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PHYSIOLOGY AND BIOCHEMISTRY

# Over-expression of a flower-specific transcription factor gene *AtMYB24* causes aberrant anther development

**X. Y. Yang · J. G. Li · M. Pei · H. Gu · Z. L. Chen · L.-J. Qu**

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**Abstract** In plants, MYB transcription factors play important roles in many developmental processes and various defense responses. *AtMYB24*, as a member of *R2R3-MYB* gene family in *Arabidopsis*, was found mainly expressed in flowers, especially in microspores and ovules using Northern blots and *in situ* hybridization. It was further found that the expression of *AtMYB24* was tightly regulated during anther development. Over-expression of *AtMYB24* in transgenic plants resulted in pleiotropic phenotypes, including dwarfism and flower development defects, in particular, producing abnormal pollen grains and non-dehiscence anthers. Further analysis showed that the anther development of the *AtMYB24-ox* lines was retarded starting from the anther developmental stages 10–11. At stages 12 and 13, the septum and stomium cells of anthers would not break, and fewer or no fibrous bands were found in the endothecium and connective cells in the *AtMYB24-ox* plants. Similar aberrant anther phenotype was also observed in the *AtMYB24-GR*-ox lines treated with dexamethasone (DEX). Quantitative real-time PCR showed expression of genes involved in phenylpropanoid biosynthetic pathway, such as *CHS* and *DFR*, and *AtGTP2* were altered in *AtMYB24-ox* lines. These results suggest an important role of *AtMYB24* in the normal development of anthers in *Arabidopsis*.

**Keywords** AtMYB24 · *Arabidopsis* · *In situ* hybridization · Anther development · Pollen defect

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X. Y. Yang · J. G. Li · M. Pei · H. Gu · Z. L. Chen · L.-J. Qu (✉)
Peking-Yale Joint Center for Plant Molecular Genetics and Agro-Biotechnology, National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University,
Beijing 100871, P. R. China
e-mail: qulj@pku.edu.cn

H. Gu · Z. L. Chen · L.-J. Qu
National Plant Gene Research Center (Beijing),
Beijing 100084, P. R. China

## Introduction

Transcription factors play important roles in the regulation of developmental and physiological processes by binding to promoters of the downstream target genes to control their expression (Pabo and Sauer 1992). Based on the nature of the DNA-binding domains, transcription factors can be categorized into many families, among which MYB superfamily is one of the largest families in *Arabidopsis* (Riechmann et al. 2000). A MYB domain is usually composed of one to three imperfect repeats, each with 51–53 amino acid residues to form a helix–turn–helix structure (Stracke et al. 2001). MYB proteins in plant are classified into three major groups: R2R3-MYB, which is plant specific, with two adjacent repeats; R1R2R3-MYB, with three adjacent repeats; and MYB-related proteins, which usually but not always contain one MYB repeat (Stracke et al. 2001; Chen et al. 2006). In plants, *MYB* genes are found to be involved in regulation of many physiological and biochemical processes such as cellular morphogenesis, meristem maintenance and lateral organ specification, floral and seed development, cell cycle, and biological circadian. Plant *MYB* genes are also involved in phytochrome and phytohormone signaling pathways (Stracke et al. 2001; Chen et al. 2006; Kranz et al. 1998).

Many *MYB* genes have been reported to play important roles in flower development, e.g., pollen and anther development. *AtMYB32* is required for normal pollen development, since *atmyb32* mutant produces more than 50% aber-

rant pollen grains, which are devoid of cytoplasmic contents (Preston et al. 2003). *AtMYB103*, specifically expressed in tapetum and trichome, is also important for pollen development. Down-regulation of *AtMYB103* expression would result in distorted pollen grains with reduced or no cytoplasmic content, and cause early tapetum degeneration at anther stage 9 (Higginson et al. 2003). *AtMYB26* is important for anther development because the expansion of the endothecium layer, which happens at late stages of the anther development, does not occur in *atmyb26* mutant. Further analysis found that the lignification did not take place in the mutant (Steiner-Lange et al. 2003). In addition, *AtMYB21* is a flower-specific gene, whose over-expression causes abnormal flower development (Shin et al. 2002).

In a previous study, we identified two groups of MYB transcription factors, which all share a conserved M/Y-MDDIW motif at their C-terminal regions, and interestingly, these genes in the group I in *Arabidopsis* seemed to be expressed predominantly in flowers (Li et al. 2006a). Here, we report the characterization of one gene from the group I, *AtMYB24* (*At5g40350*). *AtMYB24* is predominantly expressed in flowers, especially in microspore and ovules. Over-expression of *AtMYB24* caused pleiotropic phenotype including dwarfism, aberrant pollens, and non-dehiscence anthers. Real-time PCR showed that expression of genes involved in phenylpropanoid biosynthetic pathway and *AtGTP2* were altered in *AtMYB24-ox* lines. Our data indicated that AtMYB24 play an important role in anther development.

## Materials and methods

### Plant materials and growth conditions

*Arabidopsis thaliana* plants, ecotype Columbia, were used as wild-type controls and for *in situ* hybridization. Plant were germinated on MS media (pH = 5.7) containing 1% sucrose and grown under long-day light at 22 ± 2°C. For continuous dexamethasone (DEX) treatment, the plant flower bud was watered with MS containing 1% sucrose and 10 $\mu$M DEX daily for 10 days (Shin et al. 2002).

