Journal of Cotton Research

RESEARCH Open Access

Down regulation of cotton *GbTRP1* leads to accumulation of anthranilates and confers resistance to *Verticillium dahliae*

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Abstract

Background: Verticillium wilt, caused by *Verticillium dahliae*, is called a "cancer" disease of cotton. The discovery and identification of defense-related genes is essential for the breeding of Verticillium wilt-resistant varieties. In previous research we identified some possible broad-spectrum resistance genes. Here, we report a tryptophan synthesis-related gene *GbTRP1* and its functional analysis in relation to the resistance of cotton to *V. dahliae*.

Results: Expression analysis shows that *GbTRP1* is suppressed at 1 h and 6 h post *V. dahliae* infection, but activated at 12 h and 24 h, and the expression of *GbTRP1* is highly induced by treatment with salicylic acid and jasmonic acid. Sub-cellular localization studies show that GbTRP1 is localized in the chloroplast. Suppression of *GbTRP1* expression leads to lesion-mimic phenotypes and activates the immune response in cotton by showing enhanced resistance to *V. dahliae* and *B. cinerea*. Metabolomic analysis shows that anthranilic compounds significantly accumulated in *GbTRP1*-silenced plants, and these metabolites can inhibit the growth of *V. dahliae* and *B. cinerea* in vitro.

Conclusions: Our results show that suppression of *GbTRP1* expression dramatically activates the immune response and increases resistance of cotton to *V. dahliae* and *B. cinerea*, possibly due to the accumulation of anthranilate compounds. This study not only provides genetic resources for disease resistance breeding, but also may provide a basis for new chemical control methods for combatting of fungal disease in cotton.

Keywords: Cotton, *GbTRP1*, *Verticillium dahliae*, *Botrytis cinerea*, Anthranilate compounds

Background

Cotton is an important natural fiber crop in the textile industry and the economic pillar of many developing countries. Verticillium wilt is a disease caused by *Verticillium dahliae* and is becoming a worldwide threat to the production of cotton. The disease was first discovered in Virginia in 1914 (Carpenter 1914), and spread to China along with the introduction of Stoneville cotton in 1935 (Cai et al. 2009). The typical symptoms of diseased cotton are yellowing, wilting and leaf drop, which greatly reduces the yield and quality of cotton fiber. However, there are almost no disease-resistant germplasm resources in upland cotton, which accounts for the 95% of the total cotton yield in China.

The resistance mechanism of most host plants to *V. dahliae* is still obscure, with the exception of tomato. The *Ve1* gene from tomato was successfully identified through map-based cloning and shown to confer specific resistance to the race 1 strain of *V. dahliae* (Kawchuk et al. 2001). Although tomato *Ve1* conferred resistance to *V. dahliae* race 1 in *Arabidopsis* (Fradin et al. 2011), it failed to improve cotton resistance to *V. dahliae* when ectopically expressed in cotton, due to the absence of the avirulence gene *ave1* in *V. dahliae* from cotton (Liu et al. 2014a; Song et al. 2018). In the last few years, several signalling pathways have been identified to play a role in cotton defense response by using transcriptomic and proteomic approaches, including homeostasis of reactive oxygen species and hormone signalling pathways such as salicylic acid, jasmonic acid and ethylene signal pathways (Xu et al. 2011; Gao et al. 2013; Xu et al. 2014; Li et al. 2016). *GbWRKY1*(Li et al. 2014), *GhSSN* (Sun

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et al. 2014), *GhJAZ2* (He et al. 2017), *GhLAC1* (Hu et al. 2018a), *GbERF1* (Guo et al. 2016), *GhNDR1*, *GhMKK2* (Gao et al. 2011) amongst others have been identified as important genes involved in the regulation of hormone synthesis and defensive signal transduction pathways, and influence cotton resistance to *V. dahliae*. In addition, some natural products called phytoalexins, such as gossypol and flavonoids (Mace et al. 1985; Hu et al. 2018a), are chemical weapons in cotton that play an important role in inhibiting the growth and reproduction of *V. dahliae*. However, these secondary metabolites and their synthetic pathways have not been studied in detail in cotton.

Tryptophan is not only an amino acid for protein synthesis, but also an important precursor of auxin and other secondary metabolites. These metabolites play an essential role in plant growth and defense response (Chen et al. 2007; Bednarek et al. 2009; Iven et al. 2012). In a previous study, we found that downregulation of tryptophan synthase *GbTSA1* could increase cotton resistance to *V. dahliae*. The indolic metabolites derived from the tryptophan pathway have the capacity to elicit defense responses and enhance cotton resistance to *V. dahliae* (Miao et al. 2019).

In this study, we found that suppression of *GbTRP1* expression, encoding an phosphoribosylanthranilate transferase (PAT, EC2. 4. 2. 18) in tryptophan synthesis pathway, dramatically activated the expression of defense-related genes and led to a spontaneous cell death phenotype in cotton. Metabolomics analysis showed that anthranilate compounds including N-benzylformamide, methyl anthranilate, 1-hydroxy-2-indolinone and N-acetylanthranilic acid accumulated to high levels in *GbTRP1*-knockdown plants. These compounds have antifungal or cytotoxic activities and play a role in the resistance of cotton to *V. dahliae* and *B. cinerea*. This study not only provides genetic resources for disease resistance breeding, but also may provide a basis for new chemical control methods for combatting fungal disease in cotton.

Results

Expression pattern analysis of *GbTRP1*

GbTRP1 was identified previously from a data-mining strategy and showed response to a broad range of pathogens (Xu et al. 2014; Miao et al. 2019). The protein sequence of GbTRP1 is 410 amino acids in length and contains a chloroplast transit peptide (cTP) and an anthranilate phosphoribosyl transferase domain (Fig. 1a), which shares a high similarity with AtTRP1 (Additional file 1: Figure S1). Therefore, GbTRP1 was recognized as a phosphoribosyl anthranilate transferase and catalyzed the second step of tryptophan synthesis. Following inoculation of cotton plants with *V. dahliae* strain "V991", the transcript levels of *GbTRP1* decreased at 1 h and 6 h but increased at 12 h and 24 h post infection. Salicylic acid (SA) and jasmonic acid (JA) are two defensive hormones involved in resistance of cotton to *V. dahliae* (Liu et al. 2014b; He et al. 2017; Hu

