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CHINESE CABBAGE *BrMYB34.2* TRANSCRIPTION FACTOR REGULATES INDOLIC GLUCOSINOLATES BIOSYNTHESIS IN *Arabidopsis*

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ABSTRACT

Glucosinolates (GS) are a group of sulfur- and nitrogen-rich plant secondary metabolites that originate from amino acids and exist mainly in plants in the order *Brassicales*, such as *Arabidopsis thaliana* (*Arabidopsis*) and Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). To date, several regulatory components responsible for GS biosynthesis have been identified in Arabidopsis. However, the functions of GS biosynthesis regulators in Chinese cabbage have not been clarified. In our current study, a putative ATR1/MYB34 orthologous gene, *BrMYB34.2*, was isolated from Chinese cabbage leaves. To investigate the function of this gene, we engineered Arabidopsis plants that overexpress *BrMYB34.2* ectopically and phenotypic analysis was performed. Moreover, we assayed the accumulation levels of indolic GS (IGS) and aliphatic glucosinolates in transgenic plants and test the expression of key genes of IGS biosynthesis and tryptophan synthesis by Real-time quantitative PCR. And further analysed the resistance of transgenic plants in 5MT stress treatment. The results indicate that ectopic expression of the *BrMYB34.2* gene in Arabidopsis was able to up-regulate the accumulation level of IGS due to the increased expression of IGS and Trp biosynthetic genes. Moreover, overexpression of *BrMYB34.2* conferred Arabidopsis 5MT resistance. These results suggest that the *BrMYB34.2* gene may function as one of the regulators of IGS and Trp biosynthesis in Chinese cabbage.

Key words: *BrMYB34.2*, indolic glucosinolate, tryptophan, Chinese cabbage

INTRODUCTION

Glucosinolates (GS) are a group of sulfur- and nitrogen-rich plant secondary metabolites that originate from amino acids and exist mainly in plants in the order *Brassicales*, such as the model plant *Arabidopsis thaliana* (Arabidopsis) and *Brassica* vegetables such as Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) [Sønderby et al. 2010]. GS can be hydrolyzed to several degradation products (e.g. isothiocyanates, nitriles, thiocyanates, epithionitriles and oxazolidines) by myrosinase [Yan and Chen 2007]. GS and their degradation products may confer plant biotic and abiotic stress tolerance [Yan and Chen 2007, Martinez-Ballesta et al. 2013], contribute to protection against cancer in humans [Traka and Mithen 2009], and produce specific flavors in *Brassica* vegetables [Schonhof et al. 2004, Padilla et al. 2007].

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Based on the amino acid origin of GS, GS are divided into aliphatic GS (AGS), aromatic GS and indolic GS (IGS) groups [Sønderby et al. 2010]. The formation of GS involves three stages, including amino acid chain elongation, core structure formation, and secondary modifications of the amino acid side chain [Sønderby et al. 2010]. IGS derives from tryptophan (Trp). The structural genes responsible for IGS biosynthesis are clearly understood in Arabidopsis. First, Trp is catalyzed by redundant cytochrome P450 enzymes, CYP79B2 and CYP79B3, to be converted into indole-3-acetaldoxime. Then, another cytochrome P450 enzyme, CYP83B1, functions to catalyze the conversion of indole-3-acetaldoxime to 1-*aci*-nitro-2-indolyl-ethane, which subsequently forms IGS by C-S lyase SUR1, *S*-glucosyltransferases, and sulfotransferases [Yan and Chen 2007]. Genetic analyses have revealed that there is crosstalk between the IAA and IGS biosynthetic pathways, of which IAOx is considered to be the node of the metabolic branch [Celenza et al. 2005].

Several regulatory components responsible for GS biosynthesis have been identified. A calmodulin-binding nuclear protein, IQD1, was shown to positively control the biosynthesis of both IGS and AGS and then confer plant resistance against generalist herbivores [Levy et al. 2005]. SLIM1 negatively regulates IGS and AGS biosynthetic pathways under sulfate deficiency [Maruyama-Nakashita et al. 2007]. *AtDof1.1* (DNA-binding-with-one-finger) has been reported to moderately increase IGS and AGS accumulation [Skirycz et al. 2006]. Furthermore, two clades of R2R3-MYB transcription factors have been reported to directly activate the biosynthesis of GS. *MYB28*, *MYB29* and *MYB76*, which belong to a clade of R2R3-type MYB transcription factors, were shown to be direct regulators of AGS biosynthesis [Gigolashvili et al. 2007b, Gigolashvili et al. 2008]. Another clade of R2R3-type MYB transcription factors, *ATR1/MYB34*, *HIG1/MYB51* and *HIG2/MYB122*, directly up-regulate several main biosynthetic enzymes of the IGS pathway [Celenza et al. 2005, Gigolashvili et al. 2007a, Gigolashvil et al. 2009]. Interestingly, *ATR1/MYB34* not only acts as a positive regulator of IGS biosynthetic genes but also positively controls Trp biosynthetic genes [Bender and Fink 1998].