### Plasmid construction and transgenic plants

For AtMYB24-GFP fusion construct, *AtMYB24* was amplified using a primerF: (5′-CAC CAC ACT AGT GAG AAA AGA GAA-3′) and a primerR: (5′-CCC ACT AGT ATT ACC ATT ATA TAT-3′) with *Pfu* DNA polymerase, and the amplified fragments were cloned into *Spe*I site of pCAMBIA1302 vector (CAMBIA). For AtMYB24-GR fusion construct, *AtMYB24* was amplified using a primerF: (5′-CAG GAT CCA ATG GAG AAA AGA GAA-3′) and a primerR: (5′-GGA TCC GCA TTA CCA TTA TAT ATA TTC ATG G-3′) with *Pfu* DNA polymerase, and the amplified fragments were cloned into *Bam*H I site of pBIΔGR vector. All plasmids were confirmed by sequencing. All constructs mentioned earlier were transformed into the *Agrobacterium tumefaciens* GV3101 and subsequently into *Arabidopsis*. More than 45 transformed lines carrying each constructs were confirmed by PCR analysis.

### GFP fusion protein analysis

Particle bombardment was conducted as described (Li et al. 2006b).

### Trans-activation activity assays

Trans-activation activity assay was conducted as described (Li et al. 2006a). Full-length and various deletions of the *AtMYB24* coding region were amplified by PCR using *Pfu* DNA polymerase (Takara, Japan) and the following appropriate primers:

AtMYB24 (1)-F: 5′-CGT CGA CAA ATG GAG AAA AGA GAA-3′
AtMYB24 (104)-F: 5′-TGT CGA CGA AGA ACC GAC AAT GAG-3′
AtMYB24 (191)-F: 5′-AGT CGA CTA TCT GTT GAT CAA TC -3′
AtMYB24 (190)-R: 5′-AGT CGA CCA TTA TCT GTT GAT CAA-3′
AtMYB24 (214)-R: 5′-GGC GCG TCG ACC TTA ATT ACC ATT-3′

### RNA isolation, Northern analysis, and real-time PCR

Total RNA was isolated from 35-day-old *Arabidopsis* plants using Trizol solution (Tiangen, China). The Northern blots were conducted following the methods as described (Qin et al. 2005). A fragment in the *AtMYB24* coding region were amplified and labeled by PCR using primers (5′-TCA TCA AGA GCG GAG AAA CGA C-3′ and 5′-TCA TGA TCG AAC CGG ATT CAG G-3′).

For reverse transcription, 5 $\mu$g of total RNA for each samples were digested with RNase-free DNase I (Roche, Switzerland) before they were used for reverse transcription according to the manufacturer's instructions (Invitrogen GmbH, Germany). Quantitative real-time PCR was conducted as described previously (Guo et al. 2006). Primers for the quantitative real-time PCR are as follows:

PAL1-F: 5′-CCG GTG TGA ATG CTA GTA GTG A-3′
PAL1-R: 5′-CCT TGG AGG AGA GTG TTG ATT C-3′
PAL2-F: 5′-GGA GAC TTC AAG AGC CGG TGT G-3′
PAL2-R: 5′-CCG GAG TAT CCT TGG AGA AGA G-3′
CHS-F: 5′-GTG AAC ACA TGA CCG ACC TCA A-3

CHS-R: 5′-GTA GTG CAG AAG ACG ACA TGA G-3′
DFR-F: 5′-CTA GCC TTA TCA CCG CGC TCT C-3′
DFR-R: 5′-TGT CCG TCA GCT TCT TGG AAC T-3′
F3H-F: 5′-CCT CGG ACT CAA GCG TCA CAC T-3′
F3H-R: 5′-TTA GAG TTC ACC ACG GCC TGA T-3′
4CL3-F: 5′-GAA CGA TCC AGA AGC CAC TTC A-3′
4CL3- R: 5′-CAA CAG CTG CAT CGG CAA TTG A-3′
ANS1-F: 5′-GCG TGG AAC ATC AAG TGA TCG T-3′
ANS1-R: 5′-GAG AAC GGT ACT CGT CGA ACC T-3′
ANS2-F: 5′-TTC TAC GAG GGC AAA TGG GTC A-3′
ANS2-R: 5′-AAC TTA GCC GGA GAC TCA ACA C-3′
C4H-F: 5′-CAA CAG CTG GAA GAA GCC TGAA-3′
C4H-R: 5′-AGA CTG TCC TGG AGG AGG AAG A-3′
CcoAOMT-F: 5′-GCT ACG TCA ACT TCC ATG AGA G-3′
CcoAOMT-R: 5′-GAC GTC TAC AGA GCG TGA TAC C-3′
COMT-F: 5′-GCT CCT TCT CAT CCT GGT ATT G-3′
COMT-R: 5′-TTG GTT GAG AGG CTT GAG TCT G-3′
AtGPT2-F: 5′-AGA CCA GAT TTC GCC GTT AAC T-3′
AtGPT2-R: 5′-ACT GCT TCG CCT GTG AGT AGA G-3′
UBQ10-F: 5′-TCC GGA TCA GCA GAG GCT TA-3′
UBQ10-R: 5′-TCA GAA CTC TCC ACC TCA AG-3

*In situ* hybridization

Inflorescences were fixed with formaldehyde and embedded in paraplast. The tissues were sliced into 8-$\mu$m-thick slices and mounted on poly-Lys-coated glass slides. The *in situ* hybridization was conducted following the methods described previously (Qu et al. 2003). The probe used for *in situ* hybridizations was the 300-bp C-terminal end of *AtMYB24* coding region.

