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Banana bunchy top virus movement protein induces resistance in banana against *Fusarium* wilt

Weiying Wang[1,2], Wenqiang Wan[1], Qian Chen[1], Taiyun Wei[1*] and Hongxiang Zhang[1*]

Abstract

Banana *Fusarium* wilt, which is known as Panama disease and caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a destructive disease that can lead to plant death and complete loss of banana plantations. For obtaining resistant varieties against Fusarium wilt, research is required to elucidate the mechanisms of genetic resistance to *Foc*. In this research, the banana plants infected with banana bunchy top virus (BBTV) showed resistance to *Foc*. RNA-seq results showed that the infection with *Foc* upregulated many resistance-related genes in BBTV-positive plants, especially genes related to ROS production. It was also found that more H_2O_2 in BBTV-positive plants was induced during *Foc* infection. The movement protein (MP) of BBTV could increase H_2O_2 levels by promoting the accumulation of MaSGT1a (suppressor of the G-two allele of SKP1) via direct interaction. Thus, the resistance to *Foc* induced by BBTV may attribute to MP increasing MaSGT1a protein levels to induce ROS production. MaSGT1a could promote the accumulation of R proteins MaRPM1 and MaRPP8, and increase H_2O_2 levels in *Nicotiana benthamiana*. The expression of R proteins in *Nicotiana benthamiana* caused an increase in H_2O_2 levels and promoted resistance to *Foc*. Thus, the MP of BBTV could induce plant defense through interaction with MaSGT1a. We speculate that MaSGT1a is likely involved in R gene-mediated resistance and thereby promotes the resistance of banana against *Foc*.

Keywords Banana *Fusarium* wilt, BBTV, MP, ROS, SGT1, R protein

Background

Banana (*Musa nana*), belonging to the family Musaceae, is an important food crop in many countries and a significant source of income for millions of people. However, banana cultivation in different parts of the world is hindered by various pests and diseases. Banana *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is a destructive disease that can lead to complete loss of banana plantations (Ploetz 2015). *Foc* infects the xylem vessels of plants, causing wilting of the leaves until the entire plant eventually dies (Ploetz 2015). *Foc* is soil-borne disease and can persist in the soil for many years; once *Foc* is introduced into a plantation, it will be extremely difficult to control (Dita et al. 2018). Banana bunchy top disease (BBTD), caused by the banana bunchy top virus (BBTV), is a major limiting factor for banana production (Qazi 2015). BBTV is transmitted by the banana aphid (*Pentalonia nigronervos*) and infects the phloem of banana plants (Qazi 2015). Infected plants develop stunted leaves with a twisted appearance that form a rosette-like growth pattern (Kumar et al. 2015). Both BBTV and *Foc* are commonly found

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in banana-growing regions worldwide and often coexist in plantations. BBTV is a phloem-limited virus that primarily replicates in the vasculature of banana plant, and *Foc* invades the xylem vessels, making it possible for the interactions between these the two banana pathogens.

Foc is characterized by different pathogenic races, such as races 1, 2, 3, and 4, which are classified based on their ability to cause disease in different banana cultivars (Siamak and Zheng 2018). Tropical race 4 (FocTR4) from *Foc* race 4 is highly virulent and affects Cavendish bananas as well as many other locally important types (Garcia-Bastidas et al. 2014; Ordonez et al. 2015). Despite advancements in transgenic programs for bananas, there are still no commercially available varieties that show effective resistance against *Fusarium* wilt (Bubici et al. 2019). Therefore, further research is required to elucidate the mechanisms of genetic resistance in *Foc*-resistant banana varieties. Wang et al. (2015) asserted that salicylic acid (SA) plays a key role in banana resistance to FocTR4. The application of methyl jasmonate (MeJA) induces defense responses and increases resistance to *Foc* race 4 in banana (Sun et al. 2013). Reactive oxygen species (ROS) play an important role in plant immune system, including pathogen-associated molecular pattern-triggered immunity (PTI) and effector triggered immunity (ETI) (Li et al. 2021). ROS are also important signaling molecules in banana defense responses (Swarupa et al. 2014; Li et al. 2012). To maintain ROS homeostasis, ROS scavenging systems, including peroxidases, glutathione, superoxide dismutases, and catalases, are necessary in plant cells (Wu et al. 2023). ROS-scavenging enzymes have been suggested as potential biochemical markers of *Foc* resistance in banana (Anthony et al. 2017; Li et al. 2022).

The nucleotide-bounding leucine-rich repeat proteins (NLRs), known as resistant (R) proteins, play significant roles in defense against pathogens (Dangl and Jones 2001). SGT1 (suppressor of the G-two allele of SKP1) is a component of R gene-mediated resistance (Azevedo et al. 2006; Muskett and Parker 2003). Many banana NLRs have been found to play important roles in defense against *Fusarium* wilt (Chang et al. 2020). Triggering of the R protein commonly causes localized programmed plant cell death (the hypersensitive response, HR), an oxidative burst that produces ROS, and the accumulation of SA (Feys and Parker 2000). Several studies have indicated that steady-state levels of *R* genes depend on SGT1 (Botër et al. 2007; Muskett and Parker 2003). SGT1 proteins are highly conserved among eukaryotes and often interact with RAR1 (required for Mla12 resistance) and HSP90 (heat shock protein 90) to form the molecular chaperone complex HRS (HSP90, RAR1, and SGT1), which is required by NLRs for disease resistance (Takahashi et al. 2003; van Wersch et al. 2020). SGT1 has been reported to be involved in regulating the accumulation and activation of NLRs (Azevedo et al. 2002; Kadota et al. 2010).

BBTV belongs to the genus *Babuvirus* within the family *Nanoviridae*, and its genome contains at least six circular single-stranded DNA molecules (Qazi 2015). Movement protein (MP) of BBTV induces severe symptoms in plants and suppresses RNA silencing (Amin et al. 2010). In recent years, it has been observed that BBTV-infected banana plants exhibit resistance to *Foc* infection due to the fungicidal properties of MP (Zhuang et al. 2016). However, the mechanisms underlying BBTV-induced resistance to *Foc* infection are not yet completely understood. In this study, we found that BBTV induced ROS, SA, and JA-resistant responses to antagonize *Foc* infection and improve banana survival. The MP of BBTV promoted the accumulation of MaSGT1a through direct interactions. The expression of either MP or MaSGT1a could induce ROS production and activate resistance to *Foc*. MaSGT1a facilitates the accumulation of MaRPM1 and MaRPP8 which as banana NLRs could be triggered by *Foc*. The expression of MaRPM1 and MaRPP8 in *N. benthamiana* also enhanced resistance to *Foc*. Therefore, the resistance to *Foc* induced by BBTV infection may be caused by the interaction between MP and MaSGT1a which involved in R-gene mediated resistance. Our findings reveal the mechanism of a viral protein mediating plant defense against *Foc* infection.

Results

Banana infected with BBTV exhibits resistance to *Foc*

The FocTR4-susceptible Cavendish banana variety Brazilian (AAA) plantlets infected by BBTV were used to assess the resistance to *Foc* through inoculation with GFP-tagged FocTR4 (FocTR4 labeled with green fluorescent protein). The BBTV-infected plants were acquired via the inoculation with aphids carrying BBTV for 2 days. Seven days post aphids feeding, banana seedlings were inoculated with GFP-tagged FocTR4 conidia on their roots. Six days post-inoculation (dpi) of GFP-tagged FocTR4 conidia, FocTR4 began to invade intercellular spaces and extend its hyphae into the vascular bundle of secondary roots, causing browning in the control plants but not in BBTV-infected plants (Fig. 1a). Hyphal mass was observed in the vascular bundle of the primary root at 15 dpi of GFP-tagged FocTR4 conidia. The GFP fluorescence signal intensity of GFP-tagged FocTR4 was stronger in control plants than in BBTV-infected plants (Fig. 1b). Additionally, the fungal biomass in the roots of BBTV-infected plants was lower than that in control plants at both 6 and 15 dpi with GFP-tagged FocTR4 conidia (Fig. 1d). The banana plants that were not infected with BBTV showed

