Cloning and Functional Verification of Gene GmELF4-LIKE4 in Soybean [Glycine max (L.) Merr]

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Cloning and functional verification of gene *GmELF4-LIKE4* in soybean

[*Glycine max* (L.) Merr]

Yukun Jin¹, He Zhao¹, Abraham Lamboro¹, Zhifeng Xiao¹, Qi Zhang¹, Shuyan Guan², Jing Qu¹, Rengui Zhao^{1*}, Piwu Wang^{1*}

Abstract

Background: Soybean grain is an important oil crop with high-quality vegetable protein and vegetable oil. Extreme weather can cause crop yield reduction, among which drought is most likely to cause a decline in annual soybean yield. How to overcome the impact of drought on soybean yield has become a major work in current breeding research.

Results: In our study, the gene *GmELF4-LIKE4* was obtained through RNA-Seq and differential gene screening technology, and the plant over expression vector and RNAi vector were constructed. Then, the Colombian *Arabidopsis thaliana* and soybean JN18 were genetically transformed and added to the Agrobacterium-mediated method in to T2 generation. We have verified in *Arabidopsis thaliana* that over expression of *ELF4-LIKE4* will delay the flowering of *Arabidopsis thaliana*, while inhibition of *ELF4-LIKE4* expression will advance the flowering period. At the same time, we verified the regulation analysis of *GmELF4-LIKE4* on *EARLY FLOWERING 3* and *CONSTANTS-like* in soybeans, and found that *GmELF4-LIKE4* positively regulates *ELF3* and *COL*; meanwhile, over expression soybeans and RNAi soybeans were tested in drought. Stressing the relative conductivity, malondialdehyde content and peroxidase activity under 0 days and 5 days, the result indicate that the over expression of *GmELF4-LIKE4* gene can reduce the drought resistance of soybean. This study laid a theoretical foundation for the identification of *GmELF4-LIKE4* gene function and the breeding of drought-resistant varieties.

Conclusion: We found that overexpression of *GmELF4-LIKE4* will delay the flowering period of *Arabidopsis thaliana* and reduce the drought resistance of soybeans. Conversely, inhibiting *GmELF4-LIKE4* will advance the flowering of *Arabidopsis thaliana* and increase the drought resistance of soybeans.

Keywords: Soybean, RNA-Seq, *GmELF4-LIKE4*, Cloning, Drought identification

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Background

Soybean is a widely planted food crop in the world. Its production is extremely vulnerable to environmental stresses such as drought. Therefore, exploring the function of soybean drought tolerance genes and clarifying the drought resistance mechanism is of great significance to soybean breeding and stable yield. In the previous research work, we screened the soybean drought-resistant mutant M18. The research on M18 found that the root system characteristics are significantly different from wild-type JN18. Using RNA-seq to screen the differentially expressed genes and obtain a gene homologous to *EARLY FLOWERING 4(ELF4)*. In order to study the function of the differentially expressed gene, we cloned it and construct two expression vectors. Soybean plants transformation was done through *Agrobacterium*-mediated method and verified the gene features. *ELF4* family genes are usually related to photoperiod regulation of flowering [1]. Since this study was selected from drought-resistant mutants *GmELFE4-LIKE4*, we wanted to verify whether this gene can improve soybean drought resistance. At the same time, as the star genes *EARLY FLOWERING 3 (ELF3)* and *CONSTANTS-like (COL)* that regulate flowering by photoperiod, the finding verified that the changes in *ELF3* and *COL* expression levels of target genes during over expression and RNAi.

