1   **Potential function of *CbuSPL* and gene encoding its interacting protein during flowering in**

2   ***Catalpa bungei***

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12   **Running title: *SPL* during *C. bungei* flowering**

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15   **Abstract**

16   "Bairihua", a variety of the Catalpa bungei, has a large amount of flowers and a long flowering

17   period which make it an excellent material for flowering researches in trees. SPL is one of the hub

18   genes that regulate both flowering transition and development. Here, a SPL homologues *CbuSPL9*

19   was cloned using degenerate primers with RACE. Expression studies during flowering transition

20   in Bairihua and ectopic expression in Arabidopsis showed that *CbuSPL9* was functional similarly

21   with its Arabidopsis homologues. In the next step, we used Y2H to identify the proteins that could

22   interact with CbuSPL9. HMGA, an architectural transcriptional factor, was identified and cloned

23   for further research. BiFC and BLI showed that CbuSPL9 could form a heterodimer with

24   CbuHMGA in the nucleus. The expression analysis showed that *CbuHMGA* had a similar

25   expression trend to that of *CbuSPL9* during flowering in "Bairihua". Intriguingly, ectopic

26   expression of *CbuHMGA* in Arabidopsis would lead to aberrant flowers, but did not effect

27   flowering time. Taken together, our results implied a novel pathway that *ChuSPL9* regulated

28   flowering development, but not flowering transition, with the participation of *ChuHMGA*. Further

29   investments need to be done to verify the details of this pathway.

30   **Keywords:** *Catalpa bungei*; Flowering; SPL; HMGA; Architectural transcriptional factor.

31   **Introduction**

32   Flowers allow flowering plants to have a broader evolutionary relationship and extend their

33   ecological niche so that they can dominate the terrestrial ecosystem. Flowering is extremely

34   important for the development of perennial woody plants and for improving the economic value of

35   plants. However, due to complex genomes and other objective characteristics of perennial woody

36   plants, research on the flowering process in perennial woody plants remains limited. *Catalpa*

37   *bungei* is valuable as both a timber and an ornamental tree[1]. "Bairihua", which is a natural variety

38   of *C. bungei*, has been characterized for its especially short juvenile period, large number of

39   flowers and long flowering period. The flowering period of "Bairihua" is approximately 15 days,

40   and its accumulative flowering period reaches 100 days, which is very rare for woody plants

41   (http://www.forestry.gov.cn/). "Bairihua" provides an excellent opportunity to evaluate the

42   flowering process of woody plants.

43   Flowering is controlled by sophisticated regulatory networks[2–5]. Five major pathways are involved

44   in these processes, including the aging pathway[6], gibberellin pathway[7–11], photoperiod

45   pathway[12–16], vernalization pathway[17–19] and autonomous pathway[20]. The SQUAMOSA

46   promoter-binding protein-LIKE (SPL) family of transcription factors (TFs) integrate multiple

47   pathways[21–25]. *SPLs* have been shown to regulate flowering time and flower organ development in

48   both herbs and woody plants, such as *Gossypium hirsutum*[26], maize[27], birch[28], *Prunus mume*[29], and

49   *Platanus acerifolia*[30]. In the model plant Arabidopsis, AtSPLs have been shown to be a group of

50   dominant regulators of the flowering process[4,11,13,22,24,31–34]. The overexpression of *AtSPLs* leads to

51   early flowering and abnormal inflorescence, and conversely, the inhibition of *AtSPL* expression

52   delays the occurrence of floral transition[21,35–37]. As a group of TFs, SPLs regulate the expression

53   of other genes. Numerous downstream genes of SPLs have been identified; for example, *AtSPL3*

54   can directly upregulate the expression of *LFY*, *FUL* and *AP1* by binding to their promoters[38–40].

55   However, in addition to protein-DNA interactions, TFs also affect plant growth and development

56   by forming protein complexes. For example, the MYB-bHLH-WD40/WDR (MBW) complex

57   regulates late biosynthetic genes in anthocyanin biosynthesis, impacts fruit quality in apple[41,42],

58   and regulates trichome initiation in *Arabidopsis thaliana*[43]. Two TFs, AT-HOOK MOTIF

59   NUCLEAR LOCALIZED PROTEIN 3/4, regulate the formation of the tissue boundary between

60   the procambium and xylem in Arabidopsis roots[44]. Few studies have investigated whether there

61   are other factors that interact with SPLs and affect their binding ability, especially in trees. The

62   function of SPLs in woody plants is still in its infancy.

63   In an effort to study the molecular mechanism of the flowering process in "Bairihua", we

64   evaluated whether there were protein interactions involved in SPL regulation during the flowering

65   process. As a first step to address this question, we isolated and characterized the *SPL9*

66   orthologous gene from "Bairihua" and performed native in planta gene expression analysis. The

67   putative function of this gene was then tested by ectopic expression experiments. This gene

68   showed similar expression patterns and the ability to induce flower organ development and early

69   flowers in Arabidopsis. The findings of this study indicated that *CbuSPL9* is functionally

70   conserved. An architectural TF, CbuHMGA, was found via screening CbuSPL9-interacting

71   proteins. *CbuHMGA* is involved in the floral organ development of "Bairihua"', but not in the

72   regulation of flowering time. These results provide a molecular basis for studying the molecular

73   mechanism of "Bairihua" flowering and provide a research direction for the study of the floral

74   transition of perennials.

75 **Materials and methods**

76 **Plant materials**

77 *C. bungei* is a perennial tree that typically flowers over a 30-day flowering period. However,

78 "Bairihua", the new variety of *C. bungei*, was found in Henan Province, China, and confirmed by

79 the National Forestry and Grassland Administration (http://www.forestry.gov.cn/). The flowering

80 period of a single flower is approximately 15 days, and its accumulative flowering period reaches

81 100 days, which is very rare for woody plants. From January 15 to April 2, 2017, we collected the

82 first round of axillary buds of EF and NF varieties every one to two days. Samples were collected

83 every 10 days during the dormant and germinating periods. Since the floral transition of

84 "Bairihua" was completed within 7 to 10 days, samples were collected every day during the floral

85 transition period, and the samples were collected every 5 days during the reproductive growth

86 stage. The samples used for RNA extraction were washed with distilled water, frozen immediately

87 in liquid nitrogen, and stored at -80°C. Samples for histological analysis were fixed in a

88 formalin:glacial acetic acid:70% ethanol (5:5:90 vol.; FAA) solution under a vacuum for at least

89 24 h.

90 **Histological analysis**

91 For histological analysis, the samples were immersed in FAA fixative and placed under vacuum at

92 4°C. Samples were dehydrated in gradient ethanol and then embedded in paraffin. Ten-mm-thick

93 sections (RM2255 Fully Automated Rotary Microtome; Leica, Germany) were stained with

94 Safranine O and fast green FCF (Sigma-Aldrich, USA). The slices were observed and

95 photographed using a Leica DM 6000B fully automated upright microscope (Leica Microsystems

96 GmbH, Wetzlar, Germany).

97 **Cloning *CbuSPL9* and *CbuHMGA* sequences from "Bairihua"**

98 RNA from "Bairihua" was extracted from the buds of the mutant using an RNA extraction kit

99 (TaKaRa), and contaminating DNA was removed with RNase-free DNase I (TaKaRa). One or two

100 micrograms of total mRNA template was added to Oligod (dT)18 primer and reverse-transcribed

101 into single-stranded cDNA by M-MLV RTase (TaKaRa). The full-length cDNA of *CbuSPL9* was

102 cloned via 3'-RACE and 5'-RACE by using the (TaKaRa) according to the manufacturer's

103 instructions. In 3'-RACE, the *CbuSPL9* gene-specific forward primers F1/F2 were designed based

104 on published and aligned *SPL* sequences from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). F1

105   nested immediately upstream of F2. The 3'-cDNA synthesis primer was provided in a kit. The

106   PCR products were cloned into the PMD18-T vector and sequenced. *CbuSPL9s* were identified

107   using BLAST. In 5'-RACE, the gene-specific reverse primers R1/R2 (Supplementary Table S1)

108   were designed based on sequences from 3'-RACE. R2 nested immediately upstream of R1. The

109   PCR products were cloned into the PMD18-T vector and sequenced.

110   **Yeast two-hybrid (Y2H) screening of the "Bairihua" cDNA library**

111   A yeast library was constructed by Oebiotech (Shanghai, China) via cloning the full-length cDNA

112   library from mRNAs in the "Bairihua" buds into the pGADT7 vector[46]. *CbuSPL9* was inserted

113   into the pGBKT7 vector. Screening of the yeast library was performed using pGBKT7-*CbuSPL9*.

