Pak. J. Bot., 49(3): 1063-1071, 2017.

ECTOPIC EXPRESSION OF SOYBEAN *GmSBH1* CONFERS ABA SENSITIVITY DURING SEED GERMINATION AND EARLY SEEDLING ESTABLISHMENT IN TRANSGENIC *ARABIDOPSIS*

YINGJIE SHU[1,2Θ], YUAN TAO[3Θ], YULI ZHOU[1], MING CHEN[2], LIYAN HUANG[2], SHOUCHENG HUANG[1*] AND HAO MA[2*]

[1]*College of Agronomy, Anhui Science & Technology University, Fengyang 233100, China*
[2]*State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China*
[3]*InnoSpring (Kunshan) Co., Ltd, Kunshan RNAi & Biomed Park, Kunshan 215300, China*
**Corresponding authors email: huangsc@ahstu.edu.cn; lq-ncsi@njau.edu.cn*
[Θ]Authors contributed equally to this work

Abstract

The class I KNOX homeobox transcription factors are known to play an important role in maintenance of plant phenotype, especially leaves and flowers. In this study, a soybean KNOX I homeobox transcription factor, GmSBH1, was analyzed and confirmed to play important roles in the process of seed germination and developing. Real time quantitative PCR assay showed that the transcript level of *GmSBH1* in soybean seedlings was modulated by plant hormones, such as IAA, GA, MeJA and ABA.Yeast one-hybrid assay showed that GmSBH1 could bind to the ABRE cis-element. Overexpression of *GmSBH1* in *Arabidopsis* resulted in the abnormal phenotype of flowers and siliques. In *GmSBH1* transgenic lines, both seed germination and seedlings growth showed hypersensitive to ABA. Moreover, the expression of ABA-responsive genes, such as *ABI3* and *ABI5*, were increased in the transgenic line seedlings. Taken together, ectopic expression of *GmSBH1* could alter the morphology and confer ABA sensitivity during seed germination and early seedling growth in transgenic *Arabidopsis*.

Key words: Soybean, Homeobox gene, *GmSBH1*, Abscisic acid, Seed germination and seedling growth, Yeast one-hybrid.

Introduction

Transcription factors bind to the specific cis-elements in the promoter regions to activate or suppress the expression of the targeted gene. They play important roles in regulation of plant growth and development, as well as responses to biotic and abiotic stresses. Plant homeobox transcription factors, especially KNOX family members are known to actively participate in maintaining the plant shoot apical meristem (SAM), which generates the entire aboveground part of vascular plants (Hay & Tsiantis, 2010). KNOX genes encode a large super-class of homeodomain (HD) proteins, which contain a three-amino acid-loop extension (TALE) motif (Burglin, 1997; Hake *et al*., 2004). Majority of KNOX members have four conserved regions, KNOX I, KNOX II, ELK and HD. Both KNOX II and HD domains are necessary for the homodimerization and transactivation, while KNOX I and ELK domains participate in suppressing target gene expression and affect phenotype severity (Nagasaki *et al*., 2001). Based on the differences of the conserved motifs, KNOX family could be subdivided into two classes (Kerstetter *et al*., 1994). Class I KNOX genes are expressed in overlapping domains within the SAM of both monocot and eudicot plants (Hake *et al*., 2004; Scofield & Murray, 2006). Class II KNOX genes form a monophyletic group and regulate secondary cell wall biosynthesis and root development (Zhong *et al*., 2008; Truernit & Haseloff 2007). Numerous evidences have revealed the contributions of Class I to the plant growth and development through loss- and gain-of-function analysis, the traits include overall plant height, leaf shape, meristem development and floral development (Long *et al*., 1996; Endrizzi *et al*., 1996; Liu *et al*., 2008). Overexpression of *Arabidopsis STM* resulted in smaller flower and earlier flowering date than wild type (Scofield *et al*., 2007). Loss-of-function mutants of maize *kn1* lead to the formation of fewer lateral meristems but more lateral organs such as leaves and carpel (Kerstetter *et al*., 1997). The expression of *35S::GmKNT1* in *Arabidopsis* plants leads to smaller and lobed leaves, shortened internodes and smaller clustered inflorescence (Liu *et al*., 2008). However, few studies had been conducted to analyze the relationship between plant morphological change and phytohormones.

ABA is an important phytohormone that plays a pivotal role in various physiological processes during the plant life cycle, including seed dormancy, germination, and adaptive responses to various environmental stress conditions (Cutler *et al*., 2010; Qing *et al*., 2010; Umezawa *et al*., 2011). A lot of research has revealed that the plant homeobox transcription factors could modulate the expression of key genes in the ABA signaling network. For example, *ATHB5* mediated the inhibitory effect of ABA on the growth of plant during the early seedling establishment (Johannesson *et al*., 2003). Overexpression of *BPM3* leaded to the decreasing of *ATHB6* and also suppressed the phenotypic alterations caused by *ATHB6* ectopic expression (Lechner *et al*., 2011). In addition, *BLH1* and *KNAT3* could together modulate the seed germination and the early seedling development by directly regulating the *ABI3* expression in *Arabidopsis* (Kim *et al*., 2013).

GmSBH1 was the first class I KNOX homeobox transcription factor isolated from soybean and played an important role in plant embryo and leaf development (Ma *et al*., 1994; Shu *et al*., 2015). In a recent study, *GmSBH1* was found to be able to enhance the tolerance of soybean to the pre-harvest seed deterioration caused by heat and humidity stress, and its overexpression in *Arabidopsis*

could alter the leaf and stoma phenotypes (Shu *et al*., 2015). In the present study, our results indicated that *GmSBH1* was sensitively regulated by various hormones (such as IAA, GA, MeJA and ABA) in soybean seedling and played important roles in seed germination and developing process. Overexpression of *GmSBH1* in *Arabidopsis* resulted in a dramatical change in floral organ and silique development. Moreover, transgenic lines with *GmSBH1* were hypersensitive to ABA during the seed germination and the early seedling establishment. The overall results confirmed that *GmSBH1* played a key role in plant morphology and conferred ABA sensitivity during the seed germination and early seedling development in transgenic *Arabidopsis*.

Material and Methods

Plant materials and treatment: Soybean (*Glycine max* (L.) Merr.) cv. Ningzhen No. 1 was released by Wang *et al*., (2012). Seeds of cv. Ningzhen No. 1 were sown in plastic pots filled with the nutrient soil, and the pots were incubated in a growth chamber with normally management. The roots of two-week-old seedlings were immersed in the solutions including indole-3-acetic acid (IAA, 20 μM), abscisic acid (ABA, 100 μM), methyl jasmonate (MeJA, 100 μM) and gibberellin (GA, 100 μM), respectively. The control treatment was washed with water. Leaves from the treated seedlings were harvested at 0, 1, 3, 6, 12, and 24 h after treatment, respectively. Seeds were collected from different reproductive periods, including initial grain-filling stage (R5), grain-filling stage (R6), early-maturing stage (R7), and full-ripening stage (R8), respectively. The samples were frozen with the liquid nitrogen and stored at -80°C or used for RNA isolation. Three independent experiments were carried out.

