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Research Paper

The *Arabidopsis thaliana* Transcription Factor AtMYB102 Functions in Defense Against The Insect Herbivore *Pieris rapae*

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KEY WORDS

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ABSTRACT

In *Arabidopsis thaliana* the R2R3-MYB transcription factor family consists of over 100 members and is implicated in many biological processes, such as plant development, metabolism, senescence, and defense. The R2R3-MYB transcription factor gene *AtMYB102* has been shown to respond to salt stress, ABA, JA, and wounding, suggesting that AtMYB102 plays a role in the response of plants to dehydration after wounding. Here, we studied the role of AtMYB102 in the response of *A. thaliana* to feeding by larvae of the white cabbage butterfly *Pieris rapae*. *A. thaliana* reporter lines expressing *GUS* under control of the *AtMYB102* promoter revealed that *AtMYB102* is expressed locally at the feeding sites of herbivore-damaged leaves, but not systemically in uninfested plant parts. Knockout *AtMYB102* transposon-insertion mutant plants (*myb102*) allowed a faster development of *P. rapae* caterpillars than wild-type Col-0 plants. Moreover, the number of caterpillars that had developed into pupae within 14 days was significantly higher on *myb102*, indicating that in wild-type plants *AtMYB102* contributes to basal resistance against *P. rapae* feeding. Microarray analysis of wild-type Col-0 and *AtMYB102* overexpressing 35S::*MYB102* plants revealed a large number of differentially expressed genes. Besides several defense-related genes, a relatively large number of genes is associated with cell wall modifications.

INTRODUCTION

Plants possess a broad range of defense mechanisms to effectively combat invasion by microbial pathogens or attack by herbivorous insects. These mechanisms include pre-existing physical and chemical barriers, as well as inducible defense responses that become activated upon pathogen infection or insect herbivory.[1,2] A concerted action of these defensive activities helps the plant to minimize damage caused by the attacker. Many studies have indicated that jasmonic acid (JA) and its derivatives are the most important regulators of induced resistance against herbivore attack.[3,4] A classic example is the observation that following attack by insect herbivores, tomato leaves accumulate JA resulting in the activation of genes encoding proteinase inhibitors that inhibit digestive serine proteinases of herbivorous insects and reduce further insect feeding.[5,6] Genetic evidence demonstrates that JAs also play an important role in induced defense against different types of herbivores in *Arabidopsis thaliana*.[7-12] Analysis of the transcriptome of *A. thaliana* upon infestation by larvae of the cabbage white butterfly, *Pieris rapae,* revealed that the majority of the induced changes in gene expression is regulated by JA.[9,13,14] Among the JA-responsive genes that are activated, several encode transcription factors, including members of the MYB-transcription factor family. However, their role in induced resistance against insects is to a large extent unknown.

MYB genes encode transcription factor proteins that share the conserved MYB DNA-binding domain,[15] and were first identified as oncogenes derived from retroviruses in animal cells.[16] MYB proteins are categorized into subfamilies depending on the number of conserved MYB domain repeats. MYB proteins from animals generally contain three MYB repeats, which are referred to as R1, R2 and R3. Most of the *MYB*-like genes in plants have only the R2 and R3 repeats. An inventory of the *A. thaliana* genome revealed that this plant species contains approximately 125 *R2R3-MYB* genes.[17,18] R2R3-MYB proteins in plants have been implicated in a range of activities, such as plant secondary metabolism, regulation of cell death, stress tolerance (reviewed in ref. 17), and pathogen resistance, but the functions of most of them have not been determined. The family of R2R3-MYB-like transcription factors has repeatedly been implicated in JA-dependent defense responses. For instance, the *OsLTR1* gene from rice regulates JA-dependent defense

responses, whereas AtMYB15, AtMYB34, AtMYB51 and AtMYB75 are associated with the wound response or resistance against insect herbivores.[19-21] Furthermore, Mengiste et al[22] demonstrated a role for the R2R3-MYB transcription factor protein BOS1 (AtMYB108) in resistance against the necrotrophic pathogens *Botrytis cinerea* and *Alternaria brassicicola*, both of which are sensitive to JA-dependent defense responses.[23,24] Pathogen-induced expression of *AtMYB108* was impaired in the JA-response mutant *coi1*, indicating that *AtMYB108* is regulated by JA. Interestingly, *AtMYB108* knockout mutants were not only impaired in resistance against necrotrophic pathogens, but also displayed impaired tolerance against water deficit and salt stress.[22] These observations suggest that AtMYB108 is a central player in multiple stress responses in *A. thaliana*.

Another R2R3-MYB transcription factor family member, AtMYB102, was identified from an *A. thaliana* transcription factor collection.[25] The gene is upregulated in *A. thaliana* upon treatment with ABA, JA, or a combined treatment of osmotic stress and wounding.[26] Plant responses that are triggered by feeding insects partly overlap with those activated upon dehydration stress and wounding.[14] This prompted us to study the role of *AtMYB102* in the response of *A. thaliana* to feeding larvae of the specialist herbivore *P. rapae*. Here, we provide evidence that AtMYB102 plays a role in resistance against these tissue-chewing caterpillars.

