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Identification, cloning and characterization of a novel *Gossypium hirsutum* L. *GhMYBantiV* transcription factor in response to biotic and abiotic stresses

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Abstract

***Verticillium* wilt is an important worldwide limiting factor for cotton production. Improving cotton varieties' disease resistance, is of great strategic and practical importance to promote world cotton production and to improve cotton fibre quality. Taking advantage of the *Gossypium raimondii* genome sequence, we cloned a new MYB transcription factor *GhMYBantiV* (GenBank accession number: KM0407832) from upland cotton variety Lumianyan 32. Characteristic analysis indicates that *GhMYBantiV* is a MYB transcription factor, since it localises in the nucleus, and shows the transcription factor activity. *GhMYBantiV* expression is greatly enhanced by *Verticillium* wilt fungus infection and salt treatment. It is, therefore, suggested that *GhMYBantiV* possibly plays an important role in biotic and abiotic stress response in cotton.**

Key words Cotton, *Verticillium* wilt, MYB transcription factors, gene cloning

Introduction

China is one of the world's major cotton producers and consumers, and is also an important cotton trading nation. But since the 1990s, the occurrence of *Verticillium* wilt in cotton plantations increases year-on-year causing severe damage to cotton production (Zhang 2006). In recent years, *Verticillium* wilt disease has become one of the major factors limiting China's cotton yield and quality. Modern molecular biology techniques, such as transgenic breeding, should be a new way to enhance resistance to *Verticillium* wilt in cotton varieties. At present, a bottleneck for transgenic cotton breeding is the lack of an effective *Verticillium* wilt resistance gene. Studies on the cotton *Verticillium* resistance molecular mechanism, and cloning of cotton genes associated with *Verticillium* wilt resistance, are the basis of cotton breeding.

In the process of biological evolution, cotton has developed a variety of self-regulating physiological and metabolic changes to resist *Verticillium* fungus infection. One important mechanism is initiating signal transduction to activate antiviral gene expression. In the *Verticillium* fungus infection signal transduction pathways, transcription factors are of vital importance since they regulate the expression of multiple functional genes. There are more than 1,600 transcription factors in the *Arabidopsis* genome, which consists of about 6% of all genes. MYB transcription factors are one of the largest families (Riechmann et al. 2000). This family is characterised by presence of one to four highly conserved helix-turn-helix (HTH) repeat DNA binding domains and an MYB domain (Dubos et al. 2010).

In recent years, scientists conducted extensive research on plant MYB transcription factors and found that MYB transcription factors are a kind of multifunctional protein family, which are often involved in more than one life processes, such as cell cycle, secondary metabolism, organ morphogenesis, signal transduction, biotic and abiotic stress responses, and ABA sensitivity (Dubos et al. 2010). So far, researchers have identified a large number of MYB transcription

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factors from various plants, including more than 198 from *Arabidopsis*, more than 80 from maize (Rabinowicz et al. 1999), and about 200 from cotton (Cedroni et al. 2003). Functional studies show that MYB transcription factors play important regulatory role in plant stress responses (Cedroni et al. 2002; Vannini et al. 2003). Many stress-related MYB transcription factors have been cloned from *Arabidopsis*, rice and wheat. *AtMYB2*, *AtMYB13*, *AtMYB14*, *AtMYB41*, *AtMYB44*, *AtMYB61*, and *AtMYB102* are stress-responsive MYB transcription factors from *Arabidopsis*. *AtMYB2* is a drought-induced gene which plays an important role in fighting drought through the ABA-dependent signal pathway (Abe 2002); *AtMYB102* is an osmotic stress response-related gene whose expression is induced by osmotic stress, ABA and mechanical damage (Denekamp 2003); *Arabidopsis* plant with overexpressed *AtMYB96* show stronger drought resistance than wild-type plants (Seo et al. 2009); overexpression of rice *OsMYB4* in *Arabidopsis*, rice and apple enhances transgenetic plant resistance to different stress conditions (Stracke et al. 2001; Vannini et al. 2003).

