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# *GbAt11* gene cloned from *Gossypium barbadense* mediates resistance to *Verticillium* wilt in *Gossypium hirsutum*

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## Abstract

**Background:** *Gossypium hirsutum* is highly susceptible to *Verticillium* wilt, and once infected *Verticillium* wilt, its yield is greatly reduced. But *G. barbadense* is highly resistant to *Verticillium* wilt. It is possible that transferring some disease-resistant genes from *G. barbadense* to *G. hirsutum* may contribute to *G. hirsutum* resistance to *Verticillium* wilt.

**Result:** Here, we described a new gene in *G. barbadense* encoding AXMN Toxin Induced Protein-11, *GbAt11*, which is specifically induced by *Verticillium dahliae* in *G. barbadense* and enhances *Verticillium* wilt resistance in *G. hirsutum*. Overexpression in *G. hirsutum* not only significantly improves resistance to *Verticillium* wilt, but also increases the boll number per plant. Transcriptome analysis and real-time polymerase chain reaction showed that *GbAt11* overexpression can simultaneously activate *FLS2*, *BAK1* and other genes, which are involved in ETI and PTI pathways in *G. hirsutum*.

**Conclusion:** These data suggest that *GbAt11* plays a very important role in resistance to *Verticillium* wilt in cotton. And it is significant for improving resistance to *Verticillium* wilt and breeding high-yield cotton cultivars.

**Keywords:** *Gossypium barbadense*, *GbAt11*, *Verticillium* wilt, resistance

## Introduction

*Verticillium* wilt is a highly destructive soil-borne fungal disease of plants that affects an extensive range of host species, including many agricultural crops (Burpee and Bloom 1978; Fradin et al. 2011; Qi et al. 2016). *Verticillium* wilt of cotton is caused by *Verticillium dahliae*, but how the pathogen causes the disease of plants remains to be clarified (Gao et al. 2011). However, there is an accumulative evidence to show that the toxin produced by *V. dahliae* is the main pathogenic factor, for example, a protein or glycoprotein is mainly responsible for the wilt symptoms. Studies on the resistance mechanisms of plants to *Verticillium* wilt found two genetic loci, *Ve1*

and *Ve2* in tomato. The expression of both *Ve1* and *Ve2* in potato can enhance resistance to *V. dahliae* (Kawchuk et al. 2001; Simko et al. 2004). Although it has been shown that *Ve1* gene is functional only in tomato (Fradin et al. 2011; Fradin et al. 2009), *GbVe1*, which is a homolog of the tomato *Ve1* cloned from *Gossypium barbadense*, is highly resistant to *Verticillium* wilt, and its overexpression in *Arabidopsis thaliana* has confirmed the disease resistance function of this gene (Zhang et al. 2011; Zhang et al. 2013). Based on the genetic principle of immune response induction, we treated *G. barbadense* with purified *V. dahliae* filtrate protein, extracted RNA, and cloned 11 full-length cDNAs of specific genes (*GbAt1* - *GbAt11*) using suppression subtractive hybridization (SSH). The objective of this study was to analyze the molecular role of *GbAt11* gene in resistance of cotton to *Verticillium* wilt.

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Recently, considerable progress has been made in understanding the immune mechanisms underlying the response of plants to pathogen infection. Studies have shown that plants identify pathogenic microorganisms by the presence of specific molecular motifs, known as pathogen-associated molecular patterns (PAMP) (Ozinsky et al. 2000), for example, the flagellin protein Flag22 on the surface of *Pseudomonas syringae*, the bacterial elongation factor EF-Tu, and chitin (Asai et al. 2002; Wan et al. 2008). When plants contact Flag22, the receptor protein FLS2 is activated on the surface of plant cells and is up-regulated through MAPK kinase signal transduction and transcription factor WRKY29 expression (Asai et al. 2002; Gomez-Gomez and Boller 2000; Adachi et al. 2015), therefore causing the production of reactive oxygen species (ROS) and the accumulation of callose at the infected site, thus protecting against the pathogen invasion (Nurnberger et al. 2004). FLS2-mediated disease resistance pathways are also involved in the BAK1 element in the brassinosteroids (BR) pathway, which is the co-receptor of both BR and FLS2 (Albrecht et al. 2008; Lin et al. 2013). After FLS2 is activated by Flag22, BAK1 is recruited to form a complex, and then transfers the resistance signal to downstream by a process called PAMP-Triggered-Immunity (PTI) (Albrecht et al. 2012). Some bacterial pathogens have evolved effectors to inhibit PTI; however, plants have evolved proteins to counteract these effectors, which can relieve the inhibition of effectors on PTI through a process called effector-triggered-immunity (ETI). Thus, host plants and their pathogens co-evolve during this mutually antagonistic process.

Since *GbAt11* was induced in *G. barbadense* with high resistance to Verticillium wilt, we studied the function of *GbAt11* in resistance to Verticillium wilt, and the *GbAt11-G. hirsutum* lines significantly increased resistance to Verticillium wilt. Similarly, we found that overexpression of *GbAt11* can up-regulate the expression of *FLS2*, *BAK1* and other related disease resistance genes. Therefore, we speculate that *GbAt11* plays an important role in the process of resistance to Verticillium wilt and is beneficial for improving resistance to Verticillium wilt and breeding high-yield cotton cultivars. And it also has reference value for the study of the mechanism of plant antifungal diseases.