There were many studies on *COL* and *ELF3* in plants, but only few reports on the association of *ELF4* family genes with *COL* or *ELF3*. The peak expression of *ZmELF4* gene happened to occur at the peak of *ZmCOL* gene expression [2,3]. Their expression patterns were also very similar, which implies that there was a certain interaction between the two, which may jointly regulate the photoperiod of maize to induce flowering process [4]. *ELF4* family genes are usually associated with functions such as photoperiod sensitivity [5]. The transcription process of most genes in plants was regulated by the circadian clock, and some of them were involved in the stress response pathway [6-9]. In previous studies, it was found that it not only participates in the light signal transduction pathway, so as to realize the transmission of light signals to the biological clock, but also participates in the response process of plants to stress environment [10-13]. In our research, we discovered an *ELF4* family homologous gene through RNA-Seq in drought-resistant mutant soybean M18 and wild-type JN18, so we wanted to verify whether this gene can improve the drought resistance of crops. As the internal timing mechanism of plants, the circadian clock system can regulate many metabolic processes so as to promote plants to adapt to the environment of day and night. However, how the circadian clock dynamically regulates the expression of these genes, coordinates plant growth and development, improves the adaptability of biotic and abiotic stresses. The research hotspots in the core areas of the biological clock are also one of the hotspots in the field of plant science research [14-15].

Results

Screening and verification of differential gene *GmELF4-LIKE4*

According to the previous analysis results of the root RNA-Seq of the Mutant18 (M18)

and wild-type soybean JN18 in V1 stage, we screened the significantly differentially expressed gene *GmELF4-LIKE4* with an absolute value of over 2.8 as a candidate gene. Detected by 1% formaldehyde denaturation gel electrophoresis, the results shown in Figure S1 (Additional file1: Figure S1), show that the 28s and 18s bands in the RNA extracted in this study are clear, indicating that the extracted RNA has good integrity and high quality.

The root unit of JN18 was 1.00, the relative expression level of target gene in M18 root system was 1.39 times that of JN18; the relative expression level of target gene in M18 stem and leaves were 1.36, 1.19 times that of JN18 respectively. It was consistent with the RNA-seq results as a whole, and this experiment provides a further basis for differential gene screening.

Fig. 1 Analysis of *GmELF4-LIKE4* expression in various tissues of JN18 and M18

Cloning and vector construction of *GmELF4-LIKE4* gene

We successfully obtained *GmELF4-LIKE4* cDNA through the RT-PCR method by using RNA extracted from the roots of Phase V1 M18 as the template, and annealing temperature was about 54.5°C. The full length of *GmELF4-LIKE4* CDS is 345 bp. The *GmELF4-LIKE4* cDNA sequence encodes 114 amino acids as shown in Figure S2 (Additional file1: Figure S2).

We collected 20 copies of *ELF4* homologous genes to construct a phylogenetic tree. The target gene we cloned is closely related to *Cajanus cajan* *ELF4-LIKE4* (XM_020363641), so it is named *GmELF4-LIKE4*; it is relatively far related to *Lotus corniculatus* *ELF4-like* (EU916981). The evolutionary grouping of *GmELF4-LIKE4* may reveal the similarities and differences in their functions.

Fig. 2 Phylogenetic tree of *ELF4*

After the cloning vector was constructed, we used pCAMBIA3301 as the backbone vector and used seamless cloning technology to construct the plant over expression vector pCAMBIA3301-*GmELF4-LIKE4* and the RNA interference expression vector pCAMBIA3301-*GmELF4-LIKE4*-RNAi. Then PCR identification and enzyme digestion were performed, which proves that the vector construction we need was completed. The results were shown in Figure S3, S4, S5 and S6 (Additional file1: Figure S3, S4, S5 and S6).

Phenotype observation and comparison of transgenic *Arabidopsis thaliana*

The wild-type *Arabidopsis thaliana* plants and transformants were cultivated under the same conditions at the same time, and the same cultivation measures were taken. During the vegetative growth stage, wild-type plants and transformed plants did not show significant differences in morphology and developmental degrees. When wild-type transferred to reproductive growth, the flowering period of over expressed *Arabidopsis thaliana* was significantly later than that of wild-type by about 7 days. RNAi flowering period of *Arabidopsis thaliana* was significantly earlier than wild-type by 5 days. It can be seen that the over expression of *GmELF4-LIKE4* gene delayed the flowering of *Arabidopsis thaliana*.

Fig. 3 Observation and Comparison of the Phenotype of *Arabidopsis thaliana*

PCR detection of T0, T1 and T2 generation soybeans

We transformed soybean JN18 via *Agrobacterium*-mediated method, and the growth process is shown in Figure 4. In this study, *Agrobacterium*-mediated methods were used to transfer the constructed expression vector to the recipient. After germination culture, pre-culture, co-culture, screening culture, elongation culture, and rooting culture, T0 soybean plants were obtained. After PCR the test, add generations to the positive plants to obtain T2 generation of soybean .

