasatellites is a pathogenicity determinant, suppressor of transcriptional gene silencing and post-transcriptional gene silencing, and interferes directly with the host plastid homeostasis, as well as activates the host unfolded protein response to subvert host defense (Gopal et al. 2007; Li et al. 2014a, 2018; Nair et al. 2023; Zhang et al. 2023). Previous studies have found that several plant TFs (such as AS1, MYC2, WRKY20, PIFs, and bZIP60) interact with the TYLCCNB β C1 protein, facilitating virus transmission and symptom development (Yang et al. 2008; Li et al. 2014b; Zhao et al. 2019; Zhao et al. 2021; Zhang et al. 2023), while the interaction between host TF and TbCSB β C1 protein remains unclear.

In this study, we observed that *GeBP* proteins of *Nicotiana benthamiana* and *Solanum lycopersicum* were localized in the nucleus, where they interacted with TbCSB and TYLCCNB β C1 proteins. Moreover, we noted the downregulation of *NbGeBP* expression during the early

stages of TbCSV and MaYVV infection, regardless of the presence of homologous or heterologous betasatellites. Notably, similar GeBP proteins from solanaceous plants, such as *Solanum tuberosum* and *Capsicum annuum*, also showed interactions with TbCSB β C1 protein, whereas equivalent GeBP proteins from non-solanaceous plants did not exhibit this interaction. Intriguingly, the β C1 protein of betasatellite associated with malvastrum yellow vein virus failed to interact with the GeBP proteins of *N. benthamiana* and *S. lycopersicum*. Our findings highlighted the potential function of GeBP transcription factors in virus infection.

Results

SlGeBP interacts with TbCSB β C1

To investigate the mechanism of β C1 protein in the infection process mediated by tobacco curly shoot virus (TbCSV) and its associated betasatellite (TbCSB), we constructed the BK- β C1 recombinant plasmid for screening the tomato nuclear yeast library. This screening identified four potential interaction partners of β C1 in tomato, namely, SlSTPK, SlHSRP, SlA7A, and SlGeBP (Additional file 1: Table S1). Given the transcription factor nature of SlGeBP (GenBank accession no. XM_004240210), we postulated its potential transcriptional activation activity. To avoid potential transcriptional activation domain of SlGeBP from binding the DNA-binding domain of yeast two-hybrid (Y2H) plasmid BK to appear false-positive results, we constructed the recombinant plasmid AD-SlGeBP. Subsequently, we conducted a Y2H assay by co-transforming AD-SlGeBP and BK- β C1 into yeast cells. As confirming that SlGeBP exhibited sequence non-specific DNA binding activity (Additional file 2: Figure S1), we were unable to definitively determine the interaction between SlGeBP and β C1. Consequently, we constructed recombinant plasmids BK-SlSTPK, BK-SlHSRP, BK-SlA7A, BK-SlGeBP, and AD- β C1, and co-transformed into yeast cells, respectively, to perform a reversed Y2H assay. These results indicate that SlGeBP lacks transcriptional activation activity and indeed interacts with β C1 in yeast (Fig. 1a).

To elucidate the interaction between β C1 and SlGeBP in vivo, we conducted a luciferase complementation imaging (LCI) assay. β C1 and SlGeBP were fused to the N-terminus and C-terminus of the luciferase protein (Luc), respectively, and transformed into *Agrobacterium tumefaciens*. Upon co-expression of β C1-NLuc and CLuc-SlGeBP in *Nicotiana benthamiana* leaf cells, positive interaction was observed in the area of co-expression (Fig. 1b). Conversely, no positive reaction was detected in leaf areas co-expressing β C1-NLuc and CLuc, NLuc and CLuc-SlGeBP, or NLuc and CLuc (Fig. 1b). The

LCI assays demonstrate that β C1 interacts with SlGeBP in vivo.

To further investigate the interaction between β C1 and SlGeBP in vivo, we conducted a bimolecular fluorescence complementation (BiFC) assay. SlGeBP and β C1 were connected to the N-terminus and C-terminus of the yellow fluorescent protein (YFP), respectively, and transformed into *A. tumefaciens*. After co-expression of Y^N-SlGeBP and Y^C- β C1, Y^N-SlGeBP and Y^C, or Y^N and Y^C- β C1 in *N. benthamiana* leaf cells, positive interaction between Y^N-SlGeBP and Y^C- β C1 was observed within the nucleus (Fig. 1c). Conversely, no positive interaction was observed in the leaf cells co-expressing Y^N-SlGeBP and Y^C, or Y^N and Y^C- β C1 (Fig. 1c). These findings corroborate the interaction between β C1 and SlGeBP in vivo.

To explore potential interactions between SlGeBP and other proteins encoded by TbCSV, namely V1, V2, C1, C2, C3, and C4, we conducted a Y2H assay. The results indicate that SlGeBP can not interact with any of the TbCSV-encoded proteins (Additional file 2: Figure S2).

TbCSB β C1 interacts with solanaceous GeBP in vitro and in vivo

To elucidate the evolutionary relationships of SlGeBP in plants, we aligned the amino acid sequences of GeBP family proteins from plants of the families of Solanaceae (*Solanum lycopersicum*, *Solanum tuberosum*, *N. benthamiana*, and *Capsicum annuum*), Brassicaceae (*Arabidopsis thaliana*), and Poaceae (*Triticum aestivum*), and constructed a phylogenetic tree. Alignment of multiple amino acid sequences reveal that SlGeBP (Solyc05g051330.1.1) shares conserved DUF573 domain with StGeBP (PGSC0003DMP400068550), NbGeBP (Niben101Scf10342g01018.1), CaGeBP (CA05g13060), AtGeBP (AT5G28040.1), and TaGeBP (Traes_4AS_928691B39.1) (Fig. 2a). SlGeBP shares 91.3%, 55.8%, and 41.6% amino acid sequence identity (ASI) to StGeBP, NbGeBP, and CaGeBP, respectively, and they phylogenetically cluster together (Fig. 2b, Additional file 2: Figure S3). To investigate the interaction of proteins with high ASI to SlGeBP with β C1, we selected StGeBP, NbGeBP, CaGeBP, and AtGeBP for further test. Y2H assays confirmed the interaction between β C1 and NbGeBP, StGeBP, and CaGeBP (Fig. 2c, d), while AtGeBP showed no interaction with β C1 (Fig. 2e).

To validate the interaction between β C1 and NbGeBP in vivo, we conducted an LCI assay. NbGeBP were connected to the C-terminus of Luc and transformed into *A. tumefaciens*. After co-expression of β C1-NLuc and CLuc-NbGeBP, β C1-NLuc and CLuc, NLuc and CLuc-NbGeBP, or NLuc and CLuc in *N. benthamiana* leaf cells, positive interaction was observed in the leaf area co-expressing β C1-NLuc and CLuc-NbGeBP (Fig. 2f). Conversely,

