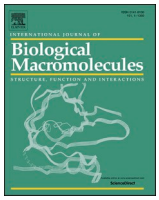
International Journal of Biological Macromolecules 245 (2023) 125464

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



A novel MORN-motif type gene *GmMRF2* controls flowering time and plant height of soybean

Jialing Zhang [1], Li Chen [1], Yupeng Cai, Qiang Su, Yingying Chen, Min Li, Wensheng Hou [*]

National Center for Transgenic Research in Plants, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

ARTICLE INFO

Keywords:
GmMRF2
Flowering time
Plant height

ABSTRACT

The flowering time of soybean is a highly important agronomic characteristic, which affects the adaptability and yield. *AtMRF1*, a MORN-repeat motif gene, acts as a floral promoter in *Arabidopsis*, its functions in soybean are not yet understood. Here, we employed qRT-PCR to analyze the tissue expression patten of *MRF1* homologs in soybean and determined that the *GmMRF2* gene, containing a MORN-motif, highly expressed in the shoot and responded to photoperiod. *GmMRF2* overexpression soybean lines exhibited earlier flowering time under long-day (LD) conditions, and increased plant height under both LD and short-day (SD) conditions compared to wild-type (WT) plants. The expression levels of gibberellic acid (GA) pathway genes that positively regulate plant height genes and flowering-promoting genes were up-regulated in the *GmMRF2* overexpression lines, were up-regulated in the *GmMRF2* overexpression lines. Further study revealed that *Gm*MRF2 interacted with *GmTCP15* to co-induce the expression of *GmSOC1b*. Together, our results preliminarily reveal the functions and mechanisms of *GmMRF2* in regulating flowering time and plant height, provide a new promising gene for soybean crop improvement.

1. Introduction

Soybean (*Glycine* max L.) is one of the world's most vital sources of protein, oil, and livestock feed [1]. As a short-day crop, soybean is particularly sensitive to photoperiod. Day length is the key factor in determining soybean flowering time-a short-day stimulates flower bud formation, whereas long-day inhibits flowering. This growing area spans nearly 30 degrees from ~20° N to ~50° N in the northern hemisphere [2]. Unfortunately, soybean is generally limited to a very narrow range of latitudes to attain the highest yields possible [3]. This limitation makes expanding the regional adaptability of soybean the key to revitalizing the soybean industry.

Flowering time is a key agronomic trait that contributes to the ability of a plant to adapt to different climates. Four major pathways have been reported to control floral transition in *Arabidopsis thaliana*, including the photoperiod, vernalization, autonomous, and GA pathways [4]. These processes converge on the integrators, then transmit these signals to floral identity genes for determining the start of flowering [5]. Furthermore, emerging evidence shows that miRNA and transposable elements also have a profound impact on flowering time [6,7]. To date, multiple genetic loci have been reported to affect flowering and maturity in soybean, such as *E1-E11* and *J*, *GmTof4*, *GmTof5*, *GmTof11*, *GmTof12*, *GmTof16*, and *GmqDTF-J* [8–11]. Of these, the soybean-specific gene *E1* is the most important gene affecting flowering and maturity [12]. *E2*, an ortholog of *Arabidopsis GI* (*GIGANTEA*), plays major roles in photoperiodic flowering and yield in soybean [13]. *E3* and *E4* were identified as homologs of *phytochrome A* (*GmPHYA3* and *GmPHYA2*, respectively), and act as major flowering promotors by activating the expression of *AP1* (*APETALA1*), *LFY2* (*LEAFY 2*) and *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CO 1*) [14,15]. Recently, several studies have characterized *FT* homologs in soybean. The *GmFT1a* was identified as a flowering inhibitor, induced by *E1* to delay flowering and maturity; *GmFT2b* was found to accelerate flowering under LD conditions; GmFT2a and GmFT5a redundantly promoted flowering through interacting with GmFDL12 and GmFDL19 [16–19]. In addition, the FT-FD complex was reported to stimulate the expression of flowering-related genes (including *FUL* (*FRUITFULL*), *SOC1* and *LFY*), induce *AP1* to initiate flowering [20,21]. In soybean, *AP1* homologs were reported to involve in regulation of flowering time and plant height [22].*GmSOC1a* and *GmSOC1b*, two homologs of *SOC1*, belong to the

* Corresponding author.
E-mail address: houwensheng@caas.cn (W. Hou).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ijbiomac.2023.125464
Received 5 March 2023; Received in revised form 9 June 2023; Accepted 13 June 2023
Available online 20 June 2023

MADS-box gene family, have been reported to contribute to floral induction through inter-regulation with *GmFT* genes [23].

In *Arabidopsis*, a novel *MRF1* (MORN-MOTIF REPEAT PROTEIN REGULATING FLOWERING 1) gene, was found to be significantly induced in the PCCs (phloem companion cells) when responding to LD conditions. The *MRF1* gene was identified as a floral promoter which induces the expression of *FT* in *Arabidopsis* [24]. The PCCs coordinated the physiological processes between the vasculature and other cell types in the leaf, and were believed to control systemic plant growth and development through the integration of information about carbohydrate availability and photoperiod [25,26]. For example, the PCC-specific proteins mediated the transportation of FT proteins (acting as long-distance signals or florigens) through SEs (sieve elements) to the SAM (shoot apical meristem), and then interacted with the basic Leu zipper transcription factor FD to induce flowering [27,28]. In addition, *BrMORN,* a gene composed of seven C-terminal MORN motifs in Chinese cabbage, could enhance the growth rate, induce acchypocotyl elongation, and increase vegetative organs size and seed productivity of *Arabidopsis* [29].

Until now, the function of *MRF* gene in soybean has not been reported. Here, we cloned a MORN motif gene, *GmMRF2*, which was involved in accelerating flowering and increasing plant height of soybean. The following study verified that GmMRF2 could interact with GmTCP15 to induce the expression of *GmSOC1b*. Our findings identified a novel type of soybean gene which promotes flowering and increases plant height, provided an insight for researching the molecular mechanisms of *GmMRF2*, and a new genetic resource for soybean adaptability and architecture improvement.

2. Materials and methods

2.1. Plant materials and cultivation

The soybean cultivar 'Jack' was used for RNA extraction, gene cloning, plant transformation, and phenotype measurement. All soybean plants were grown in a culture room maintained at 22–30 °C, with two photoperiods: SD (12 h light/12 h dark) and LD (16 h light/8 h dark).

The tissue expression analysis was performed using soybean plants after the flowering stage under LD conditions, and several tissue types were samples including root, leaf, stem, shoot, bud, and flower. For diurnal expression pattern analysis, the 12 DAE (days after emergence) soybean leaves of early-flowering variety HH27 (Heihe 27) and late-flowering variety ZGDD (Zigongdongdou) were sampled at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h after light up under SD and LD conditions, respectively.

For expression analysis of flowering-related genes, the samples were taken at 4 h after light under SD (15 DAE) and LD (25 DAE), respectively. The shoot apices were used for examining the flowering-related genes, including *GmMRF2*, *GmAP1a/b/c, GmSOC1a/b*, *GmFUL1a/b*, *GmLFY2*, and the leaves were used for examining the *GmFT2a* and *GmFT5a.* For expression analysis of GA metabolic pathway-related genes, the leaves were sampled at 4 h after light under SD (15 and 25 DAE) and LD (25 and 40 DAE), respectively. DAE, days after emergence.

2.2. RNA extraction and qRT-PCR

Total RNA was extracted using a TransZol Up plus RNA kit (Transgen, China). A reverse transcription kitHiScript® III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme, China) was used for reverse transcription of soybean RNA according to its operating procedures. Real-time quantitative PCR was performed using the method provided by ChamQ SYBR qPCR Master Mix (Vazyme, China). The primers information for qRT-PCR are listed in Supplementary Table S2. There were three replicates for each individual. The $2^{-\Delta\Delta Ct}$ method was used for data analysis.

2.3. Plasmid construction and plant transformation

In-Fusion HD Cloning kits were used to amplify the cDNAs of *GmMRF2* (*Glyma.03G106900*) and *GmTCP15* (*Glyma.13G089200*). The amplified cDNAs were then inserted into the pTF101 vector which contained a CaMV35S promoter (pTF101-GmMRF2) (Takara, Japan). The pTF101-GmMRF2 vector was transformed into EHA101 (Zhuangmeng, China), and then introduced into the 'Jack' soybean cultivar by agroinfiltration. Positive overexpression in soybean plants was detected by using PCR to amplifying the *Bar* gene, and determining the presence of the PAT protein by LibertyLink strips. Primers for plasmid construction are listed in Supplementary Table S1.

