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Identification and expression characterisation of *SbERECTA* family genes in *Sorghum bicolor*

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Abstract. ERECTAs are receptor-like kinases that regulate plant biomass and stress resistance. In this study, the wheat (*Triticum aestivum*) *TaERECTA* gene was used as a probe to identify the *SbERECTA* family genes (*SbERs*) in the sorghum (*Sorghum bicolor*) genome, analyse their subcellular localisation and characterise their expression. Results showed that the two *SbER* members, *SbER10* with three copies (*SbER10_X1*, *SbER10_X2*, and *SbER10_X3*) and *SbER4* with two copies (*SbER4_X1* and *SbER4_X2*), were found on chromosomes 10 and 4 of sorghum, respectively. *SbER10* had the highest expression level in the pedicel tissue and showed a remarkable response under treatment with abscisic acid, brassinolide, gibberellin and indole-3-acetic acid. *SbER10_X1*, functioning on the cell membrane and chloroplast, exhibited abundant transcript in only a few sorghum varieties that are grown in mountainous areas and receive strong light, heat, and water supply. Expression of *SbER10_X1* was significantly and positively correlated with plant biomass of 32 sorghum germplasm resources. These results indicate that *SbER10* genes have an important regulatory role in sorghum growth, and increasing *SbER10* transcription level offers a potential strategic target for breeding or biotechnological approaches to enhance sorghum biomass and environmental adaptability.

Keywords: correlation analysis, ERECTA family, expression characteristics, *SbER*, sorghum.

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Introduction

Sorghum bicolor L., one of the key C_4 crops, is used to produce food, forage and energy, and in brewing. It provides food and resources for more than 500 million people in arid and semiarid areas in Africa and Asia (Abdel-Ghany *et al*. 2020; Kimani *et al*. 2020). Global population growth and increases in per capita income intensify human demand for food and energy. Therefore, crop breeding is needed, drawing on traditional methods, functional gene screening, genome editing, and other technical means, to obtain new varieties with drought resistance, pest resistance, and high light efficiency. The sorghum genome contains many useful genes related to high yield, stress resistance, and high light efficiency (McCormick *et al*. 2018). Development and use of sorghum functional genes is a potential strategy to ensure global food security, provide energy, and promote the development of animal husbandry.

ERECTA (ER) is a receptor-like kinase (RLK) that regulates plant photosynthesis and transpiration efficiency, increases biomass, and improves plant stress resistance (Masle *et al*. 2005; van Zanten *et al*. 2009). Transgenic maize (*Zea mays*) with *ZmER* shows increased organ size, enhanced transpiration efficiency and drought resistance (Guo *et al*. 2011). After the wheat (*Triticum aestivum*) *TaER* gene was silenced, leaf stomatal density and conductance of the mutant plant increased (Zheng *et al*. 2019). The *AtER* gene (*Arabidopsis thaliana*) was overexpressed in tomatoes and rice, leading to the increased thermostability of transgenic lines (Shen *et al*. 2015). Interaction of *ER* and *BAK1* genes can improve the resistance of *A. thaliana* to the necrotrophic fungus *Plectosphaerella cucumerina* BMM (Jordá *et al*. 2016). These results indicate that the *ER* family has broad application prospects in regulating plant development and stress resistance.

In sorghum, *SbER2-1* (equivalent to *SbER4_X1* in this study) was isolated and introduced into maize to increase drought resistance and net photosynthetic rate (Li *et al*.

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2019). However, *SbERECTA* has two members (*SbER4* and *SbER10*), and *SbER10* was not specifically investigated and introduced into sorghum for improvement of forage biomass. In this study, the evolutionary relationship of the *SbER* family in dicotyledonous and monocotyledonous plants was clarified by analysing the characteristics of sorghum *SbER* family members and *cis*-regulatory elements. The expression pattern of the *SbER10* genes under hormone induction was examined, and *SbER10_X1* was isolated to detect its expression variation in 32 sorghum germplasm resources of China. This work provides a theoretical basis for using *SbER10_X1* to improve sorghum production potential and stress resistance.

Materials and methods

Identification of SbER *family in the sorghum genome*

Wheat *TaER* gene (JQ599261.2) was used to search in the Phytozome v12.1 database; two *SbER* gene tags of sorghum (*Sobic.004G317200* and *Sobic.010G077000*) were found and aligned against the NCBI database to obtain the *SbER* nucleotide and protein sequences, and predict the *SbER* full CDS coding frame and chromosome position information. The PROSITE database was used to analyse the amino acid composition, molecular weight, and isoelectric point of SbER. The GSDS database was used for predicting the exons of *ER* family genes, PlantCARE database for the *cis*-regulatory elements of the *SbER* promoter, and Plant-mPLoc database for the characteristics of SbER subcellular localisation. Supplementary Material Table S1 lists all databases used and their URLs (available at the journal's website).

Analysis of protein sequence and phylogenetic tree

PROSITE and SMART databases were used to predict the core conserved regions and functional domains of the *SbER* family. The ER family members of monocotyledonous and dicotyledonous plants were obtained in the NCBI database, and amino acid sequences with >80% similarity were downloaded. MEGA 5.0 software was used for alignment (Tamura *et al*. 2013). A phylogenetic tree was generated with the neighbour-joining method under the threshold of 1000 replications for bootstrap (Tamura *et al*. 2013).

