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# Expression of a tomato MYB gene in transgenic tobacco increases resistance to *Fusarium oxysporum* and *Botrytis cinerea*

Zhen Liu · Yushi Luan · Jingbin Li · Yali Yin

**Abstract** MYB transcription factors play an essential role in defense responses in various plant species. Although research has investigated the function of MYB transcription factors, relative to the research progress in model plants, limited numbers of MYB transcription factors have been studied in tomato. In our previous study, transgenic tobacco plants overexpressing *SpMYB* increased resistance to *Alternaria alternata*. In the present study, some *cis*-acting elements associated with the environmental stresses response were observed in the promoter of this gene. *SpMYB* expression was significantly induced after infection with *Fusarium oxysporum* and *Botrytis cinerea*. Furthermore, transgenic tobacco plants increased resistance to *F. oxysporum* and *B. cinerea* compared with wild-type plants, and the transgenic plants had lower malonaldehyde content, but higher peroxidase, superoxide dismutase and phenylalanine ammonia-lyase activities. This resistance was also coupled with enhanced the expression of some defense-related genes (*NtPOD*, *NtSOD* and *NtPAL*) as well as marker genes for the jasmonic acid signaling pathway (*NtPR4* and *NtPDF1.2*). Moreover, the transgenic plants also exhibited lower levels of $H_2O_2$ accumulation than wild-type plants following pathogen infection. Taken together, these results suggested that *SpMYB* positively regulates plant disease resistance; these findings will expand our knowledge on the function of tomato MYB transcription factors in defense against pathogens.

**Keywords** Tomato · MYB · Tobacco · *Fusarium oxysporum* f. sp. *nicotianae* · *Botrytis cinerea*

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## Introduction

Pathogen infection is one of the most severe biotic stresses, and causes heavy damage to the crop yield. To reduce the adverse effects of pathogen attack, plants have developed a variety of efficient defense mechanisms such as physiological and biochemical changes (Li et al. 2015a; Li et al. 2015b). Transcriptional regulation of defense-related genes is a key step in the activation of plant defense responses (Buscaill and Rivas 2014). During these processes, transcription factors play an essential role in the adaptive plasticity of plants in highly variable environments by forming a regulatory network (Eulgem and Somssich 2007). Furthermore, transcription factors can also interact with *cis*-elements that are present in the promoter region of defense-related genes, thus regulating the expression of these genes (Ciolkowski et al. 2008). As a large gene family, MYB has received increasing attention for its roles in plant defense responses.

The MYB family of genes is the largest family of transcription factors in all eukaryotes (Dubos et al. 2010). Based on the number of adjacent repeats in their

DNA binding domain, MYB transcription factors are grouped into four subfamilies: R1-MYB has one repeat, R2R3-MYB has two repeats, R1R2R3-MYB has three repeats and 4R-MYB has four repeats (Dubos et al. 2010). Among these subfamilies, R2R3-MYB transcription factors form the largest subfamily and play significant roles in regulating plant defense responses. In *Arabidopsis thaliana*, plants overexpressing the *AtMYB96* increased resistance to *Pseudomonas syringae* (Seo and Park 2010). Likewise, plants overexpressing the *AtMYB44* also increased resistance to *P. syringae* and enhanced the expression of *pathogenesis-related* (*PR*) genes (Zou et al. 2012). Furthermore, plants overexpressing the *OsMYB4* increased resistance to *P. syringae*, *tobacco necrosis virus* and *Botrytis cinerea* (Vannini et al. 2006). In wheat, plants overexpressing the R2R3-MYB gene *TaPIMP1* increased resistance to *Bipolaris sorokiniana* and enhanced the expression of a subset of defense-related genes (Zhang et al. 2012). In tomato, plants overexpressing the *OsMYB4* increased resistance to *tomato mosaic virus* (Vannini et al. 2007). To date, only a few tomato R2R3-MYB transcription factors related to abiotic stress have been isolated, and their functions have been studied (Meng et al. 2014; Zhang et al. 2011; Abuqamar et al. 2009), while research focusing on the R2R3-MYB transcription factors related to biotic stress is limited. In our previous study, transgenic tobacco plants overexpressing *SpMYB* increased resistance to *Alternaria alternata* (Li et al. 2014).

Here, we investigated the expression patterns of *SpMYB* in response to *Fusarium oxysporum* and *B. cinerea* infection in wild-type (WT) tomato plants as well as assayed for resistance to *F. oxysporum* and *B. cinerea* in transgenic tobacco plants. Moreover, changes in physiological parameters and the expression levels of defense-related genes were investigated in WT and transgenic tobacco plants after infection with *F. oxysporum*. Likewise, the expression levels of marker genes for the jasmonic acid (JA) signaling pathway were also investigated before and after infection with *F. oxysporum* and *B. cinerea*.