2.4. Subcellular localization of GmMRF2

The full length of *GmMRF2* CDS was constructed into pTF101 and formed a recombinant vector, with a GFP (green fluorescent protein) label (GmMRF2-GFP). The recombinant plasmid was transiently introduced into GV3101, an Electroporation Competent Cell (Zhuangmeng, China), and then injected into *N. benthamiana* as described by Yoo [30]. The FV3000 confocal microscope (Olympus, Japan) was used for GFP fluorescence imaging.

2.5. Phenotyping and statistical analysis

The flowering time was recorded from time of plant emergence to the R1 stage (the time of appearance of the first flower at any node). The plant height was recorded as the distance between root base and the top of the main stem [31]. Statistical analysis and significance tests were performed with SPSS (version 19.0).

2.6. Yeast two-hybrid system and library

Total RNA was extracted from soybean shoot, leave, bud, flower, stem, and root at the flowering stage to create the AD library (Oebiotech, Shanghai). The fragment of *GmMRF2* CDS was fused with pGBKT7 (GmMRF2-BD), and the resulting GmMRF2-BD was transformed into yeast strain AH109 and used as bait for yeast two-hybrid screening. The yeast two-hybrid screening assay was carried out by the Yeast Protocols Handbook (Clontech, USA). For the yeast two-hybrid assay, the full length of the *GmTCP15* coding sequence was fused with pGADT7 (GmTCP15-AD), then GmTCP15-AD and GmMRF2-BD were co-transformed into yeast strain Y2HGold. pGBKT7 and pGADT7 were used as negative controls. Transformants were grown on SD/-Trp/-Leu and SD/-Trp/-Leu/-His/-Ade yeast culture media. The primers used for yeast two-hybrid are listed in Supplementary Table S1.

2.7. Luciferase complementation imaging assay (LCI)

The CDSs full length of *GmMRF2* and *GmTCP15* were constructed in the N-terminal fragment of luciferase (nLUC) and the C-terminal fragment of luciferase (cLUC), respectively. The two recombined vectors were co-transformed into *A. tumefaciens* strain GV3101, and then infiltrated into roughly 28-day-old *N. benthamiana* leaves. After 48 h of culture, the beetle luciferin treatment was conducted following detection the light by CCD imaging apparatus (NightShade LB 985, Berthold). The primers used for LCI assay are listed in Supplementary Table S1.

2.8. Pull-down assay

Full-length CDS of *GmMRF2* was cloned into the pGEX-4T-1 vector (GST-GmMRF2), and the *GmTCP15* CDS was fused with pMAL-C5X vector (MBP-GmTCP15). MBP-GmTCP15 and GST-GmMRF2 recombinant vectors were transformed into BL21 competent cells, and the pull-down reaction was performed as described in the protocol. The purified proteins MBP/MBP-GmTCP15 and GST-GmMRF2 were incubated with

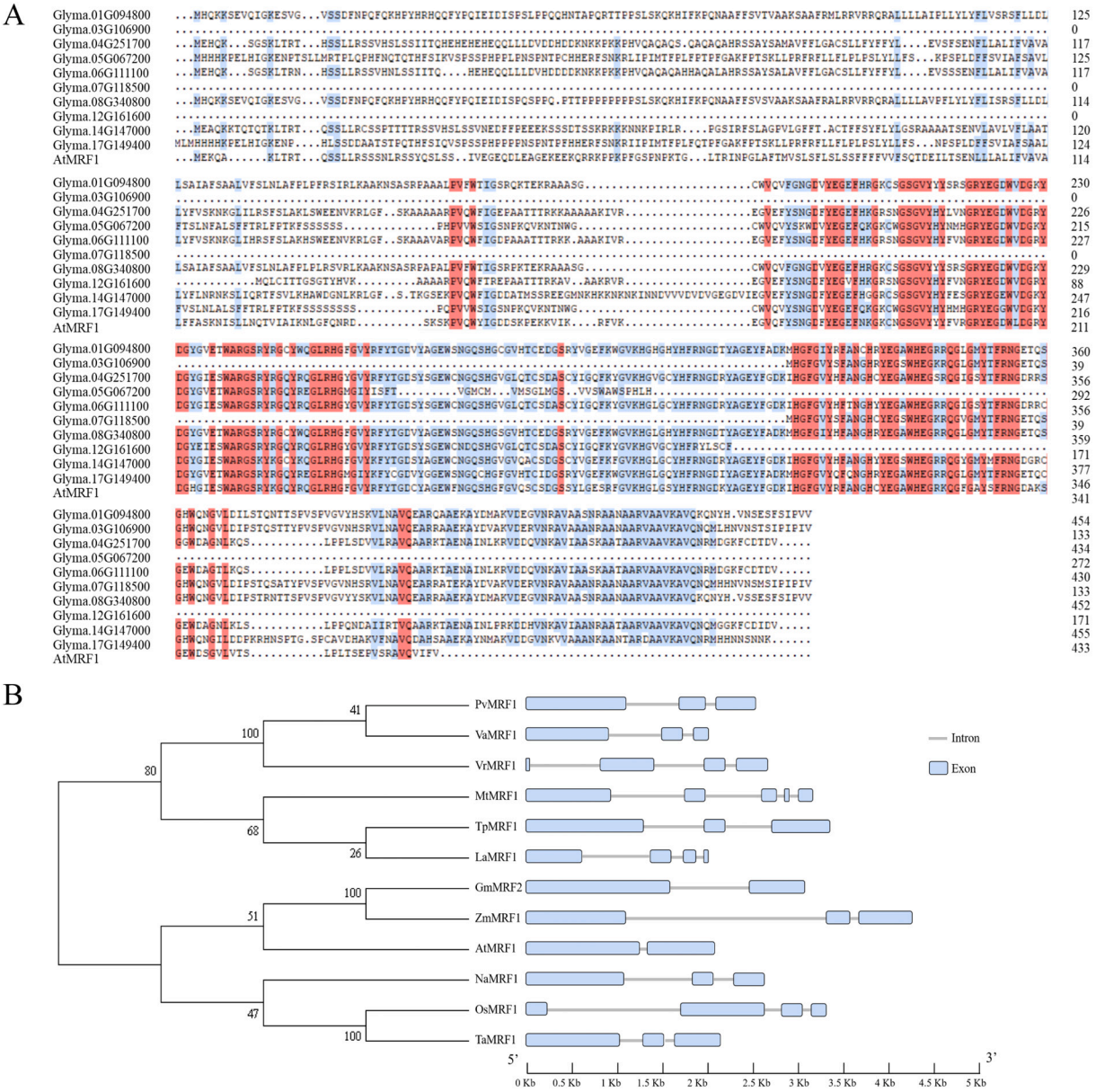


Fig. 1. Identification and sequence analysis of GmMRF2. (A) Protein sequence alignment of GmMRF2 with MRF2 of other species; AtMRF1: AT1G21920. (B) Phylogenetic and structure analysis of *MRF* homologs in different species; Pv, *Phaseolus vulgaris* L.; Va, *Vigna angularis*; Vr, *Vigna radiata*; Mt., *Medicago truncatula*; Tp, *Trifolium pratense* L.; La, *Lupinus angustifolius* L.; Gm, *Glycine* max L.; Zm, *Zea mays*; At, *Arabidopsis thaliana*; Na, *Nicotiana attenuate*; Os, *Oryza sativa*; Ta, *Triticum aestivum* L.

Amylose-Resin (Sigma, USA) and Glutathione-Beads (Sigma, USA) in PBS buffer (150 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) at 4 °C for 2 h, respectively. After washing five times with PBS buffer, the reactions were stopped by adding 50 μL PBS buffer and 10 μL 5× protein loading buffer boiled for 5 min. The immunoblot analysis used 10 % SDS-PAGE gel and detected with 1:5000 (anti-GST) and 1:10000 (anti-MBP) dilutions of antibodies.

2.9. Yeast one-hybrid system

The CDSs of *GmTCP15* and *GmMRF2* were fused into the pB42AD vectors, respectively (pB42AD-GmTCP15/GmMRF2). The P1 (4906 bp, base pairs −5068 bp to −162 bp), P (2869 bp, base pairs −3031 bp to −162 bp), and P3 (1096 bp, base pairs −1258 bp to −162 bp) fragments of the *GmSOC1b* promoter region were amplified from the soybean cultivar 'Jack', then separately cloned into pLacZ2u vectors (P1/P2/P3-pLacZ2u). Appropriate pairs of constructs were transformed into yeast strain EGY48. The yeast clones were grown on SD/-Trp/-Ura medium at 30 °C for 3 days, and then spotted onto a SD medium (lacking Trp and Ura) plus X-gal to detect interactions.