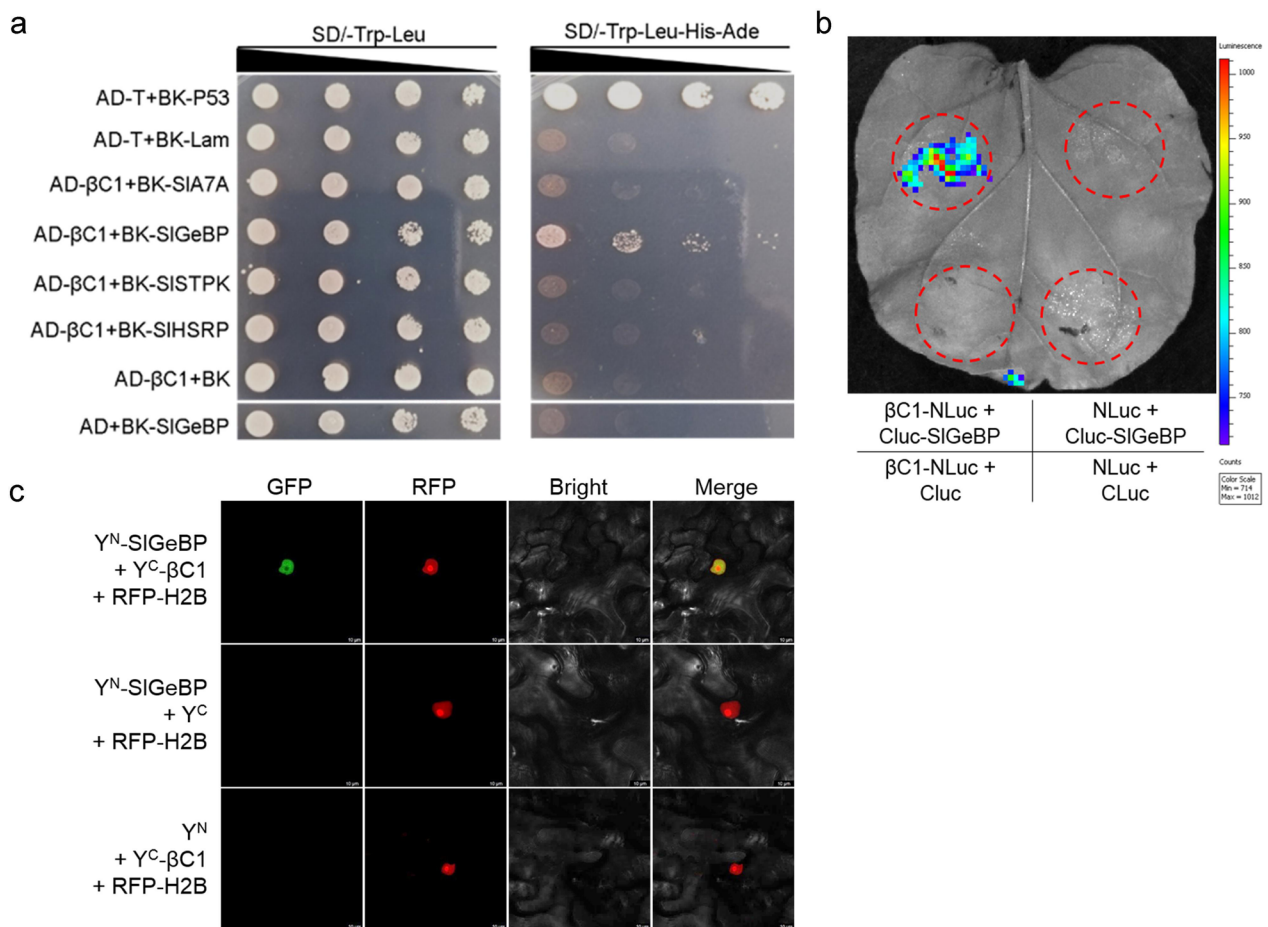


Fig. 1 SIgeBP interacts with TbCSB β C1 in vitro and in vivo. **a** Y2H analysis of the interaction between β C1 and candidate interacting proteins in vivo. The transformed yeast cells were subjected to tenfold serial dilutions and plated on SD/-Trp-Leu-His-Ade medium for 3 days. Yeast cells co-transformed with AD-T and BK-P53 were served as positive controls; yeast cells co-transformed with AD-T and BK-Lam, AD- β C1 and BK, or AD and BK-SIgeBP were used as negative controls. **b** LCI analysis of the interaction between β C1 and SIgeBP in *N. benthamiana* leaves. β C1-NLuc co-expressed with CLuc or NLuc co-expressed with CLuc-SIgeBP, and NLuc co-expressed with CLuc, were used as negative controls. **c** BiFC analysis of the interaction between SIgeBP and β C1 in *N. benthamiana* leaves. The RFP-H2B is nuclear localization marker. Co-expression of Y^N-SIgeBP and Y^C or Y^N and Y^C- β C1 were used as negative controls. Confocal imaging was performed at 48 hpi. Scale bars: 10 μ m

(See figure on next page.)

Fig. 2 TbCSB β C1 interacts with NbGeBP in vitro and in vivo. **a** Alignment of GeBP protein sequences from *S. lycopersicum*, *S. tuberosum*, *N. benthamiana*, *C. annuum*, *A. thaliana*, and *T. aestivum*. **b** Phylogenetic tree of GeBP was constructed by the Maximum-Likelihood method using the protein sequences from *S. lycopersicum*, *S. tuberosum*, *N. benthamiana*, *C. annuum*, *A. thaliana*, and *T. aestivum*. **c** Y2H assay of the interaction between the β C1 and NbGeBP in yeast cells. The transformed yeast cells were subjected to tenfold serial dilutions and plated on SD/-Trp-Leu-His-Ade medium for 3 d. Yeast cells co-transformed with AD-T and BK-P53 were served as positive controls; yeast cells co-transformed with AD-T7 and BK-Lam were used as negative controls. **d** Y2H assay of the interaction between the β C1 and CaGeBP and StGeBP in yeast cells. **e** Y2H assay of the interaction between the β C1 and AtGeBP in yeast cells. **f** LCI analysis of the interaction between β C1 and NbGeBP in *N. benthamiana* leaves. β C1-NLuc co-expressed with CLuc or NLuc co-expressed with CLuc-NbGeBP, and NLuc co-expressed with CLuc, were used as negative controls. **g** BiFC analysis of the interaction between NbGeBP and β C1 in *N. benthamiana* leaves. The RFP-H2B is nuclear localization marker. Co-expression of Y^N-NbGeBP and Y^C, or Y^N and Y^C- β C1 were used as negative controls. Confocal imaging was performed at 48 hpi. Scale bars: 50 μ m. **h** BiFC analysis of the interaction between AtGeBP and β C1 in *N. benthamiana* leaves. Co-expression of Y^N-SIgeBP and Y^C- β C1 were used as positive controls, and co-expression of Y^N- β C1 and Y^C, or Y^N and Y^C-AtGeBP were used as negative controls. Confocal imaging was performed at 48 hpi. Scale bars: 100 μ m. **i** Y2H assay of the interaction between the β C1 and NbGeBP-deletion derivatives (Δ 1–7aa, Δ 16–23aa, Δ 36–45aa, Δ 46–138aa, and Δ 338–377aa) in yeast cells. These experiments were performed with three independent biological replicates with similar results

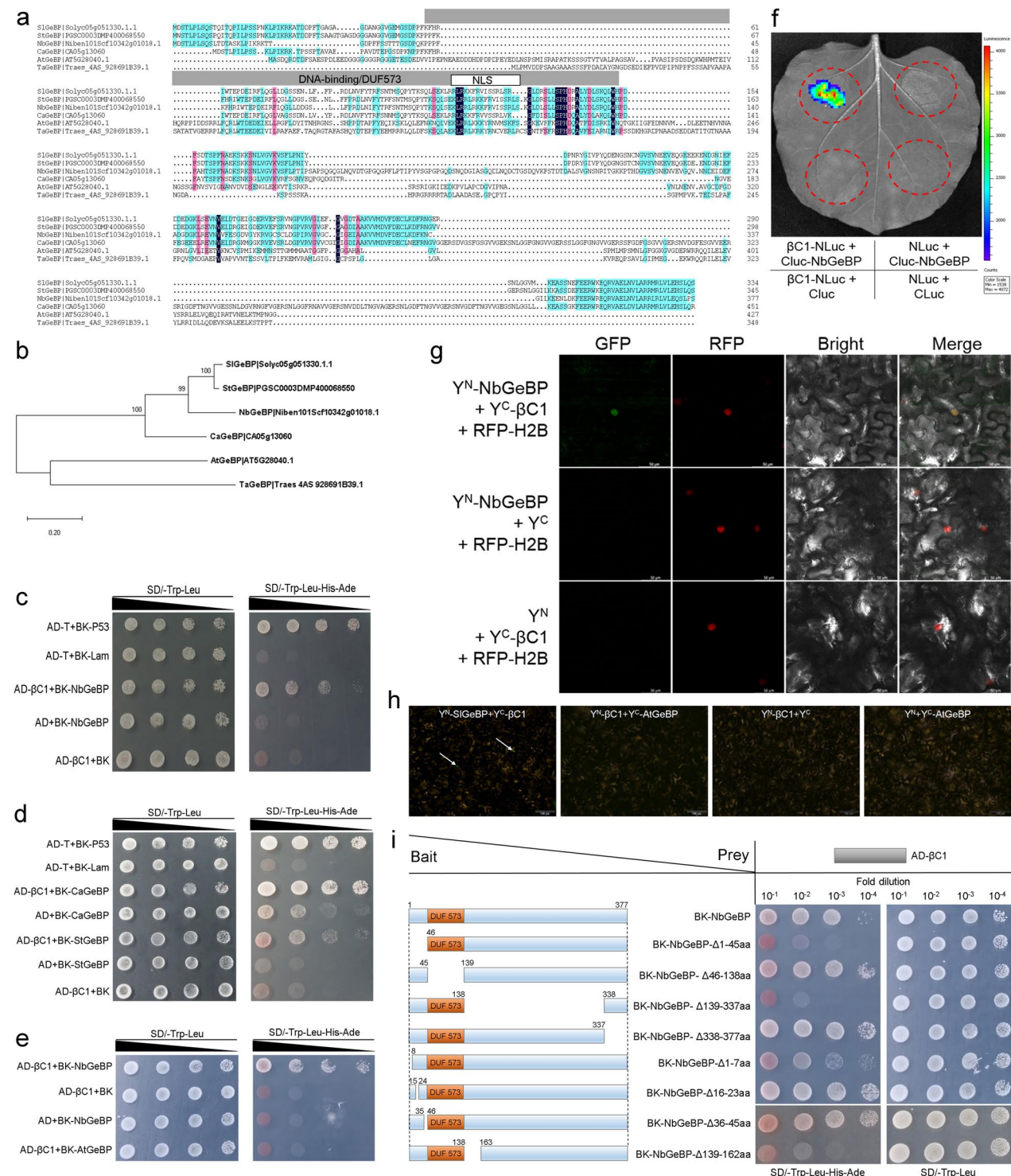


Fig. 2 (See legend on previous page.)