## Materials and methods

### Analysis of *SpMYB* promoter

The 2000 bp fragment from the 5′-upstream sequence of *SpMYB* were analyzed using PlantCARE available online (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

### Plant materials, growth conditions and stress treatments

Tomato (*Solanum pimpinellifolium* L3708) seeds were sown into plastic pots filled with soil and placed in a greenhouse at $25 \pm 3$ °C with a 16 h light and 8 h dark photoperiod cycle. Three-weeks old seedlings dedicated to hydroponics were transferred into triangular flask containing aerated quarter-strength Hoagland nutrient solution until plants have reached the five-leaf stage.

*F. oxysporum* f. sp. *nicotianae* was cultured on potato dextrose agar (PDA) medium at 25 °C for 7 days. Conidia were collected following the method as described previously (Cakir et al. 2014) and adjusted to $1 \times 10^6$ spores/ml using sterilized water. The root-dip inoculation (RDI) method was used with some modifications to inoculate the tomato seedlings with the fungus (Ahmed et al. 2013). Briefly, five-leaf stage seedlings were immersed in the conidial suspension. They were kept in the growth chamber at $25 \pm 1$ °C with a 16 h light and 8 h dark photoperiod cycle. Root samples were harvested at 0, 3, 12, 24, 36, 48, 72 and 96 h post-inoculation (hpi), respectively, immediately frozen in liquid nitrogen and stored at −80 °C. For each time point, three independent biological replicates were made and each replicate was run three times.

*B. cinerea* was also incubated on PDA medium at 20 °C for 7 days. Release of zoospores from *B. cinerea* was performed as described previously (Maruyama et al. 2013). Then, tomato seedlings at five-leaf stage were infected with *B. cinerea*. Briefly, 2 ml aliquots of zoospores suspension ($1 \times 10^6$ zoospores/ml) were sprayed onto tomato seedlings. The inoculated plants were moved to a growth room at $23 \pm 1$ °C with a 16 h light and 8 h dark photoperiod cycle. Leaf samples were harvested at 0, 3, 12, 24, 36, 48, 72 and 96 hpi, respectively, immediately frozen in liquid nitrogen and stored at −80 °C. For each time point, three independent biological replicates were made and each replicate was run three times.

### Generation of transgenic tobacco plants

The transgenic tobacco plants were obtained following the method as described previously (Li et al. 2014). In brief, the full-length coding sequence of *SpMYB* was inserted into pBI121 under the control of CaMV35S

promoter by replacing the GUS gene. The fused construct of pBI121-*SpMYB* was transformed into the leaf disk of tobacco by *Agrobacterium tumefaciens* strain EHA105. The initial transgenic tobacco plants were selected using 50 mg/l of kanamycin and further confirmed by PCR. The copy number of *SpMYB* in transgenic plants was determined by qRT-PCR following the methods as described previously (Huang et al. 2013; Li et al. 2015b). The single-copy *Ntactin* gene was selected as endogenous calibrator gene. The expression levels of *SpMYB* in these selected single-copy transgenic lines were further examined by qRT-PCR. The primers used are listed in supplementary Table S1.

*F. oxysporum* resistance assay

Four-week-old tobacco plants (WT and transgenic lines) were transferred into plastic pots filled with soil and cultured at $25 \pm 3$ °C for 7 days in a greenhouse with a 16 h light and 8 h dark photoperiod cycle. The genotypes of the plants used here were the same as those used in Li et al. 2014.

For this assay, 100 ml conidial suspension was applied to the soil of WT and transgenic lines for 4 consecutive weeks following a validated protocol (Munis et al. 2010). The resistance to *F. oxysporum* was evaluated at 4 weeks after inoculation according to a 0–4 disease severity scale: 0 = no symptoms; 1 = smaller than 25 % of leaves with wilting symptoms; 2 = between 26 and 50 % of leaves with wilting symptoms; 3 = between 51 and 75 % of leaves with wilting symptoms; 4 = larger than 75 % of leaves with wilting symptoms or plant death. The disease index (DI) was calculated from the disease severity using the following formula: DI = $\sum$ (disease severity × number of plants in that disease severity) × 100 / (total number of plants × highest rating) following a validated protocol (Bora et al. 2004). A high DI value means a low resistance. The inoculated plants were moved to a greenhouse at $25 \pm 1$ °C with a 16 h light and 8 h dark photoperiod cycle. The symptoms of tobacco plants were photographed and the key physiological parameters were measured after inoculation. Furthermore, the harvested roots were used to analyze the expression levels of defense-related genes after inoculation. Likewise, the harvested roots were also used to analyze the expression levels of marker genes for the JA signaling pathway before and after inoculation. Three independent biological replicates (20 plants of per replicate) were made and each replicate was run three times.

