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An R2R3 MYB Transcription Factor *Ghmyb18* Confers Cotton Aphid Resistance Through Regulating The Synthesis of Flavonoids in Cotton Plants

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Abstract

Accumulating researches confirmed that plant MYB transcription factors are involved in the defense response to insect herbivores. However, the target genes and signaling pathways they regulate are still unknown. Here, we identified the R2R3 MYB transcription factor *GhMYB18* from upland cotton (*Gossypium hirsutum* L.). *GhMYB18* was a functional transcription activation factor. The expression level of *GhMYB18* was significantly up-regulated under the conditions of aphid infestation and exogenous SA treatment. Overexpression of *GhMYB18* in cotton cotyledons conferred enhanced tolerance to the aphid (*Aphis gossypii* Glover), while the silence of *GhMYB18* expression led to increased susceptibility of cotton plants to aphid. Subsequent analysis showed that *GhMYB18* activated defense-related enzymes including CAT, POD, PPO and PAL, induced the synthesis of salicylic acid and flavonoids to participate in the defense response against aphid. These results collectively prove that the cotton MYB gene *GhMYB18* modulated the plant defense response to aphid attack, which may offer guide strategies for the prevention of cotton aphid.

Key Message

R2R3 MYB transcription factor *GhMYB18* is involved in the defense response to cotton aphid by participating in the synthesis of flavonoids.

1. Introduction

Cotton (*Gossypium hirsutum* L.) is a momentous industrial crop used widely as raw materials of fiber, cooking oil and biofuel in the world (Liu et al. 2021). *Aphis gossypii* Glover is one of the important insect pests in the cotton production that seriously influence cotton growth, fiber yield and lint quality (Quan et al. 2019). It is an environmentally friendly measure to use the aphid resistance of cotton to control cotton aphids. Therefore, it is importantly significant for cotton aphid control to continuously excavate the aphid resistance gene of cotton and the mechanism of cotton resistance to aphids (Yates and Michel 2018). This task has been promoting promptly with rapid development of sequencing technology (Kumar et al. 2016; Hu et al. 2019).

At present, many aphid resistance genes of cotton have been discovered. Some of them can improve cotton resistance to cotton aphids directly through regulating aphid-resistance substance synthesis, such as cotton chitinase gene *GhChi6* (Zhong et al. 2021), caryophyllene synthase gene *GhTPS1* (Zhang et al. 2020) and laccase gene *GhLac1* (Hu et al. 2018). The other genes that include mainly transcriptional factors can promote cotton resistance to cotton aphids indirectly through regulating gene expression and signal transduction under cotton aphid stress. But these genes have not been reported in cotton although it has been reported in other plants. For example, *TaMYB19*, *TaMYB29* and *TaMYB44*, which are regulated by ethylene signaling pathway, cooperatively participate in wheat phloem defense and change English grain aphid feeding behavior (Zhai et al. 2017). *CmMYB15* (An et al. 2019) and *CmMYB19* (Wang et al. 2017) provide chrysanthemum resistance to aphids by regulating the biosynthesis of lignin. The

Arabidopsis MYB transcription factors *AtMYB102* (Zhu et al. 2018) and *AtMYB44* (Lu et al. 2013) regulate resistance to *Myzus persicae* by activating ethylene defense in *Arabidopsis*. These studies have indicated that aphid resistance in cotton is a complex biologic trait that is regulated by multiple genes and varies from cotton cultivar, environmental conditions, growth developmental stages and nutritional status.

MYB transcription factor is one of the largest transcription factor families (TFs) and has a characteristic MYB domain that consist of 52 amino acid repeats that form helix–turn–helix structures required for binding to cis-elements in the promoters of target genes (Dubos et al. 2010). According to the numbers of adjacent repeats, MYB transcription factors are divided into four classes: R1-MYB including R3-type, R1/R2-type, and GARP family, R2R3-MYB, 3R-MYB including R1R2R3-type MYB, and 4R-MYB containing four R1/R2-like repeats (Cheng et al. 2016). The most common of them is R2R3-type MYB (Chen et al. 2006). R2R3-type MYB is comprised of a superfamily of gene members that have undergone a rapid expansion through repeated gene duplication and functional divergence, notably playing conserved roles in plant growth, development and metabolism regulation (Liu et al. 2021).

R2R3-type MYB proteins play diverse roles in cotton development and response to all varieties of stress. Firstly, R2R3-type MYB proteins is involved in cotton growth and development. For example, studies have shown that the developmental regulation of six newly cotton myb-domain genes (*GhMYB*) of the R2R3-MYB family is involved in the differentiation and expansion of cotton seed trichomes (Loguercio et al. 1999). Two TT2-type MYB transcription factors identified from tetraploid cotton are involved in regulating proanthocyanidins biosynthesis, providing new strategies for engineering condensed tannins in crops (Lu et al. 2017). Secondly, R2R3-type MYB proteins response to all kinds of abiotic stress. For instance, cotton MYB transcription factor *GbMYB5* is positively involved in plant adaptive response to drought stress (Chen et al. 2015). Overexpression of cotton R2R3 MYB Gene *GhMYB73* increases tolerance to salt stress in transgenic *Arabidopsis* (Zhao et al. 2019). Additionally, R2R3-type MYB proteins response to all kinds of biotic stress including pathogen and herbivore insets feeding. For example, *GhMYB36*, a gene encoding a R2R3-type MYB protein in *Gossypium hirsutum*, which confers drought tolerance and *Verticillium dahliae* resistance in both *Arabidopsis* and cotton (Liu et al. 2021). Similarly, the cotton *MYB108* forms a positive feedback regulation loop with *CML11* and participates in the defense response against *Verticillium dahliae* infection (Cheng et al. 2016). However, there are few reports on the relationship between MYB factor and insect resistance of cotton although some MYB proteins have been proved to function in defense against insect herbivores, including *AtMYB102* (De et al. 2006), *GsMYB15* (Shen et al. 2018), *AtMYB44* (Lu et al. 2013). *NaMYB8* (Kaur et al. 2010), *TaMYB19, TaMYB29, TaMYB44* (Zhai et al. 2017).

Cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is the most common sucking pest in cotton. It usually appears on the abaxial surface of leaves and feeds on the phloem, causing the infected leaves curled and wrinkled. (Kerns et al. 1992). As a result of feeding, honeydew secreted by cotton aphids contribute to a variety of plant diseases (Hegde et al. 2011). In addition, the interaction between aphids and other organisms, including plant pathogens, ants, and other herbivores, seriously influences the

plants growth and reproduction. Therefore, the massive cotton aphids due to the insecticide resistance or favorable weather condition has a negative impact on the cotton yield (Goggin et al. 2007; Ali et al. 2016).

In this study, a R2R3-type MYB genes *GhMYB18* was identified in cotton response to cotton aphid damage. The transient expression and Virus-mediated gene silencing (VIGS) strategy were employed to clarify the role of *GhMYB18* in response to the damage of *Aphis gossypii*, indicating that it participates in the defense response to cotton aphid. Subsequent studies have found that *GhMYB18* can activate defense-related enzymes including CAT, POD, PPO and PAL, induce the synthesis of salicylic acid, and further accumulate flavonoids by regulating phenylpropanoid metabolic pathway in cotton in defense response to cotton aphids.

2. Materials And Methods

2.1 Materials and culture condition

Cotton variety Zhongjixing 7 (*Gossypium hirsutum* L.), kindly provided by professor Dinguo Li in Yangtze University, was used in this study. Cotton plants were grown in pots (500 mL) filled with soil mix (vermiculite:humus, 1:1) at 25°C under 16 h light / 8 h dark conditions. Tobacco (*Nicotiana benthamiana*) is also grown at 25°C under 14 h light / 10 h dark conditions. Cotton aphids were collected from cotton experimental field in Yangtze University, inoculated on cotton plants at 25°C under 16 h light / 8 h dark conditions and transferred to fresh plants every 2 weeks.

2.2 Phylogenetic Analysis of *GhMYB18*

The homologous sequences of *GhMYB18* were retrieved and analyzed using online BLAST tools from the NCBI (National Center for Biotechnology Information, USA). The sequences of MYB proteins were aligned using DNAMAN. A phylogenetic tree of *GhMYB18* with its homologs was constructed using MEGA 7.0 with the maximum likelihood method (bootstraps = 1000).

2.3 RNA extraction and qPCR analysis

Total RNA was extracted using Spectrum™ plant total RNA kit (Sigma-Aldrich, USA) according to the manufacturer's protocol. Total RNA was reverse transcribed into first-strand cDNA with reverse transcriptase (Promega, USA), which was used as a template for quantitative real-time PCR (qPCR). Cotton gene *GhUBI1* (GenBank accession number: EU604080) served as an internal control. The relative expression level of each gene was calculated using the 2-MMCT method (Livak and Schmittgen 2001). The relative value of their expression was showed as mean values of three independent tests, and three replicates were performed for each test. All the primer sequences are shown in Table S1.

2.4 Trans-activation activity assays

The CDS of *GhMYB18* was ligated to the pGBKT7 vector (Clontech, Mountain View, CA, USA). The BD-*GhMYB18* plasmid generated was transferred into the *Yeast* strain AH109 (Clontech). The pGBKT7 empty vector served as the negative control. The transformed strains were cultured on medium at 28°C for 3

days before observation. Three types of media were used: SD/-Trp, SD/-Trp with aureobasidin A (AbA), and SD/-Trp with AbA and X-α-Gal (Jiang et al. 2021).

2.5 Expression profile of *GhMYB18* in response to aphid infestation and Phytohormone treatment

The cotton seedlings selected in the experiment had grown for 14 days with same phenotype. Each cotton seedling cotyledons were inoculated with 20 adults of cotton aphids with the same size. After the cotton aphids were feed for 24 h, 48 h, and 72 h, the expression levels of *GhMYB18* in the roots, stems and cotyledons. of cotton seedlings were analyzed. A solution of methyl jasmonate (MeJA) and salicylic acid (SA) with a concentration of 0.05 mmol/L was prepared and sprayed evenly on the cotyledons of cotton seedlings. After hormone induction for 24 h, 48 h, and 72 h, the expression levels of *GhMYB18* was analyzed in cotton cotyledons. Taking untreated cotton seedlings as a control, three biological replicates were used for the above treatments.