MATERIALS AND METHODS

Cultivation of plants. Seeds of *Arabidopsis thaliana* accession Col-0, reporter line *MYB102*::*GUS,*[14] knockout mutant *myb102* (transposon insertion line Sm_3_41654 obtained from the EXOTIC collection of the Nottingham Arabidopsis Stock Centre; ref. 27) and *AtMYB102* overexpressing 35S::*MYB102* plants (line 2.3; ref. 28) were sown in quartz sand. All genotypes were in the Col-0 background. Two-week-old seedlings were transferred to 60-mL pots containing a sand/potting soil mixture that was autoclaved twice for 20 min. Plants were cultivated in a growth chamber with an 8-hr day (200 $\mu E.m^{-2}.sec^{-1}$ at 24°C) and 16-hr night (20°C) cycle at 70% relative humidity for another 3 weeks. Plants were watered every other day and received half-strength Hoagland nutrient solution[29] containing 10 μM Sequestreen (CIBA-Geigy, Basel, Switzerland), once a week. For the microarray analysis, Col-0 and 35S::*MYB102* plants were grown until they had reached the same developmental stage (fully mature rosettes that had not started bolting; five weeks for Col-0 and eight weeks for 35S::*MYB102*) in potting soil in a growth chamber with an 8-hr day (24°C) and a 16-hr night (20°C) cycle at 70% relative humidity. Instead of line 2.3 (which was used for the insect bioassays), 35S::*MYB102* line 8.4 was used for the microarray analysis. Both *AtMYB102* overexpressing lines showed similar levels of *AtMYB102* mRNA.[28]

Insect bioassay. Tissue-chewing larvae of the small cabbage white butterfly *Pieris rapae* were reared on Brussels sprout plants *(Brassica oleracea gemmifera* cv. *Cyrus*) in a growth chamber with a 16-hr day and 8-hr night cycle (21°C; 50–70% relative humidity) as described previously.[30] Infestation of *A. thaliana* plants was carried out by transferring first-instar larvae to 5-week-old plants using a fine paintbrush. To study *P. rapae* performance, a single first-instar larva was transferred to each of 20 Col-0, *myb102*, or 35S::*MYB102* plants. At seven and ten days, the fresh weight of each larva was determined, as described.[31] After ten days, the first larvae started to pupate. Therefore, fresh weight was determined only up to ten days of feeding. To examine effects on caterpillar development, the percentage of caterpillars that had pupated within 14 days after hatching was determined.

Verification of transposon insertion site in *myb102*. Verification of the transposon insertion in transposon insertion line Sm_3_41654 (*myb102*) was performed according to the guidelines from the Genome Laboratory of the John Innes Centre from which Sm_3_41654 was obtained. In brief, seedlings from Col-0 and Sm_3_41654 were grown for ten days on Murashige and Skoog (MS) medium and subsequently harvested for isolation of genomic DNA. Line Sm_3_41654 contains a transposon insertion at the end of the first exon of the *AtMYB102* gene (At4g21440), 124 bp downstream of the translation start site. To verify disruption of the *AtMYB102* gene, *AtMYB102*-specific primers were designed ~500 bp up and downstream of the predicted transposon insertion site (At4g21440; *AtMYB102*-FW (in 5' UTR) 5'- TCA ATC CCC ATT CTA AGT AGC TTC TTT C-'3; *AtMYB102*-RV (in 3rd exon) 5'-CGT ATA GCT GCC ACA AAC GTA AAA ATA A-'3). In addition a transposon-specific primer was used (Spm32; 5'-TAC GAA TAA GAG CGT CCA TTT TAG AGT GA-'3). Gene-specific primers for *AtTUB8* (At5g23860; *AtTUB8*-FW; 5'-TCT CTA TGA CAT TTG CTT CAG AA-'3; *AtTUB8*-RV: 5'-ACG TTG TTT GGG ATC CAT TCC AC-'3) were used as an internal control. The following PCR program was used for all PCR reactions: 94°C for 3 min; 30 cycles of 94°C for 30 sec, 58°C for 45 sec, and 72°C for 1 min. A PCR with the two *AtMYB102*-specific primers and the transposon-specific primer will discriminate between wild-type Col-0, and heterozygous and homozygous transposon-inserted plants. Wild-type plants will yield a single band of ~850 bp (amplified from primers *AtMYB102*-FW and *AtMYB102*-RV), plants homozygous for the transposon insertion will yield a single band of ~550 bp (amplified from Spm32 and either *AtMYB102*-FW or *AtMYB102*-RV). When the *AtMYB102* gene is disrupted by the transposon insertion, the distance between the *AtMYB102*-FW and *AtMYB102*-RV primers will be too large to amplify a PCR product under the PCR conditions used. Heterozygous plants will yield both the ~550- and ~850-bp PCR products.