no interaction was detected in control experiments (Fig. 2f). Additionally, in BiFC assays, confocal micrographs showed that positive interaction was observed in the nucleus of *N. benthamiana* cells co-expressing

Y^N-NbGeBP and Y^C-βC1, confirming the interaction between the two proteins in vitro, and no interaction was detected in control experiments (Fig. 2g). Together, no interaction was detected in leaf cells of *N. benthamiana*

co-expressing Y^N - β C1 and Y^C -AtGeBP, confirming no interaction between the two proteins (Fig. 2h). These results reveal that NbGeBP can interact with β C1 in vitro and in vivo, but AtGeBP can not.

TbCSV β C1-NbGeBP interaction is not mediated by the DNA-binding domain of NbGeBP

GeBPs form homo- or heterodimers by the DNA-binding domain and the C-terminal domain to regulate downstream genes expression (Chevalier et al. 2008). To investigate whether NbGeBP forms homodimers by self-interaction, we performed a BiFC assay. Confocal micrographs showed that positive interaction occurred in the nucleus of *N. benthamiana* cells co-expressing Y^N -NbGeBP and Y^C -NbGeBP (Additional file 2: Figure S4). These experiments indicate that NbGeBP forms homodimers in vivo.

To explore whether β C1 disrupts the dimerization of NbGeBP to affect the downstream genes expression of NbGeBP, we constructed different NbGeBP deletion derivatives and performed Y2H assays to localize β C1-interacting domains. In accordance with the functional domain of NbGeBP and existing interaction results between β C1 and SlGeBP, StGeBP, NbGeBP, CaGeBP, or AtGeBP (Fig. 2c–h), the following deletion mutants of NbGeBP were constructed: BK-NbGeBP- Δ 1–45aa, BK-NbGeBP- Δ 46–138aa (deleting the DNA-binding/DUF573 domain), BK-NbGeBP- Δ 139–337aa, BK-NbGeBP- Δ 338–377aa, BK-NbGeBP- Δ 1–7aa, BK-NbGeBP- Δ 16–23aa, BK-NbGeBP- Δ 36–45aa, and BK-NbGeBP- Δ 139–162aa. Y2H assays indicate that the key functional domain of NbGeBP mediates the interaction with β C1 is 8–15aa, 24–35aa, and 139–162aa, not the DNA-binding domain or the C-terminal domain (Fig. 2i), implicating that the homo- or heterodimerization of NbGeBP remains unaffected during its interaction with β C1.

TbCSV β C1 colocalizes with NbGeBP and SlGeBP in the nucleus

The GeBP family proteins are localized in the nucleus, which is closely related to its function of DNA-binding (Curaba et al. 2003; Chevalier et al. 2008). To determine the subcellular localization of NbGeBP and SlGeBP, we performed co-localization assays. When co-expressing NbGeBP-GFP and RFP-H2B (nuclear localization marker), or SlGeBP-GFP and RFP-H2B, NbGeBP-GFP and SlGeBP-GFP colocalized with RFP-H2B in the nucleus of *N. benthamiana* cells at 48 h post agroinfiltration (hpa), suggesting that NbGeBP and SlGeBP should be localized in the nucleus (Fig. 3a, c).

To further explore the subcellular localization of NbGeBP and SlGeBP in the presence of β C1,

NbGeBP-GFP or SlGeBP-GFP was co-expressed with DsRed2- β C1 in *N. benthamiana* cells. We found that DsRed2- β C1 alone distributed in the cytoplasm and nucleus, and its localization did not change when co-expressed with either NbGeBP-GFP or SlGeBP-GFP; however, NbGeBP-GFP and SlGeBP-GFP co-localized with DsRed2- β C1 in the nucleus at 48 hpa (Fig. 3b, d). Furthermore, Y^N -SlGeBP and Y^C - β C1, or Y^N -NbGeBP and Y^C - β C1 were co-expressed with RFP-H2B in *N. benthamiana* cells. We demonstrate that the interaction complex of SlGeBP and β C1, or NbGeBP and β C1 colocalizes with RFP-H2B in the nucleus at 48 hpa (Fig. 1c and Fig. 2g). These results indicate that NbGeBP and SlGeBP are nuclear localization proteins, and both interact with β C1 in the nucleus.

Virus but not its betasatellite induces the downregulation of NbGeBP

Malvastrum yellow vein virus (MaYVV), a member of monopartite begomoviruses, causes yellow vein symptoms in *Malvastrum coromandelianum* plants (Guo et al. 2008). All isolates of MaYVV are associated with a betasatellite (MaYVB), an indispensable component for symptom induction (Guo et al. 2008). To clarify the effect of *NbGeBP* gene expression level on the infection of TbCSV and its heterologous betasatellite MaYVB or its homologous betasatellite TbCSB, the PVX vector was used for gene overexpression assay. The constructed recombinant plasmid PVX-NbGeBP was transferred into *Agrobacterium* and a PVX-GUS vector was used as a control to infiltrate *N. benthamiana* leaves. After 6 days, TbCSV/MaYVB and TbCSV/TbCSB infectious clones were inoculated into *N. benthamiana* plants. The symptoms were observed at 5 days post inoculation (dpi). The plants inoculated with PVX-GUS_TbCSV/MaYVB, PVX-NbGeBP_TbCSV/MaYVB, PVX-GUS_TbCSV/MaYVB, and PVX-NbGeBP_TbCSV/TbCSB showed similar mosaic and mild leaf curling symptoms (Fig. 4a). RT-qPCR detection showed that *NbGeBP* gene expression in *N. benthamiana* plants inoculated with PVX-NbGeBP was about 5000 times that of the control treatment (Fig. 4b). qPCR detection showed that there were no significant differences in TbCSV, TbCSB, and MaYVB DNA accumulations in the plants inoculated with PVX-GUS_TbCSV/MaYVB, PVX-NbGeBP_TbCSV/MaYVB, PVX-GUS_TbCSV/TbCSB, and PVX-NbGeBP_TbCSV/TbCSB at 5 dpi and 10 dpi (Fig. 4c). These results show that the overexpression of *NbGeBP* can not influence DNA concentration of virus and betasatellite in *N. benthamiana* plants after infection with TbCSV and its heterologous betasatellite MaYVB or its homologous betasatellite TbCSB.