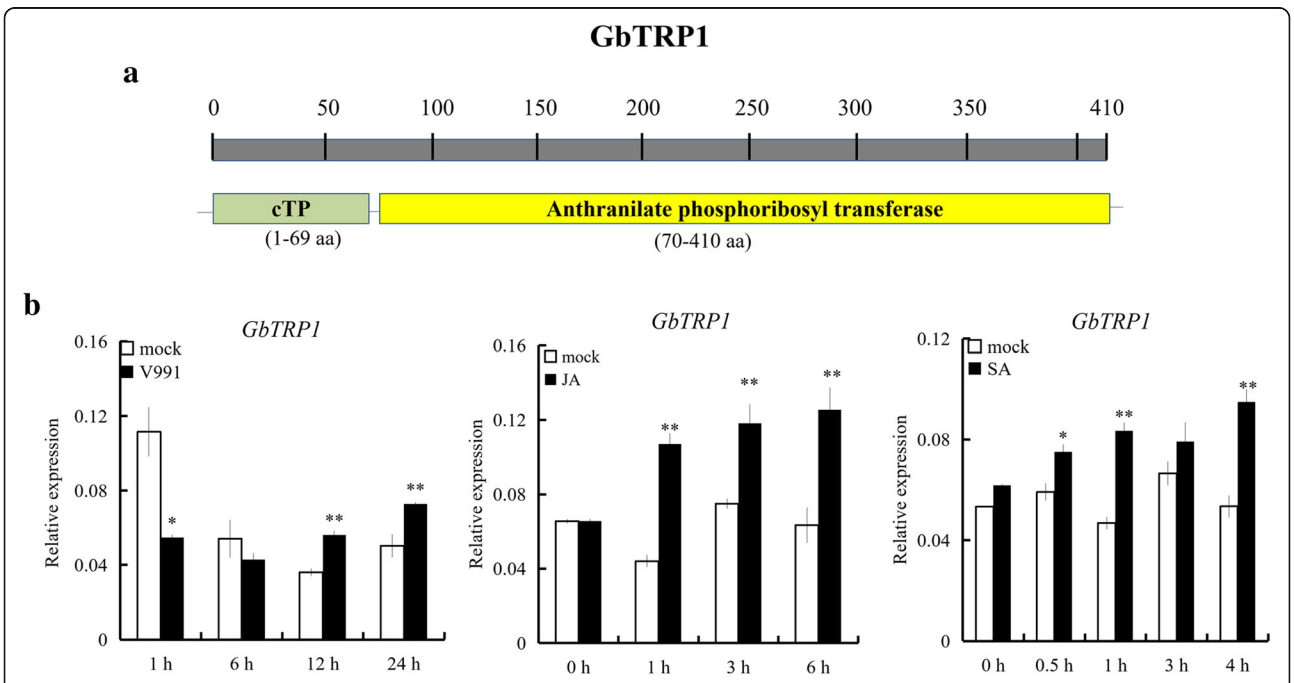

Fig. 1 Sequence and expression pattern analysis of *GbTRP1*. **a** Schematic diagram of conserved domain of GbTRP1 protein predicted by the CDD database in NCBI. **b** *GbTRP1* expression patterns were analyzed upon *V. dahliae*, SA and JA treatments at different time points using *G. barbadense* cv. Hai7124 root samples and RT-qPCR. Error bars represent the standard deviation of the mean of three biological replicates, and the transcript levels were normalized to *UB7* (* $p < 0.05$, ** $p < 0.01$, Student's *t*-test)

et al. 2018b; Miao et al. 2019). We found that *GbTRP1* was highly induced by SA and JA treatment (Fig. 1b). These results suggest that *GbTRP1* might play a role in the defense response of cotton to *V. dahliae*. GbTRP1 protein contains cTP in the N-terminus of the protein, which indicates that GbTRP1 might be localized in the chloroplast. To confirm this, we constructed the GbTRP1-GFP fusion protein driven by CaMV35S promoter and transiently expressed the vector in the tobacco leaf cells to observe the GFP fluorescence by confocal laser scanning microscope. The fluorescence of GbTRP1-GFP fusion protein co-localized with the spontaneous fluorescence of chloroplast, while the control vector containing GFP protein with no cTP mainly localized to the nucleus and membrane (Fig. 2). These results showed that GbTRP1 protein was localized in the chloroplast as predicted.

Suppression of *GbTRP1* expression triggers cell death and defense response in cotton

To validate the function of *GbTRP1* in cotton resistance to *V. dahliae*, we obtained three transgenic lines (*pi-1*, *pi-3*, *pi-4*) in which *GbTRP1* expression was suppressed by RNAi. Cell death and lesion mimic phenotypes were observed on the stems of *GbTRP1*-RNAi transgenic plants under normal (uninfected) growth conditions (Fig. 3a). The young leaves of wild type (WT) and transgenic plants were sampled to detect the expression levels of *GbTRP1* and the results showed that the mRNA amount of *GbTRP1* was greatly decreased in transgenic plants compared with WT control plants (Fig. 3b). It has been reported that many lesion mimic mutants are coupled with a high expression level of *PR* genes (Sun et al. 2014; Chai et al. 2017). To investigate this in *GbTRP1*-RNAi plants, we analysed the transcript levels for *PR1*, *PR2* and *PR5* and found that the expression levels of *PR* genes were significantly increased in the RNAi plants (Fig. 3c). We also knocked-down the expression of *GbTRP1* using the VIGS technology and the phenotypes of the *TRV:TRP1* plants were similar to the RNAi plants. The *TRV:TRP1* plants were stunted, with lesion mimics occurring on the stems and leaves (Fig. 4a). RT-PCR was used to analyse the expression levels of *GbTRP1* in *TRV:00* and *TRV:TRP1* plants, and the results showed that the expression of *GbTRP1* was greatly suppressed in *TRV:TRP1* compared with *TRV:00* plants (Fig. 4b). The expression of *PR* genes was monitored in the young leaves 2 weeks post VIGS infiltration before the appearance of lesions. All the *PR* genes examined were strongly up-regulated in *TRV:TRP1* plants compared with control plants (Fig. 4c). Therefore, suppressing the expression of *GbTRP1* activates the immune response in cotton in the absence of pathogens.

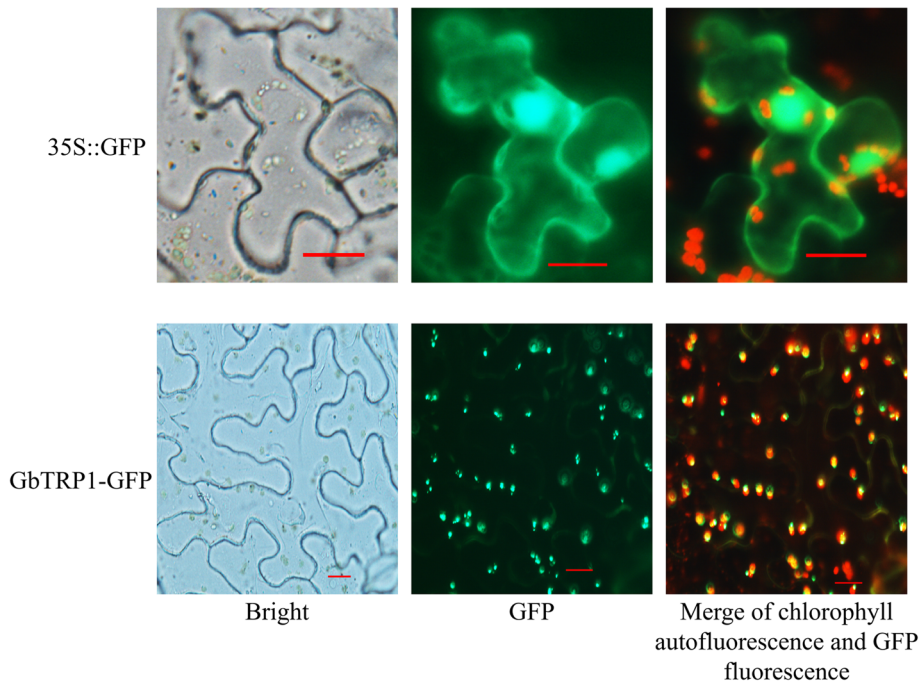

Fig. 2 Sub-cellular localization of GbTRP1. Transient expression of GFP and GbTRP1-GFP fusion proteins in tobacco leaf cells. Laser scanning confocal microscope was used to observe the green fluorescence 48 h after infiltration by Agrobacterium. Green color indicates GFP expression, and red color indicates chloroplast autofluorescence; Bars = 20 μm