114   The corresponding primers are listed in Supplementary Table S1. Transformed cells were grown

115   on SD medium supplemented with Trp. The transformants were screened on supplemented SD

116   medium lacking Leu, Trp, His and Ade and supplemented with X-a-Gal and Aureobasidin A.

117   Plates were incubated at 30°C for 48-72 h and photographed. The positive clones were verified

118   with both pGBKT7 vector consensus primers. Positive control mating was as follows: pGADT7-T

119   in Y2HGold and pGBK-53 in Y187. Negative control mating was as follows: pGADT7-T in

120   Y2HGold and pGBKT7-Lam in Y187.

121   **Subcellular localization**

122   To validate subcellular localization, the full-length coding sequences (without the stop codon) of

123   *CbuSPL9* and *CbuHMGA* were amplified from RNA of "Bairihua" buds by RT-PCR. The PCR

124   products of *CbuSPL9* and *CbuHMGA* were ligated to the vector pCAMBIA1304 using the

125   Seamless Assembly Cloning Kit (CloneSmarter, Beijing, China) to construct the

126   CbuSPL9/CbuHMGA-GFP fusion genes driven by a CaMV35S promoter (Niwa, 2003).

127   pCAMBIA1304-GFP was used as a positive control. The transient expression vectors

128   *CbuSPL9*-GFP, *CbuHMGA*-GFP and GFP-HDEL were injected into the leaf lower epidermal cells

129   of *Nicotiana tabacum* L. using Agrobacterium transformation as described by Chen et al. (2011).

130   The transformed cells were incubated for 2 days. The leaves were removed, cut into squares and

131   immersed in PBS buffer containing 1 g mL^{-1} DAPI to stain the nuclei. The transient expression of

132   the CbuSPL9/CbuHMGA-GFP fusion proteins was observed under an UltraVIEW VoX 3D Live

133   Cell Imaging System Spinning Disk confocal laser scanning microscope (PerkinElmer, Waltham,

134   MA, USA). The wavelength of excitation used was 488 nm for GFP and 405 nm for DAPI.

BiFC analysis

To confirm and visualize the interaction between CbuSPL9 and CbuHMGA in protoplasts from *Populus trichocarpa*, a BiFC assay was performed based on split EYFP. EYFP was fused to the C-terminus of CbuSPL9 and the N-terminus of CbuHMGA, resulting in CbuSPL:EYFP^C and CbuHMGA:EYFP^N. A positive EYFP signal indicates an interaction of EYFP^C and EYFP^N due to the heterodimerization of CbuSPL9 with CbuHMGA. CbuSPL:EYFP^C was cotransfected with CbuHMGA:EYFP^N and H2A:mCherry into protoplasts. The transient expression of the CbuSPL9/CbuHMGA-GFP fusion proteins was observed under an UltraVIEW VoX 3D Live Cell Imaging System Spinning Disk confocal laser scanning microscope (PerkinElmer, Waltham, MA, USA).

Biolayer interferometry assay

CbuSPL9 was cloned into pGEX6P-1 as a C-terminal GST-tagged construct, and the construct was confirmed by sequencing. CbuHMGA was cloned into pET28a as an N-terminal 6His-tagged construct, and the construct was confirmed by sequencing. The protein was purified using the procedure for EMCV-3C and RV-3C as described above. Real-time interactions between CbuHMGA and CbuSPL9 were monitored with an Octet QK (Forte-Bio) that is based on BLI[47]. BLI was used to determine dissociation constants (K_D) as well as the on- and off-rate (k_{on} and k_{off}) for HIS-CbuHMG binding to GST-CbuSPL1.

Bioinformatic analysis

Multiple sequence alignment and phylogenetic analysis were performed using MEGA6.0. After alignment, the evolutionary history was calculated using the neighbor-joining (NJ) method. The tree was inferred from 1000 bootstrap replicates to show the evolutionary history of the genes. The MEME online tool (http://meme-suite.org/tools/meme) was used to identify the motifs of the CbuSPL9 protein. MEME was run locally with the following parameters: number of repetitions = any and maximum number of motifs = 20. All candidate interacting protein sequences were examined by the domain analysis program SMART (Simple Modular Architecture Research Tool) (http://smart.embl-heidelberg.de/).

RNA extraction and quantitative real-time PCR

Total RNA was isolated from "Bairihua" buds at different developmental stages. The purity and quality of RNA was checked by NanoDrop8000 (Thermo Fisher Scientific, Waltham, MA, USA)

165 and analyzed by gel electrophoresis. First-strand cDNA synthesis was carried out with ~1 µg RNA

166 using the SuperScript III reverse transcription kit (Invitrogen) and random primers according to

167 the manufacturer's instructions. Primers were designed using Primer 3 online. The melting

168 temperature of the primers was 60°C, and the amplicon lengths were 100-200 bp. All primers are

169 listed in Supplementary Table S1. qRT-PCR was performed on a 7500 Real-Time PCR System

170 (Applied Biosystems, CA, USA) using a SYBR Premix Ex Taq™ Kit (TaKaRa, Dalian, China)

171 according to the manufacturer's instructions. Relative expression levels were calculated using the

172 2-ΔΔCt method. Actin was used as an internal control, and each reaction was conducted in

173 triplicate. The stem expression values were set to 1.

174 **Transient overexpression in *Arabidopsis thaliana***

175 Full-length CbuSPL9 and CbuHMGA were cloned into the binary vector pBI121 (BD Biosciences

176 Clontech, USA) under the control of the cauliflower mosaic virus 35S promoter in the sense

177 orientation. The transgenic plants were generated with the 35S:CbuSPL9 and 35S:CbuHMGA

178 constructs via *Agrobacterium tumefaciens* GV3101 by using the floral dip method (Clough and

179 Bent, 1998). Surface-sterilized T1 seeds were grown on a solid $0.5 \times$ MS medium containing 30

180 µg mL^{-1} hygromycin at 4°C for 2 days, which were then transferred to the greenhouse under

181 long-day conditions (16 h light/8 h dark) at 22°C for 10 days. Subsequently, the seedlings were

182 transplanted into soil. Phenotypes of the transgenic plants were observed in the T1 generation, and

183 the overexpression of *CbuSPL9* and *CbuHMGA* in the transgenic plants was confirmed by PCR

184 genotyping (Supplementary Figure S1 and S2). For each construct, at least 10 transgenic lines

185 with similar phenotypes were observed, and 3 of them were used for detailed analysis.

186 **Flowering time measurement**

187 Flowering time was measured by counting the total number of rosette leaves and the number of

188 days to flower (when the floral buds were visible). The numbers of rosette and cauline leaves of

189 ~20 plants were counted and averaged. The presence of abaxial trichomes was used to

190 differentiate between the juvenile and adult vegetative leaves. Data were classified with

191 Win-Excel and analyzed via analysis of variance (ANOVA) using the SPSS (version 8.0, SPSS

192 Inc., Chicago, IL, USA) statistical package. Comparisons between the treatment means were made

193 using Tukey's test at a probability level of $P \leq 0.05$.