Light microscopy

For pollen analysis, pollen grains were mounted by Alexander's stain (Alexander 1969). For toluidine blue stain, Arabidopsis inflorescences were fixed in 4% glutaraldehyde in 12.5 mM of phosphate buffer (pH6.8) for 2 days. The inflorescences were embedded in Spurr resin (SPI-Chem™) according to the manufacturer's instructions and sliced into 3 $\mu$m transverse sections. All slides were observed under bright field microscopy (OLYMPUS BX51, Japan) equipped with a CCD camera (Eastman Kodak, Rochester, NY).

## Results

Characterization of *AtMYB24* as a transcription factor

*AtMYB24*, which displays 68.5% sequence identity to that of *AtMYB21*, encodes an R2R3-MYB protein comprising 214 amino acids (Fig. 1A). Based on the conserved motifs outside the MYB domains, *AtMYB24* was found sharing a W-MDDIW motif at their C-terminal regions with four other *Arabidopsis* members (AtMYB21, AtMYB57, AtMYB62, AtMYB116), two snapdragon proteins (AmMYB305 and AmMYB340), one protein from pea (PsMYB26) and three rice MYB proteins (9633.m04622, 9633.m00347, 9640.m03662) (Kranz et al. 1998; Li et al. 2006a).

To test whether AtMYB24 is localized to nuclei, we generated an AtMYB24-GFP fusion protein construct in

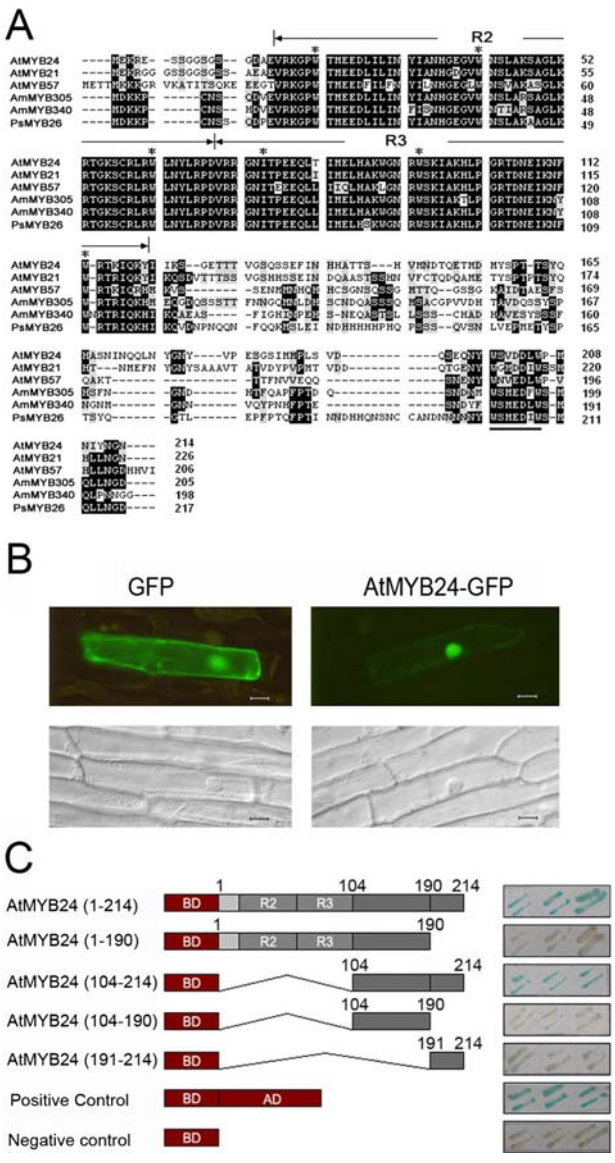


**Fig. 1** **A** Amino acid alignment of AtMYB24 and other MYB proteins. **B** Subcellular localization of AtMYB24-GFP. Each image was inspected by both a fluorescence microscope (*upper panels*) and a differential–interference–contrast microscope (*bottom panels*). Bar = 50 $\mu$m. **C** $\beta$-Galactosidase activity assay. Three independent transformants were shown. Empty pYF503 vector was used as the negative control, and pYF504, which harbors the full-length *GAL4* gene, was used as the positive control (Ye et al. 2004)

which transient gene expression was driven by the strong CaMV35S promoter, and bombarded into onion epidermal cells. AtMYB24-GFP fusion protein was found exclusively localized in nuclear, as visualized by fluorescence microscopic and differential interference contrast (DIC) images (Fig. 1B). This result suggests that the AtMYB24 protein is targeted to the nuclei, consistent with the proposed role of AtMYB24 as a transcription factor.

To determine whether AtMYB24 acts as a transcriptional activator, we fused a series of deletion of AtMYB24 with the GAL4 DNA-binding domain and co-transformed them with the reporter vector (Ye et al. 2004). As shown in Fig. 1C, the entire AtMYB24 protein significantly activated the *LacZ* reporter gene expression in yeast cells, suggesting that AtMYB24 is a transcriptional activator. Furthermore, deletion of a 24-bp region including the W-MDDIW motif from the C-terminus completely abolished the trans-activation activity, indicating that this conserved motif is essentially important for the activation activity (Fig. 1C). This is consistent with the results of AtMYB21 (Li et al. 2006a). However, although the 110 amino acid residues at the C-terminus is with transcriptional activity and the region 104–190 amino acid has no activity, this 24-bp region alone is of no activation activity at all (Fig. 1C), suggesting that the region 104–190 amino acid is also required for the transactivation activity.