2.6 Transient expression

The vector pBI121-*GhMYB18* and empty vector were ligated into *Agrobacterium tumefaciens* GV3101 (Sun et al. 2015). The transformed strains were grown in a lysogeny broth (LB) liquid medium (pH = 7.0) containing 50 ug/mL kanamycin and rifampin at 28°C, 220 rpm. After centrifugation, the pelleted bacteria were resuspended (OD_{600} = 0.8). The resuspension is injected into the cotton cotyledons by a needle-less syringe followed by the expression detection of *GhMYB18* (Yue et al. 2012).

2.7 Virus-induced gene silencing

The pTRV1 and pTRV2 vectors were used for VIGS experiments. The CDS of *GhMYB18* of *GhMYB18* was inserted into pTRV2 (Liu et al. 2002). *Agrobacterium tumefaciens* cultures (OD_{600} = 1.5) harboring pTRV1 and that containing pTRV2-*GhMYB18* were mixed at a 1:1 ratio and inoculated into cotton plants by vacuum infiltration as described by Qu et al (Qu et al. 2012).

2.8 The resistance of *GhMYB18*-overexpressing and *GhMYB18*-silenced cottons against cotton aphids

No-choice and choice assays were performed between wild-type plants, control plants and treatment group plants. For no choice assay, 10 adult cotton aphids with the same individual size were inoculated on the cotton cotyledons, and the cotyledons were fixed with insect cages to prevent the aphids from escaping. The number of cotton aphids on the cotyledons after 24 h, 48 h, and 72 h were investigated respectively. For choice assay, the detached cotton cotyledons were placed symmetrically on the inner edge of a petri dish, and the petioles are soaked with MS nutrient solution to prevent the leaves from withering. The leaves were inoculated with 30 adult cotton aphids with the same individual size and investigate the number of aphids on the cotyledons after 24 h.

2.9 Quantification of aphid excreted honeydew

The sterilized agar medium was poured into a glass petri dish with a diameter of 9 cm, and the front of the leaves were placed on the medium without leaving a gap. The cotyledons were inoculated with 10 adult of cotton aphids with the same individual size, the petri dish was inverted, and the aphid honeydew was collected with whatman filter paper for three days. The determination method of aphid honeydew was as follows: the filter papers with collected honeydew were immersed in 0.1% (w/v) ninhydrin solution. After drying, the filter papers were torn into pieces, and stain were extracted in 1 mL of 90% (v/v) methanol for 1 h at 4°C with continuous oscillation. After centrifugation at 5000×g for 1 min, the absorbance was measured at 500 nm (Kim and Jander 2007; Nisbet et al. 1997).

2.10 Determination of enzyme activity

0.3 g leaves were placed in a pre-cooled mortar, liquid nitrogen was added and the leaves were quickly ground. After grinding uniformly, the extraction buffer was added and the mixture was collected in a centrifuge tube. After centrifugation at 4°C and 14000×g for 10 min, the supernatant is the crude enzyme solution. The activities of CAT, POD, PAL and PPO were determined according to the guide of enzyme activity kit (Nanjing Jiancheng Bioengineering Institute, China).

2.11 Determination of salicylic acid content

The SA content is extracted and purified according to the method in the previous study (Fu et al. 2012). 0.1 g cotton cotyledons were fully ground in liquid nitrogen, suspended in 1 mL of methanol, and incubated at -20°C overnight. The mixture was centrifuged at 14000×g for 10 min at 4°C and purified with a 0.25 um needle nylon filter. 200 µL of filtrate was taken and dried with a nitrogen gas, then 200 µL of sodium acetate buffer (0.1 M, pH = 5.5) was added. The mixture was shaken at 4°C for 1 h and centrifuged at 14000 ×g for 10 min at 4°C. The suspension was treated with 10 µL of β-glucosidase, incubated in a water bath at 37°C for 2 h, and then boiled in water for 5 min to stop the enzymatic reaction. After that, the mixture was centrifuged at 14000×g for 10 min at 4°C, and then the supernatant was subjected to LC-MS to analyze the total SA content.

2.12 Determination of total flavonoids content

0.1 g leaves were placed in a centrifuge tube, added 1 ml 80% (v/v) methanol, and shaken them overnight at 4 ℃. After centrifugation at 12000×g in at 4 ℃ for 15 min, the supernatant was collected. Total flavonoids were determined according to the method described previously (Sultana et al. 2009). 1 mL of 80% methanol was Added into the precipitation again, and extracted at 4 ℃ for 1 h, centrifuged at 12000×g for 15 min. The supernatants of the two extractions were combined for subsequent research. 100 µL supernatants, 500 µL deionized water, and 30 µL 5% $NaNO_2$ were mixed. After 6 min, 200 µL 1 M NaOH was added. After 10 min, 140 µL deionized water was added to adjust the volume to 1 mL. 200 µL reaction solution was used to determine the absorbance at 420 nm.

2.13 Statistical Analysis

SPSS v26.0 was used for all statistical analysis, and all data was shown as mean ± SD of one representative experiment. Student's t-test was used to compare the significant differences of *GhMYB18* expression levels after aphid infestation and phytohormone treatment. The p-value was shown as $p < 0.05$ or $p < 0.01$ to indicate significant differences. In case of other experiments, Tukey's multiple range test ($p < 0.05$) and one-way analysis of variance were performed to determine the significance of the data. English letters a, b, c indicated the significance levels ($p < 0.05$).

3. Results

3.1 Sequence Analysis of *GhMYB18*

GhMYB18 was isolated from upland cotton 'Zhongjixing 7', and sequence analysis showed that it was a new cotton MYB family gene. The *GhMYB18* gene is composed of 981 bp and encoding a polypeptide of 326 amino acids (File S1). *GhMYB18* belongs to the R2R3-MYB subfamily, as it has two conserved MYB domains including an R2MYB domain between aa 13–63 and an R3MYB domain between aa 66–114. Alignment analysis indicated that this gene has 67% similarity with GhMYB30 (Fig. 1A). Phylogenetic trees of GhMYB18 and MYB proteins from other plant species show that GhMYB18 are closely related to GhMYB30 (Fig. 1B).

3.2 *GhMYB18* was induced by cotton aphid infestation, SA and MeJA

GhMYB18 was expressed in roots, stems and cotyledons in cotton plants. At 24 h, 48 h and 72 h after sustained infestation of cotton aphids, the expression levels of *GhMYB18* were significantly higher than that of the control in roots, stems and cotyledons in cotton plants. Furthermore, *GhMYB18* presented higher gene expression levels in roots, stems and cotyledons at 72 h under cotton aphid infestation. Their expression levels were respectively 4.03, 3.66, and 3.21 times more than that in the wild type plants (WT) at 72 h (Fig. 2A). Secondly, *GhMYB18* transcripts were significantly up-regulated in leaves at 24 h, 48 h and 72 h after salicylic acid induction (Fig. 2B). In addition, after methyl jasmonic acid induction, the expression level of *GhMYB18* was inhibited and was significantly down-regulated at 48 h compared with control (Fig. 2C). Those results show that aphid damage or exogenous SA induction can promote the expression levels of *GhMYB18*, while exogenous MeJA induction may decrease the expression level of *GhMYB18*.

3.3 *GhMYB18* was a functional transcription activation factor

Yeast harboring pGBKT7-*GhMYB18* or pGBKT7 (negative control) grew normally on SD/-Trp medium (Fig. 3B). But on SD/-Trp with AbA medium, only the yeast strain of pGBKT7-*GhMYB18* grew normally (Fig. 3C). As the same time, it was no more than yeast transferred into pGBKT7-*GhMYB18* turned blue on the chromogenic medium (SD/-Trp with AbA and X-α-Gal medium) (Fig. 3D). These results illustrate that *GhMYB18* is a functional transcription activation factor.

3.4 *GhMYB18* overexpression enhances cotton plant tolerance to *Aphis gossypii*

The *GhMYB18* expression level in transiently overexpressed *GhMYB18* cotton seedlings was measured by qPCR. Compared with wild type cotton plants (WT) and cotton plants containing pBI121(pBI121 plants), the expression levels of *GhMYB18* in cotton plants containing pBI121-*GhMYB18* (pBI121-*GhMYB18* plants) significantly increased at 24 h, 48 h 72 h after *Agrobacterium tumefaciens* infection. The pBI121-*GhMYB18* plants presented a higher expression level of *GhMYB18* at 48 h, and its expression level was 6.26 times higher than that of pBI121 plants (Fig. 4A). The choice assay showed that the cotton aphids were more widely distributed on pBI121 plants and WT than pBI121-*GhMYB18* plants at 24 h, 48 h and 72 h after sustained feeding of cotton aphids (Fig. 4B). Obviously, WT and pBI121 plants are more attractive to cotton aphids. Simultaneously, we conducted no-choice assay to examine the aphid resistance of *GhMYB18*. Consistently, compared WT and pBI121 plants, the number of cotton aphids on pBI121-*GhMYB18* plants was lower at 24 h, 48 h, and 72 h after sustained feeding of cotton aphids (Fig. 4C). Assays of honeydew secretion from *Aphis gossypii* was consistent with the above conclusions (Fig. S1). Assays of honeydew secretion from *Aphis gossypii* showed that the amount of honeydew secreted on pBI121-*GhMYB18* plants was significantly lower than that of WT and pBI121 plants at 48 h and 72 h (Fig. 4D). These results indicate that *GhMYB18* overexpression may enhance cotton plant tolerance to *Aphis gossypii*.