## Materials and methods

### Materials

*G. barbadense* variety H7124 seeds were purchased from the National Cotton Seeds Repository (China). Seeds of *G. hirsutum* variety JM169 were provided by the Institute of Cotton, Hebei Academy of Agriculture and Forestry Sciences (Shijiazhuang, Hebei). *G. barbadense* and *G. hirsutum* were grown in greenhouse at 25 °C with a

12 h/12 h (day/night) cycle, and *G. hirsutum* JM169 was used as the wild type (WT) control. All transgenic plants used in this study are in JM169 background.

### Methods

#### *GbAt11* gene cloning and sequencing

The *G. barbadense* seedlings were divided into two groups, and one was soaked in a solution of *V. dahliae* toxin, while the other was soaked in water as the control. Three plants were sampled at 6, 12, 18, and 24 h of treatment, respectively, while their total RNA was extracted, and mRNA was purified, then used as SSH (Diatchenko et al. 1996). A total of 11 specific gene fragments were obtained, cloned into T-vector and sequenced by Sangon Biotech Co. Ltd. From the sequencing results, they were named *GbAt1* to *GbAt11*. This study focused on exploring the role of *GbAt11* gene. Basic local alignment search tool (Blast) searches were performed against GenBank to find the full-length cDNA sequence. The chromosomal location of the *GbAt11* gene was obtained from an analysis at the website.

#### Specific real-time PCR detection of *GbAt11* expression in *G. barbadense* and *G. hirsutum*

Seedlings of *G. barbadense* and *G. hirsutum* were grown in a greenhouse. Leaves were collected at the adult-plant stage from the same parts of the plants, soaked in *Vd991* spore suspension ( $1 \times 10^6$  per mL), sampled after 0 h, 4 h, 8 h, 12 h, and 24 h, respectively, and total RNA was extracted from 100 mg of powder. Then we used real-time polymerase chain reaction (RT-PCR) (Salin et al. 2005) to quantify the level of *GbAt11*-specific mRNA at different times. RNA was extracted with the EASYspin plant RNA rapid extraction kit (Beijing Aidlab biotechnologies Co. Ltd), quantified, and the relative expression of *GbAt11* mRNA was measured using RT-PCR after cDNA synthesis by reverse transcription. PCR primer sequences were designed as follows: forward primer 5'-AGGGTTTCGTCGTCTACT-3', and reverse primer 5'-GATGCTTCTCCTCATAGG-3'. Amplification reactions (20  $\mu$ L) contained 10  $\mu$ L SYBR Premix Ex Taq, 0.6  $\mu$ L of each primer (F + R), 0.4  $\mu$ L ROX, 1–2  $\mu$ L cDNA, and ddH<sub>2</sub>O to 20  $\mu$ L.

#### Construction of the *GbAt11* overexpression binary vector (the concentration of primary proteins quantified with spectrophotometer)

We replaced the Hyg<sup>R</sup> gene with the *tdA* gene in the pCAMBIA1300 binary vector to make a vector called pSPT. We designed primers containing *Sal1/KpnI* restriction enzyme sites on both ends of the *GbAt11* open reading frame (ORF); AT11–2-*Sal1*-F: 5'-CGGTCGACATGTCGATCGCGTTGGAACG-3', and AT11–2-*KpnI*-R: 5'-CGGGTACCGTTATATTCACGTACAT

CAGCC-3', and obtained the target gene by reverse transcription of *G. barbadense* AXMN mRNA. The pSPT vector was digested with *SalI* and *KpnI*, then ligated with the target gene fragment using T4 DNA ligase to construct the recombinant binary vector containing the *GbAt11* gene.

#### **Verticillium dahliae inoculations**

*V. dahliae* inoculations was conducted according to Fradin et al. (2009). The 2~ 3-week-old cotton plants were uprooted, and then the roots and (or) adult-stage cotton leaves were rinsed in water. Subsequently, the roots or petioles were dipped for 24–72 h in a suspension of  $10^6$  conidia per mL of Czapek's medium dextrose broth (Difco) and harvested from 2-week-old *Verticillium* cultures on Czapek's medium dextrose agar (Oxoid). Control plants were treated similarly, but their roots were dipped in Czapek's medium without conidiospores. After dipping, the disease phenotype was recorded and photographed from 1 h to 72 h.

#### **Screening of transgenic positive cotton lines**

Transformation of *G. hirsutum* was performed using the pistil drip method (Zhang and Chen 2012). Transgenic seeds were sown in the field, sprayed with 2,4-D at 30 mg·kg<sup>-1</sup> after sprouting, and the plants with a completely unfolded true leaf were screened. DNA was extracted and plants were screened for the *tfdA* gene and the target gene. Primers for *tfdA* amplification were *tfdA*-F:5'-ATGAGATCCATGGGTGAGCG-3', and *tfdA*-R:5'-AGAACG CAGCGGTTGTCC-3'. PCR was used to identify single copy transgenic plants based on the segregation ratio of the T<sub>2</sub> generation against 2,4-D. We first identified disease resistance in the single copy lines in the disease nursery (cotton stalks inoculated with *Vd991* were crushed and evenly spreaded in the field every year, to ensure the uniformity of *V. dahliae* in the disease nursery), and then we selected lines L-213, L-214, and L-235 with good field resistance, treated with a *V. dahliae* spore suspension (concentration of  $1 \times 10^6$  per mL) for resistance identification in a disease assay. We next extracted total proteins from L-214 leaves, concentrated the GbAT11-Flag protein fusion using the Flag tag, fractionated the proteins on a denaturing polyacrylamide gel, and then detected the anti-Flag antibody by western hybridization.

#### **Transcriptome analysis**

Transcriptome analysis was performed by Beijing Biomarker Technologies Co. Ltd.