*AtMYB24* is a flower-specific gene

To understand the expression characteristics of *AtMYB24*, we examined the expression of this gene in different tissues by Northern blots. The result showed that the transcripts of *AtMYB24* were only detected in flowers, suggesting that *AtMYB24* is a flower-specific gene (Fig. 2A).

To further determine the expression pattern of *AtMYB24* during the flower development, *in situ* hybridization was adopted with a 300-bp coding region of *AtMYB24* as the probe which shows low sequence homology to *AtMYB21*. The results showed that, in early flower development stages before floral stage 6 (Smyth et al. 1990), *AtMYB24* is expressed in the primordia of the flower organs (Fig. 2B). From floral stages 7 and 8 to floral stages 9 and 10 (Smyth et al. 1990), high level expression of *AtMYB24* was detected in male and female gametophytes, especially in microspores (Fig. 2C and 2D). At floral stages 11 and 12 (corresponding to anther stages 10 and 11), the expression of *AtMYB24* was greatly reduced in the anthers (Fig. 2E). At about floral stages 13 and 14, transcripts of *AtMYB24* were only detected in ovules but not in carpels (Fig. 2F). These results suggest that the expression of *AtMYB24*, although constitutively detected in the ovules, is tightly regulated with the development of anthers.

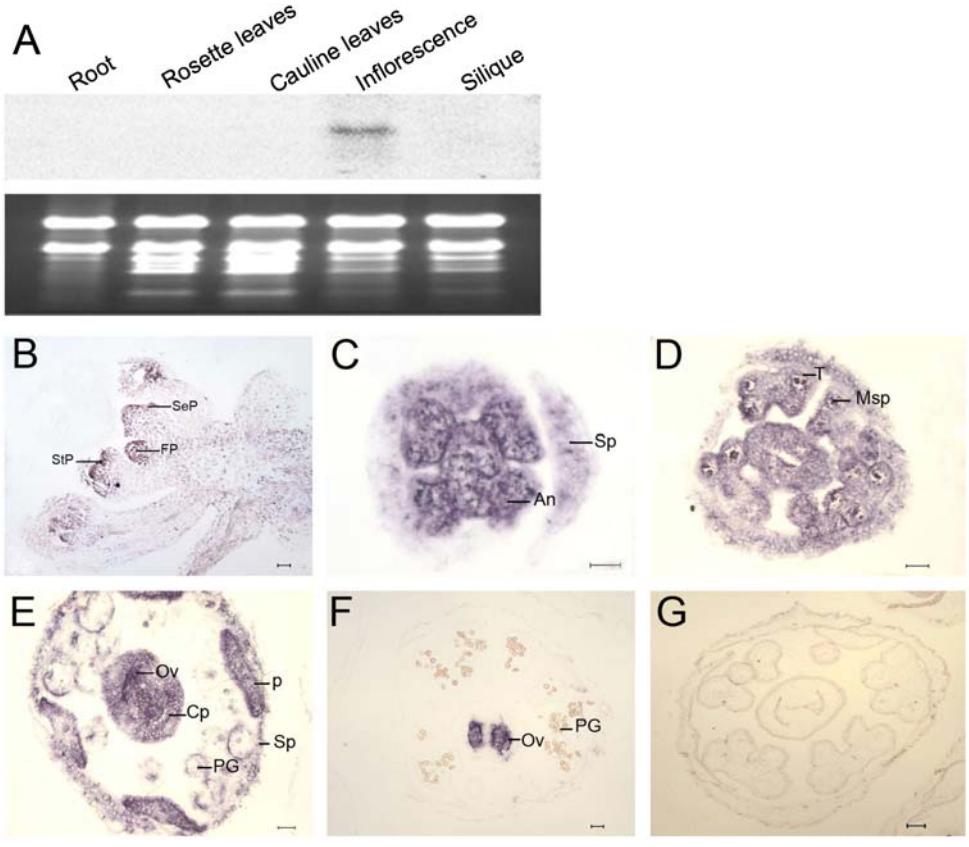


**Fig. 2** Expression pattern of *AtMYB24* in flowers. **A** RNA gel blot analysis of *AtMYB24* in different organs. Fifteen micrograms of RNA samples were loaded per lane. **B–G** In situ hybridization of *AtMYB24*, using DIG-labeled anti-sense probe (**B–F**) and sense probe (**G**). **B** Inflorescences before floral stage 6. **C** Floral stages 7–8. **D** Floral stage 9. **E** Floral stages 11–12. **F** Floral stages 13–14. **G** Sense probe control. An, anther; Sp, sepal; T, tapetum; Msp, microspore; Ov, ovule; Cp, carpel; PG, pollen grain; P, petal. Bar = 20 $\mu$m

