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Ectopic expression of soybean methionine synthase delays flowering time in transgenic tobacco plants

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Abstract

A photoperiod-sensitive soybean [*Glycine max* (L.) Merr] cv. ZhongDou 24 (ZD24) exhibiting delayed flowering when grown under long-days (LD, a 16-h photoperiod) was used to identify the genetic control of flowering delay. A differential expression profiling technique enabled identification of a gene fragment that was up-regulated under LD. This fragment was homologous to a gene encoding methionine synthase (MS) in soybean and was named *GmMS*. The RNA content confirmed that *GmMS* was expressed in roots, stems, and leaves of soybean grown under LD. The highest expression was in stems. Full length *GmMS*, encoding 763 amino acids, was transferred into tobacco plants. The ectopic expression of *GmMS* in tobacco resulted in delayed flowering. Other effects included stunting, an increased MS activity and methionine content, a higher content of alcohol-soluble proteins and of chlorophylls, and a lower content of anthocyanins.

Additional key words: anthocyanin, chlorophyll, ectopic expression, *Glycine max*, photoperiod response, protein.

Introduction

Flowering represents the transition from the vegetative to reproductive phase in plants. This process is regulated by numerous endogenous and environmental signals in an intricate network of genetic and protein interactions (Simpson *et al*. 1999, Jung and Müller 2009, Andrés and Coupland 2012). Photoperiod is one essential cue affecting flowering time (Thomas and Vince-Prue 1997). There are three major photoperiod responses: short-day (SD) plants, long-day (LD) plants, and day-neutral (DN) plants (Jackson 2009). Several key molecular elements are involved in the photoperiod regulatory pathway, such as CONSTANS (CO), FLOWERING LOCUS T (FT), and TWIN SISTER OF FT (TSF) (Jung and Müller 2009). CO is regulated at the transcriptional level by circadian clock associated genes and at the protein level by photoreceptors (Izawa *et al*. 2002, 2003, Hayama *et al*. 2003, Hayama and Coupland 2004, Hecht *et al*. 2005). High *CO* transcript accumulation and high CO protein stability coincide at the end of light phase to induce flowering under LD in *Arabidopsis* (Valverde *et al*. 2004, Jackson 2009). However, the *CO* ortholog *HEADING DATE 1* (*Hd1*) stimulates flowering by promoting the expression of the *FT* ortholog *Hd3a* under SD, whereas it represses *Hd3a* under LD in the SD plant *Oryza sativa* probably by post-translational modification of Hd1 (Izawa 2000, Kojima *et al*. 2002). *CO* and *FT* expressions can be influenced by environmental conditions, *e.g.*, by cadmium (Wang *et al.* 2012). In addition to *CO*, other genes are involved in flowering regulation, such as vernalization requirement (VR) gene *VRN1,* and MADS-box genes

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Abbreviations: AFLP - amplified fragment length polymorphism; AP1 - APETALA1; CaMV - cauliflower mosaic virus; CDS - coding DNA sequence; CGS - cystathionine γ-synthase; CO - CONSTANS; CRY1a - cryptochrome 1a; FT - FLOWERING LOCUS T; GFP - green-fluorescent protein; Gm - *Glycine max*; Hd1 - HEADING DATE 1; LD - long-day; MS - methionine synthase; NP - neutral photoperiod; PHYB1 - phytochrome B1; RAV- related to ABI3/VP1 gene; RT- PCR - reverse transcription polymerase chain reaction; SAMS - S-adenosylmethionine synthase; SD - short-day; TSF - TWIN SISTER OF FT; VR - vernalization requirement; ZD24 - ZhongDou 24; ZTL3 - ZEITLUPE 3
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(Ergon *et al*. 2013).