#### **Subcellular localization of the GbAT11-GFP fusion protein**

We constructed the pCAMBIA:GbAT11-GFP recombinant expression vector and used it to transform

*Arabidopsis thaliana*. T<sub>2</sub> generation transgenic seeds were sown on murashige and skoog basal medium (MS) medium, and after the GbAT11-GFP-expressing seedlings grew for 1 week, GFP fluorescence was observed with a laser scanning confocal microscope. The excitation and emitting wavelengths for GFP were 488 and 525 nm, respectively. This allowed us to see whether GbAT11 was localized to the cell wall after treated with *V. dahliae* toxin. We treated the roots of transgenic Arabidopsis with 20% sucrose solution to plasmolyze the cells in order to observe the membrane localization of GbAT11.

#### **Field disease resistance identification and examination of yield traits**

The test on Verticillium wilt resistance of plants carrying the endogenous *At11* gene, transgenic plants overexpressing *GbAt11*, and *G. hirsutum* carrying a TALENT knockout of *GhAt11* was carried out in a field disease nursery inoculated with the *Vd991* in Shandong province. There were two lines of each variety, a total of 60 plants. Plants were examined for symptoms of Verticillium wilt on June 20 and August 20, and were classified into five levels based on the degree of morbidity (ratings of 0 to 4). We calculated the Verticillium wilt disease index according to the formula (1), for every line, and counted the average number of bolls per plant on September 10. And the same test was carried out at the test base of China Agricultural University (Hebei province, in north-central China).

## **Results**

### **Screening of *Gossypium barbadense*-response genes induced by Verticillium wilt using suppressive subtraction hybridization**

*Gossypium barbadense* possesses the highest level of resistance to verticillium wilt among the four cotton species, while the widely cultivated *G. hirsutum* is highly susceptible. We soaked seedlings of *G. barbadense* and *G. hirsutum* in a solution of purified proteins secreted by *V. dahliae*, and necrotic lesions were found present on the leaves of *G. barbadense* after 72 h, while the entire leaves of *G. hirsutum* were withered (data not shown). We induced the verticillium wilt-resistant *G. barbadense* with a solution of purified *V. dahliae* proteins, and obtained 11 specific genes (*GbAt1-GbAt11*) using SSH.

We initially focused on *GbAt11* because its expression appeared to be strongest in *G. barbadense* induced by *V. dahliae*. The full-length *GbAt11* cDNA is 768 bp, and the predicted molecular mass of the protein is about 27 kd. The N-terminal ORF from *G. barbadense* has an additional 17 amino acids than the predicted protein from *G. hirsutum*. In addition, BLAST searches confirmed that no *GbAt11*-like gene was present in *A. thaliana*.

Further DNA sequence comparison and amino acid sequence analysis predicted that GbAT11 has 49.08% and 48.76% amino acid sequence homology with MTD1 (Methylene-tetrahydrofolate dehydrogenase [NAD(+)] and *Jatropha curcas* serine/arginine repetitive matrix protein 2-like (SRRM2-like), respectively. But the strange thing is that the primary amino acid sequence of GbAT11 is closely similar to Damaged DNA-binding 2, putative isoform 1 (DDB2), but the gene has not yet been reported, and the most similar homolog gene is related with the uncharacterized protein LOC105800652 in NCBI (National Center for Biotechnology Information). A further search of the COTTONGEN (<http://www.cottongen.org>) indicated that the *GbAt11* gene is located on Chromosome 9 in *G. raimondii*, so *GbAt11* is a new gene which may be located on chromosome 9 in *G. hirsutum*.

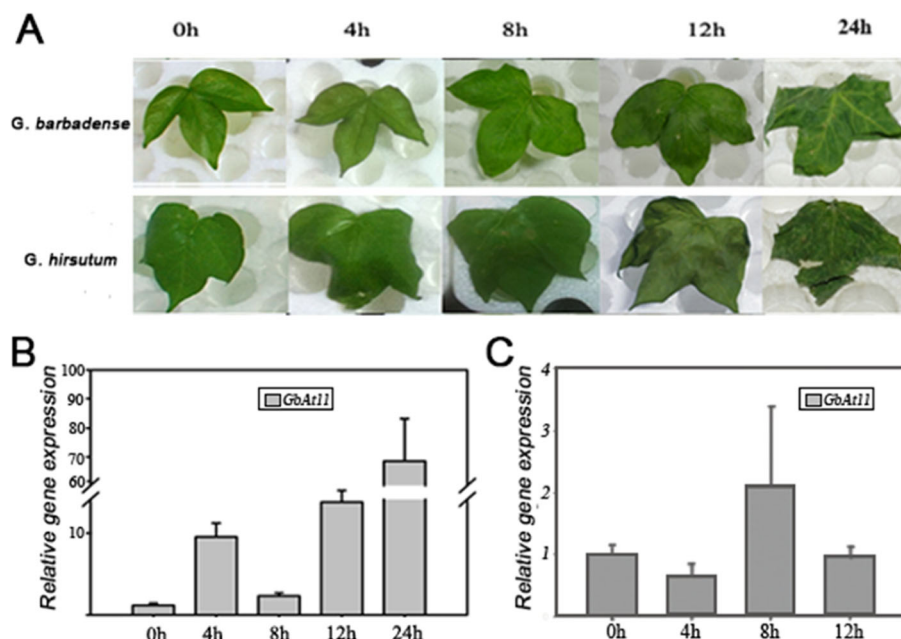
**GbAt11 gene expression is specifically induced by *Verticillium dahliae* in *Gossypium barbadense***