194 **Result**

A flowering-related *SPL* homologous gene was isolated in "Bairihua"

The SPL homologous genes in *C. bungei* were isolated by using degenerate primers targeting the homeodomain. The isolated homeodomain sequences were extended by genome walking to acquire the full genomic sequences (exons and introns). One of the isolated sequences encoded a protein with a typical SPL protein structure, which included a highly conserved SBP-box domain bearing two zinc-binding sites and one bipartite nuclear localization signal[45] (Supplementary Table S2). The first Zn-finger-like structure (ZN-1 in Figure 1a) was C3H-type, and the second (Zn-2 in Figure 1a) was C2HC-type. The nuclear localization sequence (NLS) is a highly conserved bipartite domain located at the C-terminus of SBP. Phylogenetic analysis showed that the isolated sequence clustered with AtSPL9 and AtSPL15 (Figure 1b). Since it shared more sequence similarity with *AtSPL9*, we named the *SPL* homologous gene *CbuSPL9*.

Expression of *CbuSPL9* during the flowering process

Intensive sampling was performed to investigate the changes in *CbuSPL9* expression during the flowering process (Figure 2). T1-T3 was the dormant period during which no significant differences in morphology could be observed between the flowering buds (FBs) and the leaf buds (LBs). T4-T5 was the germination period. During this period, the internal morphology was similar between the FBs and LBs, but the external morphology of the FBs and LBs had a pale green appearance compared with that of the FBs and LBs during the dormant period. T6-T9 followed the short germination period and was the floral transition period, during which flower primordium and leaf primordium were developed in the FBs and LBs, respectively. Finally, T10-T12 was the reproductive growth period. The expression level of *CbuSPL9* was significantly higher in the FBs than in the LBs, especially during the dormant period and the reproductive growth period. Overall, the expression study supported that *CbuSPL9* was an SPL homologous gene and involved in flowering regulation.

The overexpression of *CbuSPL9* in Arabidopsis

As there is no available transformation system in *C. bungei*, *CbuSPL9* was overexpressed in Arabidopsis (Columbia ecotype, col). The flowers from *col* were tetradynamous and had four petals distributed in cross type (Figure 3aI). In contrast, the *oe-spl9* transgenic plants showed aberrant flower organs (Supplementary Table S3). The *oe-spl9* transgenic lines exhibited flower organs with altered numbers and locations, such as shrunken petals, increased stamens, and

225   overlapping petals (Figure 3aII-VI). In addition to the evident changes in floral organ morphology,

226   an acceleration in flowering time was observed in the *oe-spl9* lines (Figure 3b, Supplementary

227   Table S4). The *col* line initiated flowering when 14 rosette leaves were present (Figure 3c).

228   However, *oe-spl9* possessed less than 9 rosette leaves at the time of bolting (Figure 3d). This result

229   indicated that the function of *CbuSPL9* was conserved. The regulatory mechanism of *CbuSPL9* in

230   "Bairihua" might be similar to that in Arabidopsis.

231   **Screening *CbuSPL9*-interacting proteins**

232   As a TF, SPL-DNA interaction studies have been extensively performed. However, TFs can also

233   fine-tune specific biological processes through protein interactions. We constructed a *C. bungei*

234   yeast two-hybrid cDNA library to explore the proteins that interact with *CbuSPL9*. A total of 809

235   blue colonies representing potential positive clones were obtained on QDO/Aba/X-a-Gal plates.

236   The potential positive clones were subsequently tested by PCR for the library plasmid, and 406

237   clones were positive. The resulting PCR products were sequenced, and the sequences were aligned

238   using the NCBI BLASTp search function. Finally, 12 interacting protein candidates were

239   identified (Table 1). These predicted proteins included HMGA, aquaporins, bHLH48,

240   GATA-related protein, PHD finger protein ALFIN-LIKE 4, heavy metal-associated protein, etc.

241   **Cloning the *HMGA* gene**

242   Among these candidates, HMGA, which is an AT-hook rich protein, belongs to the most abundant

243   nonhistone protein family in the nucleus. We cloned the full-length *HMGA* homologous gene via

244   rapid amplification of cDNA ends. Phylogenic analysis revealed that this protein has a close

245   relationship with AtHMGA. Therefore, this protein was renamed CbuHMGA. *CbuHMGA*

246   encoded a H15 domain and 4 AT-hook motifs (Figure 4a). The expression analysis showed that the

247   expression trend of *CbuHMGA* was similar to that of *CbuSPL9* in the four periods in "Bairihua"

248   (Figure 4b). The expression of *CbuHMGA* was elevated in the dormant period, and the highest

249   expression level was detected in the reproductive growth period. However, the increase in the

250   expression of *CbuHMGA* before the reproductive growth period occurred slightly earlier than that

251   of *CbuSPL9*.

252   **Localization of the CbuSPL9 and CbuHMGA proteins**

253   We fused GFP at the C-terminus of CbuSPL9 and CbuHMGA and transformed them into

254   *Nicotiana benthamiana* leaf epidermal cells to determine the localization of the CbuSPL9 and

255   CbuHMGA proteins. 35S:GFP was used as a control. The GFP fluorescence in CbuSPL9-GFP and

256   CbuHMGA-GFP was exclusively observed in the nucleus, whereas the fluorescence of GFP in the

257   control was distributed throughout the entire cell (Figure 5a). These results showed that CbuSPL9

258   and CbuHMGA were located in the nucleus. This result was in agreement with the prediction

259   based on the protein structure.

Protein interaction analysis

261   Biolayer interferometry (BLI) was used to determine dissociation constants (K_D) as well as the on-

262   and off-rate (k_{on} and k_{off}) for HIS-CbuHMGA binding to GST-CbuSPL9 (Table 2). Five different

263   concentrations of GST-CbuSPL9 (2381.00 nm, 1191.00 nm, 595.30 nm, 297.60 and 148.80 nm)

264   were evaluated, and the K_D was 311 nm for each tested concentration. These results suggested a

265   strong interaction between CbuSPL9 and CbuHMGA (Figure 5b).

266   To confirm these interactions, the full-length cDNA of *CbuSPL9* was inserted into the vector

267   pGBKT7 (BD-CbuSPL9) as bait, and the full-length cDNA of *CbuHMGA* was inserted into the

268   vector pGADT7 (AD-CbuHMGA) as prey. Yeast strains containing AD-CbuHMGA and

269   BD-CbuSPL9 were positive for X-α-gal activity when grown on synthetically defined

270   (SD)/-Trp/-Leu/-His/-Ade medium (Figure 5c). These results showed that CbuSPL9 interacted

271   with HMGA in yeast. Finally, a bimolecular fluorescence complementation (BiFC) assay was

272   performed. CbuSPL:EYFP^C was cotransfected with CbuHMGA:EYFP^N and H2A:mCherry into

273   protoplasts. The signal of enhanced yellow fluorescent protein (EYFP) was colocalized with

274   mCherry (Figure 5d). Collectively, we demonstrated that CbuSPL9 formed a heterodimer with

275   CbuHMGA in the nucleus.

The overexpression of *CbuHMGA* in Arabidopsis

277   To characterize the potential function of *CbuHMGA*, we overexpressed *CbuHMGA* in Arabidopsis.

278   The *oe-hmga* transgenic lines developed aberrant flowers with abnormal petals and stamens

279   (Figure 6aI-VI). This mutant phenotype was similar to the phenotype of floral organs when

280   *CbuSPL9* was overexpressed (Supplementary Table S5). However, flowering time was not

281   affected in the *oe-hmga* transgenic lines compared to that in the col line (Supplementary Table S6).