2.10. Dual-luciferase assay

According to the results of the yeast one-hybrid (Y1H) assay, the CDSs of *GmTCP15* and *GmMRF2* were inserted into pGreenII 62-SK (GmTCP15/GmMRF2-pGreenII 62-SK) as effectors. P1 segment of *GmSOC1b* was cloned into pGreenII 0800-LUC to active the LUC reporter gene (P1-LUC). The Renilla luciferase gene (REN) was used as an internal control. The relative constructs were transferred into the roughly 4-week-old tobacco leaves. The LUC/REN activity was determined by a dual-luciferase reporter system. All primers used are listed in Supplementary Table S1.

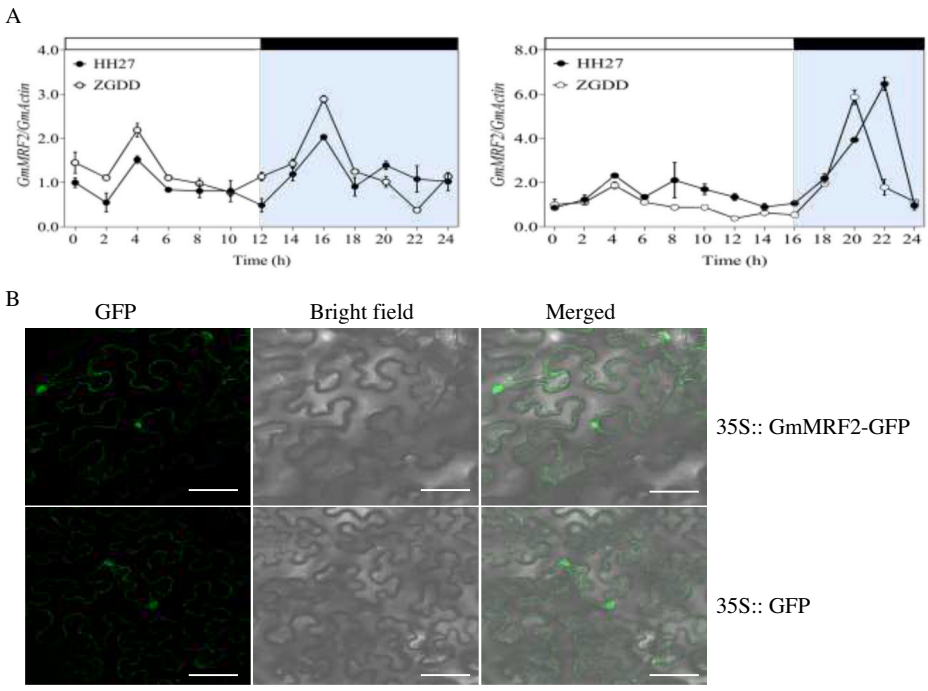


Fig. 2. Expression analysis. (A) Diurnal expression pattern of *GmMRF2* in leaves. White and blue bars represent dark and light periods, respectively. The expression analysis used *GmActin* as an internal control. The data are means ± SE of three technical replicates. (B) Subcellular localization of GmMRF2 in *N. benthamiana.* Bar = 10 μm.

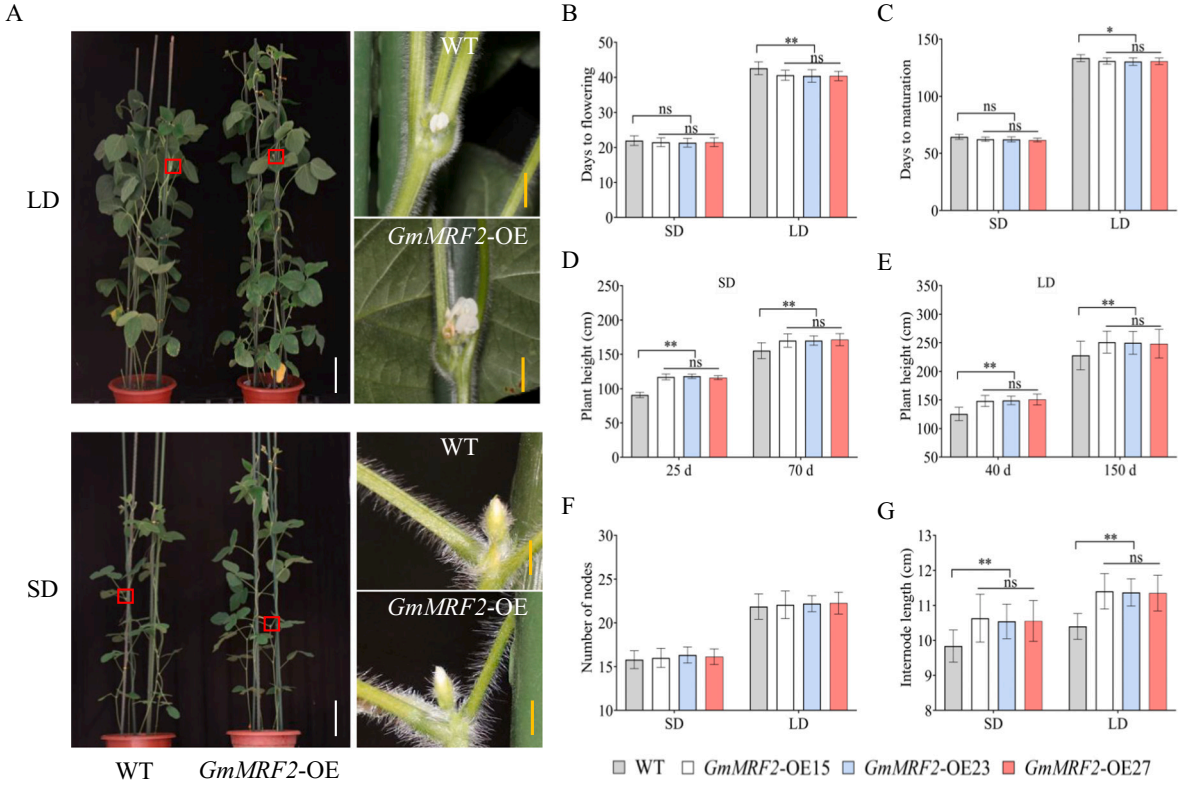


Fig. 3. Overexpression of *GmMRF2* in soybean promotes flowering and increases plant height. (A) Phenotypes of *GmMRF2* overexpressing lines and WT under LD and SD conditions. White bar = 10 cm, Yellow bar =0.5 cm. (B) Days to flowering. (C) Days to maturity (R7 stage). (D) Plant height under SD conditions. (E) Plant height under LD conditions. (F) Number of nodes. (G) Internode length. LD, long day (16 h light/8 h dark); SD, short day (12 h light/12 h dark). *, ** indicate statistically significant differences at the level of $P < 0.05$, $P < 0.01$, respectively. Student's *t*-test, two-tailed, $n = 13$.