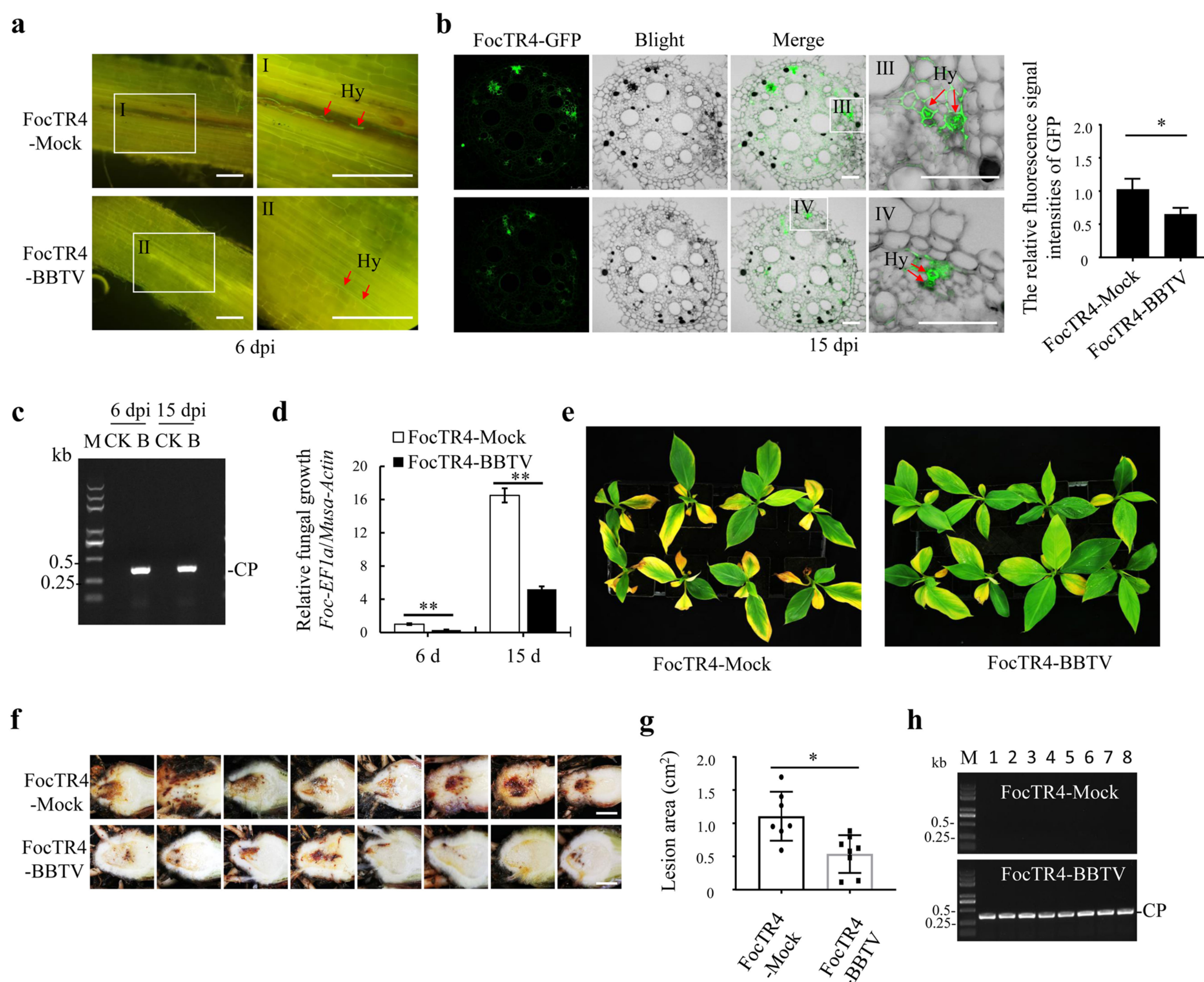

Fig. 1 BBTV suppressed *Foc* infection. **a** and **b** Observation of FocTR4 in the secondary roots at 6 dpi and in the primary roots at 15 dpi. The relative fluorescence intensities of GFP in panels B were analyzed using Image J software. Panels I, II, III, and IV present enlarged images of the boxed area in panel A and B respectively. **c** Detection of BBTV in the roots of bananas at 6 and 15 dpi using specific primer pairs of BBTV coat protein (CP). CK, samples from control bananas inoculated with FocTR4; B, samples from BBTV-infected bananas inoculated with FocTR4. **d** RT-qPCR analysis of the relative fungal growth in inoculated banana roots at 6 and 15 dpi. Relative fungal growth was evaluated using the FocEF1a/MusaActin ratios. **e** Symptom of aboveground banana caused by *Foc*. **f** Disease symptoms in the rhizomes and pseudostems of banana plantlets at 30 dpi. **g** Lesion of the pseudostem caused by *Foc* at 30 dpi. **h** Detection of BBTV in the roots of banana at 30 dpi using specific primer pairs of *CP* gene of BBTV. FocTR4 strain was labeled with GFP. FocTR4-Mock, control bananas inoculated with FocTR4. FocTR4-BBTV, BBTV-infected bananas inoculated with FocTR4. Mean ± SD in panels **b**, **d** and **g** are shown for ten banana plants and represent three biological replicates. The significance of differences was determined using a two-tailed Student's t-test. *$P < 0.05$; **$P < 0.01$. **a** Scale bars: 500 μm, **b** Scale bars: 75 μm, **f** Scale bars: 1 cm

significant browning and yellowing of the pseudostem, and discoloration from the margin of the leaf laminas at 30 dpi of GFP-tagged FocTR4 conidia, which was much more severe than that in the plants infected with BBTV (Fig. 1e–g). The BBTV-infected plants had been detected by PCR (Fig. 1c, h). These observations suggest that a prior BBTV infection strengthens banana resistance to *Foc*.

The presence of BBTV heightened banana's resistance responses to *Foc*

To investigate the mechanism of resistance of BBTV-infected plant to *Foc*, RNA-sequencing (RNA-seq) was performed to analyze the gene expression profiles of banana pseudostems at 15 dpi, when *Foc* began to infect pseudostems. Compared with BBTV-free plants, 1146 differentially expressed genes (DEGs) were upregulated