*B. cinerea* resistance assay

For detached leaves inoculation assay, 20 µl zoospores suspension was infiltrated into leaves that had been detached from WT and transgenic lines using a toothpick. The inoculated leaves were wrapped with freekeeping film in order to moisture and maintained in the dark at high humidity for 24 h and then moved to a greenhouse at $23 \pm 1$ °C with a 16 h light and 8 h dark photoperiod cycle. The diameter of lesions was measured at 3 days after inoculation. For this experiment, three leaves from three tobacco plants from each line were used and the experiment was repeated six times. For whole plant inoculation assay, 2 ml aliquots of zoospores suspension were applied on tobacco leaves using a hand-held sprayer until run off. The infected plants were moved to a greenhouse at $23 \pm 1$ °C with a 16 h light and 8 h dark photoperiod cycle. Relative humidity was maintained at 100 % by covering the plants with clear plastic. Harvested leaves were photographed at 5 days after inoculation and the lesion size were determined by Image J software in randomly selected areas of taken pictures following the method as described previously (Wang et al. 2014). After inoculation, the leaves of WT and transgenic lines were harvested for histochemical staining. Moreover, the harvested leaves were used to analyze the expression levels of marker genes for the JA signaling pathway before and after inoculation. Three independent biological replicates (20 plants of per replicate) were made and each replicate was run three times.

3, 3′–diaminobenzidine (DAB) and trypan blue staining assays

For $H_2O_2$ staining, leaves that had been detached from WT and transgenic lines after infection with *B. cinerea* were incubated in a DAB solution (1 mg/ml, pH 3.8) at 25 °C for 24 h in the dark. After staining, the leaves were soaked 95 % ethanol overnight to remove the chlorophyll (Shi et al. 2014). Trypan blue staining was also performed as described previously (Dang et al. 2013) with a minor modification. In brief, leaves were boiled in a trypan blue solution (10 ml lactic acid, 10 ml glycerol, 10 ml phenol, 10 ml distilled water and 10 mg trypan blue) for 2 min, and then shaken at low speed for overnight. After changing the solution several times to reduce the background, the leaves were preserved in fresh ethanol at room temperature and

photographed. At least six leaves were used for each staining assay.

Measurement of physiological parameters

All enzyme extraction procedures were conducted at 4 °C. After being infected with *F. oxysporum* for 4 weeks, the roots of WT and transgenic lines were collected to measure the MDA content, peroxidase (POD), superoxide dismutase (SOD) and phenylalanine ammonia-lyase (PAL) activities as described previously (Sun et al. 2013). For each experiment, three independent biological replicates were made and each replicate was run three times.

Gene expression analysis

The expression levels of genes were determined using quantitative real-time polymerase chain reaction (qRT-PCR) with specific primers (supplementary Table S1) on a Rotor-Gene 3000 PCR instrument (Corbett Research, Australia) using the SYBR Premix Ex Taq™ kit (TaKaRa, Dalian, China) with the following conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. The primers for qRT-PCR were designed using the website http://www. idtdna.com/primerquest/Home/Index. To assess the amplification specificity, melting curve analysis was performed at the end of each experiment. Standard curve was also constructed by serially diluting PCR product. Data acquisition and analysis were performed by using Rotor-gene 6 software. Relative expression levels of every gene were calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The expression of *actin* was quantified separately to normalize the control gene. The Ct value for gene-of-interest and *actin* was calculated for all samples and used to compute the significant of induction levels. For each sample, three independent biological replicates were made and each replicate was run three times.

Statistical analysis

All of the above numerical data expressed as mean ± standard deviation (SD) of three independent experiments performed. Statistical analyses were carried out with SPSS version 17.0 software using one-way analysis of variance (ANOVA). Significant differences were further analyzed by Duncan's multiple range test ($P < 0.05$).

## Results

### *SpMYB* promoter analysis

To determine whether the *SpMYB* is induced by stress, a 2000 bp fragment from the 5′-upstream region of *SpMYB* were analyzed using the PlantCARE database. Several elicitor-related elements were found in the promoter region of the *SpMYB* gene such as ARE, Box-W1, CGTCA-motif, ELI-box3, HSE, LTR, MBS and TGACG-motif. In addition, *cis*-acting regulatory elements associated with development-related genes also existed in this region, including CAT-box, RY-element and Skn-1_motif. All of the identified *cis*-elements are listed in Table 1. These data implied that *SpMYB* might play a role in response to several biotic and abiotic stresses as well as plant development.

Expression patterns of *SpMYB* under pathogen infection in tomato.

