

Role for the AtMYB102 transcription factor in the defense of Arabidopsis against tissue-chewing insects

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Abstract

In Arabidopsis 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 Arabidopsis to feeding by larvae of the white cabbage butterfly *Pieris rapae*. Arabidopsis 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. Knock-out *AtMYB102* T-DNA 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. *AtMYB102* over-expressing 35S:*MYB102* plants did not show an enhanced resistance to feeding by *P. rapae* larvae. To analyze the effect of constitutive *MYB102* gene expression, transcript profiling of wild-type and over-expressor 35S:*MYB102* plants was performed. A total of 268 genes was found to be differentially expressed. A relatively large proportion of genes that were up-regulated in the over-expressor appeared to be associated with the cell wall, suggesting that MYB102 plays a role in regulating the capacity for cell wall remodeling.

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. 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. A classic example is the observation that following attack by larvae of the tobacco hornworm, *Manduca sexta*, 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 (Howe, 2005; Ryan, 2000). Genetic evidence demonstrates that JAs also play an important role in induced defense against

different types of herbivores in *Arabidopsis* (Ellis *et al.*, 2002; McConn *et al.*, 1997; Reymond *et al.*, 2004; Stintzi *et al.*, 2001; Stotz *et al.*, 2002; Van Poecke and Dicke, 2004). Analysis of the transcriptome of *Arabidopsis* 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 (De Vos *et al.*, 2005; Reymond *et al.*, 2000; 2004). Among the JA-responsive genes that are activated several encode transcription factors, including several members of the MYB-transcription factor family. However, their role in induced resistance against insects is unknown.

MYB genes encode transcription factor proteins that share the conserved MYB DNA-binding domain (Jin and Martin, 1999), and were first identified as oncogenes derived from retroviruses in animal cells (Klempnauer *et al.*, 1982). 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 *Arabidopsis* genome revealed that this plant species contains approximately 125 R2R3-MYB genes (Stracke *et al.*, 2001). 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 Stracke *et al.*, 2001), 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* and *AtMYB51* are associated with the wound response (Cheong *et al.*, 2002). In addition, Mengiste *et al.* (2003) 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 (Thomma *et al.*, 1998; Ton *et al.*, 2002). 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 (Mengiste *et al.*, 2003). These observations suggest that *AtMYB108* is a central player in multiple stress responses in *Arabidopsis*. Recently, *AtMYB72* was demonstrated to be essential for the onset of rhizobacteria-induced systemic resistance (ISR), a JA-dependent induced defense response that is effective against a broad spectrum of plant pathogens (Pieterse *et al.*, 2002; Van Loon *et al.*, 1998). Colonization of the roots by ISR-inducing *Pseudomonas fluorescens* WCS417r bacteria led to the activation of the *AtMYB72* gene in the roots (Verhagen *et al.*, 2004).

AtMYB72 knockout mutants were no longer able to express ISR in the leaves, indicating that AtMYB72 is an important regulator of ISR (Verhagen, 2004).

Another R2R3-MYB transcription factor family member (AtMYB102) was identified from an Arabidopsis transcription factor collection (Quaedvlieg *et al.*, 1996). The gene is up-regulated in Arabidopsis upon treatment with ABA, JA, or a combined treatment of osmotic stress and wounding (Denekamp and Smeekens, 2003). Plant responses that are triggered by feeding insects partly overlap with those activated upon dehydration stress and wounding (Reymond *et al.*, 2000). This prompted us to study in how far *AtMYB102* is involved in the response of Arabidopsis 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 and may regulate multiple genes that are involved in cell wall modification.

Results and discussion

***AtMYB102* expression upon herbivore attack**

Herbivore-infested plants undergo substantial transcriptional reorganization in which the plant hormone jasmonic acid (JA) plays an important regulatory role (De Vos *et al.*, 2005; Reymond *et al.*, 2000; 2004). The transcription factor gene *AtMYB102* is induced by dehydration and wounding (Denekamp and Smeekens, 2003). Because herbivore-damaged plants also suffer from water loss, we investigated the role of AtMYB102 in defense against caterpillar feeding. Wild-type Arabidopsis 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 (De Vos *et al.*, 2005), 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 (Denekamp, 2001). Figure 1C shows that m-glucuronidase (GUS) activity was strongly 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.