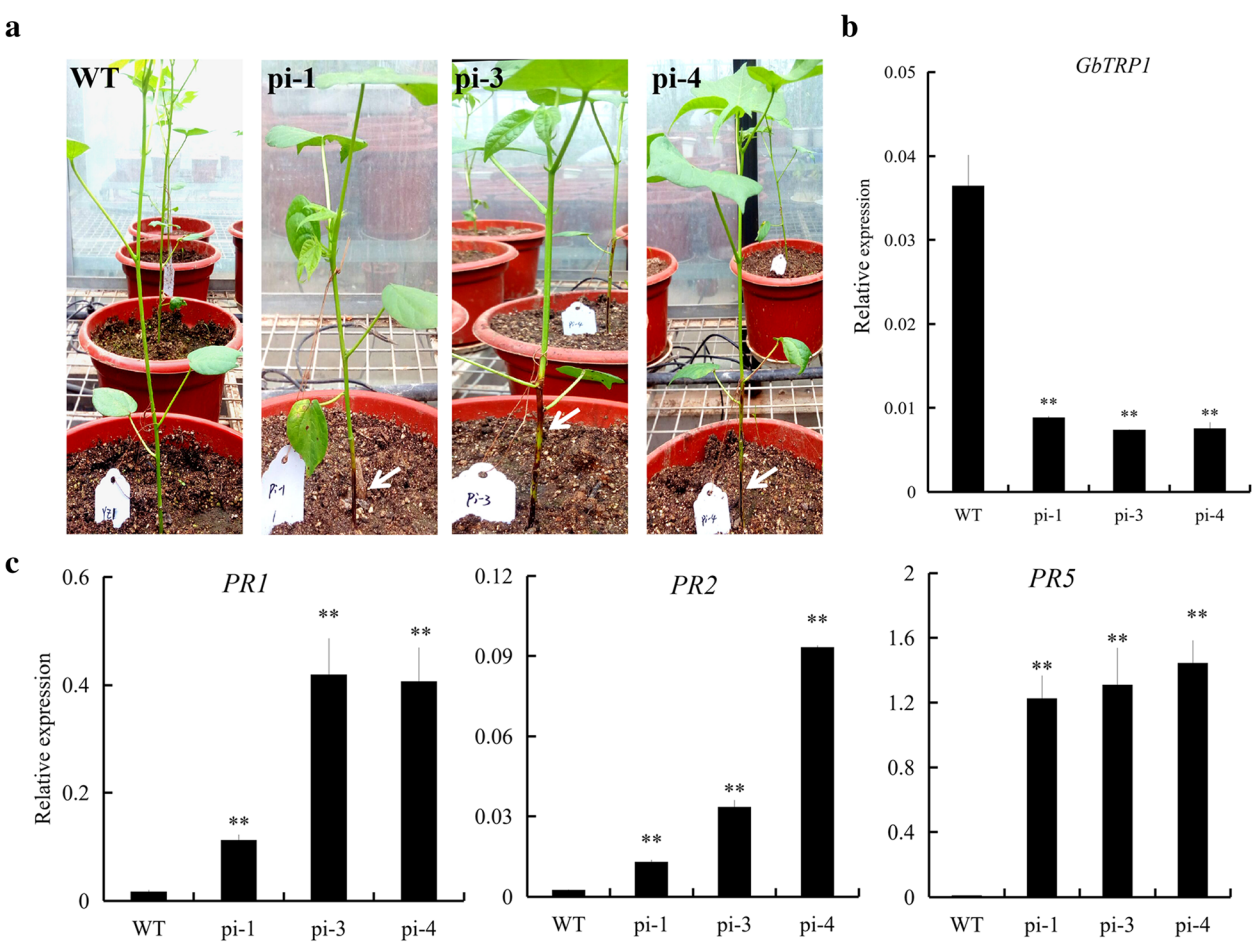

Fig. 3 Phenotypic observation and expression analysis of *GbTRP1*-RNAi plants. **a** Lesion-mimic phenotypes were observed on the stems of *GbTRP1*-RNAi transgenic lines. Plants were grown in the greenhouse for 2 months and the white arrows indicate the sites of necrosis. **b** and **c** RT-qPCR analysis of the expression levels of *GbTRP1* and *PR* genes in WT and *GbTRP1*-RNAi lines in 10 day-old plants before the lesions appeared on the plants. Error bars represent the standard deviation of the mean of three biological replicates (** $p < 0.01$, Student's *t*-test)

Suppression of *GbTRP1* expression enhances resistance of cotton to *V. dahliae* and *B. cinerea*

To examine pathogen resistance of *GbTRP1*-knockdown plants, we selected the plants with relatively mild symptoms of necrosis and inoculated these with *V. dahliae* or *B. cinerea*. The results show that resistance of the transgenic plants (*pi-1*, *pi-3*, *pi-4*) to *V. dahliae* was enhanced, with the plants showing reduced leaf chlorosis, fewer necrotic vascular bundles and a lower disease index compared with control plants (Fig. 5a, b, c).

TRP1 was also responsive to *B. cinerea* infection in *Arabidopsis* (Xu et al. 2014). To investigate the role of *GbTRP1* in cotton resistance to *B. cinerea*, the leaves of *TRV:00* and *TRV:TRP1* plants were collected at 16 days post VIGS infiltration and inoculated with *B. cinerea,* and disease symptoms were observed 7 days post inoculation. The detached leaves from *TRV:00* plants exhibited severe necrosis while leaves from *TRV:TRP1* showed less necrosis; the disease symptom area measured by Image J software also supported this observation (Fig. 6a, b). These results show that suppressing the expression of *GbTRP1* enhances the resistance of cotton to both *V. dahliae* and *B. cinerea*.