Quantitative Real-Time PCR. Q-RT-PCR analysis was performed basically as described previously.[32] Two micrograms of RNA was digested with Turbo DNA-free™ (Ambion, Huntingdon, UK) according to the manufacturer's instructions. To check for genomic DNA contamination, a PCR with primers designed on intron sequences of *AtACT7* (At5g09810; *ACT7*-FOR: 5'-GAC ATG GAA AAG ATA TGG CAT CAC AC-3'; *ACT7*-REV: 5'-AGA TCC TTC CTG ATA TCG ACA TCA C-3') was carried out. Subsequently, DNA-free total RNA was converted into cDNA using oligo-dT_{20} primers (Invitrogen, Breda, the Netherlands), 10 mM dNTPs, and SuperScript™ III Reverse Transcriptase (Invitrogen, Breda, the Netherlands) according to the manufacturer's instructions. Efficiency of cDNA synthesis was assessed by Q-RT-PCR, using primers of the constitutively expressed gene *AtUBI10* (At4g05320; *UBI10*-FOR: 5' AAA GAG ATA ACA GGA ACG GAA ACA TAG T-3'; *UBI10*-REV: 5'-GGC CTT GTA TAA TCC CTG ATG AAT AAG-3'). Gene-specific primers were designed for *AtMYB102* (At4g21440; *AtMYB102*-FOR: 5'-GTT GCC AGA AGA ACG GAC TC-3'; *AtMYB102*-REV: 5'-GGG AGG GTT CTC CAG TTA CC-3'). Q-RT-PCR analysis was done in optical 96-well plates with a MyIQ™ SingleColor Real-Time PCR Detection System (Bio-Rad, Veenendaal, the Netherlands), using SYBR® Green to monitor dsDNA synthesis. Each reaction contained 1 μL of cDNA, 0.5 μL of each of the two gene-specific primers (10 $pmol.\mu L^{-1}$),

and 10 µL of 2x IQ SYBR® Green Supermix reagent (Bio-Rad, Veenendaal, the Netherlands) in a final volume of 20 µl. The following PCR program was used for all Q-RT-PCR reactions: 95°C for 3 min; 40 cycles of 95°C for 30 sec, 59.5°C for 30 sec, and 72°C for 30 sec. C_T (threshold cycle) values were calculated using Optical System Software, version 1.0 for MyIQ™ (Bio-Rad, Veenendaal, the Netherlands). Subsequently, C_T values were normalized for differences in dsDNA synthesis using the *AtUBI10* C_T values. Normalized transcript levels of *AtMYB102* were compared to untreated controls and the fold change in expression level was calculated after 24 hr of feeding by *P. rapae*.

GUS assay. Larvae of *P. rapae* were transferred to five-week-old *MYB102*::*GUS* plants. After 24 hr of caterpillar feeding, leaf tissue was harvested and GUS activity assessed by transferring the leaves to GUS staining solution (1 mM X-Gluc, 100 mM NaP_i buffer, pH 7.0, 10 mM EDTA, 0.1% (v/v) Triton X-100, 1 mM potassium ferrocyanide and 1 mM potassium ferricyanide). After overnight incubation at 37°C, the leaves were destained by repeated washes in 70% ethanol and evaluated for staining intensity as described.[33]

Microarray preparation. The Arabidopsis 6K microarray used consisted of 6008 cDNA fragments, of which 5834 were from the Incyte Unigene collection (Arabidopsis Gem I; Incyte, Palo Alto, CA), and 408 positive and negative controls, of which 384 were from the Lucidea Microarray ScoreCard v1.1 (GE Healthcare Bio-Sciences, Little Chalfont, UK). cDNA fragments were spotted in duplicate and distantly from each other; for details see the VIB MicroArray Facility (MAF) website (www.microarrays.be).

Sample preparation, target labeling and hybridizations. For isolation of RNA from leaf tissue, shoots of untreated wild-type Col-0 and 35S::*MYB102* line 8.4 plants were harvested. Total RNA (5 µg) of each sample was reverse transcribed to double-stranded cDNA and further amplified. Subsequent Cy5 and Cy3 (GE Healthcare Bio-Sciences) labeling, hybridization, post-hybridization washing and scanning were performed as described previously.[34] All protocols for Cy3 and Cy5 labeling, hybridization and scanning can be accessed through the VIB MAF (www.microarrays.be/service.htm/protocols).

Experimental design and statistical analysis of the microarray data. We constructed an experimental design consisting of two replicated dye-swap Arabidopsis 6K microarray experiments, in which two independent pools of untreated wild-type Col-0 and 35S::*MYB102* line 8.4 shoots were directly compared. The expression data were analyzed in two steps: (1) a within-slide analysis aimed at removing variation associated with differential dye responses to binding and scanning, as noise; and (2) a between-slide analysis aimed at estimating the mean differences between treatments (i.e., genotypes) and their standard error. For the within-slide analysis we used the robust scatter plot smoother LOWESS[35] as implemented in Genstat,[36] where the response variable is the $\log_2$ ratio of the artefact-removed total foreground Cy5 (R) and Cy3 (G) fluorescence intensities (M) measured at the 6008 Arabidopsis spots and a total of 24 negative control spots containing a *Bacillus subtilis*-specific cDNA. The fraction of the data used for estimating the local LOWESS fit was set at 20%. Once the adjusted $\log_2$ ratios (M') for each gene were obtained, adjusted $\log_2 R$ and $\log_2 G$ signal intensities were calculated. Positive signals were selected as described previously[34] based on the 48 adjusted $\log_2 R$ and $\log_2 G$ signal intensities of the 24 negative controls spots printed in duplicate. One hundred eighty five genes were below the signal threshold in all observations for each genotype and were subsequently removed from the data set.