Prokaryotic expression and western blot analysis of SbER10 family

The common Ser/Thr kinase segment of the *SbER10* genes was amplified with primers of *SbER10-KF* and *SbER10-KR* (Table S2), using the PCR reaction system (50 μL): 25 μL 2 × PCR buffer, 10 μL dNTP (2 mM), 1.5 μL Primer-F (10 μM), 1.5 μL Primer-R (10 μM), 1 μL KOD FX (1.0 U μL^{-1}, KFX-101, Toyobo, China), 5 μL cDNA as template (the peduncle of sorghum variety Tx623B), 6 μL ddH_2O. The PCR procedure was: 94°C for 2 min, 40 cycles (98°C for 10 s, 61°C for 30 s for renaturation, 68°C for 1 min for extension), 68°C for 10 min.

PCR products were introduced into pET-32a-c(+) vector via restriction site (Table S2), transformed into *Escherichia coli* (DH5α), and then cultured at 37°C until OD_{600} 0.6–0.8. Isopropyl β-d-1-thiogalactopyranoside (IPTG) was added into the culture with a final concentrations of 0, 0.4 and 0.8 mM. The cells cultured at 37°C for 3 h were harvested and split by repeated freezing and thawing with lysozyme. The supernatant (~50 μg) was run on SDS–PAGE and transferred onto nitrocellulose filter membrane (NC membrane) by wet transblot apparatus (Bio-Rad, USA). Primary and secondary antibodies were anti-histidine (His) (mouse monoclonal antibody) and IgG antibody (horseradish peroxidase IgG). Protein marker was pre-dyed protein maker II (MP203, Tiangen, China). Immune complexes were visualised with scanner system (Canon, Japan).

Gene isolation and subcellular localisation of SbER10_X1

SbER10_X1 (XM_002437978.2) had an open reading frame of 2949 bp, encoding a protein of 983 amino acids (Table 1). The specific primers (*SbER10_X1-2F*/*SbER10_X1-2R* in Table S2) were designed to isolate the *SbER10_X1* gene from the peduncle cDNA of sorghum variety Tx623B by using the KOD FX enzyme. The PCR reaction system and procedure were as above, except for 64°C for 30 s for renaturation and 68°C for 4 min for extension.

The code fragment of *SbER10_X1* (without the stop codon for development of fusion protein in Supplementary materials Fig. S1) was amplified with primers *GFP-SbER10_X1-F1* and *GFP-SbER10-X1-R1* (Table S2), using the PCR procedures and reaction system as above, except for 62°C for 30 s for renaturation and 68°C for 3 min for extension. The PCR fragment was fused into the N-terminus of green fluorescent protein (GFP) containing the promoter of CaMV35S as 35S::*SbER10_X1*-GFP, and the 35S::GFP vector was used as the control (Shi *et al*. 2018), The fusion construct was confirmed by DNA sequencing. Vectors 35S::GFP and 35S::*SbER10_X1*-GFP were introduced into wheat mesophyll protoplasts to estimate their transient expression. The transformed cells were incubated in darkness at 22°C for 18–20 h, and then photographed under a confocal laser scanning microscope (LSM700; CarlZeiss, Germany).

Plant materials and treatments

For detection of expression profiles, sorghum Tx623B and 32 sorghum germplasm resources were planted in the experimental field of Anhui Science and Technology University in 2018 (32°52′N, 117°33′E; 43 m elevation). For expression profiles analysis at different growth stages, the latest expanded leaves of Tx623B were collected at seedling (stage 2), jointing (stage 3), and booting (stage 4); and flag leaves were collected at heading (stage 5), flowering (stage 6), filling (stage 7), and maturity (stage 8). For tissue specific expression, tissue samples of root (stage 3), stem (stage 4), leaf and leaf sheath (stage 5), panicle, pedicel, anther and ovary (stage 6), and kernel (stage 7) were collected. Details of the sampling period can be found in Fig. S2.

In 32 germplasm resources, the flag leaves were collected at only stage 5, in addition to investigation of photosynthetic index and agronomic traits. Nine plants of each of the 32 germplasm resources were evaluated as replications of each

Table 1. Characteristics of putative *SbER* genes in *Sorghum bicolor*
pI, Isoelectric point; MW is the molecular weight of amino acids

Name	Locus (JGI) DNA	Gene ID (NCBI) DNA	Nucleotide Gene length (bp)	Locus (NCBI) mRNA	No. of exons	Protein accession (NCBI)	pI	Protein subcellular location	Protein length (AA)	MW (kDa)	Location
SbER10_X1	Sobic.010G077000	LOC8077321	7715	XM_002437978.2	27	XP_002438023.1	5.99	Cell membrane	983	107	Chr. 10
SbER10_X2				XM_021448730.1	27	XP_021304405.1	6.05	Cell membrane	981	107	Chr. 10
SbER10_X3				XM_021448731.1	26	XP_021304406.1	6.06	Cell membrane	959	105	Chr. 10
SbER4_X1	Sobic.004G317200	LOC8081937	7319	XM_021458593.1	27	XP_021314268.1	5.73	Cell membrane	991	108	Chr. 4
SbER4_X2				XM_021458594.1	27	XM_021458594.1	5.74	Cell membrane	990	108	Chr. 4

trait. Table S3 displays the source and names of 32 sorghum germplasm resources.