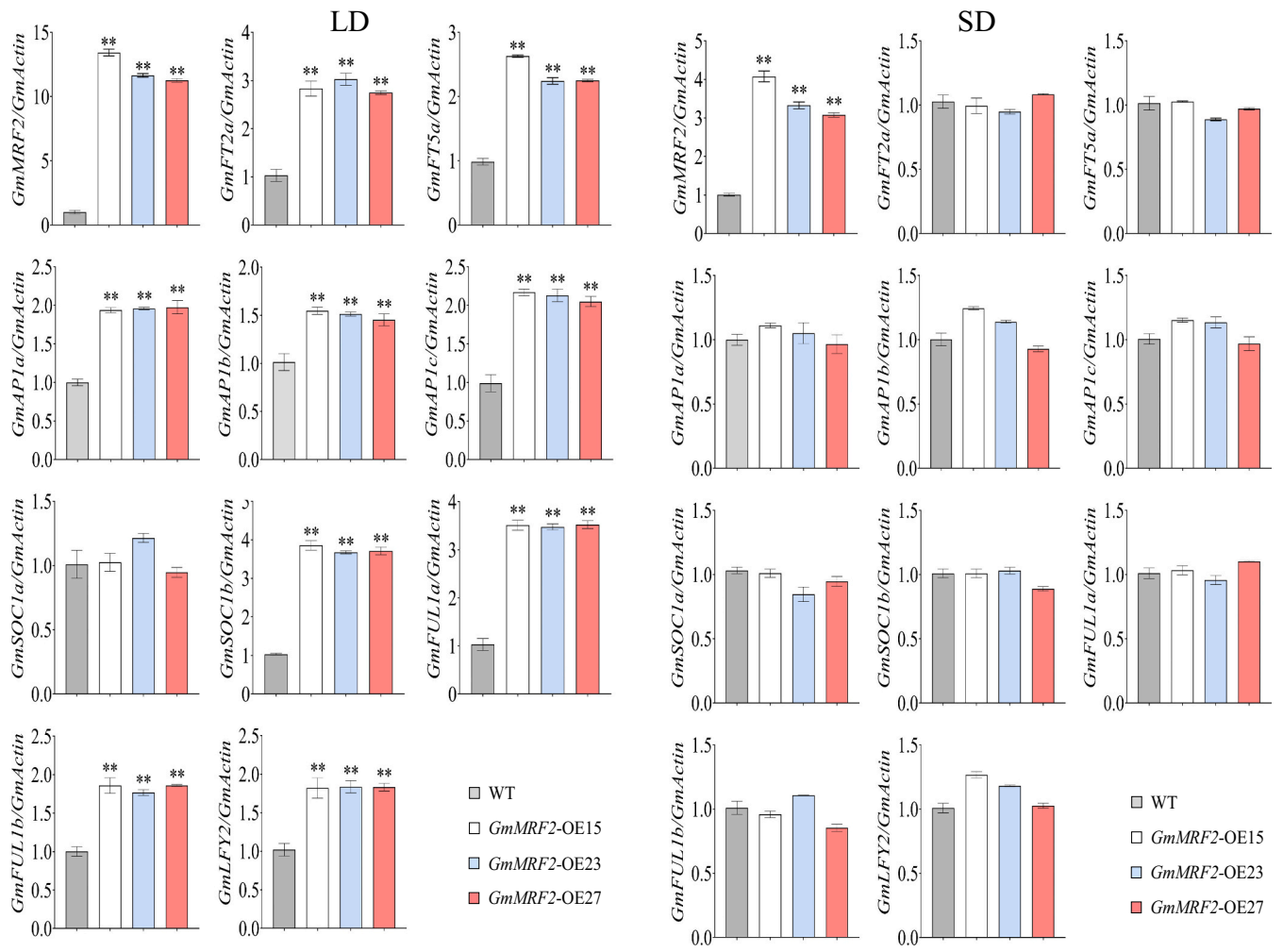


Fig. 4. Expression levels of flowering-related genes of *GmMRF2* overexpressing lines and WT. RNA was extracted at 25 DAE (LD) and 15 DAE (SD). *GmMRF2*: *Glyma.03G106900*; *GmFT2a*: *Glyma.16G150700*; *GmFT5a*: *Glyma.16 g04830*; *GmAP1a*: *Glyma.16 g13070*; *GmAP1b*: *Glyma.01 g08150*; *GmAP1c*: *Glyma08g36380*; *SOC1a*: *Glyma.18G224500*; *SOC1b*: *Glyma.09G266200*; *FUL1a*: *Glyma.04G159300*; *FUL1b*: *Glyma.06G205800*; *LFY2*: *Glyma.06G163600*. DAE, days after emergence. Relative expression levels are the means ± SE of three replications. LD, long day (16 h light/8 h dark); SD, short day (12 h light/12 h dark). ** indicates statistically significant differences at the level of $P < 0.01$. Student's t-test, two-tailed.

3. Result

3.1. Cloning of GmMRF2

Ten homologs of *GmMRF* genes were identified in a soybean genome database (Phytozome 2017), including *Glyma.01G094800*, *Glyma.03G106900*, *Glyma.04G251700*, *Glyma.05G067200*, *Glyma.06G111100*, *Glyma.07G118500*, *Glyma.08G340800*, *Glyma.12G161600*, *Glyma.14G147000*, and *Glyma.17G149400*. The multiple sequence alignments of *GmMRFs* showed that GmMRF protein sequence varied greatly in soybean (Fig. 1A). To better investigate the functions of *MRF* in soybean, we detected the expression levels of all 10 selected *GmMRFs* in different organs (root, stem, shoot, leaf, bud, flower, and pod). The results showed that the expression level of *Glyma.03G106900* was the highest among all analyzed *GmMRF* genes in various tissues and organs, with the highest levels recorded in the shoot (Fig. S1). Sequence analysis showed that *Glyma.03G106900* gene shared 53.76 % amino acid sequence identity with *AtMRF1*, and the annotation of protein domain structure revealed that the Glyma.03G106900 protein contained a MORN-motif (Fig. S2). Thereby, *Glyma.03G106900* was chosen to be cloned from 'Jack', and hereafter named as *GmMRF2*. *GmMRF2* comprises 3070 bp, and includes 1 intron and 2 exons, a 402 bp CDS region, and 133 responding amino acids. We also performed phylogenetic analysis of GmMRF2 with other MRFs in multiple species. The results revealed that GmMRF2 was closely related to *Zea mays* (Fig. 1B).

3.2. Expression patterns and subcellular localization

In this study, we evaluated the diurnal expression pattern of *GmMRF2* in early-flowering variety HH27 and late-flowering variety ZGDD in leaves sampled under SD and LD conditions. Under SD conditions, the expression of *GmMRF2* peaked at 4 h after dark in both HH27 and ZGDD. Results were comparable under LD conditions, although *GmMRF2* levels peaked 2 h earlier in ZGDD than in HH27 (Fig. 2A). These results demonstrated that *GmMRF2* is regulated in response to circadian rhythms under both SD and LD conditions.

To determine the subcellular localization of GmMRF2, we constructed a recombinant vector, GmMRF2-GFP, with a GFP-label. We then transiently introduced the vector into *N. benthamiana* by *Agrobacterium* infection. As shown in Fig. 2B, GmMRF2 protein was localized in both the cytosol and nucleus, similar to the cellular localization of the GFP alone.

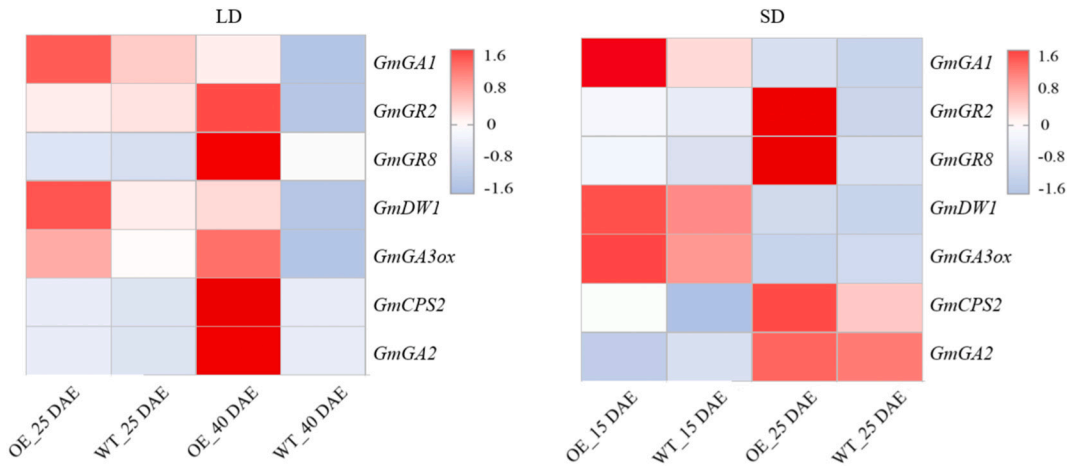


Fig. 5. Expression levels of GA metabolic pathway-related genes of *GmMRF2* overexpressing lines and WT. Under SD conditions, at 15 and 25 DAE; under LD conditions at 25 and 40 DAE. *GmGA1*: *Glyma.09G149200*; *GmGR2*: *Glyma.20G230600*; *GmGR8*: *Glyma.11G216500*; *GmGA3ox*: *Glyma.17G205300*; *GmDW1*: *Glyma.08G163900*; *GmCPS2*: *Glyma.19G157000*; *GmGA2*: *Glyma.20G153400*. DAE, days after emergence. Relative expression levels are the means ± SE of three replications. LD, long day (16 h light/8 h dark); SD, short day (12 h light/12 h dark). Student's t-test, two-tailed.

3.3. *GmMRF2 positively regulates flowering time and plant height*

To further identify the functions of *GmMRF2*, the gene was genetically transformed into the soybean cultivar 'Jack' by using the CaMV35S promoter. By employing *Bar* gene and PAT protein detection, we identified three *GmMRF2* transgenic soybean lines (Fig. S3). Under LD conditions, transgenic lines OE15, OE23, OE27, and the WT plants flowered at 39.2 DAE, 39.0 DAE, 39.5 DAE and 43.3 DAE, respectively; and the flowering time of the transgenic lines were 4.1 d, 4.3 d, and 3.8 d earlier than the WT plants, respectively. Under SD conditions, the flowering time of all tested transgenic lines (OE15, 22.8 DAE; OE23, 22.6 DAE; OE27, 23.0 DAE) were almost the same as that of the WT (23.2 DAE) (Fig. 3A, B). Moreover, the maturity time in *GmMRF2* transgenic soybean lines were earlier than WT under LD conditions, and similarly with that of the WT under SD conditions (Fig. 3C). The results demonstrated that *GmMRF2* significantly promote soybean flowering and maturity under LD conditions.