For the between-slide analysis, we applied an ANOVA model to the 46584 LOWESS fits to the spot measurements as described previously.[37] Briefly, to account for the multiple sources of variation in the microarray experiment, we applied the linear normalization ANOVA model of the form:

response = µ + array + dye + genotype + gene + (gene.array) + (gene.dye) + (gene.genotype) + error

where the response variable represents the 46584 corrected $\log_2$-transformed Cy3 and Cy5 fluorescence intensity measurements. Array models the hybridization effects of each of the four microarrays, dye models the effects of each of the two dyes, genotype represents the effect of each of the two genotypes, gene represents the effect of each of the 5823 genes, gene.array represents the spot effect, gene.dye represents the gene-specific dye effects and gene.genotype represents the interaction between the genotypes and the genes, which are the effects of interest. Nonzero differences in the genotype by gene effects for a given gene indicate differential expression. To determine which of these differences in the genotype by gene interactions are significantly different from zero, we estimated 95.0% confidence intervals for these differences employing a bootstrap analysis of the residuals as described in reference 37 and based on 1000 bootstrap data sets. Since $e^{0.48} = 1.395$ means a fold-change of approximately 40 % appears significant at the 0.05 level. No further multiple testing has been taken into account.

Functional analysis. Clones were annotated using the MIPS Arabidopsis thaliana Genome Database (MatDB; http://mips.gsf.de/proj/thal/db/index.html). Biological function and predicted subcellular localization of the proteins was assessed using the same Internet facilities and the Gene Ontology tool at TAIR.

RESULTS

AtMYB102 expression upon herbivore attack. The transcription factor gene *AtMYB102* is induced by dehydration and wounding.[26] Because herbivore-damaged plants also suffer from water loss, we investigated the role of AtMYB102 in defense against caterpillar feeding. Wild-type *A. thaliana* Col-0 plants were infested with larvae of *P. rapae* and the expression of *AtMYB102* was analyzed 24 hr later. Q-RT-PCR analysis of *AtMYB102* mRNA levels showed a 2.3-fold induction of *AtMYB102* in *P. rapae*-damaged tissue compared to untreated Col-0 plants (Fig. 1A), indicating that insect feeding induced the expression of *AtMYB102.* This result was confirmed by data from a previously published whole-genome GeneChip array experiment,[13] in which *AtMYB102* mRNA levels were increased at both 12 hr and 24 hr after infestation by *P. rapae* (Fig. 1B).

To further study the herbivore-induced expression of *AtMYB102*, we made use of a transgenic *AtMYB102*::*GUS* reporter line, containing a translational fusion of the *uidA* reporter gene with the promoter of the *AtMYB102* gene.[28] Figure 1C shows that β-glucuronidase (GUS) activity was induced around the feeding sites of *P. rapae*. All together, these results indicate that wounding caused by feeding of *P. rapae* triggers the expression of *AtMYB102,* predominantly in the cells surrounding the feeding sites.

Role of AtMYB102 in resistance against P. *rapae.* To investigate the role of AtMYB102 in resistance against *P. rapae,* a defective *Suppressor-mutator* (d*Spm*) transposon insertion line with a d*Spm* element inserted in the first exon of the *AtMYB102* gene 124 bp from the translation start site (line Sm_3_41654 designated