Brassica rapa is an important class of *Brassica* vegetables, and it comprises a variety of subspecies, such as Chinese cabbage, broccoli, pak choi, and turnip types. Chinese cabbage is highly consumed in Asia, especially in China, Korea, and Japan. Chinese cabbage is rich in GS, however, undetectable levels of glucoraphanin exist in many Chinese cabbage cultivars [Padilla and Velasco 2007, Zang et al. 2009]. *Brassica* and Arabidopsis diverged from a common ancestor, and *Brassica* has recently undergone genome triplication [Mun et al. 2010, Wang et al. 2011, Seo et al. 2017]. Thus, the biosynthesis and regulation of GS in *Brassica rapa* might be different from those in Arabidopsis, and corresponding studies are needed. With the completion of the *Brassica rapa* genome sequence, orthologs of GS biosynthetic genes and transcription factors in *Brassica rapa* have been identified by comparative genomic analysis between *Brassica rapa* and Arabidopsis [Zang et al. 2009, Wang et al. 2011]. Subsequently, several investigations related to GS biosynthetic regulators have been conducted. Seo et al. [2017] reported the expression profiles of 13 *BrMYB* transcription factors related to glucosinolate biosynthesis in eight subspecies of *Brassica rapa* [Seo et al. 2017]. In addition, studies have shown that the expression of some *BrMYB* transcription factors, such as *BrMYB28.2*, *BrMYB28.3*, and *BrMYB29.1,* were highly responsive to various abiotic and biotic stressors [Justen and Fritz 2013, Baskar and Park 2015, Wang et al. 2015, Seo et al. 2017]. Moreover, the function of three *BrMYB28* transcription factors in controlling the biosynthesis of GS has been analyzed [Seo et al. 2016]. However, the function of *BrMYB* transcription factors related to GS biosynthesis, except for *BrMYB28,* has not been identified yet.

In this study, the *BrMYB34.2* gene (Bra013000), an Arabidopsis *ATR1/MYB34* ortholog from Chinese cabbage, was cloned, and its regulatory function was identified via transformation into Arabidopsis. Our results showed that ectopic expression of the *BrMYB34.2* gene in Arabidopsis was able to up-regulate the accumulation level of IGS due to the increased expression of IGS and Trp biosynthetic genes. Moreover, overexpression of *BrMYB34.2* conferred Arabidopsis 5MT resistance, at least partially in a way that co-regulates the expression of Trp and IGS biosynthetic genes. Our results provide useful information on *MYB34* transcription factor in Chinese cabbage.

MATERIALS AND METHODS

Plant materials and growth conditions. Chinese cabbage seeds (highly inbred lines A5-2-1) were kindly provided by prof. Yaowei Zhang at Northeast Agricultural University (Harbin, China). The seeds were sown in a 1 : 1 mixture of soil and vermiculite in growth chambers. The chambers were kept at 4°C in the dark for 20 days before they were transferred to an incubator with a photosynthetic flux of 150 μmol·M^{-2}·s^{-1}, a photoperiod of 16 h light at 25°C and 8 h dark at 23°C, and a relative humidity of approximately 50–70%.

The Arabidopsis plants used in this study are in the Columbia-0 background. The seeds were surface-sterilized and grown on MS medium in 9 cm Petri dishes. After being kept at 4°C in the dark for 3 days, the seedlings were cultured in a growth chamber at 22°C, with 8 h light at 150 μmol·m^{-2}·s^{-1} and 70% relative humidity. Seven days after germination, the seedlings were transferred to growth chambers (5 cm in diameter and 6 cm in depth) filled with a 1 : 2 mixture of soil and vermiculite.

Construct preparation and plant transformation. To create the P_{35S}*::BrMYB34.2*^{OE} construct, *BrMYB34.2* cDNA was amplified with the forward primer 5′-CGGGATCCGTCGAGATGGTGAGAACAC-3′ and reverse primer 5′-GACTAGTCCAACGTGTAATTGATTTTGC-3′ by RT-PCR and subcloned downstream of the 35S promoter in a ProkII vector. The P_{35S}*::BrMYB34.2*^{OE} construct was transformed into *Agrobacterium tumefacien*s (*A. tumefaciens*) GV3101. The *A. tumeofaciens*-mediated transformation of Arabidopsis was performed via vacuum infiltration [Bechtold et al. 1993]. The harvested seeds were screened on 1/2 MS agar plates containing 50 mg/ml kanamycin sulfate, and the resistant plants were further verified by reverse transcription-PCR (RT-PCR). Homozygous T3 generations of transgenic plants were used for subsequent analyses.

Semi-quantitative RT-PCR and quantitative RT-PCR (qRT-PCR). Total RNA from leaves was extracted using the TRIzol reagent according to the manufacturer's procedures (Invitrogen, China). Total RNA was treated with DNase I (TaKaRa, China). The first-strand cDNA was synthesized by using an oligo (dT) primer and M-MLV reverse transcriptase (TaKaRa). The primers for amplifying the partial fragment of *BrMYB34.2* were forward primer 5′-GGTTTCATATCGATTCTTACG-3′ and reverse primer 5′-CCAACGTGTAATTGATTTTGC-3′. Arabidopsis *Actin2* served as an internal control, and the specific primers were 5′-GTGACAATGGAACTGGAATGG-3′ and 5′-AGACGGAGGATAGCGTGAGG-3′. Thirty cycles were performed for the semi-quantitative analysis. Experiments were independently replicated three times under identical conditions.

Total RNA extraction for qRT-PCR was identical to the protocol for semi-quantitative RT-PCR. After digesting genomic DNA with RNase-free DNase I (TaKaRa), first-strand cDNA was synthesized from 500 ng of total RNA using the PrimeScript RT reagent Kit (TaKaRa). Quantitative assays were performed using the SYBR Green Master mix (TaKaRa) with an ABI 7500 sequence detection system according to the manufacturer's protocol (Applied Biosystems, USA). Relative transcript levels were calculated using the relative standard curve method, and *ACTIN2* was used as the reference gene. The details of gene accession numbers and the oligonucleotide primers can be found in Table 1.