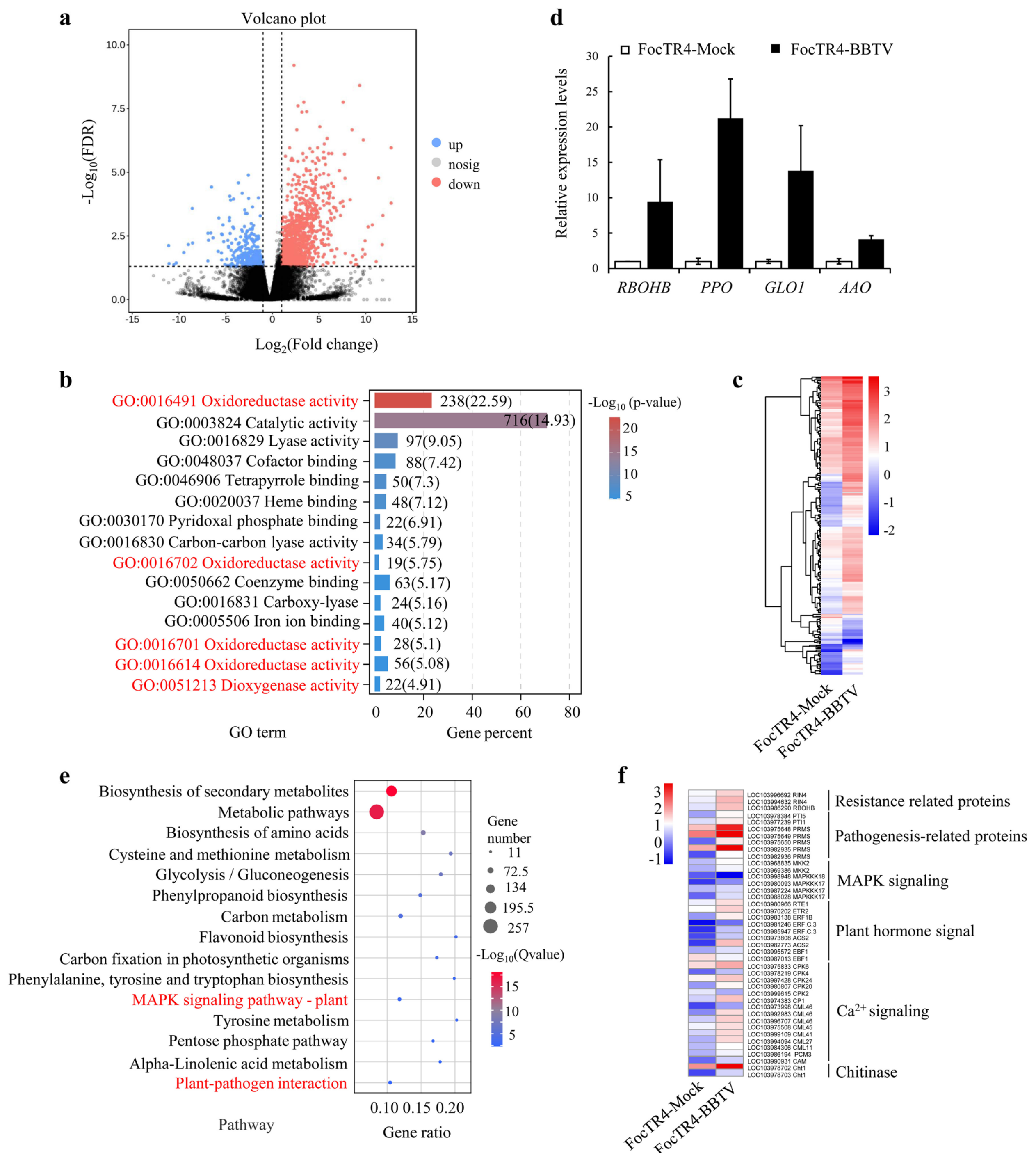

Fig. 2 BBTV induced the upregulated expression of resistant genes in pseudostems at 15 dpi, as assayed by RNA-seq. **a** Volcano map of DEGs in BBTV-infected bananas compared with control plants after inoculation with FocTR4 at 15 dpi. **b** Functional analysis of DEGs using GO. Only the top 15 enriched GO terms are presented. **c** Expression profiles of DEGs enriched in oxidoreductase activity terms in BBTV-infected and uninfected bananas. **d** Relative expression levels of *RBOHB*, *GLO1*, *PPO*, and *AAO* in the pseudostems of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 15 dpi. RBOHB, respiratory burst oxidase homolog protein B (LOC103986290). GLO1, peroxisomal (S)-2-hydroxy-acid oxidase (LOC103988537). PPO, polyphenol oxidase (LOC103995522). AAO, L-ascorbate oxidase (LOC103983914). **e** KEGG pathway enrichment of DEGs. **f** Expression profiles of DEGs enriched in MAPK signaling and plant-pathogen interaction pathways. DEGs were identified based on a 2-fold change threshold from the comparison of BBTV-infected bananas to control plants after inoculation with FocTR4 at 15 dpi and FDR < 0.05. A heat map was created using the expression levels, which were measured using the average RPKM of the treatment sample

and 346 DEGs were downregulated in BBTV-infected plants (Fig. 2a). Gene ontology (GO) enrichment analysis revealed that many DEGs associated with molecular function were enriched in oxidoreductase activity terms closely related to ROS production (Fig. 2b). Gene expression analysis showed that most genes in the oxidoreductase activity terms of BBTV-infected plants were upregulated compared with those in uninfected plants (Fig. 2c). In particular, the expression of *RBOHB*, *GLO1*, and *AAO* genes involved in generating ROS were significantly up-regulated (Fig. 2d). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of DEGs revealed that many DEGs were enriched in MAPK signaling and plant-pathogen interaction pathways, which play important roles in plant systemic resistance (Fig. 2e). It was found that most of genes related to pathogenesis, MAPK signal transduction, plant hormone, ROS and hypersensitive response, and chitinase were upregulated in BBTV-infected banana (Fig. 2f). This result suggests that BBTV likely induces higher expression of a series of resistance-related genes during *Foc* infection.

BBTV infection induces ROS, SA, and JA production

The pathogenicity analysis of *Foc* were used to detect the resistance of bananas induced by BBTV infection. At 2 and 5 dpi, BBTV-infected banana plants showed reduced lesion size caused by *Foc* compared with the control plants (Fig. 3a, b). The inoculation with *Foc* caused a significant increase in H_2O_2 levels in the leaves or roots (Fig. 3c–e). 3,3′-diaminobenzidine (DAB) staining and H_2O_2 content assays revealed that banana plants infected with BBTV exhibited higher H_2O_2 levels than the control plants in leaves at 2 and 5 dpi (Fig. 3c, d). The same result was measured in roots at 15 dpi by H_2O_2 content assays (Fig. 3e). Furthermore, the levels of salicylic acid (SA) and jasmonate (JA), which are important resistance-related plant hormones, were significantly affected by BBTV and *Foc* infection in the roots at 15 dpi (Fig. 3f, g). BBTV-infected plants produced more SA and JA than the control plants upon infection with *Foc* (Fig. 3f, g). Thus, BBTV promotes the production of ROS, SA, and JA during *Foc* infection.

MP of BBTV induces the production of H_2O_2 and promotes the accumulation of MaSGT1a

A previous study reported that the MP of BBTV could manipulate host immune defenses (Zhuang et al. 2016). To explore the mechanism of BBTV infection-boosting banana defenses against *Foc*, we ectopically expressed the MP protein in *Nicotiana benthamiana* (35S:MP) plants. 35S:MP transgenic plants exhibited a dwarfing phenotype compared with the wild-type (WT) controls (Fig. 4a). Transgenic lines #1 and #2, which accumulated high levels of MP, were selected for further analysis (Fig. 4b). H_2O_2 levels were higher in 35S:MP plants than in WT plants, either in the presence or absence of *Foc* (Fig. 4c, d). Moreover, more H_2O_2 was induced in 35S:MP and WT plants inoculated with *Foc* at 3 dpi (Fig. 4c, d). Meanwhile, the 35S:MP plants showed significant resistance to *Foc* compared with WT plants after inoculation with FocTR4-GFP, as assayed by lesion measurement and western blot assays for GFP (Fig. 4e, f). Thus, MP expression in *N. benthamiana* could cause the increase of the H_2O_2 level to suppress the *Foc* infection.

SGT1 facilitates steady-state levels of the R protein and plays an important role in R protein-triggered plant defenses (Azevedo et al. 2006). Yeast two-hybrid (Y2H) assay showed that MP interacts with SGT1 of bananas (MaSGT1a) (Fig. 5a). Bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (Co-IP) assays further confirmed that MP directly interacted with MaSGT1a (Fig. 5b, c). Co-expression in *N. benthamiana* showed that MP and MaSGT1a co-localized in cell membrane and the cytoplasm (Additional file 1: Figure S1). The expression level of *MaSGT1a* in banana was increased by BBTV and *Foc* co-infection (Fig. 5d), whereas its homologous gene *MaSGT1b* and *MaSGT1c* were not (Additional file 1: Figure S2). These results suggest that the MP-MaSGT1a interaction may involve in the resistance of bananas against *Foc*. When co-infected with both BBTV and *Foc*, the expression of *MaSGT1a* was higher than that in banana infected with either pathogen alone (Fig. 5d). This suggests that either BBTV or *Foc* infection can activate the expression of *MaSGT1a*, and this activation is cumulative. To investigate the effect of MP on MaSGT1a via protein-protein interactions, MP tagged with GFP (MP-GFP) and MaSGT1a tagged with His (MaSGT1a-His) were transiently expressed in *N. benthamiana* leaf cells through agroinfiltration to examine the relationship between MP and MaSGT1a in plants. Western blot assays showed that the protein accumulation level of MaSGT1a increased with increasing MP (Fig. 5e). This result demonstrates that MP expression promoted the accumulation of MaSGT1a. The expression of MaSGT1a in *N. benthamiana* also induced the production of H_2O_2 like MP, especially in the leaves of *N. benthamiana* inoculated with *Foc* for 2 days, as assayed by DAB staining and H_2O_2 content assay kits (Fig. 5f, g). Co-expression of MP and MaSGT1a caused elevated H_2O_2 production than single expression after inoculation with *Foc* (Fig. 5f, g). Thus, the elevated production of H_2O_2 induced by MP may be due to its interaction with MaSGT1a.