Soybean is a typical SD plant and SD is a critical regulatory factor for flowering induction which has been extensively studied (Garner and Allard 1920, Bernier *et al*. 1981, Liu *et al*. 2009). The seven loci controlling flowering belong to the *E*-gene series (Bernard 1971, Buzzell 1971, Buzzell and Voldeng 1980, McBlain 1987, Cober 2001). Several genes controlling flowering have been identified in soybean. Up-regulation of *cryptochrome 1a* (*CRY1a*), *Phytochrome B1* (*PHYB1*), *FT* orthologs, and *APETALA1* (*AP1*), and down-regulation of *RAV* (the relative to *ABI3/VP1* gene) can promote early flowering (Zhang *et al*. 2008, Thakare *et al*. 2010, 2011, Chi *et al*. 2011, Wu *et al*. 2011, Zhao *et al*. 2012), whereas up-regulation of *GmRAV*, *ZEITLUPE 3* (*ZTL3*), and *E1* genes delay flowering (Zhao *et al*. 2008, Xia *et al*. 2012, Xue *et al*. 2012). *GmFT2a* and *GmFT5a* coordinately control flowering and enable the adaptation of soybean to a wide range of photoperiodic environments (Kong *et al.* 2010).

Methionine synthase (MS) catalyzes the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine, generating H4-folate and methionine (Banergee and Matthews 1990). Methionine is an essential amino acid for protein synthesis. It also functions as the precursor of S-adenosylmethionine, the primary methyl-group donor and the precursor of polyamines and ethylene. It regulates many aspects of plant growth, such as cell proliferation and differentiation, apoptosis, and homeostasis (Ravanel *et al*. 1998, Amir 2010). Increased or decreased methionine content leads to abnormal plant phenotypes.

We identified a photoperiod-sensitive soybean cultivar ZD24 which flowered after 22 d under SD, after 24 d under a 13.5-h photoperiod, and after 53 d under LD (Yang *et al*. 2001). In this study, we used amplified fragment length polymorphism (AFLP) to screen differentially expressed genes in ZD24 when subjected to different day-lengths. From the differentially expressed genes, one encoding methionine synthase delayed flowering under LD conditions in transgenic tobacco.

Materials and methods

Plants and cultivation: Seeds of soybean [*Glycine max* (L.) Merr] cv. ZD24 were grown in the field of the Institute of Oil Crops Research, CAAS, Wuhan, China. After the appearance of the first trifoliate leaf, plants were transferred to greenhouses and grown in pots under SD (an 8-h photoperiod), NP (a 13.5-h photoperiod), or LD (a 16-h photoperiod) (Yang *et al*. 2001). An irradiance was 114 μmol m^{-2} s^{-1}, a temperature of 25 - 28 °C, and a relative humidity of 50 %. There were three independent repetitions of this process. After 10 d, young leaves from 5 plants grown under each photoperiod were collected at 10 a.m., immediately frozen in liquid nitrogen, and maintained at -80° C until used for AFLP and reverse transcription polymerase chain reaction **(**RT-PCR) analyses.

Isolation of total RNA, and AFLP analysis: Total RNA was extracted using a *Tripure* isolation reagent (*Roche Applied Science*, Rotkreuz, Switzerland) according to the manufacturer's instructions. RNA extracted from leaves was subjected to the cDNA-AFLP analysis. The synthesis of double-stranded cDNA was performed using a *SMART*™ cDNA synthesis kit (*Clontech*, Palo Alto, CA, USA). The cDNA-AFLP analysis was performed as described by Bachem *et al*. (1996). Double-stranded cDNA (500 ng) was used and the restriction enzymes were *Eco*RI and *Mse*I (*Fermentas Life Sciences*, Vilnius, Lithuania). For pre-amplifications, *Mse*I primer and *Eco*RI primer without selective nucleotides were combined. The amplification mixtures were diluted 100-fold, and 0.005 cm^3 was used for final selective amplifications. All 256 possible primer combinations with 2 additional selective nucleotides were used for transcript profiling. Selective amplifications were resolved on 6 % sequencing gels followed by visualization with silver staining (Chalhoub *et al*. 1997).

Differentially expressed fragments were excised from the gel with a razor blade. The gel slices were hydrated in 0.1 cm^3 of water and incubated at 95 °C for 15 min. Eluted DNA was amplified using the same primers and conditions as used for the selective amplification. Sequence information was obtained by cloning the fragments in pMD-18 vector (*Takara*, Dalian, China) and by sequencing individual clones.