Extraction and detection of GS. Leaves were dissected from 5-week-old plants and were frozen in liquid nitrogen. Then, 0.1 g frozen leaf samples were homogenized in a tissue grinding instrument. GS were extracted in preheated 70% methanol after the addition of benzyl glucosinolate (Phytoplan, Germany) as an internal standard. Extracts were transferred to a DEAE Sephadex A25 column (Amersham Biosciences, Sweden). Then, sulfatase (type H-1, from *Helix pomatia*, Sigma, USA) was added to the extraction and incubated overnight in order to convert the GS into desulfoglucosinolates. After eluting with ultrapure water, the desulfoglucosinolates were freeze-dried to a powder and dissolved with ultrapure water.

For analysis of desulfoglucosinolates, samples were subjected to UPLC analysis on an ACQUITY UPLC H CLASS chromatograph (Waters, USA) with a quaternary pump and a TUV UV detector. Elution was accomplished on an ACQUITY UPLC HSS T3 column (50 × 2.1 mM, 1.8 μM, Waters) with a gradient (solvent A, water; solvent B, methanol) of 0–60% B (7.6 min), 60–100% B (0.6 min), then a 0.6-min hold, followed by a gradient of 0–100% A (0.8 min). Peaks

Table 1. Sequences of the primers used for quantitative RT-PCR

Genes	Accession numbers	Primers（5'–3'）
ACTIN2	At3g18780	ATTCAGATGCCCAGAAGTCTTGTTCC
		ACCACCGATCCAGACACTGTACTTCC
ASA1	At5g05730	CGAATTCCTCAGTCATGGT
		ATGTCTAGCGTTGGTCGTT
TSB1	At5g54810	CGTCTCGCTCCAAGTCAT
		CCAAACCGACCGAACGAA
CYP83B1	At4g31500	TCACGCCATATCTACCAGC
		TGGACGTCATGACTGGAC
CYP79B2	At4g39950	GTAACTTCGGAGCATTCGT
		TCGCCGGATATCACATCC
CYP79B3	At2g22330	AGTCACTTCCGAACACTCA
		TCGCAGGTTACCATATTCC
MAM1	At5g23010	TTGAGGAGGTCGTGATGG
		CTGATGAATGCCGCTCTC
CYP83A1	At4g13770	ATAGTATATGTTCCTCCAGTGTATTC
		GAGAAAGATAGAGAGACGATTGC
SUR1	At2g20610	ACAATCCCTGTGGAAATGTCTACTC
		ACAACCCATCCCTTAGATATGCC
ST5a	At1g74100	CGAAGTCGATCTCAACGTA
		ACCAACTGATTTCCTCGTC
UGT74B1	At1g24100	TATCTTGATGATCGGATGGA
		TACTGACTGAGCCTGCTTAG

were quantified by the peak area at 229 nm relative to the area of the internal standard peak. Data presented are the mean ± standard error for at least three replicates per sample.

5-methyl-tryptophan resistance analysis. To test the resistance of transgenic plants to 5-methyl-tryptophan (5MT), seeds were grown aseptically on MS medium containing 0 μM, 5 μM, 10 μM or 15 μM 5MT and incubated at 22°C (with 16/8 h day/night cycles) for 15 days. Seedlings were monitored for root growth relative to the wild-type control on the same plate.

RESULTS AND DISCUSSION

***BrMYB34.2* ectopic expression affects GS accumulation in Arabidopsis.** To determine the biological functions of *BrMYB34.2*, we transferred this gene to Arabidopsis by a sense plasmid (P_{35S}::*BrMYB34.2*^{OE}). Forty-one P_{35S}::*BrMYB34.2*^{OE} kanamycin resistance lines were obtained, and they were further confirmed by semi-quantitative RT-PCR analysis (Fig. 1a). No significant difference in plant size or organ phenotype was found between the wild-type and the P_{35S}::*BrMYB34.2*^{OE} transgenic lines (data not shown). To confirm whether *BrMYB34.2* ectopic expression affects GS accumulation in Arabidopsis, GS accumulation pattern analysis was further performed in P_{35S}::*BrMYB34.2*^{OE} transgenic plants. Transgenic line 2, line 3 and line 4 were selected for study. Twelve GS were detected: indol-3-ylmethyl glucosinolate (I3M), 1-methoxyindol-3-ylmethyl glucosinolate (1MOI3M), 4-methoxyindol-3-ylmethyl glucosinolate (4MOI3M), and 4-hydroxyindol-3-ylmethyl glucosinolate (4OHI3M), 3-methylsulfinylpropyl glucosinolate (3MSOP), 4-methylsulfinylbutyl glucosinolate (4MSOB), 4-methylthiobutyl glucosinolate (4MTB),

5-methylsulfinylpentyl glucosinolate (5MSOP), 6-methylsulfinylhexyl glucosinolate (6MSOH), 7-methylthiohepthyl glucosinolate (7MTH), 8-methylsulfinyloctyl glucosinolate (8MSOO), and 8-methylthiooctyl glucosinolate (8MTO). The first four are IGS, and the last eight are AGS.