As an important cash crop, the study of important functional genes has been the focus of cotton research. In recent years, some cotton MYB genes with important functions were identified. However, most cotton MYB genes cloned are associated with cotton fibre development (Wang et al. 2004). *GhMYB25* is upregulated in the fibre cell initiation period (Wu et al. 2006). Transgenic tobacco leaves overexpressing *GhMYB25* with increased trichomes indicating that *GhMYB25* can regulate epidermal cell differentiation and growth (Machado et al. 2009). The *GhMYB109* gene is specifically expressed in cotton fibres undergoing their initiation and elongation phases (Suo et al. 2003). Inhibition of *GhMYB109* expression leads to reduced expression of some fibre elongation-related genes and shortened cotton fibre (Pu et al. 2008). Cotton stress responsive MYB genes are rarely reported. Zhu et al. (2013) compared cotton transcriptome data before, and after, the ABA, cold, salt, drought, and alkaline treatments, and found that MYB transcription factors are involved in response to various stresses.

Taking advantage of the *Gossypium raimondii* genome database, we cloned a new MYB transcription factor, *GhMYBantiV*, from upland cotton Lumianyan 32 (*G. hirsutum*). Its open reading frame length is 702 bp. After comparing it with the MYB protein amino acid sequences of the model plant Arabidopsis (*Arabidopsis thaliana*) and wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), it was found that the *GhMYBantiV* protein shows very high similarity (an E-value of 7e-83 and 1e-90 respectively) with *Arabidopsis* protein AtMYB13/14, which are important abscisic acid (abscisic acid, ABA) synthesis and signal transduction genes. *GhMYBantiV* shows two highly conserved R2R3 domains and a transcriptional activation domain. Yeast transcriptional activation analysis showed that *GhMYBantiV* has transcriptional activation activity. Transient expression analysis showed that *GhMYBantiV* localises in the nucleus. Real-time quantitative PCR analysis showed that *GhMYBantiV* gene expression is significantly upregulated after *Verticillium* fungus (*Verticillium dahliae*) infestation, drought, and oxidative stress treatment. So we speculate that *GhMYBantiV* gene plays an important regulatory role in cotton biotic and abiotic stress responses. Therefore, in this study cloning of *GhMYBantiV* was done and characterization was attempted.

Materials and methods

Plant materials

Lumianyan 32 is a cotton variety selected from the population derived from cross between (Lumianyan 18 and 1565 line) and (Stone 368 × Australia A93-14). Lumianyan 32 shows high resistance to *Fusarium* wilt, *Verticillium* wilt, and strong adaptability to salinity environment (Li 2008). The seeds of Lumianyan 32 were taken after planting the crop for three consecutive generations. We planted the seeds of Lumianyan 32 in pots and grew them at 25°C in a cultivation cabinet. Two-week old seedling roots and leaves were collected for gene cloning and expression characterisation.

RNA extraction and gene cloning

Using roots and leaves from two-week old seedlings for total RNA extraction with an improved CTAB method (White et al. 2008), DNA digestion and first-strand cDNA synthesis were conducted according to the TransScript II One-Step gDNA Removal and cDNA Synthesis SuperMix kit (Transgen Biotech Company) instructions. Cloning primers were designed according to the phytozome database diploid myb-like sequence with Primer Premier 6.0. Sense and antisense primer sequences are: TGGTGAGAGCTCCTTGCTG and GGTCAATGTGGTGGTTCTCC, respectively and 2×TransTaq High Fidelity (HiFi) PCR SuperMix I (-dye) from Transgen Co. was used for gene cloning. The PCR amplification system used a sample volume

of 20 uL, with a total of 38 cycles: 95°C 3 min, 95°C 30 s, 56°C 30 s, 72°C 1 min 20 s, and finally 72°C 7 min. The PCR product was run on 1% agarose gel. The desired DNA band was recovered and purified, and then ligated on the pEASY-T1 cloning vector (provided by Beijing Transgen Biotechnology Co., Ltd) and transformed into *E. coli* competent cells Trans5á. Positive colonies were sent to the Beijing Genomics Institute for sequencing.

GhMYBantiV phylogenetic tree and protein sequence analysis

We predicted the *GhMYBantiV* open reading frame with ORF Finder (http: //www.ncbi nlm.nih.gov /gorf/gorf.html) and compared the GhMYBantiV amino acid sequence with that of MYB from *Arabidopsis* and important crops such as rice, wheat and maize using MEGA6.0 We constructed the phylogenetic tree and multiple sequence alignment map by neighbour-joining method, and predicted the function of GhMYBantiV with information from this analysis.