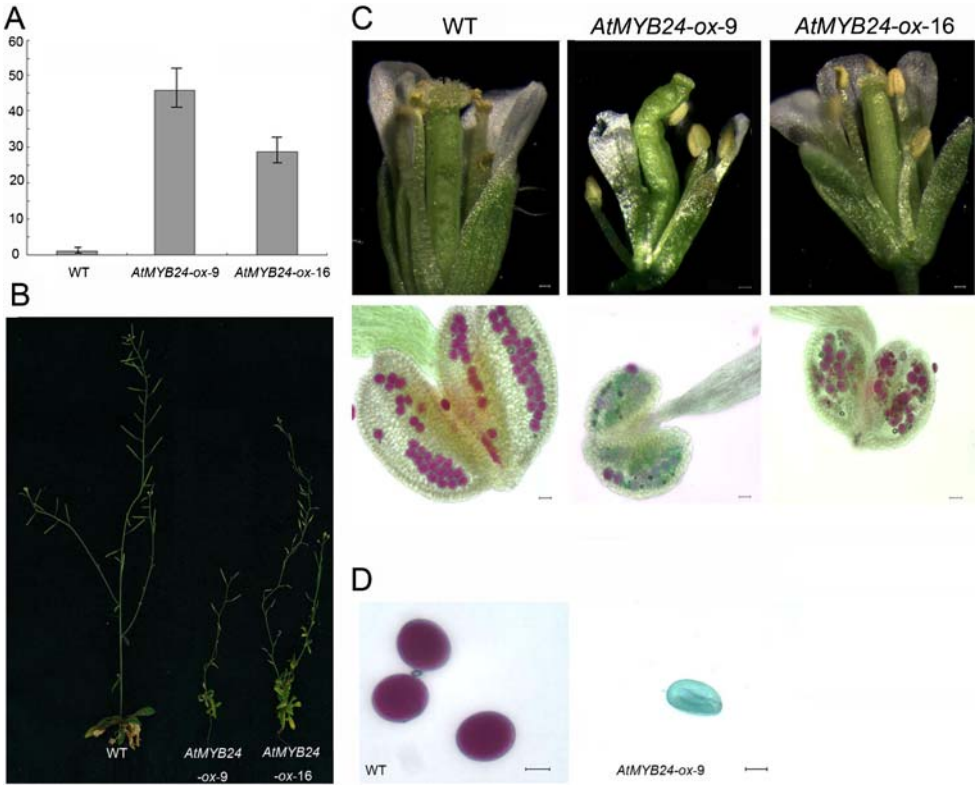


**Fig. 3** Phenotypes of *AtMYB24*-ox plants. **A** Relative expression level of *AtMYB24* in *AtMYB24*-ox lines revealed by quantitative real-rime PCR. The number of *y*-axis indicates the relative expression level of *AtMYB24* to that of wild-type. **B** A 7-week-old wild-type plant and two independent *AtMYB24-ox* plants. **C** Comparison of flowers and pollen grains of wild-type and the two *AtMYB24-ox* lines. Bar = 0.5 mm. **D** Alexander's staining of pollens from wild-type and *AtMYB24-ox* lines. Pollen grains of wild-type are stained red inside and the outer wall is stained green (Alexander 1969). The abnormal pollen grains of *AtMYB24-ox* lines were stained green. Bar = 20 $\mu$m

## Over-expression of *AtMYB24* causes dwarfism, pollen defects, and male sterility

To investigate the function of *AtMYB24* in anther development, we generated transgenic plants over-expressing *AtMYB24* under the control of a CaMV 35S promoter. Two independent homozygous lines, *AtMYB24-ox*-9 and *AtMYB24-ox*-16, were identified, in which the transcript level of *AtMYB24* was elevated by 45- and 28-fold, respectively (Fig. 3A). These two lines were subjected to subsequent analysis.

As shown in Fig. 3B, the *AtMYB24-ox* lines exhibited dwarf phenotype, with severely decreased fertility, and the dwarf phenotype correlates with the expression levels of *AtMYB24*. The *AtMYB24-ox* plants had smaller flowers, with narrow petals and malformed carpels (Fig. 3C, top panel). Alexander staining of the anthers showed that large proportions of pollens (almost 100% for *AtMYB24-ox*-9 and 50% for *AtMYB24-ox*-16) were found aberrant in the *AtMYB24-ox* plants (Fig. 3C, bottom panel). Further analysis revealed that the aberrant pollens from both the over-expression lines were collapsed and devoid of cytoplasmic contents (Fig. 3D).

To further investigate the mechanism of sterility, we pollinated the stigmas of *AtMYB24-ox* plants with wild-type pollens. The result showed that the sterility phenotype of *AtMYB24-ox*-16 and *AtMYB24-ox*-9 plants were rescued by the wild-type pollens (Table 1), suggesting that the sterility phenotype was due to pollen defects of *AtMYB24-ox* lines.

**Table 1** The sterility phenotype is rescued by pollination with wild-type pollens

| Transgenic lines | Percentage of filled siliques[a] | |
|---|---|---|
| | Self-crossed (%) | Pollinated by WT pollens (%) |
| *AtMYB24*-ox-16 | 15.4 | 97.8 |
| *AtMYB24*-ox-9 | 5.2 | 95.3 |

[a] 50 siliques for each line were examined.

This is different from *AtMYB21-ox* lines, in which the sterile phenotype was not rescued either by pollinating the transgenic stigma with wild-type pollens, or by pollinating the wild-type stigma with transgenic pollens (Shin et al. 2002).

To examine at which developmental stage the pollens of the *AtMYB24-ox* plants become abnormal, we compared the anther development of the *AtMYB24-ox* lines with that of the wild-type plants. The result showed that no obvious difference was found until anther stage 9, when an exine wall was generated and microspores become vacuolated (Sander et al. 1999). At anther stages 10 and 11, mitotic divisions occur in pollens and tapetum degenerate in the wild-type plants (Sander et al. 1999). However, in the *AtMYB24-ox* plants, the pollen grains generated from microspore were devoid of cytoplasm and could not be stained by toluidine blue (Fig. 4). At anther stages 12 and 13 in wild-type plants (Sander et al. 1999), the septum was degenerated so that the anthers become bilocular, and the stomium of the anthers was broken so that pollens were released from the locules.