Plasmid construction and the transformation of tobacco: A search of the soybean genome database (http://www.phytozome.net/soybean) with the nucleic acid sequence of a 1 280 bp differentially expressed fragment identified from the cDNA-AFLP analysis yielded the sequence of *Glyma16g04240* encoding methionine synthase. Primers 5'-ATGGCATCT CACATCGTTGG-3' and 5'-GCAACGAACTTGCC AAGTGA-3' were designed to amplify the coding sequence (CDS) region of *Glyma16g04240*. The amplicons were inserted into pGWC vector (Chen *et al*. 2006) and confirmed by sequencing. The ectopic expression vector *GmMS*-pK7WG2D was established by an LR recombination reaction according to the *Gateway*® cloning protocol (*Invitrogen*, Carlsbad, CA, USA). The vector pK7WGD2 contains the GATEWAY site between the 2X cauliflower mosaic virus (CaMV) 35S promoter and 35S terminator which is replaced by the target fragment. It also contains the rolD promoter fused to the coding sequences of the enhanced green-fluorescent protein (GFP) linked to the endoplasmic reticulum-targeting signal (EgfpER) and 35S terminator (Karimi *et al*. 2002). The resultant construct *GmMS*-pK7WG2D was introduced into *Agrobacterium tumefaciens* GV3101 *via* the freeze-thaw method, and then introduced into

tobacco (*Nicotiana tabacum* L. cv. Samsung) by the leaf disc transformation method (Horsch *et al*. 1985). The empty vector pK7WG2D was introduced into tobacco as negative control. Transformants were selected by growing on a Murashige and Skoog medium containing 100 mg dm^{-3} kanamycin. Tobacco transgenic and wild-type plants were grown in a growth room at LD and other conditions mentioned above. The transgenic progeny was grown in a greenhouse at a 13.5-h photoperiod and the time until the appearance of the first flower was recorded.

Homologs of *Glyma16g04240* were obtained by searching in the *NCBI* databases using *BlastX* (http://www.ncbi.nlm.nih.gov/). Multiple alignments of the sequences were conducted with *Clustal X v. 2.0* and viewed with *GeneDOC*. A phylogenetic tree was constructed using *MEGA 4* using the neighbor-joining method (Tamura *et al*. 2007).

RT-PCR analysis: Total RNA was firstly treated with DNase I (*Promega*, Madison, WI, USA) and 2 µg of it was used in combination with *M-MLV* reverse transcriptase (*Promega*) to synthesize the first strand cDNA following the manufacturer's instructions. A volume of 0.002 cm^3 of 1:10 diluted first strand cDNA was used as template in a 0.02-cm^3 reaction mixture. Gene-specific primers 5'-TGTCCATGCCTTCAGAATCA-3'and 5'-AGTCAG GGTTCACCCACAAG-3' were used for the RT-PCR analysis. Primers 5'-CCCTCCCACATGCTATTCT-3' and 5'-AGAGCCTCCAATCCAGACA-3' were amplified for *actin* to normalize the reaction. All reactions were carried out in a *PTC-200* DNA engine cycler (*Bio-Rad*, Hercules, CA, USA).

Determination of MS activity and content of amino acids, proteins, chlorophylls, and anthocyanins in T_0 transgenic plants: Leaves of T_0 transgenic plants were scraped, pelleted, and frozen at -80 °C prior to assay for MS activity. Approximately 200 mg of leaf powder was resuspended in 0.8 cm^3 of a homogenization buffer containing 50 mM KH_2PO_4 (pH 7.2), 10 mM dithiotreitol, and 100 µM $MgSO_4$. The homogenate was centrifuged at 1 200 *g* for 5 min. Assays were performed under anaerobic conditions as described by Jarrett *et al*. (1997). Each 1 cm^3 of the reaction mixture was transferred to a 1.5 cm^3 microcentrifuge tube and centrifuged at room temperature at 12 000 g for 5 min to precipitate proteins. Absorbance was then measured at 350 nm using a spectrophotometer *UV-1800* (*Shimadzu*, Kyoto, Japan).

For analysis of free amino acids, polar metabolite fractions were extracted from a 30-mg leaf sample as described by Nikiforova *et al.* (2005). The samples were dried in *Speed-vac* (*SPD1010*, *Thermo Fisher Scientific*, Waltham, MA USA). Then 0.1 cm^3 of 6 M HCl was added to the dried samples and incubated at 100 °C with shaking at 500 rpm for 16 h. The solvent was then evaporated at 100 °C. To the dry material, 0.075 cm^3 of methanol, 0.025 cm^3 of chloroform, and 0.1 cm^3 of water were added and mixed by vortexing. The mixtures were centrifuged at 12 000 *g* and 4 °C for 5 min, and the upper phase dried in the *Speed-vac*.