Fig. 4 Agrobacterium-mediated tissue culture of soybean A: Germination culture; B: Pre-culture; C: Co-culture; D: Screening culture; E: Elongation culture; F: Rooting culture; G: Regenerated plants

We use T0 soybean gDNA as a template, plasmid as a positive control, wild-type JN18 gDNA as a negative control, and detect all the functional elements *35s*, *Bar*, *NOS* and target genes on the T-DNA region of the plant expression vector pCAMBIA3301. The result was shown in Figure S7(Additional file1: Figure S7).

After that, we cultivated soybeans that tested positive from T0 generation to T2 generation and performed PCR detection. The result was shown in Figure S8 and S9(Additional file1: Figure S8, Figure S9). After PCR detection, 9 soybeans with positive over expression of T2 generation and 10 soybeans with positive RNAi were obtained.

Southern Blot Detection of Transgenic Soybean

In order to identify the integration of *GmELF4-LIKE4* gene in T1 generation soybeans, we performed Southern blot detection on plants that were positive by PCR. Since the target gene is an endogenous soybean gene, so we selected marker *Bar* as a probe, the recombinant plasmid was used as a positive control, and the wild-type JN18 gDNA was used as a negative control. The result was shown in Figure S10(Additional file1: Figure S10). There was no obvious hybridization signal for wild-type JN18. Among the tested plants, 5 over expression plants and 5 RNAi plants showed obvious hybridization signals, and the hybridization bands were single and different in position. This shows that the target gene is integrated at different sites in the soybean genome as a single copy.

qPCR Detection of *GmELF4-LIKE4* Transgenic Soybean

We used a real-time fluorescence quantitative analyzer and the fluorescent dye SYBR Green to determine the Ct values of the *GmELF4-LIKE4* gene and β -*Actin* gene in the roots, leaves and flowers of the transformed positive plants and the control soybean JN18 in the R2 stage, according to $2^{-\Delta\Delta CT}$ calculates the relative expression of target genes in different tissues. The result is shown in Figure 5.

Among the three selected transgenic *GmELF4-LIKE4* over expression soybean plants, we found that the relative expression of the target gene in the flowers increased the most. Transgenic soybeans were 4.35 times, 6.23 times, and 5.86 times that of the wild type soybeans, respectively. The relative expression levels of target genes in the leaves in transgenic soybeans were 4.23 times, 3.94 times, and 3.73 times that of the wild type soybeans, respectively. The increase in roots was the smallest, and the relative expression levels of target genes in transgenic soybeans were 2.81 times, 2.58 times, and 2.89 times that of wild type soybeans, respectively.

Among the three selected *GmELF4-LIKE4*-RNAi soybeans, the transgenic soybeans in the root system are 0.70 times, 0.52 times, and 0.53 times that of wild-type soybeans respectively; the genetically modified soybeans in the leaves are 0.42 times, 0.44 times, and 0.44 times that of wild-type soybeans respectively; transgenic soybeans in flowers are 0.29 times, 0.33 times, and 0.33 times that of wild-type soybeans.

From this we concluded that in transgenic *GmELF4-LIKE4* over expression soybeans, the relative expression in flowers was higher; in RNAi plants, the relative expression in flowers was lower. Different plants have different gene expression levels in the same tissue, and different tissues in the same plant have different gene expression levels. This may be related to the site of gene integration in soybeans.

Fig. 5 Analysis of relative expression of *GmELF4-LIKE4* in tissues of transgenic soybean

A: Analysis of Relative Expression of Over expressed soybean in Roots

B: Analysis of Relative Expression of Over expressed soybean in Leaves;

C: Analysis of Relative Expression of Over expressed soybean in Flowers;

D: Analysis of Relative Expression of RNAi soybean in Roots;

E: Analysis of Relative Expression of RNAi soybean in Leaves;

F: Analysis of Relative Expression of RNAi soybean in Flowers.