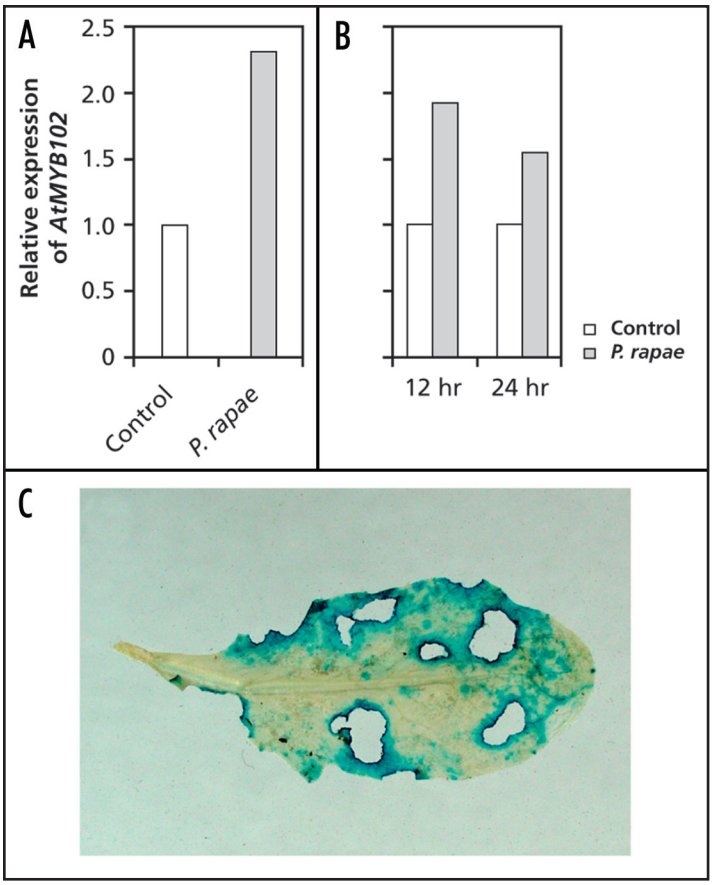

Figure 1. *Pieris rapae*-induced expression of *AtMYB102*. (A) Q-RT-PCR analysis of *AtMYB102* mRNA levels in Col-0 plants 24 hr after feeding by first-instar larvae of *P. rapae*. Uninfested control is set at 1. (B) Relative level of *AtMYB102* mRNA in Col-0 plants 12 and 24 hr after *P. rapae* feeding. Values are derived from an Affymetrix ATH1 GeneChip experiment.[13] Uninfested control is set at 1. (C) Histochemical staining of β-glucuronidase (GUS) activity in leaves of transgenic *A. thaliana* line *MYB102*::*GUS* 24 hr after feeding by *P. rapae*.

myb102; Fig. 2A), and a 35S::*MYB102* overexpressing line (line 2.3; ref. 28) were used. To confirm disruption of *AtMYB102* in the *myb102* knockout mutant, two PCR reactions were performed on Col-0 and *myb102* genomic DNA. One reaction with non-target *AtTUB8* gene-specific primers, and one reaction with a transposon-specific primer (Spm32) and two *AtMYB102*-specific primers (*AtMYB102*-FW and *AtMYB102*-RV located ~500 bp up and downstream of the transposon insertion, respectively). The *AtTUB8* primers amplified a similar PCR product of ~500 bp on Col-0 and *myb102* genomic DNA, confirming that equal amounts of template genomic DNA were used in the PCR reactions (Fig. 2A; *TUB*). The PCR reaction with the two *AtMYB102*-specific primers and the Spm32 transposon-specific primer amplified a ~850-bp fragment on Col-0 DNA, which corresponds to the ~850-bp region of the *AtMYB102* gene that is located between the *AtMYB102* primers (Fig. 2A; *MYB102*). A similar PCR reaction with *myb102* genomic DNA yielded a ~550-bp fragment, which corresponds to the ~550-bp region between the Spm32 binding site in the d*Spm* transposon and the *AtMYB102*-specific primer binding site in the *AtMYB102* gene. The ~850-bp *AtMYB102* DNA fragment was not amplified on *myb102* DNA because this fragment contains the d*Spm* transposon and is, thus, too large to be amplified under the PCR conditions used. Overexpression of *AtMYB102* in 35S::*MYB102* line 2.3 was confirmed by northern blot analysis of RNA that was isolated from uninduced wild-type and transgenic plants (Fig. 2B).

To study herbivore performance in the knockout mutant and the overexpressor in comparison to wild-type Col-0, five-week-old plants were each infested with one first-instar *P. rapae* larva. Subsequently, larval performance was monitored over a period up to ten days by determining larval weight gain. In addition, we determined the percentage of larvae that pupated within 14 days of infestation. Figure 3A shows that on days seven and ten, the weight of the larvae that fed on *myb102* was significantly higher (approx. 1.5-fold) than that of the larvae feeding on wild-type Col-0 plants. This increased caterpillar weight was associated with a greater percentage of larvae that had entered pupation by day 14. About 50% of the larvae feeding from *myb102* plants had developed into pupae on day 14, while only 5% of the larvae feeding from wild-type Col-0 plants had pupated at that time (Fig. 3B). Surprisingly, overexpression of *AtMYB102* did not result in a reduction of larval performance. Caterpillar growth on 35S::*MYB102* plants did not differ significantly from that on Col-0 plants (Fig. 3A). Also the percentage of larvae feeding from 35S::