282   The expression of endogenous *AtSPL9* was thoroughly studied to further monitor flower

283   development in the transgenic lines. Within the sampling period, the expression level of *AtSPL9*

284   continuously increased in *oe-hmga* and wild type. However, in *oe-spl9,* the expression of *AtSPL9*

285   reached a peak in T6 and then decreased (Figure 6b). The shift in *AtSPL9* expression in *oe-spl9,*

286   but not in *oe-hmga,* further confirmed the observation that *CbuHMGA* cannot accelerate flower

287   development, while *CbuSPL9* can accelerate flower development.

288   **Discussion**

289   Flowering is a very complex process, a qualitative change in the life history of higher plants, and a

290   central link in plant development. "Bairihua", a variety of the flowering perennial woody plant *C.*

291   *bungei*, has a large amount of flowers and a long flowering period. "Bairihua" is an excellent

292   material for evaluating the flowering process in trees.

293   *SPL*s play an important role in regulating flowering in many plants, most notably in Arabidopsis.

294   In woody plants, *SPLs* are studied extensively, but limited information about flowering is

295   known[29,48–51]. Here, we cloned the SBP-domain-encoding gene *CbuSPL9* in *C. bungei.* Its

296   conserved structure and flower development-related expression trends suggest that *CbuSPL9*

297   might have conserved *SPL9* functions in *C. bungei.* Furthermore, heterogeneous overexpression of

298   *CbuSPL9* in Arabidopsis accelerated flower development and lead to aberrant flower organs.

299   Although we could not generate homogenous transgenic plants due to technology and time, these

300   results are sufficient to demonstrate that *CbuSPL9* is an SPL homolog and participates in flower

301   development.

302   TFs can affect biological processes by forming complexes with other TFs. However, most of the

303   studies on SPLs have focused on their interactions with DNA motifs[38–40]. The proteins that could

304   directly interact with SPLs and participate in flower regulation are largely unknown. For further

305   insight, we screened the yeast hybridization library for CbuSPL9 in buds. Many flowering-related

306   proteins were detected, for example, the GATA-related proteins[52–57], bHLH48[58], FLA[59–62], and

307   PHD finger protein ALFIN-LIKE[63–65]. Furthermore, a high mobility group (HMG) protein,

308   CbuHMGA, was identified to interact directly with CbuSPL9. ChuHMGA contains an H15

309   domain and 4 AT-hook motifs. The BiFC assay and BLI assay further confirmed their interaction.

310   The BiFC assay suggested that CbuSPL9 and CbuHMGA could form protein complexes in the

311   nucleus. HMGA proteins have a highly conserved structure[66,67]. Unlike the HMGAs in the animal

312   kingdom, which invariably contain three AT-hook motifs, most plant HMGA proteins have four

313   AT-hook motifs[67,68]. Additionally, the amino-terminal region of plant HMGA proteins shares

314   remarkable homology with the DNA-binding domain of histone H1[69,70]. A similar structural

315   description was confirmed in CbuHMGA, which encoded a H15 domain and four AT-hook motifs.

316   HMGA is an architectural TF[70–73]. It regulates gene expression in vivo by controlling the

317   formation of multiprotein complexes on the AT-rich regions of certain gene promoters[68,70,81,73–80].

318   To date, some plant HMGA proteins have been isolated, such as those in soybean[82], rice[83], maize[84]

319   and Arabidopsis[67,73,75,80,85–87]. The Arabidopsis HMGA gene was detected in flowers, and

320   developing siliques had the highest expression[87]. However, studies of the interacting proteins and

321   the function of HMGA in woody plants are rare. In "Bairihua", the expression of *CbuHMGA* was

322   elevated in the dormant period, and the highest expression level was detected in the reproductive

323   growth period. The result was consistent with that in Arabidopsis. Although the *oe-hmga*

324   transgenic lines did not show a flowering time phenotype, the floral organ mutation of *oe-hmga*

325   was similar to that of the *oe-spl9* transgenic lines.

326   The HMGA proteins have an important role in biological processes and interact with different

327   TFs[70,73,88]. Their intrinsic flexibility allows the HMGA proteins to participate in specific

328   protein-DNA and protein-protein interactions that induce structural changes in chromatin and the

329   formation of stereospecific complexes called 'enhanceosomes' on the promoter/enhancer regions

330   of genes whose transcription they regulate. The chromatin structure changes affect the ability of

331   TFs to bind with the promoter/enhancer regions[68,74,77,78,80,85]. From the results in this study, we

332   suggest that the interaction of CbuHMGA with CbuSPL9 might strengthen or weaken the binding

333   ability of CbuSPL9 with the corresponding DNA sequences or downstream proteins, thus

334   affecting the flowering process. Further experiments are needed to test this hypothesis.

335   **Conclusion**

336   "Bairihua" provides us with a valuable opportunity to gain a deeper understanding of the

337   flowering process of woody plants. Our study showed that *SPLs* may have similar structures and

338   regulatory mechanisms in perennial trees and Arabidopsis. Screening CbuSPL9-interacting

339   proteins revealed not only additional proteins for research on the regulatory pathway of CbuSPL9

340   but also the CbuSPL9 interaction with CbuHMGA. *oe-hmga* showed a phenotype that affected

341   floral organ development but did not change flowering time. This result suggests that the

342   mechanism by which *CbuSPL9* affects the flowering process is a complex process, and

343   *CbuHMGA* is involved in the development of flower organs but not in the regulation of flowering

344   time.

Conflict of interest

None declared

Funding

Author contributions

J.H.W., S.G.Z., G.Z.Q., L.I.S., Z.W. and W.J.M. designed the experiments. Z.W. and W.J.M. analyzed the RNA-seq data and wrote the manuscript. Z.W., and T.Q.Z. detected the expression of genes using qRT-PCR. Z.W., N.L., F.Q.O.Y., N.W. and G.J.Y. collected the samples used in the experiment. All the authors have read the paper and agreed to list their names as co-authors.

Supplementart Data

Supplementary Table S1 The list of the all Primers used in this paper.

Supplementary Figure S1 Verification of *oe-SPL9* positive Arabidopsis.

Supplementary Figure S2 Verification of *oe-HMGA* positive Arabidopsis.

Supplementary Table S2 Details of motif-sequences of CbuSPL9 were identified by MEME.

Supplementary Table S3 Statistics of mutant of floral organs in *oe-SPL9* Arabidopsis.

Supplementary Table S4 Statistics of mutant of flowering time in *oe-SPL9* Arabidopsis.

Supplementary Table S5 Statistics of mutant of floral organs in *oe-HMGA* Arabidopsis.

Supplementary Table S6 Statistics of mutant of flowering time in *oe-HMGA* Arabidopsis.