GhMYBantiV expression analysis

After sterilisation, Lumianyan 32 seeds were sown in sterilised quartz sands and cultivated in a greenhouse until three or four true leaves appeared. Then the following treatments were applied: CK, (control, Hogland culture medium); 10% PEG6000 solution (simulated drought stress); 150 mmol/L NaCl solution (simulated salt stress); *Verticillium* fungus spore solution (using high-pathogenicity strain *Verticillium* VD8 at a spore suspension concentration of approximately 1×10^7 mL^{-1}); 4°C low-temperature treatment (simulated cold stress); and 200 mmol/L H_2O_2 solution (simulated oxidative stress). After 2 hours inoculation in the aforementioned solutions, the root and young leaves were removed and frozen in liquid nitrogen, then stored at –80°C for future use. Real-time quantitative PCR primers were designed as detailed at: http://www.idtdna.com/primerquest/Home/Index. Sense and the antisense primer sequences are GGACTCATG AAGAAG ACCAGA TT and GCAACTCT TCCCGCA TC TTA, respectively. Real-time quantitative PCR was conducted according to Takara Company SYBR®Premix Ex TaqTM (Tli RNaseH Plus) kit instructions. Cotton ubiquitin (Ubiquitin) gene UBQ7 was used as a reference gene. The PCR program was as follows: 95°C 30 s, 40 cycles of 95°C 5 s, 60°C 30 s, and finally the fusion program was run (2-ΔΔCt was used for subsequent data analysis).

GhMYBantiV sub-cellular localisation analysis

We amplified the *GhMYBantiV* full-length cDNA sequence with primers with additional restriction sites. We ligated the amplified fragment onto pMD 18-T Vector. Using pBSK-GFP as a transient expression vector, we then ligated the *GhMYBantiV* cDNA fragment onto a pBSK-GFP vector, to obtain GhMYBantiV-GFP fusion protein driven by the 35S promoter. We conducted onion cell sub-cellular localisation with pBSK-GFP fusion protein as a positive control.

Analysis of GhMYBantiV transcriptional activity

Using pGBKT7 as a negative control, we transformed pGBKT7 and pGBKT7-GhMYBantiV into yeast competent cells AH109. We applied 1:1, 1:10, and 1:100 dilutions evenly on an SD/-Trp single-deficiency plate and an SD/-Trp/-His double-deficiency plate. These were cultured at 28°C for 2 to 3 days to allow observation of colony growth. Yeast positive clones were selected and cultured on YPD medium containing X-Gal and IPTG: the β-galactosidase activity was then detected.

Results

Cloning and expression analysis of GhMYBantiV

Taking advantage of the Myb4ike gene (Gorai.011G173900.1) sequence from the *G. raimondii* genome sequence database, we designed primers and amplified a fragment from Lumianyan 32 cDNA (Fig. 1). The fragment size was about 950 to 1000 bp. After cloning and sequencing, we obtained a coding sequence (CDS) of 705 bp, encoding a peptide of 234 amino acids (Fig. 2).

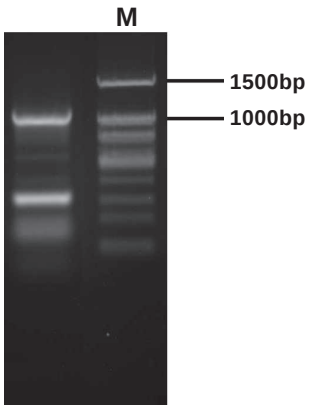

Fig. 1. Agarose gel electrophoresis of the *GhMYBantiV* cDNA fragment, M: 100 bp DNA ladder; 1: amplified band of gene *GhMYBantiV*