As shown in Figure 1b, there was no significant difference in the total GS content between P_{35S}::*BrMYB34.2*^{OE} transgenic plants and wild-type plants. However, the transgenic lines contained significantly increased accumulation of total IGS compared to wild-type plants, while the level of total AGS content was significantly reduced in P_{35S}::*BrMYB34.2*^{OE} strains compared with that in wild-type plants. Therefore, *BrMYB34.2* ectopic expression promotes Trp-derived IGS metabolites in Arabidopsis, and *BrMYB34.2* might play opposite roles in IGS and AGS accumulation. The increased IGS level, accompanied by a compensatory decrease in AGS content after overexpressing *BrMYB34.2,* implies a homeostatic control of IGS and AGS biosynthesis, which is consistent with the 'limiting electron hypothesis' [Grubb and Abel 2006]. It has been reported that the paralogous genes of Arabidopsis *ATR1/MYB34* and *HIG1/MYB51* also possess opposite functions in the biosynthetic pathways of IGS and AGS [Gigolashvili et al. 2007a], which is similar to *BrMYB34.2* in our study, indicating that gene function might have been redistributed after *Brassica* and Arabidopsis diverged from the common ancestor.

Further analysis showed that all the transgenic lines had significantly elevated levels of I3M, 4MOI3M, and 4OHI3M compared with wild-type plants (Fig. 2). I3M levels in the transgenic plants (line 3) were increased by 3-fold (line 3) compared with those in the wild-type plants. Likewise, the transgenic lines contained 7-fold-increased levels of 4OHI3M (line 2) compared with wild-type plants. These indicated that *BrMYB34.2* possesses an important regulatory function in controlling IGS biosynthesis in Arabidopsis. The results that *BrMYB34.2* overexpression lines contained significantly higher levels of total IGS than wild-type plants due to the accumulation of the major IGS I3M were consistent with Arabidopsis *ATR1/MYB34* overexpression lines [Celenza et al. 2005, Gigolashvili et al. 2007a]. The altered level of I3M-derived IGS in our *BrMYB34.2* transgenic lines was different from

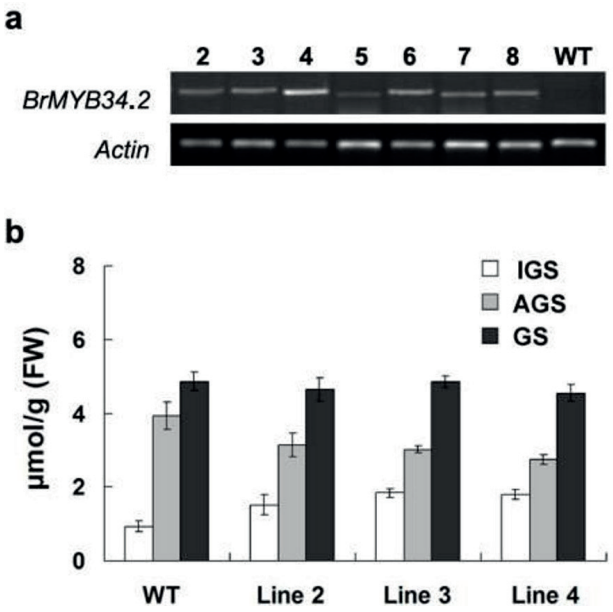

Fig. 1. RT-PCR analysis of the *BrMYB34.2* gene (a) and glucosinolate accumulation (b) in P_{35S}::*BrMYB34.2*^{OE} transgenic Arabidopsis lines; IGS – total indolic glucosinolates, AGS – total aliphatic glucosinolates, GS – total glucosinolates

that in *ATR1/MYB34* transgenic plants [Celenza et al. 2005, Gigolashvili et al. 2007a], indicating that the function of *BrMYB34.2* in promoting Trp-derived IGS metabolites might be conserved but somewhat divergent with *ATR1/MYB34*.

Ectopic expression of *BrMYB34.2* in Arabidopsis up-regulates IGS biosynthetic genes with irregular expression of AGS biosynthetic genes. To better visualize the role of *BrMYB34.2* in IGS accumulation, the expression of genes involved in IGS biosynthesis was examined in P_{35S}::*BrMYB34.2*^{OE} transgenic lines using qRT-PCR. As shown in Figure 3, the expression of *CYP79B2*, *CYP79B3*, *CYP83B1*, *SUR1*, *UGT74B1*, and *ST5a* was obviously stronger in all the transgenic lines than in wild-type plants. Furthermore, the increase in the expression levels of almost all the IGS biosynthetic genes detected correlated well with the accumulation of transcripts of *BrMYB34.2* in the transgenic lines: plants (line 4) showing the highest expression of *BrMYB34.2* also possessed the highest activation potential towards the target genes (Fig. 3).