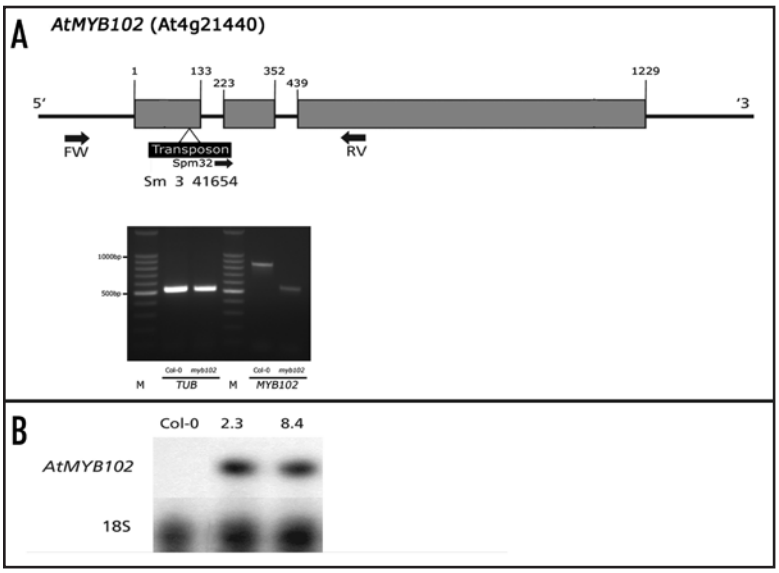

Figure 2. Molecular analysis of knockout mutant *myb102* and *AtMYB102* overexpressor 35S::*MYB102* lines. (A) Structure of the *AtMYB102* gene and position of the transposon insertion in the *myb102* knockout mutant. Exons are indicated as gray boxes. The nucleotide numbers above indicate the start and the end of the exons. A transposon insertion in *myb102* is located in the first exon of the *AtMYB102* open reading frame. The primers used for the verification of the position of the transposon insertion are indicated by arrows (FW, RV, and Spm32). To verify the transposon insertion, PCR amplification of genomic DNA of Col-0 and *myb102* plants was performed using the transposon-specific primer Spm32 and the *AtMYB102*-specific primers FW and RV. Specific primers for *AtTUB8* were designed as internal loading control. FW, *AtMYB102* forward primer; RV, *AtMYB102* reverse primer; Spm32, transposon-specific primer; M, 100-bp DNA ladder. (B) Northern blot analysis of *AtMYB102* mRNA levels in 35S::*MYB102* lines 2.3 and 8.4. The blot was hybridized with a gene-specific probe for *AtMYB102*. The probe for 18S rRNA was used to check for equal loading.

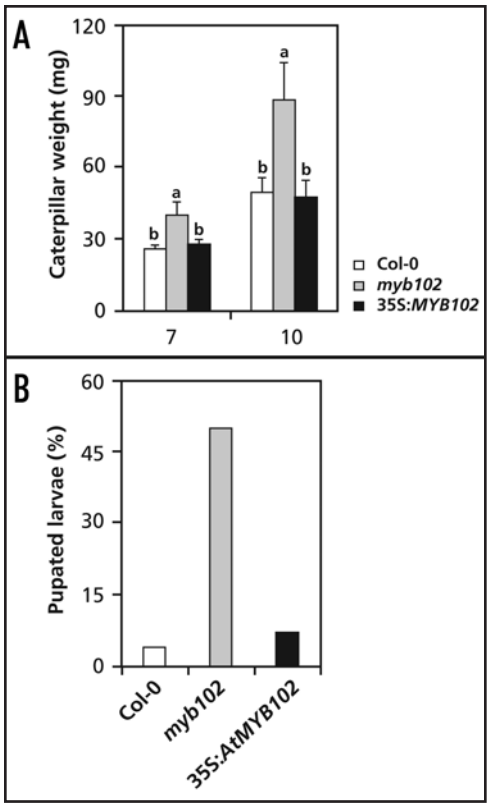

Figure 3. Effect of herbivore-induced resistance on *P. rapae* performance. (A) Growth of *P. rapae* larvae on wild-type Col-0, mutant *myb102,* and *AtMYB102* overexpressing 35S::*MYB102* plants. Larval fresh weight (FW) was measured after seven and ten days of feeding. The values presented are means (±SE) of 20 larvae on each plant genotype. Different letters indicate statistically significant differences between treatments (Fisher's LSD test; α = 0.05). (B) Percentage of *P. rapae* larvae (n = 20) that had developed into pupae by 14 days after infestation.

MYB102 plants that had pupated by day 14 did not differ significantly from those feeding on wild-type Col-0 plants (Fig. 3B). These data indicate that *AtMYB102* contributes to basal resistance against *P. rapae* feeding. However, overexpression of *AtMYB102* does not increase resistance above the basal level.