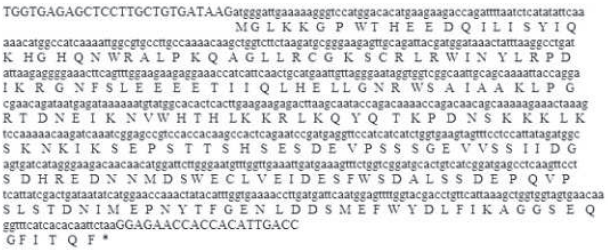

Fig. 2. Coding sequences and protein sequences of *GhMYBantiV*

Two-week old seedlings of Lumianyan 32 were treated with culture medium (CK)/10% PEG6000 solution (simulated drought stress)/150 mM NaCl solution (simulated salt stress)/*Verticillium* fungus spore solution (at a spore suspension concentration of approximately 1×10^7/mL)/4°C low-temperature treatment (simulated cold stress)/200 mM H_2O_2 solution (simulated oxidative stress). Alterations in expression of *GhMYBantiV* were detected in the seedling root and leaf by real-time quantitative PCR (Fig. 3). The results showed that, after Verticillium

GhMYBantiV phylogenetic tree and protein sequence analysis

The *GhMYBantiV* amino acid sequence was compared with that of the model plant *Arabidopsis* and important crops, such as wheat, rice, and corn for phylogenetic analysis. We established the evolutionary tree using a neighbour-joining method. *GhMYBantiV* showed closest similarity with the *Arabidopsis* R2R3 type MYB transcription factors *AtMYB13*/*14*/*15* (Supplementary Fig. 1; http://epubs.icar.org.in/journal/index.php/IJGPB). They all belong to the R2R3 subfamily, which shows a highly-conserved DNA binding domain (Supplementary Fig. 2). The measured *AtMYB13* and *AtMYB14* responses to GA, jasmonic acid, and salicylic acid treatment, which are plant hormones closely related to abiotic stress response and development. *AtMYB13* and *AtMYB14* are also involved in salt stress reaction processes. *Arabidopsis* plants overexpressing *AtMYB15* show stronger abscisic acid (ABA) sensitivity and higher salt/drought tolerance when compared with wild types. In Arabidopsis plants overexpressing *AtMYB15*, the

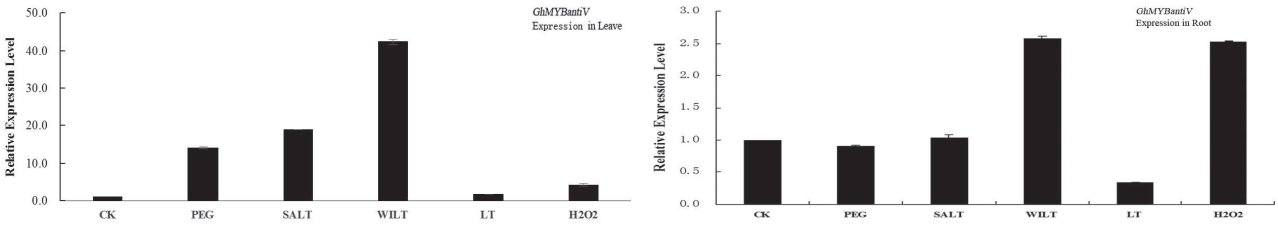

Fig. 3. qRT-PCR analysis of the gene *GhMYBantiV*. The horizontal axis represents six treatments: CK = the control treatment; PEG = 10% PEG6000 solution treatment as simulated drought stress; SALT = 150 mM NaCl solution treatment as simulated salt stress; WILT = *Verticillium* fungus spore solution treatment to cause biotic stress; LT = low temperature, 4°C low-temperature treatment as simulated cold stress); H2O2 = 200 mM H_2O_2 solution treatment as simulated oxidative stress. The longitudinal axis represents the relative expression value under different treatments. The left graph indicates the GhMYBantiV expression level in Lumianyan 32 leaves. The right graph indicates the GhMYBantiV expression level in Lumianyan 32 root

fungal infection, *GhMYBantiV* expression in leaves was 42.3 times that of the control ($P < 0.0001$). Though there was no such huge change in *GhMYBantiV* expression in leaves after low-temperature and H_2O_2 treatments, but *GhMYBantiV* expression level was also altered significantly by PEG and NaCl treatment indicating that *GhMYBantiV* is responsive to both biotic stress (*Verticillium* wilt fungus infection) and abiotic stresses (PEG and NaCl treatment). However, after the various treatments described above, there was no significant change in *GAMYB12* expression in Lumianyan 32 seedling root (Supplementary Table 1).

expression of genes involved in ABA synthesis (*ABA1*, *ABA2*), signal transduction (*ABI3*) and drought response (*AtADH1*, *RD22*, *RD29B*, *AtEM6*) are significantly increased (Chen et al. 2006; Katiyar et al. 2012). It is suggested that *GhMYBantiV* may play an important role in cotton response to biotic and abiotic stresses.