For hormone induction, sorghum variety Tx623B was planted in flowerpots (35 by 35 cm). Each flowerpot was sown with 35 seeds pre-germinated for 4 days and placed in a light incubator for growth (humidity 60%, 23°C/20°C day/night, 16 h/8 h light/dark culture, light intensity 525 μmol s^{-1} m^{-2}). After 15 days of growth, the seedlings were collected including the roots, rinsed, dried on filter paper for instant drying, and then placed in hormone solution with deionised water (control) for recovery growth. Hormone solutions were abscisic acid (ABA) (100 μM), brassinolide (BR) (0.75 μM), gibberellin (GA_3) (30 mM), and the auxin indole-3-acetic acid (IAA) (10 μM) (Zheng and Hu 2016). Samples (mixture with organs of roots, stems and leaves) were taken at different times: 0, 1, 2, 4, 6, 12, 24, 48, and 60 h. Three individual plants were selected as biological replicates each time.

All samples were immediately frozen in liquid nitrogen after collection and stored at –80°C for later use.

RNA extraction, RT-qPCR and data analysis

Total RNA of sorghum samples was extracted using RNAprep Pure Kit (Tiangen Biochemical Technology, China), and reverse transcribed to obtain cDNA samples by using PrimeScript 1st Strand cDNA Synthesis Kit (Takara, Japan). Five cDNA sequences of *SbER10* and *SbER4* genes were aligned to design specific primers for *SbER10* expression, selecting regions with high similarity among the three copies of *SbER10* genes but with diversity between *SbER10* and *SbER4*. The primers of *SbER10* genes (*SbER10-2QF*/*SbER10-2QR*) and reference genes (*SbActin-F*/*SbActin-R*) are listed in Table S2. The *SbER10* expression level was detected by using RT-qPCR methodology and the ABI Prism 7500 system (Applied Biosystems, USA) (Liu *et al*. 2013).

The RT-qPCR data were analysed in accordance with procedure of Zheng *et al*. (2015) and Zheng and Hu (2016). Error analysis was conducted with the SPSS Statistics Software version 18.0 (IBM, USA) based on the biological replicates of three individual plants. Correlation analysis between *SbER10* expressions and measured agronomy traits was performed with SPSS 18.0 by using the Pearson product moment correlation test.

Results

Characteristics of the sorghum SbER gene family

The *SbER* family has two members, *SbER10* (LOC8077321) and *SbER4* (LOC8081937), located on sorghum chromosomes 10 and 4, respectively. *SbER10* had three copies, namely, *SbER10_X1* (XM_002437978.2), *SbER10_X2* (XM_021448730.1) and *SbER10_X3* (XM_021448731.1), and *SbER4* had two copies, namely, *SbER4_X1* (XM_021458593.1) and *SbER4_X2* (XM_021458594.1) (Table 1). Among the five copies, four had 27 exons, except for *SbER10_X3*, in which exon 21 was deleted, and all members were predicted to be transmembrane proteins. In exon 26 of *SbER4* and *SbER10* genes, *SbER4_X2* encoded one fewer alanine (Ala) than *SbER4_X1*, whereas *SbER10_X2* encoded one fewer lysine

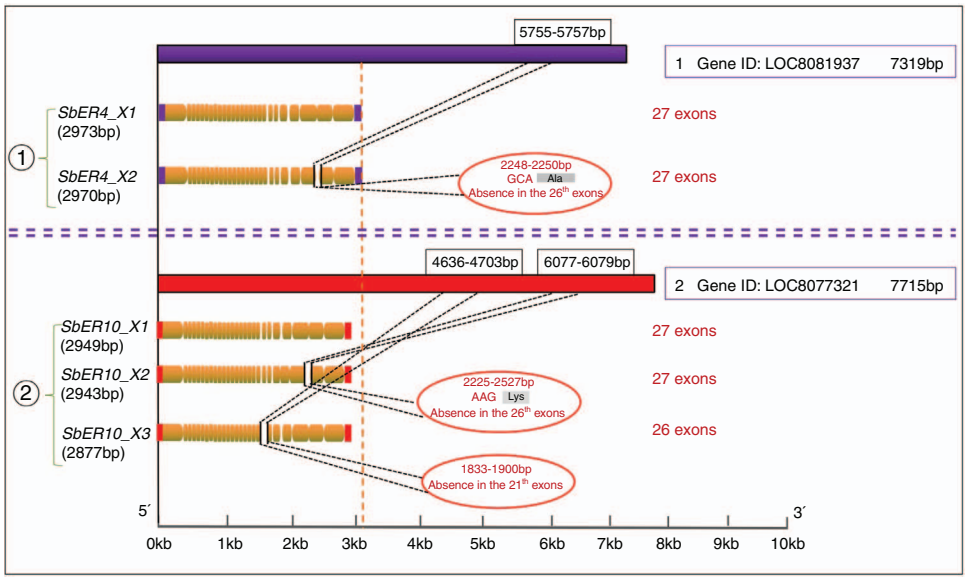

Fig. 1. Nucleotide sequence characteristics of *SbER* family genes.