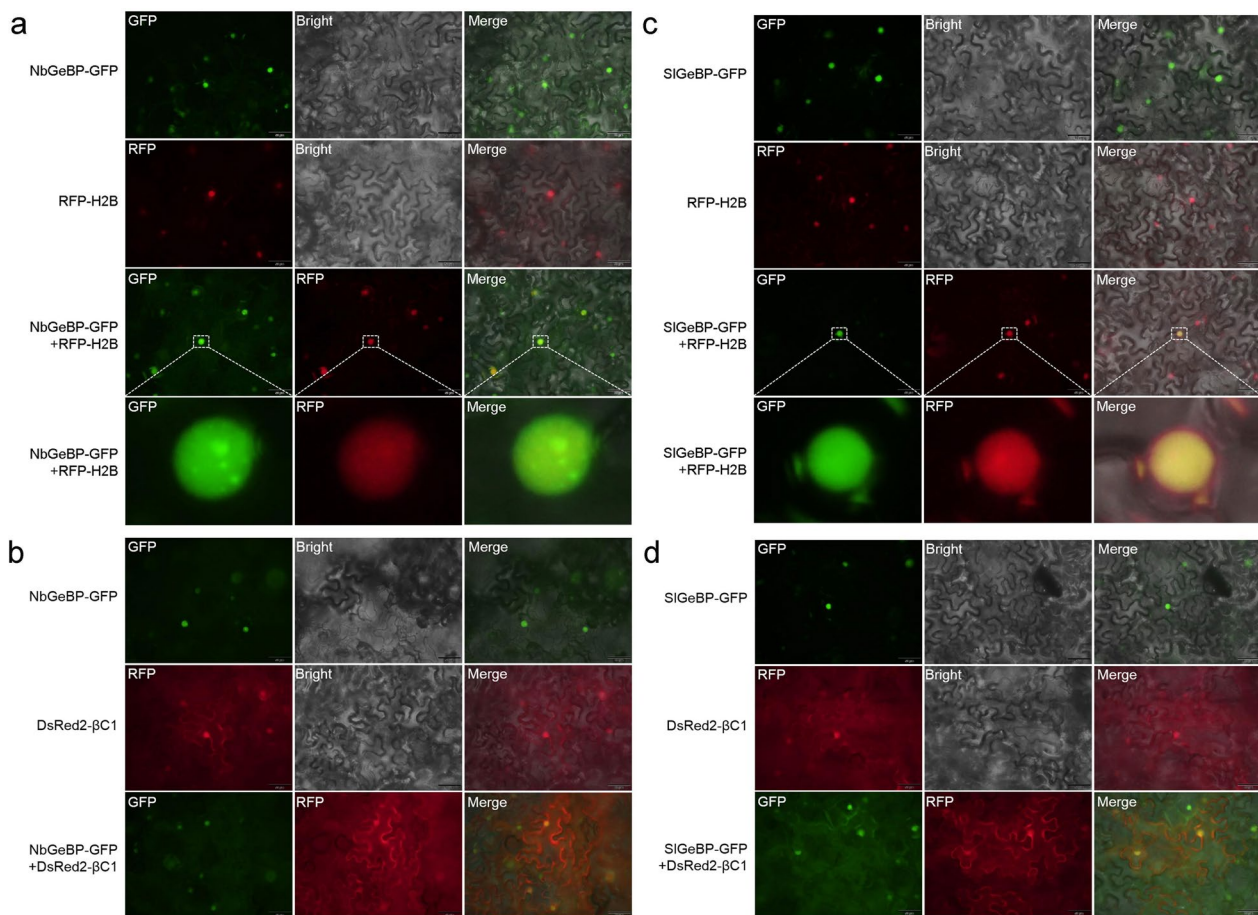


Fig. 3 DsRed2-βC1 and NbGeBP-GFP/SiGeBP-GFP co-localized in the nucleus. **a** NbGeBP localized in the nucleus. Confocal micrographs of *N. benthamiana* leaf cells co-expressing NbGeBP-GFP and RFP-H2B (nuclear localization marker) at 48 hpi. **b** DsRed2-βC1 and NbGeBP-GFP co-localized in the nucleus. Confocal micrographs of *N. benthamiana* leaf cells co-expressing NbGeBP-GFP and DsRed2-βC1 at 48 hpi. **c** SiGeBP-GFP localized in the nucleus. Confocal micrographs of *N. benthamiana* leaf cells co-expressing SiGeBP-GFP and RFP-H2B at 48 hpi. **d** DsRed2-βC1 and SiGeBP-GFP co-localized in the nucleus. Confocal micrographs of *N. benthamiana* leaf cells co-expressing SiGeBP-GFP and DsRed2-βC1 at 48 hpi. Scale bars: 50 μm. These experiments were performed with three independent biological replicates with similar results

To verify whether SiGeBP is required for TbCSV/TbCSB infection in tomato, we chose the PVX vector for *SiGeBP* gene overexpression assay. The constructed recombinant plasmid PVX-SiGeBP was transferred into *Agrobacterium* and a PVX-GUS vector was used as control to infiltrate tomato leaves. After 19 days, the plants inoculated with PVX-GUS_TbCSV/TbCSB and PVX-SiGeBP_TbCSV/TbCSB showed similar mosaic symptoms (Additional file 2: Figure S5a). RT-qPCR detection showed that *SiGeBP* gene expression in tomato inoculated with PVX-SiGeBP was about 100 times that of the control treatment (Additional file 2: Figure S5b). The TbCSV/TbCSB infectious clones were inoculated into tomato plants. The plants inoculated with PVX-GUS_TbCSV/TbCSB and PVX-SiGeBP_TbCSV/TbCSB showed no obvious symptom difference (data not shown), but the *βC1* gene could be detected by PCR in

the systemically infected leaves of tomato plants infected with TbCSV/TbCSB at 12 dpi (Additional file 2: Figure S5c). Quantitative PCR detection showed that there were no significant differences in TbCSV and TbCSB DNA accumulations of the plants inoculated with PVX-GUS_TbCSV/TbCSB and PVX-SiGeBP_TbCSV/TbCSB (Additional file 2: Figure S5d). These results suggest that the overexpression of *SiGeBP* can not influence virus concentration in tomato after TbCSV/TbCSB infection.

AtGeBP shares 19.5% identity to SiGeBP. To test the biological roles of the *AtGeBP* gene (AT5G28040.1) on TbCSV/TbCSB infection, *Arabidopsis gebp* mutant, the Col-0 SALK_129879C line homozygous for T-DNA insertions in *AtGeBP*, was used (Additional file 2: Figure S6a, b). TbCSV/TbCSB infectious clones were inoculated into four-week-old *Arabidopsis* plants. *Arabidopsis* Col-0 and *gebp* mutant plants showed

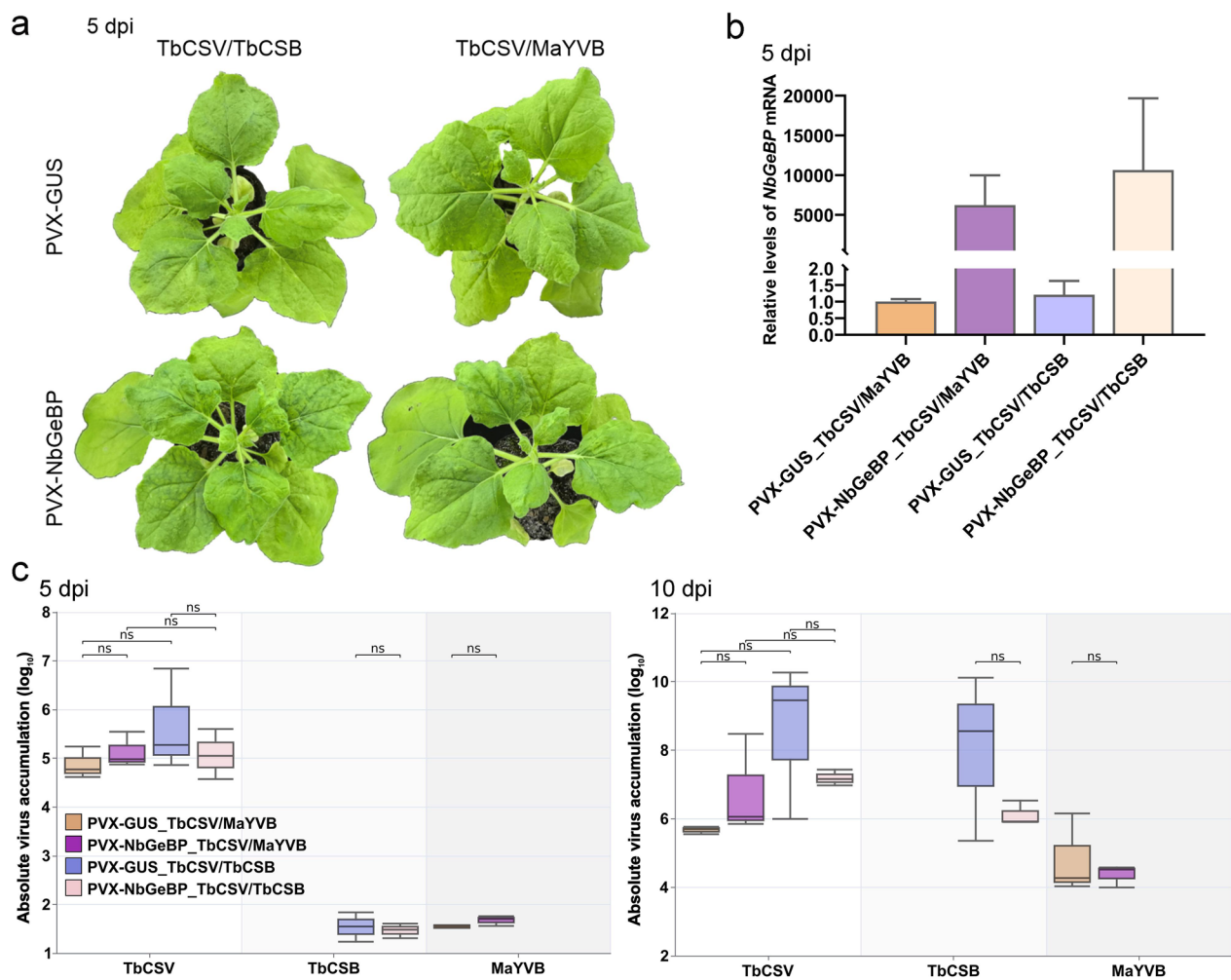


Fig. 4 *NbGeBP* is not required for the symptom development and DNA accumulations of TbCSV/TbCSB and TbCSV/MaYVB. **a** Symptoms of *N. benthamiana* plants inoculated with TbCSV/TbCSB, or TbCSV/MaYVB at 5 dpi. **b** RT-qPCR analysis *NbGeBP* mRNA levels in the systemic leaves of *N. benthamiana* plants. **c** qPCR analysis of TbCSV, TbCSB, and MaYVB DNA accumulation in the systemic leaves of *N. benthamiana* plants at 5 dpi and 10 dpi. Data are the mean of three independent biological replicates. Error bars represent SEM. The significant difference was analyzed by unpaired Student's *t* test (two-tailed)

similar symptoms at 7 dpi (Additional file 2: Figure S6c), and qPCR detection showed that TbCSV and TbCSB DNA accumulations in *gebp* plants decreased significantly (Additional file 2: Figure S6d). The results indicated that the knockout of *AtGeBP* decreased virus concentration in *Arabidopsis* plants after TbCSV/TbCSB infection.