GhMYBantiV sub-cellular localisation analysis

A *GhMYBantiV-GFP* onion epidermal transient expression vector was constructed on the basis of the pBSK-GFP expression vector. After bombardment

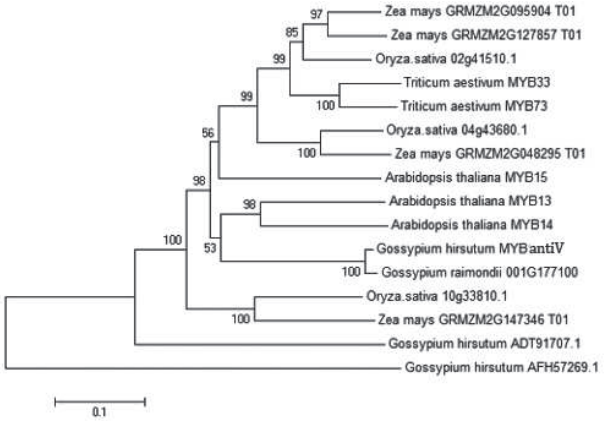

Fig. 4. Phylogenetic analysis of *GhMYBantiV*

transformation into the onion skin; the fluorescence signal using confocal microscopy at an excitation wavelength of 488 nm was detected and also the GFP green fluorescence from the empty vector control showed an even distribution across the nucleus, cytoplasm, and cell membrane (Figs. 5A and 5B) and the GFP green fluorescence distribution of the fusion protein GhMYBantiV-GFP (Figs. 5C and 5D) was found only in the nucleus. This meant that the *GhMYBantiV* is a nuclear protein.

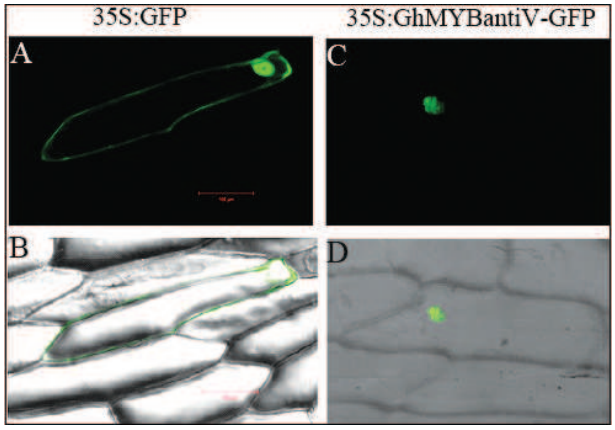

Fig. 5. GhMYBantiV-GFP localises in the nucleus

GhMYBantiV transcriptional activity analysis

The pGBKT7-GhMYBantiV vector was transformed and the empty vector into yeast strain AH109 and found that both the yeast strain with the empty vector, and the yeast strain with pGBKT7-*GhMYBantiV* can grow normally on SD/Trp-plates, while only pGBKT7-*GhMYBantiV* recombinant yeast strains can grow on SD/His-medium. The pGBKT7-*GhMYBantiV* recombinant yeast strain can also show as blue spots in the presence of X-Gal (Fig. 6), indicating that *GhMYBantiV* works as a transcriptional factor in yeast.

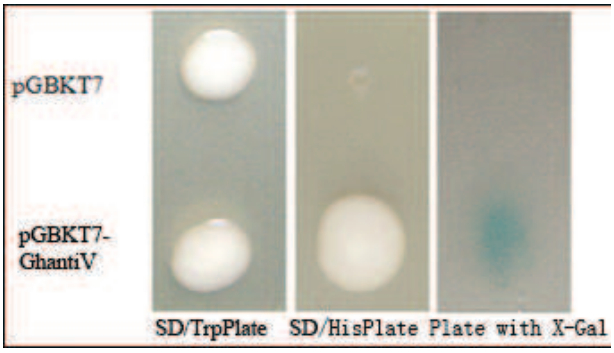

Fig. 6. GhMYBantiV activates the expression of yeast genes

Discussion

The adaptability of certain crops plants, to various stresses has a significant influence on their growth and development. Improving crop stress-resistance has become an important task for crop breeders. Research into the stress response mechanisms in crops and exploration of the crop genetic resources related to stress tolerance forms the basis for improving crop stress-resistance has practical significance in crop breeding. MYB transcription factors are a kind of multi-functional gene family, which plays an important role in plant development and stress-resistance. In *Arabidopsis*, rice, wheat, maize, etc. a number of MYB transcription factors with key regulatory functions have been cloned. Cloning of MYB transcription factor and the exploration of their function and mechanism, has important significance for molecular breeding of cotton.