Expression profiling of 35S::*MYB102* plants. To study downstream effects of upregulation of *AtMYB102* by *P. rapae* we performed a microarray experiment to identify the genes that are affected by overexpression of the transcription factor gene *AtMYB102*. To this end, wild-type Col-0 and 35S::*MYB102* line 8.4[28] plants were compared in a replicated, dye-swapped microarray experiment using a cDNA micro-array consisting of 6008 *A. thaliana* cDNA fragments. To identify genes of which the expression was substantially affected we applied two selection criteria: i) the changes in gene expression should be statistically significant, and ii) the changes should be above the arbitrarily chosen level of 2-fold. Although statistically significant changes in gene expression below the threshold level of 2-fold may be biologically relevant, we have chosen to disregard all changes below this threshold level to limit the number of false positives. Overexpression of *AtMYB102* significantly increased the expression (>2-fold) of 151 genes, while 117 genes showed an at least 2-fold reduction (supplementary data Table S1). Among the genes that are upregulated in the 35S::*MYB102* plants, were *AtTHI2.1* (At1g72260) encoding an anti-microbial thionin,[38] an ethylene biosynthesis gene (At2g19590) encoding a putative 1-aminocyclopropane-1-carboxylate oxidase, an anti-microbial thaumatin-like gene (At4g38660) with high homology to *AtTLP1* that is activated in *A. thaliana* upon colonization of the roots by resistance-inducing rhizobacteria,[39] and *AtVSP1* (At5g24780), encoding a vegetative storage protein.[40] Recently, AtVSP2 (At5g24770), which shares 82% amino acid sequence identity with AtVSP1, was shown to possess anti-insect properties that could be

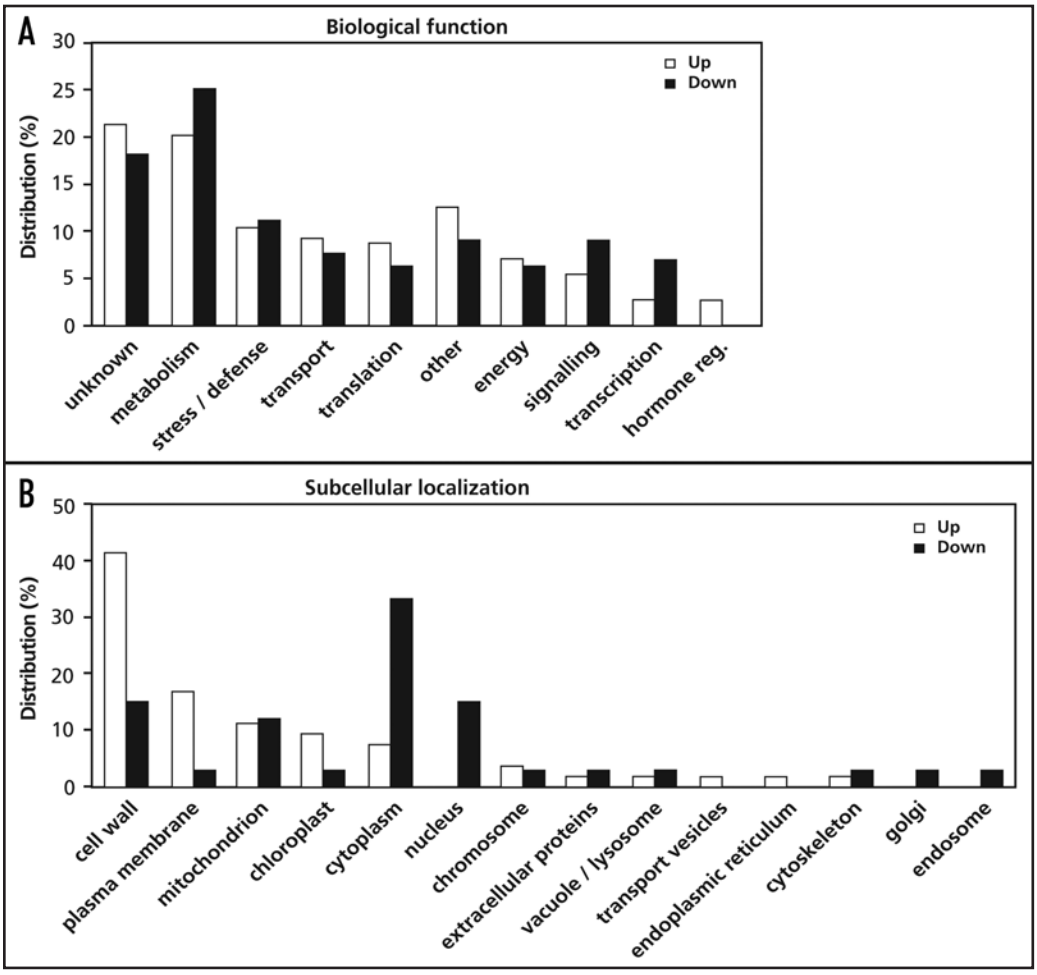

Figure 4. Biological function and predicted cellular localization of the proteins encoded by the differentially expressed genes in 35S::*MYB102* plants. (Supplementary Table S1). Selected genes showing a statistically significant and at least 2-fold difference in expression between Col-0 and *AtMYB102* overexpressing (35S::*MYB102*) plants.