Derivatization and metabolite analysis of free amino acids was carried out as described by Nikiforova *et al.* (2005). Ribitol was used as internal standard. Samples were injected in splitless mode (0.001 cm^3 per sample) and analyzed using a *Waters 2695* amino acid analyzer (Milford, MA, USA).

To analyze water and alcohol soluble proteins, a total of 100 mg leaf tissue was ground in liquid nitrogen. The powder was mixed vigorously with 0.4 cm^3 of 0.1 M Na-phosphate buffer (pH 7.8), followed by centrifugation at 12 000 *g* and 4 °C for 30 min. The concentration of total water-soluble proteins in the supernatant was determined by the Bradford protein assay (Bradford 1976). The alcohol-soluble protein fraction was extracted from the pellet using 0.4 cm^3 of a solution containing 70 % (v/v) ethanol and 1 % (v/v) 2-mercaptoethanol by incubation at 65 °C for 30 min with shaking at 1000 rpm. The extracts were centrifuged at 12 000 *g* and 4 °C for 30 min (Dancs 2008).

Chlorophylls were extracted from 60 mg of seedling tissue collected in pools of 5 - 6 leaves using 1 cm^3 of dimethylformamide. Absorbance values were measured at 647 nm (A_{647}) and 664.5 nm ($A_{664.5}$) on a *UVIKON 942* spectrophotometer (*KONTRON Instruments*, Milan, Italy) in 1-cm cuvettes. The total chlorophylls were calculated using the formula (17.90 A_{647} + 8.08 $A_{664.5}$)/60 (Inskeep and Bloom 1985).

Anthocyanins were extracted from 1 g of leaves with 10 cm^3 of 1 % (m/v) HCl in methanol at 4 °C for 12 h. Concentrations of the chloride forms of the anthocyanin pigments were determined spectrophotometrically by measuring the absorbance at 540 nm.

Statistics: The analysis of variance (*ANOVA*) was performed using the *SAS* package, and means were compared using the Tukey's test.

Results

The seeds of ZD24 were sown on April 14, 2010 and grown under SD, NP, and LD photoperiods. The first ternate compound leaf was observed on April 23. Flowering was observed and recorded every 2 d. The emergence of the first flower of ZD24 under SD, NP, and LD occurred at 24, 26, and 55 d after sowing. Compared to NP, the flowering time was not significantly affected by SD, but was significantly delayed by LD.

As the flowering time of ZD24 under LD was much later than under SD and NP, we considered that LD might induce changes in the expression of genes involved in the regulation of the transition from the vegetative to generative phase. One 280 bp fragment was markedly up-regulated in the leaves of soybean under LD. This was

amplified from the primers combination *Eco*RI+TA/*Mse*I+GG using cDNA-AFLP (Fig. 1). After sequencing and a search of the *NCBI* database, the fragment was found to be 99 % homologous to the gene encoding methionine synthase of *Glycine max* (NP_001235794.1). Therefore, the fragment was named *GmMS*.

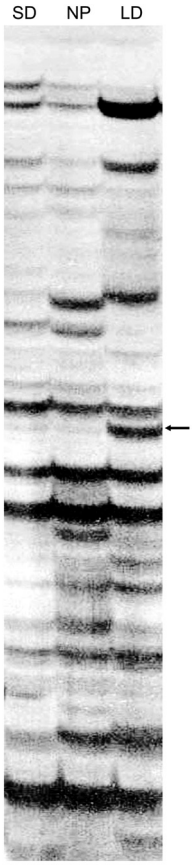

Fig. 1. The expression of a 280 bp fragment detected *via* cDNA-AFLP in leaves of cv. ZD24 grown under different day-lengths (SD - short day, NP - neutral photoperiod, LD - long day). The *arrow* indicates the differential expression of the 280 bp fragment. The primers combination used in cDNA-AFLP was *Eco*RI+TA/*Mse*I+GG.

To validate the expression pattern of *GmMS*, semi-quantitative RT-PCR was used to measure its expression in leaves of ZD24 under SD, NP, and LD. This analysis revealed that the *GmMS* expression was up-regulated in the leaves of ZD24 grown in LD (Fig. 2*A*). This coincided with the cDNA-AFLP analysis.