Previous studies have shown that some members of the MYB family play an important role in *Arabidopsis* response to biotic and abiotic stresses. These members contain two MYB domains belonging to the R2R3-type MYB transcription factor sub-family. By regulating signal transduction processes and inducing stress-resistance genes, these transcription factors thereby improve *Arabidopsis* resistance to various stresses. So far extensive research on *Arabidopsis* and the rice MYB family has been conducted, and a number of MYB transcription factors with important functions have been identified (Dubos et al. 2010); however, there are few studies of cotton MYB genes. In this study, using the *G. raimondii* genomic dataset, we cloned *GhMYBantiV* from Lumianyan 32 (a cotton variety showing high resistance

to *Fusarium* wilt and *Verticillium* wilt). *GhMYBantiV* shows high similarity to *Arabidopsis* AtMYB13/14/15, and also belongs to the R2R3 MYB sub-family. Yeast transcriptional activity analysis shows that *GhMYBantiV* has transcriptional activation activity. Sub-cellular localisation analysis show that *GhMYBantiV* localises in the nucleus. Overexpression of *AtMYB15* greatly enhances the sensitivity to abscisic acid (ABA) and tolerance to salt and drought stress. In *Arabidopsis* plants overexpressing *AtMYB15*, expression of genes involved in ABA synthesis (*ABA1*, *ABA2*), signal transduction (*ABI3*), and drought response genes (*AtADH1*, *RD22*, *RD29B*, and *AtEM6*) all significantly increased (Chen et al. 2006; Katiyar et al. 2012). *AtMYB13* and *AtMYB14* are involved in responses to plant hormones, such as GA, jasmonic acid, salicylic acid and abiotic stress (Chen et al. 2013; Hanh et al. 2017). It is suggested that *GhMYBantiV* may presumably be responsible for cotton stress responses, especially biotic stresses.

Plants have evolved overlapping signal transduction pathways to sense outside stress factors. There are intersections of biotic and abiotic stress signalling pathways at different levels (O'Donnell et al. 2003; Qiu et al. 2008). In recent years, research into stress tolerance-related transcription factors found that a transcription factor may be activated by various stresses indicating that various stress signals can be integrated via the transcription factor genes (Ma and Bohnert 2007; Qiu et al. 2008). Using real-time quantitative PCR, we analysed *GhMYBantiV* expression before and after, *Verticillium* wilt fungus infection, PEG, NaCl, cold, and H_2O_2 treatments. It was found that *GhMYBantiV* expression in cotton leaves was significantly upregulated after *Verticillium* fungus infection and drought, salt treatment, though at a lower level. Although salt, drought, and *Verticillium* wilt fungal treatment belong to different types of external stress, they all break the redox balance inside the plant cells causing reactive oxygen species formation and ultimately leading to cell death (Shao et al. 2008). The upregulation of *GhMYBantiV* after the imposition of these stresses may promote the expression of downstream functional genes. These genes produce a series of protective enzymes to maintain cell redox balance, so as to reduce, or compensate for, the damage caused by them. In addition, *GhMYBantiV* expression in cotton root was not significantly changed, while that in leaves it was greatly upregulated. This may indicate the presence of a tissue-specific promoter that can efficiently enhance the expression of *GhMYBantiV* in the leaves.

Authors' contribution

Conceptualization of research (YY); Designing of the experiments (YY, LF); Contribution of experimental materials (LZ, FM, LF, HX, HK); Execution of field/lab experiments and data collection (LF, LZ); Analysis of data and interpretation (LF, TM, YZ); Preparation of manuscript (LF, CX, HX, HK, YZ).

Declaration

The authors declare no conflict of interest.