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599

600   **Table 1 Details of the proteins interacting with the CbuSPL9 protein**

Annotation	Quantity
Sesamum indicum HMG-Y-related protein B-like (LOC105159884), mRNA	4
PREDICTED: Sesamum indicum aquaporin PIP2-4-like (LOC105174310), mRNA	1
PREDICTED: Sesamum indicum GATA transcription factor 8 (LOC105166606), transcript variant X2, mRNA	1
PREDICTED: Sesamum indicum heavy metal-associated isoprenylated plant protein 33-like (LOC105156967), mRNA	2
PREDICTED: Sesamum indicum probable E3 ubiquitin ligase SUD1 (LOC105169523), mRNA	4
PREDICTED: Erythranthe guttatus transcription factor bHLH148-like (LOC105966580), mRNA	1
PREDICTED: Sesamum indicum aquaporin TIP1-1-like (LOC105170758), mRNA	1
PREDICTED: Sesamum indicum probable GTP diphosphokinase RSH2, chloroplastic (LOC105157806), transcript variant X1, mRNA	1
Arabidopsis thaliana proline-rich family protein mRNA （(PRPs）	1
PREDICTED: Sesamum indicum fasciclin-like arabinogalactan protein 17 (LOC105161692), mRNA	1
PREDICTED: Erythranthe guttatus MAG2-interacting protein 2 (LOC105963338), mRNA	1

601

602 **Table 2 Interaction between HIS-CbuHMGA and GST-CbuSPL9 by BLI**

Protein1	Ptotein2	Conc. of Protein2/nM	K_{on}/Ms	K_{off}/s	K_D/nM
HIS-CbuHMGA	GST-CbuSPL9	2381	5.40E-04	1.74E+03	311
HIS-CbuHMGA	GST-CbuSPL9	1191	5.40E-04	1.74E+03	311
HIS-CbuHMGA	GST-CbuSPL9	595.3	5.40E-04	1.74E+03	311
HIS-CbuHMGA	GST-CbuSPL9	297.6	5.40E-04	1.74E+03	311
HIS-CbuHMGA	GST-CbuSPL9	148.8	5.40E-04	1.74E+03	311

603

604 **Figure 1 The phylogenetic relationship and motif composition analysis of the CbuSPL9 gene**

605 **in *C. bungei*.** a) Multiple sequence alignment and sequence logo of the *C. bungei* SBP-box

606 domain. Sequence alignment was performed with DNAMAN. The two conserved zinc fingers and

607 NLS are indicated. The sequence logo was obtained from MEME online software. The overall

608 height of the stack indicates the sequence conservation at that position. b) The phylogenetic tree

609 was constructed with MEGA 6.0 by the neighbor-joining (NJ) method with 1000 bootstrap

610 replicates. Bootstrap support is indicated at each node. *A. thaliana* (At), *C. bungei* (Cbu).

611 **Figure 2 Expression profile of *CbuSPL9* in the flower buds and leaf buds during the**

612 **developmental periods of *C. bungei*.** T1-T3 represents the flowering buds and leaf buds collected

613 in the dormant period; T4-T5 represents the flowering buds and leaf buds collected in the

614 germination period; T6-T9 represents the flowering buds and leaf buds collected in the floral

615 transition period; and T10-T12 represents the flowering buds and leaf buds collected in the

616 reproductive period. D1: Image of flowering buds in the dormant period; D2: Image of leaf buds

617 in the dormant period; G1: Image of flowering buds in the germination period; G2: Image of leaf

618 buds in the germination period; F1: Section of flowering buds in the floral transition period; note

619 the flat generative apex (Ga); F2: Section of leaf buds in floral transition period, note the bulged

620 vegetative apex (V); R1: Image of flowering buds in the reproductive period; and R2: Image of

621 leaf buds in the reproductive period. Notably, even though floral transition was never observed in

622 the leaf buds, the leaf buds collected in the period corresponding to floral transition are henceforth

623 called F2 and R2 for convenience. Error bars indicate SD from three independent biological

624 replicates. *Difference between flowering buds (black) and leaf buds (gray) is significant

625 (Student's test; p<0.05). **Difference between flowering buds (black) and leaf buds (gray) is

626 highly significant (Student's test; p<0.01).

627 **Figure 3 Phenotype of overexpression mutants of *CbuSPL9*.** a) The change in floral organs

628 occurred in the CbuSPL9-overexpressing transgenic Arabidopsis lines. Image of a normal flower

629 from col as the control (a/I); and the floral organ mutant from oespl9 (a/II-VI). b) Comparison of

630 the flowering phenotype in both the col and oespl9 transgenic plants. From top to bottom: col

631 (control) and oespl9 (transgenic plants). c) and d) are the statistical data of the time of flower bud

632 emergence and the number of rosette leaves. The oespl9 transgenic plants (black), the oe-hmga