Analysis of *GmELF4-LIKE4* in Soybean Regulating *ELF3* and *COL*

We measured the relative expression of *ELF3* and *COL* genes under the same sunlight

conditions (8 hours of light and 16 hours of dark growth room) on the leaves and flowers of *GmELF4-LIKE4* transgenic soybean JN18 at the R2 stage, and then analyzed *GmELF4-LIKE4* and *ELF3*, *COL* expression influence. The result was shown in Figure 6.

In wild type soybeans, the relative expression levels of *GmELF4-LIKE4*, *ELF3*, and *COL* were 1.00, 1.50, and 1.56, respectively. In the leaves of transgenic *GmELF4-LIKE4* gene over expressing soybeans, the relative expression level of *ELF3* was approximately 1.96 times that of wild type soybean JN18, and the relative expression level of *COL* was approximately 1.83 times that of wild type soybean JN18. In the flowers of transgenic *GmELF4-LIKE4* gene over expressing soybeans, the relative expression level of *ELF3* was approximately 1.47 times that of wild-type soybean JN18, and the relative expression level of *COL* was approximately 1.57 times that of wild-type soybean JN18.

Fig. 6 Relationship of expression of *GmELF4-LIKE4*, *ELF3* and *COL*

A: Relative expression of each gene in leaves; B: Relative expression of each gene in flowers

At the same time, we studied the relationship between *GmELF4-LIKE4* and *ELF3* and *COL* genes in the RNAi soybean JN18. The relative expression levels of *GmELF4-LIKE4*, *ELF3*, and *COL* in wild type soybean JN18 were 1.00, 1.49, and 1.35, respectively.

The relative expression of *ELF3* in the leaves of transgenic *GmELF4-LIKE4* RNAi soybeans was about 0.55 times that of wild type soybean JN18, and the relative expression of *COL* was about 0.51 times that of wild type soybean JN18. The relative expression level of *ELF3* in the flowers of transgenic *GmELF4-LIKE4* RNAi soybean is about 0.55 times that of wild type soybean JN18, and the relative expression of *COL* is about 0.48 times that of wild type soybean JN18.

The experimental results showed that in *GmELF4-LIKE4* over expression soybeans, the relative expression levels of *ELF3* and *COL* genes also increased in leaves and flowers; in RNAi soybeans, the expression levels of *ELF3* and *COL* genes also declined. So we speculate that *GmELF4-LIKE4* positively regulates *ELF3* and *COL*.

Fig. 7 Relationship of expression of *GmELF4-LIKE4*, *ELF3* and *COL*

A: Relative expression of each gene in leaves;

B: Relative expression of each gene in flowers

Analysis of Drought Resistance of Transgenic Soybean with *GmELF4-LIKE4*

The results shown in Table 1. Under non-stress conditions, there was no significant difference between the *GmELFE-LIKE4* gene transgenic soybean and wild type JN18 in relative conductivity, malondialdehyde (MDA) content, and peroxidase (POD) activity.

After drought stress for 5D, the relative conductivity of the transgenic *GmELF4-LIKE4* over expression soybean was 7.90%, 5.68%, 10.12% lower than that of the wild type JN18, respectively; the relative conductivity of the transgenic *GmELF4-LIKE4*-RNAi soybean was higher than that of the wild type JN18, respectively 8.64%, 9.38%, 12.02%. In terms of MDA content, after stress treatment for 5D, the MDA content of *GmELF4-LIKE4* over expression soybeans was 10.59%, 9.87%, 9.00% lower than that of wild type JN18, respectively; the MDA content of *GmELF4-LIKE4*-RNAi soybeans was lower than that of wild type JN18, respectively 10.81%, 12.56%, 12.56% higher. In terms of POD activity, the POD activity of *GmELF4-LIKE4* over expression soybean was 10.40%, 6.37%, 11.58% higher than that of wild type JN18, respectively; the POD activity of *GmELF4-LIKE4*-RNAi soybean was 9.03%, 11.11% lower than that of wild type JN18, respectively, 9.27%.

After drought stress treatment for 5D, the difference between transgenic soybean and wild-type JN18 in the above three physiological indicators reached a significant level, indicating that the expression of *GmELF4-LIKE4* gene can improve the drought resistance of plants. Figure 8 shows the soybean under drought stress 5D.