To test if *SpMYB* is involved in plant response to pathogen infection, the expression levels of *SpMYB* were measured by qRT-PCR after the treatment of tomato seedlings with *F. oxysporum* and *B. cinerea*. *SpMYB* expression reached its peak at 48 h with 5.01-fold, when infected with *F. oxysporum* (Fig. 1a). After being infected with *B. cinerea*, *SpMYB* expression was more rapidly induced and reached a maximum at 36 h with 4.87-fold (Fig. 1b). These results suggested that the expression of *SpMYB* might play a role in regulating plant necrotrophic pathogens disease responses.

### Transgenic tobacco plants overexpressing *SpMYB* increased resistance to *F. oxysporum*

The analysis of promoter and expression patterns implied that *SpMYB* might be involved in plant defense responses. To further analyze the putative function of *SpMYB* in plant, full-length coding sequence of *SpMYB* was cloned into the plant binary vector pBI121 by replacing the GUS gene, and transgenic plants were generated. Kanamycin-tolerant plants were further verified by PCR. QRT-PCR analysis revealed that transgenic lines 4, 5, 6 and 8 having single insertion event [reflected in *nptII* to *NtActin* ratios close to 1 as calculated in (Li et al. 2015b)]. Three lines (line 5, line 6 and line 8) with a single transgene event and expressing high levels of *SpMYB* were selected for further analysis (supplementary Fig. S1).

**Table 1** Putative *cis*-acting regulatory elements identified in *SpMYB*

| *Cis*-element | Position | Sequence (5′- 3′) |
|---|---|---|
| **Light responsive elements** | | |
| Box 4 | −505 (+), −1068 (+) | ATTAAT |
| CAAT-motif | −144 (−) | GCATTC |
| GA-motif | −885(+) | ATAGATAA |
| GATA-motif | −490 (−), −668 (−) | AAGGATAAGG/GATAGGA |
| GT1-motif | −59 (−) | GGTTAA |
| Sp1 | −587 (+), −1483 (−), −639 (+) | CC (G/A) CCC |
| Chs-CMA1a | −989 (−) | TTACTTAA |
| **Elicitor-related elements** | | |
| ARE | −231 (+), −1160 (−) | TGGTTT |
| Box-W1 | −375 (−) | TTGACC |
| CGTCA-motif | −723 (+), −1402 (+) | CGTCA |
| ELI-box3 | −1160 (+) | AAACCAATT |
| HSE | −1358 (+), −1373 (+) | AAAAAATTTC |
| LTR | −1286 (+), −1324 (+) | CCGAAA |
| MBS | −940 (+) | TAACTG |
| TGACG-motif | −723 (−), −1402 (−) | TGACG |
| **Development-related elements** | | |
| CAT-box | −1200 (−) | GCCACT |
| RY-element | −1258 (+) | CATGCATG |
| Skn-1_motif | -610 (−), −1431 (−), −1403 (+) | GTCAT |

In order to understand *SpMYB* functions in disease resistance, WT and transgenic lines were infected with *F. oxysporum*. Disease symptoms of roots were photographed after 4 weeks and the degree of resistance was calculated by the DI. As shown in Fig. 2a, symptoms of lower leaves yellowing and dark-brown necrotic lesions on the roots were observed in the WT plants, whereas the transgenic lines were generally healthy with no clear symptoms of Fusarium wilt. Moreover, the DI of transgenic lines was also lower than WT plants (Fig. 2b), suggesting that transgenic tobacco plants overexpressing *SpMYB* increased resistance to *F. oxysporum*.
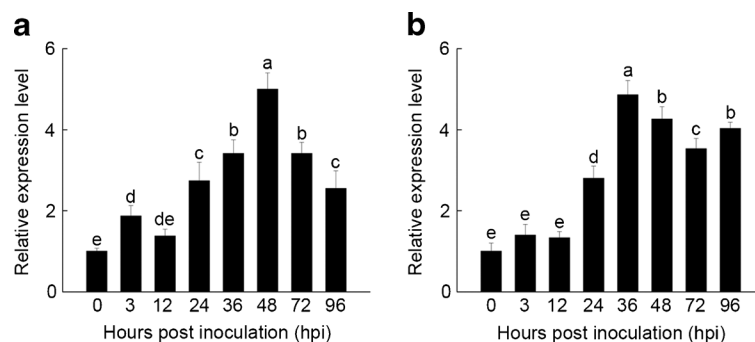