3.5 Silencing of *GhMYB18* increased cotton plant susceptibility to *Aphis gossypii.*

The virus-mediated gene silencing (VIGS) strategy was also employed to clarify the role of *GhMYB18* in cotton response to the infestation of *Aphis gossypii*. Compared with the wild-type plants (WT) and control group plants (*TRV:00*), the expression level of *GhMYB18* was obviously reduced in *GhMYB18*-silenced plants (*TRV: GhMYB18*) at 18 d and 24 d after *Agrobacterium tumefaciens* infection. The *GhMYB18*-silenced plants presented a lowest expression level of *GhMYB18* at 18 d after the silencing treatment, which was only 49.5% of control group plants (Fig. 5A). The choice tests showed that cotton aphids were more inclined to feed on *GhMYB18*-silenced cottons than WT and control group plants at 18 d (Fig. 5B). In the no-choice assay, the number of cotton aphids in the *GhMYB18*-silenced plants was significantly higher than that of WT and control group plants after 18 d (Fig. 5C). The quantity of honeydew secreted on *GhMYB18*-silenced cotton was significantly higher than others at 18 d and 24 d after aphid infestation (Fig. 5D). These results suggest that silencing of *GhMYB18* increased cotton plant susceptibility to *Aphis gossypii.*

3.6 Influence of altering *GhMYB18* expression on enzyme activities

Enzyme activities of PAL, CAT, PPO and POD go hand in hand with plant resistance to insect pests. Therefore, these enzyme activities and their gene expression levels were firstly measured. The gene

expression level and activities of PAL, CAT, PPO and POD in pBI121-*GhMYB18* plants were always higher than those in pBI121 plants (Fig. 6A, B). In the same way, the gene expression levels and activities of these enzymes in *GhMYB18*-silenced plants were always lower than those in WT and control group plants (*TRV:00*) (Fig. 6C, D). These results prove that the expression level of *GhMYB18* is closely related to PAL, CAT, PPO and POD.

3.7 *GhMYB18* increase cotton resistance to *Aphis gossypii* via the SA pathway

Salicylic acid is irreplaceable in plant response to aphid infestation. Therefore, analyzed the expression levels of the genes related to salicylic acid signaling pathway (*EDS1*, *PAD4*, *ICS*, *PA*L, *NPR1*) and the content of salicylic acid in *GhMYB18* transiently overexpressed cotton plants and *GhMYB18*-silenced cotton plants. Compared with WT and pBI121 plants, EDS1 and PAL transcripts were significantly higher in the *GhMYB18*-overexpressing plants, but PAD4 transcript had no significant difference. Compared with WT plants, the expressed levels of ICS and NPR1 significantly decreased in the *GhMYB18*-overexpressing plants (Fig. 7A). A similar pattern was observed in *GhMYB18*-silenced plants, the expression levels of EDS1, ICS, PAL were down-regulated than that WT and control group plants (*TRV:00*), but the expression levels of PAD4 and NPR1 had no significant difference (Fig. 7B). The SA content was significantly increased in plants overexpressing *GhMYB18*, but decreased in *GhMYB18*-silenced plants (Fig. 7C, D). To sum up, *GhMYB18* may participate in the defense response to cotton aphid by regulating the SA signaling pathway.

3.8 *GhMYB18* stimulates the production of total flavonoids by PAL pathway

Previous studies have shown that PAL is involved in the biosynthesis of polyphenolic compounds, including flavonoids in plants (Rohde et al. 2004). To further explore the relationship between flavonoids content and expression level of *GhMYB18*, we detected related indicators in *GhMYB18* transiently overexpressed cottons and *GhMYB18*-silenced cottons. Firstly, we examined the expression of genes related to the flavonoid synthesis pathway. The expression level of CHS, CHI, FLS, ANS was up-regulated significantly in plants overexpressing *GhMYB18*, but compared with WT plants, the transcripts of F3'H and FLS significantly decreased in plants overexpressing *GhMYB18*. Even more interesting is that all the genes significantly down-regulated obviously in the *GhMYB18*-silenced plants (Fig. 8A, B). As the same time, the total flavonoid content was significantly increased in plants overexpressing *GhMYB18*, but decreased in *GhMYB18*-silenced plants (Fig. 8C, D). These results indicate that *GhMYB18* regulates the defense response of cotton to *Aphis gossypii* by influencing the synthesis of flavonoids.

Discussion

As one of the largest transcription factors (TFs) family in plants, MYBs play vital roles in regulating abundant plant-specific physiological processes (Jiang et al. 2020). Compared with extensive studies on the roles of MYB transcription factors in plant growth and abiotic stress, little is known about MYB proteins regarding their functions in resisting agricultural pests such as aphids. Existing studies have

only confirmed the resistance of MYB proteins to aphids in some plants including *Triticum aestivum* L. (Zhai et al. 2017), chrysanthemum (An et al. 2019; Wang et al. 2017), *Arabidosis* (Zhu et al. 2018; Lu et al. 2013). However, knowledge is lacking about the roles of MYBs in cotton defense against cotton aphids. Therefore, in this study, *GhMYB18*, a novel resistance-related R2R3-MYB gene was cloned and identified, and the function and mechanism of its resistance to cotton aphids were studied.

Unlike most MYB transcription factors, *GhMYB18* is a functional transcription activation factor. Sequence analysis showed that the cotton gene *GhMYB18* belongs to the plant R2R3-MYB family. Phylogenetic analysis showed that *GhMYB18* is closely related to *GhMYB30*, *AtMYB94*, and *GhMYB60*. These genes are related to the plant immune system. In this study, compared with WT plants, *GhMYB18* expression levels significantly increased under aphid infestation and SA treatment. However, they declined under MeJA treatment, and decreased significantly at 48 h. These preliminary evidences suggested that *GhMYB18* may be involved in SA-related signaling pathway and related to cotton immune response to cotton aphids. To verify the *GhMYB18* important role in response to cotton aphid infestation, VIGS and transient overexpression were used to verify the *GhMYB18* function. The results showed that *GhMYB18* overexpressed plants have a negative effect on aphids, inhibiting the reproduction rate and feeding rate of cotton aphids. The opposite result appeared on the *GhMYB18*-silenced plants. These results indicated that *GhMYB18* contributed to improve cotton resistance to cotton aphids.

Aphids are a most destructive group of agricultural pests that cause severe injury to plants by ingesting nutrients from sieve tubes (Zust and Agrawal 2016). In response to aphid infestation, plants have evolved multiple strategies to resist aphid, including trichome formation, callose deposition, and the production of secondary metabolites such as phenols and alkaloids (Goggin 2007). Although there are many related studies, our understanding of plant defense mechanisms against aphid is far from enough (Nalam, Louis and Shah 2019). In this study, only the expression level of PAL increased in *GhMYB18* overexpressed plants and decreased in *GhMYB18* silenced plants, while the transcript of CAT, POD and PPO decreased in *GhMYB18* overexpressed plants. However, all the enzyme activities of all the genes increased in *GhMYB18* overexpressed plants and decreased in *GhMYB18* silenced plants, whose mechanism needs further study. It is known that POD and CAT belong to the cleaning system of the ROS, which can effectively inhibit the reactive oxygen species from damaging organisms (Zhang et al. 2021). Polyphenol oxidase (PPO) catalyses oxidation of phenolics, and it's mainly involved in the researches concerning plant-pathogen relation (Araji et al. 2014). As the first committed enzyme in phenylpropanoid pathway, phenylalanine ammonia-lyase is mainly involved in the defense against sap sucking herbivores such as aphid and brown planthopper (Chaman, Copaja and Argandona 2003). In addition, Consistent with our findings, the cotton gene *GhRac6* improves the plant defense response to aphid feeding by improving activities of PAL (Yang et al. 2018). Rice MYB transcription factor *OsMYB30* conferred brown planthopper resistance by regulating expression of *OsPAL6* and *OsPAL8* (He et al. 2020). This suggested that *GhMYB18* could involve in cotton plant response to cotton aphid infestation through mediating PAL.

PAL is the first enzyme induced by the phenyl-propanoid pathway in the biosynthesis of various phenolic compounds with structural and defense-related functions such as lignin, pigment, flavonoid, and

phytoalexin (Solekha et al. 2020). These compounds have a significant effect on plant growth, development, reproduction, defense, and environmental responses (Qian et al. 2019). Lots of studies have reported the potential role of flavonoids in aphid defense response. For example, when cotton is subjected to salt stress, the content of flavonoids in the cotton increases, which promotes the cotton's defense response to aphids (Wang et al. 2015). Zahedi believes that when melons are infested by aphids, more flavonoids will accumulate while reducing the amount of chlorophyll (Zahedi et al. 2019). In this study, the results showed that the content of total flavonoids and the expression levels of flavonoid biosynthetic pathway-related genes are significantly increased in the plants overexpressing *GhMYB18*, but reduced in the plants *GhMYB18* RNAi plants. Above all, the results suggested that *GhMYB18*, regulates defense response to aphids through influencing the synthesis of flavonoids.

Salicylic acid (SA) plays a crucial role in minimizing infections and effects of biotic and abiotic stresses (Zhang et al. 2019). The salicylic acid signaling pathway is activated when plants are exposed to piercing-sucking insects (Kaloshian and Walling 2005), such as *Diuraphis noxia* (Mohase and Westhuizen 2002), *Nilaparvata lugens* (He et al. 2020) and *S. graminum* (Chaman, Copaja and Argandona 2003). By exogenous treatment with plant hormones on cotton seedlings, the transcription of *GhMYB18* was significantly up-regulated after SA induction, and MeJA induction inhibited the expression level of *GhMYB18*. The reciprocal antagonism of SA and JA signaling pathways has been demonstrated in numerous studies (Koornneef and Pieterse 2008). For example, the feeding by *B. tabaci* nymphs on *Arabidopsis* suppresses downstream JA-responsive defenses by inducing SA (Zarate, Kempema and Walling 2007); exogenous SA reduced the amount of JA that Egyptian cotton worm induced in both npr1 mutant and wild-type *Arabidopsis* (Stotz et al. 2002). In these discoveries, *GhMYB18* increased the SA content and the expression levels of EDS1, ICS and PAL in SA signaling pathway. By inhibiting the expression of *GhMYB18*, we have reached the same conclusion: *GhMYB18* may be involved in the defense response to aphids by regulating the SA signal pathway. As for how the expression of *GhMYB18* affects the JA signaling pathway, further research is still needed.