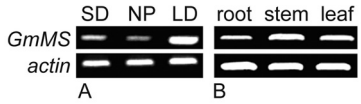

Fig. 2. The expression of *GmMS* detected by semi-quantitative RT-PCR. *A* - The expression of *GmMS* in the leaves of ZD24 growing under SD, NP, and LD. *B* - The expression of *GmMS* in roots, stems, and leaves of ZD24 growing under LD. *Actin* is loading control. Twenty-eight and 35 PCR cycles were run for *actin* and *GmMS*, respectively.

To further investigate whether *GmMS* is expressed in a tissue-specific pattern, total RNA was extracted from the leaves, roots, and stems of ZD24. The semi-quantitative RT-PCR analysis shows that *GmMS* was expressed in all the detected tissues and the highest expression was found in the stems (Fig. 2*B*).

The total length of *GmMS* was 2292 bp and the sequence encoded 763 amino acids (Fig. 1 Suppl.). The bioinformatic analysis of the sequence indicated that GmMS was highly hydrophilic protein, had no trans-membrane domain, no signal peptide, and no disulfide bonds. GmMS contained two highly conserved domains, the tetrahydrofolate binding domain and the catalytic domain. Phylogenetic analyses show that GmMS clustered closely with the methionine synthase protein of *Cicer arietinum* to form a branch. It also closely clustered to many other related plant species (Fig. 3).

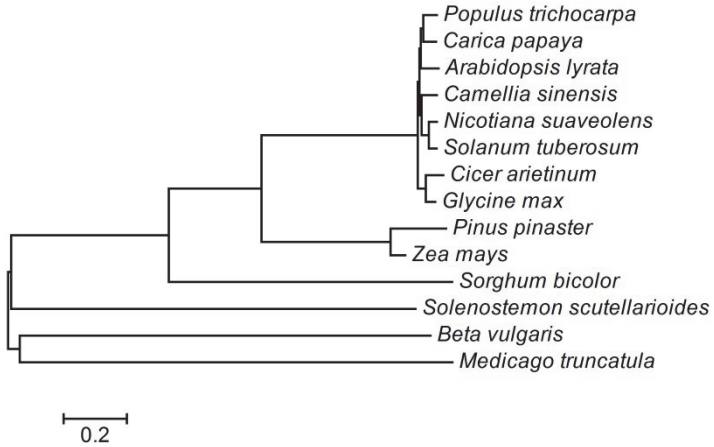

Fig. 3. Phylogenetic relationships between methionine synthase from *Glycine max* and other species: *Pinus pinaster* (acc. No. CCC42221.1), *Sorghum bicolor* (AF466201_8), *Zea mays* (AAL33589.1), *Beta vulgaris* (BAE07181.1), *Solenostemon scutellarioides* (CAA89019.1), *Arabidopsis lyrata* (XP_002871787.1), *Medicago truncatula* (XP_003624677.1), *Nicotiana suaveolens* (ABI95860.1), *Camellia sinensis* (AEO86798.1), *Populus trichocarpa* (XP_002325856.1), *Solanum tuberosum* (AAF74983.1), *Carica papaya* (ABS01352.1), and *Cicer arietinum* (ACL14488.1).

To investigate the effect of *GmMS* expression on flowering time, the ectopic expression vector GmMS-pK7WG2D was introduced into tobacco through *Agrobacterium*-mediated transformation. A total of 20 kanamycin resistant plants were obtained, 13 of which were identified by PCR as containing the expression vector sequence. The semi-quantitative RT-PCR analysis showed that two tobacco transgenic lines had the *GmMS* expression enhanced compared to the control. The time to flowering of the control wild type plants was 91 d compared to 138 and 154 d for the transgenic MS2 and MS4 lines, respectively (Fig. 4).