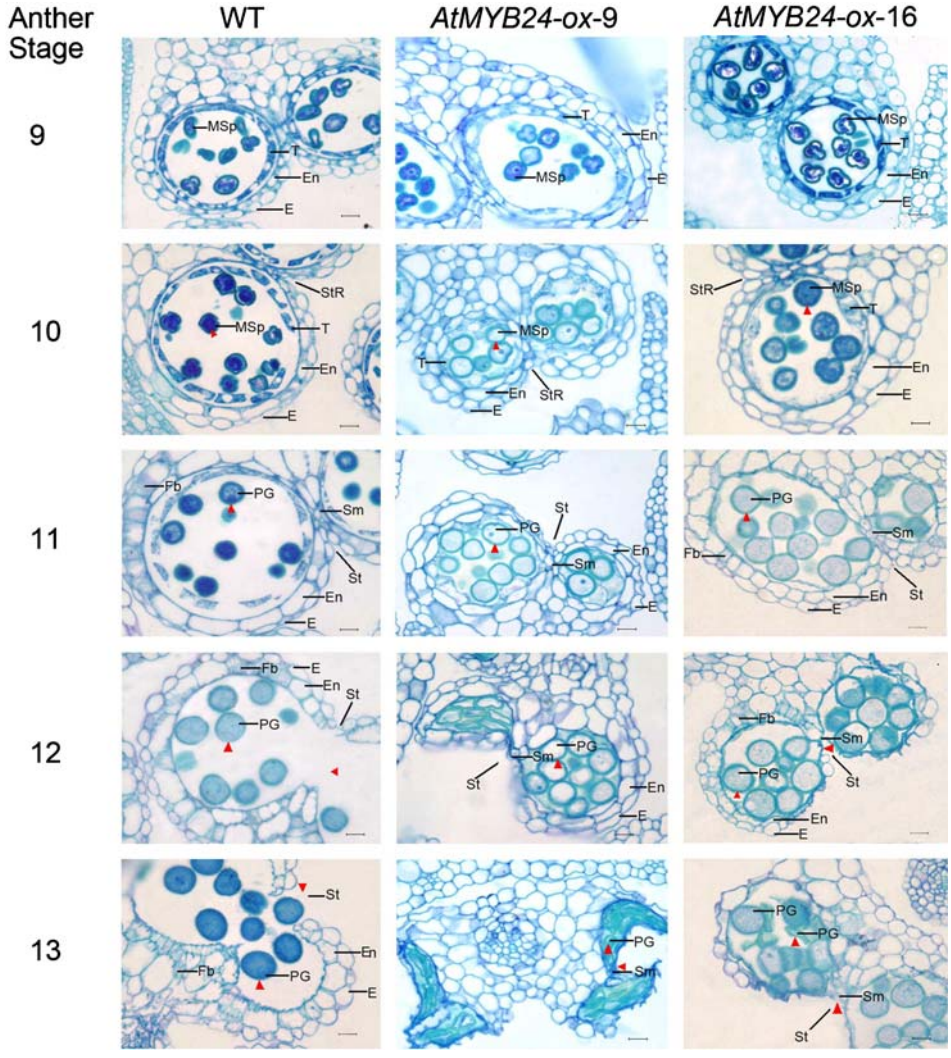


**Fig. 4** Comparison of anther development between *AtMYB24-ox* lines and wild-type. T, tapetum; MSp, microspore; Sm, septum; StR, stomium region; St, stomium; E, epidermis; En, endothecium; Fb, fibrous bands; PG, pollen grain Bar = 20 $\mu$m

At the same time, fibrous bands, which may help the anthers "flip open" at dehiscence (Sander et al. 1999), appear in the endothecium and connective cells of the anthers. However, in *AtMYB24-ox*, the septum and the stomium were not broken, and very few and even no fibrous bands were found in the anthers of *AtMYB24-ox*-16 and *AtMYB24-ox*-9 plants (Fig. 4). Interestingly, the septum, the stomium, and the endothecial cells underwent a cell-death program that is different from that of the wild-type plants. These aberrant pollens and anther abnormities ultimately resulted in anthers with non-dehiscent phenotype, leading to sterility of the *AtMYB24-ox* plants. Since, in wild-type plants, the expression of *AtMYB24* was greatly reduced in the male gametophytes during anther stages 10 and 11 (Fig. 2E), the abnormal anther development of the *AtMYB24-ox* plants should be due to the over-expression of *AtMYB24* at these stages.

To exclude the possibility that the phenotypes were the result of constitutive expression of *AtMYB24*, we generated transgenic plants over-expressing AtMYB24-GR fusion protein, in which, without DEX, the GR-fusion proteins would localize in the cytoplasm, whereas, when treated with DEX, the fusion protein would translocate into nucleus (Lloyd et al. 1994). Two independent homozygous lines, *AtMYB24-GR-ox*-1 and *AtMYB24-GR-ox*-9, were obtained, in which expression of *AtMYB24* were elevated by 403- and 115-fold, respectively (Fig. 5A). In the absence of DEX, *AtMYB24-GR-ox* lines were indistinguishable from wild-type plants, suggesting that AtMYB24-GR was not functional (Figs. 5B and C). When the inflorescences of the 5-week-old plants were treated with DEX solution daily continuously for 10 days, the *AtMYB24-GR-ox* lines displayed smaller flower phenotype with narrower petals, aberrant anther development, and severe sterility, whereas wild-type plants grew normal. When treated with DEX, the phenotype severity of the *AtMYB24-GR-ox* lines was correlated with the expression level of the *AtMYB24* (Figs. 5B and C). These data confirmed that *AtMYB24* plays important roles in flower development, and that over-expression of *AtMYB24* even