In addition, overexpression of *GmMRF2* was found to increase plant height under both SD and LD conditions. At 25 DAE when flowering was initiated, the height of *GmMRF2* overexpressed lines (OE15, 116.9 cm; OE23, 115.8 cm; OE27, 113.4 cm) was significantly higher than the WT (90.9 cm), and the difference increased at 70 DAE under SD conditions (Fig. 3D). Under LD conditions, we found that the transgenic soybean lines were taller than WT at 40 (OE15, 148.1 cm; OE23, 147.6 cm; OE27, 147.8 cm; WT, 125.3 cm) and 150 DAE (OE15, 251.1 cm; OE23, 249.4 cm; OE27, 249.2 cm; WT, 227.7 cm) (Fig. 3E). These results suggested

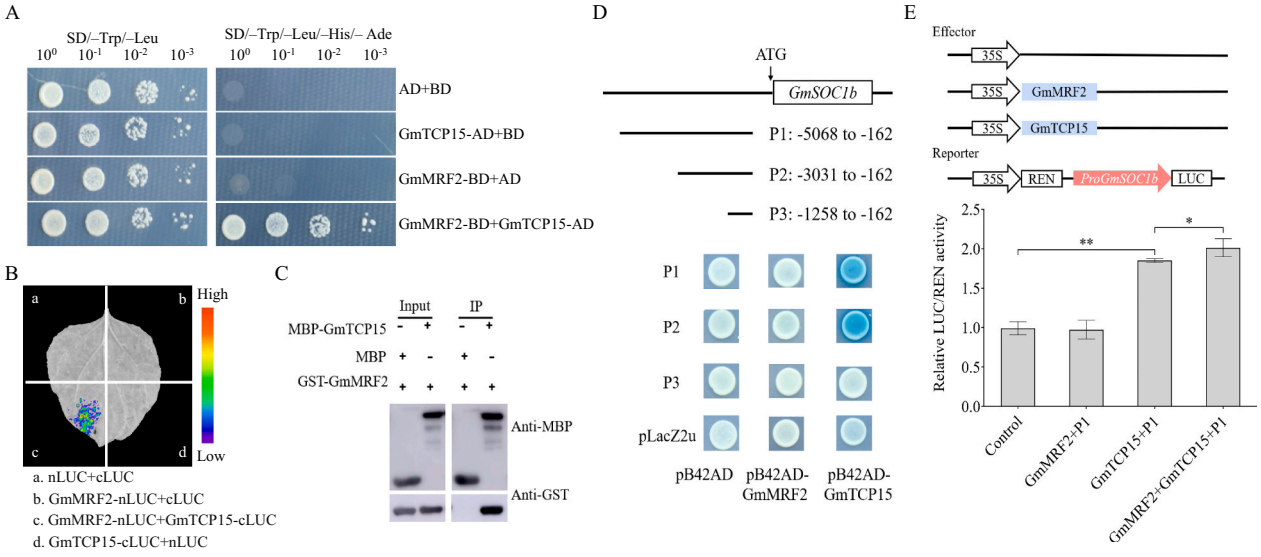


Fig. 6. GmMRF2 interacts with GmTCP15 co-regulate the expression of *GmSOC1b*. (A) GmMRF2 interacted with GmTCP15 in yeast. The transformants were assessed on SD/−Trp/−Leu and SD/−Trp/−Leu/-His/−Ade media. Empty vectors were negative control. AD: pGADT7; BD: pGBKT7. (B) GmMRF2 associated with GmTCP15 in LCI assays. (C) GmMRF2 interacted with GmTCP15 in pull-down assays. MBP-TCP15 was used as the bait to pull down glutathione-labeled GmMRF2. MBP tag used as negative control. (D) The association of GmMRF2 and GmTCP15 with the *GmSOC1b* promoter by Y1H assay. The *GmMRF2* and *GmTCP15* CDSs were inserted into pB42AD vector and 3 fragments of *GmSOC1b* promoter were fused with pLacZ2u vector. The transformants were assessed on SD/−Trp/-Ura media plus 20 mM X-gal. Empty vectors and were negative control. (E) The association of GmMRF2 and GmTCP15 with the *GmSOC1b* promoter by dual-luciferase assay. The *GmMRF2* and *GmTCP15* were inserted into the effector construct pGreenII 62-SK, and P1 fragment of *GmSOC1b* promoter was fused with the reporter vector pGreen II 0800-LUC. Empty pGreenII 62-SK was used as negative control. Values are means ± SE ($n = 3$). *, ** indicate statistically significant differences at the level of $P < 0.05$, $P < 0.01$, respectively.

that *GmMRF2* positively regulates plant height in soybean. Moreover, the internode length of transgenic lines was notably increased compared with WT (Fig. 3F, G). These results suggested that in addition to acting as a floral promotor, *GmMRF2* also regulates plant height and other agronomic traits in soybean.

3.4. Expression levels of downstream flowering-related and GA metabolic pathway-related genes

To integrate *GmMRF2* into the photoperiodic flowering regulation network of soybean, qRT-PCR was performed to measure the expression levels of several genes known to be important in the flowering time pathway of *GmMRF2* transgenic lines and the wild-type. These included two representative *GmFT* genes (*GmFT2a*: *Glyma.16G150700*, *GmFT5a*: *Glyma.16G044100*), *GmAP1* genes (*GmAP1a*: *Glyma.16g13070*, *GmAP1b*: *Glyma.01g08150*, *GmAP1c*: *Glyma.08g36380*), *GmFUL1* genes (*GmFUL1a*: *Glyma.06g22650*, *GmFUL1b*: *Glyma.04g31847*), *GmSOC1* genes (*GmSOC1a*: *Glyma.18G224500*, *GmSOC1b*: *Glyma.09G266200*), and *GmLFY2* (*Glyma.06g17170*). Under LD conditions, the expression levels of *GmFT2a*, *GmFT5a*, *GmAP1a*, *GmAP1b*, *GmAP1c*, *GmFUL1a*, *GmFUL1b*, *GmSOC1b*, and *GmLFY2* in *GmMRF2*-OE plants were significantly higher compared with wild-type. While, no significant expression differences were observed under SD conditions (Fig. 4). These results suggest that the *GmMRF2* regulates the expression of downstream flowering-related genes to promote flowering under LD conditions.

Previous studies have shown that GA is able to increase plant height by promoting cell elongation, playing a key role in determining plant height [32]. In this study, the internode lengths of *GmMRF2* transgenic lines were found to be longer than in the wild-type. We therefore determined the relative expression levels of several GA biosynthesis pathway genes in *GmMRF2* transgenic lines, including GA-20 oxidase, copalyl pyrophosphate synthase, ent-kaurene synthase, and GA-responsive genes, which corresponds to *GmGA1* and *GmGA2*, *GmCPS2*, *GmDW1*, *GmGR2*, and *GmGR8*. The expression levels of these genes in *GmMRF2* transgenic lines were higher compared to WT under both LD and SD conditions (Fig. 5). These results indicate that *GmMRF2* has a positive regulation on the expression of GA signal transduction pathway genes which control plant height.

3.5. GmMRF2 interacts with GmTCP15 to directly induce GmSOC1b expression

To further determine the role of *GmMRF2* in flowering time and plan height, we performed a yeast two-hybrid screening assay. At first, the CDS full length of *GmMRF2* was fused with pGBKT7 (pGBKT7-GmMRF2) to detect the transactivation activity. The results revealed no detection of transactivation activity (Fig. S4). Afterwards, the pGBKT7-GmMRF2 vector was used to conduct a yeast two-hybrid library screening. As a result, seven candidate proteins were identified that might interact with GmMRF2 (Table S3). To confirm the interaction between GmMRF2 and candidate proteins, we isolated the CDSs of the 8 genes and constructed them into pGADT7. We then transformed each into the yeast host AH109, along with pGBKT7-GmMRF2. As shown in Fig. 6A, GmMRF2 could interact with GmTCP15 in the yeast colony growth assay. To identify the occurrence of these interactions in plants, LCI assay was performed. *GmMRF2* was constructed in the N-terminal part of LUC (GmMRF2-nLUC), and *GmTCP15* was constructed in the C-terminal part of LUC (GmTCP15-cLUC). The result showed when GmMRF2-nLUC and GmTCP15-cLUC were co-expressed in tobacco leaves, significant fluorescence signals were observed; and no signal was detected in the control zones (Fig. 6B). We also verified the physical interaction between GmMRF2 and GmTCP15 by pull-down assay. The GST-GmMRF2, MBP-GmTCP15, and MBP proteins were purified and isolated from *Escherichia coli*. The pull-down assay using MBP beads suggested that GST-GmMRF2 pulled down MBP-GmTCP15, but not MBP alone (Fig. 6C). Altogether, these results indicated that GmMRF2 interacts with GmTCP15 both in planta and in vitro.