633 transgenic plants (gray) and the col plants (white). A total of 30 plants were averaged to obtain the
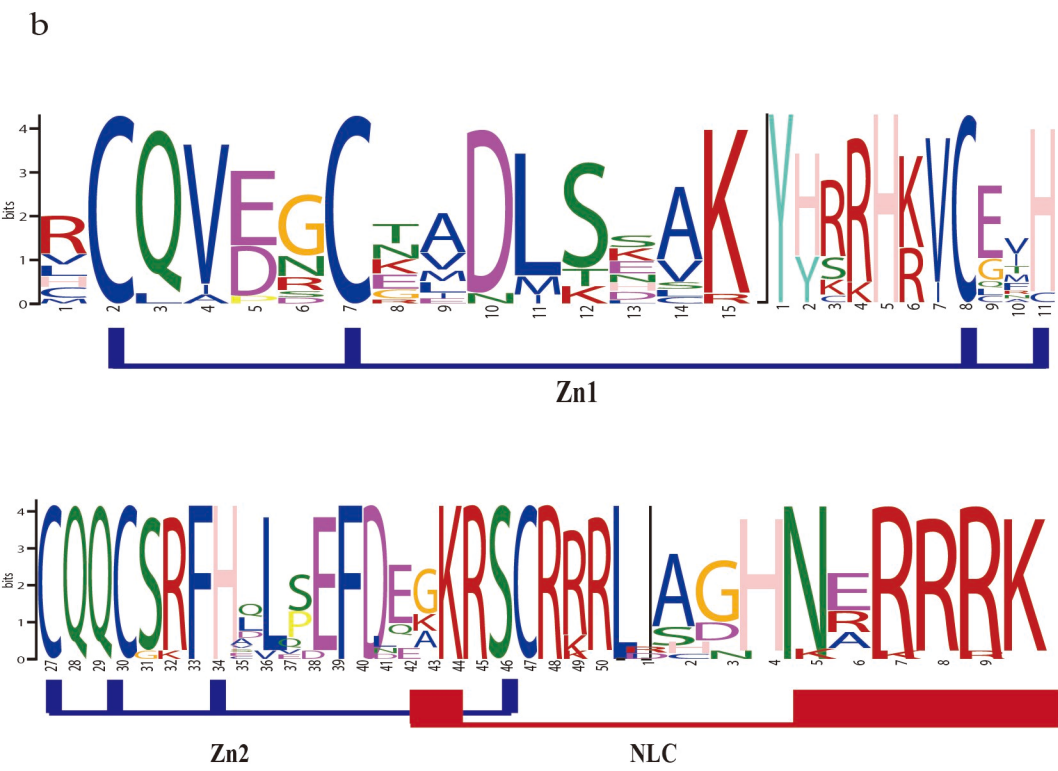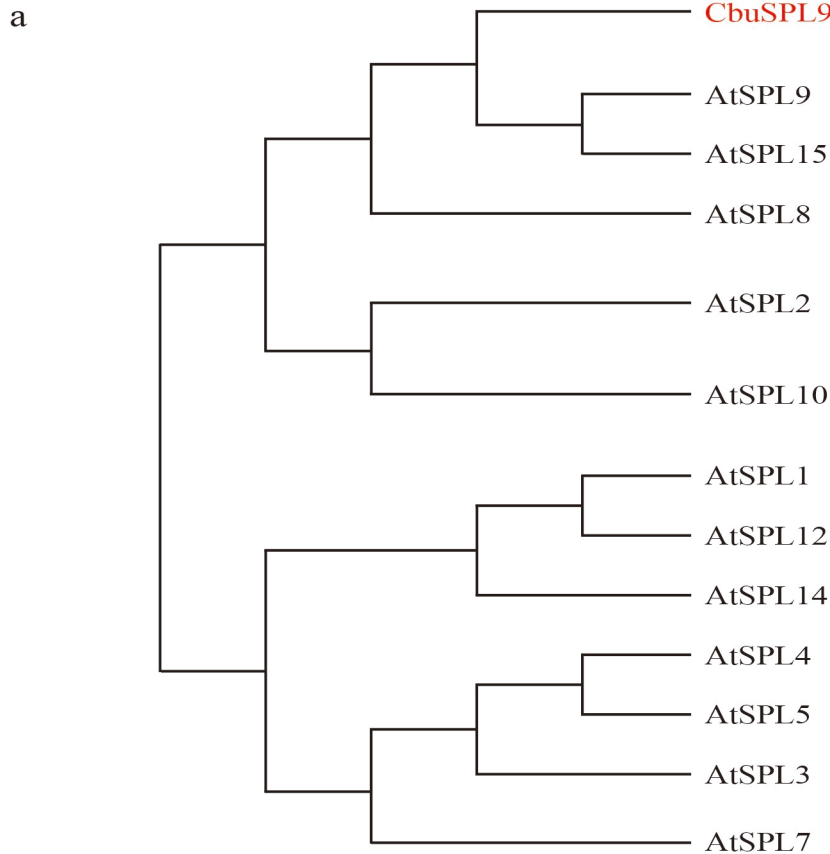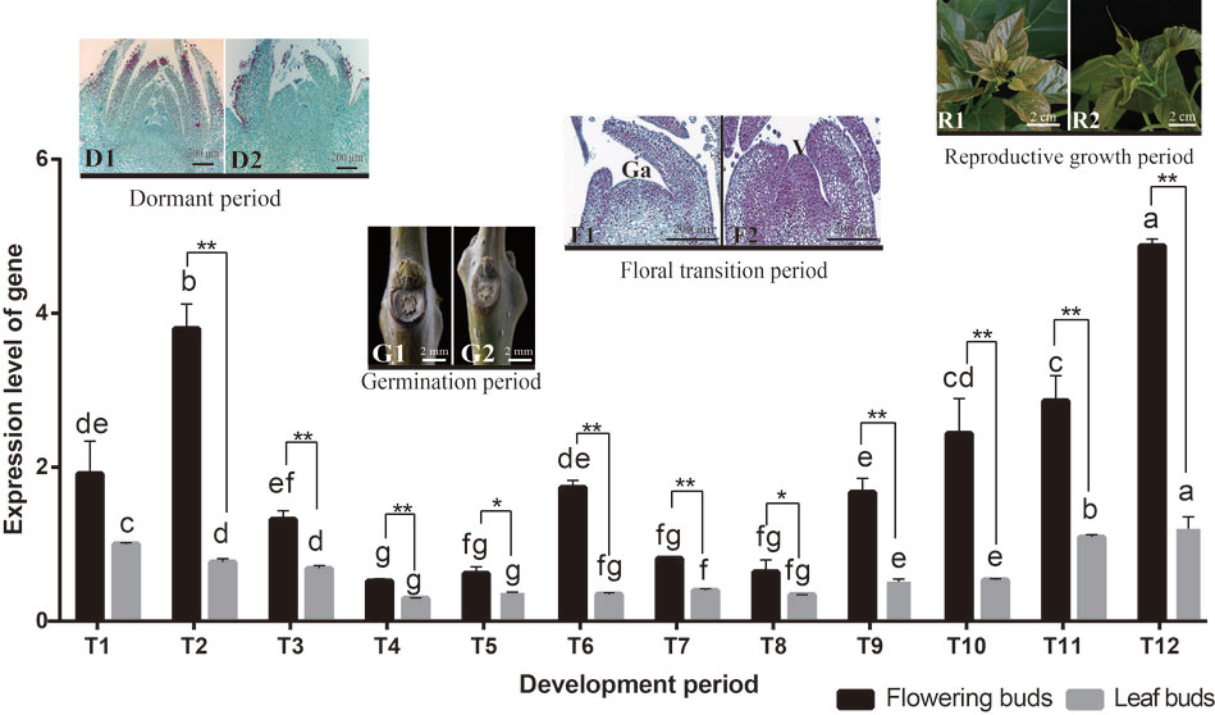
634   mean. Error bars indicate SD.

635   **Figure 4 The sequence and expression profile analysis of *CbuHMGA*.** a) Sequence analysis of

636   the CbuHMGA protein by SMART. b) The expression analysis of *CbuHMGA* between flowering

637   buds and leaf buds during the development periods. T1-T3 represents the flowering buds and leaf

638   buds collected in the dormant period; T4-T5 represents the flowering buds and leaf buds collected

639   in the germination period; T6-T9 represents the flowering buds and leaf buds collected in the

640   floral transition period; and T10-T12 represents the flowering buds and leaf buds collected in the

641   reproductive period. Notably, even though floral transition was never observed in the leaf buds,

642   the leaf buds collected in the period corresponding to floral transition are henceforth called the

643   floral transition period and reproductive period for convenience. Error bars indicate SD from three

644   independent experiments. *Difference between flowering buds (black) and leaf buds (gray) is

645   significant (Student's test; $p < 0.05$). **Difference between flowering buds (black) and leaf buds

646   (gray) is highly significant (Student's test; $p < 0.01$).

647   **Figure 5 Interaction between the two proteins CbuSPL9 and CbuHMGA.** a) Nuclear

648   localization of the CbuSPL9 protein and CbuHMGA protein. The GFP (control) gene,

649   CbuSPL9-GFP fusion gene and CbuHMGA-GFP fusion gene were expressed transiently in

650   *Nicotiana benthamiana* leaf epidermal cells and observed with confocal microscopy. DAPI, DAPI

651   for nuclear staining image; GFP, GFP green fluorescence image; Merge, the merged images of

652   bright-field, GFP and DAPI staining. b) Binding of GST-CbuSPL9 to HIS-CbuHMGA, with

653   GST-CbuSPL9 concentrations of 2381.00 nm, 1191.0 nm, 595.30 nm, 297.60 nm, 148.80 nm

654   assessed by real-time biolayer interferometry. c) Yeast two-hybrid assays for the interactions

655   between CbuSPL9 and CbuHMGA. CbuHMGA (as prey) was fused with the GAL4 activation

656   domain (AD) in pGADT7, while CbuSPL9 (as bait) was fused with the GAL DNA-binding

657   domain (BD) in pGBKT7. The positive control was as follows: pGADT7-T and pGBK-53. The

658   negative control was as follows: pGADT7-T and pGBK-Lam. Interactions are indicated by the

659   blue color on SD/-Trp/-Leu/-His/-Ade/X-α-gal medium. d) Confocal images of the BiFC analysis

660   in protoplasts from *P. trichocarpa*. CbuSPL9 was fused with $EYFP^C$, and CbuHMGA was fused

661   with $EYFP^N$. EYFP signal was detected in the nucleus of the S1-21 protoplasts transfected by

662   H2A:mCherry and $CbuSPL9:EYFP^C$ with (A) $CbuHMGA:EYFP^N$. Bars=50 μm.

663   **Figure 6 Phenotype of overexpression mutants of *CbuHMGA*.** a) The change in floral organs

664   occurred in the overexpression of the CbuHMGA transgenic Arabidopsis lines. The images are the

665   floral organ mutant from oe-hmga transgenic Arabidopsis lines (a/I-VI). b) Expression profiles of

666   atSPL9 homologous genes in the oespl9 transgenic plants, oe-hmga transgenic plants and col.