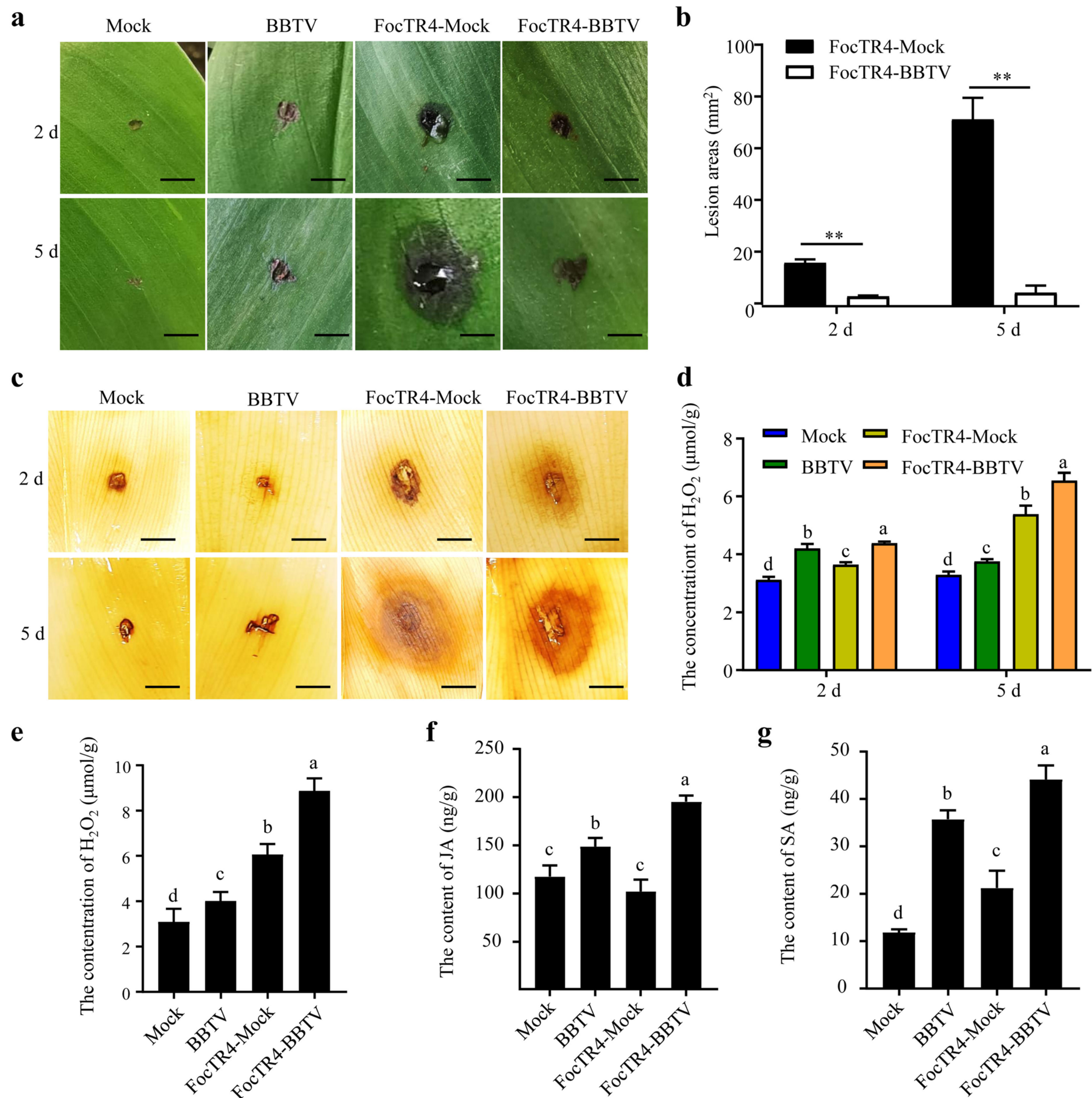

Fig. 3 BBTV infection induces the production of H_2O_2, JA, and SA. **a** Lesions caused by FoTR4 in the leaves of BBTV-infected and uninfected bananas at 2 and 5 dpi. Scale bars: 2 mm. **b** Calculation of the lesion area in **a**. **c** DAB staining determined the H_2O_2 levels of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 2 and 5 dpi. Scale bars: 2 mm. **d** H_2O_2 content levels of BBTV-infected and uninfected bananas after inoculation at 2 and 5 dpi, as determined by the H_2O_2 content assay kit. **e**, **f**, and **g** H_2O_2, JA, and SA levels in the pseudostems of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 15 dpi. Mock, the healthy banana as a control. BBTV, BBTV-infected bananas. FocTR4-Mock, healthy banana plants inoculated with FocTR4. FocTR4-BBTV, BBTV-infected bananas inoculated with FocTR4. Five leaf-stage banana seedlings were selected for inoculation. Mean ± SD in panels **b**, **d**, **e**, **f** and **g** are shown for ten banana plants and represent three biological replicates. The significance of differences in **b** was determined using a two-tailed Student's t-test. *$P < 0.05$; **$P < 0.01$. The significance levels in **d**–**g** were tested based on multiple comparisons using two-way ANOVA. Different lowercase letters at the top of each column indicate significant differences ($P \leq 0.01$)

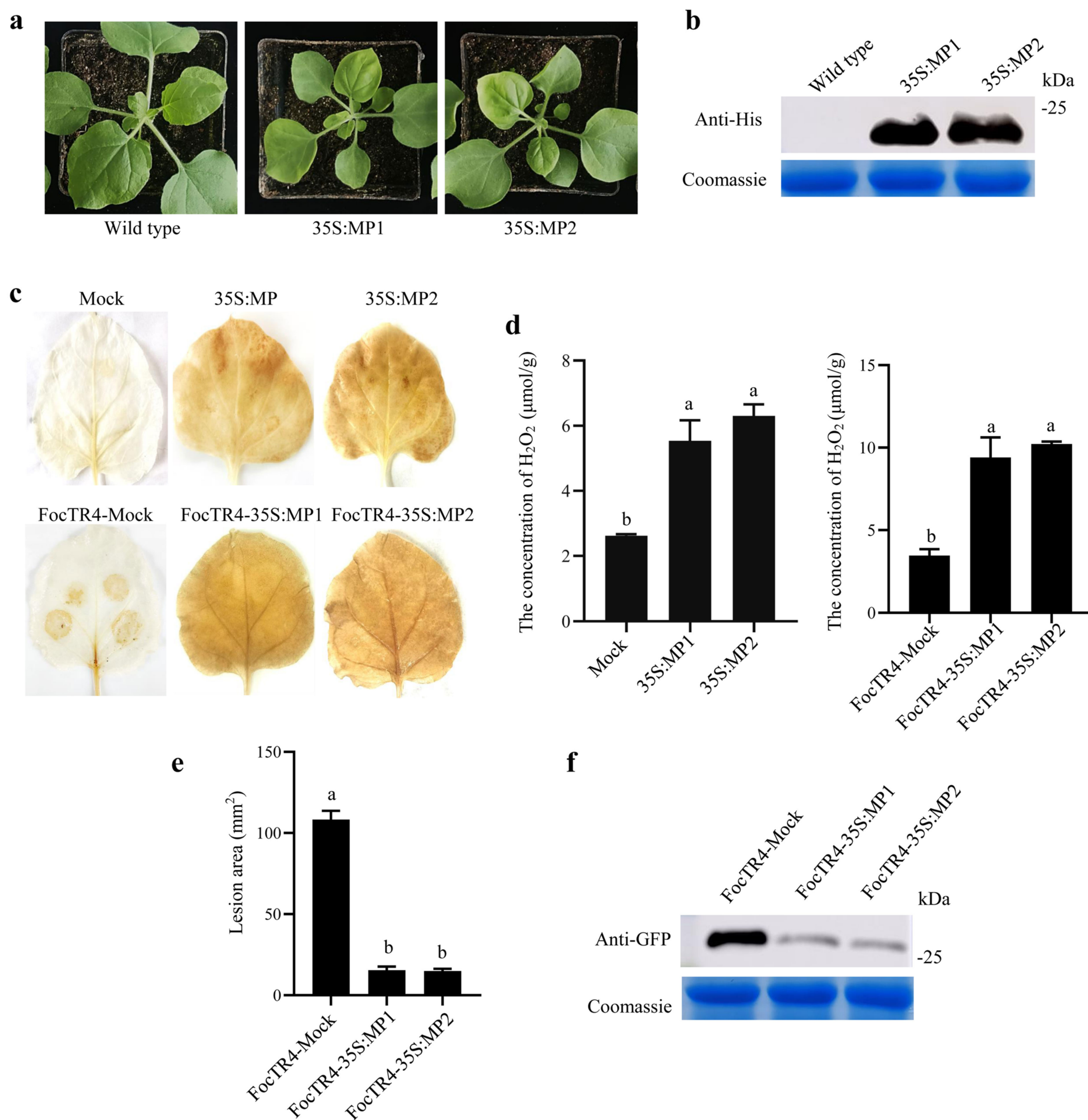

Fig. 4 Overexpression of MP in *N. benthamiana* increases H_2O_2 levels and suppresses *Foc* infection. **a** Phenotype of 35S:MP compared with that of wild-type plants. **b** Accumulation levels of MP in 35S:MP, as determined by western blot assays. Wild-type plants served as the controls. Coomassie blue staining for RuBisCO was used as the loading control. **c**, **d** H_2O_2 levels of wild-type (mock) and 35S:MP *N. benthamiana* plants which had been inoculated with FocTR4 for 3 days, as determined by DAB staining and H_2O_2 content assay. **e** Lesion size of wild-type (mock) and 35S:MP caused FocTR4 at 3 dpi. **f** Western blot assays of the relative fungal growth of FocTR4 in inoculated banana leaves at 3 dpi using an antibody against GFP. The FocTR4 strain was labeled with GFP. Mock, wild-type. FocTR4-Mock, wild-type plants inoculated with FocTR4. FocTR4-35S:MP, 35S:MP plants inoculated with FocTR4. Five leaf-stage *N. benthamiana* seedlings were inoculated with FocTR4. Mean ± SD are shown for ten *N. benthamiana* plants and represent three biological replicates. Significance levels were tested based on multiple comparisons using two-way ANOVA. Different lowercase letters at the top of each column indicate significant differences ($P \leq 0.01$)