Anthranilate compounds accumulate to high levels in *TRV:TRP1* plants

We found that the transgenic RNAi plants (*pi-1*, *pi-3*, *pi-4*) and *TRV:TRP1* plants have an aromatic smell under normal conditions and exhibit blue fluorescence under UV light (Fig. 7a). These phenotypes are similar to the fluorescent and aromatic *trp1* mutants which accumulate a large amount of anthranilic acid and anthranilate β-glucoside (Last and Fink 1988). Gas chromatography-mass spectrometry (GC-MS) were used to investigate the possible changes in metabolite accumulation in *TRV:00* and *TRV:TRP1* seedlings. The results show that anthranilic acid and its

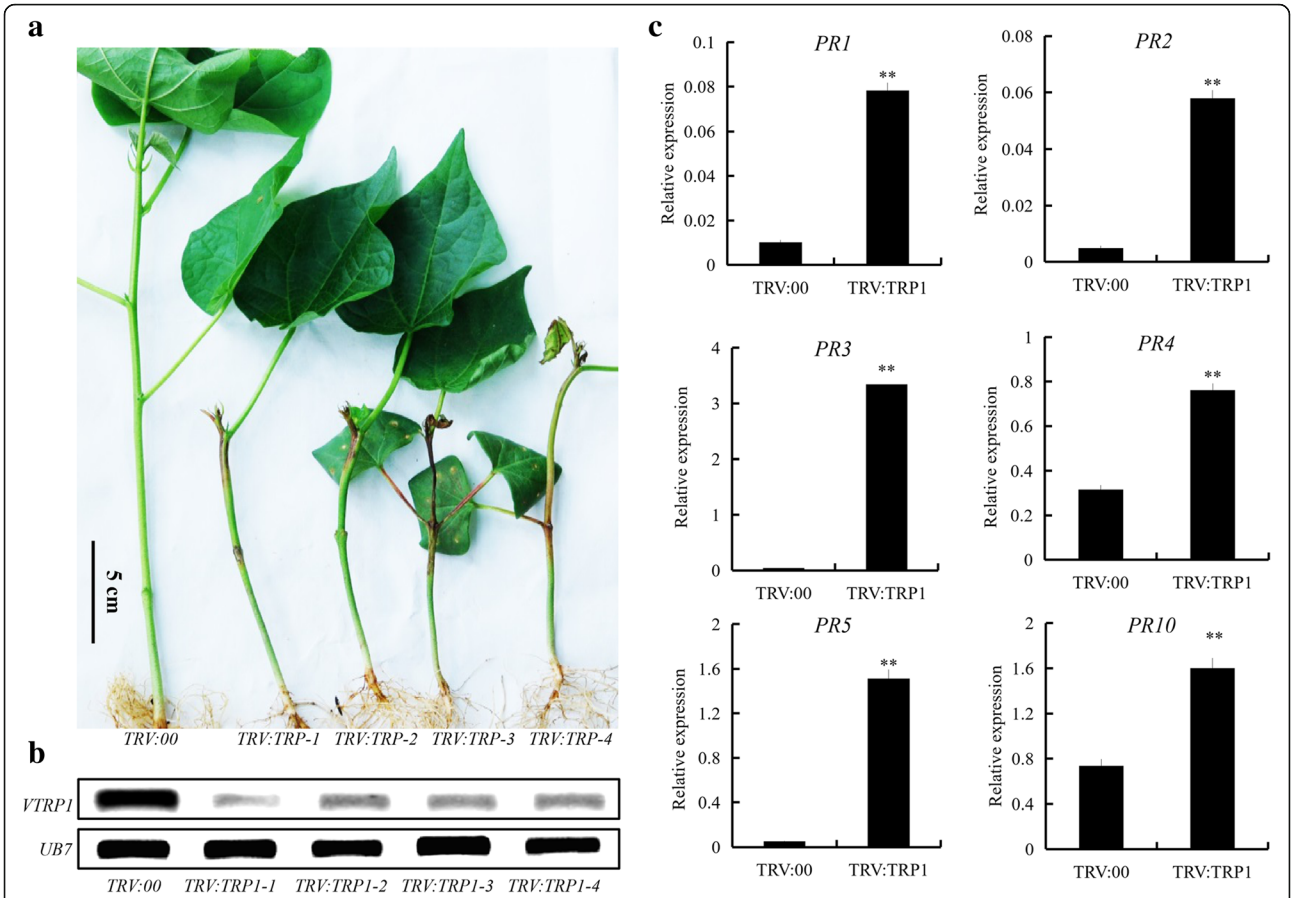

Fig. 4 Phenotypic observation and expression analysis of *TRV:TRP1* plants. **a** Spontaneous lesion formation on the stems and young leaves of *TRV:TRP1* cotton seedlings. Images were obtained 21 days after VIGS; Bars = 5 cm. **b** RT-PCR analysis indicates that the transcripts of *GbTRP1* are reduced at 14 days after infiltration (** $p < 0.01$, Student's *t*-test). **c** RT-qPCR to detect the transcript levels of *PR* genes in *TRV:TRP1* and control plants 14 days after infiltration, before the appearance of lesions. Error bars represent the standard deviation of the mean of three biological replicates(** $p < 0.01$, Student's *t*-test)

derivatives, including N-benzylformamide, methyl anthranilate, 1-hydroxy-2-indolinone and N-acetylanthranilic acid, were accumulated to high levels in *TRV:TRP1* transgenics, while these compounds were not detected in the control plants (Fig. 7b, c).

Anthranilate compounds inhibit the growth of *V. dahliae* and *B. cinerea*

To explore the possible biological function of elevated anthranilate compounds in *TRV:TRP1* plants, we tested the toxicity of methyl anthranilate both to *V. dahliae* and *B. cinerea*. *V. dahliae* and *B. cinerea* were inoculated onto the potato dextrose agar (PDA) medium containing different concentrations (0, 100 μmol·L^{-1}, 500 μmol·L^{-1}, 1 mmol·L^{-1}) of methyl anthranilate, respectively. It was found that methyl anthranilate application greatly reduced the growth of *B. cinerea* at 100 μmol·L^{-1} concentration and totally blocked the growth of *B. cinerea* at 500 μmol·L^{-1} and 10 mmol·L^{-1} (Fig. 8a, b). Six isolates of *V. dahliae* were selected to test the antifungal properties of methyl anthranilate. These strains can be divided into two groups according to their pathogenicity: the first group, comprising T9, 4TM6-15 and V991, are strong pathogenic isolates; the second, 1 cd3-2, BP2, and 1HN-1, are weak pathogenic isolates. The results show that methyl anthranilate is toxic to all the six isolates of *V. dahliae* at 1 mmol·L^{-1} concentration. The *V. dahliae* pathogens with low pathogenicity (1 cd3-2, BP2, and 1HN-1) were more susceptible to methyl anthranilate treatment than the strains with strong pathogenicity (T9, 4TM6-15 and V991) (Fig. 8c, d). These results show that anthranilate compounds may contribute to cotton resistance to *V. dahliae* and *B. cinerea*.