(Lys) than *SbER10_X1*. *SbER10_X3*, with a deleted exon, encoded 24 fewer amino acids (Fig. 1).

SbER protein functional domain and phylogenetic relationship

The SbER family had typical characteristics of RLKs, including the N-terminal signal peptide area, leucine repeat region (LRR), transmembrane region, and C-terminal serine/threonine kinase region (Fig. S3). In particular, the amino acid sequences of the SbER family and other species in the LRR region and kinase region were relatively conserved, whereas the amino acids in the N-terminal signal peptide region and transmembrane region were different (Fig. S4).

The ER proteins of common monocotyledonous and dicotyledonous plants can be clearly divided into four categories (Fig. 2). The first category is Gramineae crops, which can be divided into two subgroups: sorghum SbER10 family, wheat, barley, maize, millet and other crops constitute one subgroup; and SbER4 family, barley HvERL1 and rice ER family constitute the other subgroup. The second category consists of monocotyledonous plants such as pineapple, banana and *Phalaenopsis*. The third category contains dicotyledonous plants including the ER family of soybeans, grapes, sesame, poplar, cocoa and *A. thaliana*. The fourth category includes only AtERL1 and AtERL2, indicating that the *A. thaliana* AtERL has the farthest evolutionary relationship. SbER10_X1, SbER10_X2 and SbER10_X3 have the closest genetic relationship with maize, and SbER4_X1 and SbER4_X2 have a close relationship with rice, implying that the evolutionary relationship of SbER10 system is close to modern field xerophytes.

SbER *gene structure and* cis*-regulatory elements*

The *cis*-regulatory elements of the *SbER* family promoter regulate plant seed-specific development, light response,

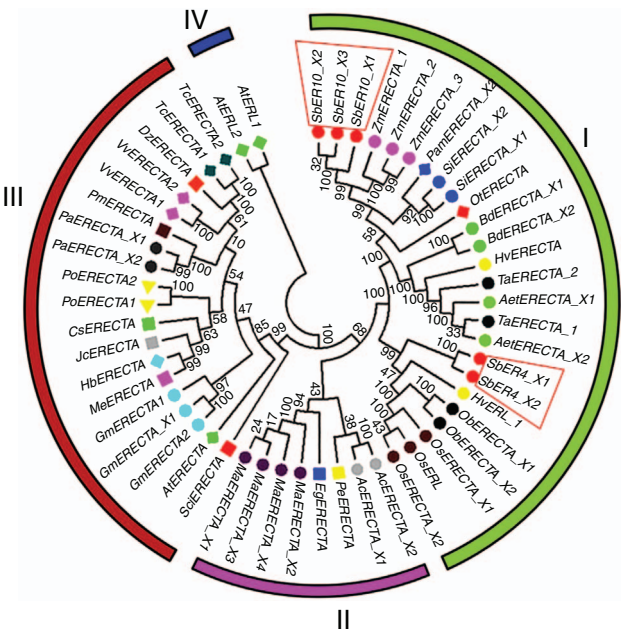

Fig. 2. Phylogenetic tree of ER family proteins in monocots and dicots. Each species is represented by the same symbol with the same colour. Numbers beside the branches represent bootstrap values based on 1000 replications. Plant species and NCBI accession numbers of proteins in phylogenetic tree are listed in Table S4.

drought, low temperature induction, and signal responses mediated by methyl jasmonate and ABA (Table 2). Therefore, the *SbER* family may have a role in regulating plant resistance. In addition, *SbER10* regulates the plant's defence response to anaerobic induction reaction and the cell division cycle, suggesting that its regulatory function may be stronger than that of *SbER4*. Therefore, *SbER10_X1* was used to analyse the

Table 2. *Cis*-acting elements of *SbER* family promoters in *Sorghum bicolor*

Functional characteristics of *cis*-acting elements of *SbER* promoters were predicted in the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)

Code	Functional elements of *SbER* promoters		Functional characteristics
	SbER10 (LOC8077321)	*SbER4* (LOC8081937)	
1	RY-element	RY-element, TCCC-motif	Seed-specific regulation
2	GA-motif, Box4, ATCT-motif, G-box, GT1-motif, AE-box, LAMP element, GATA-motif, AT1-motif, BoxII, MRE, TCT-motif, G-Box	TCT-motif, Box4, G-box	Light responsive
3	MBS	MBS	Drought-inducibility
4	TC-rich repeats		Defence and stress responsiveness
5	CGTCA-motif, TGACG-motif	TGACG-motif, CGTCA-motif	MeJA-responsiveness
6	LTR	LTR	Low-temperature responsiveness
7	ABRE	ABRE	Abscisic acid responsiveness
8	ARE	ARE, GT1-motif, GC-motif	Regulatory of the anaerobic induction
9	TGA-element		Anoxic specific inducibility
10	MSA-like		Involved in cell cycle regulation