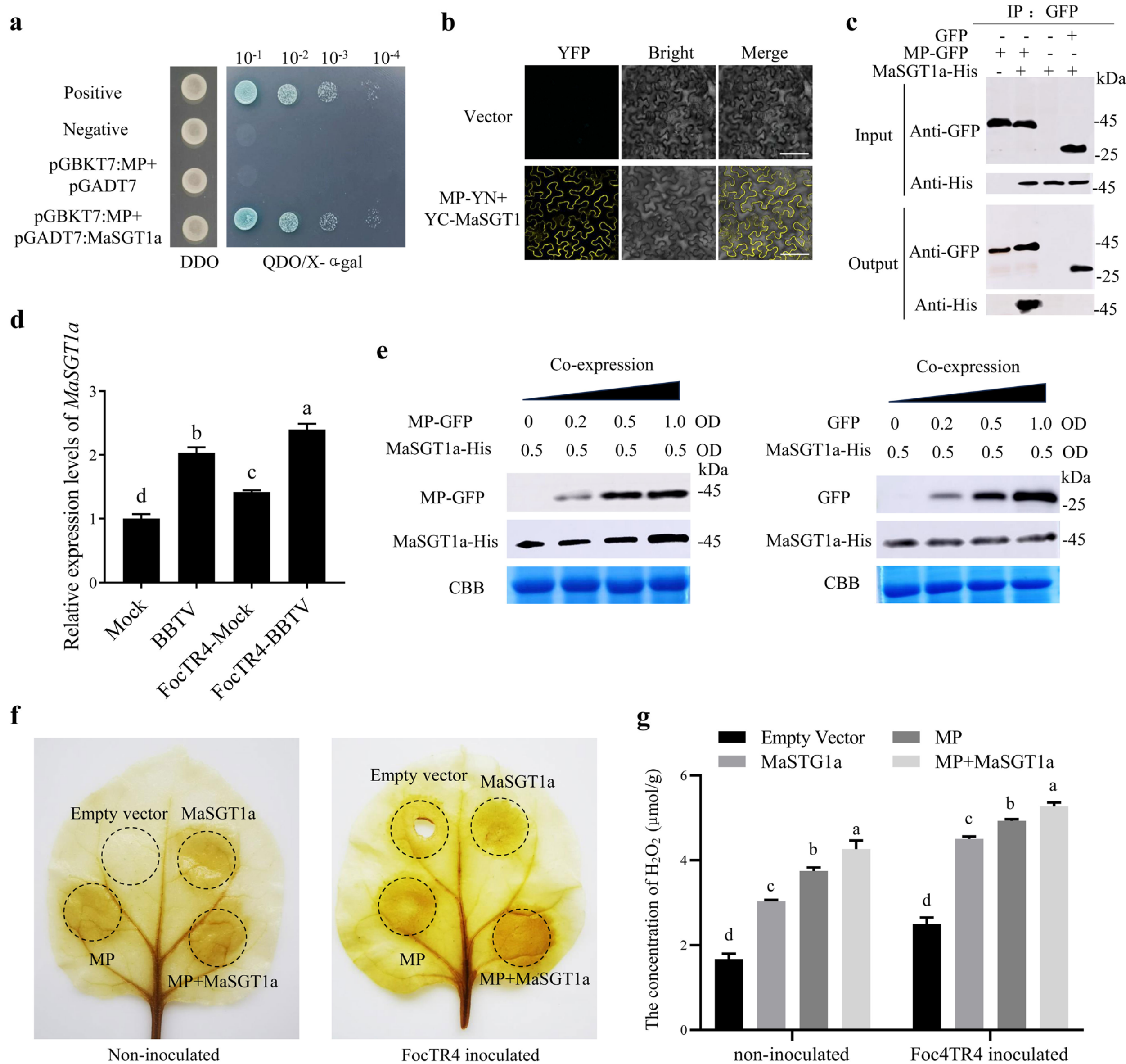

Fig. 5 MP promotes the accumulation of MaSGT1a protein through direct interactions. **a**–**c** Yeast two-hybrid, BiFC, and CO-IP assays showing the interaction between MP and MaSGT1a. DDO, SD-Trp-Leu medium. QDO, SD-Trp-Leu-His-Ade medium with 2.5 mM 3-AT. **b** MP fused with the N-terminus of YFP (MP-YN) and MaSGT1a fused with the C-terminus of YFP (YC-MaSGT1a) were co-expressed in *N. benthamiana* for BiFC. Scale bars: 150 μm. **c** MP fused with GFP (MP-GFP) as bait, whereas MaSGT1a fused with His (MaSGT1a- His) as the prey. The bait or GFP control was co-expressed with MaSGT1a- His in *N. benthamiana*. Input and affinity-isolation samples were detected by western blot assays using antibodies against GFP or His. **d** The expression levels of MaSGT1a in the pseudostem of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 15 dpi. Mock, the healthy banana as a control. BBTV, BBTV-infected banana plants. FocTR4-Mock, healthy bananas inoculated with FocTR4. FocTR4-BBTV and BBTV-infected bananas inoculated with FocTR4. Five-leaf stage banana seedlings were selected for inoculation. **e** Expression of MP promotes the protein accumulation of MaSGT1a, as assayed by western blot assays. OD (0.5) *Agrobacterium* expressing MaSGT1a-His was co-infiltrated with *N. benthamiana* leaves at different concentrations (0, 0.2, 0.5, and 1.0 OD) *Agrobacterium* expressing MP-GFP and with expressing GFP as control. Coomassie blue staining for RuBisCO was used as the loading control. **f**, **g** H_2O_2 levels of MP and MaSGT1a expressed *N. benthamiana* leaves that had been inoculated with FocTR4 for 2 days or not, as determined by DAB staining and H_2O_2 content assay. Non-inoculation, leaves of *N. benthamiana* were not inoculated. FocTR4-inoculated leaves of *N. benthamiana* were inoculated with FocTR4 at 1-day post-co-infiltration. Mean ± SD are shown for ten *N. benthamiana* plants and represent three biological replicates. The significance levels were tested based on multiple comparisons using two-way ANOVA. Different lowercase letters at the top of each column indicate significant differences ($P \leq 0.01$)

MaSGT1a facilitates the accumulation of R proteins

SGT1 proteins are highly conserved among eukaryotes and often interact with RAR1 and HSP90 to form the molecular chaperone complex which is required by NLRs for disease resistance (Takahashi et al. 2003; van Wersch et al. 2020). Y2H and BIFC were used to test the interaction between MaSGT1a and MaRAR1 (Additional file 1: Figure S3). Four homologs of the NLR genes *RPS2, RPM1, RPP8*, and *RPP13,* which are involved in *R* gene-mediated plant defense against other *F. oxysporum* species, was found in banana genome database. To confirm whether the four *R* genes respond to BBTV and *Foc* infection, the expression levels of *R* genes induced by BBTV or *Foc* infection were tested. It was showed that BBTV infection increased the expression of all four *R* genes, whereas only the expression of *MaRPM1* and *MaRPP8* was upregulated in the roots at 15 dpi compared with the control plants (Fig. 6a). Co-infection with BBTV and *Foc* induced higher expression of the four *R* genes than infection with BBTV or *Foc* alone (Fig. 6a). Thus, *MaRPM1* and *MaRPP8* showed transcriptional stimulation during *Foc* infection, suggesting that they may play an important role in defense against *Foc* infection. The co-expression experiments in *N. benthamiana* revealed the co-localization of MaSGT1a with MaRPM1 and MaRPP8 (Additional file 1: Figure S4). The protein levels of MaRPM1 and MaRPP8 were increased with the increasing expression of MaSGT1a, as determined by western blot assays (Fig. 6b). This finding suggests that MaSGT1a stimulated the accumulation of MaRPM1 and MaRPP8 proteins. Additionally, the expression of MaRPM1 and MaRPP8 led to increased levels of H_2O_2, as detected by DBA staining and the H_2O_2 content assay kit (Fig. 6c–f). Co-expression of MaRPM1-MaSGT1a and MaRPP8-MaSGT1a caused more H_2O_2 than single expression when infected with *Foc* (Fig. 6c-f). Therefore, *Foc* infection induced MaRPP8- and MaRPM1-related defense which caused ROS accumulation. Simultaneously, the expression of MaRPP8 and MaRPM1, especially co-expression with MaSGT1a, effectively suppressed *Foc* infection (Fig. 6g). Thus, MaRPP8 and MaRPM1 may be the potential *R* genes in banana that respond to *Foc* infection. This finding suggests that MaSGT1a promotes the accumulation of some R-proteins and plays an important role in *R*-related resistance. The resistance to *Foc* induced by MP in BBTV-infected bananas may be caused by interacting with MaSGT1a to facilitate the accumulation of R proteins.