Discussion

Reduced expression of *GbTRP1* leads to lesion-mimic phenotypes and activates the immune response in cotton

Research on the tryptophan synthesis pathway was first carried out in *E. coli*, from which the tryptophan operon and attenuator models were proposed (Crawford 1989),

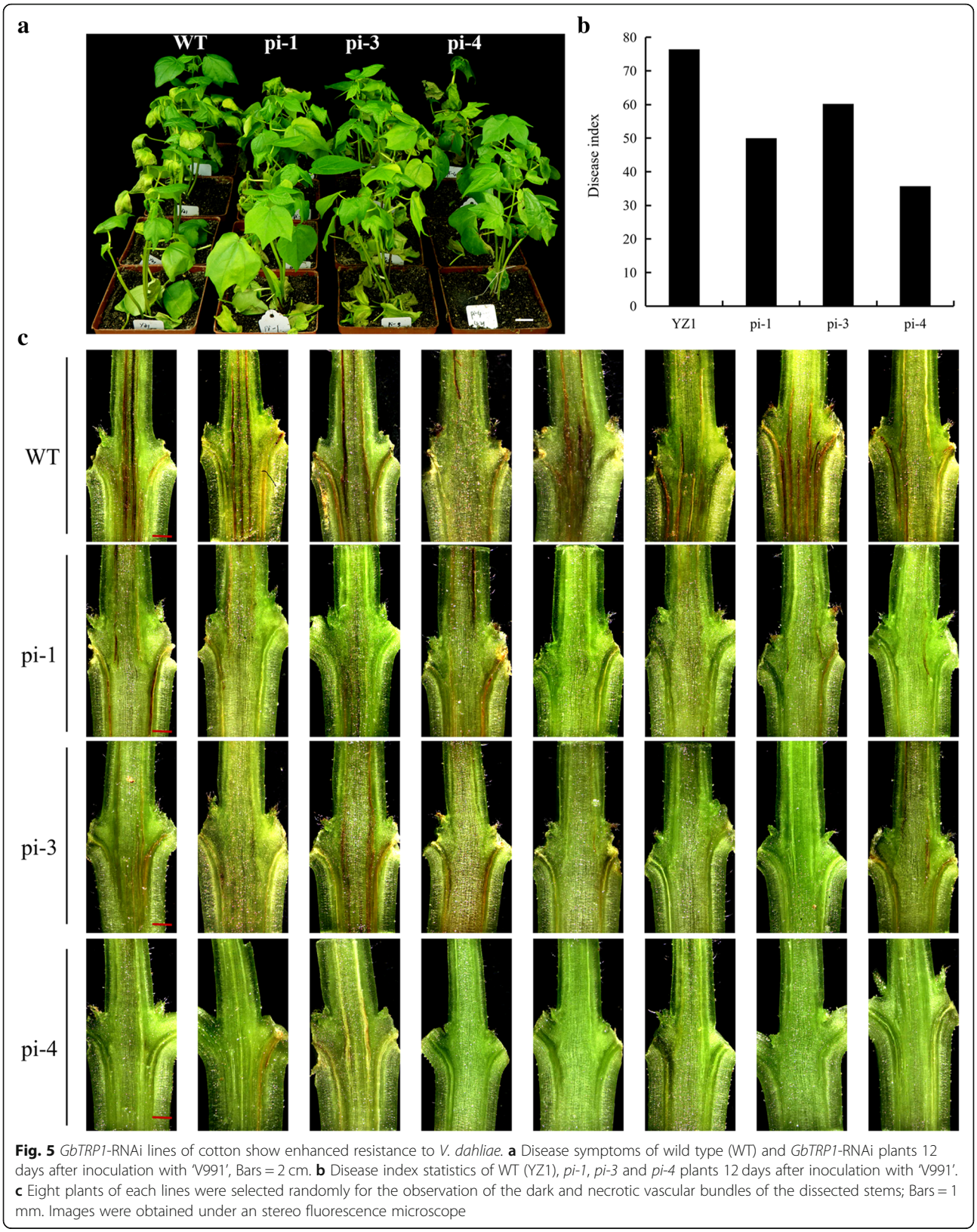

Fig. 5 *GbTRP1*-RNAi lines of cotton show enhanced resistance to *V. dahliae*. **a** Disease symptoms of wild type (WT) and *GbTRP1*-RNAi plants 12 days after inoculation with 'V991', Bars = 2 cm. **b** Disease index statistics of WT (YZ1), *pi-1*, *pi-3* and *pi-4* plants 12 days after inoculation with 'V991'. **c** Eight plants of each lines were selected randomly for the observation of the dark and necrotic vascular bundles of the dissected stems; Bars = 1 mm. Images were obtained under an stereo fluorescence microscope

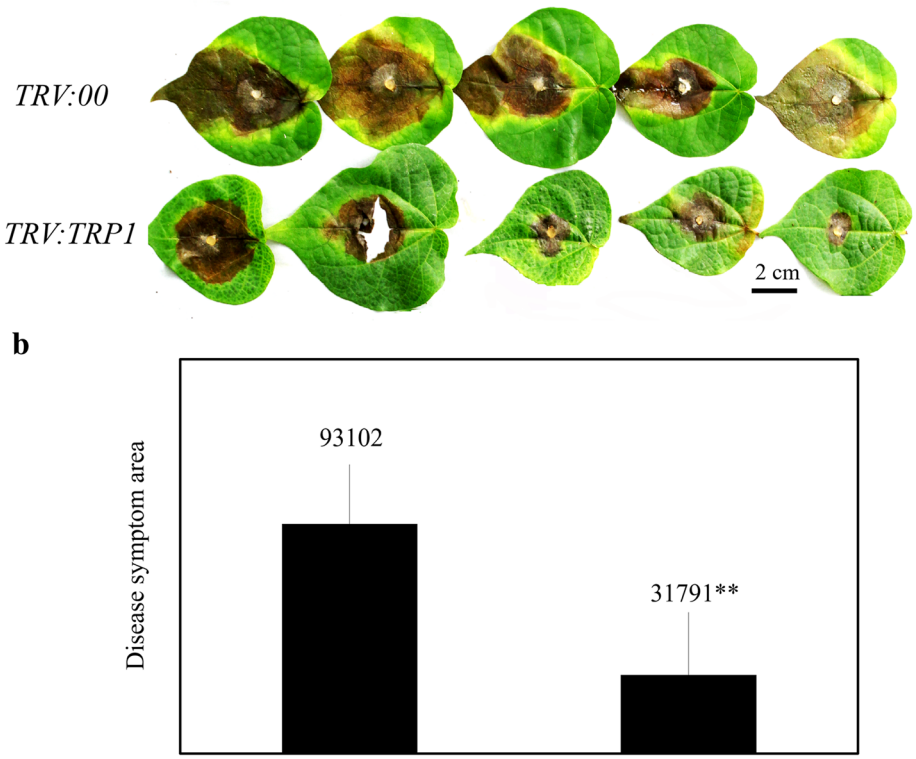

Fig. 6 *TRV:TRP1* plants of cotton show enhanced resistance to *B. cinerea*. **a** Disease symptoms of *TRV:00* and *TRV:TRP1* leaves 7 days post inoculation with *B. cinerea*, Bars = 2 cm. **b** The statistics of disease area by Image J software. The values represent the means from three biological replicates (** $P < 0.01$, Student's *t*-test)

and have become classic models of gene regulation in the history of molecular biology. Although the importance of tryptophan and its metabolites has been well understood for a long time in *E. coli* and in fungi, detailed studies of the tryptophan synthesis pathway in plants were not conducted until the 1990s. In *Arabidopsis* there are seven genes involved in Trp synthesis and all seven genes have been cloned (Radwanski and Last 1995). *AtTRP1* was the first gene studied in tryptophan synthesis through mutant selection. The *trp1* mutant of *Arabidopsis* is defective in phosphoribosyl anthranilate transferase (PAT) activity, and exhibits blue fluorescence under UV light because of the accumulation of anthranilate compounds. The *trp1* mutant is also small and bushy, consistent with a defect in IAA biosynthesis (Last and Fink 1988; Rose et al. 1992).