**Fig. 1** Expression profiles of *SpMYB* in response to **a** *F. oxysporum* infection, **b** *B. cinerea* infection. The samples were collected at 0, 3, 12, 24, 36, 48, 72, 96 hpi, respectively. The tomato *actin* gene was used as an internal control of gene expression. The expression of control treatment (0 h) is set as one. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test
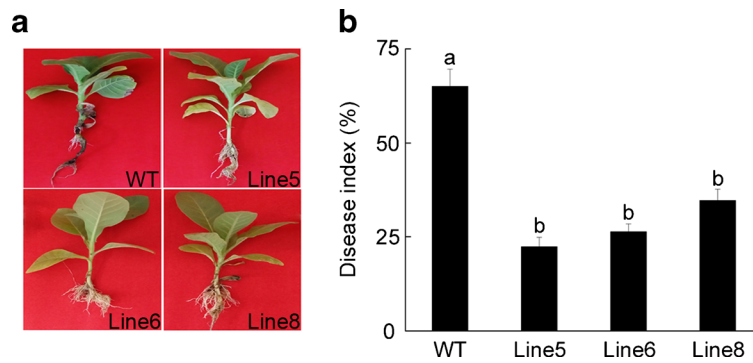
**Fig. 2** Evaluation of transgenic tobacco plants resistance against *F. oxysporum*. **a** Disease symptoms were observed at 4 weeks after inoculation, **b** Disease index was measured at 4 weeks after inoculation. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences (*P* < 0.05) according to Duncan's Multiple Range Test

Some key physiological parameters were measured in the WT and transgenic lines after inoculation. The MDA content represents the degree of cell membrane damage (Li et al. 2012; Li et al. 2015a). The MDA content of transgenic lines was significantly lower than WT plants after inoculation (Fig. 3a), indicating that membrane injury degree of transgenic lines was lesser than WT plants. Plants have developed complex antioxidant defense systems, including POD and SOD, to control intracellular ROS homeostasis (Panda 2007). The activities of these enzymes were higher in transgenic lines than WT plants after inoculation (Fig. 3b, c). PAL is the first enzyme in the phenylpropanoid pathway and plays an important role in the regulation of antimicrobial compound biosynthesis in plants as a response to pathogen infection (Liu et al. 2011a). After inoculation, the activity of PAL enzyme was also higher in transgenic lines than WT plants (Fig. 3d). Together, these results suggested that transgenic tobacco plants overexpressing *SpMYB* cause physiological changes, conferring transgenic plants greater resistance to *F. oxysporum*.

**Fig. 3** Physiological changes in WT and transgenic tobacco plants after infection with *F. oxysporum*. **a** MDA content, **b** POD activity, **c** SOD activity, **d** PAL activity. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences (*P* < 0.05) according to Duncan's Multiple Range Test
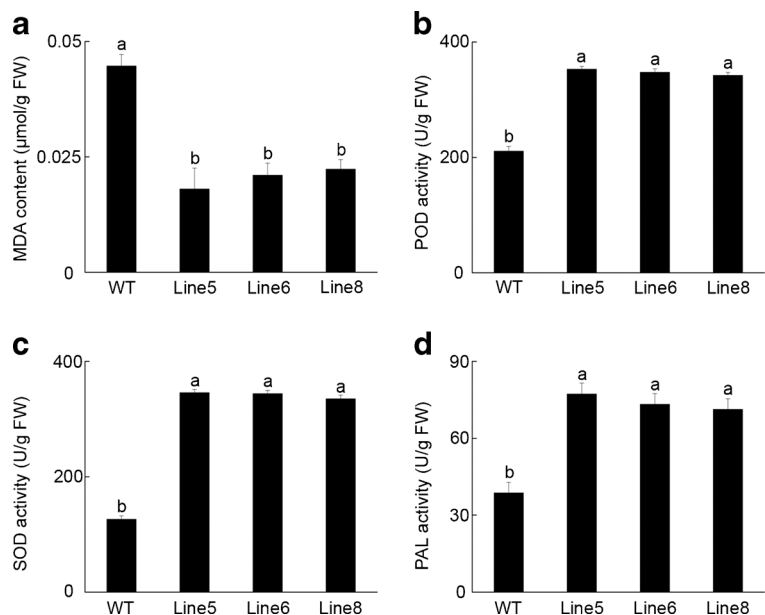
Transgenic tobacco plants overexpressing *SpMYB* altered gene expression in response to *F. oxysporum* infection

To gain further insight into the role of *SpMYB* in defense against *F. oxysporum* and elucidate its possible molecular mechanisms, qRT-PCR was used to investigate the expression levels of some well-known genes such as defense-related genes (*NtPOD*, *NtSOD* and *NtPAL*) and marker genes for the JA signaling pathway (*NtPR4* and *NtPDF1.2*). The expression levels of defense-related genes were significantly higher in transgenic lines than WT plants after inoculation (Fig. 4). Likewise, the expression levels of marker genes for the JA signaling pathway were also higher in transgenic lines than WT plants before and after inoculation (Fig. 5). The results suggested that *SpMYB* functions upstream and directly or indirectly regulates the expression of these genes.