In order to investigate the relationship between *GbAt11* and *V. dahliae*, and to explore transcriptional difference between *G. barbadense* and *G. hirsutum*, we collected young leaves at the adult-plant stage from the same parts of wild *G. barbadense* and wild *G. hirsutum*. Leaves were dipped in filtration of *Vd991* (spore concentration of  $1 \times 10^6$  per mL). Total RNA was extracted from samples at different induction times, then

reversely transcribed into cDNA to determine the relative level of *GbAt11* transcription by RT-PCR. Results showed that: (1) transcription of *GbAt11* increased 10-fold in seedlings treated with *Vd991* for 4 h in *G. barbadense*, and then declined to the initial level. Transcription of *GbAt11* increased significantly when induced for 8 h, and peaked at 24 h at more than 70-fold. These results indicated that the *GbAt11* gene is specifically induced by *V. dahliae* in *G. barbadense*. (2) Transcription of *GhAt11* increased slightly in *G. hirsutum*, but the leaves were heavily wilted at 12 h, and RNA were failed to be extrated. These results showed that expression of *GbAt11* is specifically induced by *V. dahliae*, and is closely related to the host resistance response to pathogenic bacteria. In response to inducing by *V. dahliae*, *GbAt11* is specifically overexpressed in Verticillium wilt-resistant *G. barbadense*, but the homolog of this gene was not specifically induced in *G. hirsutum* which is susceptible to Verticillium wilt, and its leaves appeared serious wilting within 12 h (Fig. 1).

**GbAt11 overexpression enhances resistance to Verticillium wilt in *Gossypium hirsutum***

Because *GbAt11* is specifically induced in highly Verticillium wilt-resistant *G. barbadense*, we speculated that overexpression of *GbAt11* could improve the resistance to Verticillium wilt in susceptible *G. hirsutum*. To test this hypothesis, we transformed the wilt-susceptible *G.*



**Fig. 1** *GbAt11* expression is specifically induced by *Verticillium dahliae* toxin in *Gossypium barbadense*. **a** The representative *G. barbadense* and *G. hirsutum* leaves after treated with *V. dahliae* spore suspension ( $1 \times 10^6$  per mL) for indicated times. **b-c** Transcription changes of *GbAt11* gene in *G. barbadense* (**b**) and *G. hirsutum* (**c**) after treated with *V. dahliae* spores ( $1 \times 10^6$ /mL) for indicated times

*hirsutum* variety JM169 (WT) with *GbAt11* and obtained 52 transgenic plants. Verticillium wilt-resistance ratings of field and greenhouse in a disease nursery were performed on the T<sub>2</sub> generation plants of 17 transgenic lines. L-214 is a high-yielding line with good disease resistance. As shown in Fig. 2a, compared with the parental line (WT), L-214 showed the highest disease resistance, followed by L-213, but L-235 showed poor disease resistance.

In order to further confirm the role of *GbAt11* gene in disease resistance, we extracted total proteins from leaves of transgenic L-214 (the flag tag fused to the C-terminal end of GbAT11) and the parental line WT (No flag tag). Anti-flag antibody-coated magnetic beads were incubated with the total protein to capture the flag tagged GbAT11 (IP). Then we used anti-flag antibody for Western blot detection, which showed that transgenic L-214 had one hybridizing band between 25 and 35 kd, which was absent in JM169, confirming the presence of GbAT11 protein in L-214 (Fig. 2b). To shed light on the relationship between the level of GbAt11 transcription and disease resistance, we quantified GbAt11 expression in transgenic lines L-213, L-214, and L-235 using real-time PCR. The results showed that GbAt11 expression was the highest in L-214 with the best

Verticillium wilt resistance, and successively decreased in lines L-213 and L-235 (Fig. 2c), corresponding to the relative levels of Verticillium wilt resistance in the two lines (Fig. 2c and d). In subsequent experiments, L-214 was selected as its excellent disease resistance.