We generated knock-down mutants of *GbTRP1* through RNAi and VIGS technologies in cotton. Consistent with the *Arabidopsis* mutant phenotype, the *GbTRP1*-knock-down plants were also small and displayed blue fluorescence under UV light (Figs. 4a, and 7a). Interestingly, there were some different phenotypes observed in cotton from those of the tryptophan auxotroph mutants in *Arabidopsis*. *GbTRP1*-silenced plants exhibited spontaneous lesion mimics on the stems without pathogen infection (Figs. 3a and 4a), which was not reported in *Arabidopsis trp1* mutants. We also found that several *PR* genes including, *PR1*, *PR2*, and *PR5*, were significantly activated in *GbTRP1* RNAi and VIGS plants (Figs. 3c and 4c). These *PR* genes are usually associated with the activation of SA synthesis and signalling pathways (Van Loon and Van Strien 1999). However, the content of SA in *TRV:TRP1* was less than that in *TRV:00* plants in this study (Additional file 1: Figure S2) and in our previous study (Miao et al. 2019). There were three extra peaks in the SA chromatograms in *TRV:TRP1* leaf samples, indicating that there may be new derivatives of SA accumulated in these mutants (Additional file 1: Figure S2). The mechanism for lesion-mimic phenotypes and activated immune response needs further study. We also found that *GbTRP1* RNAi and *TRV:TRP1* plants showed enhanced disease resistance to both *V. dahliae* and *B. cinerea* (Figs. 5 and 6), which suggested that down regulation of *GbTRP1* may confer broad-spectrum resistance in cotton.

Anthranilates have antifungal properties

Plants produce large amounts of secondary metabolites and many of them are chemical weapons for plants to

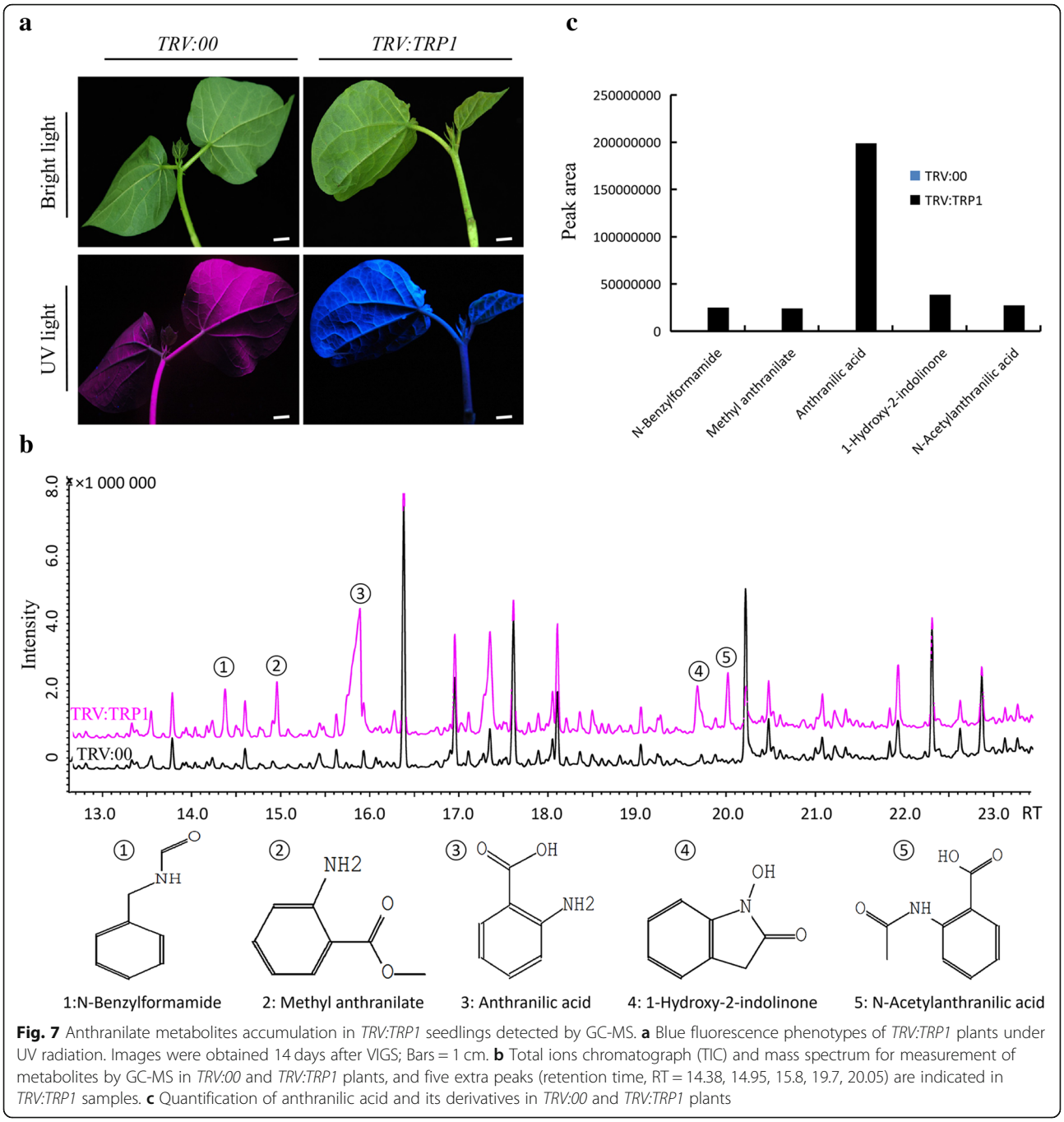

Fig. 7 Anthranilate metabolites accumulation in *TRV:TRP1* seedlings detected by GC-MS. **a** Blue fluorescence phenotypes of *TRV:TRP1* plants under UV radiation. Images were obtained 14 days after VIGS; Bars = 1 cm. **b** Total ions chromatograph (TIC) and mass spectrum for measurement of metabolites by GC-MS in *TRV:00* and *TRV:TRP1* plants, and five extra peaks (retention time, RT = 14.38, 14.95, 15.8, 19.7, 20.05) are indicated in *TRV:TRP1* samples. **c** Quantification of anthranilic acid and its derivatives in *TRV:00* and *TRV:TRP1* plants

defend against the adverse environmental stresses. These secondary metabolites are usually derived from primary metabolites, including amino acids (Zeier 2013). Some compounds derived from amino acid metabolic pathways can act as defense signals, such as the well known methionine-derived hormone ethylene and the recently identified lysine catabolites pipecolic acid (Pip) and N-hydroxypipecolic acid (NHP) (Chae and Kieber 2005; Hartmann et al. 2018; Shan and He 2018). Other products derived from amino acid metabolic pathways, the phytoalexins, have antimicrobial and toxic activities that can directly protect plants from pathogen and insect attack (Ahuja et al. 2012).