Collectively, our research provides new insight into the mechanism the function and mechanism of cotton *MYB* gene in plant defense response to aphid, and offer valuable evidence upland cotton breeding. However, on account of the complex interaction between plants and aphid, more studies on *GhMYB18* resistance to cotton aphid will be performed in the future.

Conclusion

This study suggests that *GhMYB18* functions as a positive regulator of aphid tolerance. *GhMYB18* acted as a transcription activation factor. Exogenous salicylic acid induction and aphid induction significantly increased *GhMYB18* expression levels. The results of choice assay, no-choice assay and assays of honeydew secretion from *Aphis gossypii* confirmed the aphid-resistance function of *GhMYB18*. Subsequent analysis showed that *GhMYB18* could participated in salicylic acid and phenylpropane signaling pathway to improving upland cotton resistance to *Aphis gossypii*. Our results provide a

significant advancement in figuring out the functions of R2R3-MYB transcription factors in aphid resistance.

Declarations

Author contributions statement

JM Zhang conceived and designed the research. ZW Hu, X Zhong and HR Zhang performed the experiments, ZW Hu, WZ Feng, XC Luo and YX Wang analyzed the data and wrote the manuscript. Y Wang, T Liu and Y Zhang provided helpful comments and discussions. All authors read and approved the final manuscript.

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Conflict of interest

The authors do not have any conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Ali A, Desneux N, Lu Y, Liu B, Wu K (2016) Characterization of the natural enemy community attacking cotton aphid in the Bt cotton ecosystem in Northern China. Sci Rep 6(1):1–9
2. An C, Sheng L, Du X, Wang Y, Zhang Y, Song A, Jiang J, Guan Z, Fang W, Chen F, Chen S (2019) Overexpression of *CmMYB15* provides chrysanthemum resistance to aphids by regulating the biosynthesis of lignin. Hortic Res 6(1):1–10
3. Araji S, Grammer TA, Gertzen R, Anderson SD, Mikulic-Petkovsek M, Veberic R, Phu ML, Solar A, Leslie CA, Dandekar AM, Escobar MA (2014) Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut. Plant physiol 164(3):1191–1203
4. Chaman ME, Copaja SV, Argandoña VH (2003) Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. J Agric Food Chem 51(8):2227–2231

5. Chen T, Li W, Hu X, Guo J, Liu A, Zhang B (2015) Cotton MYB transcription factor *GbMYB5*, is positively involved in plant adaptive response to drought stress. Plant Cell Physiol 56(5):917–929
6. Chen YH, Yang XY, He K, Liu MH, Li JG, Gao ZF, Lin ZQ, Zhang YF, Wang XX, Qiu XM, Shen YP, Zhang L, Deng XH, Luo JC, Deng XW, Chen ZL, Gu HY, Qu LJ (2006) The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol Biol 60(1):107–124
7. Cheng HQ, Han LB, Yang CL, Wu XM, Zhong NQ, Wu JH, Wang FX, Wang HY, Xia GX (2016) The cotton *MYB108* forms a positive feedback regulation loop with *CML11* and participates in the defense response against *Verticillium dahliae* infection. J Exp Bot 67(6):1935–1950
8. De Vos M, Denekamp M, Dicke M, Vuylsteke M, Van Loon L, Smeekens SC, Pieterse CM (2006) The *Arabidopsis thaliana* transcription factor *AtMYB102* functions in defense against the insect herbivore *Pieris rapae*. Plant Signal Behav 1(6):305–311
9. Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in *Arabidopsis*. Trends Plant Sci 15:573–581
10. Fu J, Chu J, Sun X, Wang J, Yan C (2012) Simple, rapid, and simultaneous assay of multiple carboxyl containing phytohormones in wounded tomatoes by UPLC-MS/MS using single SPE purification and isotope dilution. Anal Sci 28(11):1081–1087
11. Goggin FL (2007) Plant–aphid interactions: molecular and ecological perspectives. Curr Opin Plant Biol 10(4):399–408
12. He J, Liu Y, Yuan D, Duan M, Liu Y, Shen Z, Yang C, Qiu Z, Liu D, Wen P, Huang J, Fan D, Xiao S, Xin Y, Chen X, Jiang L, Wang H, Yuan L, Wan J (2020) An R2R3 MYB transcription factor confers brown planthopper resistance by regulating the phenylalanine ammonia-lyase pathway in rice. Proc Natl Acad Sci 117(1):271–277
13. Hegde M, Oliveira JN, Da-Costa JG, Bleicher E, Santana AE, Bruce TJ, Caulfield J, Dewhirst SY, Woodcock CM, Pickett JA, Birkett MA (2011) Identification of semiochemicals released by cotton, *Gossypium hirsutum*, upon infestation by the cotton aphid, *Aphis gossypii*. J Chem Ecol 37(7):741–750
14. Hu Q, Min L, Yang X, Jin S, Zhang L, Li Y, Li YY, Ma YZ, Qi XW, Li DQ, Liu HB, Lindsey K, Zhu LF, Zhang XL (2018) Laccase *GhLac1* modulates broad-spectrum biotic stress tolerance via manipulating phenylpropanoid pathway and jasmonic acid synthesis. Plant physiol 176(2):1808–1823
15. Hu Y, Chen J, Fang L, Zhang Z, Ma W, Niu Y, Ju L, Deng J, Zhao T, Lian J, Baruch K, Fang D, Liu X, Ruan YL, Rahman MU, Han J, Wang K, Wang Q, Wu H, Mei G, Zang Y, Han Z, Xu C, Shen W, Yang D, Si Z, Dai F, Zou L, Huang F, Bai Y, Zhang Y, Brodt A, Ben-Hamo H, Zhu X, Zhou B, Guan X, Zhu S, Chen X, Zhang T (2019) *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin and evolution of allotetraploid cotton. Nat Genet 51:739–748.
16. Jiang C, Wang D, Zhang J, Xu Y, Zhang C, Zhang J, Wang X, Wang Y (2021) *VqMYB154* promotes polygene expression and enhances resistance to pathogens in Chinese wild grapevine. Hortic Res 8(1):151

17. Jiang CK, Rao GY (2020) Insights into the diversification and evolution of R2R3-MYB transcription factors in plants. Plant physiol 183(2):637–655
18. Kaloshian I, Walling LL (2005) Hemipterans as plant pathogens. Annu Rev Phytopathol 43:491–521
19. Kaur H, Heinzel N, Schöttner M, Baldwin IT, Gális I (2010) R2R3-*NaMYB8* regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in *Nicotiana attenuate*. Plant Physiol 152(3):1731–1747
20. Kerns DL, Gaylor MJ (1992) Insecticide resistance in field populations of the cotton aphid (Homoptera:Aphididae). J Econ Entomol 85(1):1–8
21. Kim JH, Jander G (2007) *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. Plant J 49(6):1008–1019
22. Koornneef A, Pieterse CMJ (2008) Cross talk in defense signaling. Plant Physiol 146 (3):839–844
23. Kumar S, Kanakachari M, Gurusamy D, Kumar K, Narayanasamy P, Venkata PK, Solanke A, Gamanagatti S, Hiremath V, Katageri IS, Leelavathi S, Kumar PA, Reddy VS (2016) Genome-wide transcriptomic and proteomic analyses of bollworm-infested developing cotton bolls revealed the genes and pathways involved in the insect pest defence mechanism. Plant Biotechnol J 14(6):1438–1455
24. Liu TL, Chen TZ, Kan JL, Yao Y, Guo DS, Yang YW, Ling XT, Wang JY, Zhang BL (2021) The *GhMYB36* transcription factor confers resistance to biotic and abiotic stress by enhancing PR1 gene expression in plants. Plant Biotechnol J
25. Liu Y, Schiff M, Dinesh-Kumar SP (2002) Virus-induced gene silencing in tomato. Plant J 31(6):777–786
26. Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408
27. Loguercio LL, Zhang JQ, Wilkins TA (1999) Differential regulation of six novel MYB-domain genes defines two distinct expression patterns in allotetraploid cotton (*Gossypium hirsutum L.*). Mol Gen Genet 261(4):660–671
28. Lu BB, Li XJ, Sun WW, Li L, Gao R, Zhu Q, Tian SM, Fu MQ, Yu HL, Tang XM, Zhang CL, Dong HS (2013) *AtMYB44* regulates resistance to the green peach aphid and diamondback moth by activating EIN2-affected defenses in *Arabidopsis*. Plant Biol 15(5):841–850
29. Lu N, Roldan M, Dixon RA (2017) Characterization of two TT2-type MYB transcription factors regulating proanthocyanidin biosynthesis in tetraploid cotton, *Gossypium hirsutum*. Planta 246(2):323–335
30. Mohase L, Westhuizen AJ (2002) Salicylic acid is involved in resistance responses in the Russian wheat aphid-wheat interaction. J Plant Physiol 159(6):585–590
31. Nalam V, Louis J, Shah J (2019) Plant defense against aphids, the pest extraordinaire. Plant Sci 279:96–107