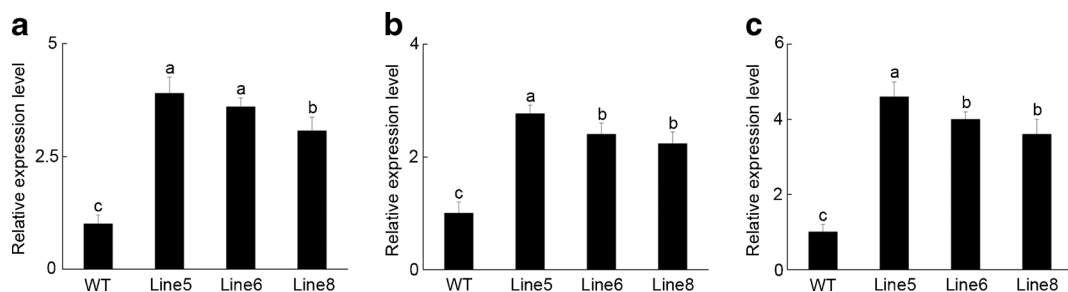Transgenic tobacco plants overexpressing *SpMYB* increased resistance to *B. cinerea*

To further investigate the function of *SpMYB* in defense against necrotrophic pathogens, WT and transgenic lines were infected with *B. cinerea*. In detached leaves inoculation assay, typical disease symptoms, e.g. necrotic lesions, were observed in the leaves from WT and transgenic lines after 3 days, but the lesions in the leaves from WT plants expanded much rapidly than those in the transgenic lines (Fig. 6a). Moreover, the diameter of lesions in the leaves from WT plants was larger than transgenic lines (Fig. 6b), which is in accordance with the above-observed results. In whole plant inoculation assay, severe disease symptoms were observed in WT plants at 5 days after inoculation, whereas the transgenic

lines showed weaker symptoms by evaluation of necrosis sizes (Fig. 6c). In addition, trypan blue staining revealed that the WT leaves had a more serious cell death phenotype than the transgenic lines leaves (Fig. 6d). These results indicated that transgenic tobacco plants overexpressing *SpMYB* increased resistance to *B. cinerea*.

Different defense pathways can be activated when plants are subjected to pathogen attack (Eulgem and Somssich 2007). Pathogen invasion is often correlated with production of reactive oxygen species (ROS) which plays an essential role in plant defense responses (Kotchoni and Gachomo 2006). To examine whether the enhanced resistance of the transgenic plants was related to ROS accumulation, we detected the content of $H_2O_2$ by DAB staining. After inoculation, the transgenic lines exhibited lower content of $H_2O_2$ than WT plants (Fig. 6e). The results demonstrated that transgenic plants show inhibited $H_2O_2$ accumulation in response to *B. cinerea* infection.
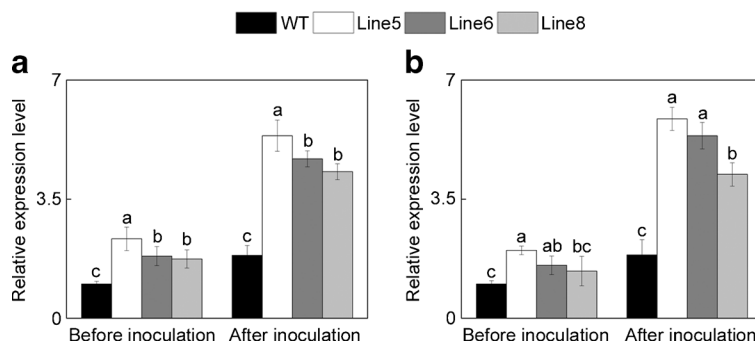
Transgenic tobacco plants overexpressing *SpMYB* altered gene expression in response to *B. cinerea* infection

To elucidate the molecular components involved in defense responses in transgenic plants, qRT-PCR was used to determine the expression levels of marker genes for the JA signaling pathway (*NtPR4* and *NtPDF1.2*). The expression levels of these genes were significantly higher in transgenic lines than WT plants before and after inoculation (Fig. 7). Therefore, our results further support the view that *SpMYB* functions in defense against necrotrophic pathogens through JA signaling pathway.



**Fig. 4** Relative expression levels of some defense-related genes in the roots from WT and transgenic tobacco plants after infection with *F. oxysporum*. **a** *NtPOD*, **b** *NtSOD*, **c** *NtPAL*. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test

**Fig. 5** Relative expression levels of some marker genes for the JA signaling pathway in the roots from WT and transgenic tobacco plants before and after infection with *F. oxysporum*. **a** *NtPR4*, **b** *NtPDF1.2*.