Table 1. **Fold-change ratio of upregulated genes that are associated with modification of the cell wall upon overexpression of *AtMYB102* in *A. thaliana***

Function[1]	Annotation	AGI No.	Fold-change[2]
Biosynthesis			
Cuticle biosynthesis	β-keto acyl reductase	At1g67730	2.04
Cell wall organization	Copia-like retrotransposon family	At2g06950	3.34
	Protodermal factor 1	At2g42840	2.36
Degradation			
Cell wall loosening	Expansin (EXP11)	At1g20190	5.48
	Expansin (EXP10)	At1g26770	4.10
	Expansin (EXP8)	At2g40610	3.21
	Expansin (EXP4)	At2g39700	2.69
Pectin degradation	Pectinesterase	At1g14890	2.52
	Pectinesterase	At1g11580	2.39
	Pectate lyase	At1g04680	2.05
Xyloglucan cleavage	Endo-xyloglucan transferase	At2g06850	3.58
Cellulase	Endo-1,4-β-glucanase	At1g70710	2.86
β-glucosidase	β-glucosidase activity	At3g09260	3.07

[1]Described functions are based on the Gene Ontology tool at the TAIR internet facilities. [2]Fold-change ratios (35S::*MYB102*/Col-0) are based on gene expression profiles of 5-week-old leaf tissue from 35S::*MYB102* line 8.4 and wild-type Col-0 plants.

contributed to the acid phospatase activity of the protein.[41] When incorporated into the diets of different insects, AtVSP2 significantly delayed development of the insects and increased their mortality.

We categorized the differentially expressed genes according to biological function (Fig. 4A) and predicted subcellular localization (Fig. 4B) using internet tools from the MIPS *Arabidopsis thaliana* Genome Database (MatDB; http://mips.gsf.de/proj/thal/db/index.html) and the Gene Ontology tool at TAIR (http://arabidopsis.org/tools/bulk/go/index.jsp). Classification according to biological functions indicates that a substantial percentage of the differentially expressed genes in the 35S::*MYB102* overexpressing line encode proteins involved in metabolism. However, this is not surprising because of all annotated genes in the *A. thaliana* genome, metabolism is the largest category of genes with known biological function. Moreover, 10% of the genes up and downregulated by overexpression of *AtMYB102* have been shown to be involved in stress and defense reactions. Classification according to predicted subcellular localization of the proteins revealed that a large proportion of the upregulated genes encode proteins that are thought to function in the cell wall or at the plasma membrane. Among the upregulated genes are several that code for cell wall-modifying proteins, such as EXPANSIN4, 8, 10 and 11, and pectolytic enzymes (Table 1).

DISCUSSION

Large scale expression analysis of the *A. thaliana MYB* transcription factor gene family revealed that several MYB transcription factor genes are regulated by JA.[18] Because JA plays an important role in insect resistance, several members of the MYB transcription factor family may play a role in insect resistance. Previously, a few of them have already been implicated in resistance against insect herbivores. For instance, manipulation of transcript levels of the *A. thaliana* MYB transcription factor gene *ATR1* (*ALTERED TRYPTOPHAN REGULATION 1*; *AtMYB34*[17]), which encodes a MYB transcription factor that regulates the expression of genes involved in the tryptophan pathway,[42] severely altered the production of indolic glucosinolate anti-herbivore compounds.[20] In another study, a transgenic line of *A. thaliana* constitutively expressing *PAP1* (*PRODUCTION OF ANTHOCYANIN PIGMENT 1*; *AtMYB75*[17]), which encodes a MYB transcription factor that activates the phenylpropanoid biosynthetic pathway,[43] showed a significantly increased resistance to feeding by fall armyworms (*Spodoptera frugiperda*).[21] In this study we showed that damage caused by *P. rapae* induced the expression of *AtMYB102* around the feeding sites. On knockout mutant *myb102* plants, *P. rapae* caterpillars developed significantly faster than on Col-0 plants, indicating that in wild-type plants, *AtMYB102* plays a role in defense against this herbivore. Overexpression of the *AtMYB102* gene did not further enhance the level of resistance against *P. rapae* feeding, suggesting that the level of *AtMYB102* that is induced upon herbivore feeding is already fully effective.

Transcript profiling of wild-type Col-0 and *AtMYB102* overexpressing plants revealed enhanced expression of several defense-related genes in 35S::*AtMYB102* plants, including *AtVSP1*, which possesses acid phosphatase activity that is associated with enhanced insect resistance.[41] Furthermore, a relatively large number of the genes that were upregulated in the *AtMYB102* overexpressor encode proteins that are predicted to exert their function in the cell wall or the plasma membrane. Several of these genes are involved in cell wall remodeling. Our findings that AtMYB102 plays a role in resistance against *P. rapae*, and regulates genes that are associated with cell wall modification, raises the question to what extent a causal relationship exist between these two processes. The speed of tissue consumption by *P. rapae* suggests that the cell wall modifications that are induced upon activation of *AtMYB102* are unlikely to contribute to inhibition of growth of the caterpillars. Hence, the AtMYB102-mediated cell wall modifications may reflect repair mechanisms that are initiated upon wounding and dehydration. However, knockout mutant *myb102* clearly allows a faster development of *P. rapae* larvae, indicating that AtMYB102-regulated genes contribute to resistance against this herbivore. Hence, future research will be focused on understanding the role of AtMYB102 in resistance against insect feeding.

Note

Supplemental Table S1 can be found at www.landesbioscience.com/journals/psb/devosPSB1-6-sup.pdf.

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