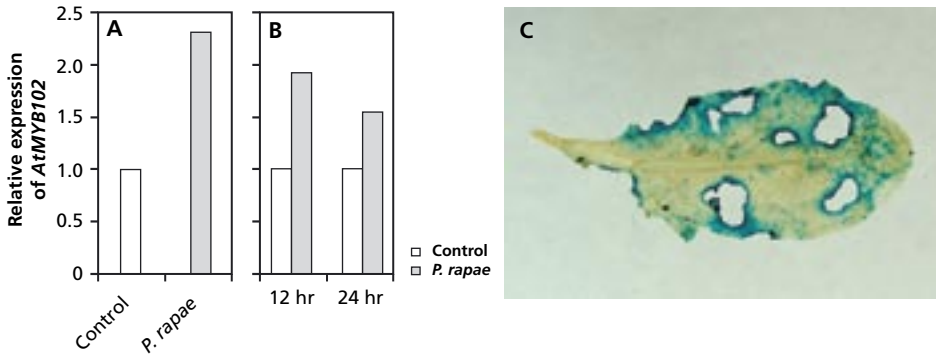


Figure 1. *Pieris rapae*-induced expression of AtMYB102

(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 (De Vos *et al.*, 2005). Uninfested control is set at 1.

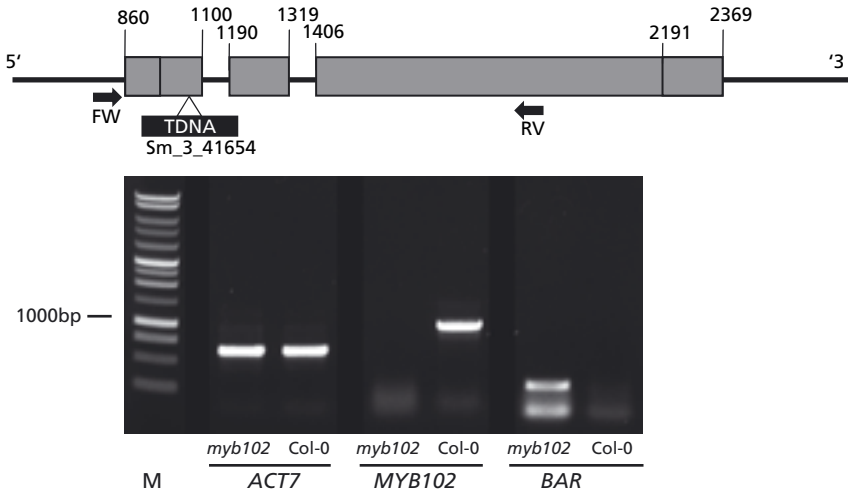
(C) Histochemical staining of β -glucuronidase (GUS) activity in leaves of transgenic Arabidopsis line *MYB102:GUS* 24 hr after feeding by *P. rapae*.

Role of AtMYB102 in resistance against *P. rapae*

To investigate the role of AtMYB102 in resistance against *P. rapae*, an *AtMYB102* T-DNA insertion line (designated *myb102*), and a 35S:*MYB102* over-expressing line (line 2.3; Denekamp, 2001) were used. Knockout mutant *myb102* contains a T-DNA insertion with a selectable marker for resistance against the herbicide glufosinate (*BAR*) in the first exon of the *AtMYB102* gene (Fig 2A). To confirm disruption of *AtMYB102* in *myb102*, gene-specific primers for *AtMYB102*, the non-target gene *AtACT7*, and the *BAR* gene were used to amplify the respective target sequences in Col-0 and *myb102*. The *AtACT7* gene was detected in the *myb102* mutant as well as in wild-type Col-0 plants (Fig. 2A). The *AtMYB102* primers did not amplify a PCR product in the *myb102* mutant, presumably because of the presence of the large T-DNA insert. The PCR reaction with primers for the *BAR* gene confirmed the presence of a T-DNA insertion in *myb102*. Over-expression 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 over-expressor in comparison to wild-type Col-0, 5-week-old plants were infested with 1 freshly hatched *P. rapae* larva. Subsequently, larval performance was monitored over a period up to 10 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 7 and 10, 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