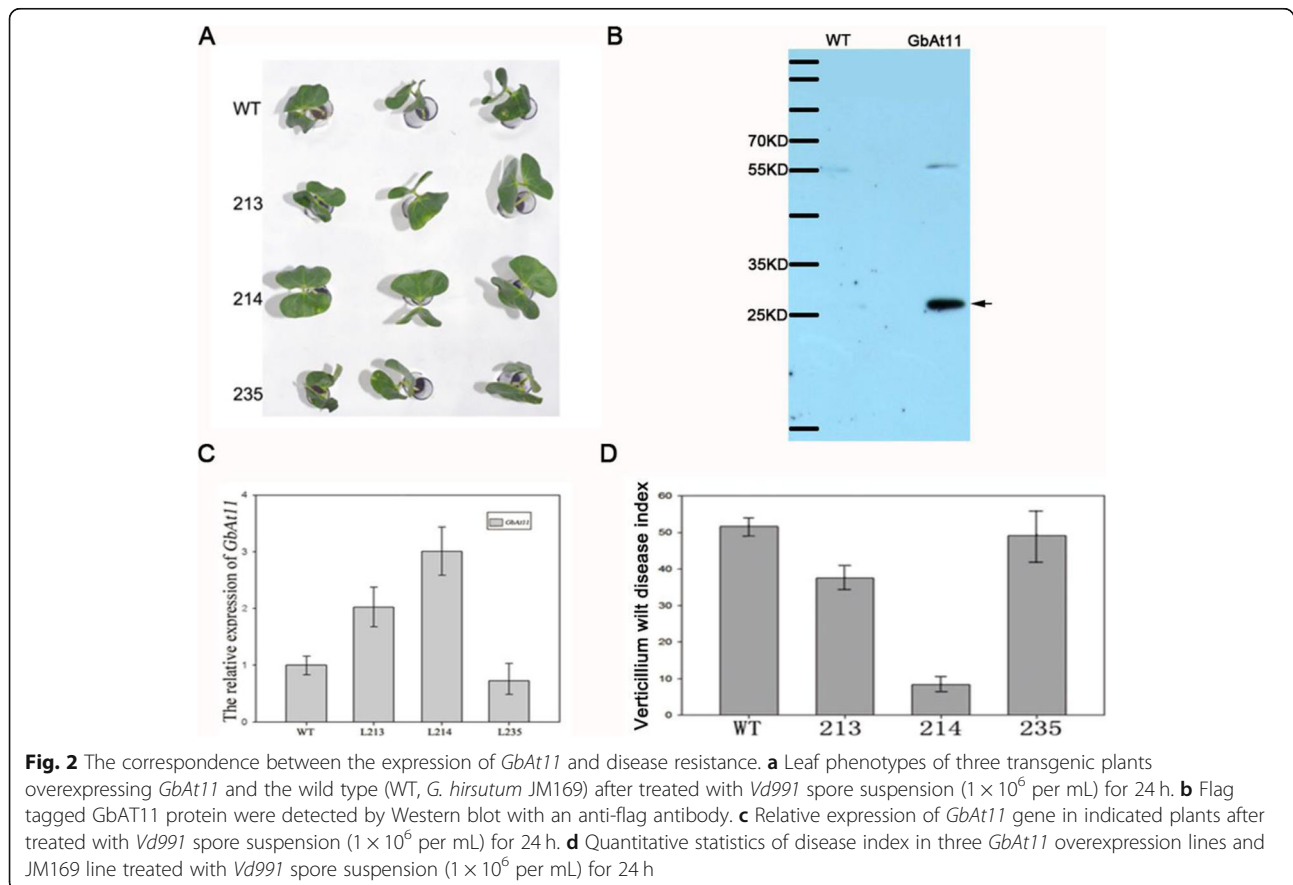
The disease index was calculated as formula (1):

$$\text{Index} = \frac{\sum \text{disease rating } c \times \text{plant number } c}{4 \times \text{total plant number}} \times 100.$$

Here, disease rating *c* was scored in five grades (0 to 4) based on the severity of Verticillium wilt symptoms in cotton. Grade 0, health, no disease symptom of leaves; grade 1, leaves have slight disease symptoms; grade 2, leaves have disease symptoms in moderate degree; grade 3, leaves have remarkable disease symptoms; And grade 4, leaves have severe disease symptoms. Plant number *c* was the number of plants corresponding to the rating of disease severity. The '4' was grade 4 which was the highest grade.

***GbAt11* overexpression in *Gossypium hirsutum* up-regulates transcription of several key genes in the immunity pathways**

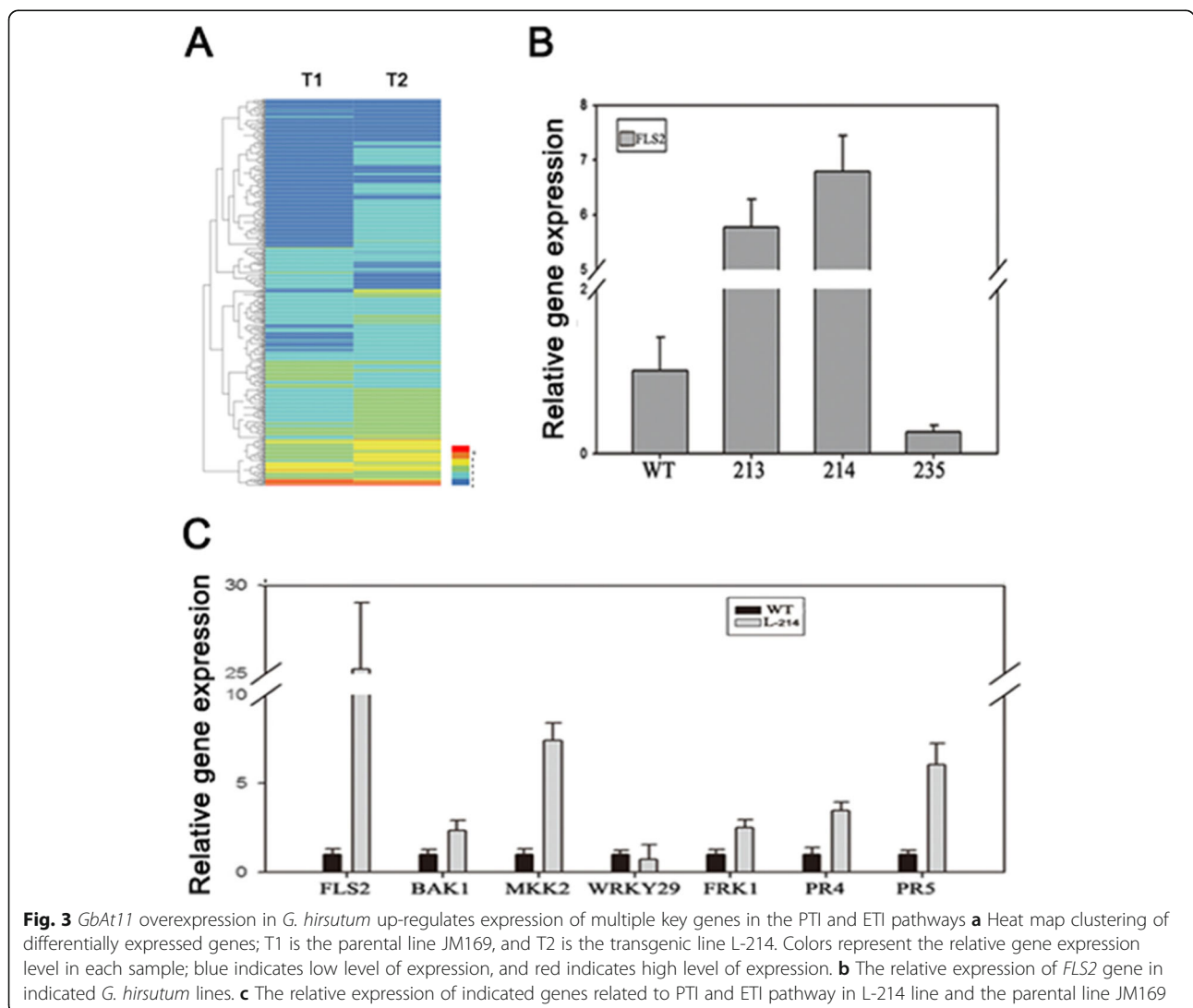
Given that *GbAt11* overexpression can improve disease resistance in plants, further transcriptome analyses