Isolation of GmSBH1 and the real time quantitative PCR (qRT-PCR) assay: *GmSBH1* was isolated from soybean seed as described by Shu *et al*. (2015). qRT-PCR was carried out on Bio-rad CFX96 Touch Real Time PCR System. The qRT-PCR mixture of 20 μL was prepared using the SYBR® Premix Ex Taq™ (Takara). Expression of gene was quantified using the comparative CT method. Experiments were performed in triplicate and the results were represented by means ± SE of three replicates. The primers sets for *GmSBH1* gene were: 5′-TTA AAG GGT CAG CTT TTG CG-3′ and 5′-TTC TGG GAT TCG GAT GGG T-3′. Soybean *Actin* gene was used as standard. The primers sets for *Actin* gene were: 5′-CCT CAA CCC AAA GGT CAA CAG-3′ and 5′-GAC CAG CGA GAT CCA AAC GAA-3′.

Yeast one-hybrid assay: Yeast one-hybrid assay was performed using the Yeastmaker™ Yeast transformation system 2 (Clontech, Palo Alto, CA, USA). The DNA fragment of three tandem copies of each element, two tandem copies of each element, one element, and three tandem copies of each mutant element were synthesized and inserted directly into the multiple cloning sites of reporter plasmids of pHis2, respectively. The whole coding sequence of GmSBH1, which removed terminated codes, was ligated into the *SmaI*-digested pGADT7-Rec2 vector to generate pGADT7-Rec2-GmSBH1 vector. These two constructs (GmSBH1 was combined with three other plasmids, respectively) were integrated into the genome of yeast strain Y187. The dual reporter strain was selected and maintained on synthetic dextrose SD-TL, SD-TLH, and SD-TLH containing 200 mM 3-AT medium, respectively (Zhou & Li, 2016).

Seed germination assay: Three T_3 homozygous transgenic lines (L11, L14, and L15) of *GmSBH1* gene were used in the study which were referred by Shu *et al*. (2015). Mature seeds from transgenic lines and wild-type were collected at the same day, respectively. Surface-sterilized seeds were planted on half-strength MS medium containing 0.5% sucrose and 0.8% agar (pH 5.7), supplemented with different concentrations of ABA (0, 0.5 and 1.0 μM). Plates were incubated at 4°C without light for 2 d, and transferred to a culture room set at 23°C under long-day conditions. Radicle emergence was scored at the indicated time intervals.

Primary root length and fresh weight measurement: Seeds were germinated on half-strength MS medium containing 0.5% sucrose and 1.5% agar (pH 5.7) and grown in a vertical position. Three-day-old seedlings were transferred to the fresh medium with or without ABA (0, 30 and 50 μM), and incubated for 2 weeks before taking the measurement of primary root length and fresh weight. The experiment was repeated three times and 20-30 plants were analyzed in each experiment.

Scanning electron microscopy: For scanning electron microscopy assay, siliques from wild-type and transgenic lines were harvested and fixed with 2.5% glutaraldehyde solution at 4°C overnight. The fixed siliques were dehydrated in gradual ethanol series, and then critical point dried. A S-3000N scanning electron microscope was used for observation of siliques at an accelerating voltage of 25 kV (Hitachi, Japan).

Expression analysis of ABA-related genes: Transgenic lines and wild-type seedlings were treated with 100 μM ABA for 2 and 5 h, respectively. The real time quantitative PCR technique was used to analyze the expression of ABA-related genes, with *Arabidopsis Tubulin* being used as a reference gene. The following primers sets were used for qRT-PCR: 5′-GAG AAA CTG ACA ACT GAA GA-3′ and 5′-CAC ATA AAC ATC CAA AGT GA-3′ for *RD29A*, 5′-GAC GGC TAA AAA TTA TAT AT-3′ and 5′-GTT CAC AAA CAG AGG CAT CAT AC-3′ for *RD29B*, 5′-CAT GGA GAT TCC ATT AGA CAG-3′ and 5′-GGT GTC AAA GAA CTC GTT GCT ATC-3′ for *ABI3*, 5′-CAA TAA GAG AGG GAT AGC GAA CGA G-3′ and 5′-CGT CCA TTG CTG TCT CCT CCA-3′ for *ABI5*, and 5′-CTC AAG AGG TTC TCA GCA GTA-3′ and 5′-TCA CCT TCT TCA TCC GCA GTT-3′ for *Tubulin*.

Statistics: Values were presented as mean ± SD. Statistical differences were evaluated using Student's t test for unpaired samples. The level of statistical significance was shown as *$p<0.05$ and **$p<0.01$.

Results

Expression of *GmSBH1* in soybean seedlings regulated by the phytohormones: Plant hormones such as auxin, gibberellin (GA), methyl jasmonate (MeJA) and abscisic acid (ABA) have been shown to be involved in the regulation of plant development-related genes expression (Ni *et al*., 2008; Qin *et al*., 2010; Cheng *et al*., 2014). To reveal whether the *GmSBH1* was regulated by plant hormones, the expression profiles of *GmSBH1* in two-week-old soybean seedlings were analyzed after treated with 100 μM of MeJA, GA_3, ABA and 20 μM of IAA, respectively. The results showed that the transcription level of *GmSBH1* was significantly regulated by phytohormones. The quantity of *GmSBH1* mRNA was increased drastically after treated with ABA and reached a maximum level at 6 h (Fig. 1A). On the contrary, the transcriptional expression of *GmSBH1* in soybean seedlings was inhibited by GA3 (Fig. 1B). Under IAA treatment, the transcript level of *GmSBH1* was increased and reached to the highest level at 6 h after the treatment followed by a rapid decline (Fig. 1C). Interestingly, the transcript levels of *GmSBH1*were higher than those of the control under the treatment of MeJA, however, its transcript level tended to significantly decrease as the treatment time prolonged (Fig. 1D). These results suggested that *GmSBH1* might be involved in response to signaling pathways of some phytohormones at early seedling development stage in soybean.