32. Nisbet AJ, Woodford JAT, Strang RHC (1994) Quantifying aphid feeding on non-radioactive food sources. Entomol Exp Appl 72:85–89
33. Qian Y, Lynch JH, Guo L, Rhodes D, Morgan JA, Dudareva N (2019) Completion of the cytosolic post-chorismate phenylalanine biosynthetic pathway in plants. Nat commun 10(1):1–15
34. Quan QM, Hu X, Pan BH, Zeng BS, Wu NN, Fang GQ, Cao YH, Chen XY, Li X, Huang YP, Zhan S (2019) Draft genome of the cotton aphid *Aphis gossypii*. Insect Biochem Mol Biol 105:25–32
35. Qu J, Ye J, Geng YF, Sun YW, Gao SQ, Zhang BP, Chen W, Chua NH (2012) Dissecting functions of KATANIN and WRINKLED1 in cotton fiber development by virus-induced gene silencing. Plant Physiol 160(2):738–748
36. Rohde A, Morreel K, Ralph J, Goeminne G, Hostyn V, De Rycke R, Kushnir S, Van Doorsselaere J, Joseleau JP, Vuylsteke M, Van Driessche G, Van Beeumen J, Messens E, Boerjan W (2004) Molecular phenotyping of the pal1 and pal2 mutants of *Arabidopsis thaliana* reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism. Plant Cell 16(10):2749–2771
37. Shen SJ, Wang YY, Zhang YX, Guo W, Jiao YQ, Zhou XA (2018) Overexpression of the wild soybean R2R3-MYB transcription factor *GsMYB15* enhances resistance to salt stress and *Helicoverpa Armigera* in transgenic *Arabidopsis*. Int J Mol Sci 19(12):3958
38. Solekha R, Susanto FA, Joko T, Nuringtyas TR, Purwestri YA (2020) Phenylalanine ammonia lyase (PAL) contributes to the resistance of black rice against Xanthomonas oryzae pv. oryzae. J Plant Pathol 102(2):359–365
39. Stotz HU, Koch T, Biedermann A, Weniger K, Boland W, Mitchell-Olds T (2002) Evidence for regulation of resistance in *Arabidopsis* to Egyptian cotton worm by salicylic and jasmonic acid signaling pathways. Planta 214(4):648–652
40. Sultana B, Anwar F, Ashraf M (2009) Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 14(6):2167–2180
41. Sun X, Gong SY, Nie XY, Li Y, Li W, Huang GQ, Li XB (2015) A R2R3-MYB transcription factor that is specifically expressed in cotton (*Gossypium hirsutum*) fibers affects secondary cell wall biosynthesis and deposition in transgenic *Arabidopsis*. Physiol Plant 154(3):420–432
42. Wang Q, Eneji AE, Kong X, Wang K, Dong H (2015) Salt stress effects on secondary metabolites of cotton in relation to gene expression responsible for aphid development. PloS one 10(6):e0129541
43. Wang Y, Sheng L, Zhang H, Du X, An C, Xia X, Chen F, Jiang J, Chen S (2017) *CmMYB19* over-expression improves aphid tolerance in *Chrysanthemum* by promoting lignin synthesis. Int J Mol Sci 18(3):619
44. Yang Z, Liu JJ, Luo L, Ye S, Yang YZ, Zhang GH, Wang XP, Zhang JM (2018) The cotton *GhRac6* gene encoding a plant ROP/RAC protein improves the plant defense response to aphid feeding. Plant Mol Biol Rep 36(5):888–896
45. Yates AD, Michel A (2018) Mechanisms of aphid adaptation to host plant resistance. Curr Opin Insect Sci 26:41–49.

46. Yue Y, Zhang M, Zhang J, Tian X, Duan L, Li Z (2012) Overexpression of the *AtLOS5* gene increased abscisic acid level and drought tolerance in transgenic cotton. J Exp Bot 63(10):3741–3748
47. Zahedi A, Razmjou J, Rafiee-Dastjerdi H, Leppla NC, Golizadeh A, Hassanpour M, Ebadollahi A (2019) Tritrophic interactions of cucumber cultivar, *Aphis gossypii* (Hemiptera: Aphididae), and its predator *Hippodamia variegata* (Coleoptera: Coccinellidae). J Econ Entomol 112(4):1774–1779
48. Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol 143(2): 866–875
49. Zhai Y, Li P, Mei Y, Chen M, Chen X, Xu H, Zhou X, Dong H, Zhang C, Jiang W (2017) Three MYB genes co-regulate the phloem-based defense against English grain aphid in wheat. J Exp Bot 68(15):4153–4169
50. Zhang LH, Lu GQ, Huang XZ, Guo HM, Su XF, Han L, Zhang YJ, Qi Z, Xiao YT, Cheng HM (2020) Overexpression of the caryophyllene synthase gene *GhTPS1* in cotton negatively affects multiple pests while attracting parasitoids. Pest Manag Sci 76(5):1722–1730
51. Zhang S, Liu J, Xu B, Zhou JJ (2021) Differential responses of *Cucurbita pepo* to *Podosphaera xanthii* reveal the mechanism of powdery mildew disease resistance in pumpkin. Front Plant Sci 12:306
52. Zhang Y, Li X (2019) Salicylic acid: biosynthesis, perception, and contributions to plant immunity. Curr Opin Plant Biol 50:29–36
53. Zhao Y, Yang Z, Ding Y, Liu L, Han X, Zhan J, Wei X, Diao Y, Qin W, Wang P, Liu P, Sajjad M, Zhang X, Ge X (2019) Over-expression of an R2R3 MYB Gene, *GhMYB73*, increases tolerance to salt stress in transgenic *Arabidopsis*. Plant Sci 286:28–36
54. Zhong X, Feng P, Ma QQ, Zhang Y, Yang YZ, Zhang JM (2021) Cotton Chitinase gene *GhChi6* improves the *Arabidopsis* defense response to aphid attack. Plant Mol Biol Rep 39(1):251–261.
55. Zhu L, Guo J, Ma Z, Wang J, Zhou C (2018) *Arabidopsis* transcription factor *MYB102* increases plant susceptibility to aphids by substantial activation of ethylene biosynthesis. Biomolecules 8(2):39
56. Züst T, Agrawal AA (2016) Mechanisms and evolution of plant resistance to aphids. Nat plants 2(1):1–9

Figures

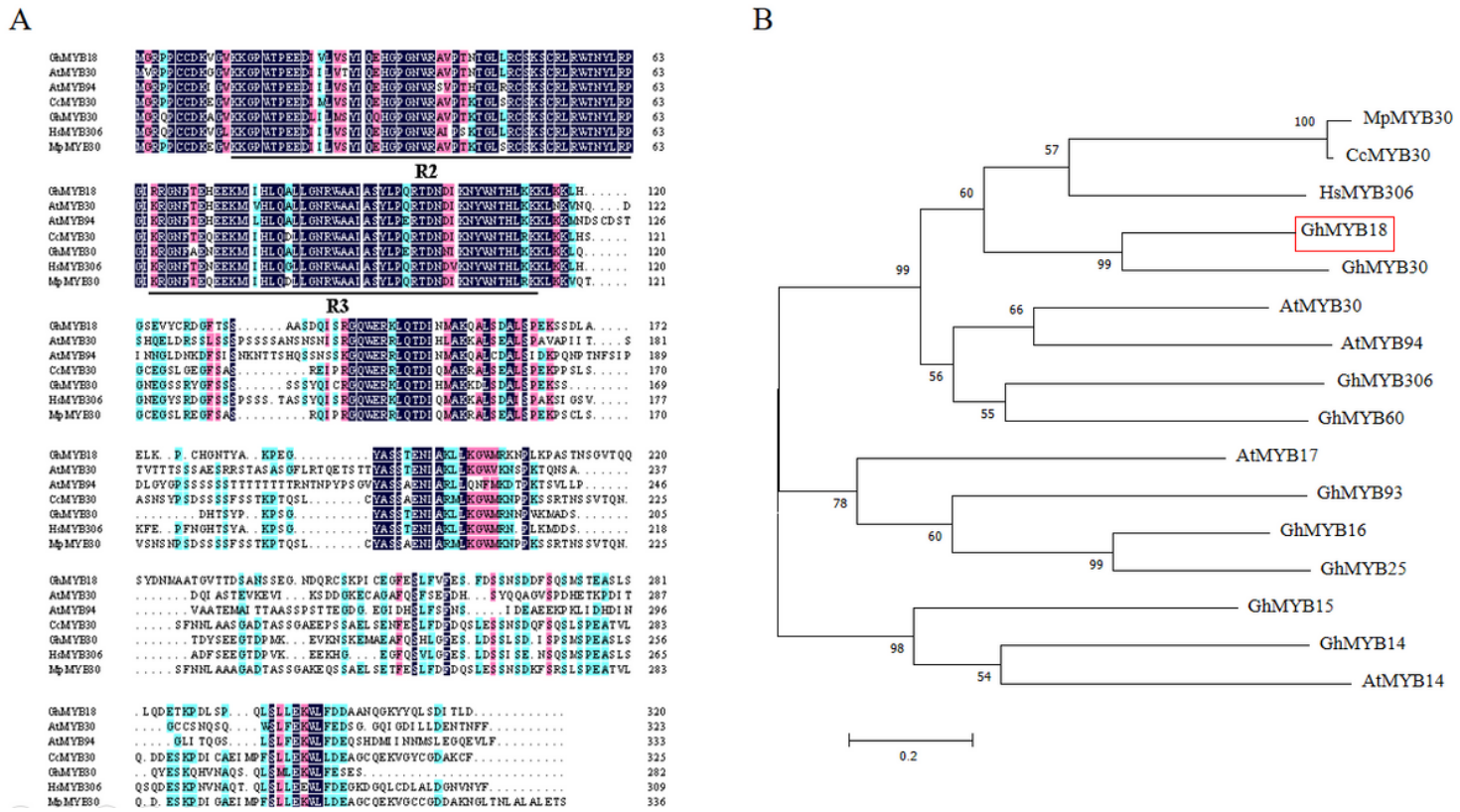


Figure 1

Putative peptide sequences of *GhMYB18* and other MYB proteins. (A) Alignment of the putative amino acid sequence of *GhMYB18*(boxed) with that of related MYB proteins that feature an R2MYB domain or an R3MYB domain (underlined), (B) Phylogenetic analysis of the relationships between *GhMYB18* and other MYB proteins. The sequence details are as follows: *MpMYB30* (RDX58629.1); *CcMYB30* (XP_020203788.1); *HsMYB306* (KAE8709998.1); *GhMYB30* (XP_016746699.1); *AtMYB30* (OAP03831.1); *AtMYB94* (OAP04445.1); *GhMYB306* (XP_016695690.1); *GhMYB60* (XP_016725886.1); *AtMYB17*(OAP04938.1); *GhMYB93* (XP_0409504 20.1); *GhMYB16* (XP_016677671.1); *GhMYB25* (AAK19616.1); *GhMYB15* (XP_ 016699485.1); *GhMYB14* (XP_016743564.2); *AtMYB14* (OAP10376.1).