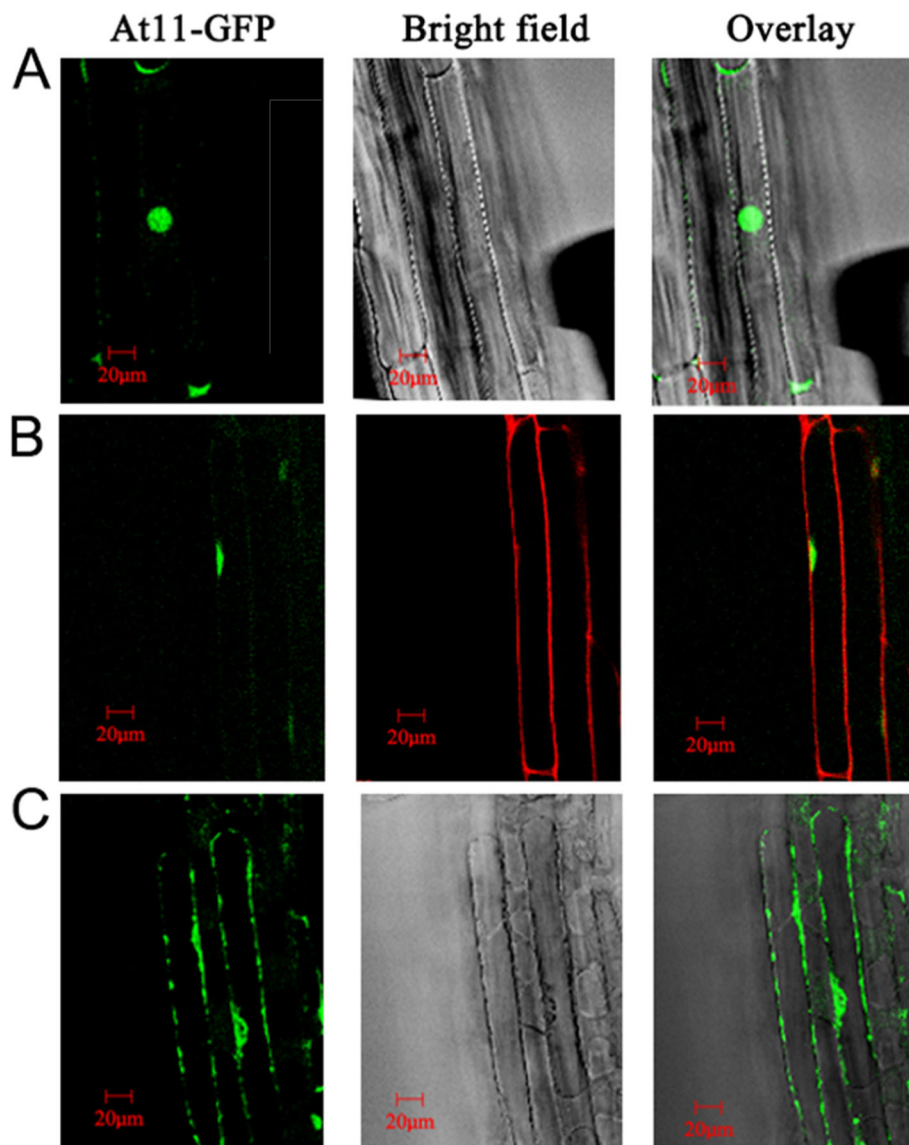
(Wang et al. 2009) were performed in L-214 line and WT line. Clustering differentially expressed genes showed that gene transcription was significantly altered in the transgenic *GbAt11*-expressing line compared with the parent line. A total of 472 genes showed significant expression differences ( $FDR < 0.01$ ) (Fig. 3a). Further transcriptome analysis showed that many genes involved in “plant-pathogen interactions” (KEGG Pathway) were up-regulated in transgenic plants of *GbAt11*, including *FLS2* and the calcium dependent protein kinase CDPK (Harmon et al. 1994; Rigo et al. 2013); *RPS2*, *RIN4* (Rigo et al. 2013) and *PBS1* (Joys 1965), which are disease-resistance genes in ETI; and *HSP90*, the downstream gene in ETI. Therefore, it is reasonable to speculate that the transgenic *GbAt11* line can activate ETI by affecting post-translational modifications, protein transporting and folding, relieving the inhibition of the pathogen on

PTI-based immunity and improving resistance to Verticillium wilt in cotton. To confirm the result of transcriptome, real-time PCR was conducted in three lines, and the data showed that *FLS2* in L214 and L213 is extremely significant higher than WT (Fig. 3b). Next, 7 genes involved in ETI and PTI process were assayed via real-time PCR in L214. The results confirmed that *FLS2*, *BAK1*, *MKK2*, *FRK1*, *RIN4*, *RPS2* genes were significantly up-regulated in transgenic line L-214, and the *FLS2* gene showed the highest up-regulation (Fig. 3c).