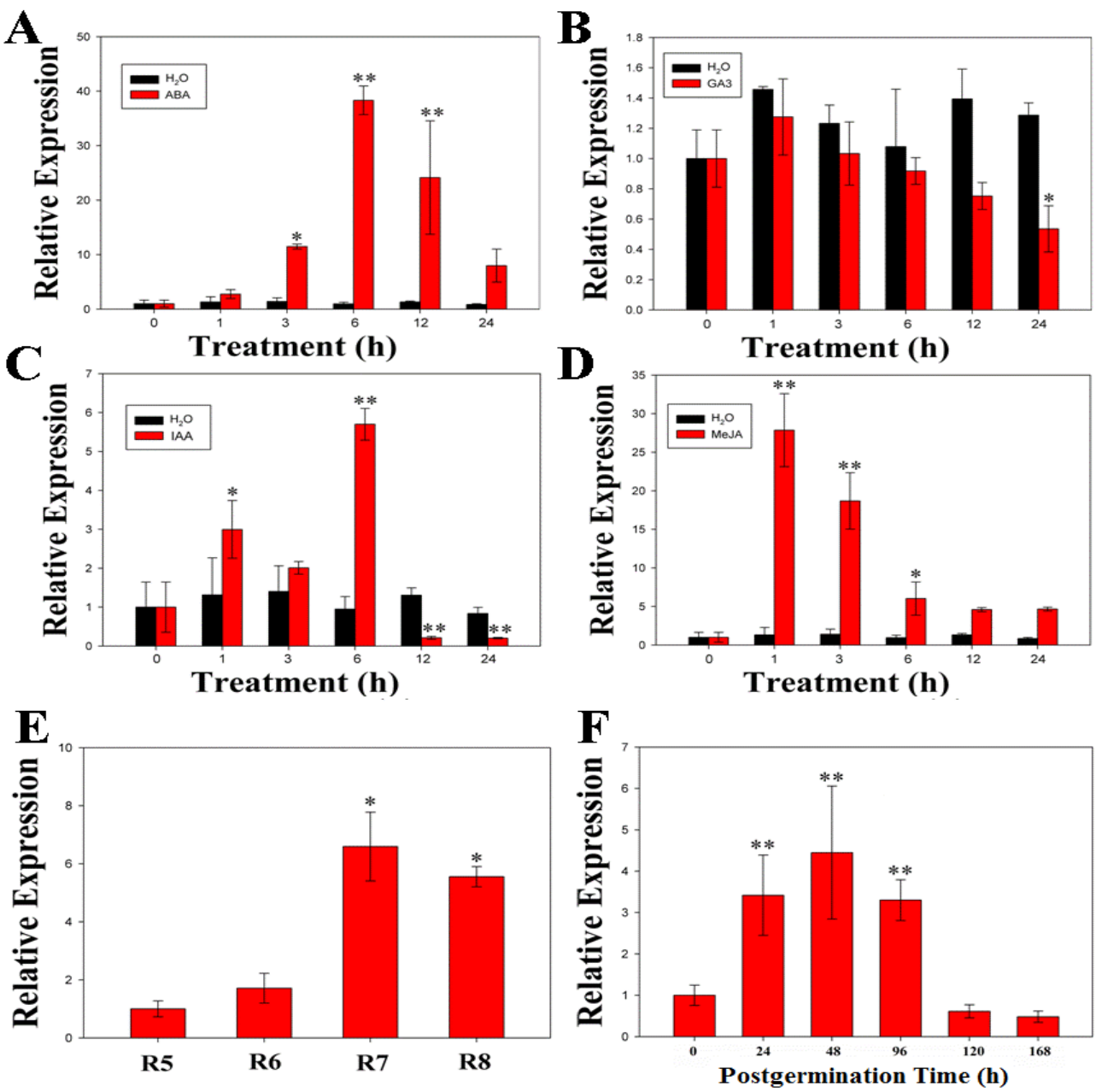

Fig. 1. Expression patterns of *GmSBH1* in soybean during germination and seedling stage. (A) to (D), Expression of *GmSBH1* in soybean seedlings with 100 μM of ABA, GA_3, MeJA and 20 μM of IAA, respectively, and H_2O as a control. (E), Expression of *GmSBH1* in different periods of soybean seeds developing process. (F), Expression of *GmSBH1* during soybean seeds germination. Error bars indicate SD (n=3). Single and double asterisks indicate statistically significant differences between the treatment and control at p values less than 0.05 and 0.01, respectively.

***GmSBH1* involved in soybean seed developing and germination processes:** The seeds from different reproductive periods (R5, R6, R7, and R8) of soybean were collected for qRT-PCR. The results showed that the transcription level of *GmSBH1* reached the highest at R7 and then slightly decreased at R8 (Fig. 1E). To characterize the role of *GmSBH1* in soybean seed germination, its expression levels were examined at 24, 48, 96, 120, and 168 h after germination (Fig. 1F). *GmSBH1* transcription reached a maximum level at 48 h and then decreased in the process of seed germination. These results suggested that *GmSBH1* might be involved in the process of seed developing and germination.

***GmSBH1* specifically bound ABRE cis-element in yeast system:** In order to clearly know the relationship between GmSBH1 and ABA, yeast one-hybrid system was used for DNA-binding ability investigation. The effector plasmids harboring the full length of GmSBH1 was made in pGADT7-Rec2 vector, and the reporter plasmid was made by inserting three tandem repeats of various cis-DNA elements into the pHis2, which contained a reporter gene *HIS3*. The effector plasmids and the reporter plasmids were transfected into yeast strain Y187 and the transformants were growth on SD-TL (used as control) and SD-TLH plus 3-AT to indicate binding ability of GmSBH1 to the different ABRE cis-DNA elements. As shown in Fig. 2, 3×ABRE element showed stronger binding ability with GmSBH1 than the others.

Overexpression of *GmSBH1* in Arabidopsis alters phenotypes: In *Arabidopsis*, silique dehiscence and floral organ abscission are two major molecular research fields (Lewis *et al*., 2006). Previous results showed that *GmSBH1* played an important role in plant leaf and stoma development (Shu *et al*., 2015). In this study, the results indicated that there were several phenotype changes in transgenic *Arabidopsis* plants compared to the wild type, such as long shaped cotyledons (Fig. 3A, B), clustered inflorescences (Fig. 3C, D), short and crooked siliques (Fig. 3F, G). Meanwhile, the growth period of transgenic lines was extended (Fig. 3E). Scanning electron microscopy showed that overexpression of *GmSBH1* resulted in abnormal replums on the *Arabidopsis* siliques, compared to those of wild types (Fig. 3H, I). These results suggested that the *GmSBH1* played important roles in vegetative and reproductive growth of plant.

Transgenic Arabidopsis plants sensitive to ABA during seed germination and early seedling development: To further analyze the functional roles of *GmSBH1* in transgenic plants, the seed germination under low ABA concentration (0.5 μM and 1 μM) (Fig. 4) and seedling root growth under high ABA concentration (30 μM and 50 μM) were examined (Fig. 5), respectively. Under non-ABA treatment, the germination potential and rate of the three transgenic lines (L11, L14, and L15) showed no significant difference compared to the wild type (Fig. 4A, B). However, on ABA-containing medium, the germination potential of L11 and L15 lines was significantly lower than that of wild-type (Fig. 4C, D). In addition, the green seedling rate of transgenic lines decreased significantly compared to that of the wild type after treatment with 0.5 μM and 1μM ABA for 6 d (Fig. 4E). The growth of transgenic *Arabidopsis* roots was inhibited by high ABA concentration (Fig. 5A, B). The fresh weight of transgenic lines was lower than that of wild type under higher ABA concentration (Fig. 5C). These results indicated that *GmSBH1* played an important role in ABA signaling pathway during seed germination and early seedling development.