667   ▬■▬ represents the PCR results in oe-hmga; ▬■▬ represents the PCR results in oespl9; and ▬▲▬

668   represents the PCR results in col. Three independent biological replicates were performed, and

669   each replicate was measured in triplicate. The error bars show the standard deviation of the results

670   of three technical replicates.

a

b

Zn1

Zn2 NLC

Dormant period

Germination period

Floral transition period

Reproductive growth period

Expression level of gene (y-axis, 0–6)

Development period (x-axis): T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12

Legend: ■ Flowering buds ☐ Leaf buds

a

I, II, III, IV, V, VI

b

col-1   col-2   col-3

35::CbuSPL9-1   35::CbuSPL9-2   35::CbuSPL9-3

c

Time of flower buds emergence/d

oespl9   col

d

Number of rosette leaves

oespl9   col

a

b

a

	DAPI	GFP	Bright	Merge
CbuSPL9				
CbuHMGA				
1304-GFP				

b

CbuSPL9 association

Disassociation

[*CbuSPL9*]

2381.00nm

1191.00nm

595.30nm

297.60nm

148.80nm

Kd=311nm

c

	BD		
AD	*CbuSPL9-1*	*CbuSPL9-2*	*CbuSPL9-3*
CbuHGMA			

53   Lam

T

SD/-T-L-H-A/x-α-gal

d

S:EYFP^C
H:EYFP^N H2A:mChery   Merged

Supplementary Table S1 The list of the all Primers used in this paper.

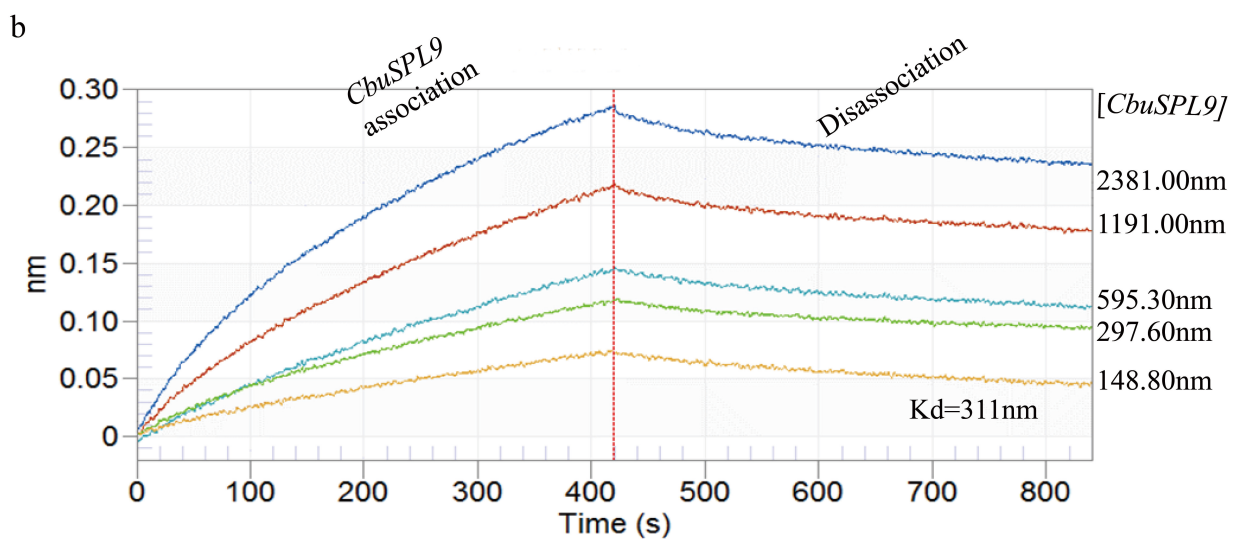|	Gene name	Primer sequence (5'-3')
Primers used in homology-basd cloningof SPL and RACE of homologous gene CbuSPL	SPLF	CAGGTGGAGGGTTGTAAGGT
	SPLR	CCAAGACTCAAGGATCGGGT
	3'RSPLF1	GCAAAGGTTTTGCCAGCAGTG
	3'RACE Out	TACCGTCGTTCCACTAGTGATTT
	3'RSPLF2	CGCCTTGCTGGCCATAACGAGCGTA
	3'RACE Inner	CGCGGATCCTCCACTAGTGATTTCACTATAGG
	5'RSPLR1	AGAAGAGAGAGAGCACCGGTG
	5'RACE Out	CATGGCTACATGCTGACAGCCTA
	5'RSPLR2	CCTGATATGGAAGTTGATACTTT
	5'RACE Inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG
	QCCbuSPLF	ATGCACTGATTGTATGAGGGAG
	QCCbuSPLR	GACACACAGAAAGCAACAACAT
Primers used in PCR of cDNA from homologous gene CbuSPL9 and CbuHMGA	CbuSPL9F	ATGGAAAAGGGTTCTTCCTC
	CbuSPL9R	TCAGATAGAGGATTTTGTGAGTTT
	CbuHMGAF	ATGGCGAGCGAAGAAGTACA
	CbuHMGAR	CTACAATGATCCATAAATTAAAAAAC
Primers used in overexpression analysis	121CbuSPL9F	TCTAGAATGGAAAAGGGTTCTTCCTC
	121CbuSPL9R	CCTAGGTCAGATAGAGGATTTTGTGAGTTT
	121CbuHMGAF	TCTAGAATGGCGAGCGAAGAAGTACA
	121CbuHMGAR	CCTAGGCTACAATGATCCATAAATTAAAAAAC
Primers used in analysis for the presence of the transgene	CbuSPL9F	GCCATCATTGCGATAAAGGAAA
	CbuSPL9R	ATCCAGACTGAATGCCCACAGG
	CbuHMGAF	GCCATCATTGCGATAAAGGAAA
	CbuHMGAR	ATCCAGACTGAATGCCCACAGG
Primers used in subcellular localization analysis	CbuSPL9-GFPF	CCATGGATGGAAAAGGGTTCTTCCTC
	CbuSPL9-GFPR	TCTAGATCAGATAGAGGATTTTGTGAGTTT
	CbuHMGA-GFPF	CCATGGATGGCGAGCGAAGAAGTACA
	CbuHMGA-GFPR	TCTAGACTACAATGATCCATAAATTAAAAAAC
Primers used in yeast two-hybrid	BD-CbuSPL9F	CATATGATGGAAAAGGGTTCTTCCTCCT
	BD-CbuSPL9R	GTCGACGTCAGATAGAGGATTTTGTGAGTTTCC
	AD-CbuHMGAF	CATATGATGGCGAGCGAAGAAGTACA
	AD-CbuHMGAR	GTCGACGCTACAATGATCCATAAATTAAAAAAC
Primers used in prokaryoticexpre	CbuSPL9-6P-F	GGATCCATGGAAAAGGGTTCTTCCTC
	CbuSPL9-6P-R	CTCGAGTCAGATAGAGGATTTTGTGAGTTT

	Gene name	Primer sequence (5'-3')
ssion	CbuHMGA-HIS-F	CGCGGATCCATGGCGAGCGAAGAAGTACAG
	CbuHMGA-HIS-R	CGCAAGCTTCTAAGCCCCTACGGGTGCGGC
	CbuSPL9F	CACCATGGAAAAGGGTTCTTCCTCCTC
	CbuSPL9R	AAGTGACCAGTGCACAGAAGAATC
Primers used in BIFC	CbuHMGAF	CACCATGGCGAGCGAAGAAGTACAGG
	CbuHMGAR	AGCCCCTACGGGTGCGG
	M13F	GTTGTAAAACGACGGCCAG
	M13R	CAGGAAACAGCTATGAC
	CaMV35S-F	GACGCACAATCCCACTATCC

Supplementary Table S2 Details of motif-sequences of CbuSPL9 were identified by MEME