Discussion

Fusarium oxysporum, a soil-borne fungal pathogen, causes significant economic losses in various crop plants, particularly banana, by inducing necrosis and wilting. This pathogen causes banana *Fusarium* wilt, also known as 'Panama disease' which results in significant economic losses to banana plantations worldwide (Ploetz 2015). BBTV causes bunchy top disease in banana, which is considered one of the most economically destructive viral diseases (Qazi 2015). The MP of BBTV is responsible for the systemic transport of BBTV through the xylem and phloem of banana (Wanitchakorn et al. 2000), enabling interactions with *Foc* and among themselves. The antagonistic relationship between BBTV and *Foc* is commonly observed in the field, making BBTV-infected plants showed more tolerance to *Foc* (Zhuang et al. 2016). However, the functional mechanisms underlying this antagonism remain unclear. Although previous studies have found that MP displays fungicidal properties to suppress *Foc* infection (Zhuang et al. 2016), plant defenses induced by MP are still important for studying the antagonism of BBTV against *Foc*. In this study, we found that a series of resistance-related genes, which are part of either the pathogen-triggered immunity (PTI) or ETI system, were upregulated in *Foc*-infected plants that were previously infected with BBTV. Therefore, BBTV infection induced a comprehensive systemic resistance response to *Foc*. Notably, we discovered that MP expression induced plant resistance to *Foc*. MP interacts with MaSGT1a, which plays an important role in *R* gene-mediated plant defense. MaRPM1 and MaRPP8 protein accumulation

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Fig. 6 MaSGT1a expression promotes MaRPM1 and MaRPP8 accumulation in *N. benthamiana*. **a** Expression levels of MaRPS2, MaRPM1, MaRPP8, and MaRPP13 in the pseudostems of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 15 dpi. Mock, the healthy banana as a control. BBTV, BBTV-infected bananas. FocTR4-Mock, healthy bananas inoculated with FocTR4. FocTR4-BBTV, BBTV-infected bananas inoculated with FocTR4. Five leaf-stage banana seedlings were selected for inoculation with FocTR4. **b** Expression of MaSGT1a promotes the accumulation of MaRPM1 and MaRPP8, as assayed by western blot assays. OD (0.5) *Agrobacterium* expressing MaRPM1-GFP or MaRPP8-GFP was co-infiltrated into *N. benthamiana* leaves at different concentrations (0, 0.2, 0.5, and 1.0 OD) of *Agrobacterium* expressing MaSGT1a-His. Coomassie-blue staining for RuBisCO served as a loading control. **c**–**f** H_2O_2 levels of MaSGT1a expressed with MaRPM1 or MaRPP8 in *N. benthamiana* leaves that had been inoculated with FocTR4 for 2 days or not, as determined by DAB staining and H_2O_2 content assay. Mean ± SD were shown for ten *N. benthamiana* plants and represent three biological replicates. The significance levels were tested based on multiple comparisons by two-way ANOVA. Different lowercase letters at top of each column indicate significant differences ($P \leq 0.01$). **g** Western blot assays of the relative fungal growth of FocTR4 in inoculated banana leaves at 2 dpi using an antibody against GFP. Coomassie-blue staining for RuBisCO served as the loading control. FocTR4 strain was labeled with GFP. Non-inoculation, Leaves of *N. benthamiana* were not inoculated. FocTR4-inoculated, leaves of *N. benthamiana* were inoculated with FocTR4 1-day post-coinfiltration

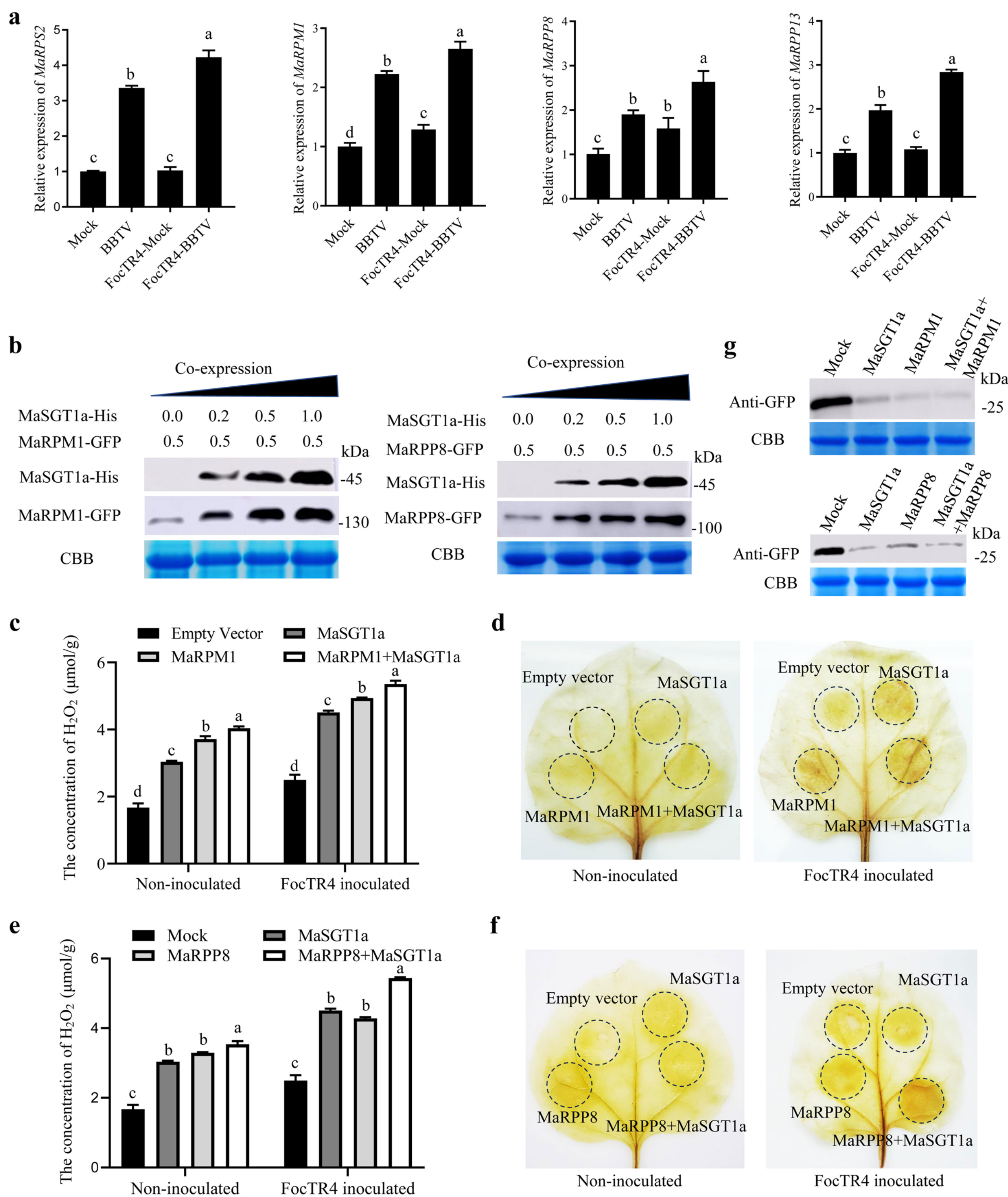

Fig. 6 (See legend on previous page.)

was promoted by MaSGT1a expression, which triggered plant immune responses, including ROS production. This suggests that MP may enhance plant defenses mediated by *R* genes through its interaction with MaSGT1a (Fig. 7).

A recent study suggested that ROS plays an important role as signaling molecules in the banana defense response against *Foc* (Swarupa et al. 2014). In addition, BBTV stimulates the production of H_2O_2 during *Foc*

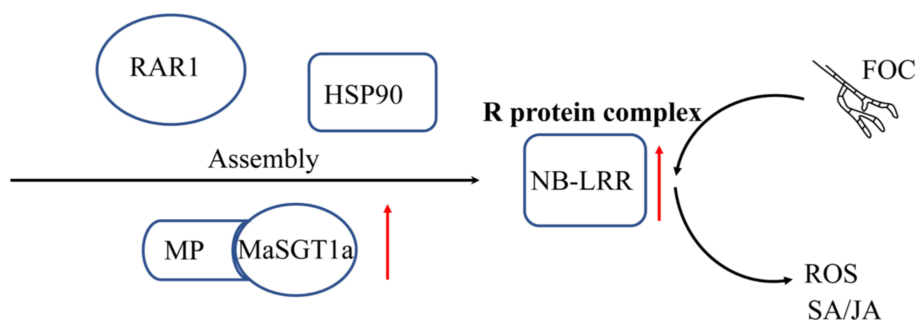

Fig. 7 Proposed model of BBTV inducing the immunity of bananas to antagonize *Foc* infection. BBTV infection increases the protein accumulation of MaSGT1a by the direct interaction between MaSGT1a and MP. MaSGT1a interacts with RAR1 (required for Mla12 resistance) and HSP90 (heat shock protein 90) to form the molecular chaperone complex that play important role in facilitating steady-state levels of the R protein. The increasing MaSGT1a induced by BBTV promote the accumulation of R protein to trigger stronger immune response such as ROS, SA, and JA, during *Foc* infection

infections. MP expression can lead to elevated levels of H_2O_2 in *N. benthamiana*, implying that MP may be responsible for promoting H_2O_2 production. In response to pathogen-associated molecular patterns (PAMPs) and effector-triggered immunity (ETI), ROS are rapidly produced in an oxidative burst upon pathogen infection, predominantly owing to the activity of membrane-localized NADPH oxidases (Torres et al. 2006). Previous studies have shown that SA and JA play important roles in plant defense against *Fusarium* wilt (Sun et al. 2013; Wang et al. 2015). The production of ROS, SA, and JA and the expressions of genes involved in plant-pathogen interactions, such as MAPK signal transduction, plant hormone, ROS and hypersensitive response, suggested that the resistance to *Foc* induced by BBTV is a result of a complex process, including PTI and ETI, determined by MP mediated *R*-gene related resistance and quantitative resistance. The defense responses of PTI triggered by viruses or fungal pathogens are always similar and easily broken by fungal pathogen as the first level of defence of the plant innate immunity (Calil and Fontes 2016). Thus, we presume that the R-related resistance mediated by MP of BBTV and MaSGT1a may play vital roles in resistance to FOC infection. However, the DEGs related to resistance in BBTV-infected plants inoculated with *Foc* still served a valuable gene resource for exploring resistance genes.