**Subcellular localization of GbAT11 in *Arabidopsis thaliana***  
 We used *Arabidopsis thaliana* to explore the functional site of GbAT11 at the cellular level. We first produced transgenic plants of *Arabidopsis thaliana* expressing *GbAt11-gfp*, and then observed the subcellular localization of the GbAT11-GFP fusion protein in the



**Fig. 3** *GbAt11* overexpression in *G. hirsutum* up-regulates expression of multiple key genes in the PTI and ETI pathways **a** Heat map clustering of differentially expressed genes; T1 is the parental line JM169, and T2 is the transgenic line L-214. Colors represent the relative gene expression level in each sample; blue indicates low level of expression, and red indicates high level of expression. **b** The relative expression of *FLS2* gene in indicated *G. hirsutum* lines. **c** The relative expression of indicated genes related to PTI and ETI pathway in L-214 line and the parental line JM169



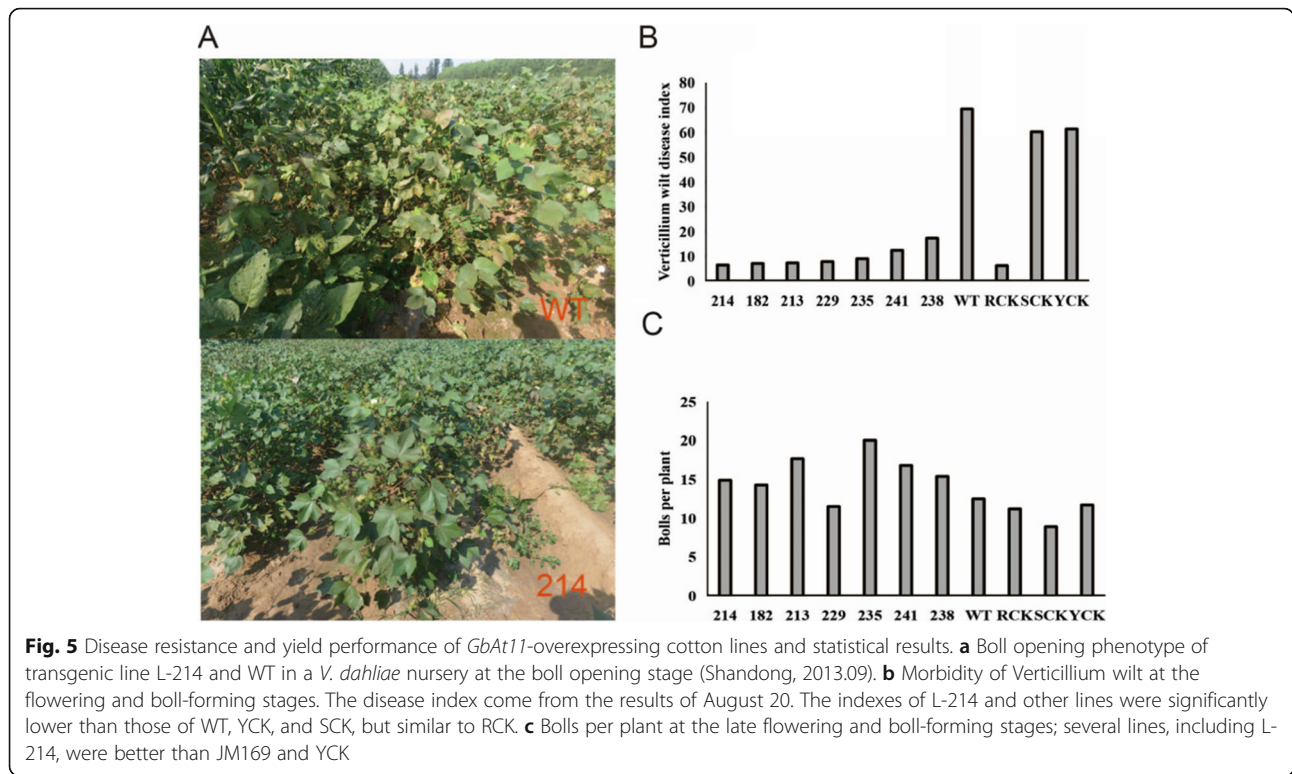
**Fig. 4** Subcellular localization of GbAT11 in roots of transgenic *Arabidopsis thaliana*. **a** This image shows the localization of GFP-GbAT11 fusion protein in *Arabidopsis* cells, and green fluorescence appears under laser scanning confocal microscopy. **b** Laser scanning confocal microscopy showing the GbAT11 protein was localized in the nucleus after PI treatment. **c** Laser scanning confocal microscopy observed that GbAT11 protein was localized in the nucleus, and localized on the cell membrane after plasmolysis with 20% sucrose solution treatment of transgenic *Arabidopsis thaliana* roots

root tip by laser scanning confocal microscopy. The results showed that GbAT11 is mainly localized to the nucleus (Fig. 4a), and a small amount presents on the cell wall (Fig. 4b). To clarify whether GbAT11 is also located on the cell membrane in addition to the nucleus, we treated *Arabidopsis* root tips with a 20% sucrose solution to separate cytoplasm from the cell wall, and then observed them under a fluorescence microscope. We found that the GbAT11-GFP protein was indeed located on the cell membrane (Fig. 4c). We also observed that the fluorescence intensity of the cell membrane was enhanced in Fig. 4c, which could be caused by shrinkage of

the cell membrane, or the transfer of the GbAT11-GFP fusion protein from the nucleus to the membrane under osmotic stress.