Tryptophan is an essential amino acid that is a precursor of the hormone auxin and a large array of phytoalexins, such as glucosinolates, alkaloids and camalexin (Bednarek et al. 2009; Iven et al. 2012; Møldrup et al. 2013). Recently, Trp-derived serotonin has been reported that may facilitate insect performance on rice (Lu et al. 2018). These compounds are all derived from the tryptophan metabolic

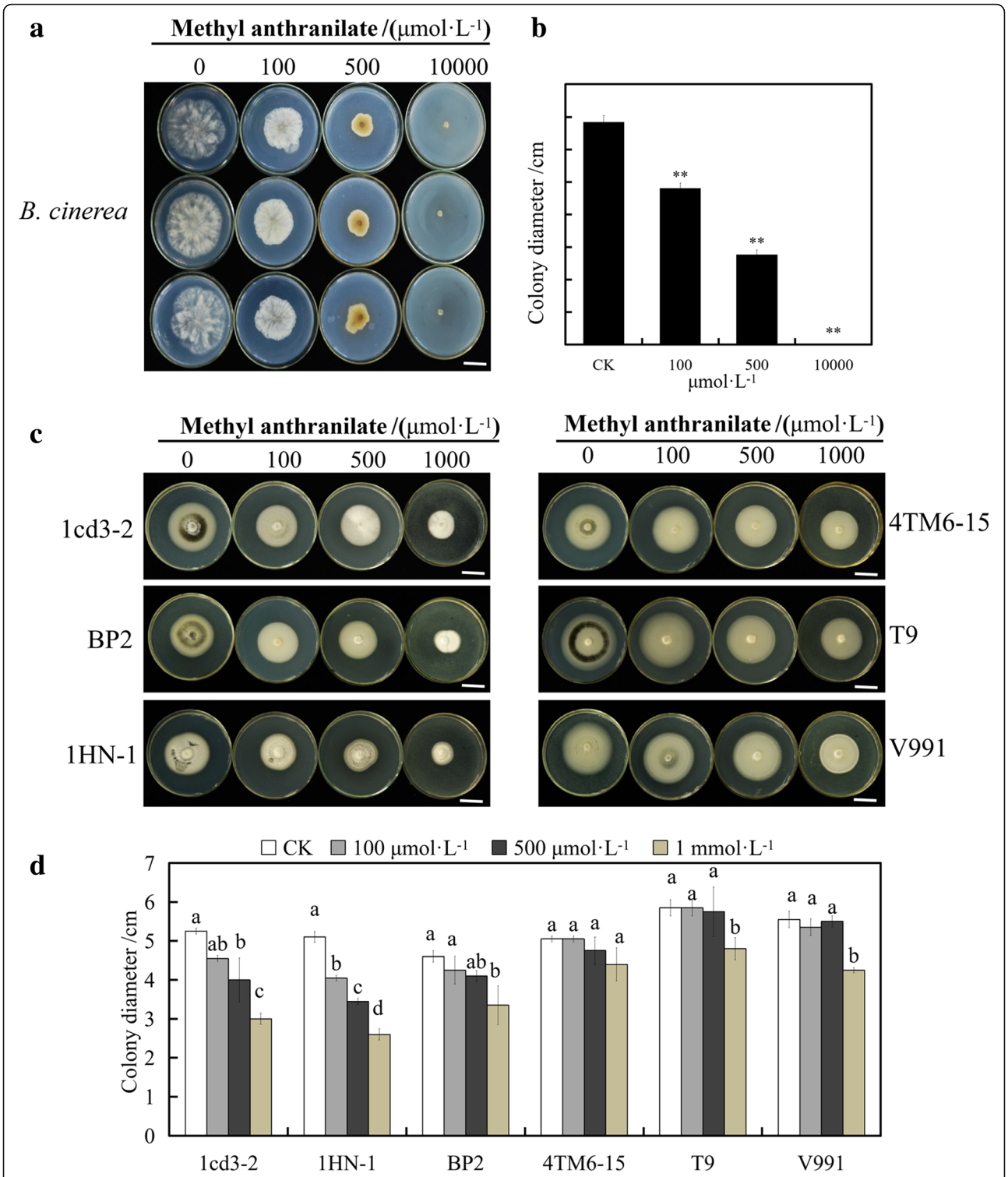

Fig. 8 Methyl anthranilate inhibits the growth of *B. cinerea* and *V. dahliae*. **a** and **b** Effect of methyl anthranilate on the growth of *B. cinerea*. Colony diameter was determined 7 days post incubation. Values represent the means ± SD from three biological replicates (** $p < 0.01$, Student's *t*-test); Bars = 2 cm. **c** and **d** Effect of methyl anthranilate on the growth of *V. dahliae*. Colony diameter was determined 7 days post incubation. Values represent the means ± SD from three biological replicates; Bars = 2 cm

pathway, while, compounds from the tryptophan synthetic pathway have been less studied. Previously we found that knockdown of the expression of *GbTSA1* and *GbTSB1* led to the accumulation of indole and indolic metabolites, which can trigger the immune response during cotton resistance to *V. dahliae* (Miao et al. 2019). In this study, we show that suppression of *GbTRP1* expression results in the accumulation of anthranilates with antifungal activity against *V. dahliae* and *B. cinerea* (Fig. 8). To our knowledge, the function of anthranilate compounds has not been studied in relation to plants resistance to pathogens. Further, these compounds may provide a basis for the chemical control of fungal disease in cotton. In addition to anthranilic acid, other anthranilic derivates including anthranilate β-glucoside, N-benzylformamide, methyl anthranilate and N-acetylanthranilic acid were also increased in *GbTRP1* knockdown plants (Fig. 7). These results suggest that the activity of some enzymes such as glycosyltransferase, methyltransferase and acyltransferase may be activated in *GbTRP1* knockdown plants and the genes encoding these enzymes require further study. These studies will help us to understand better the regulation of the aromatic secondary metabolic synthesis pathway, through the identification of key rate-limiting enzymes and new metabolic products may contribute to the engineering of pathogen-resistant crops.