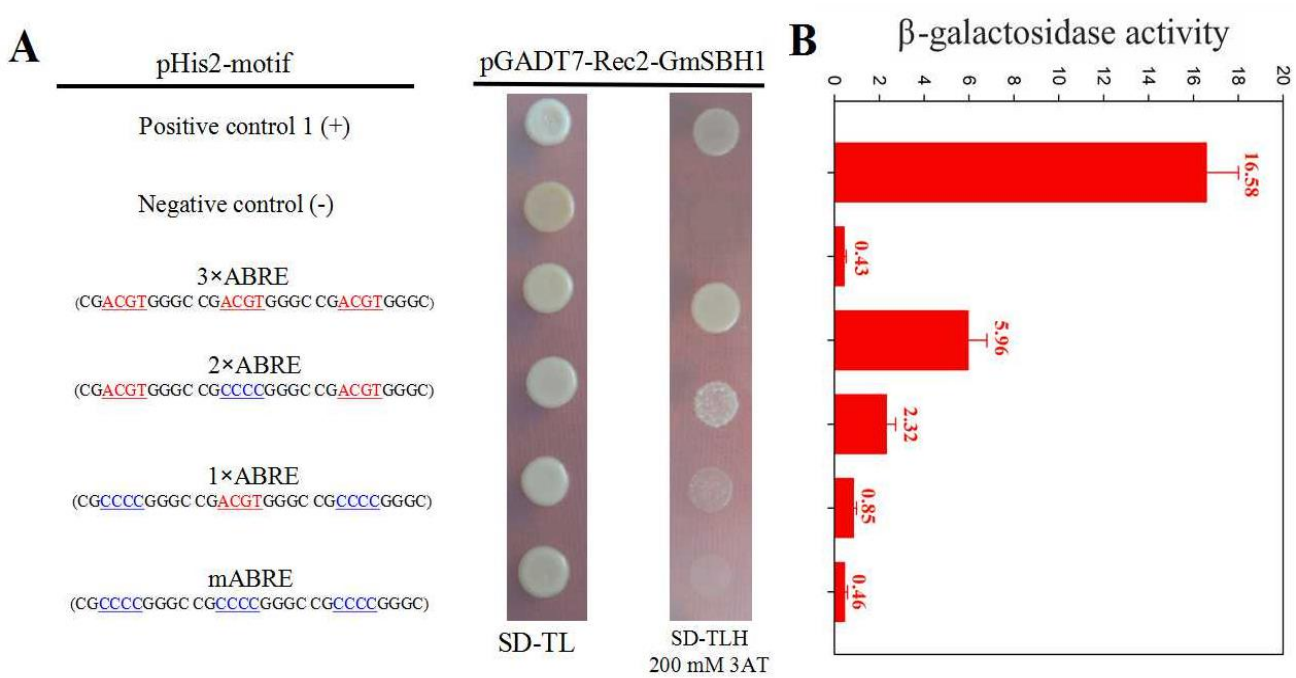

Fig. 2. Interaction between ABRE cis-element and GmSBH1.(A), Yeast one-hybrid assay, yeast transformants expressing pHis2-ABRE and the pGADT7-Rec2-GmSBH1 constructs were assayed for growth on SD-Trp-Leu (SD-TL) and SD-Trp-Leu-His (SD-T-L-H) with 200 mM 3-AT. (B), Quantitative analysis of Lac Z activity of the yeast strains in liquid culture. Values are the means of data taken from three independent experiments. Error bars indicate the standard deviation.