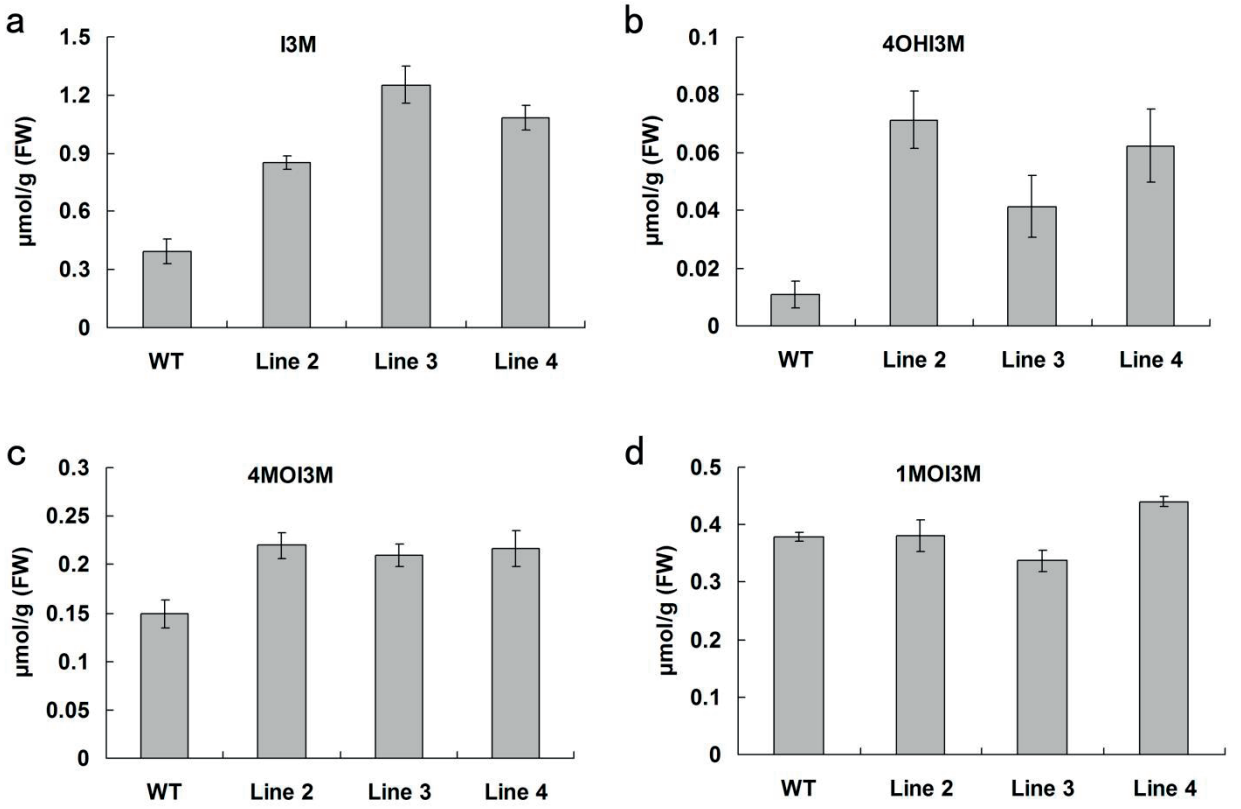

Fig. 2. Glucosinolate content in rosette leaves of P_{35S}::*BrMYB34.2*^{OE} transgenic Arabidopsis plants: (a) I3M content, (b) 4OHI3M content, (c) 4MOI3M content, (d) 1MOI3M content; I3M – indole-3-ylmethyl-glucosinolate, 4OH-I3M – 4-hydroxy-indole-3-ylmethyl-glucosinolate, 4MO-I3M – 4-methoxy-indole-3-ylmethyl-glucosinolate, 1MO-I3M – 1-methoxy-indole-3-ylmethyl-glucosinolate

In addition, this is correlated with the highest content of total IGS (Fig. 1).

These results indicate that *BrMYB34.2* appears to serve as a general positive activator of IGS biosynthetic pathway genes. Celenza et al. [2005] had reported the same regulation pattern of three CYP genes (*CYP79B2*, *CYP79B3*, and *CYP83B1*) by *ATR1/MYB34* using Arabidopsis *atr1D* overexpression mutants, 35S-ATR1 overexpressing plants, and an *atr1* loss-of-function mutant, indicating that *BrMYB34.2* possesses similar function with *ATR1/MYB34* in regulating IGS biosynthetic genes.

To further study whether the *BrMYB34.2* gene plays roles in the biosynthesis of AGS, the accumulation levels of transcripts of AGS biosynthetic genes (*MAM1* and *CYP83A1*) were also analyzed using qRT-PCR. It has been shown previously that the MAM1 protein is capable of catalyzing the condensation reactions of the first two elongation cycles of methionine-derived glucosinolates [Textor et al. 2007], and *CYP83A1* is involved in the biosynthesis of both short-chain and long-chain AGS [Hemm et al. 2003]. It is evident from Figure 4 that the *MAM1* gene was significantly reduced only in transgenic line 4 and slightly but not significantly reduced in other *BrMYB34.2* transgenic lines, whereas *CYP83A1* was slightly increased in transgenic lines. Therefore, the steady-state transcript levels of genes encoding AGS biosynthetic enzymes were also disturbed by overexpressing the *BrMYB34.2* gene, and it might thus result in the altered AGS content in P_{35S}::*BrMYB34.2*^{OE} transgenic lines. *BrMYB34.2* overexpression in Arabidopsis wild-type plants led to an expected increase in the level of IGS biosynthetic genes, with AGS biosynthetic genes re-