**GbAt11 overexpression increase cotton yield**

A common misconception is that it is difficult to have both disease resistance and high yield in the same plant. However, our results showed that overexpression of *GbAt11* not only improved disease resistance, but also increased boll-setting, which is the most important factor in cotton production. To identify the high yielding transgenic lines that overexpress *GbAt11*, we



investigated disease severity of the transgenic lines in a *V. dahliae* nursery and also analyzed the basic yield traits, such as boll number per plant, boll opening phenotype in the field (Fig. 5a). The disease indexes of Verticillium wilt were 6.52, 7.35, and 9.09 in L-214, L-213, and L-235, respectively, and 69.44, 60.23, 62.25, and 61.36 in the wild type control (WT), the susceptible control (SCK), the disease resistant control (RCK), and the high yield control (YCK), respectively. Through analysis, it was found that the disease indexes of all transgenic lines were lower than the WT, YCK, and SCK, moreover, L-214 was extremely close to the RCK (Fig. 5b). The results showed that the bolls per plant of L-214, L-213 and L-235 were 14.9, 17.6 and 20.0, respectively, and 12.5, 8.9, 11.2, and 11.7 in WT, SCK, RCK, and YCK, respectively. Boll-setting in all three transgenic lines was higher than the parent lines and other controls. Other than these three lines, the bolls per plant in many other different lines were also better than YCK (Fig. 5c). In conclusion, overexpression of the *GbAt11* gene in *G. hirsutum* not only improved its resistance to disease caused by *V. dahliae*, but also increased the number of bolls per plant in a field that was severely infested with Verticillium wilt.

**Discussion**

In this study, *GbAt11* gene expression experiments showed that *GbAt11* up-regulation started at 4 h after

induction in *G. barbadense*, then decreased at 8 h, and increased to 70-fold at 24 h. This result is a normal response of plants to pathogen infection: the expression of genes associated with disease resistance will increase, followed by a process of adaptation, and then expression increases again as the infection becomes severe (Denance et al. 2013). This pattern of *GbAt11* expression was observed in the response of *G. barbadense* to infection by *V. dahliae*. As for the highly susceptible *G. hirsutum*, expression of genes relevant to disease resistance are not induced, but occurs systemic infection and results in leaf wilt. Thus it appears that *GbAt11* plays an important role in determining Verticillium wilt resistance in *G. barbadense*.

Previous studies have shown that after low temperature treatment, dehydrated protein WZY2 will leave the dehydration point and disperse into the cytoplasm, indicating that low temperature will affect the distribution of wheat dehydrated protein WZY2 in cells (Lv 2014). Observing the localization of GbAT11, we can find that the cell membrane leaves the cytoplasm after the plasma wall separation occurs, and the fluorescence is localized in the cell membrane. The fluorescence brightness of the cell membrane in Fig. 4c is enhanced, which may be caused by shrinkage of the cell membrane, or when the cell is stressed, the *GbAt11*-GFP fusion protein localized in the nucleus is transferred to the cell membrane.



Typically, overexpression of transcription factors, such as DRE, can improve stress tolerance or disease resistance in plants, but it may also cause dwarfing and yield reduction, etc. (Narusaka et al. 2003). We found that the cotton strain transferred to *GbAt11* had a significant effect against Verticillium wilt. In addition, later production of cotton showed that the yield of transgenic cotton was higher than wild type. Although we demonstrated that the transgenic lines overexpressing *GbAt11* had better resistance to Verticillium wilt, the mutant strain was failed to be constructed. Because cotton is a heterotetraploid plant, and AT11 has two copies in *G. barbadense*, it is difficult to knock them out together with the same vector. In addition, because of the limitation of cotton growth cycle, it is impossible to obtain double mutant in a short time. So next we will use the virus induced gene silencing system to silence the *GbAt11* gene, and then further investigate the cotton resistance to Verticillium wilt. Unfortunately, we were unable to identify any proteins that directly interact with GbAT11 by co-immunoprecipitation and yeast two hybrid assays, so this remains to be further investigated in the future. Although its function needs to be further researched, the effect of increasing yield and resistance to Verticillium wilt has guiding significance to cotton production practice.

## Conclusion

*GbAt11* has been identified as a new gene which is specifically induced by *V. dahliae*. Its overexpression in *G. hirsutum* not only significantly improves resistance to Verticillium wilt, but also up-regulates transcription of several key genes in the immunity pathways. These results show that *GbAt11* plays a role in disease resistance. Furthermore, overexpression of the *GbAt11* gene in *G. hirsutum* can also increase the number of bolls per plant to increase cotton yield.

## Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s42397-020-00047-3>.

**Additional file 1.**

**Additional file 2.**

**Additional file 3.** (XLS 215535 kb)

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## Authors' contributions

Qi JS conceived and initiated the research. Qi JS, Qiu TT, Wang YJ and Jiang J performed the experiments. Qi JS, Qiu TT, Wang YJ and Zhao J analyzed

the data. Qi JS, Qiu TT, Wang YJ and Jiang J wrote the article. The authors read and approved the final manuscript.

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## Availability of data and materials

The source data underlying Figs. 1, 2, 3, 4 and 5 are provided as a Source Data file. All other data that support the findings of this study are available from the corresponding author upon request.

## Ethics approval

This study was approved by the local ethics committee.

## Consent for publication

We accept publication.

## Competing interests

The authors declare that they have no competing interests.

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