A AtMYB102 (At4g21440)



B

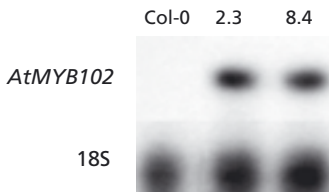


Figure 2. Molecular analysis of knockout mutant *myb102* and *AtMYB102* over-expressor *35S:MYB102* lines.

(A) Structure of the *AtMYB102* gene and position of the T-DNA insertion in the *myb102* mutant allele. Exons are indicated as gray boxes. The nucleotide numbers above indicate the start and the end of the exons. A T-DNA 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 T-DNA insertion are indicated by arrows (FW and RV). The expected size of the PCR products for Col-0 is 1002 bp. FW, *AtMYB102* forward primer; RV, *AtMYB102* reverse primer. To verify the T-DNA insertion, PCR amplification of genomic DNA of Col-0 and *myb102* plants was performed using the *AtMYB102*-specific FW and RV. Specific primers for *AtACT7* were designed as internal loading control. M = 1000bp 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.

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 on that time point (Fig. 3B). Surprisingly, over-expression 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

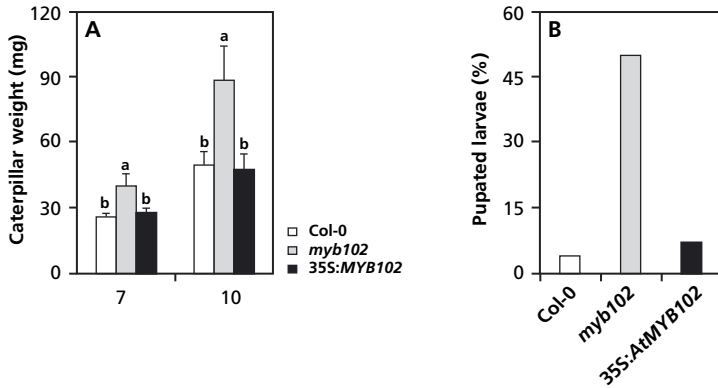


Figure 3. Effect of herbivore-induced resistance on *P. rapae* performance

(A) Growth of *P. rapae* larvae on wild-type Col-0, mutant *myb102*, and *AtMYB102* over-expressing *35S:MYB102* plants. Larval fresh weight (FW) was measured after 7 and 10 days of feeding. The values presented are means (\pm SE) of 20 larvae on each plant genotype. Different letters indicate statistically significant differences between treatments (Fisher's LSD test; $\alpha=0.05$).

(B) Percentage of *P. rapae* larvae ($n=20$) that had developed into pupae by 14 days after infestation (DAI).

35S: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, over-expression of *AtMYB102* does not increase resistance above the basal level.

Expression profiling of *35S:MYB102* plants

To study downstream effects of up-regulation of *AtMYB102* by *P. rapae* we performed a microarray experiment to identify the genes that are regulated by the transcription factor *AtMYB102*. To this end, wild-type Col-0 plants and *35S:MYB102* line 8.4 (Denekamp, 2001) were compared in a dedicated cDNA micro-array consisting of approximately 6,000 Arabidopsis cDNA fragments. Over-expression 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). 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* over-expressing line encode proteins involved in metabolism. However, this is not surprising because of all annotated genes in the Arabidopsis genome, metabolism is the largest category of genes with known biological function. Moreover, 10% of the genes up- and