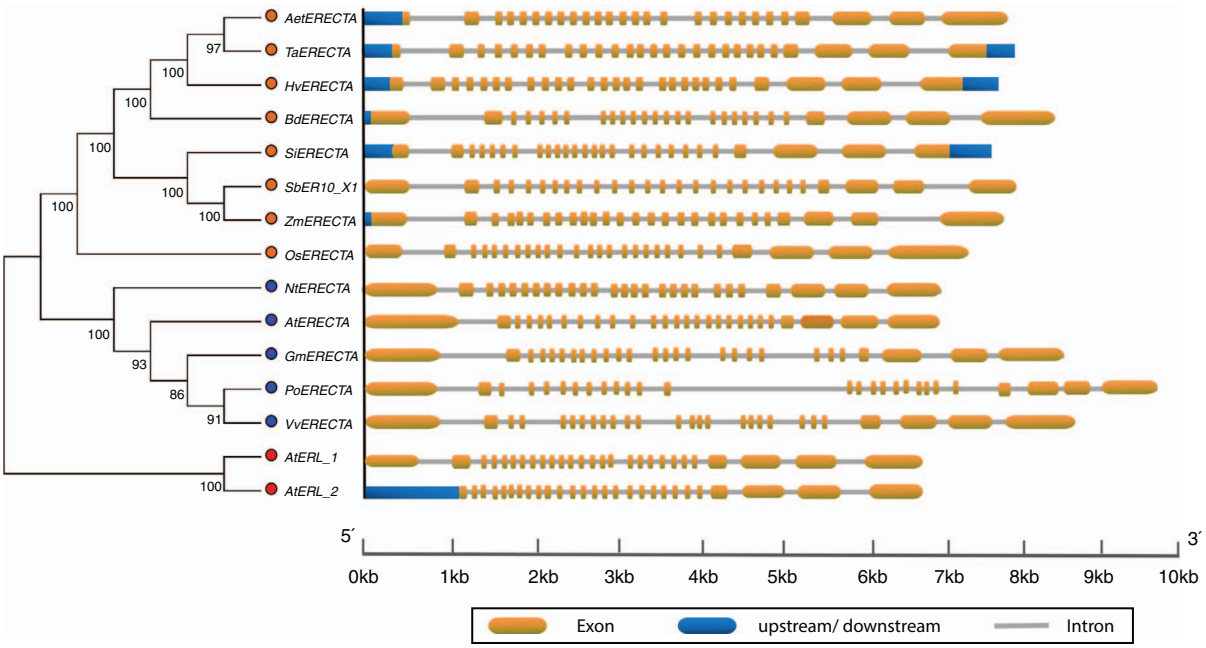

Fig. 3. Intron–exon structures of *ER* genes in monocots and dicots. Each same species is represented by the same symbol with the same colour. Numbers beside the branches represent bootstrap values based on 1000 replications. Plant species and NCBI accession numbers of *ER* genes in phylogenetic tree are listed in in Table S5.

ER gene family structure of Gramineae crops and published soybean, poplar, grape and *A. thaliana*. Figure 3 shows that the *ER* family nucleotide sequence can also be divided into monocotyledonous plants, dicotyledonous plants and the *AtERL* family. The selected members of the *ER* family all contained 27 exons, of which exons 25, 26 and 27 were large and mainly encode the threonine/serine kinase domain. Exon 1 of the dicotyledon *ER* family was large, and exon 1 of the monocotyledon was small. Exon 1 encoded the N-terminal signal peptide region or part of the LRR repeat region. *AtERL* family genes were relatively short and had separate branches. From this finding, it was speculated that the *ER* genes of different species have various regulatory functions in receiving upstream signals that are transmitted into the cell, owing to the different elements of the N-terminal signal peptide region.

Expression pattern of SbER10 *gene family in different organs and at developmental stages*

In different organs of sorghum Tx623B (Fig. 4), the *SbER10* family was highly expressed in aboveground organs but hardly expressed in roots. With the expression level of root organs as a reference, *SbER10* expression in the pedicels was the highest, at 1500 times, followed by that in the panicles. The expression levels in young stems, leaf sheaths and ovary were close and >40 times that of roots, and the expression level in anthers and

kernels was lower at only ~16 times. Of the different developmental stages (Fig. 5), *SbER10* showed the highest expression level at booting stage, 37 times higher than at seedling stage, followed by heading and flowering stages, but low expression at grain-filling and maturation stage. This finding indicates great variations in the functional mechanism of *SbER10* when regulating the development of different sorghum organs during growth stages.