Table 1 Relative conductivity, MDA content and POD activity statistics table

Plants	Relative conductivity (μ S/cm)		MDA (μ mol/g Fw)		POD (U/g • min)	
	OD	5D	OD	5D	OD	5D
RNAi-1	32.63 $\pm$ 0.39 a	37.30 $\pm$ 0.12 de	0.1477 $\pm$ 0.0002 a	0.1630 $\pm$ 0.0002 f	21.38 $\pm$ 0.027 a	28.23 $\pm$ 0.061 b
RNAi -2	32.87 $\pm$ 0.55 a	38.20 $\pm$ 0.12 d	0.1477 $\pm$ 0.0002 a	0.1643 $\pm$ 0.0007 e	21.43 $\pm$ 0.022 a	27.20 $\pm$ 0.021 c
RNAi -3	32.80 $\pm$ 0.45 a	36.40 $\pm$ 0.20 e	0.1474 $\pm$ 0.0002 a	0.1659 $\pm$ 0.0005 d	21.46 $\pm$ 0.104 a	28.53 $\pm$ 0.056 a
WT	33.40 $\pm$ 0.36 a	40.50 $\pm$ 0.67 c	0.1476 $\pm$ 0.0003 a	0.1823 $\pm$ 0.0003 c	21.50 $\pm$ 0.023 a	25.57 $\pm$ 0.038 d
OE-1	32.67 $\pm$ 0.47 a	44.00 $\pm$ 0.21 b	0.1470 $\pm$ 0.0003 a	0.2020 $\pm$ 0.0005 b	21.40 $\pm$ 0.020 a	23.26 $\pm$ 0.046 e
OE-2	32.67 $\pm$ 0.21 a	44.30 $\pm$ 0.21 b	0.1470 $\pm$ 0.0001 a	0.2052 $\pm$ 0.0004 a	21.45 $\pm$ 0.076 a	22.73 $\pm$ 0.040 f
OE-3	32.83 $\pm$ 0.47 a	45.37 $\pm$ 0.18 a	0.1475 $\pm$ 0.0002 a	0.2052 $\pm$ 0.0002 a	21.56 $\pm$ 0.073 a	23.20 $\pm$ 0.046 e

Note: Difference lowercase letters indicate significant difference ($P < 0.05$)

Fig. 8 Soybeans after 5D of drought stress

Discussion

In this experiment, the homologous gene *GmELF4-LIKE4* of *ELF4* was functionally verified in *Arabidopsis thaliana* and soybean. Transgenic *Arabidopsis thaliana* over expressing *GmELF4-LIKE4* was delay flowering, while transgenic *Arabidopsis thaliana* with *GmELF4-LIKE4*-RNAi bloom early. This result is in line with Harriet G. McWatters [16] and Mark R. Doyle [17] studied the function of *ELF4* gene in *Arabidopsis thaliana* there are similarities. It can be speculated that the homologous genes of *ELF4* we cloned are functionally in common with other genes in the *ELF4* family.

The circadian clock can not only affect the response process of plant hormones, but different hormonal pathways can also interact with the circadian clock to feedback and regulate the rhythm of the circadian clock [18,19]. Artificially changing the expression patterns of key components of the biological clock in plants will increase the sensitivity of plants to stresses such as salt, osmotic potential, drought and high temperature, indicating that the biological clock plays an important role in the process of plants resisting external environmental stresses. The conclusion is consistent with our research [20-22].

Habte reported that under normal conditions, *ELF4* does not affect abscisic acid (ABA) synthesis, but under ABA treatment and drought conditions, *ELF4* responds to external environmental signals to promote ABA biosynthesis. *ELF4* directly promotes the synthesis of ABA, thereby regulating the stomata opening and improving the stress resistance of plants. In our functional verification, the over expression of *GmELF4-LIKE4* can improve the drought resistance of soybeans, while inhibiting *GmELF4-LIKE4* will reduce the drought resistance of soybeans. This conclusion is corroborated with Habte 's [23].