To explore the effect of $\beta C1$ on *NbGeBP* gene expression, we detected the expression level of *NbGeBP* by RT-quantitative PCR (RT-qPCR) in $\beta C1$ -transgenic and wild-type *N. benthamiana*. The expression levels of *NbGeBP* were not significantly different in $\beta C1$ -transgenic or wild-type *N. benthamiana* plants (Fig. 5a), suggesting that $\beta C1$ does not transcriptionally regulate *NbGeBP*.

To further investigate the effect of virus infection on *NbGeBP* gene expression, we inoculated *N. benthamiana* plants with infectious clones of TbCSV, MaYVV, TbCSV/TbCSB, TbCSV/MaYVB, MaYVV/TbCSB, or MaYVV/MaYVB and examined the relative expression levels of *NbGeBP* at 3, 5, 7, 14, and 21 dpi, respectively, using RT-qPCR. Our findings are as follows: (1) At 3 dpi, TbCSV infection significantly downregulated *NbGeBP* expression. There were no significant differences in *NbGeBP* expression between plants infected with TbCSV and those co-infected with TbCSV/TbCSB or TbCSV/MaYVB. (2) Similarly, at 3 dpi, MaYVV infection also significantly downregulated *NbGeBP* expression. There were no significant differences in *NbGeBP* expression among the plants infected with MaYVV, MaYVV/MaYVB, or

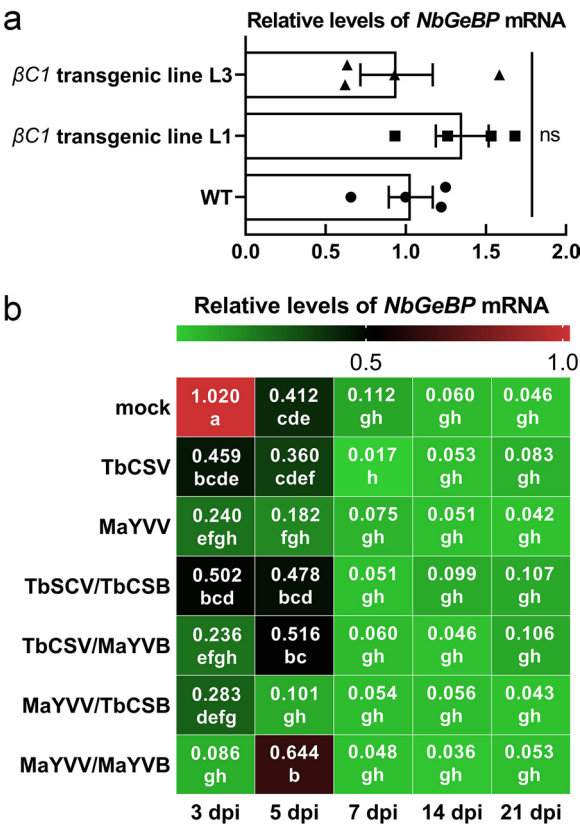


Fig. 5 *NbGeBP* mRNA levels in tobacco analyzed by RT-qPCR. **a** Relative levels of *NbGeBP* mRNA in wild-type *N. benthamiana* leaves and β C1-transgenic *N. benthamiana* leaves were analyzed by RT-qPCR. **b** TbCSV-, MaYVV-, TbCSV/TbCSB-, TbCSV/MaYVB-, MaYVV/TbCSB-, MaYVV/MaYVB-, and non-infiltrated *N. benthamiana* leaves at 3, 5, 7, 14, 21 dpi were analyzed by RT-qPCR. Relative mRNA levels in leaf tissue were normalized using *NbActin* mRNA as a reference. Values are means of four independent experiments. Error bars represent SEM. The significant difference was analyzed by Duncan's multiple range test and different letters denote significant differences ($p < 0.05$)

MaYVV/TbCSB. (3) At 5 dpi, MaYVV infection continued to significantly downregulate *NbGeBP* expression. There were no significant differences in *NbGeBP* expression between plants infected with MaYVV and MaYVV/TbCSB. However, the plants infected with MaYVV/MaYVB showed a significant upregulation of *NbGeBP* compared with those infected with MaYVV or the mock-inoculation control. (4) At 5, 7, 14, and 21 dpi, there were no significant differences in *NbGeBP* expression among the mock-inoculated plants and the plants infected with TbCSV, TbCSV/TbCSB, or TbCSV/MaYVB. (5) Similarly, at 7, 14, and 21 dpi, there were no significant differences in *NbGeBP* expression among the mock-inoculated plants and plants infected with MaYVV, MaYVV/MaYVB, or MaYVV/TbCSB (Fig. 5b). These results suggest that both TbCSV and MaYVV could induce the downregulation of *NbGeBP* in the early stages of infection, and the

presence of a homologous or heterologous betasatellite does not affect this effect.

TYLCCNB β C1 but not MaYVB β C1 interacts with SiGeBP and NbGeBP

To delve deeper into the interaction between GeBPs of solanaceous plants and different β C1 proteins, we firstly performed sequence alignment and conducted a phylogenetic analysis of 134 β C1 proteins, encoded by different monopartite begomoviruses associated betasatellites. Our results reveal that β C1 proteins are divided into two distinct clades and TbCSB Y35 β C1 is located in Clade II (Additional file 2: Figure S7). Thus, we wondered whether these phylogenetically separated β C1 proteins have the same ability to interact with GeBP proteins of solanaceous plants. To answer this question, we selected MaYVB Y47 β C1 located in Clade I and TYLCCNB Y10 β C1 located in Clade II for further investigation (Additional file 2: Figure S7). TbCSB Y35 β C1 shares 71.2% ASI with TYLCCNB Y10 β C1 and 28.8% ASI with MaYVB Y47 β C1 (Fig. 6a, b). We performed BiFC assays to investigate the interaction between Y10 β C1 and SiGeBP, Y10 β C1 and NbGeBP, Y47 β C1 and SiGeBP, Y47 β C1 and NbGeBP in vivo. The results showed positive interaction in the nucleus of *N. benthamiana* cells co-expressing Y^N-SiGeBP and Y^C-Y10 β C1, or Y^N-NbGeBP and Y^C-Y10 β C1, while no interaction was observed with Y^C-Y47 β C1 (Fig. 6c). These results demonstrate that β C1 proteins of TbCSB and TYLCCNB can interact with Solanaceae GeBP proteins in the nucleus, whereas MaYVB β C1 can not. Hence, these phylogenetically clustered β C1 proteins in Clade II might have the same ability to interact with Solanaceae GeBP proteins.