The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test

## Discussion

The MYB transcription factor is a member of a transcription factor family that plays vital roles in plants. Recently, increasing attention has been focused on MYB transcription factors which are involved in the regulation of plant defense responses (Seo and Park 2010; Zou et al. 2012; Vannini et al. 2006; Zhang et al. 2012; Vannini et al. 2007; Li et al. 2014). However, functional analysis of MYB transcription factors has been mostly focused on model plants and little progress has been made toward understanding the function of
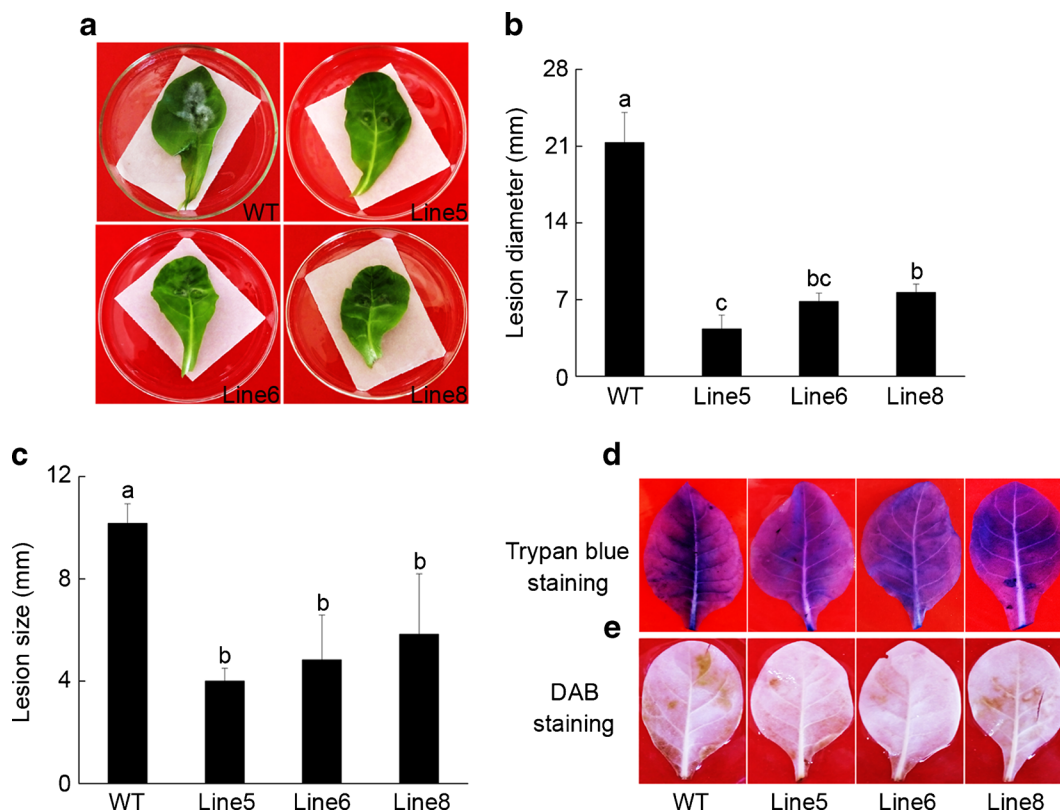


**Fig. 6** Evaluation of transgenic tobacco plants resistance against *B. cinerea*. **a** Disease signs on the detached leaves from WT and transgenic lines at 3 days after inoculation, **b** Lesion diameter was measured at 3 days after inoculation, **c** Lesion size was measured at

5 days after inoculation, **d** Trypan blue staining, **e** DAB staining. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test

**Fig. 7** Relative expression levels of some marker genes for the JA signaling pathway before and after infection with *B. cinerea*. **a** *NtPR4*, **b** *NtPDF1.2*. The data are presented as the mean ± SD of three independent experiments. Different letters above the columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test
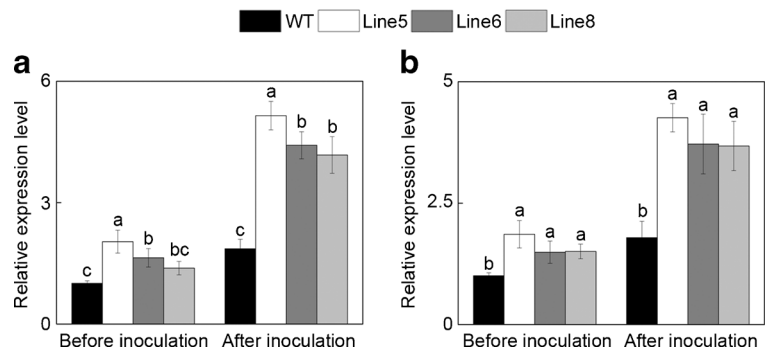


tomato MYB transcription factors. In our previous study, transgenic tobacco plants overexpressing *SpMYB* increased resistance to *A. alternata* (Li et al. 2014). In the present study, *cis*-elements involved in defense responses such as fungal elicitor and JA were found in the promoter region, suggesting that *SpMYB* may play positive roles in defense against pathogens.