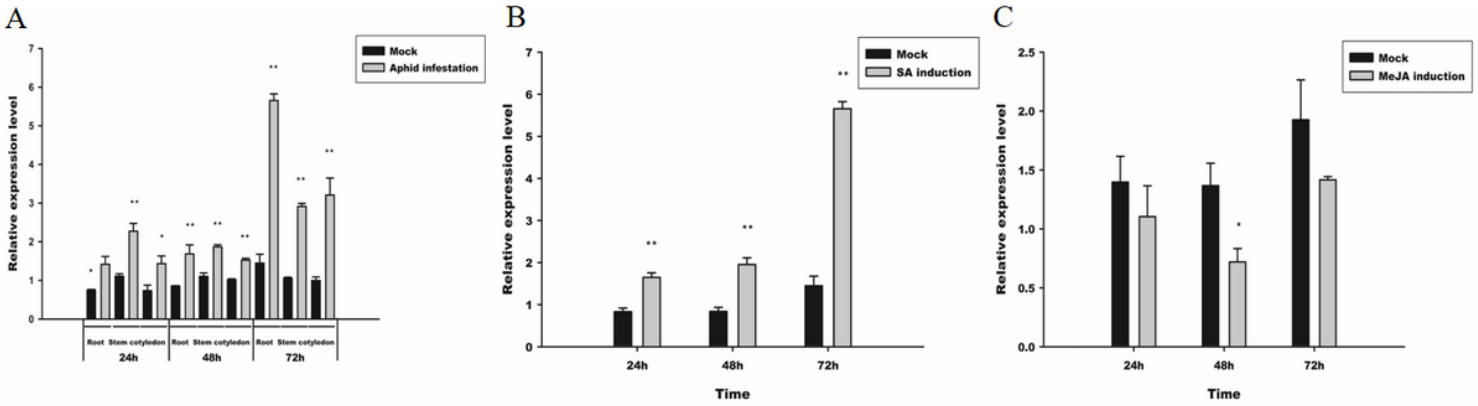


Figure 2

Expression levels of *GhMYB18* during cotton aphid infestation and SA/MeJA induced. (A) *GhMYB18* expression in different cotton tissues infested with cotton aphids. (B) *GhMYB18* expression in cotton cotyledons following SA induced. (C) *GhMYB18* expression in cotton cotyledons following MeJA induced. Mean values and SD bars are provided (* *p<0.05*, ** *p<0.01*; Student's t-test).

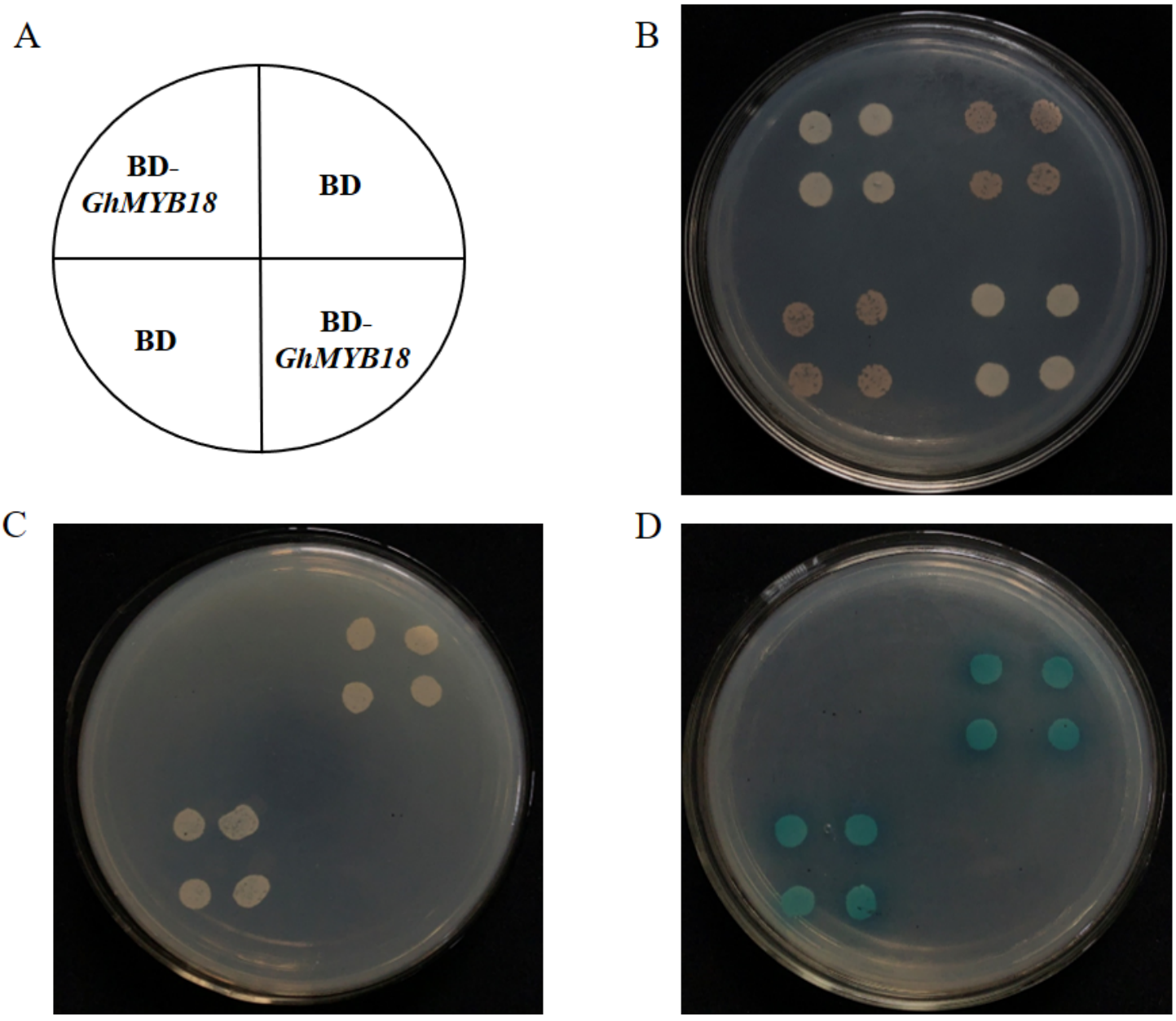


Figure 3

Transactivation assay of *GhMYB18* in yeast. *Yeast* strains harboring BD- *GhMYB18* and the BD control (A) were grown on SD/-Trp (B), SD/-Trp with AbA (C) and SD/-Trp with AbA and X-a-Gal medium (D) and cultivated at 28 ℃ for 3 days.

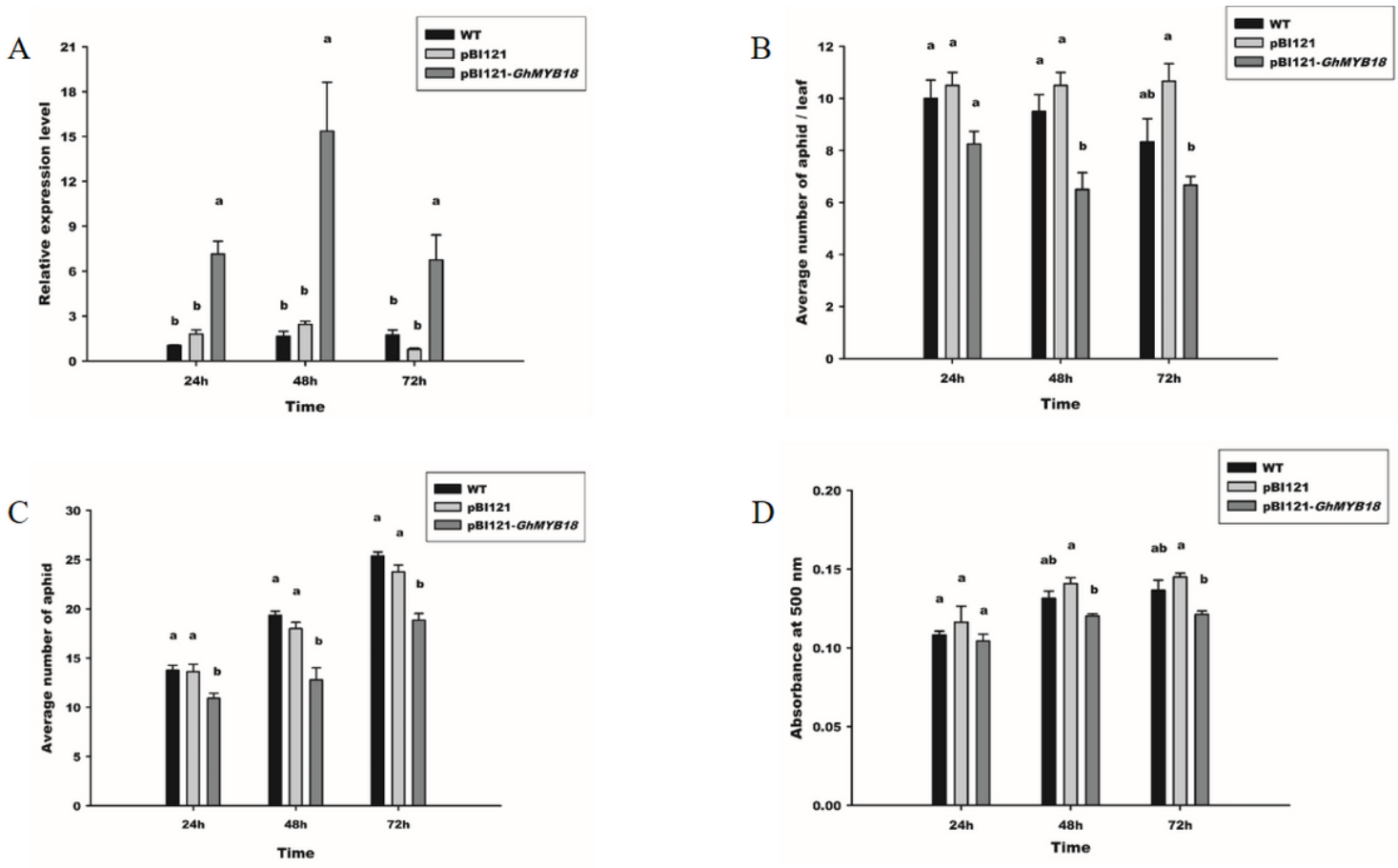


Figure 4

The aphid resistance of wild-type cotton, control cotton and *GhMYB18*-overexpressing cotton. (A) Expression level of *GhMYB18* in transgenic cotton at 24 h,48 h and 72 h. (B) The number of aphids on each cotyledon at different times in the choice tests. (C) The number of aphids on each plant at different times in the no-choice tests. (D) The quantity of honeydew secreted by aphids on each cotyledon at different times at 500 nm. Those bars indicate the standard errors, the alphabets represent the level of significant difference (*p□0.05*).