No.	Sequence（5'-3'）	Quantity of motif site
1	YYCRHKVCGMHSKSPKVIVAGLEQRFCQQCSRFHQLPEFD QGKRSCRRRL	16
2	RCQVEGCKVDLSDAK	16
3	AGHNERRRKP	16
4	RTGRIVFKLFGKEPNEFPIVLRGQILDWLSHSPTDMESYIRP GCIVLTIY	4
5	PAGLTPLHIAAGKDGSEDVLDALTEDPAMVGIEAWKTCRD STGFTPEDYA	3
6	LGLKLGKRTYFEDFW	7
7	AYRPAMLSMVAIAAVCVCVALLFKSCPEVLYVFQ	3
8	FFPFLVVEDDDVCSEIRILETTLEFTGTDSAKQAMDFIHEIG WLLHR	2
9	FPLIRFQWLIEFSMDREWCAVIRKLLNMFFD	3
10	QAETAWEELSDDLGFSLGKLLDLSDDPLWTTGWIYVRVQN QLAFVYNGQV	3
11	GKRSVEWDLNDWKWD	5
12	ATLSELCLLHRAVRKNSKPMVEMLLRY	5
13	EKAQFTVKGMNLRQRGTRLLCSVEGKYLIQETT	4
14	GRDSWPNTTSERGLGNQSATTGKYQLPYQGNSQNP	2
15	DHHHQSRRQYMEDENTRAYDSSSHHTNW	2
16	VNFSCDMPILSGRGFMEIEDQ	2
17	FDNHSRSGGFMMDFSAY	2
18	MECNAKPPFQWELENLISFGTSTAEVPRKLKPMEWEI	2
19	DSNCALSLLSN	7
20	DDQTSNYMLITLLKILSNIHSNQSDQ	2

Supplementary Table S3 Statistics of mutant of floral organs in *oe-SPL9* Arabidopsis

Type	overlapping petals	Change in petals number	shrunken petals
CbuSPL9-1	-	*	*
CbuSPL9-2	-	*	-
CbuSPL9-3	-	-	-
CbuSPL9-4	*	-	-
CbuSPL9-5	-	*	-
CbuSPL9-6	*	-	-
CbuSPL9-7	*	*	-
CbuSPL9-8	-	*	-
CbuSPL9-9	-	-	-
CbuSPL9-10	-	-	*
CbuSPL9-11	*	*	-
CbuSPL9-12	-	*	*
CbuSPL9-13	*	*	-
CbuSPL9-14	*	*	-
CbuSPL9-15	-	-	*
CbuSPL9-16	-	-	*
CbuSPL9-17	*	*	-
CbuSPL9-18	*	-	-
CbuSPL9-19	-	*	-
CbuSPL9-20	-	*	
CbuSPL9-21	-	-	*
CbuSPL9-22	-	-	-
CbuSPL9-23	-	*	-
CbuSPL9-24	-	-	*
CbuSPL9-25	-	*	-
CbuSPL9-26	-	*	*
CbuSPL9-27	*	-	-
CbuSPL9-28	-	*	-
CbuSPL9-29	*	*	-
CbuSPL9-30	-	*	-
CbuSPL9-31	-	-	*
CbuSPL9-32	*	-	-
CbuSPL9-33	-	*	-
CbuSPL9-34	-	-	*
CbuSPL9-35	*	*	-
CbuSPL9-36	-	*	-

Supplementary Table S4 Statistics of mutant of flowering time in *oe-SPL9* Arabidopsis

Type	DAY	Type	DAY
CbuSPL9-01	22	WT-01	27
CbuSPL9-02	17	WT-02	27
CbuSPL9-03	23	WT-03	26
CbuSPL9-04	21	WT-04	27
CbuSPL9-05	22	WT-05	25
CbuSPL9-06	16	WT-06	28
CbuSPL9-07	22	WT-07	28
CbuSPL9-08	23	WT-08	26
CbuSPL9-09	23	WT-09	25
CbuSPL9-10	22	WT-10	27
CbuSPL9-11	21	WT-11	25
CbuSPL9-12	22	WT-12	27
CbuSPL9-13	23	WT-13	27
CbuSPL9-14	22	WT-14	26
CbuSPL9-15	22	WT-15	27
CbuSPL9-16	24	WT-16	27
CbuSPL9-17	22	WT-17	25
CbuSPL9-18	18	WT-18	29
CbuSPL9-19	23	WT-19	27
CbuSPL9-20	21	WT-20	28
CbuSPL9-21	24	WT-21	27
CbuSPL9-22	23	WT-22	26
CbuSPL9-23	21	WT-23	26
CbuSPL9-24	24	WT-24	27
CbuSPL9-25	21	WT-25	27
CbuSPL9-26	23	WT-26	25
CbuSPL9-27	21	WT-27	26
CbuSPL9-28	18	WT-28	26
CbuSPL9-29	21	WT-29	27
CbuSPL9-30	20	WT-30	26

Supplementary Table S5 Statistics of mutant of floral organs in *oe-HMGA* Arabidopsis.

Type	Overlapping petals	Change in petals number	shrunken petals
CbuHMGA-1	*	*	
CbuHMGA-2			*
CbuHMGA-3		*	
CbuHMGA-4	-	-	-
CbuHMGA-5		*	*
CbuHMGA-6		*	
CbuHMGA-7	*	*	
CbuHMGA-8		*	
CbuHMGA-9			*
CbuHMGA-10	*	*	
CbuHMGA-11	-	-	-
CbuHMGA-12	*	*	
CbuHMGA-13		*	
CbuHMGA-14		*	
CbuHMGA-15			*
CbuHMGA-16		*	
CbuHMGA-17		*	*
CbuHMGA-18		*	
CbuHMGA-19	-	-	-
CbuHMGA-20	-	-	-
CbuHMGA-21		*	
CbuHMGA-22	*		
CbuHMGA-23			*
CbuHMGA-24		*	
CbuHMGA-25			*
CbuHMGA-26	*	*	
CbuHMGA-27			*
CbuHMGA-28	-	-	-
CbuHMGA-29			*
CbuHMGA-30	*	*	
CbuHMGA-31			*
CbuHMGA-32		*	
CbuHMGA-33			*
CbuHMGA-34		*	
CbuHMGA-35	-	-	-
CbuHMGA-36	*	*	

Supplementary Table S6 Statistics of mutant of flowering time in *oe-HMGA* Arabidopsis.

Type	Time	Type	Time
CbuHMGA-01	25	WT-01	27
CbuHMGA-02	25	WT-02	27
CbuHMGA-03	26	WT-03	26
CbuHMGA-04	24	WT-04	27
CbuHMGA-05	27	WT-05	25
CbuHMGA-06	28	WT-06	28
CbuHMGA-07	26	WT-07	28
CbuHMGA-08	24	WT-08	26
CbuHMGA-09	28	WT-09	25
CbuHMGA-10	27	WT-10	27
CbuHMGA-11	24	WT-11	25
CbuHMGA-12	28	WT-12	27
CbuHMGA-13	27	WT-13	27
CbuHMGA-14	26	WT-14	26
CbuHMGA-15	25	WT-15	27
CbuHMGA-16	28	WT-16	27
CbuHMGA-17	25	WT-17	25
CbuHMGA-18	24	WT-18	29
CbuHMGA-19	28	WT-19	27
CbuHMGA-20	27	WT-20	28
CbuHMGA-21	26	WT-21	27
CbuHMGA-22	26	WT-22	26
CbuHMGA-23	26	WT-23	26
CbuHMGA-24	25	WT-24	27
CbuHMGA-25	27	WT-25	27
CbuHMGA-26	25	WT-26	25
CbuHMGA-27	27	WT-27	26
CbuHMGA-28	26	WT-28	26
CbuHMGA-29	26	WT-29	27
CbuHMGA-30	28	WT-30	26