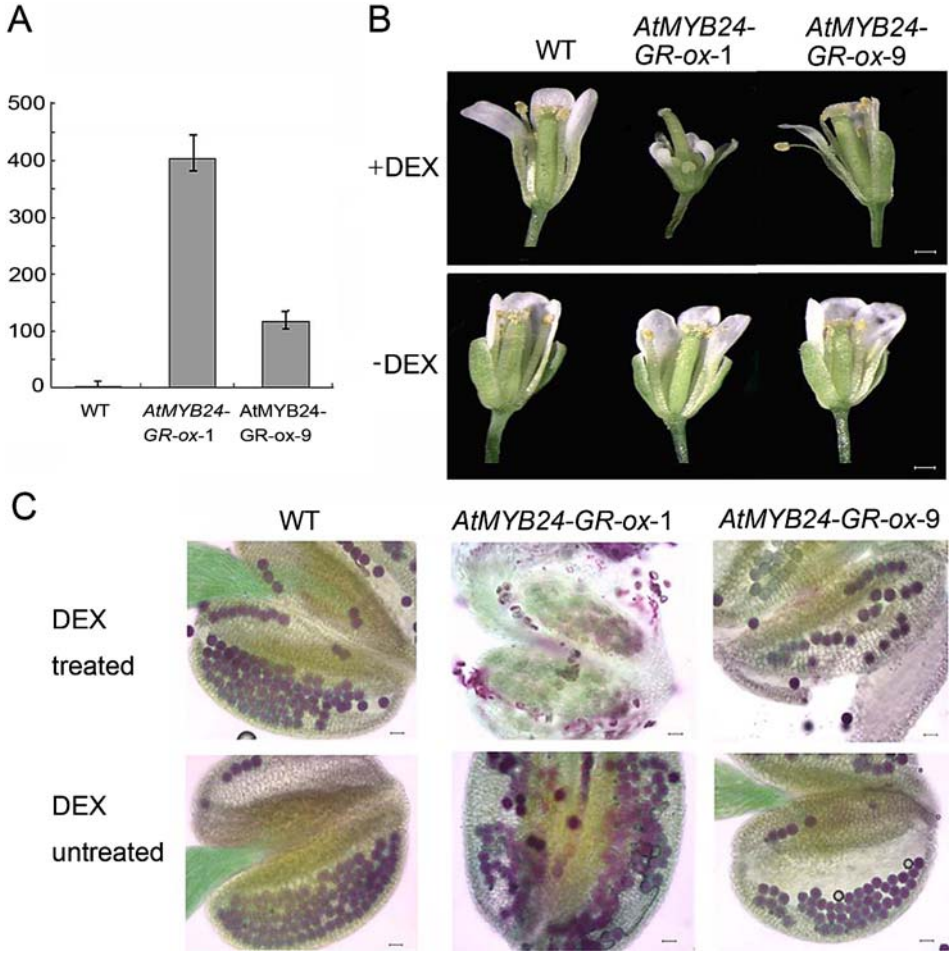


**Fig. 5** Comparison of flowers from wild-type and *AtMYB24-GR*-ox plants. **A** Relative expression level of *AtMYB24* in *AtMYB24-GR-ox* lines revealed by quantitative real-rime PCR. The number of *y*-axis indicates the relative expression level of *AtMYB24* to that of wild-type. **B** Flowers of wild-type and *AtMYB24-GR-ox* lines treated with DEX. Bar = 0.2 mm **C** Pollen grains of wild-type and *AtMYB24-GR-ox* lines stained by Alexander's dye when treated with DEX. Bar = 20 $\mu$m

for a short period could significantly affect normal flower development.

Expression of genes involved in phenylpropanoid biosynthetic pathway and *AtGTP2* were altered in *AtMYB24-ox* lines

It was reported that AmMYB305, AmMYB340, PsMYB26, and AtMYB21 could bind to the promoters and activate the expressions of several genes involved in phenylpropanoid biosynthetic pathway (Shin et al. 2002; Moyano et al. 1996; Uimari and Strommer 1997). To test whether AtMYB24 regulates genes in this pathway as well, we adopted quantitative real-time PCR to assess transcript levels of these genes both in *AtMYB24*-ox and wild-type flowers. As shown in Fig. 6, transcript levels of *CHS* (encoding chalcone synthase) and *DFR* (encoding dihydroflavonol 4-reductase) were substantially decreased, whereas those of *PAL2*, *ANS1*, and *C4H* were increased by 50% in *AtMYB24-ox* plants compared to the wild-type plants, suggesting that the phenylpropanoid biosynthetic pathway is affected. In the meantime, the expression of *AtGPT2*, coding for a glucose-6-phosphate/phosphate-translocator, was also significantly down-regulated by ninefold in *AtMYB24-ox* plants. Interestingly, the closely related homologue of this gene, *AtGPT1*, had been reported to be involved in the pollen maturation and embryo sac development in *Arabidopsis* (Niewiadomski et al. 2005). It would be interesting to investigate the mechanism that affects the normal development of anthers by regulating the phenylpropanoid biosynthetic pathway and other metabolic pathways by *AtMYB24* in the future.