In *Arabidopsis*, TCP15 was reported to induce flowering by binding to the promoter of *SOC1*, a flowering pathway integrator [33]. As the expression level of *GmSOC1b* was up-regulated in *GmMRF2*, we conducted a yeast one-hybrid assay to confirm whether *GmSOC1b* was a direct target of GmTCP15. The coding sequences of *GmTCP15 and GmMRF2* were cloned into vector pB42AD (pB42AD-GmTCP15/GmMRF2). Three fragments of the *GmSOC1b* promoter were constructed into vector pLacZ2u (P1/P2/P3-pLacZ2u). The results of this assay showed that only GmTCP15 bound to the P1 and P2 of *GmSOC1b* promoter, but not P3. This suggests that the *GmSOC1b* promoter is the direct target of GmTCP15 (from −1258 bp to −3031 bp region), whereas GmMRF2 couldn't directly bind to the promoter of *GmSOC1b* (Fig. 6D). To explore whether GmTCP15 and GmMRF2 could co-regulate the expression of *GmSOC1b,* we conducted a dual-luciferase reporter assay. The results showed that only GmTCP15 could directly activate the expression of *GmSOC1b* alone, but the co-transformation of GmTCP15 and GmMRF2 had a stronger promoting effect than GmTCP15 *alone* (Fig. 6E). These results suggest that GmTCP15 and GmMRF2 co-regulate the expression of *GmSOC1b.*

4. Discussion

Flowering time is a crucial trait that directly affects the ability of soybean to adapt to different latitudes [34]. *MRF1* (*MORN-MOTIF REPEAT PROTEIN REGULATING FLOWERING1*) is a novel gene that positively regulates flowering in *Arabidopsis*. However, the biologic mechanisms of *AtMRF1* have not been reported, and the functions of *MRF* homologous genes in soybean remained unknown [24]. In this study, *GmMRF2*, a new MORN-motif gene in soybean, was cloned and identified. Expression pattern analysis showed that this gene was expressed in various tissues at flowering stage, reached the highest expression level in the shoot, and responded to the photoperiod, suggesting that *GmMRF2* might be involved in soybean growth and development. To verify the functions of *GmMRF2* in regulating soybean flowering time, we generated *GmMRF2* overexpression lines. The phenotypic analysis showed that these altered lines flowered significantly earlier than WT in LD conditions. The expression analysis of downstream flowering-related genes showed that *GmFT2a*, *GmFT5a*, *GmAP1s*, *GmFUL1s*, *GmSOC1b* and *GmLFY2* were induced in *GmMRF2* overexpression soybean lines under LD conditions. Therefore, we speculated that *GmMRF2* promotes soybean flowering by up-regulating the expression of several flowering-related genes under LD conditions.

The TCPs (TEOSINTE BRANCHED1-CYCLOIDEA-PCF) family is a group of plant-specific transcription factors which is grouped into two classes. They exert their biological functions by interacting with each other or other functional proteins, allowing them to play an important role in plant growth and development processes [35–39]. For example, class I TCP7 could integrate the *SOC1* gene to promote *Arabidopsis* flowering by interacting with Nuclear Factor-Ys (NF-Y) [40]. In contrast, class II TCP20 and TCP22 interact with LWD1 (LIGHT-REGULATED WD1) and bind to the TCP-binding site of *CCA1* (*CIRCADIAN CLOCK ASSOCIATED1*) to delay flowering in *Arabidopsis* [41]. Meanwhile, *TCP15*, belonging to the TCP 1 subfamily, was reported to accelerate flowering in *Arabidopsis* [33]. In our study, GmTCP15, a homolog of AtTCP15, was verified to interact with GmMRF2 through yeast two-hybrid assay, LCI assay, and pull-down assay. Additionally, previous reports showed that the TCP15 could induce flowering by binding to the promoter of *SOC1* in *Arabidopsis* [33]. *SOC1* is a MADS-box transcription factor gene, which plays a pivotal role in flowering-time regulation [42]. In soybean, two *SOC1* homologs, *GmSOC1a* and *GmSOC1b*, were verified to affect flowering time in soybean [23]. Then, we investigated whether GmTCP15 and GmMRF2 could bind to the promoter of *GmSOC1a* and *GmSOC1b*. The results of yeast one-hybrid assay verified that the GmTCP15 or GmMRF2 could not bind to the promoter of *GmSOC1a* (Fig. S5), while GmTCP15 could directly bind to the promoter of

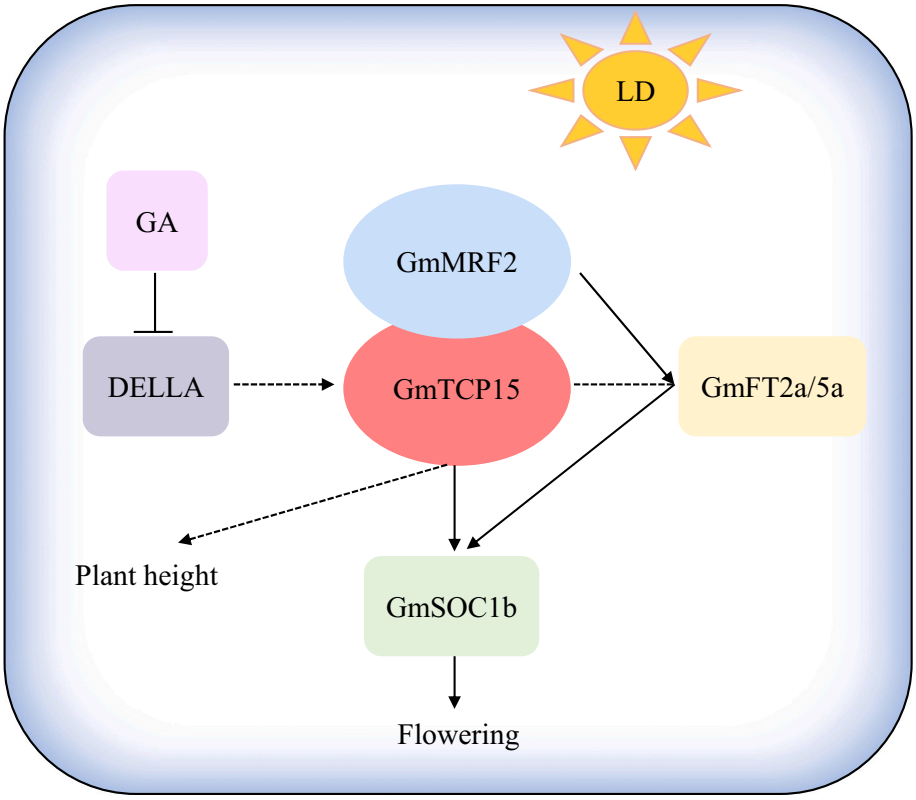


Fig. 7. A proposed model illustrating *GmMRF2*-invoveled regulatory mechanisms of soybean flowering and plant height. GmMRF2 physically interacts with GmTCP15 and activates the expression of *GmSOC1b* and GA-related genes. The lines, arrows, and T-shaped symbols represent protein-protein interactions, positive and negative effects, respectively. Dashed lines represent unverified regulatory mechanism.

GmSOC1b. The expression of *GmSOC1b* was up-regulated in *GmMRF2* overexpression soybean lines under LD condition. A further study showed that the interaction between GmTCP15 and GmMRF2 could induce the expression of *GmSOC1b*. Thus, we infer that GmMRF2 and GmTCP15 co-regulate the expression of *GmSOC1b* to promote flowering. In addition, previously report showed that *MRF1* was identified as a floral promoter which induces *FT* in *Arabidopsis* [24]. In our study, the expression of flowering promoters *GmFT2a* and *GmFT5a* was up-regulated in *GmMRF2* overexpression soybean lines. Therefore, we speculated that *GmMRF2* may also indirectly induce the expression of *GmFT2a* and *GmFT5a* to promote flowering.

In addition to early flowering, the *GmMRF2* transgenic soybean lines exhibited significantly longer plant height and internode length compared to WT. There was no difference in node number and the expression levels of several GA pathway genes were up-regulated in the overexpression lines. The results indicate that *GmMRF*2 might positively regulate plant height through the GA signal transduction pathway. In *Arabidopsis, TCP14* and *TCP15* were able to co-regulate plant height by promoting cell division in young internodes [43]. The *Arabidopsis* TCP15 was also verified to determine plant height by interacting with DELLA [44]. Thus, we believe that GmMRF2 might interact with GmTCP15 to increase plant height through the GA pathway. Our study showed that the number of pods and seeds produced by the *GmMRF2* overexpression lines was increased under SD and LD conditions (Fig. S6). Therefore, we speculate that GmMRF2 may act in coordination with GmTCP15 to promote flowering, increase plant height, and benefit yield. The final verification of how these two genes interact to regulate soybean flowering and plant height will be the focus of follow-up research (Fig. 7). Our findings provide a useful candidate gene for soybean architecture breeding.