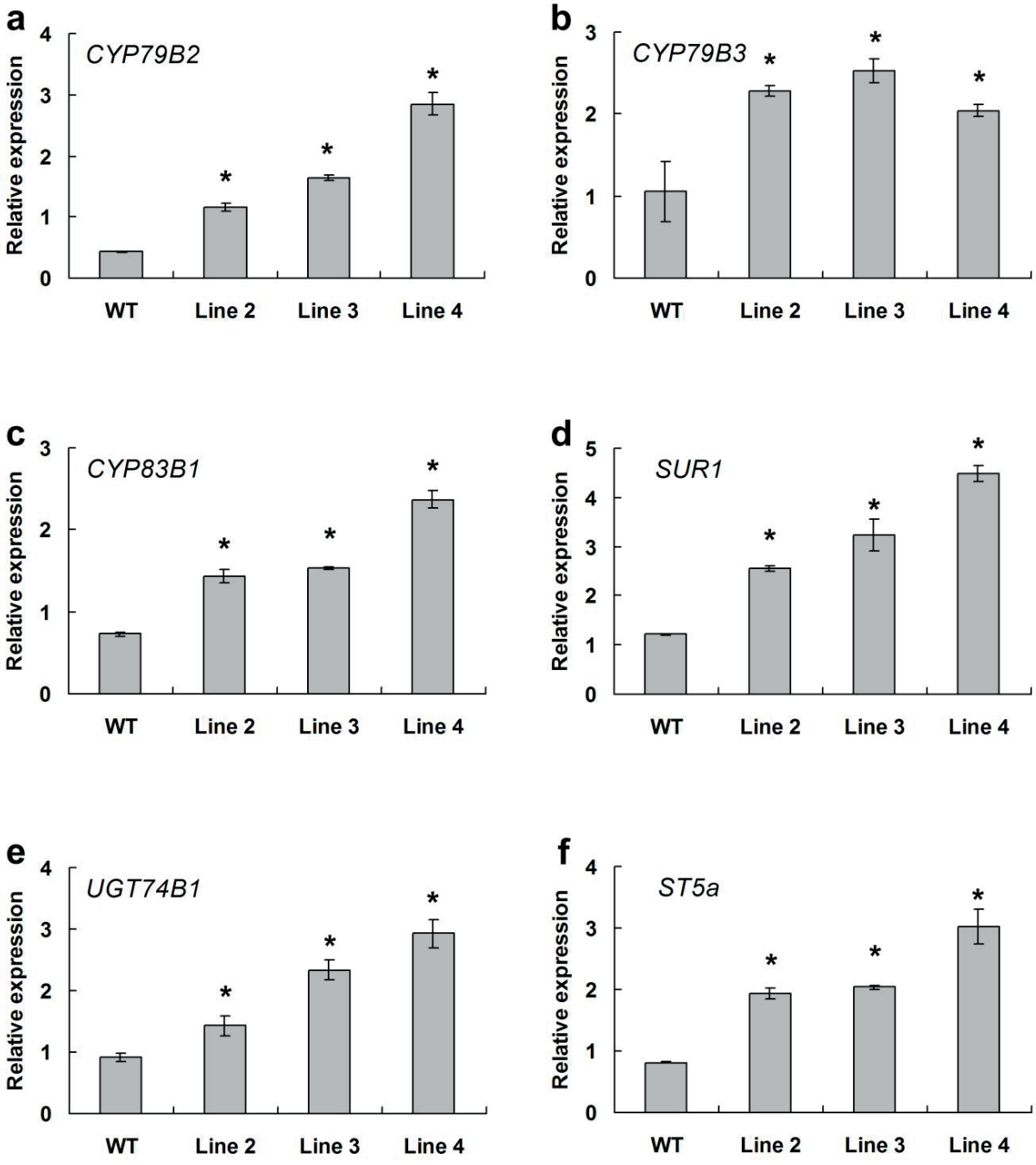

Fig. 3. Transcript levels of indolic glucosinolate pathway genes in rosette leaves of P_{35S}::*BrMYB34.2*^{OE} transgenic Arabidopsis plants: (a) the expression of *CYP79B2*, (b) the expression of *CYP79B3*, (c) the expression of *CYP83B1*, (d) the expression of *SUR1*, (e) the expression of *UGT74B1*, (f) the expression of *ST5a*. Expression data are presented relative to *Actin2*. Data are the mean ± SE of three replicates

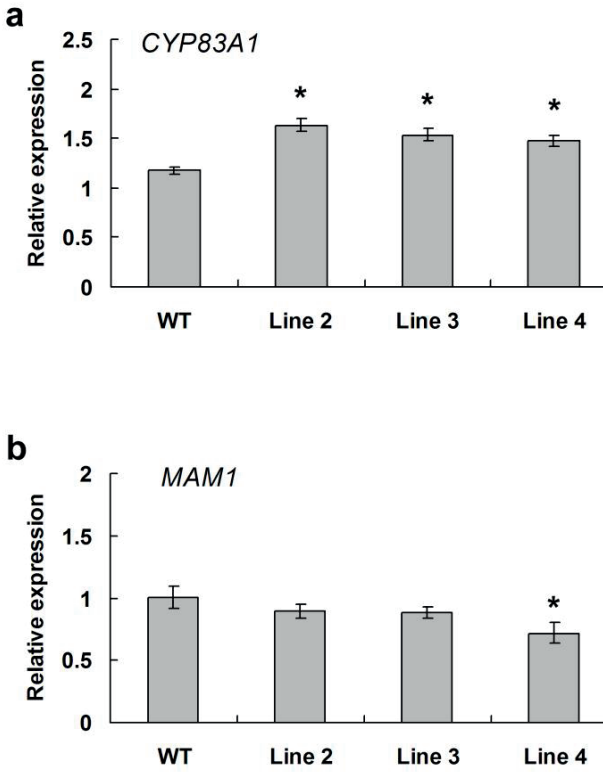

Fig. 4. Transcript levels of aliphatic glucosinolate pathway genes in rosette leaves of P_{35S}*::BrMYB34.2*^{OE} transgenic Arabidopsis plants: (a) the expression of *CYP83A1*, (b) the expression of *MAM1*. Expression data are presented relative to *Actin2*. Data are the mean ± SE of three replicates