Due to the induction of *SpMYB* expression in response to *F. oxysporum* and *B. cinerea*, we hypothesized that *SpMYB* might be involved in defense responses. To test the role of *SpMYB* in pathogen infection, transgenic plants were inoculated with *F. oxysporum* and *B. cinerea*. The transgenic tobacco plants overexpressing *SpMYB* increased the resistance to necrotrophic pathogen *F. oxysporum* and *B. cinerea*. Moreover, the expression of defense-related genes showed higher levels in transgenic lines than WT plants. These results strongly suggested that *SpMYB* functions as a positive regulator to pathogens resistance in transgenic plants. It is recently reported that most of the MYB transcription factors have positive roles in plant disease resistance. For example, transgenic tobacco plants overexpressing R2R3-MYB gene *TaPIMP1* increased resistance to *Ralstonia solanacearum* (Liu et al. 2011a). Similarly, transgenic wheat plants overexpressing *TiMYB2R-1* increased resistance to take-all disease (Liu et al. 2013). These results provide further evidence for MYB transcription factors playing significant roles in regulating plant disease resistance.

The deteriorative effects of pathogen on plant may include generation of MDA, resulting in membrane damage (Li et al. 2015b). Thus, we performed an experiment to analyze the MDA content of WT and transgenic lines, and the results showed that transgenic lines had lower content of MDA than WT plants under pathogen infection. Furthermore, the transgenic lines also exhibited lower levels of $H_2O_2$ accumulation than WT plants

under pathogen infection. To protect cells against ROS, plants have formed complex antioxidant defense systems such as induction of POD and SOD enzyme activities (Li et al. 2015b). Transgenic lines had higher POD and SOD activities than WT plants in response to pathogen infection. In addition, the expression levels of *NtPOD* and *NtSOD* encoding ROS-scavenging enzymes were also higher in transgenic lines. Collectively, these data demonstrated that transgenic tobacco plants overexpressing *SpMYB* may function in activation of antioxidant defense systems to decrease production of ROS or more effective scavenging of excessive ROS. PAL catalyzes the first step of the phenylpropanoid pathway and plays an essential role in defense responses (Liu et al. 2011a). The activity of PAL was significantly higher in transgenic lines than WT plants under pathogen infection. Similarly, the expression levels of *NtPAL* in transgenic lines were also higher. The improved activity of PAL may lead to the accumulation of antifungal secondary metabolites, thus increasing resistance against pathogens.

As one might expect, JA signaling pathway plays a key role in plant defense against necrotrophic pathogens (Mur et al. 2006). For instance, transgenic *Arabidopsis* plants overexpressing *AtERF1* and *AtERF2* increased resistance to *F. oxysporum* and enhanced the expression of marker genes for the JA signaling pathway (Berrocal-Lobo and Molina 2004; McGrath et al. 2005). Likewise, transgenic *Arabidopsis* plants overexpressing *AaERF1* and *BkERFs* increased resistance to *B. cinerea* and enhanced the expression of marker genes for the JA signaling pathway (Lu et al. 2013; Liu et al. 2011b). In this study, the expression levels of marker genes for the JA signaling pathway were higher in transgenic lines than WT plants before and after inoculation. These results support the existing evidence that JA-mediated defense against necrotrophic pathogens.

Growing evidence suggests that MYB transcription factors participate in plant defense responses and plant development. For example, *MYBH* played a critical role in developmentally regulated and dark-induced leaf senescence in *Arabidopsis* (Huang et al. 2015). Similarly, *MYB82* played an essential role in regulation of trichome development in *Arabidopsis* (Liang et al. 2014). In this study, *cis*-acting regulatory elements associated with development-related genes were found in the promoter region. We speculated that *SpMYB* might participate in plant defense responses as well as plant growth or development, and further experiment need to verify the possibility.

In conclusion, our results suggested that transgenic tobacco plants overexpressing *SpMYB* increased resistance to necrotrophic pathogen *F. oxysporum* and *B. cinerea*. The increased resistance may be connected with the JA-, ROS-and PAL-mediated defense pathways. The study provides evidence for the positive regulatory role of *SpMYB* in defense against pathogens and the mechanisms underlying the function of *SpMYB* should ideally be elucidated in transgenic tomato. As we learn more about *SpMYB* and its regulation, the design of efficient strategies for crop improvement should become possible.

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