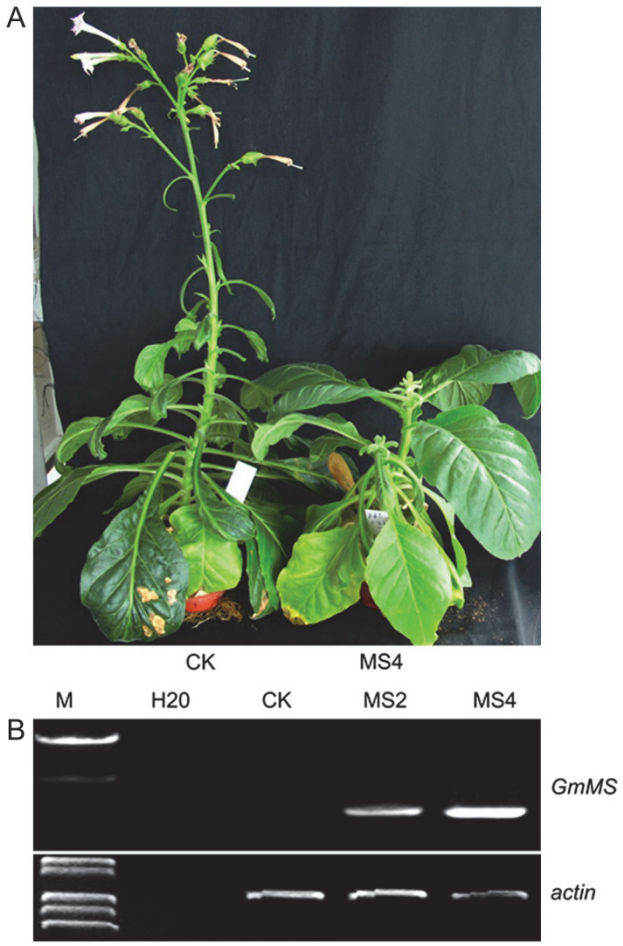

Fig. 4. The flowering phenotype (*A*) and RT-PCR analysis of *GmMS* expression (*B*) in a T_0 transgenic tobacco plant. CK - a control plant transformed with a blank vector, M - a DNA marker; H_2O - a blank control, MS2 and MS4 - transgenic tobacco lines. *Actin* is loading control. Twenty-eight and 35 PCR cycles were run for *actin* and *GmMS*, respectively.

Table 1. Time from sowing to the first flower appearance in T_1 transgenic tobacco plants. MS4-2 to MS4-17 were derived from the MS4 T_0 line. CK - control plants transformed with a blank vector.

Lines	Flowering time [d]	PCR confirmation
CK	94	+
MS4-2	124	+
MS4-4	124	+
MS4-5	129	+
MS4-7	116	+
MS4-6	106	+
MS4-3	92	-
MS4-8	96	-
MS4-13	96	-
MS4-15	97	-
MS4-16	99	-
MS4-17	96	-

The T_0 MS2 transgenic plant was accidently destroyed after flowering time, so the measurement of flowering time for the T_1 plants is unavailable. The flowering time for the T_1 transgenic plants of MS4 was measured to investigate whether the phenotype can be inherited. Out of 11 MS4 T_1 transgenic plants, 5 plants (MS4-2, MS4-4, MS4-5, MS4-6, and MS4-7) containing the transgene had flowering delays of 12 to 35 d compared to the control plants (Table 1). The flowering time was not significantly different among the other five transgenic plants in which no transgene was identified (Table 1). These results show that the ectopic expression of *GmMS* in tobacco delayed flowering time.

The MS activity increased 6- to 7-fold in the transgenic lines MS2 and MS4 (Table 2). The content of free methionine in the MS2 and MS4 lines was 1.4- and 1.8-fold higher, respectively, than that of the control plants (Table 2). The analyses of alcohol-soluble proteins and

Table 2. The content of anthocyanin [ng g^{-1}(f.m.)], chlorophyll, methionine, alcohol-soluble protein, and water-soluble proteins, and the MS activity in leaves of T_0 transgenic tobacco lines MS2 and MS4. CK - control plants transformed with a blank vector. Means ± SE, $n = 3$, different letters indicate significant differences at $P \leq 0.01$ (the Tukey's test).