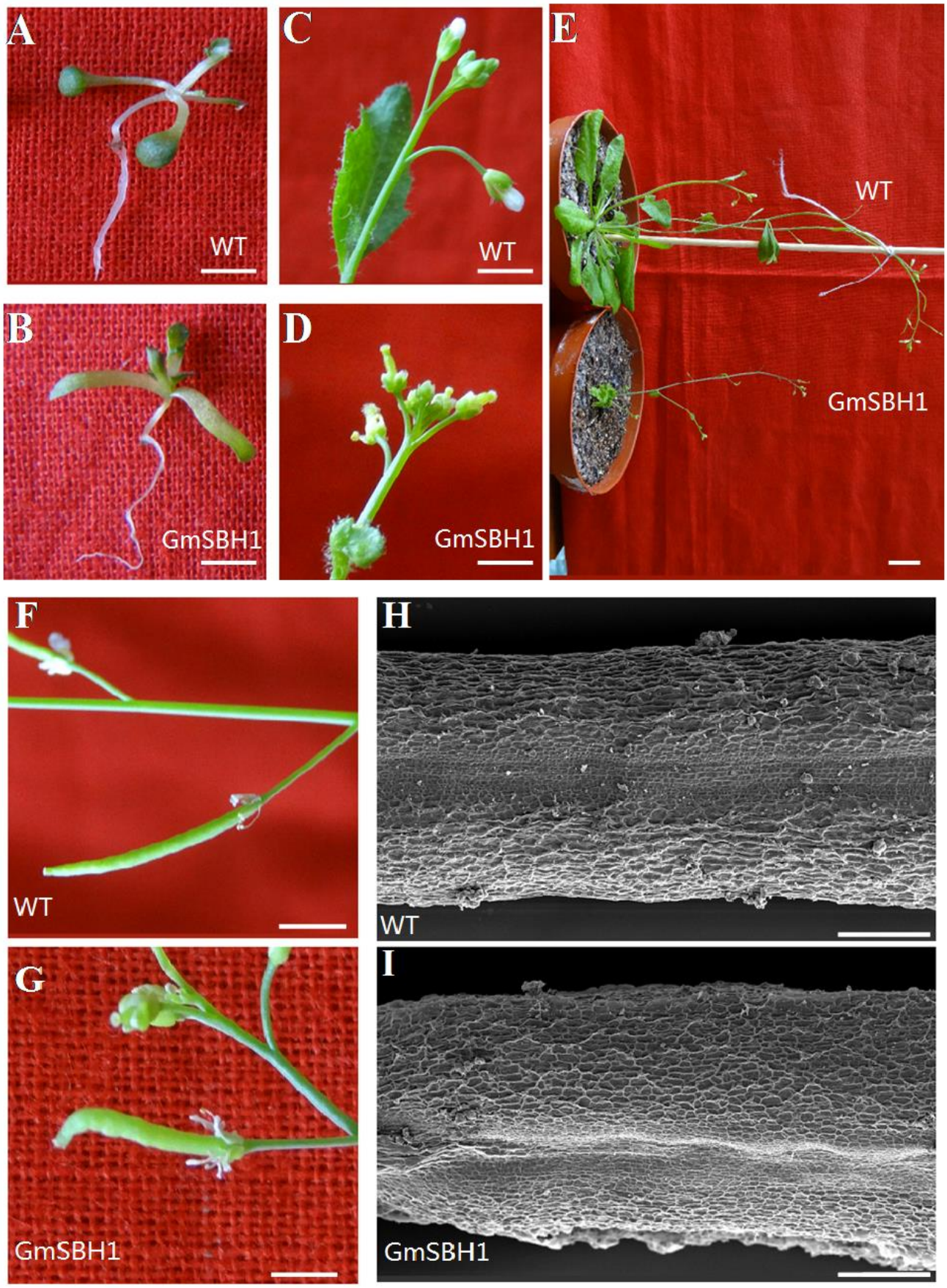

Fig. 3. Phenotypic differentiations between the transgenic lines and the wild type. (A) and (B), The phenotype of WT (A) and transgenic lines (B) seedling cotyledon. (C) and (D), The phenotype of WT (C) and transgenic lines (D) flowers. (E), The whole plant of WT and transgenic lines. (F) and (G), The phenotype of WT (F) and transgenic lines (G) silique. (H) and (I), Scanning electron micrographs of silique cells of the wild type (H) and transgenic lines (I). Bars=100 μm.

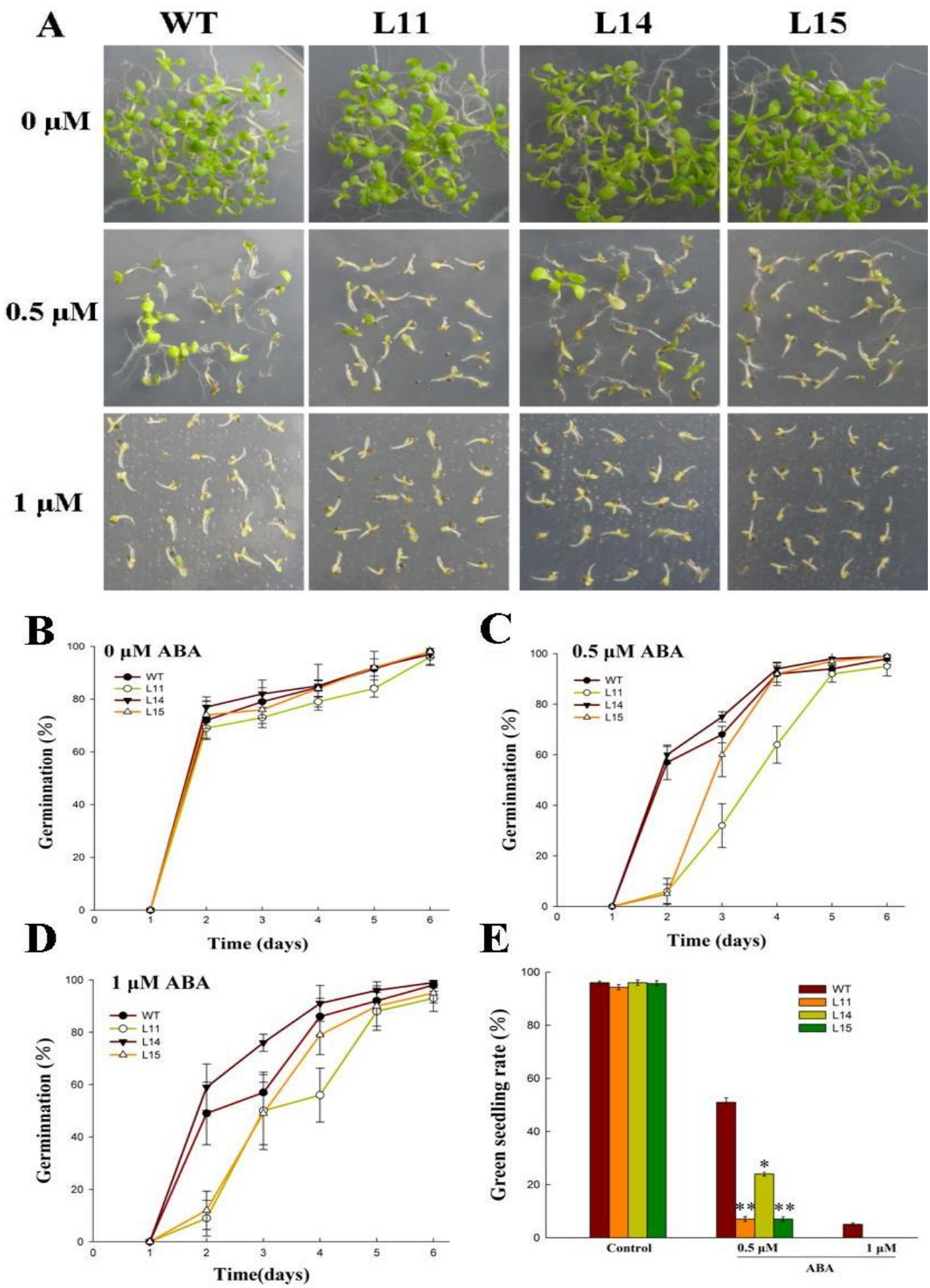

Fig. 4. *GmSBH1* modulates the ABA sensitivity during the germination. (A), The seeds germination condition of WT and transgenic lines on MS medium with or without low concentration of ABA. (B) to (D), The germination potential of WT and transgenic lines on MS medium with or without low concentration of ABA during 6 days. (E), Green seedling rate of WT and transgenic lines.