Overall, our findings demonstrate that *GmMRF2* is an essential positive regulator of flowering time and plant height in soybean, provide new insights for the simultaneous breeding of improved flowering time and plant height.

CRediT authorship contribution statement

H WS, C L designed the experiments; Z JL, L M, S Q conducted the experiments; Z JL, C YY, C YP analyzed the data; H WS, Z JL, C L wrote the manuscript; All the authors agreed on the contents of the paper and post no conflicting interest.

Declaration of competing interest

The authors declare that they have no conflicts of interest in relation to the content of this manuscript.

Data availability

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31871644) and the CAAS (Chinese Academy of Agriculture Sciences) Agricultural Science and Technology Innovation Project (S2022ZD03).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2023.125464.

References

[1] P.H. Graham, C.P. Vance, Legumes: importance and constraints to greater use, Plant Physiol. 131 (3) (2003) 872–877, https://doi.org/10.1104/pp.017004.

[2] V. Carpentleri-Pipolo, L.A. de Almeida, R.A.D. Kiihl, Inheritance of a long juvenile period under short-day conditions in soybean, Genet. Mol. Biol. 25 (4) (2002) 463–469, https://doi.org/10.1023/A:1003927817278.

[3] A.Q. Parvez, F.P. Gardner, Daylength and sowing date responses of soybean lines with "juvenile" trait 1, Crop Sci. 27 (2) (1987) 305–310, https://doi.org/10.2135/cropsci1987.0011183X002700020037x.

[4] F. Fornara, A. de Montaigu, G. Coupland, SnapShot: control of flowering in *Arabidopsis*, Cell 141 (3) (2010) 550–550.e2, https://doi.org/10.1016/j.cell.2010.04.024.

[5] J. Adrian, S. Torti, F. Turck, From decision to commitment: the molecular memory of flowering, Mol. Plant 2 (4) (2009) 628–642, https://doi.org/10.1093/mp/ssp031.

[6] J. Lee, I. Lee, Regulation and function of SOC1, a flowering pathway integrator, J. Exp. Bot. 61 (9) (2010) 2247–2254, https://doi.org/10.1093/jxb/erq098.

[7] S. Waheed, L.H. Zeng, The critical role of miRNAs in regulation of flowering time and flower development, Genes (Basel) 11 (3) (2020), https://doi.org/10.3390/genes11030319, 319.

[8] L. Dong, C. Fang, Q. Cheng, T. Su, K. Kou, L. Kong, C. Zhang, H. Li, Z. Hou, Y. Zhang, L. Chen, L. Yue, L. Wang, K. Wang, Y. Li, Z. Gan, X. Yuan, J.L. Weller, S. Lu, F. Kong, B. Liu, Genetic basis and adaptation trajectory of soybean from its temperate origin to tropics, Nat. Commun. 12 (1) (2021) 5445, https://doi.org/10.1038/s41467-021-25800-3.

[9] L. Dong, S. Li, L. Wang, T. Su, C. Zhang, Y. Bi, Y. Lai, L. Kong, F. Wang, X. Pei, H. Li, Z. Hou, H. Du, H. Du, T. Li, Q. Cheng, C. Fang, F. Kong, B. Liu, The genetic basis of high-latitude adaptation in wild soybean, Curr. Biol. 33 (2) (2023), https://doi.org/10.1016/j.cub.2022.11.061, 252-262 e4.

[10] L.P. Kong, S.J. Lu, Y.P. Wang, C. Fang, F.F. Wang, H.Y. Nan, T. Su, S.C. Li, F. G. Zhang, X.M. Li, X.H. Zhao, X.H. Yuan, B.H. Liu, F.J. Kong, Quantitative trait locus mapping of flowering time and maturity in soybean using next-generation sequencing-based analysis, Front. Plant Sci. 9 (2018) 995, https://doi.org/10.3389/fpls.2018.00995.

[11] S. Lu, L. Dong, C. Fang, S. Liu, L. Kong, Q. Cheng, L. Chen, T. Su, H. Nan, D. Zhang, L. Zhang, Z. Wang, Y. Yang, D. Yu, X. Liu, Q. Yang, X. Lin, Y. Tang, X. Zhao, X. Yang, C. Tian, Q. Xie, X. Li, X. Yuan, Z. Tian, B. Liu, J.L. Weller, F. Kong, Stepwise selection on homeologous *PRR* genes controlling flowering and maturity during soybean domestication, Nat. Genet. 52 (4) (2020) 428–436, https://doi.org/10.1038/s41588-020-0604-7.

[12] Z.J. Xia, S. Watanabe, T. Yamada, Y. Tsubokura, H. Nakashima, H. Zhai, T. Anai, S. Sato, T. Yamazaki, S.X. Lu, H.Y. Wu, S. Tabata, K. Harada, Positional cloning and characterization reveal the molecular basis for soybean maturity locus *E1* that regulates photoperiodic flowering, Proc. Natl. Acad. Sci. U. S. A. 109 (32) (2012) E2155–E2164, https://doi.org/10.1073/pnas.1117982109.

[13] L. Wang, H. Li, M. He, L. Dong, Z. Huang, L. Chen, H. Nan, F. Kong, B. Liu, X. Zhao, GIGANTEA orthologs, E2 members, redundantly determine photoperiodic flowering and yield in soybean, J. Integr. Plant Biol. 65 (1) (2023) 188–202, https://doi.org/10.1111/jipb.13398.

[14] X. Lin, L. Dong, Y. Tang, H. Li, Q. Cheng, H. Li, T. Zhang, L. Ma, H. Xiang, L. Chen, H. Nan, C. Fang, S. Lu, J. Li, B. Liu, F. Kong, Novel and multifaceted regulations of photoperiodic flowering by phytochrome a in soybean, Proc. Natl. Acad. Sci. U. S. A. 119 (41) (2022), e2208708119, https://doi.org/10.1073/pnas.2208708119.

[15] M. Xu, Z. Xu, B. Liu, F. Kong, Y. Tsubokura, S. Watanabe, Z. Xia, K. Harada, A. Kanazawa, T. Yamada, J. Abe, Genetic variation in four maturity genes affects photoperiod insensitivity and PHYA-regulated post-flowering responses of soybean, BMC Plant Biol. 13 (2013) 91, https://doi.org/10.1186/1471-2229-13-91.

[16] Y. Cai, L. Wang, L. Chen, T. Wu, L. Liu, S. Sun, C. Wu, W. Yao, B. Jiang, S. Yuan, T. Han, W. Hou, Mutagenesis of GmFT2a and GmFT5a mediated by CRISPR/Cas9 contributes for expanding the regional adaptability of soybean, Plant Biotechnol. J. 18 (1) (2020) 298–309, https://doi.org/10.1111/pbi.13199.

[17] L. Chen, Y.P. Cai, M.G. Qu, L.W. Wang, H.B. Sun, B.J. Jiang, T.T. Wu, L.P. Liu, S. Sun, C.X. Wu, W.W. Yao, S. Yuan, T.F. Han, W.S. Hou, Soybean adaption to high-latitude regions is associated with natural variations of *GmFT2b*, an ortholog of *FLOWERING LOCUS T*, Plant Cell Environ. 43 (4) (2020) 934–944, https://doi.org/10.1111/pce.13695.

[18] X.M. Li, C. Fang, Y.Q. Yang, T.X. Lv, T. Su, L.Y. Chen, H.Y. Nan, S.C. Li, X.H. Zhao, S.J. Lu, L.D. Dong, Q. Cheng, Y. Tang, M.L. Xu, J. Abe, X.L. Hou, J.L. Weller, F. J. Kong, B.H. Liu, Overcoming the genetic compensation response of soybean florigens to improve adaptation and yield at low latitudes, Curr. Biol. 31 (17) (2021) 3755–3767, https://doi.org/10.1016/j.cub.2021.06.037.

[19] R. Takeshima, H.Y. Nan, K. Harigai, L.D. Dong, J.H. Zhu, S.J. Lu, M.L. Xu, N. Yamagishi, N. Yoshikawa, B.H. Liu, T. Yamada, F.J. Kong, J. Abe, Functional divergence between soybean *FLOWERING LOCUS T* orthologues *FT2a* and *FT5a* in post-flowering stem growth, J. Exp. Bot. 70 (15) (2019) 3941–3953, https://doi.org/10.1093/jxb/erz199.