Plants have developed *R* genes that specifically identify the corresponding avirulence (avr) genes to activate plant defenses via a mechanism known as gene-for-gene resistance (Dangl and Jones 2001). The highly variable LRR portions of plant R proteins have been shown to play a role in pathogen recognition and signal transduction (Dangl and Jones, 2001). SGT1 is a highly conserved eukaryotic protein that positively regulates the disease resistance conferred by many R proteins in plants (Azevedo et al. 2006; Muskett and Parker 2003; Takahashi et al. 2003). MaSGT1a is also involved in banana resistance by facilitating the accumulation of the R proteins MaRPM1 and MaRPP8. The MP of BBTV indirectly mediates R-related resistance through promoting the accumulation of MaSGT1a via direct interaction. However, it is not clear whether MaSGT1 has a general role in maintaining the stabilities of NLRs in banana. Further research is needed to clarify the function of MaSGT1 in resistance against *Foc*. Furthermore, although the expression of MaRPM1 and MaRPP8 in *N. benthamiana* promoted the resistance to *Foc*, MaRPM1 and MaRPP8 still cannot be identified as the effective *R*-genes of banana against *Foc* infection. Despite the absence of any commercially available cultivar with resistance to *Fusarium* wilt and lack of effective *R*-genes, proteins like SGT1 that play a role in assembling R protein complexes and maintaining R protein stability, could serve as potential options for genetic improvement and developing resistance against *Fusarium*.

Conclusions

In this study, we reveal the mechanism of resistance induced by BBTV against banana *Fusarium* wilt through the interaction between MP and MaSGT1a to trigger NLRs-mediated resistance. The infection of BBTV promote the resistance of banana to *Foc* by up-regulating resistance-related genes and inducing ROS production. MP of BBTV increased H_2O_2 levels by promoting the accumulation of MaSGT1a via direct interaction. MaSGT1a could promote the accumulation of R proteins MaRPM1 and MaRPP8. The expression of MaSGT1a or R proteins in *N. benthamiana* caused an increase in H_2O_2 levels and increased resistance to *Foc*. Thus, SGT1 as a component in assembling R protein complexes would be potential options for genetic improvement and developing resistance against *Fusarium*.

Methods

Fungal strains and plants

The experimental materials used were 'Brazilian' (Musa AAA), provided by the Subtropical Agriculture Research Institute of the Fujian Academy of Agricultural Sciences. The banana wilt pathogen strain *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (FocTR4), was tagged with GFP using the protoplasts transformation system with the plasmid pCT74 containing a *GFP* gene (Guo et al. 2015). FocTR4 strains were cultivated on potato dextrose agar (PDA) at 28 °C. The banana plantlets were grown in a greenhouse at 26–28 °C under an 8:16 h light:dark cycle.

Preparation and treatment of banana seedlings

The banana seedlings at the five-leaf stage were fed on by ten viruliferous *Pentalonia nigronervos* for 2 days to acquire BBTV-infected plants. Seven days after aphid feeding, banana seedlings were inoculated with GFP-tagged FocTR4 conidia on the root, as described by Wang et al. (2023), with banana plants without viruliferous aphid feeding (mock) as a control. The banana roots were slightly wounded, immersed in a 1×10^5 spores/mL suspension, and placed on a shaker for 8 h at 50 rpm/min before being planted in pots. At 6, 15, 30 dpi, mock-inoculated and BBTV-infected plants inoculated with FocTR4 were used to assay the pathogenicity, and the levels of JA, SA, and H_2O_2. BBTV-infected plants were confirmed by PCR detection of *CP* genes before inoculation. Each treatment consisted of 20 banana plants.

Calculation of lesion area, estimation of fungal biomass, and confocal microscopy observation of infection

At 30 dpi, the banana pseudostems with different treatments were symmetrically cut along the central line of the corms, photographs were taken with a ruler aside from the corms, and the lesion area was calculated using the ImageJ software.

Fungal biomass was determined at 6 and 15 dpi using a previously described method (Thatcher et al. 2009). Total RNA was extracted from different tissues using the TRIzol reagent (Thermo Fisher Scientific, 15596026). RT-qPCR was performed using 2×RealStar Fast SYBR qPCR Mix (Genstar, A303) in a QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). Elongation factor 1-a (*EF1a*) from FocTR4 was used to quantify fungal colonization, and the banana actin gene (*Musa-Actin*) was used as an endogenous reference control. Experiments were performed in triplicate and a pool of total RNAs from five inoculated plants was used for each replicate. The primers used in the RT-qPCR assays are listed in Additional file 1: Table S1.

For microscope observation, banana roots were prepared and observed under Leica TCS SP5II confocal microscope. To determine the fungal hyphae inside the roots, longitudinal sections of secondary roots of inoculated plants at 6 dpi and transverse sections of primary roots of inoculated plants at 15 dpi were used to observe the appearance of fungi with GFP (488 nm). The fluorescence intensity was calculated by Image J software.

RNA-seq and data analysis

To understand the changes in transcription profiles of FocTR4-inoculated banana plants induced by BBTV infection, the roots of the mock-inoculated (FocTR4-Mock) and BBTV-infected (FocTR4-BBTV) banana plants that were re-inoculated with FocTR4 at 15 dpi were sampled for sequencing. RNA extracted from the roots of five plants constituted one replicate with three replicates per treatment. RNA samples were sequenced using Illumina Novaseq 6000 (Gene Denovo Biotechnology Co., Guangzhou, China). The reference sequence was obtained from *Musa acuminata* subsp. *malaccensis* ASM31385v2. The mapped reads of each sample were assembled using the StringTie v1.3.1. For each transcription region, a fragment per kilobase of transcript per million mapped reads (FPKM) value was calculated to quantify the expression abundance and variations using RSEM software. DEGs were detected using DESeq2 with a $|\mathrm{Log}_2$ (fold change)$|>1$ and a false discovery rate (FDR)-adjusted $P<0.01$. GO and KEGG analyses of the DEGs were performed using the Gene Denove online web server (https://www.omicshare.com/tools/). Heatmaps of DEG expression profiles were generated using R 3.6.0. with the average FPKM of FocTR4-Mock and FocTR4-BBTV treatments, respectively.

SA and JA content analysis

The roots of mock and BBTV-infected plants inoculated with FocTR4 at 15 dpi were used to determine the SA and JA content. Fresh samples were ground into a uniform powder using a mortar and pestle in liquid nitrogen. Approximately 50 mg (±0.5 mg) of the sample powder was weighed for analysis. The JA and SA contents of the roots were analyzed by high-performance liquid chromatography-mass spectrometry (UPLC-MS/MS) using labeled internal standards. Experiments were performed in triplicate and samples mixed with five inoculated plants were used for each replicate.

RT-qPCR and western blot assays

To compare the relative transcript levels of *MusaSGT1*, *MusaRPS2*, *MusaRPM1*, *MusaRPP8*, and *MusaRPP13* in the roots of banana plants induced by BBTV or *Foc* infections, the roots of mock-inoculated and BBTV-infected plants that were re-inoculated with FocTR4 at 15 dpi were used for RT-qPCR analysis. Total RNA was extracted from the banana plants using a polysaccharide

and polyphenol removal kit. RT-qPCR was performed using 2×RealStar Fast SYBR qPCR Mix (Genstar, A303) in the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). The primers used in the RT-qPCR are listed in Additional file 2: Table S1. The transcript level of the banana actin gene (*Musa-Actin*) served as an internal reference for normalization of gene expression levels. The relative genes expression levels were estimated using the $2^{-\Delta\Delta CT}$ (cycle threshold) method. Experiments were performed in triplicate and a pool of total RNAs from five inoculated plants was used for each replicate.

The protein expression levels of GFP which represent the biomass of FocTR4 tagged with GFP in different *N. benthamiana* leaves inoculated with FocTR4 at 3 dpi, were analyzed using western blot assays. Total protein from *N. benthamiana* leaves was extracted using RIPA lysis buffer (Thermo Fisher). Extracted proteins were analyzed by western blot assays. Antibodies against GFP (0.5 μg/μL) served as primary antibodies, and goat anti-mouse IgG-peroxidase (Sigma-Aldrich, A0545) (0.5 μg/μL) served as secondary antibodies. The experiments were performed in triplicate. The Rubisco large subunit was used as a loading control and stained with Coomassie Brilliant Blue. Protein band intensities were quantified using the ImageJ software (version 1.53e; https://imagej.nih.gov/ij/). Bands were detected using the Luminata Classico Western HRP Substrate (Millipore, WBKLS0500) and imaged with a highly sensitive CCD imaging system in Amersham Imager 600 (General Electric Company, USA).