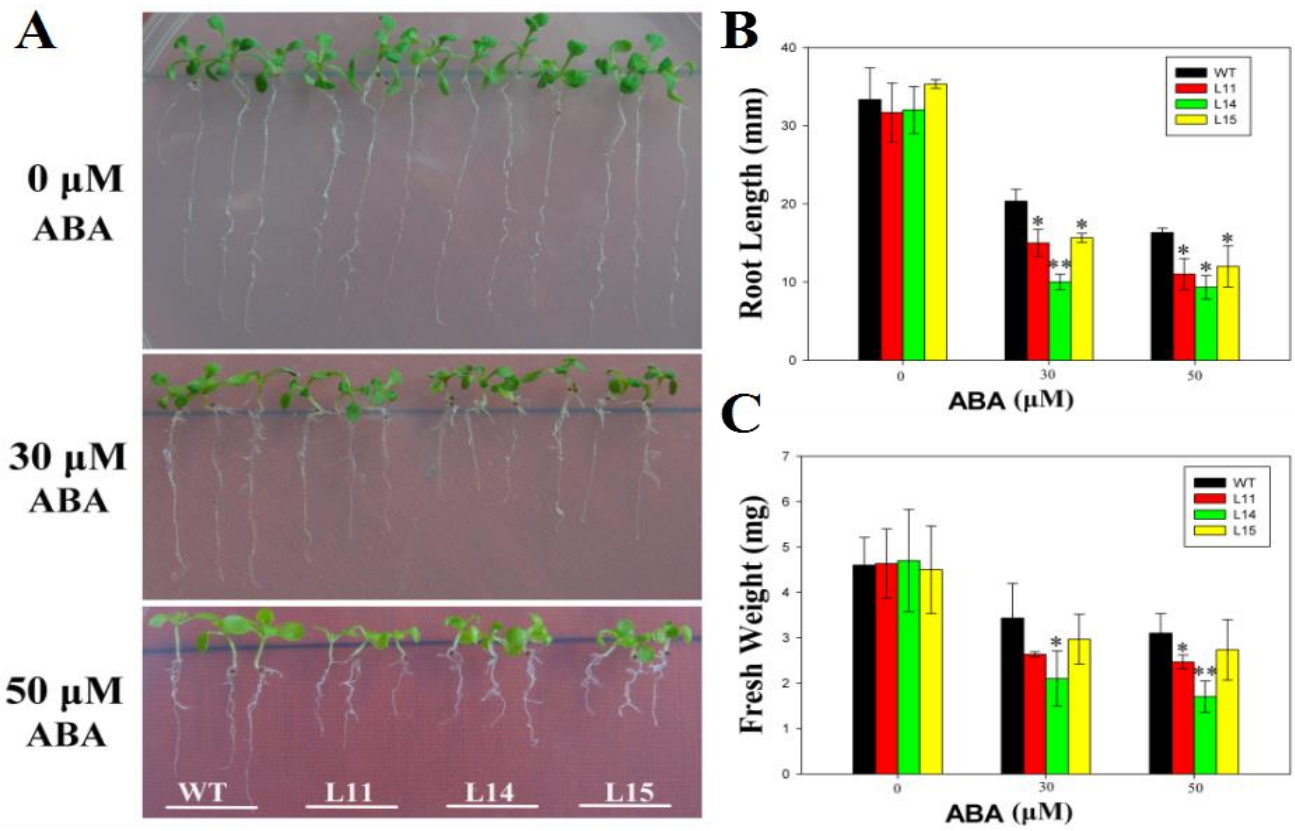

Fig. 5. *GmSBH1* modulates the ABA sensitivity during the early seedling stage. (A), The roots induction assay of WT and transgenic lines on MS medium with or without high concentration of ABA. (B), The roots length of WT and transgenic lines. (C), The fresh weight of WT and transgenic lines.

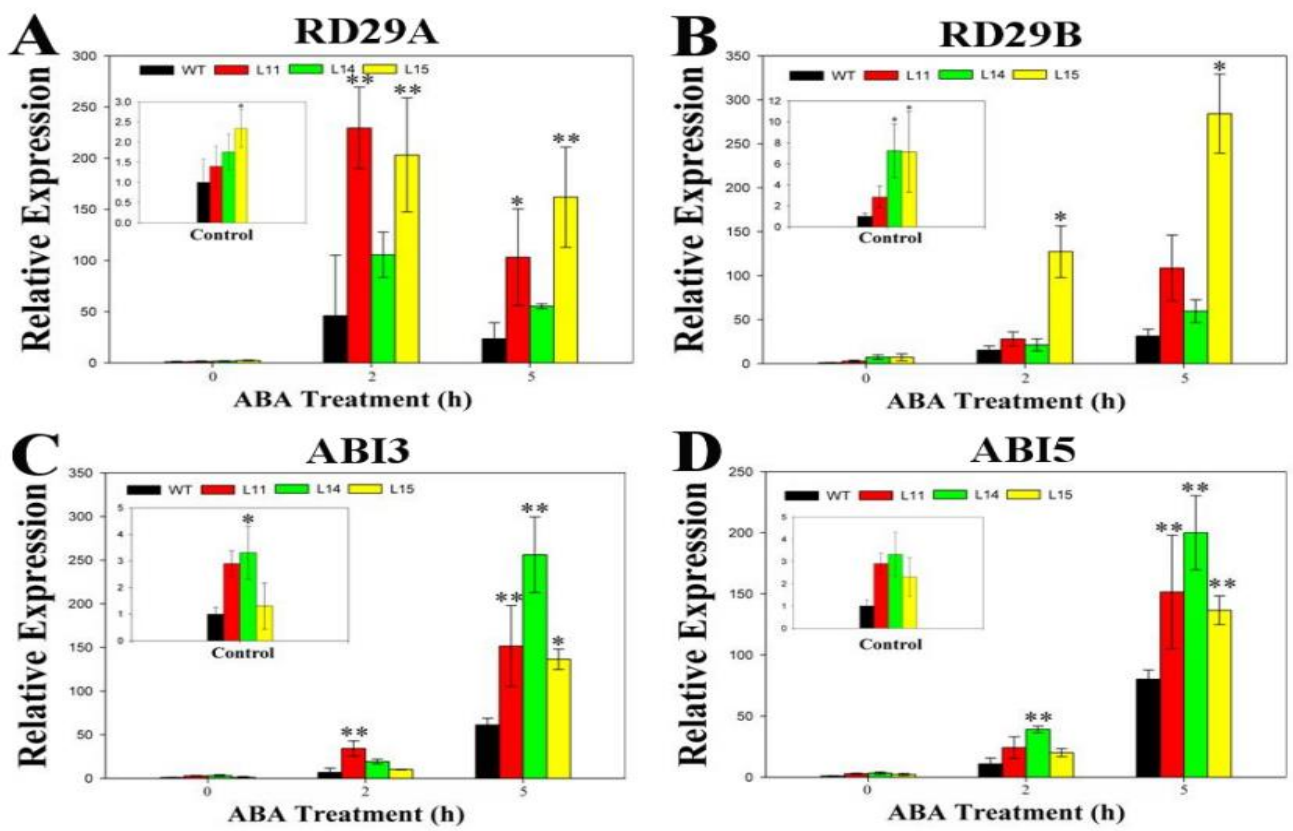

Fig. 6. *GmSBH1* regulated the expression of ABA-inducible genes. The expression patterns of ABA signaling-related gene (*RD29A, RD29B, ABI3* and *ABI5)* were determined in two-week-old WT and transgenic seedlings. All seedlings were treated with 100 μM ABA for 2 h and 5h. Values are means and SE (n = 3).