Conclusions

Our results showed that suppression of *GbTRP1* expression dramatically activated the immune response and increased resistance of cotton to *V. dahliae* and *B. cinerea* possibly due to the accumulation of anthranilate compounds. This study not only provides gene resources for disease resistance breeding, but also may provide a theoretical basis for chemical control of fungal disease in cotton.

Materials and methods

Plant material and fungus proliferation

Cotton plants, *G. hirsutum* cv. YZ1, *G. barbadense* cv. H7124 and transgenic plants derived from YZ1, were grown in the greenhouse under a controlled 14 h light/10 h dark cycle at 28 °C. For fungus proliferation, *V. dahliae* strain "V991" were first grown on potato dextrose agar (PDA) medium for 3 days, then the activated mycelia were cultured in Czapek's medium for 3–5 days at 25 °C for spore production. *B. cinerea* were cultivated twice on PDA medium for activation.

Stresses treatment and sample collection

For *V. dahliae* treatment, seedlings of H7124 were cultivated in Hoagland solution for 2 weeks; the plants were root-dip-infected with *V. dahliae* conidial suspensions (1×10^6 conidia·mL^{-1}) and the roots were harvested at 1, 6, 12, and 24 h after inoculation with control seedlings treated with sterile water. For hormone treatments, 1 mmol·L^{-1} SA and 100 μmol·L^{-1} methyl jasmonate (MeJA) were applied in Hoagland solution independently, and root samples were collected at different time points after hormone treatment for analysis. At least 5 plants were sampled for each time point, with three biological repeats. All the samples were frozen in liquid nitrogen immediately after collection and stored at −80 °C for subsequent RNA extraction.

RT-qPCR analysis

RNA was extracted according to the methods described previously (Zhu et al. 2005), and 3 μg of total RNA was reverse-transcribed into cDNA for gene expression analysis. Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR) were performed using the ABI Prism® 7 500 system (Applied Biosystems, Foster City, CA, USA) by the methods described by Miao et al. (2019).

Vectors construction and genetic transformation

To study the function of *GbTRP1*, a 464 bp fragment of *GbTRP1* was amplified from the cDNA made from *G. barbadense* H7124 root samples and the PCR products were cloned into pHellsgate4 and pTRV2 to generate *GbTRP1*-RNAi and *TRV:TRP1* vectors, respectively. For subcellular localization of GbTRP1 protein, the full-length cDNA sequence lacking stop codon was fused into the N-terminus of green fluorescent protein (GFP) to construct *35S::GbTRP1-GFP*. The primer sequences are listed in Additional file 1: Table S1. All vectors were transferred to *Agrobacterium tumefacien* (GV3101).

For genetic transformation, a *GbTRP1*-RNAi vector was used to transform hypocotyl sections of YZ1 using the *Agrobacterium tumefaciens* (GV3101)-mediated transformation methods described by Jin et al. (2006).

Subcellular localization analysis

A. tumefaciens harboring *35S::GbTRP1-GFP* vector were transiently transformed into tobacco leaf cells using the methods described previously (Miao et al. 2019). The fluorescence of GFP was observed by confocal laser scanning microscopy (Olympus FV1200).

Virus-induced gene silencing and pathogen inoculation

A. tumefaciens harboring *TRV:TRP1* and *TRV:00* control vector were infiltrated into the cotyledons of 10 day-old H7124 seedlings using methods described previously (Gao et al. 2013). Leaf samples were then taken at 16 days post-VIGS infiltration for *B. cinerea* inoculation. The activated *B. cinerea* hyphal discs (5 mm) were inoculated onto the excised leaves at 25 °C and then covered with black plastic wrap to maintain sufficient moisture.

The lesion areas were measured by Image J software after 7 days post inoculation.

Anthranilate assays

Leaf samples were harvested from *TRV:00* and *TRV:TRP1* plants at 16 days post VIGS infiltration and immediately frozen in liquid nitrogen for determination of metabolites by gas chromatography-mass spectrometry (GC-MS). The methods for extraction and measurement of anthranilates are the same as the methods for indole determination (Miao et al. 2019).

Determination of toxicity of methyl anthranilate to *V. dahliae* and *B. cinerea*

To study the antifungal properties of anthranilates, methyl anthranilate was used as an exemplar. PDA medium was supplemented with different methyl anthranilate concentrations (0, 100 μmol·L^{-1}, 500 μmol·L^{-1}, 1 mmol·L^{-1}, 10 mmol·L^{-1}) of methyl anthranilate. 5 μL conidial suspension (1×10^6 conidia·mL^{-1}) of *V. dahliae* and hypha discs (5 mm) of *B. cinerea* were inoculated on the center of PDA medium containing methyl anthranilate. The fungi were then cultured in an incubator at 25 °C. After 1 week, colony diameters were measured.

Accession numbers

Sequence data from this article can be found in the CottonGen database (http://www.cottongen.org) or GenBank databases under the following accession numbers: *GbTRP1*, Gbar_D12G029450; *GhUB7*, Gh_A11G0969; *GhPR1*, Gh_A12G0274; *GhPR2*, Gh_D06G2277; *GhPR3*, Gh_D01G1683; *GhPR4*, Gh_D13G1816; *GhPR5*, Gh_D12G2247; *GhPR10*, Gh_D04G1399.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42397-019-0034-1.

Additional file 1: Table. S1. Primers used in this study. The bold and underlined parts are adaptor sequence; the primers for RT-qPCR are marked by 'RL'. **Figure S1.** Alignment of the amino acid sequences of GbTRP1 (Gbar_D12G029450) with AtTRP1 (AT5G17990). Yellow box indicates chloroplast transit peptide (cTP). **Figure S2.** Detection of SA contents in the leaves of *TRV:00* and *TRV:TRP1* plants. A: High-performance liquid chromatography (HPLC) profiles of SA extracted from leaf samples of *TRV:00* and *TRV:TRP1* seedlings. The number superimposed on each peak is the retention time (RT), and three extra peaks (RT = 5.35, 6.74, 7.11) are indicated in the *TRV:TRP1* samples. B: Detection of SA contents in *TRV:00* and *TRV:TRP1* plants 14 days after infiltration. The values represent the means ± SD from three biological replicates (** $p < 0.01$, Student's *t*-test).

Acknowledgments

We would like to thank Prof. THOMMA Bart (Laboratory of Phytopathology, Wageningen University and Research Center, The Netherlands) for providing VIGS vectors and Prof. JIAN Guiliang (Institute of Plant Protection, Chinese Academy of Agricultural Sciences, China) for providing *V. dahliae* strain 'V991'. Language revision by Prof. LINDSEY Keith (Department of Biosciences, Durham University, Durham, UK) is appreciated.

Authors' contributions

Zhang XL and Zhu LF designed the experiment. Miao YH performed the research, analyzed the data and drafted the manuscript. Zhang XL revised the manuscript. All authors read and approved the final manuscript.

Funding

Supports by China Agricultural Research System (CARS-15-04) are appreciated.

Availability of data and materials

Not applicable.

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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Received: 14 May 2019 Accepted: 23 September 2019
Published online: 12 November 2019

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