Treatment	Anthocyanin [ng g^{-1}(f.m.)]	Chlorophyll [μg dm^{-2}]	MS activity [pmol g^{-1}(f.m.) min^{-1}]	Free methionine [pmol g^{-1}(f.m.)]	Alcohol-soluble proteins [mg g^{-1}(f.m.)]	Water-soluble proteins [mg g^{-1}(f.m.)]
MS2	35 ± 1.0 a	32 ± 1.0 a	1.75 ± 0.10 b	4.6 ± 0.1 b	7.0 ± 1.6 a	5.0 ± 0.0 b
MS4	45 ± 2.0 b	31 ± 0.3 a	2.10 ± 0.30 a	5.7 ± 0.4 a	9.0 ± 0.5 a	6.3 ± 0.5 a
CK	88 ± 2.0 c	12 ± 2.0 b	0.30 ± 0.01 c	3.2 ± 0.4 c	1.4 ± 0.2 b	3.6 ± 0.1 b

water-soluble protein show that the content of total proteins of the MS2 and MS4 was higher than that of the control, and the proportion of alcohol-soluble proteins in the total proteins increased more than 2-fold in both the transgenic lines (Table 2). The chlorophyll content also increased significantly in the transgenic lines, to a level about
2.5 times greater than in the control plants (Table 2). The anthocyanin content in the transgenic tobacco lines decreased significantly, with more than a 50 % reduction in both the transgenic lines (Table 2).

Discussion

In this study, we examined the gene expression of the photoperiod-sensitive soybean cv. ZD24 under different day-lengths. The analysis of 10-d-old plants using cDNA-AFLP revealed a fragment that encoded methionine synthase. Semi-quantitative RT-PCR indicates that the expression of *GmMS* was up-regulated when the plants were grown under LD (Fig. 2*A*), and its expression was highest in stems (Fig. 2*B*). The flowering time was delayed and the plants were dwarf in the *GmMS* overexpressing transgenic tobacco plants (Fig. 4, Table 1). The transcription of *GmMS*, the MS activity, as well as the methionine content in tobacco leaves increased (Fig. 4*B*, Table 2). These results suggest that an increased expression of *GmMS* can affect the flowering time in plants.

Methionine regulates many aspects of plant growth, such as cell proliferation and differentiation, apoptosis, and is involved in methyl-group transfer and syntheses of proteins, ethylene, polyamines, biotin, and chlorophylls (Ravanel *et al*. 1998, Amir 2010). A previous analysis demonstrated that an increase or decrease of methionine content in plant cells results in abnormal plant development. Changes in methionine content by increasing cystathionine γ-synthase (CGS) or decreasing S-adenosylmethionine synthase (SAMS) produce abnormal phenotypes, such as stunted growth, changes in leaf architecture, size, and shape, a slow developmental rate, loss of apical dominance, deformed flowers, numerous apical shoots, and a reduction of anthocyanin content (Boerjan *et al*. 1994, Hacham *et al*. 2002, Kim *et al*. 2002, Dancs *et al*. 2008). Reduced methionine content by down-regulating *CGS* also lead to severe growth retardation with reduced apical dominance and stacked floral organs, as well as decreased chlorophylls content in *Arabidopsis* (Gakiere *et al*. 2000a,b, Kim and Leustek 2000). In our work, the transgenic lines with the increased MS activity and methionine content not only showed the delayed flowering time but also dwarfism. The modification of *GmMS* transcription also increased the ethanol-soluble proteins and chlorophylls content and decreased the anthocyanin content. The results indicate that MS affected many facets of plant development and metabolism, in addition to the flowering time.

MS is the final enzyme in the methionine biosynthesis pathway catalyzing the methylation of homocysteine to methionine with 5-methyltetrahydrofolate as methyl group donor. It also serves to generate the methyl group of S-adenosylmethionine (SAM; Amir 2010), and 80 % of methionine is involved in the formation of SAM (Ravanel *et al*. 1998). Reports in several other plant species indicate that MS is involved in the massive turnover of methionine in the methyl transfer reaction through SAM and the methyl cycle (Amir 2010). SAM is precursor for the biosynthesis of ethylene which is involved in many aspects of plant growth and development (Abeles *et al*. 1992). Ethylene accelerates flowering in *Arabidopsis thaliana*, pineapple, and rice. Reduction of ethylene production by regulating the expressions of the genes involved in the ethylene signal pathway delays flowering (Ogawara *et al*. 2003, Trusov and Botella 2006, Wuriyanghan *et al*. 2009). In our study, the overexpression of *GmMS* led to delayed flowering in transgenic tobacco. Further investigation will be required to determine if the delayed flowering observation in our study is associated with the change of ethylene content since MS can regulate SAM which is precursor for the ethylene biosynthesis.

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