Our research finding shows that when *GmELF4-LIKE4* is over expressed, the *COL* gene will also increase the expression level. At the same time, the *COL* gene has been confirmed to be involved in regulating plants to resist biotic and abiotic stresses. Liu

used RNA-Seq to obtain the differentially expressed rapeseed gene *BnCOL2* that was significantly induced by abiotic stress, and found that *BnCOL2* was mainly expressed in the cotyledons and leaves of rape seedlings. The expression profile showed that *BnCOL2* was induced by NaCl, PEG6000 and ABA. In addition, the drought resistance of *Arabidopsis thaliana* over expressing *BnCOL2* under PEG6000 treatment was significantly reduced, which is consistent with our research result [24]. It has also been confirmed in rice that *OsCOL9*, a member of the *COL* family, can positively enhance rice resistance to rice blast [25].

In previous studies, Casal once found that abscisic acid (ABA) strongly induced the expression of *AtCOL* in *Arabidopsis thaliana*, and ABA is an important regulator of plant tolerance to abiotic stress [26]. Song found 8 *COL* family genes through genome-wide association analysis, and proved that the 8 genes showed different expression patterns in maize leaves. Either inhibited by ABA treatment, or induced by ABA treatment [27].

Here, we conclude that a feedback mechanism linking the circadian clock with plant responses to biotic and abiotic stress. It laid the foundation for exploring how the biological clock-related genes regulate the mechanism of plants against external stress.

Materials and Methods

Screening and verification of differential gene *GmELF4-LIKE4*

The Ct values of *GmELF4-LIKE4* gene and internal reference gene β -Actin (NM_001252731) in the roots, stems and leaves of wild-type JN18 and root mutant M18 were determined by real-time fluorescence quantitative PCR, and *GmELF4-LIKE4* was calculated according to $2^{-\Delta\Delta CT}$. The relative expression level of the gene using GraphPad Prism 6.

Cloning and vector construction of *GmELF4-LIKE4* gene

According to the results of RNA-Seq, Primer Premier5.0 was used to design specific primers ACGGTGATATATTTGGA and TTCTCTTCTGGTTGACTT for this gene. Using soybean M18 V1 root cDNA as a template, *GmELF4-LIKE4* was cloned by RT-PCR. After that, we connected the target gene to the pMD-18T cloning vector and sequenced it. Then the results were successfully compared, we used seamless cloning technology to construct the pCAMBIA3301-*GmELF4-LIKE4* over expression vector and pCAMBIA3301-*GmELF4-LIKE4*-RNAi expression vector [28].

Transformation of *GmELF4-LIKE4* into *Arabidopsis thaliana* and *Glycine max* (L.) Merr

In order to verify the function of *GmELF4-LIKE4*, we transformed the *GmELF4-LIKE4* gene into Colombian *Arabidopsis thaliana* and soybean JN18 by inflorescence infection method and Agrobacterium-mediated method, respectively. Observe the morphology of T2 generation *Arabidopsis thaliana*. And for soybeans, we performed PCR detection on T0, T1 and T2 generation soybeans, Southern Blot

detection, and fluorescence quantitative PCR detection on the roots, leaves and flowers of confirmed positive soybeans in R2. At the same time, we also studied the relationship between *GmELF4-LIKE4* and *ELF3*, *CONSTANS-like* expression in transgenic soybeans under the conditions of 8 hours of light and 16 hours of dark in the growth room.

Analysis of Drought Resistance of Transgenic Soybean with *GmELF4-LIKE4*

The stress method of this experiment is to use 10% PEG6000 solution to osmotic stress on soybeans [29]. Under drought stress 0D and 5D conditions, we measured the relative conductivity, MDA content and POD activity of the leaves of the *GmELF4-LIKE4* genetically modified soybeans and control materials. For specific experimental methods, we referred to Rahman and Li [30,31].

Additional file 1:

Figure S1. Electrophoresis of total RNA of M18 roots.

Figure S2. Cloning of *GmELF4-LIKE4*.

A: Electrophoresis detection of cDNA amplification *GmELF4-LIKE4* gene, M: DNA Marker DL2000, 1-5:cDNA; B: PCR detection of pMD-18T-*GmELF4-LIKE4* N: Water, M: DNA Marker DL2000, 1-4: Recombinant plasmid.