GmSBH1 regulates ABA-responsive genes in transgenic Arabidopsis seedlings: Transcription factors (TFs) play key roles in regulating gene expression at the transcriptional level, controlling many biological processes such as plant growth, development, cell division, and responses to environmental stimuli (Wang *et al*., 2005; Rueda *et al*., 2005). *RD29A* and *RD29B* are typical marker genes induced by ABA and abiotic stress in *Arabidopsis* (Yamaguchishinozaki & Shinozaki, 1993), while *ABI3* and *ABI5* are key genes of ABA signaling modulator (Lopez-Molina *et al*., 2002; Yotsui *et al*., 2013). To characterize the function of GmSBH1 in regulating the expression of ABA-related genes, the expression level of these four genes was examined in 10-day-old seedlings with or without ABA (100 μM). The results showed that the expressions of *RD29A*, *RD29B*, *ABI3* and *ABI5* were significantly up-regulated under ABA treatment (Fig. 6). Moreover, the expression levels of these genes in transgenic lines were higher than that of wild type under ABA treatment (Fig. 6). These results indicated that *GmSBH1* might positively regulate the expression of ABA-inducible genes and involve in ABA pathway during early seedling development.

Discussion

GmSBH1 was the first homeobox gene isolated from soybean. It has been confirmed to be involved in maintaining the embryo and leaf development as well as response to high temperature and humidity (HTH) stress in soybean (Ma *et al*., 1994; Shu *et al*., 2015). However, the relationship between *GmSBH1* and plant hormones has not been clearly understood. Abundant evidences demonstrated that a close relationship exists between KNOX I genes and hormones in the SAM formation and maintenance (Shani E *et al*., 2006). Previous studies showed that the expression of KNOX I genes could directly repress the *GA20-oxidase* (Negasaki *et al*., 2001), which plays a key role in the GA3 biosynthesis. *GmKNT1* was found to be regulated by GA3, CTK, IAA, ABA and JA in soybean leaves (Liu *et al*., 2008). In this study, the expression of *GmSBH1* was also found to be regulated by GA3, IAA, MeJA and ABA in the leaves of soybean seedlings. The results suggested that *GmSBH1* might be involved in response to signaling pathways of some plant hormones at early seedling development stage in soybean.

It has been proposed that class I KNOX homeobox genes were critical for the maintenance of plant shoot apical meristem (SAM). And the overexpressing of KNOX I genes in most plants would result in the abnormal flowers that might contain wrinkled or shortened organs. For example, *GmKNT1* overexpressed in *Arabidopsis* led to the clustered and abnormal flowers, and in addition, the inflorescence turned shorter than that of the wild type (Liu *et al*., 2008), the overexpression of *GmSBH1*in *Arabidopsis* could alter the plant leaf and stoma phenotypes (Shu *et al*., 2015). In some cases, flowers presented short, thickened stamens in *FaKNOX1* over-expressing *Arabidopsis* (Chatterjee *et al*., 2011). Similar phenotype has also been observed in this study. Our study indicated that the changes of phenotype in transgenic *Arabidopsis* appeared not only in leaf and stoma but also in the flower, silique and plant growth period compared to the wild type (Fig. 3G, H).

ABA plays a central role in seed germination and seedling growth in response to environmental stresses. Many homeobox transcription factors have been proved to be involved in ABA signaling pathway. For example, the homeodomain-leucine zipper (HD-Zip) transcription factor family was found to be dependent on ABA-signal for their transcriptional regulation (Qin *et al*., 2010). Overexpression of *ATHB5* and *ATHB6* were hypersensitive to ABA-induced growth inhibition during early seedling establishment (Johannesson *et al*., 2003; Lechner *et al*., 2011); two ABA-inducible genes, *ABI3* and *ABI5*, were typical and key genes in plant ABA signal pathway, which were significant up-regulated in the transgenic lines treated with ABA (Lopez-Molina *et al*., 2002; Yotsui *et al*., 2013; Fang *et al*., 2014). BLH1 and KNAT3 were found to modulate ABA responses by directly regulating *ABI3* expression during the germination and the early seedling development in *Arabidopsis* (Kim *et al*., 2013). Moreover, more evidences have showed that exogenous ABA could promote stomatal closure. Our previous study indicated that the overexpression of *GmSBH1* in *Arabidopsis* could alter the size and number of stomas (Shu *et al*., 2015). In the present study, the GmSBH1 specifically bound ABRE cis-element in yeast system and the lines overexpressing *GmSBH1* were found to be sensitive to ABA during the seed germination and early seedling development. All these results might implied that the stomatal phenotype has some relationship with ABA sensitivity in transgenic lines. However, the molecular regulatory mechanism of *GmSBH1* in plant hormones signaling pathway, especially in ABA pathway, needs to be future investigated.

Conclusions

In this study, a class I KNOX homeobox transcription factor,GmSBH1, involved in ABA responses during seedling development insoybean. Overexpression of *GmSBH1* in *Arabidopsis* altered its flower and silique phenotypes. Moreover, in *GmSBH1* transgenic lines, both seed germination and seedlings growth showed hypersensitive to ABA. These results will contribute to the understanding of *GmSBH1* functions in soybean.

Acknowledgements

This research was financially supported by the national natural science foundation of China (No. 31101212, 31171572, 31371711), the national natural science foundation of Anhui province (No.1608085MC69, KJ2016A073), the youth talent major project of college and university of Anhui (No.gxyqZD2016216, gxyq2017043) and the stable talent project of Anhui Science & Technology University.

References

Burglin, T.R. 1997. Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res.*, 25(21): 4173-4180.

Cutler, S.R., P.L. Rodriguez, R.R. Finkelstein and S.R. Abrams. 2010. Abscisic Acid: Emergence of a Core Signaling Network. *Annu. Rev. Plant Biol.*, 61(1): 651-679.

Chatterjee, M., C.L. Bermudez-Lozano, M.A. Clancy, T.M. Davis and K.M. Folta. 2011. A strawberry KNOX gene regulates leaf, flower and meristem architecture. *PloS one.*, 6(9): e24752.

Cheng, S., Y. Huang, N. Zhu and Y. Zhao. 2014. The rice WUSCHEL-related homeobox genes are involved in reproductive organ development, hormone signaling and abiotic stress response. *Gene*., 549(2):266-274.

Endrizzi, K., B. Moussian, A. Haecker, J.Z. Levin and T. Laux. 1996. The SHOOT MERISTEMLESS gene is required for maintenance of undifferentiated cells in Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristem genes WUSCHEL and ZWILLE. *Plant J.*, 10(6): 967-979.

Fang, Y.H., X.K. Dong, K.Y. Ping, I. Abdurazak, L.J. Xiang and Z.L. Jun. 2014. Identification and functional analysis of ABA-insensitive3 from *rosa canina. Pak. J. Bot.*, 46(3): 803-810.

Hake, S., H.M.S. Smith, H. Holtan, E. Magnani, G. Mele and J. Ramirez. 2004. The role of knox genes in plant development. *Annu. Rev. Cell Dev. Bi.*, 20(1): 125-151.

Hay, A. and M. Tsiantis. 2010. KNOX genes: versatile regulators of plant development and diversity. *Development.*, 137(19): 3153-3165.

Johannesson, H., Y. Wang, J. Hanson and P. Engstrom. 2003. The Arabidopsis thaliana homeobox gene ATHB5 is a potential regulator of abscisic acid responsiveness in developing seedlings. *Plant Mol. Biol.*, 51(5): 719-729.