Response pattern of SbER10 *gene family under hormone treatments*

For the study of treatment with hormones ABA, BR, GA_3 and IAA, the expression level of *SbER10* genes was stable in control samples but significantly ($P < 0.01$) increased in the treated samples, thus showing a strong response (Fig. 6). After 2 h of ABA treatment, the expression level of *SbER10* gradually increased, with the highest expression 14 times that of the control at 48 h (Fig. 6*a*). After 2 h of BR treatment, the expression level of *SbER10* increased rapidly, with the highest expression 18 and 17 times that of the control at 12 and 24 h, respectively (Fig. 6*b*). After 2 h of GA_3 treatment, the expression level of *SbER10* increased slowly, with highest expression 14 times that of that control at 12 h, and then decreased rapidly (Fig. 6*c*). After IAA treatment, the *SbER10* genes showed a positive response, with highest expression level 36 times that of the control at 2 h, and the expression level gradually decreased after 2 h (Fig. 6*d*). This finding indicates that *SbER10* can actively respond to hormone induction and may regulate physiological processes related to sorghum development and stress resistance.

SbER10_kinase prokaryotic expression

For the study of treatment with IPTG at constant temperature (37°C) for 3 h, the target protein (pET-32a-c (+)-SbER10_kinase) without induction by IPTG slightly

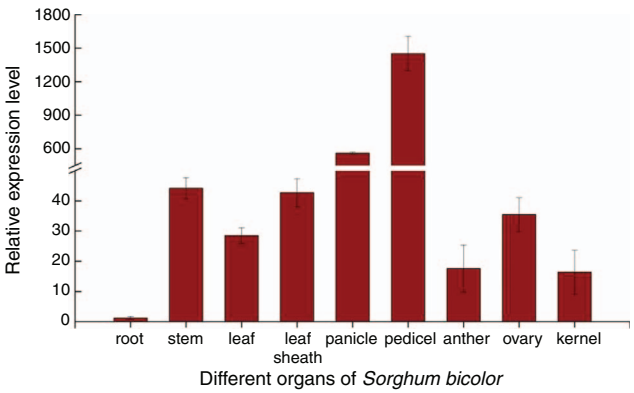

Fig. 4. Tissues specific expression profiles of *SbER10* gene in sorghum. Sorghum Tx623B cDNA was used to detect expression patterns of the gene. The primers of *SbER10* gene (*SbER10-2QF*/*SbER10-2QR*) and reference gene (*SbActin-F*/*SbActin-R*) are listed in Table S2.

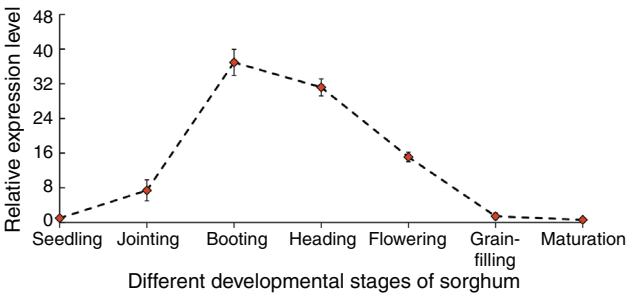

Fig. 5. Expression profiles of *SbER10* gene during sorghum growth stages. Sorghum variety Tx623B cDNA was used to detect expression patterns of the gene. The primers of *SbER10* gene (*SbER10-2QF*/*SbER10-2QR*) and reference gene (*SbActin-F*/*SbActin-R*) are listed in Table S2.

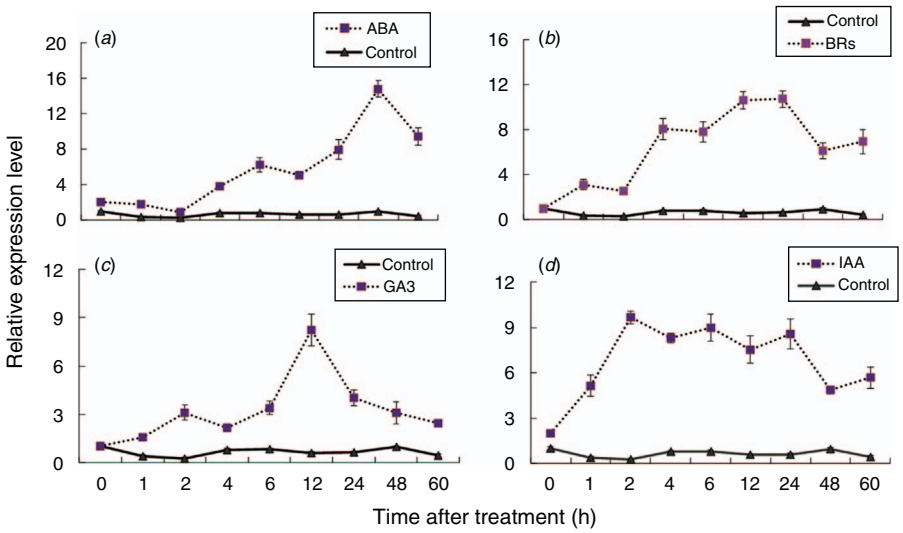

Fig. 6. Expression patterns of *SbER10* gene after hormone-induction. Sorghum variety Tx623B cDNA was used to detect expression patterns of the gene: (*a*) abscisic acid (ABA) treatment (100 μM); (*b*) brassinolide (BRs) treatment (0.75 μM); (*c*) gibberellin (GA_3) treatment (30 mM); (*d*) auxin (IAA) treatment (10 μM).