Figure S3. Identification of recombinant vector pCAMBIA3301-*GmELF4-LIKE4*.

A: Identification of PCR, P: pMD-18T-*GmELF4-LIKE4*, N:Water, 1-5: Recombinant plasmid
B: Enzyme digestion analysis, 1-2: Recombinant plasmid

Figure S4. The structure of overexpression vector.

Figure S5. Identification of recombinant vector pCAMBIA3301-*GmELF4-LIKE4*-RNAi. A: Identification of PCR, M: DNA Marker DL2000, 1-4: Sense, 5-9: Antisense, 9-12: Introns fragment. B: Enzyme digestion analysis, 1-2: Recombinant plasmid.

Figure S6. The structure of RNA interference vector.

Figure S7. PCR detection of transgenic soybean T0.

A: PCR detection of *35s*; B: PCR detection of *Bar*; C: PCR detection of *NOS*; M: M:DNA Marker DL2000; P: pCAMBIA3301-*GmELF4-LIKE4*; N: Water; WT: Wild Type; 1-5:Transgenic soybean.

Figure S8. PCR detection of transgenic soybean T1.

A: PCR detection of *35s*; B: PCR detection of *Bar*; C: PCR detection of *NOS*; M: M:DNA Marker DL2000; P: pCAMBIA3301-*GmELF4-LIKE4*; N: Water; WT: Wild Type; 1-5: Transgenic soybean

Figure S9. Southern blotting detection of transgenic soybean T2.

A: PCR detection of *35s*; B: PCR detection of *Bar*; C: PCR detection of *NOS*; M: M:DNA Marker DL2000; P: pCAMBIA3301-*GmELF4-LIKE4*; N: Water; WT: Wild Type; 1-5: Transgenic soybean

Figure S10. Southern blotting detection of transgenic soybean T2.

A: *GmELF4-LIKE4* Over expression Soybean; B: *GmELF4-LIKE4* RNAi Soybean; M: Southern Marker; P: pCAMBIA3301-*GmELF4-LIKE4*; WT: Wild Type; 1-5: Transgenic soybean

Abbreviations

ABA: abscisic acid (ABA); CDS: Coding sequences; *COL*: CONSTANTS-like; *ELF3*: *Early flowering 3*; *ELF4*: *Early flowering 4*; MDA: Malondialdehyde; POD: Peroxidase; RNAi: RNA interference; SYBR: Synergy Brands

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Availability of data and materials

All the data which is generated and analyzed during our study are included within the article and additional files.

Authors' contributions

JY, ZH and AL identified problems, carried out laboratory activities, analyzed the data, and prepared manuscript. ZQ, XZ, GS and QJ worked in laboratory activities and preparing the manuscript. WP and ZR supervised the study and revised the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared no conflict interests.

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Figures

Figure 1

Analysis of GmELF4-LIKE4 expression in various tissues of JN18 and M18

Figure 2

Phylogenetic tree of ELF4

Figure 3

Observation and Comparison of the Phenotype of *Arabidopsis thaliana*

Figure 4

Agrobacterium-mediated tissue culture of soybean A: Germination culture; B: Pre-culture; C: Co-culture; D: Screening culture; E: Elongation culture; F: Rooting culture; G: Regenerated plants

Figure 5

Analysis of relative expression of GmELF4-LIKE4 in tissues of transgenic soybean A: Analysis of Relative Expression of Over expressed soybean in Roots B: Analysis of Relative Expression of Over expressed soybean in Leaves C: Analysis of Relative Expression of Over expressed soybean in Flowers D: Analysis of Relative Expression of RNAi soybean in Roots E: Analysis of Relative Expression of RNAi soybean in Leaves F: Analysis of Relative Expression of RNAi soybean in Flowers.

Figure 6

Relationship of expression of GmELF4-LIKE4 , ELF3 and COL A: Relative expression of each gene in leaves B: Relative expression of each gene in flowers

Figure 7

Relationship of expression of GmELF4-LIKE4, ELF3 and COL A: Relative expression of each gene in leaves B: Relative expression of each gene in flowers

Figure 8

Soybeans after 5D of drought stress

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