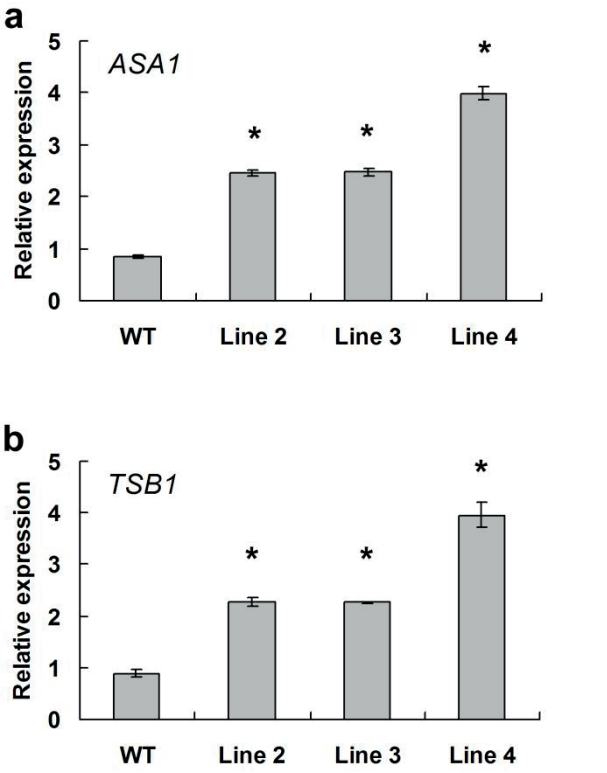

Fig. 5. Transcript levels of tryptophan biosynthesis genes in rosette leaves of P_{35S}*::BrMYB34.2OE* transgenic Arabidopsis plants: (a) the expression of *ASA1*, (b) the expression of *TSB1*. Expression data are presented relative to *Actin2*. Data are the mean ± SE of three replicates

maining at varied levels (Figs 3, 4). The reason for this is yet unknown, but the results may suggest that a regulatory interaction between *BrMYB34.2* and other factors might participate in the control of both IGS and AGS biosynthetic genes and that the activity of putative regulators of AGS biosynthetic genes might be altered in the P_{35S}*::BrMYB34.2*^{OE} transgenic lines.

BrMYB34.2 ectopic expression in Arabidopsis induced the transcription of Trp biosynthetic genes. Arabidopsis *ATR1/MYB34* plays a physiological role in IGS biosynthesis as well as Trp biosynthesis [Bender and Fink 1998, Celenza et al. 2005]. To identify whether *BrMYB34.2* acts as a regulator in Trp biosynthesis, the transcription levels of Trp biosynthesis genes, *anthranilate synthase α-subunit* (*ASA1*) and *Tryptophan synthase beta 1* (*TSB1*), were detected. In qRT-PCR analysis, P_{35S}*::BrMYB34.2*^{OE} transgenic lines displayed elevated accumulation of *ASA1* and *TSB1* transcripts (Fig. 5). *ASA1* encodes the alpha subunit of anthranilate synthase [Niyogi 1993], which catalyzes the first step of the Trp biosynthetic pathway, and *TSB1* encodes the beta subunit of Trp synthase [Pruitt and Last 1993]. The up-regulation of *ASA1* and *TSB1* genes in line 4 transgenic strains was stronger than that detected in line 2 and line 3 transgenic plants (Fig. 5), which is consistent with the transcriptional accumulation of exogenesis *BrMYB34.2* (Fig. 1). Thus, these results demonstrate a physiological role for *BrMYB34.2* in Trp biosynthetic gene control, which is identical to Arabidopsis *ATR1/MYB34* [Bender and Fink 1998, Smolen and Bender 2002, Celenza et al. 2005].

***BrMYB34.2* ectopic expression in Arabidopsis confers 5MT resistance.** 5-Methyl-tryptophan (5MT) is a type of toxic Trp analog that inhibits plant growth through feedback inhibition of Trp biosynthesis without substituting for the nutritional role of Trp [Smolen and Bender 2002]. To further understand whether *BrMYB34.2* overexpression accelerates the overall rate of Trp metabolism, 5MT resistance analysis of *BrMYB34.2* overexpressed Arabidopsis was performed. Line 4 strain of P_{35S}*::BrMYB34.2*^{OE} transgenic plants was selected for the detection. As is shown in Figure 6, the transformants did not display any obvious morphological abnormalities with wild-type plants without 5MT. However, the length of the roots in transformants was increased by approximately 2-fold compared with the wild-type plants when 5 μM 5MT was added. Under the 10 μM 5MT conditions, the wild-type plants were more sensitive to 5MT growth inhibition, while the transgenic plants displayed approximately 2-fold longer roots than that of wild-type plants. When 5MT was added to 15 μM, the root growth of wild-type plants was almost arrested, and there was almost no significant effect in root growth of transformants (Fig. 6). These results suggest that *BrMYB34.2* overexpression in Arabidopsis confers 5MT resistance, which is consistent with the role of the Arabidopsis *ATR1/MYB34* gene [Bender and Fink 1998, Smolen and Bender 2002, Celenza et al. 2005].