## Discussion

*AtMYB24* encodes a group I MYB protein. The genes in this group are all flower-specific, and the encoded proteins share a conversed W-MDDIW motif in *Arabidopsis* and *Antirrhinum* (Li et al. 2006a). For instance, *AmMYB305* and *AmMYB340* are found highly expressed in nectary, developing ovules and placental tissue (Moyano et al. 1996). The *in situ* hybridization result in this study presents a more detailed expression pattern of *AtMYB24* in flowers. *AtMYB24* is expressed in gynoecium from young flower

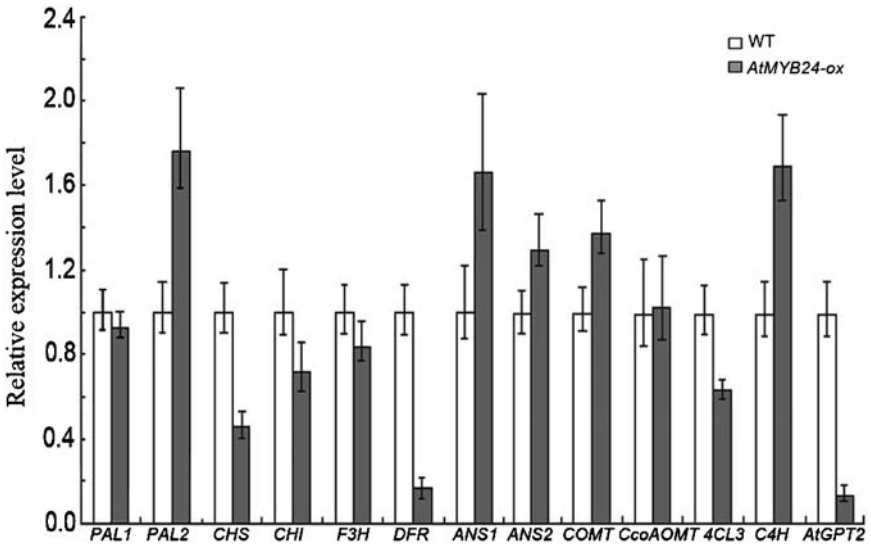


**Fig. 6** Expression analysis of the genes involved in anthocyanin biosynthetic pathway in *AtMYB24-ox* lines by quantitative real-time PCR

buds to mature flowers, similar to those of *AmMYB305* and *AmMYB340* (Jackson et al. 1991) and expressed in anthers until anther stage 10, especially in microspores. In addition, the expression of *AtMYB24* depends on anther development.

Anther dehiscence in *Arabidopsis* includes the endothecial cell expansion, degeneration of septum, stomium breaking, and releasing of pollen grains (Sander et al. 1999). Several *Arabidopsis* mutants that are reported have defects in anther dehiscence process. Some of these mutated genes, i.e., *DELAYED DEHISCENCE1*, *DAD1*, and *AOS*, are involved in jasmonic acid biosynthesis pathway, and mutants of these genes result in dehiscence-delayed anthers (Sanders et al. 2000; Ishiguro et al. 2001; von Malek et al. 2002; Park et al. 2002). Other mutants, e.g., *atmyb26* mutant and *nst1 nst2* double mutant, exhibit non-dehiscent anther phenotype (Steiner-Lange et al. 2003; Mitsuda et al. 2005). Nevertheless, all these mutants would have their septum degenerated and stomium broken and produced bilocular anthers from stage 12 onward. In *AtMYB24-ox* lines, breaking of septum and stomium did not occur, producing anthers with four locules through the development. This suggests that overexpression of *AtMYB24* has greater affects on the anther dehiscence process at the earlier stages than do those mutants that are described above. It was reported that ectopic expression of *PCS1* caused septum and stomium cells to survive but to die in wild-type anthers (Ge et al. 2005). It will be interesting to study whether AtMYB24 and PCS1 are involved in the same regulation route and, if so, whether they are interacted.

The expression level of *CHS* and *DFR* involved in phenylpropanoid metabolism pathway were down-regulated in flowers of *AtMYB24-ox* lines. Although CHS was found to be involved in pollen germination (Napoli et al. 1999), it was reported that *atmyb32* mutants produced abnormal pollens devoid of cytoplasm and *DFR* was also down-regulated in the mutant (Preston et al. 2003), similar to the pollen phenotypes in *AtMYB24-ox* lines. Many products of phenylpropanoid metabolism pathway and its branch pathways are important components of pollen coat and precursors for sporopollenin, which are required for normal pollen development (Piffanelli et al. 1998), for example, knock-down the expression of *PAL* genes in tapetum resulted in pollen defects in tobacco (Matsuda et al. 1996). Changes in the expression of genes involved in these metabolism pathways due to the over-expression of *AtMYB24* may account for the abnormal pollens and anther defects in those transgenic *AtMYB24-ox* lines. The evidences presented in this study suggest an important role of AtMYB24 in the development of anther in *Arabidopsis*.

Two *atmyb24* mutants were obtained from ABRC (SALK_030452 and SALK_017221). Unfortunately, the phenotypes of these two mutants are indistinguishable with the wild-type plants (data not shown). It is probably due to the gene redundancy of *AtMYB21* and/or other related MYB transcription factor genes, since *AtMYB21*-ox plants also exhibited malformed flower development and sterility (Shin et al. 2002). This is further supported by a recent report that *atmyb21 atmyb24* double mutant displayed a more serious male sterile phenotype than *atmyb21* did (Mandaokar et al. 2006). Although *atmyb21* and *atmyb24* double mutant had non-dehiscent anthers and closed petals, the anthers seemed to be morphologically normal up to stage 13 (Mandaokar et al. 2006). Considering the fact that both elevation of expression of either these two genes and simultaneous knock-out of these two genes would result in defects in anther development, we suggests that *AtMYB24* and its homolog *AtMYB21*, partially redundant in their functions, play important roles in anther development, especially in anther dehiscence. Future work would be focused on dissecting the

distinct function of *AtMYB24* with *AtMYB21* during anther development.

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