H_2O_2 content assay and DAB staining of H_2O_2

The H_2O_2 content in banana roots and *N. benthamiana* leaves was examined using a Hydrogen Peroxide assay kit (Beyotime-S0038), according to the manufacturer's instructions. Absorbance was measured at 560 nm, and a series of known H_2O_2 concentrations and absorbance were measured at 240 nm to establish a standard curve. The absorbance of the samples and standard substances was measured using a SPARK 10M microplate spectrophotometer (TECAN, Austria). The roots of mock-inoculated and BBTV-infected plants which were then inoculated with FocTR4 at 15 dpi and the *N. benthamiana* leaves that expressed different proteins and were inoculated with FocTR4 were used to assay the H_2O_2 content level. Experiments were performed in triplicate and samples mixed with five inoculated plants were used for each replicate.

The stain DAB (Sigma) was used to determine the H_2O_2 location and visualize its accumulation in *N. benthamiana* leaves. *N. benthamiana* leaves expressing different proteins and inoculated with FocTR4 were infiltrated with 1 mg/mL LDAB (dissolved in 10 mM Tris-HCl, pH 6.5) solution at 37°C in the dark for 16 h. The leaf samples were then washed with a decolorization solution (ethanol:acetic acid = 3:1) to bleach chlorophyll at 70°C for 30 min. Leaves were photographed using an optical microscope equipped with a stereoscope.

Overexpressed MP in *N. benthamiana* transgenic lines

Full-length MP-His coding sequence was amplified and cloned into the restriction enzyme *Xcm*I-digested site of PcxSN. The recombinant plasmid PcxSN-MP-His was freeze-thawed in *Agrobacterium* strain GV3101 and leaf disc transformation was used to transform *N. benthamiana* leaves. Overexpressed MP (35S:MP) transgenic *N. benthamiana* lines were detected by western blot assays using a His-tag antibody. Wild-type *N. benthamiana* was used as the control. The leaves of the six-leaf stage transgenic seedlings were inoculated with FocTR4. At 2 dpi, FocTR4-inoculated leaves were analyzed for H_2O_2 levels using the H_2O_2 content assay and DAB staining, and the lesion area was calculated using ImageJ software. The biomass of FocTR4 strains was detected by western blot assays using a GFP-tagged antibody.

Yeast two-hybrid assay

To test the interaction between the MP of BBTV and MusaSGT1 in banana plants, a yeast two-hybrid assay was performed using the Matchmaker Gal4 Two-Hybrid System 3 (Clontech). BBTV-MP gene was constructed using the bait plasmid pGBKT7. MusaSGT1 genes from banana plants were amplified and constructed using the prey plasmid, pGADT7. The bait and prey plasmids were used to co-transform the yeast strain AH109. The transformants were cultured on SD/-Leu/-Trp (DDO; Clontech, 630,417) plates, and positive clones were transferred and grown on SD/-Leu/-Trp/-His/-Ade/X-a-gal (QDO; Clontech, 630,428). The interaction of pGBKT7-53 and pGADT7-T served as a positive control and the interaction of pGBKT7-Lamp and GADT7-T served as a negative control.

BIFC assay

Full-length MP were cloned into the *Bam*HI sites of pEarleyGate-YN containing the N-terminal end of YFP to obtain MP-YN and MusaSGT1 was cloned into the *Bam*HI site of pEarleyGate-YC containing the C-terminal fragment of YFP to generate SGT1-YC. All constructs were verified by sequencing. *Agrobacterium* strain GV3101 harboring the corresponding constructs (SGT1-YC/MP-YN and YN/YC) was co-infiltrated into *N. benthamiana*. At 48 h post infiltration (hpi), the fluorescence signal was observed using a scanning confocal microscope. Each experiment was repeated at least three times.

Co-immunoprecipitation (CoIP) assay

The full MP-GFP and MusaSGT1-His coding sequences were cloned into the plant binary expression vector pYFP-N104at the *Bam*HI site to generate pYFPN104-SGT1-His and pYFPN104-MP-GFP. *Agrobacterium* strain GV3101 harboring the corresponding constructs pYFPN104-SGT1-His and pYFPN104-MP-GFP was co-infiltrated into *N. benthamiana*, with the co-infiltration of pYFPN104-SGT1-His and pYFPN104-GFP as controls. At 48 hpi, total protein was extracted from the infiltrated *N. benthamiana* leaves and incubated with GFP-Trap agarose beads (Beyotime). Immunoprecipitated proteins analyzed by western blot assays using His and GFP antibodies (Proteintech). Each experiment was repeated three times.

Transient expression in *N. benthamiana*

Full-length MaSGT1a-His and MP-GFP were fused at the *Bam*HI sites of the pYFPC104 vector to obtain pYFPN104-SGT1a-His and pYFPN104-MP-GFP vectors. These constructs were individually transformed into *Agrobacterium* strain GV3101 and infiltrated into *N. benthamiana*. The injection optical density of pYFPN104-MP-GFP was at OD_{600} values of 0, 0.2, 0.5, and 1.0, whereas pYFPN104-SGT1a-His was at OD_{600} of 0.5. At 48 hpi, the accumulation of SGT1a-His and MP-GFP proteins was detected by western blot assays using His-tagged and GFP-tagged antibodies. *N. benthamiana* leaves with single expression and co-expression of MaSGT1a and MP were inoculated with FocTR4 tagged with GFP at the injection site at 24 hpi. Infiltration with pYFPN104 was used as a control (empty vector). Transiently expressed leaves inoculated with FocTR4 at 2 dpi and non-inoculated leaves were used to determine H_2O_2 levels by H_2O_2 content assay and DAB staining.

Full-length MaRPM1 and MaRPP8 genes were amplified and cloned into the pYFPN104 vector to obtain the pYFPN104-MaRPM1-GFP and pYFPN104-MaRPP8-GFP vectors. These constructs were individually transformed into *Agrobacterium* strain GV3101 and infiltrated into *N. benthamiana*. After co-injection with pYFPN104-SGT1a-His, the injection optical density of pYFPN104-SGT1a-His was at OD_{600} values of 0, 0.2, 0.5, and 1.0, whereas that of pYFPN104-MaRPM1-GFP or pYFPN104-MaRPP8-GFP was at an OD_{600} of 0.5. At 48 hpi, the accumulation of protein levels of SGT1a-His, MaRPM1-GFP, and MaRPP8-GFP was detected by western blot assays using His-tagged and GFP-tagged antibodies. MaRPM1-GFP and SGT1a-His, MaRPP8-GFP and SGT1a-His were individually expressed and co-expressed in *N. benthamiana* leaves with an empty vector as a control. The transiently expressed leaves were inoculated with FocTR4 tagged with GFP on injection location at 24 hpi. At 2 dpi, FocTR4 inoculated transiently expressed leaves were assayed by western blot assays using a GFP-tagged antibody to detect the biomass of FocTR4 tagged with GFP. The transiently expressed leaves inoculated with FocTR4 at 2 dpi and non-inoculated leaves were used to determine H_2O_2 levels by H_2O_2 content assay and DAB staining.

Statistical analysis

Data were analyzed using Microsoft Excel and GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA). The two-tailed Student's t-test and two-way analysis of variance (2-way ANOVA) used for statistical analyses were performed as indicated in the figure legends.

Abbreviations

BBTV	Banana bunchy top virus
BBTD	Banana bunchy top disease
DAB	3,3′-Diaminobenzidine
DEGs	Differentially expressed genes
Dpi	Days post-inoculation
ETI	Effector triggered immunity
Foc	*Fusarium oxysporum* f. sp. *cubense*
GO	Gene ontology
HSP90	Heat shock protein 90
JA	Jasmonate
KEGG	The kyoto encyclopedia of genes and genomes
MeJA	Methyl jasmonate
MP	Movement protein
NLRs	Nucleotide-bounding leucine-rich repeat proteins
PTI	Pattern-triggered immunity
RAR1	Required for Mla12 resistance
ROS	Reavtive oxygen species
SA	Salicylic acid
SGT1	Suppressor of the G-two allele of SKP1
TR4	Tropical race 4
WT	Wild-type

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00242-z.

Additional file 1: Figure S1. Co-localization of MaSGT1a with MP expressed in *N. benthamiana*. **Figure S2.** The expression levels of MaSGT1b and MaSGT1c in the pseudostem of BBTV-infected and uninfected bananas after inoculation with FocTR4 at 15 dpi. **Figure S3.** MaSGT1a interacts with MaRAR1. **Figure S4.** Co-localization of MaSGT1a with MaRPM1 and MaRPP8 expressed in *N. benthamiana*.

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

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Authors' contributions
HZ and TW designed the research. WW, WW, and HZ performed the research. WW, HZ, QC, and TW analyzed the data. HZ and TW wrote the paper. All authors read and approved the manuscript.

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Availability of data and materials
The datasets generated in the current study are available in NCBI repository: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1065648.

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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