accumulated compared with the control (pET-32a-c(+) empty vector) in the prokaryotic system. After induction by IPTG (0.4 and 0.8 mM), the target protein accumulated in large amounts, with a size of ~45 kDa. After the His tag was removed for 14 kDa, the size of SbER10_kinase was close to 31 kDa, which is consistent with the predicted size of the target protein (Fig. 7*a*). Western blot analysis was conducted to confirm the reliability of the target protein; Fig. 7*b* shows that no band appeared after hybridisation of the empty vector pET-32a-c(+) protein, and the hybrid bands of the target protein without IPTG induction were weak. After IPTG induction, the target protein hybrid band pattern was evident, indicating that the SbER10_kinase sequence can completely encode the target protein for translation.

SbER10_X1 *clone and subcellular localisation*

SbER10_X1 gene fragment with size 3376 bp was isolated using the pedicel of sorghum Tx623B as material (Fig. S5). The 35S:*SbER10_X1*-GFP fusion vector was transferred into wheat leaf protoplasts and cultured using PEG to clarify the role of the *SbER10_X1* protein in cells. Observation showed that the fluorescent signal of the *SbER10_X1*-GFP fusion protein was located on the cell membrane and chloroplast of wheat mesophyll protoplasts (Fig. 8). Meanwhile, the control pCAM35-GFP was located on the cell membrane, cytoplasm, and nucleus, indicating that *SbER10_X1* mainly functions on the cell membrane and chloroplast. This finding is consistent with the above structural characteristics of predicted SbER10 as a transmembrane protein.

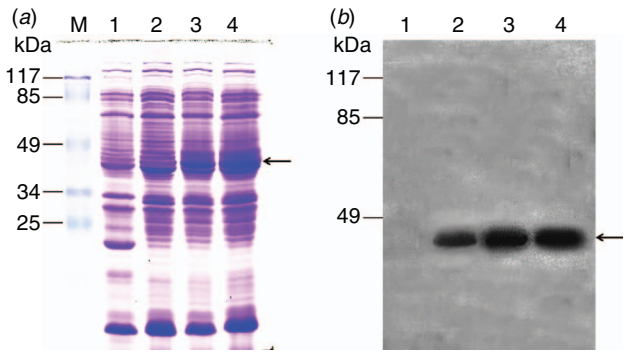

Fig. 7. Detection of SbER10_kinase protein under IPTG induction: (*a*) SDS-polyacrylamide gel electrophoretogram; (b) immunoblot. Target protein is indicated by black arrow. M, Protein marker. 1, pET-32a-c (+) empty vector; 2–4, pET-32a-c(+)-SbER10_kinase protein induced by IPTG concentrations of 0, 0.4 and 0.8 mM, respectively.

Correlation of expression of SbER10_X1 *with photosynthetic index and agronomic traits*

Thirty-two sorghum germplasm resources in different provinces of China were selected to identify the expression level *of SbER10_X1*. The results showed that the expression level was high in only a few sorghums and low in most materials (Fig. 9*a*). The resources can be clearly divided into three groups, with highly significant ($P < 0.01$) difference among the three categories. The first group contained three germplasm resources (No. 8, 22 and 29) and showed the highest expression of *SbER10_X1* with an average of 10.9. The photosynthetic index and agronomic traits were greatly different among the 32 sorghum germplasm resources, and negative linear correlations were found for *SbER10_X1* expression with transpiration rate (E), stomatal conductance (gs) and stomatal number per unit (SN), whereas positive linear correlations were detected for *SbER10_X1* expression with 1000-kernel weight (TKW), main stem panicle grain yield (MSPGY), main stem diameter (MSD), main stem fresh weight (MSFW) and main stem drought weight (MSDW) (Table 3). Regression analyses confirmed that *SbER10_X1* expression was significantly ($P < 0.05$) and negatively correlated with E and gs, and highly significantly ($P < 0.01$) and positively correlated with MSFW and MSDW (Fig. 9*b*). This finding shows that only a few varieties during sorghum natural evolution have high expression levels of *SbER10_X1*,