[20] S. Quiroz, J.C. Yustis, E.C. Chavez-Hernandez, T. Martinez, M.P. Sanchez, A. Garay-Arroyo, E.R. Alvarez-Buylla, B. Garcia-Ponce, Beyond the genetic pathways, flowering regulation complexity in *Arabidopsis thaliana*, Int. J. Mol. Sci. 22 (11) (2021) 5716, https://doi.org/10.3390/ijms22115716.

[21] L. Yue, X. Li, C. Fang, L. Chen, H. Yang, J. Yang, Z. Chen, H. Nan, L. Chen, Y. Zhang, H. Li, X. Hou, Z. Dong, J.L. Weller, J. Abe, B. Liu, F. Kong, FT5a interferes with the Dt1-AP1 feedback loop to control flowering time and shoot determinacy in soybean, J. Integr. Plant Biol. 63 (6) (2021) 1004–1020, https://doi.org/10.1111/jipb.13070.

[22] L. Chen, H. Nan, L. Kong, L. Yue, H. Yang, Q. Zhao, C. Fang, H. Li, Q. Cheng, S. Lu, F. Kong, B. Liu, L. Dong, Soybean *AP1* homologs control flowering time and plant height, J. Integr. Plant Biol. 62 (12) (2020) 1868–1879, https://doi.org/10.1111/jipb.12988.

[23] K. Kou, H. Yang, H. Li, C. Fang, L. Chen, L. Yue, H. Nan, L. Kong, X. Li, F. Wang, J. Wang, H. Du, Z. Yang, Y. Bi, Y. Lai, L. Dong, Q. Cheng, T. Su, L. Wang, S. Li, Z. Hou, S. Lu, Y. Zhang, Z. Che, D. Yu, X. Zhao, B. Liu, F. Kong, A functionally divergent *SOC1* homolog improves soybean yield and latitudinal adaptation, Curr. Biol. 32 (8) (2022) 1728–1742, e6, https://doi.org/10.1016/j.cub.2022.02.046.

[24] Y. You, A. Sawikowska, J.E. Lee, R.M. Benstein, M. Neumann, P. Krajewski, M. Schmid, Phloem companion cell-specific transcriptomic and epigenomic analyses identify MRF1, a regulator of flowering, Plant Cell 31 (2) (2019) 325–345, https://doi.org/10.1105/tpc.17.00714.

[25] M. Endo, H. Shimizu, M.A. Nohales, T. Araki, S.A. Kay, Tissue-specific clocks in *Arabidopsis* show asymmetric coupling, Nature 515 (7527) (2014) 419–422, https://doi.org/10.1038/nature13919.

[26] H. Shimizu, K. Katayama, T. Koto, K. Torii, T. Araki, M. Endo, Decentralized circadian clocks process thermal and photoperiodic cues in specific tissues, Nat. Plants 1 (2015) 15163, https://doi.org/10.1038/nplants.2015.163.

[27] M. Abe, Y. Kobayashi, S. Yamamoto, Y. Daimon, A. Yamaguchi, Y. Ikeda, H. Ichinoki, M. Notaguchi, K. Goto, T. Araki, FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex, Science 309 (5737) (2005) 1052–1056, https://doi.org/10.1126/science.1115983.

[28] F. Turck, F. Fornara, G. Coupland, Regulation and identity of florigen: *FLOWERING LOCUS T* moves center stage, Annu. Rev. Plant Biol. 59 (2008) 573–594, https://doi.org/10.1146/annurev.arplant.59.032607.092755.

[29] J. Lee, C.T. Han, Y. Hur, Overexpression of *BrMORN*, a novel 'membrane occupation and recognition nexus' motif protein gene from Chinese cabbage, promotes vegetative growth and seed production in *Arabidopsis*, Mol. Cells 29 (2) (2010) 113–122, https://doi.org/10.1007/s10059-010-0006-2.

[30] S.D. Yoo, Y.H. Cho, J. Sheen, *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis, Nat. Protoc. 2 (7) (2007) 1565–1572, https://doi.org/10.1038/nprot.2007.199.

[31] Q. Su, L. Chen, Y. Cai, Y. Chen, S. Yuan, M. Li, J. Zhang, S. Sun, T. Han, W. Hou, Functional redundancy of *FLOWERING LOCUS T 3b* in soybean flowering time regulation, Int. J. Mol. Sci. 23 (5) (2022) 2497, https://doi.org/10.3390/ijms23052497.

[32] X. Li, Z. Guo, Y. Lv, X. Cen, X. Ding, H. Wu, X. Li, J. Huang, L. Xiong, Genetic control of the root system in rice under normal and drought stress conditions by genome-wide association study, PLoS Genet. 13 (7) (2017), e1006889, https://doi.org/10.1371/journal.pgen.1006889.

[33] L.E. Lucero, P.A. Manavella, D.E. Gras, F.D. Ariel, D.H. Gonzalez, Class I and class II TCP transcription factors modulate SOC1-dependent flowering at multiple levels, Mol. Plant 10 (12) (2017) 1571–1574, https://doi.org/10.1016/j.molp.2017.09.001.

[34] S. Watanabe, K. Harada, J. Abe, Genetic and molecular bases of photoperiod responses of flowering in soybean, Breed. Sci. 61 (5) (2012) 531–543, https://doi.org/10.1270/jsbbs.61.531.

[35] N.G. Uberti-Manassero, L.E. Lucero, I.L. Viola, A.C. Vegetti, D.H. Gonzalez, The class I protein AtTCP15 modulates plant development through a pathway that overlaps with the one affected by CIN-like TCP proteins, J. Exp. Bot. 63 (2) (2012) 809–823, https://doi.org/10.1093/jxb/err305.

[36] Y.F. Lin, Y.Y. Chen, Y.Y. Hsiao, C.Y. Shen, J.L. Hsu, C.M. Yeh, N. Mitsuda, M. Ohme-Takagi, Z.J. Liu, W.C. Tsai, Genome-wide identification and characterization of *TCP* genes involved in ovule development of Phalaenopsis equestris, J. Exp. Bot. 67 (17) (2016) 5051–5066, doi: 10.1093/jxb/erw273.

[37] Z.M. He, X.Y. Zhao, F.N. Kong, Z.C. Zuo, X.M. Liu, TCP2 positively regulates *HY5/HYH* and photomorphogenesis in *Arabidopsis*, J. Exp. Bot. 67 (3) (2016) 775–785, https://doi.org/10.1093/jxb/erv495.

[38] S. Carrara, M.C. Dornelas, *TCP* genes and the orchestration of plant architecture, Trop. Plant Biol. 14 (1) (2021) 1–10, https://doi.org/10.1007/s12042-020-09274-z.

[39] K. Sarvepalli, U. Nath, CIN-TCP transcription factors: transiting cell proliferation in plants, IUBMB Life 70 (8) (2018) 718–731, https://doi.org/10.1002/iub.1874.

[40] X. Li, G. Zhang, Y. Liang, L. Hu, B. Zhu, D. Qi, S. Cui, H. Zhao, TCP7 interacts with nuclear factor-Ys to promote flowering by directly regulating *SOC1* in *Arabidopsis*, Plant J. 108 (5) (2021) 1493–1506, https://doi.org/10.1111/tpj.15524.

[41] J.F. Wu, H.L. Tsai, I. Joanito, Y.C. Wu, C.W. Chang, Y.H. Li, Y. Wang, J.C. Hong, J. W. Chu, C.P. Hsu, S.H. Wu, LWD-TCP complex activates the morning gene *CCA1* in *Arabidopsis*, Nat. Commun. 7 (2016) 13181, https://doi.org/10.1038/ncomms13181.

[42] R.G. Immink, D. Pose, S. Ferrario, F. Ott, K. Kaufmann, F.L. Valentim, S. de Folter, F. van der Wal, A.D. van Dijk, M. Schmid, G.C. Angenent, Characterization of SOC1's central role in flowering by the identification of its upstream and downstream regulators, Plant Physiol. 160 (1) (2012) 433–449, https://doi.org/10.1104/pp.112.202614.

[43] M. Kieffer, V. Master, R. Waites, B. Davies, *TCP14* and *TCP15* affect internode length and leaf shape in *Arabidopsis*, Plant J. 68 (1) (2011) 147–158, https://doi.org/10.1111/j.1365-313X.2011.04674.x.

[44] J.M. Daviere, M. Wild, T. Regnault, N. Baumberger, H. Eisler, P. Genschik, P. Achard, Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height, Curr. Biol. 24 (16) (2014) 1923–1928, https://doi.org/10.1016/j.cub.2014.07.012.