Discussion

The monopartite begomovirus betasatellite-encoded β C1 protein is a pathogenicity determinant (Cui et al. 2004), inducing growth defects in transgenic plants (Briddon et al. 2003; Cui et al. 2004; Yang et al. 2008; Cheng et al. 2011; Bhattacharyya et al. 2015; Nair et al. 2020), and suppressing various plant defense mechanisms, including transcriptional gene silencing, post-transcriptional gene silencing, and host immunity (Cui et al. 2005; Li et al. 2014a, 2018). Recent research has shed light on additional roles of β C1, including its ability to interfere directly with the host plastid homeostasis and activate the host unfolded protein response to promote virus infection (Nair et al. 2023; Zhang et al. 2023). However, β C1 can be targeted by many host defense-related factors for post-translational modifications and protein degradation (Shen et al. 2011, 2016; Haxim et al. 2017). Previous research has identified interactions between β C1 and numerous plant TFs, resulting in altered symptom

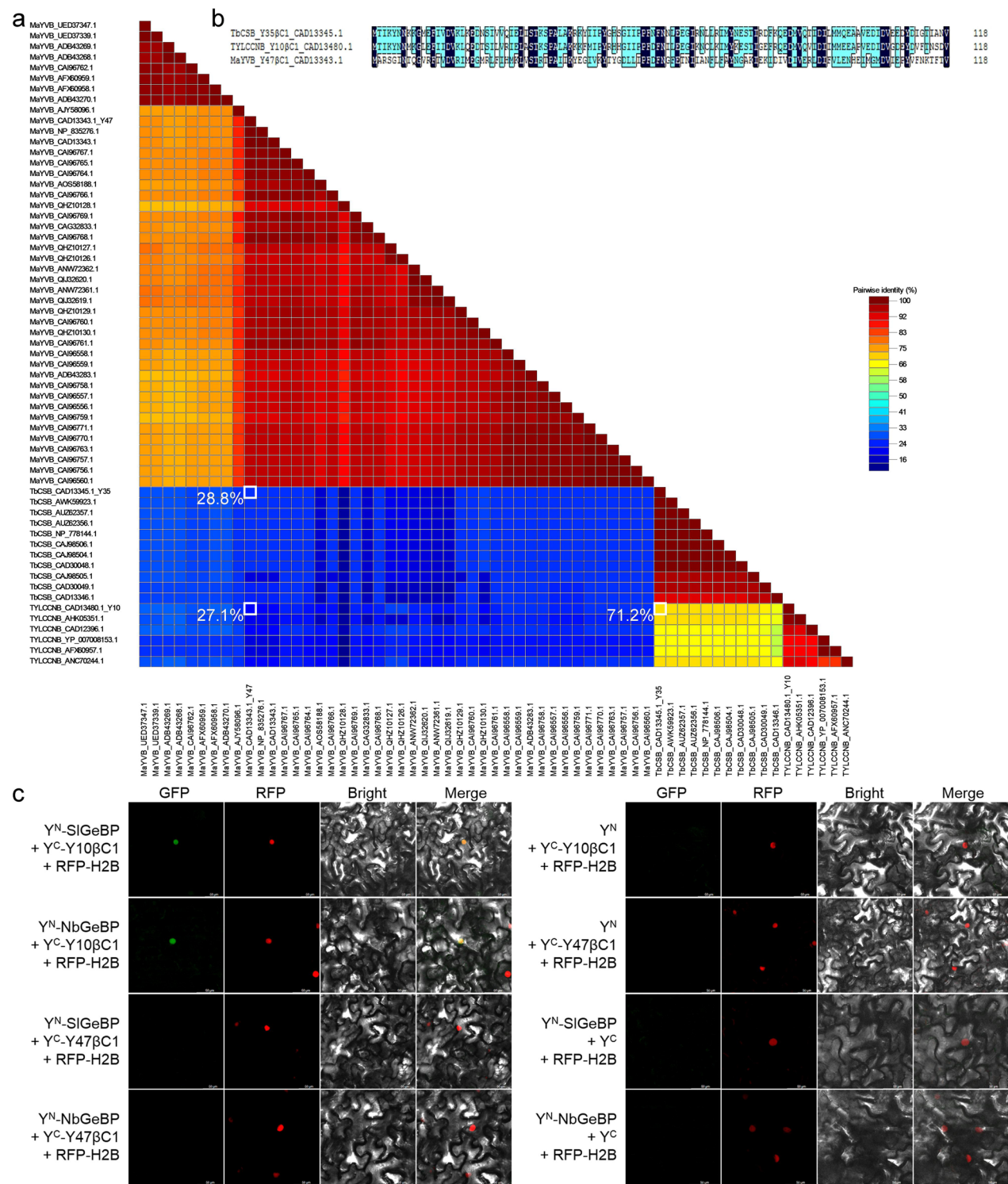


Fig. 6 TYLCCNB βC1 but not MaYVB βC1 interacts with SiGeBP and NbGeBP in the nucleus. **a** The amino acid sequences identity of βC1 proteins encoded by tobacco curly shoot betasatellite (TbCSB), tomato yellow leaf curl China betasatellite (TYLCCNB), and malvastrum yellow vein betasatellite (MaYVB). **b** Alignment of βC1 protein sequences from TbCSB Y35, TYLCCNB Y10, and MaYVB Y47. **c** BiFC analysis of the interaction between SiGeBP/NbGeBP and Y10βC1 or SiGeBP/NbGeBP and Y47βC1 in *N. benthamiana* leaves. The RFP-H2B is nuclear localization marker. Co-expression of Y^N and Y^C-Y10βC1, Y^N and Y^C-Y47βC1, Y^N-SiGeBP and Y^C, or Y^N-NbGeBP and Y^C were used as negative controls. Confocal imaging was performed at 48 hpi. Scale bars: 50 μm. These experiments were performed with three independent biological replicates with similar results

development, enhanced virus infection, and improved vector performance (Yang et al. 2008; Li et al. 2014a; Li et al. 2014b; Li et al. 2017a; Li et al. 2017b; Zhao et al. 2019; Zhao et al. 2021; Zhang et al. 2023), our study introduces a novel Solanaceae TF, GeBP, which specifically interacts with β C1 proteins encoded by TbCSB and TYLCCNB.

The nuclear localization is important for TFs to regulate the expression of downstream genes. A recent research reported that TYLCCNB Y10 β C1 induces the nuclear export of NbbZIP60 TF to manipulate the expression of NbbZIP60 downstream genes associated with unfolded protein response for facilitating virus infection (Zhang et al. 2023). GeBPs, including AtGeBPs, typically localize in the nucleus (Curaba et al. 2003; Chevalier et al. 2008), with the exception of VFP4 (AT5G28040), which displays dual localization in the nucleus and cytoplasm (Garcia-Cano et al. 2018). VFP4 has been reported to interact with VirF (an *Agrobacterium* F-box protein effector) and VBF (an *Arabidopsis* F-box protein) in the nucleus (Garcia-Cano et al. 2018). Consistent with these findings, we confirmed the nuclear localization of NbGeBP and SlGeBP, and observed that their localization remained unaffected in the presence of β C1.

GeBP belongs to an evolutionarily conserved plant TF family, characterized by a conserved DNA-binding domain (DUF573, domain architecture ID 10517430) (Garcia-Cano et al. 2018). Our analysis revealed that SlGeBP, similar to AtGeBPs, displays DNA-binding activity but lacks transcriptional activation activity. The DNA-binding domain and C-terminal noncanonical Leu-zipper domain are crucial for homo- or heterodimerization of GeBP to regulate the downstream genes expression (Curaba et al. 2003; Chevalier et al. 2008). In this study, we found that NbGeBP has a typical DNA-binding domain and forms homodimers in the nucleus, and the key β C1-interacted domain for NbGeBP is neither the DNA-binding domain nor the C-terminal domain, indicating that the ability of GeBP to form homo- or heterodimers might remain unaffected after interaction with β C1. Since the homo- or heterodimerization is crucial for the DNA-binding ability of GeBP, we proposed that the function of GeBP to bind DNA and regulate downstream gene expression remains intact during interaction with β C1.

GeBPs have been implicated in pathogen resistance of plants. For example, the transgenic *Arabidopsis* plants overexpressing *GPL2* exhibit enhanced resistance against *Pst* DC3000 (Perazza et al. 2011). However, our findings suggest that *GeBP* may function differently in TbCSV/TbCSB infection. VFP4 regulates a set of biotic stress-related genes to control a spectrum of plant defenses against pathogens including *A. tumefaciens* which utilizes its F-box protein effector VirF to recognize and

target VFP4 for proteasomal degradation, thereby mitigate the VFP4-based defense (Garcia-Cano et al. 2018). In this study, we found that TbCSV/TbCSB induced similar symptoms in *N. benthamiana* and tomato plants overexpressing *GeBP* to that in wild-type plants, and there was no significant change in viral DNA accumulation (Additional file 2: Figure S5). Interestingly, we found that knockout of the *AtGeBP* gene decreased viral DNA accumulation after TbCSV/TbCSB infection compared with wild-type *Arabidopsis* plants (Additional file 2: Figure S6), suggesting that *AtGeBP* is a susceptibility gene for the TbCSV/TbCSB infection. *AtGeBPs* are mainly expressed in leaf primordium, meristem, and vascular tissue (Curaba et al. 2003). We found that the expression patterns of *SlGeBP* and *NbGeBP* differ from *AtGeBP*, and *SlGeBP* was predominantly expressed in roots and fruits, especially in collenchyma and vascular tissue of fruit (Additional file 2: Figure S8), while *NbGeBP* mainly expressed in flowers, roots, stem, apical region, and mature leaves (Additional file 2: Figure S9). Although plant GeBP family proteins have conserved DNA-binding domain, we found that the ASI between *SlGeBP* and *NbGeBP* is 51.8%, whereas the ASI between *SlGeBP* and VFP4 is merely 19.5%, suggesting that there are significant differences in GeBP protein sequences. Hence, we speculate that the observed variations in genetic evolution and expression patterns between *SlGeBP*/*NbGeBP* and VFP4 may contribute to the different biological functions and responses to TbCSV/TbCSB infection seen among GeBP family proteins in various plant families, including Solanaceae and Cruciferae.