Kerstetter, R., E. Vollbrecht, B. Lowe, B. Veit, J. Yamaguchi and S. Hake. 1994. Sequence-Analysis And Expression Patterns Divide the Maize Knotted1-Like Homeobox Genes into 2 Classes. *The Plant Cell.*, 6(12): 1877-1887.

Kerstetter, R.A., D. LaudenciaChingcuanco, L.G. Smith and S. Hake. 1997. Loss-of-function mutations in the maize homeobox gene, knotted1, are defective in shoot meristem maintenance. *Development.*, 124(16): 3045-3054.

Kim, D., Y.H. Cho, H. Ryu, Y. Kim, T.H. Kim and I. Hwang. 2013. BLH1 and KNAT3 modulate ABA responses during germination and early seedling development in Arabidopsis. *Plant J.*, 75(5): 755-766.

Lechner, E., N. Leonhardt, H. Eisler, Y. Parmentier, M. Alioua, H. Jacquet, J. Leung and P. Genschik. 2011. MATH/BTB CRL3 Receptors Target the Homeodomain-Leucine Zipper ATHB6 to Modulate Abscisic Acid Signaling. *Dev Cell.*, 21(6): 1116-1128.

Lewis, M.W., M.E. Leslie and S.J. Liljegren. 2006. Plant separation: 50 ways to leave your mother. *Curr. Opin. Plant Biol.*, 9(1): 59-65.

Liu, J., D. Ha, Z.M. Xie, C.M. Wang, H.W. Wang, W.K. Zhang, J.S. Zhang and S.Y. Chen. 2008. Ectopic expression of soybean GmKNT1 in Arabidopsis results in altered leaf morphology and flower identity. *J. Genet Genomics.*, 35(7): 441-449.

Long, J.A., E.I. Moan, J.I. Medford and M.K. Barton. 1996. A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature.*, 379(6560): 66-69.

Lopez-Molina, L., B. Mongrand, D.T. McLachlin, B.T. Chait and N.H. Chua. 2002. ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J.*, 32(3): 317-328.

Ma, H.C., M.D. Mcmullen and J.J. Finer. 1994. Identification Of a Homeobox-Containing Gene with Enhanced Expression during Soybean (Glycine-Max L) Somatic Embryo Development. *Plant Mol. Biol.*, 24(3): 465-473.

Nagasaki, H., T. Sakamoto, Y. Sato and M. Matsuoka. 2001. Functional analysis of the conserved domains of a rice KNOX homeodomain protein, OSH15. *The Plant Cell*., 13(9): 2085-2098.

Ni, Y.X., X.L. Wang, D.D. Li, Y.J. Wu, W.L. Xu and X.B. Li. 2008. Novel cotton homeobox gene and its expression profiling in root development and in response to stresses and phytohormones. *Acta Bioch. Bioph. Sin.*, 40(1): 78-84.

Qin, Y.F., D.D. Li, Y.J. Wu, Z.H. Liu, J. Zhang, Y. Zheng and X.B. Li. 2010. Three cotton homeobox genes are preferentially expressed during early seedling development and in response to phytohormone signaling. *Plant Cell Rep.*, 29(10): 1147-1156.

Qing, Q.M. and Z. Hao. 2010. Sensitivity to abscisic acid regulates stomatal oscillation and closure in *Arabidopsis thaliana*. *Pak. J. Bot.*, 42(1): 353-359.

Rueda, E.C., C.A. Dezar, D.H. Gonzalez and R.L. Chan. 2005. Hahb-10, a sunflower homeobox-leucine zipper gene, is regulated by light quality and quantity, and promotes early flowering when expressed in Arabidopsis. *Plant Cell Physiol.*, 46(12): 1954-1963.

Scofield, S., W. Dewitte and J.A.H. Murray. 2007. The KNOX gene SHOOT MERISTEMLESS is required for the development of reproductive meristematic tissues in Arabidopsis. *Plant J.*, 50(5): 767-781.

Scofield, S.and J.A.H. Murray. 2006. KNOX gene function in plant stem cell niches. *Plant Mol. Biol.*, 60(6): 929-946.

Shani, E., O. Yanai and N.Ori. 2006. The role of hormones in shoot apical meristem function. *Curr. Opin. Plant Biol.*, 9(5): 484-489.

Shu, Y.J., Y. Tao, S. Wang, L.Y. Huang, X.W. Yu, Z.K. Wang, M. Chen and H. Ma. 2015. GmSBH1, a homeobox transcription factor gene, relates to growth and development and involves in response to high temperature and humidity stress in soybean. *Plant Cell Rep.*, 34(11): 1927-1937.

Truernit, E. and J. Haseloff. 2007. A Role for KNAT Class II Genes in Root Development. *Plant signaling & Behavior.*, 2(1): 10-12.

Umezawa, T., T. Hirayama, T. Kuromori and K. Shinozaki. 2011. The Regulatory Networks of Plant Responses to Abscisic Acid. *Adv. Bot. Res.*, 57(57): 201-248.

Wang, Y.J., Y.D. Li, G.Z. Luo, A.G.Tian, H.W. Wang, J.S. Zhang and S.Y. Chen. 2005. Cloning and characterization of an HDZip I gene GmHZ1 from soybean. *Planta*, 221(6): 831-843.

Wang, L.Q., H. Ma, L.R. Song, Y.J. Shu and W.H. Gu. 2012. Comparative proteomics analysis reveals the mechanism of pre-harvest seed deterioration of soybean under high temperature and humidity stress. *J. Proteomics.*, 75(7): 2109-2127.

Yamaguchishinozaki, K. and K. Shinozaki. 1993. Characterization of the expression of a desiccation-responsive *RD29* gene of *Arabidopsis-thaliana* and analysis of its promoter in transgenic plants. *Mol. Gen. Genet.*, 236(2): 331-340.

Yotsui, I., M. Saruhashi, T. Kawato, T. Taji, T. Hayashi, R.S. Quatrano and Y. Sakata. 2013. Abscisic acid in sensitive 3 regulates abscisic acid-responsive gene expression with the nuclear factor Y complex through the ACTT-core element in Physcomitrella patens. *New Phytol.*, 199(1): 101-109.

Zhong, R.Q., C.H. Lee, J.L. Zhou, R.L. McCarthy and Z.H. Ye. 2008. A battery of transcription factors involved in the regulation of secondary cell wall Biosynthesis in *Arabidopsis*. *The Plant Cell.*, 20(10): 2763-2782.

Zhou, C.G. and C.H. Li. 2016. A novel R2R3-MYB transcription factor BpMYB106 of birch (Betulaplatyphylla) confers increased photosynthesis and growth rate through up-regulating photosynthetic gene expression. *Frontiers in Plant Science*.,7: 315. DOI:10.3389/fpls.2016.00315.

(Received for publication 15 March 2016)