The mechanism for 5MT resistance is either a creation of feedback resistance mutations in the *ASA1* gene or transcriptional up-regulation of *ASA1* and other tryptophan metabolism genes [Smolen and Bender 2002]. In our study, the 5MT resistance of *BrMYB34.2*-overexpressing plants was conferred by the activation of Trp biosynthetic genes and IGS biosynthetic genes observed in our study (Figs 3, 5), and this suggests that

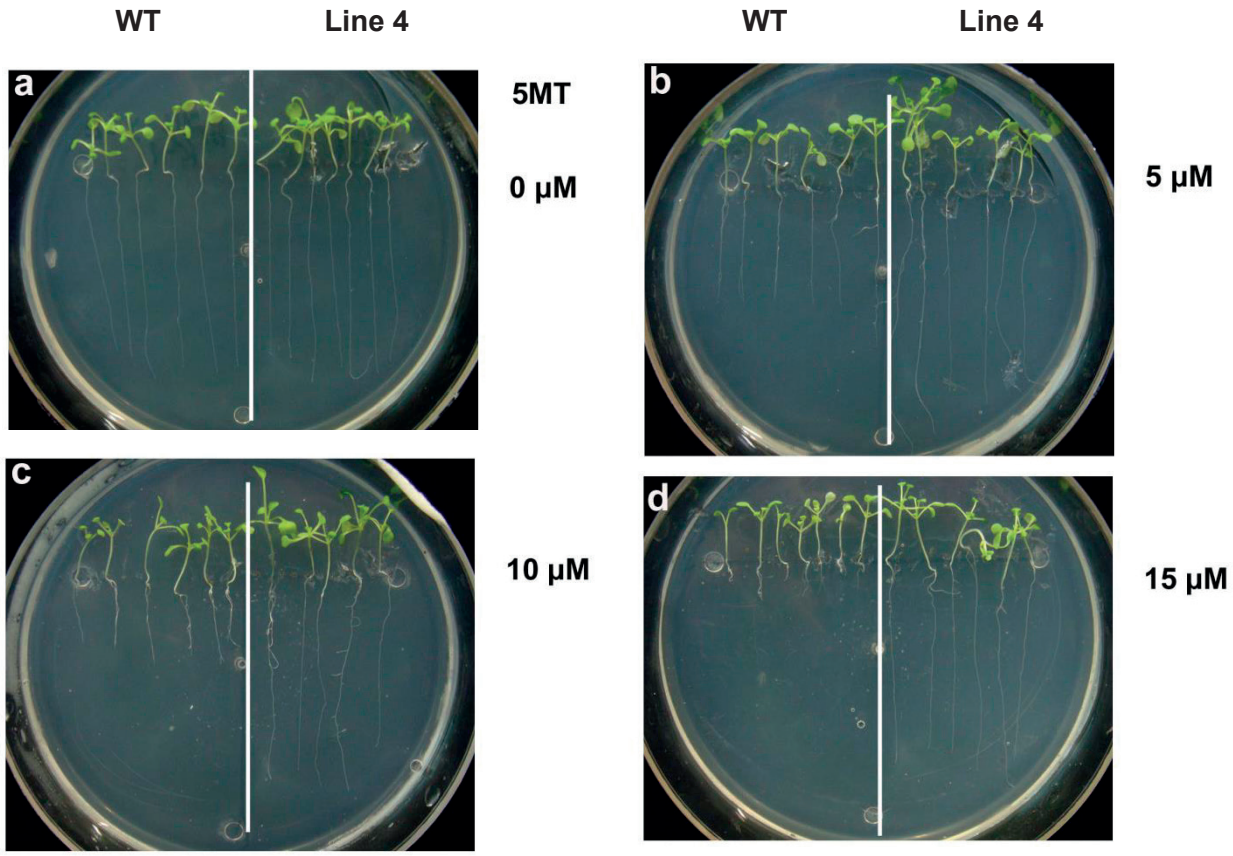

Fig. 6. 5MT resistance analysis of P_{35S}*::BrMYB34.2*^{OE} transgenic Arabidopsis seedlings

BrMYB34.2 co-regulates the expression of Trp and IGS biosynthetic genes. The co-regulated pattern of Trp and IGS biosynthetic genes by *BrMYB34.2* indicates that the up-regulation of Trp biosynthetic pathway genes might synthesize adequate amounts of the precursor Trp for the activated IGS biosynthesis.

CONCLUSIONS

In this study, we isolated *BrMYB34.2* gene, a putative Arabidopsis *ATR1/MYB34* orthologous gene, from Chinese cabbage and transferred it into Arabidopsis. The results showed that ectopic expression of *BrMYB34.2* in Arabidopsis altered GS accumulation. The accumulation levels of IGS components in transgenic plants were significantly elevated compared with those in wild-type plants, resulting in an increased total IGS level in transgenic plants. The increased IGS accumulation was due to up-regulated expression of IGS biosynthetic genes. Nevertheless, overexpression of *BrMYB34.2* did not interfere with the accumulation of total aliphatic GS because of irregular expression changes in aliphatic GS biosynthetic genes. Moreover, ectopic expression of *BrMYB34.2* increased the transcription levels of tryptophan biosynthesis genes and conferred Arabidopsis 5MT resistance. These results suggest that the *BrMYB34.2* gene may function as one of the regulators of IGS and tryptophan biosynthesis in Chinese cabbage. Our results provide useful information on *MYB34* transcription factor in Chinese cabbage.

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