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References

Abe H. 2002. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) Function as Transcriptional Activators in Abscisic Acid Signaling. The Plant Cell Online, **15**: 63-78.

Cedroni M. L., Cronn R. C., Adams K. L., Wilkins T. A. and Wendel J. F. 2002. Association of decreased expression of a Myb transcription factor with the TPD (tapping panel dryness) syndrome in Hevea brasiliensis. Plant Molecular Biology, **51**: 51-58.

Cedroni M. L., Cronn R. C., Adams K. L., Wilkins T. A. and Wendel J. F. 2003. Evolution and expression of MYB genes in diploid and polyploid cotton. Plant Mol. Biol., **51**: 313-328.

Chen Y., Chen Z., Kang J., Kang D., Gu H. and Qin G. 2013. AtMYB14 Regulates Cold Tolerance in Arabidopsis. Plant Mol. Biol. Report., **31**: 87-98.

Chen Y., Zhang X., Wu W., Chen Z., Gu H. and Qu L.-J. 2006. Overexpression of the Wounding-Responsive Gene AtMYB15 Activates the Shikimate Pathway in *Arabidopsis*. J. Integrative Plant Biol., **48**: 1084-1095.

Denekamp M. 2003. Integration of Wounding and Osmotic Stress Signals Determines the Expression of the AtMYB102 Transcription Factor Gene. Plant Physiology, **132**: 1415-1423.

Dubos C., Stracke R., Grotewold E., Weisshaar B., Martin C. and Lepiniec L. 2010. MYB transcription factors in Arabidopsis. Trends Plant Sci., **15**: 573-581.

Hanh H. T. M., Nha N. D. and Sik C. W. 2017. Identification of R2R3- MYB Transcription Factor (AtMYB13) as a Novel Substrate of *Arabidopsis* MPK3 and MPK6. VNU J. Sci. Natural Sci. Tech., **32**: 220-226.

Katiyar A., Smita S., Lenka S. K., Rajwanshi R., Chinnusamy

V. and Bansal K. C. 2012. Genome-wide classification and expression analysis of MYB transcription factor families in rice and *Arabidopsis*. BMC Genomics, **13**: 544-562.

Ma S. and Bohnert H. J. 2007. Integration of Arabidopsis thaliana stress-related transcript profiles, promoter structures, and cell-specific expression. Genome Biol., **8**: R49.

Machado A., Wu Y., Yang Y., Llewellyn D. J. and Dennis E. S. 2009. The MYB transcription factor GhMYB25 regulates early fibre and trichome development. Plant J., **59**: 52-62.

O'Donnell P. J., Schmelz E., Block A., Miersch O., Wasternack C., Jones J. B. and Klee H. J. 2003. Multiple hormones act sequentially to mediate a susceptible tomato pathogen defense response. Plant Physiol., **133**: 1181-1189.

Pu L., Li Q., Fan X., Yang W. and Xue Y. 2008. The R2R3 MYB transcription factor GhMYB109 is required for cotton fiber development. Genetics, **180**: 811-820.

Qiu J. L., Zhou L., Yun B. W., Nielsen H. B., Fiil B. K., Petersen K., Mackinlay J., Loake G. J., Mundy J. and Morris P. C. 2008. *Arabidopsis* mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4 and MKS1. Plant Physiol., **148**: 212-222.

Rabinowicz P. D., Braun E. L., Wolfe A. D., Bowen B. and Grotewold E. 1999. Maize R2R3 Myb Genes_ Sequence Analysis Reveals Amplification in the Higher Plants. Genetics, **153**: 427-444.

Riechmann J. L., Heard J., Martin G., Reuber L., Z. C., Jiang, Keddie J., Adam L., Pineda O., Ratcliffe O. J., Samaha R. R., Creelman R., Pilgrim M., Broun P., Zhang J. Z., Ghandehari D., Sherman B. K. and L. Yu G. 2000. *Arabidopsis* Transcription Factors: Genome-Wide Comparative Analysis Among Eukaryotes. Science, **290**: 2105-2110.

Seo P. J., Xiang F., Qiao M., Park J. Y., Lee Y. N., Kim S. G., Lee Y. H., Park W. J. and Park C. M. 2009. The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in *Arabidopsis*. Plant Physiol., **151**: 275-289.