Currently, GeBP family proteins have been identified in many crops, including *Glycine max* (Liu et al. 2022), *Brassica rapa* (Wang et al. 2023), and graminaceous crops (Huang et al. 2021), whereas the function of GeBP family proteins in solanaceous crops remains unclear. In *N. benthamiana*, we found that NbGeBP exhibits ASI differences with other 11 family proteins and shares 13.9% ASI with its closest intra-species homologous protein, suggesting that ASI is relatively low among these intra-species GeBP family proteins and NbGeBP might have specific function (Additional file 2: Figure S10). In this study, both TbCSV and MaYVV induced the downregulation of *NbGeBP* during the early stages of infection, regardless of the presence of homologous or heterologous betasatellites, thus we presumed that *NbGeBP* has a crucial role in virus infection. We attempted to knock down *NbGeBP* through a tobacco rattle virus (TRV)-based virus-induced gene silencing system (VIGS), but we detected no downregulation of *NbGeBP* in the leaves and roots of the treated plants in three independent biological experiments (data not shown). We speculated that it is attributed to the tissue-specific expression and low

expression levels of *NbGeBP* (Additional file 2: Figure S9). In the future, we consider creating the *N. benthamiana* lines with *NbGeBP* expression knockout by using the CRISPR/Cas9 gene-editing system to elucidate the biological function of *NbGeBP* and its roles in virus infection. Overall, our findings shed light on the potential role of GeBP in virus infection and highlight the importance of understanding the biological functions of GeBP family proteins in different plant species.

Prior research has demonstrated that the betasatellite associated with ageratum yellow vein virus can alter the host range of Sri Lankan cassava mosaic virus (Saunders et al. 2002). To elucidate the role of GeBP- β C1 interaction in virus infection, we conducted a phylogenetic analysis of 134 β C1 proteins encoded by betasatellites. Our findings reveal that β C1 proteins are divided into two distinct clades (Additional file 2: Figure S7). Among the primary hosts as initially discovered for betasatellites, the solanaceous plants constitute a significant proportion (50/134 betasatellites), and solanaceous plants account for 10.9% of the primary hosts within clade I betasatellites (5/46 betasatellites), but more than half of the primary hosts are solanaceous plants within clade II betasatellites (45/88 betasatellites) (Additional file 2: Figure S7). This disparity unveils a potentially more intimate interaction between clade II betasatellites and solanaceous plants, which might have implications for elucidating the host adaptation mechanisms of betasatellites.

Moreover, we found that the ASI between Y35 β C1 and Y10 β C1 is up to 71.2%, while the ASI between Y35 β C1 and Y47 β C1 is merely 28.8%. Notably, Y35 β C1 and Y10 β C1, but not Y47 β C1, interacts with *NbGeBP* and *SlGeBP*; and only Solanaceae GeBP proteins interact with Y35 β C1, while *AtGeBP* from plants of Cruciferae does not interact with Y35 β C1. *TbCSV/TbCSB* and *TYLCCNV/TYLCCNB* have been detected in many solanaceous crops, including tomato, pepper, and tobacco, while there are almost no reports about natural infection of *MaYVV/MaYVB* in solanaceous plants (Additional file 1: Table S2). Intriguingly, Zhang documented a field occurrence of *MaYVV* infecting cultivated tomatoes, which was unexpectedly linked to the heterologous betasatellite *TYLCCNB*, as opposed to the native betasatellite *MaYVB* (Zhang 2010), suggesting that the interaction between *MaYVV* and *TYLCCNB* might be crucial for *MaYVV* to infect tomato. Therefore, we raise the question of whether the GeBP- β C1 interaction is necessary for the begomoviruses which have a betasatellite to infect solanaceous plants successfully. In future studies, based on the *NbGeBP* loss-of-function mutant of *N. benthamiana*, the functional exploration of *NbGeBP* will help us to understand why certain begomoviruses can infect solanaceous plants whereas others can not.

Conclusions

In summary, we identified the interaction between monopartite begomovirus betasatellite-encoded β C1 protein and Solanaceae GeBP transcription factor. We observed that *NbGeBP* and *SlGeBP* localize in the nucleus regardless of the presence of *TbCSB* β C1. Moreover, Solanaceae GeBP proteins form homodimers and exhibit DNA-binding activity, with their DNA-binding ability seemingly unaffected by interaction with β C1. Significantly, we found that both *TbCSV* and *MaYVV* induce the downregulation of *NbGeBP* during the early stages of infection, regardless of the presence of homologous or heterologous betasatellites. While *TbCSV/TbCSB* and *TYLCCNV/TYLCCNB* infect various solanaceous plants, there is minimal natural infection of *MaYVV/MaYVB* in solanaceous plants. Intriguingly, Solanaceae GeBP proteins interact with the β C1 from *TbCSB* and *TYLCCNB* but not from *MaYVB*, and these GeBP- β C1 interactions are exclusive to Solanaceae. This research raises intriguing questions about why certain begomoviruses can infect solanaceous plants while others cannot. In the future, we plan to create a *NbGeBP* loss-of-function mutant of *N. benthamiana* to delve deeper into the function of *NbGeBP* in virus infection.

Methods

Plant materials and growth conditions

Wild-type *Arabidopsis thaliana* Col-0 plants, *Arabidopsis* SALK_129879C line, wild-type *Nicotiana benthamiana*, and β C1-transgenic *N. benthamiana* plants were grown in a greenhouse set at 23°C and 16 h light/8 h dark photoperiod with 60% relative humidity.

Plasmid construction

For Y2H screening, the full-length *TbCSB* β C1 gene was cloned into the pGBKT7 vector to generate a bait vector pBK- β C1. For Y2H assays, the full-length *SlSTPK*, *SlHSRP*, *SlA7A*, and *SlGeBP* of tomato, *N. benthamiana* *GeBP*, *C. annuum* *GeBP*, *S. tuberosum* *GeBP*, *A. thaliana* *GeBP*, were amplified and cloned individually into the pGBKT7 vector to produce pBK-*SlSTPK*, pBK-*SlHSRP*, pBK-*SlA7A*, pBK-*SlGeBP*, pBK-*NbGeBP*, pBK-*CaGeBP*, pBK-*StGeBP*, and pBK-*AtGeBP*. The full-length *V1*, *V2*, *C1*, *C2*, *C3*, and *C4* of *TbCSV*, along with *TbCSB* β C1 were amplified and cloned into the pADT7 vector to produce pAD-*V1*, pAD-*V2*, pAD-*C1*, pAD-*C2*, pAD-*C3*, pAD-*C4*, and pAD- β C1. Together, yeast expression plasmid pAD-*SlGeBP* was constructed. In addition, eight deleting mutants of *NbGeBP* (referred to as *NbGeBP*- Δ 1–45aa, *NbGeBP*- Δ 46–138aa, *NbGeBP*- Δ 139–337aa, *NbGeBP*- Δ 338–377aa, *NbGeBP*- Δ 1–7aa,

NbGeBP-Δ16–23aa, NbGeBP-Δ36–45aa, and NbGeBP-Δ139–162aa) were constructed based on the pBK-NbGeBP through plasmid mutagenesis protocol (Liu and Naismith 2008).

For LCI assay, the full-length *βC1* and *SlGeBP/NbGeBP* were amplified and cloned individually into p35S:nLUC and p35S:cLUC vector to generate p β C1-nLuc and pCLuc-SlGeBP/pCLuc-NbGeBP.

For BiFC assays, the full-length *SlGeBP*, *NbGeBP*, and *βC1* were amplified and cloned individually into pCV-nYFP vector to generate pY^N-SlGeBP, pY^N-NbGeBP, and pY^N-*βC1*. Together, the full-length *AtGeBP*, *NbGeBP*, *βC1*, *Y10βC1*, and *Y47βC1* were amplified and cloned individually into pCV-cYFP vector to generate plasmids pY^C-AtGeBP, pY^C-NbGeBP, pY^C-*βC1*, pY^C-Y10βC1, and pY^C-Y47βC1, respectively.