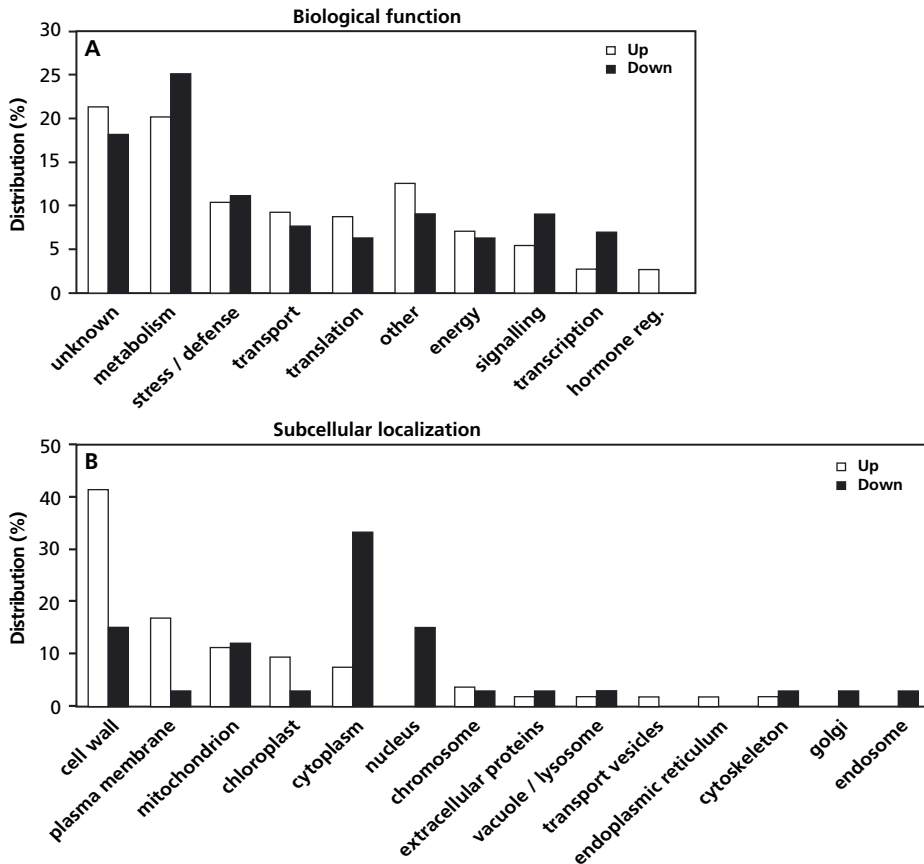


Figure 4. Biological function and predicted cellular localization of the proteins encoded by the differentially expressed genes in 35S:MYB102 plants.

down-regulated by over-expression 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 up-regulated genes encode proteins that are thought to function in the cell wall or at the plasma membrane. Among the up-regulated genes are several that code for cell wall-modifying proteins, such as EXPANSIN4, 8, 10 and 11, and pectolytic enzymes (Table 1).

Conclusions

In this study we showed that damage caused by feeding larvae of *P. rapae* induced the expression of *AtMYB102* around the feeding sites. On knockout *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

Table 1. Fold-change ratio of up-regulated genes that are associated with modification of the cell wall upon over-expression of *AtMYB102* in Arabidopsis.

Function ¹	Annotation	AGI No.	Fold-change ²
Biosynthesis			
- Cuticle biosynthesis	α -keto acyl reductase	At1g67730	2.04
- Cell wall organization	Copia-like retrotransposon family Protodermal factor 1	At2g06950	3.34
		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- α -glucanase	At1g70710	2.86
- α -glucosidase	α -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.

against this herbivore. Over-expression 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* over-expressing plants revealed that a large proportion of genes that were significantly up-regulated in the *AtMYB102* over-expressor are predicted to exert their function in the cell wall or the plasma membrane. Moreover, several genes involved in cell wall remodeling were up-regulated in the *AtMYB102* over-expressor. 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 in how far 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. Clearly, more research

is required to understand the role of AtMYB102 in resistance against insect feeding.

Materials and methods

Cultivation of plants

Seeds of *Arabidopsis thaliana* accession Col-0, *MYB102:GUS* (Denekamp and Smeekens, 2003), knockout mutant *myb102* (T-DNA insertion line Smm3m41654 obtained from the EXOTIC collection of the Nottingham Arabidopsis Stock Centre; Tissier *et al.*, 1999) and *AtMYB102* over-expressing 35S:*MYB102* plants (line 2.3; Denekamp, 2001) 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 a 8-hr day (200 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ 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 (Hoagland and Arnon, 1938) 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; 5 weeks for Col-0 and 8 weeks for 35S:*MYB102*) in potting soil in a growth chamber with a 8-hr day (24°C) and a 16-hr night (20°C) cycle at 70% 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* over-expressing lines showed similar levels of *AtMYB102* mRNA (Denekamp, 2001). Line 2.3 was used in the insect bioassays because at the time of the insect experiments, no viable seeds could be recovered from line 8.4.