Shao H.-b., Chu L.-y., Shao M.-a., Jaleel C. A. and Hong-mei M. 2008. Higher plant antioxidants and redox signaling under environmental stresses. Comptes Rendus Biologies, **331**: 433-441.

Stracke R., Werber M. and Weisshaar B. 2001. The R2R3-MYB gene family in *Arabidopsis thaliana*. Curr. Opinion Plant Biol., **4**: 447-456.

Suo J., Liang X., Pu L., Zhang Y. and Xue Y. 2003. Identification of GhMYB109 encoding a R2R3 MYB transcription factor that expressed specifically in fiber initials and elongating fibers of cotton (*Gossypium hirsutum* L.). Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression, **1630**: 25-34.

Vannini C., Locatelli F., Bracale M., Magnani E., Marsoni M., Osnato M., Mattana M., Baldoni E. and Coraggio I. 2003. Overexpression of the rice Osmyb4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant J., **37**: 115-127.

Wang S., Wang J. W., Yu N., Li C. H., Luo B., Gou J. Y., Wang L. J. and Chen X. Y. 2004. Control of plant trichome development by a cotton fiber MYB gene. Plant Cell, **16**: 2323-2334.

White E. J., Venter M., Hiten N. F., Burger J. T. 2008. Modified cetyltrimethylammonium bromide method improves robustness and versatility: the benchmark for plant RNA extraction. Biotechnol J., **3**: 1424-1428.

Wu Y., Machado A. C., White R. G., Llewellyn D. J. and Dennis E. S. 2006. Expression profiling identifies genes expressed early during lint fibre initiation in cotton. Plant Cell Physiol., **47**: 107-127.

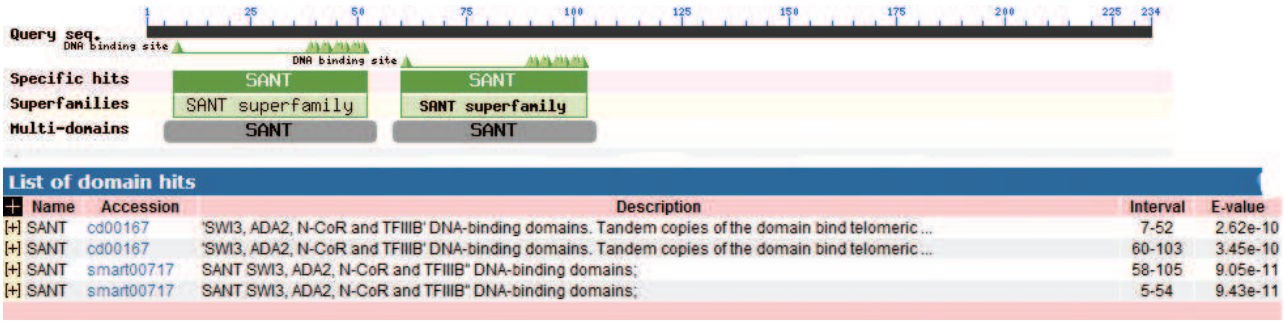
Zhang L. 2006. Study on the occurrence rule of cotton Verticillium wiltand resistance of cotton varieties in Xinjiang(D). Shihezi University.

Zhu Y. N., Shi D. Q., Ruan M. B., Zhang L. L., Meng Z. H., Liu J. and Yang W. C. 2013. Transcriptome analysis reveals crosstalk of responsive genes to multiple abiotic stresses in cotton (*Gossypium hirsutum* L.). PLoS One, **8**: e80218.

Supplementary Table 1. Quantitative real time PCR data for the *GhMYBantiV* gene (data calculated by 2-ΔΔCt method)

	GhMYBantiVL	Standard deviation	*GhMYBantiVR*	Standard deviation
CK	1	0	1	0
PEG	14.05634307	0.256726116	0.901922175	0.012254397
SALT	18.93268976	0.067719809	1.02948742	0.050325224
WILT	42.30440653	0.747471583	2.575929242	0.037186373
LT	1.664714413	0.018530355	0.334812386	0.001514888
H2O2	4.239986571	0.189670768	2.533943591	0.006644281

Supplementary Figure 1 Comparison between the amino acid sequences of *GhMYBantiV* and *AtMYB13/14*



Supplementary Figure 2 Conserved domains of *GhMYBantiV*