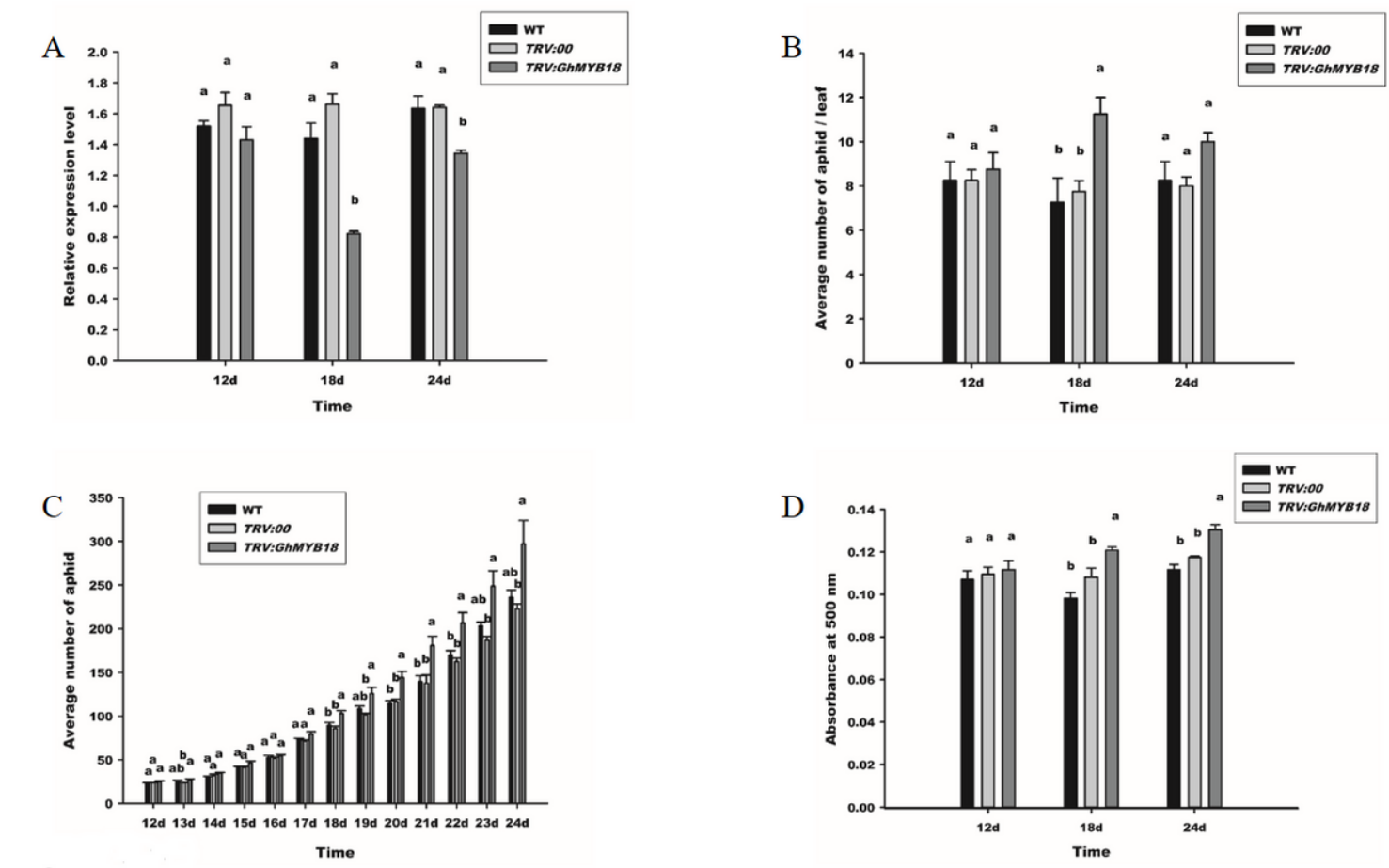


Figure 5

Increased susceptibility of *GhMYB18*-silenced cottons to *Aphis gossypii*. (A) Analysis of *GhMYB18* expression level at 12 d, 18 d and 24 d. (B) The number of aphids on each cotyledon at different times in the choice assay. (C) The number of aphids on each plant at different times in the no-choice assay. (D) The quantity of honeydew secreted by aphids on each cotyledon at different times at 500 nm. Those bars indicate the standard errors, the alphabets represent the level of significant difference ($p \leq 0.05$).

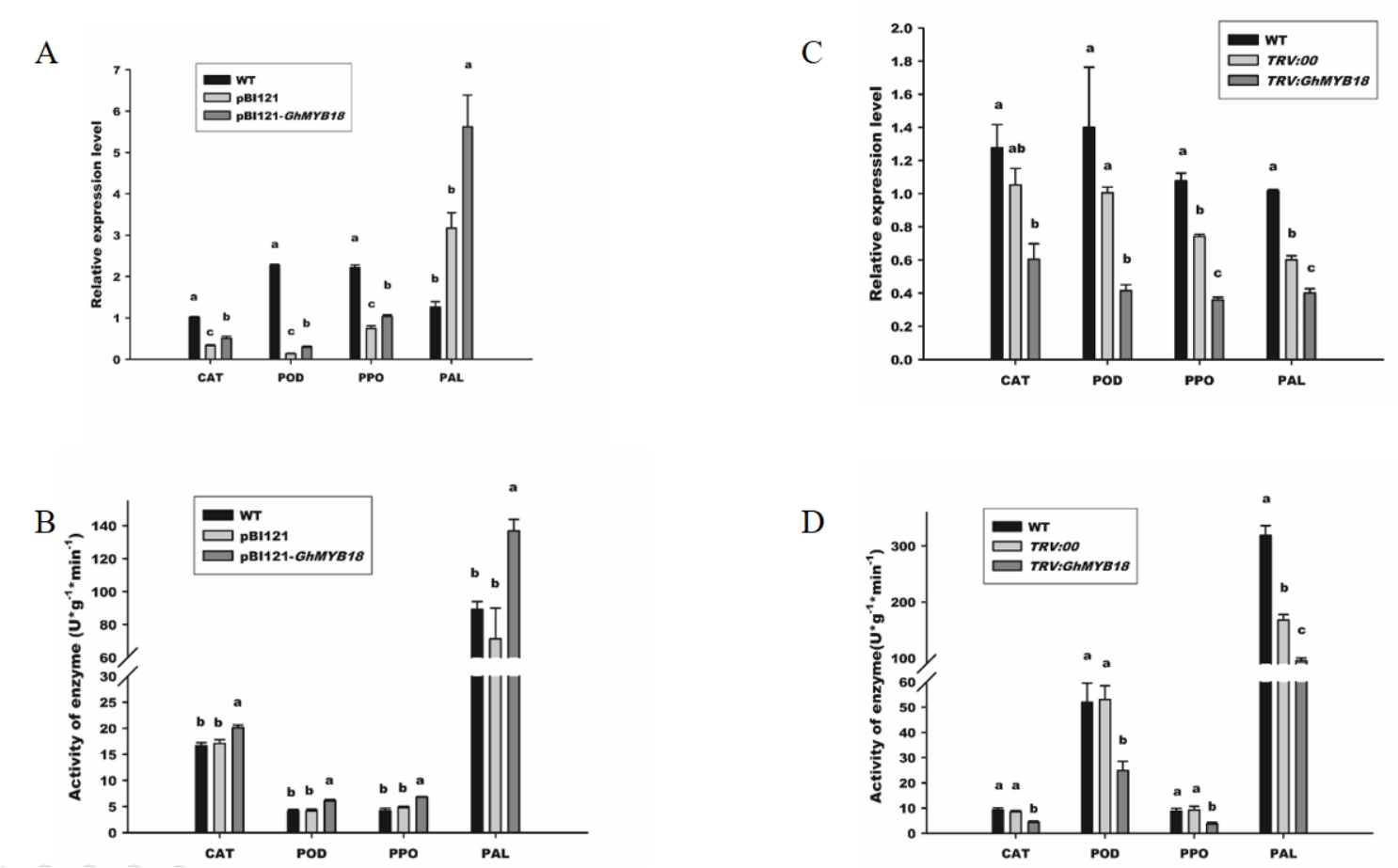


Figure 6

The expression level and activities of PAL, CAT, PPO and POD in *GhMYB18* transiently expressed cottons and *GhMYB18*-silenced cottons. (A) Research on expression level of four enzymes in wild-type cottons (WT), control group cottons(pBI121) and *GhMYB18*-overexpressing cottons (pBI121-*GhMYB18*). (B) Research on activities of four enzymes in wild-type cottons (WT), control group cottons(pBI121) and *GhMYB18*-overexpressing cottons (pBI121-*GhMYB18*). (C) Research on expression level of four enzymes in wild-type cottons (WT), control group cottons (pTRV2) and *GhMYB18*-silenced cottons (pTRV2-*GhMYB18*). (D) Research on activities of four enzymes in wild-type cottons (WT), control group cottons (pTRV2) and *GhMYB18*-silenced cottons (pTRV2-*GhMYB18*). Those bars indicate the standard errors, the alphabets represent the level of significant difference ($p \leq 0.05$).