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 (Van Poecke *et al.*, 2001). Infestation of Arabidopsis 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 freshly hatched first-instar larva was transferred to each of 20 Col-0, *myb102*, or 35S:*MYB102* plants. At 7 and 10 days, the fresh weight of each larva was determined. After 10 days, the first larvae started to pupate. Therefore, fresh weight was determined only up to 10 days of feeding. To examine effects on

caterpillar development, the percentage of caterpillars that had pupated within 14 days after hatching was determined.

Confirming T-DNA insertion in *AtMYB102*

Seedlings from Col-0 and T-DNA insertion line Sm_3_41654 were grown for two weeks on Murashige and Skoog (MS) medium supplemented or not with 20 mg.L⁻¹ BASTA and subsequently harvested for isolation of genomic DNA. Disruption of the *AtMYB102* gene was checked by PCR using gene-specific primers for *AtMYB102* (At4g21440; FOR 5'-TTC CCC TTA CGG ACC CTA CGA-3'; REV 5'-TGG TGG CAT GGA AGA TTG GAG T-3') located on opposite sites of the predicted T-DNA insertion. Gene-specific primers for *AtACT7* (At5g09810; FOR; 5'-GAC ATG GAA AAG ATA TGG CAT CAC AC-3'; REV; 5'-AGA TCC TTC CTG ATA TCG ACA TCA C-3'), and *BAR* (FOR; 5'-ACT TCA GCA GGT GGG TGT AGA G-3'; REV; 5'-ATC GTC AAC CAC TAC ATC GAG AC-3') were used as controls. The following PCR program was used for all PCR reactions: 95 °C for 3 min; 40 cycles of 92 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min.

Quantitative real-time PCR

Q-RT-PCR analysis was performed basically as described previously (Czechowski *et al.*, 2004). Two µg of RNA was digested with Turbo DNA-free™ (Ambion, Huntingdon, United Kingdom) 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-dT₂₀ 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 an 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.µL⁻¹), and 10 mL of 2x IQ SYBR® Green Supermix reagent (Bio-Rad, Veenendaal, the Netherlands) in

a final volume of 20 μ l. The following PCR program was used for all 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 5-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 NaPi 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.

Sample preparation and microarray analysis

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 μ g) of each sample was reverse transcribed and amplified according to a modified protocol for *in vitro* transcription (<http://www.microarrays.be/service.htm>), labeled with fluorescent Cy5 or Cy3 (Amersham Biosciences, Roosendaal, the Netherlands), and subsequently hybridized to a dedicated Arabidopsis 6K microarray consisting of 6,008 cDNA fragments. Fragments and controls were obtained from the Incyte Unigene collection (Arabidopsis Gem I; Incyte, Palo Alto, CA) and the Universal Score Card spike set (Amersham BioSciences, Little Chalfont, UK), respectively. Clones were spotted in duplicate, distant from each other (for details see <http://www.microarrays.be/service.htm>).

Hybridization and washing were performed in an automated hybridization station (Amersham Biosciences, Roosendaal, the Netherlands). The arrays were scanned at 532 and 635 nm by a Generation III scanner (Amersham BioSciences, Roosendaal, the Netherlands) and images were analyzed with an ArrayVision (Imaging Research Inc, Ontario, Canada). Genes showing at a least 2-fold change in expression (increase or decrease in line 8.4 compared to wild-type plants) 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.

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Supplementary Table 1: Selected genes showing an at least 2-fold difference in expression between Col-0 and *AtMYB102* over-expressing (35S:*MYB102*) plants. This supplementary table can be found at http://www.bio.uu.nl/~fytopath/GeneChip_data.htm.