For the subcellular localization assay in *N. benthamiana* leaf cells, the full-length *SlGeBP*, *NbGeBP*, and *βC1* were amplified and cloned individually into pCV-GFP vector to generate pSlGeBP-GFP, pNbGeBP-GFP, and p β C1-GFP. Together, the full-length *βC1* was amplified and cloned into pGDR vector to generate pDsRed2-*βC1*.

For PVX-mediated overexpression assays, the full-length *SlGeBP* and *NbGeBP* were amplified and cloned individually into pGR106 vector to generate pPVX-SlGeBP and pPVX-NbGeBP.

All recombinant plasmids were transferred into *Escherichia coli* DH5α competent cells and were validated by Sanger sequencing. Primers used are listed in Additional file 1: Table S3.

Agroinfiltration and virus inoculation

pPVX plasmids were transformed into *A. tumefaciens* strain GV3101 (pSoup). Other plant expression vector plasmids were transformed into *A. tumefaciens* strain GV3101. The transformants were cultured in Luria–Bertani liquid medium with appropriate antibiotics at 28 °C overnight and individually resuspended in infiltration buffer (10 mM MgCl₂, 10 mM MES (pH 5.6), 150 μM acetosyringone). The suspensions were incubated at 28 °C for 3 h before agroinfiltration.

For virus inoculation following VOX, *A. tumefaciens* cultures carrying pPVX-GUS (PVX-GUS) or pPVX-NbGeBP (PVX-NbGeBP) at an OD₆₀₀=0.5 were inoculated into 3-week-old *N. benthamiana* plants. Six days later, *A. tumefaciens* cultures harboring infectious clones of TbCSV/TbCSB or TbCSV/MaYVB at an OD₆₀₀=1.0 were inoculated into PVX-GUS- or PVX-NbGeBP-infiltrated plants. Systemically infected leaves were harvested at 5 dpi and 10 dpi.

For virus inoculation in wild-type *N. benthamiana*, 4-week-old *N. benthamiana* plants were directly agroinoculated with infectious clones of TbCSV, MaYVV, TbCSV/TbCSB, TbCSV/MaYVB, MaYVV/TbCSB, or MaYVV/MaYVB at an OD₆₀₀=1.0. Systemically infected leaves were harvested at different stages post inoculation.

For virus inoculation in wild-type *A. thaliana* Col-0 and SALK_129879C line plants, 4-week-old *Arabidopsis* plants were directly agroinoculated with infectious clones of TbCSV/TbCSB at an OD₆₀₀=1.0. Systemically infected leaves were harvested at 7 dpi.

RNA extraction and RT-qPCR

Total RNA was extracted from leaves with RNAiso Plus (TaKaRa, Cat# 9109) and 1000 ng of total RNA was reversely transcribed by HiScriptII Q RT SuperMix kit (Vazyme, R223-01). qPCR was conducted using Universal SYBR qPCR Master Mix (Biosharp, BL697A). *NbActin* was used for internal control. Primers used are listed in Additional file 1: Table S3.

DNA extraction and qPCR

Total DNA was extracted from plant leaves by the CTAB method. For detection of AtGeBP T-DNA insertion alleles in *Arabidopsis* plants, we performed Tri-Primer-PCR. For detection of viral DNA accumulation, we performed qPCR using Universal SYBR qPCR Master Mix (Biosharp, BL697A) and analyzed the results by the absolute quantification method as described previously (Du et al. 2020). Primers used are listed in Additional file 1: Table S3.

Y2H screening and interaction assay

A tomato Y2H prey library was screened to identify the proteins capable of binding to *βC1*. For the Y2H interaction assay, the Y2H system was used according to the manufacturer's instructions (Protein Interaction). The yeast strain Y2HGold was transformed with pairs of plasmids and grown on a selective SD/-Trp-Leu medium for 3 days. The co-transformants were transferred and cultured onto a selective SD/-Trp-Leu-His-Ade medium to test for possible interactions for 3 d.

Fluorescence microscopy

For BiFC assay, 4-week-old *N. benthamiana* leaves were infiltrated with a combination of *Agrobacterium* cultures harboring the corresponding plasmids. The GFP interaction signal was detected using upright fluorescence microscope BX43 (Olympus, Japan) or super resolution fluorescence lifetime imaging system (Leica, Germany) at 48 hpi. RFP was excited at 543 nm and captured at 590–630 nm. EGFP was excited at 488 nm and captured at 510–550 nm. RFP-H2B signals were used to mark the nucleus. For subcellular localization assay, epidermal cells of the assayed leaves were

examined by fluorescence microscopy 48 hpi as described above. Three independent experiments were performed.

LCI assay

The LCI assay was performed as described previously (Chen et al. 2008). Four-week-old *N. benthamiana* leaves were infiltrated with a combination of *Agrobacterium* cultures harboring the corresponding plasmids. An equal volume of each *Agrobacterium* culture ($OD_{600}=1.0$) was mixed before co-infiltration into *N. benthamiana* leaves. The infiltrated leaves were captured after the addition of luciferin at 48 hpi. A low-light cooled CCD imaging apparatus IVIS Lumina Series III (PerkinElmer, US) was used to capture the luciferase image.

Protein sequence alignment

Sequences of GeBP proteins from *N. benthamiana*, *Solanum lycopersicum*, *S. tuberosum*, *C. annuum*, *Triticum aestivum* and *A. thaliana* were downloaded from the Plant Transcription Factor Database. <http://planttfdb.gao-lab.org/family.php?fam=GeBP>. Accessed 4 March 2022. Sequences of β C1 proteins were downloaded from the National Center for Biotechnology Information (NCBI) Database (<https://www.ncbi.nlm.nih.gov/>). Accessed on 24 January 2024). Multiple sequence alignments were performed through the ClustalW program in BioEdit, using the default parameters. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model (Jones et al. 1992). A phylogenetic tree was constructed in MEGA X (Kumar 2018). Conserved domains in GeBP proteins are identified from the NCBI Database (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Accessed on 4 March 2022).

Abbreviations

ASI	Amino acid sequence identity
BiFC	Bimolecular fluorescence complementation
<i>CPR5</i>	Constitutive expressor of pathogenesis-related gene-5
dpi	Days post inoculation
GeBP	<i>GLABROUS1</i> Enhancer binding protein
hpi	Hours post agroinfiltration
LCI	Luciferase complementation imaging
Luc	Luciferase protein
MaYVB	Malvastrum yellow vein virus associated betasatellite
MaYV	Malvastrum yellow vein virus
<i>Pst</i>	<i>Pseudomonas syringae</i> P _v . <i>tomato</i>
SRBSDV	Southern rice black-streaked dwarf virus
TbCSB	Tobacco curly shoot virus associated betasatellite
TbCSV	Tobacco curly shoot virus
TF	Transcription factor
TYLCCNB	Tomato yellow leaf curl China virus associated betasatellite
TYLCCNV	Tomato yellow leaf curl China virus
Y2H	Yeast two-hybrid

YFP Yellow fluorescent protein

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-025-00319-3>.

Additional file1: Table S1. The candidate interacting proteins of β C1 identified in tomato cDNA library through Y2H screening. **Table S2.** The host range of TbCSV, TbCSV/TbCB, TYLCCNV/TYLCCNB, and MaYV. **Table S3.** Primers used in this study. **Table S4.** The detailed information of 134 β C1 proteins.

Additional file2: Figure S1. SiGeBP has sequence-non-specific DNA binding activity. **Figure S2.** No interaction between SiGeBP and viral protein (V1, V2, C1, C2, C3, and C4) encoded by TbCSV. **Figure S3.** Phylogenetic analysis of GeBP protein family. **Figure S4.** NbGeBP protein has self-interaction. **Figure S5.** SiGeBP is not required for TbCSV/TbCSB infection in tomato. **Figure S6.** AtGeBP is required for TbCSV/TbCSB infection in *Arabidopsis*. **Figure S7.** Phylogenetic analysis of β C1 proteins encoded by betasatellites of monopartite begomoviruses. **Figure S8.** The tissue-specific expression of SiGeBP (Solyc05g051330) in Tomato eFP Browser. **Figure S9.** The tissue-specific expression of NbGeBP (Niben101Scf10342g01018.1) in Nb Browser. **Figure S10.** NbGeBP (Niben101Scf10342g01018.1) is different from the other *N. benthamiana* GeBP family proteins.

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Author contributions

MZ, ML, GW, and LQ conceived the research project and designed the experiments. MZ, LZ, NW, XT, XY, and MW performed the experiments. MZ prepared the Figures and the manuscript. All authors analyzed and reviewed the experimental results. MZ, ML, HG, and LQ co-wrote and edited the article. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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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