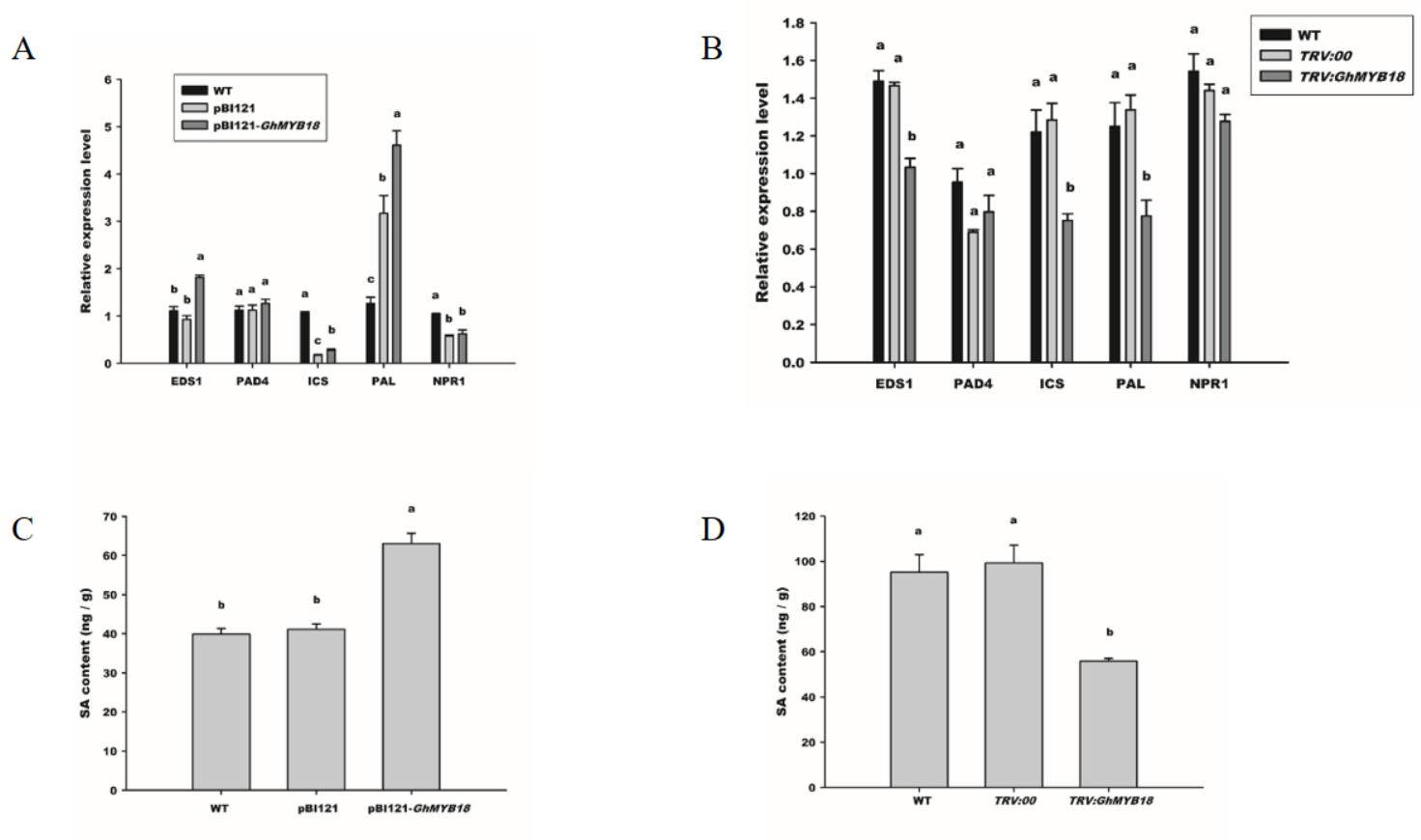


Figure 7

The salicylic acid content and expression level of genes related to SA signaling pathway in *GhMYB18* transiently expressed cottons and *GhMYB18*-silenced cottons. (A) The expression level of five genes related to the SA pathway in wild-type cottons (WT), control group cottons (pBI121) and *GhMYB18*-overexpressing Cottons (pBI121-*GhMYB18*). (B) Five gene transcripts in the SA signaling pathway in wild-type cottons (WT), control group cottons (pTRV2) and *GhMYB18*-silenced cottons (pTRV2-*GhMYB18*). (C) The salicylic acid content of wild-type cottons, control group cottons and *GhMYB18*-overexpressing/ silenced plants. (D) The salicylic acid content of wild-type cottons, control group cottons and *GhMYB18*-silenced plants. Those bars indicate the standard errors, the alphabets represent the level of significant difference ($p \leq 0.05$).

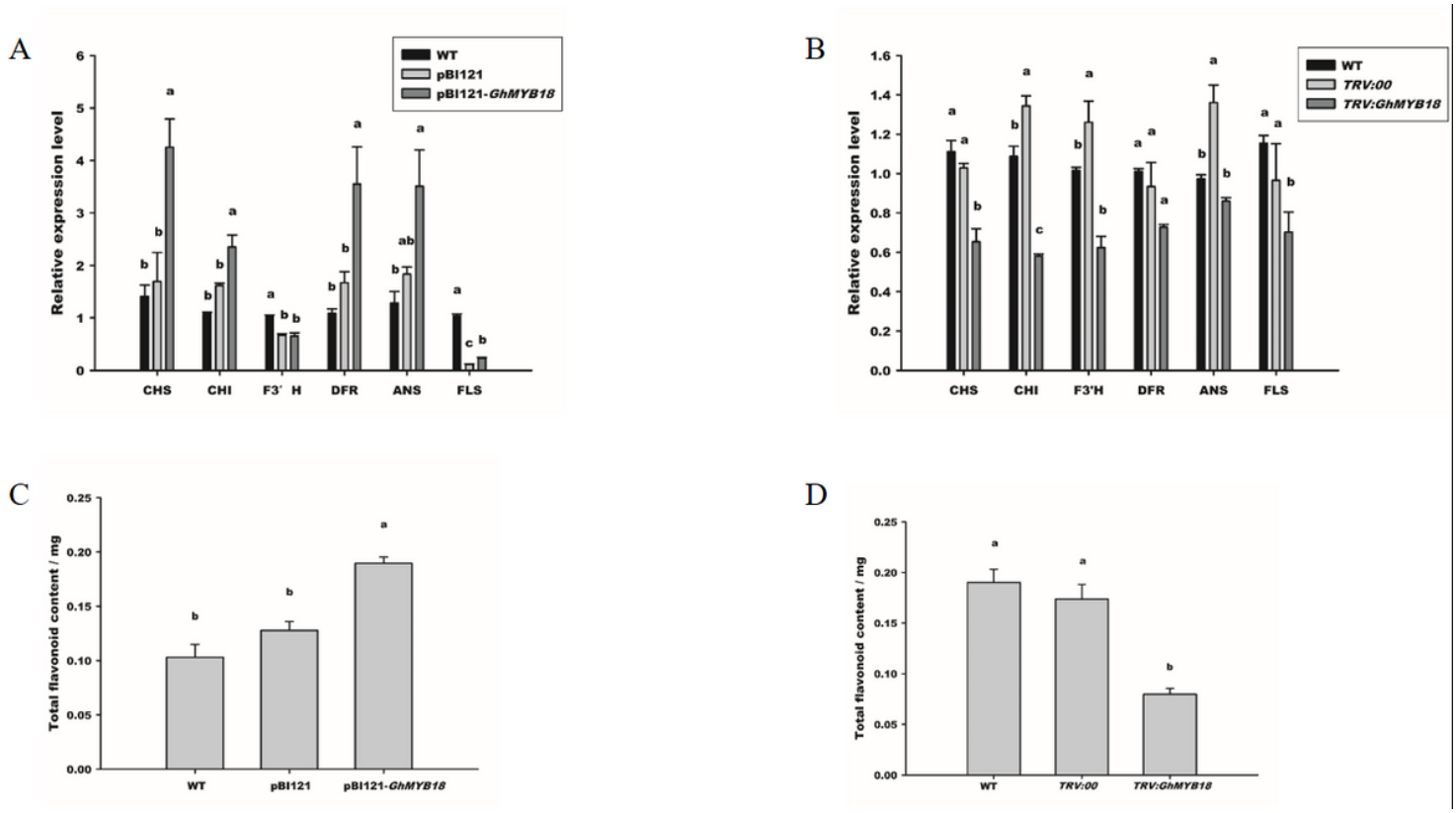


Figure 8

The expression level of genes related to PAL signaling pathway and total flavonoids content in *GhMYB18* transiently expressed cottons and *GhMYB18*-silenced cottons. (A) The expression level of six genes related to the flavonoids synthesis pathway in wild-type cottons (WT), control group cottons (pBI121) and *GhMYB18*-overexpressing cottons (pBI121-*GhMYB18*). (B) Six gene transcripts in flavonoids synthesis pathway in wild-type cottons (WT), control group cottons (pTRV2) and *GhMYB18*-silenced cottons (pTRV2-*GhMYB18*). (C) Total flavonoids content of wild-type cottons, control group cottons and *GhMYB18*-overexpressing plants. (D) Total flavonoids content of wild-type cottons, control group cottons and *GhMYB18*- silenced plants. Those bars indicate the standard errors, the alphabets represent the level of significant difference($p \leq 0.05$).

Supplementary Files

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