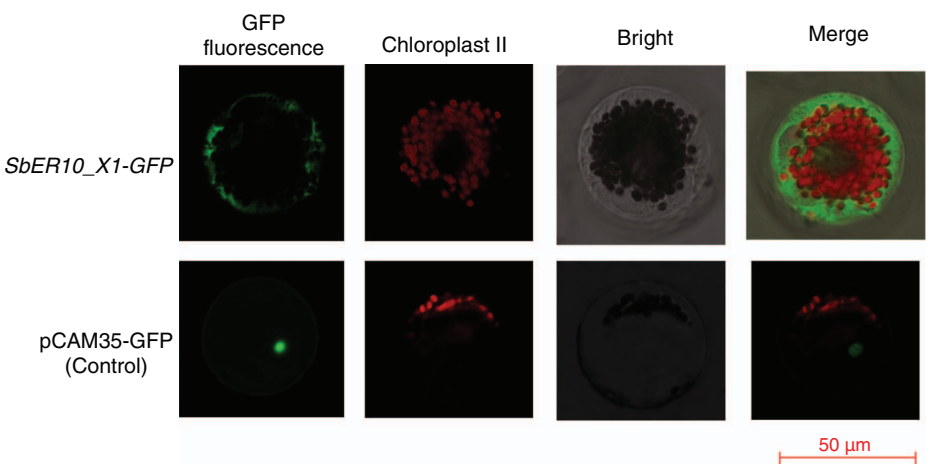

Fig. 8. Subcellular localisation of *SbER10_X1* fusion proteins in wheat mesophyll protoplasts. Note: *SbER10_X1*-GFP and pCAM35-GFP (control) were transiently expressed in wheat mesophyll protoplasts. Images captured using confocal microscopy (scale bar = 50 μm).

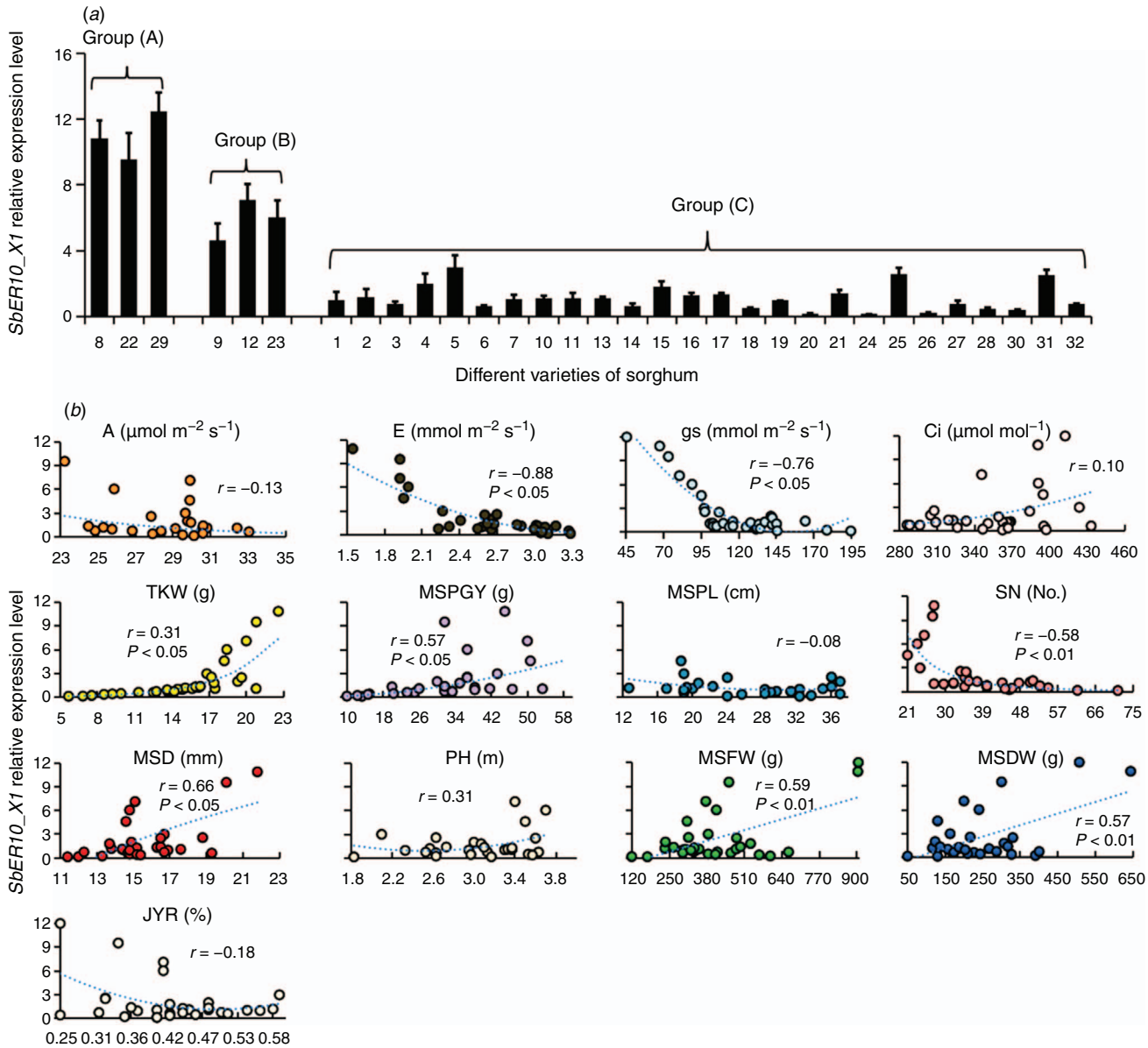

Fig. 9. Regression analysis of *SbER10_X1* expression with photosynthetic index and agronomic traits: (*a*) *SbER10_X1* gene expression level; (*b*) regression analysis. For the source and names of 32 sorghum germplasm resources, see Table S3. In (*a*): different letters indicate that the expression level of *SbER10_X1* among the three groups was highly significantly different ($P < 0.01$). In (*b*): A, photosynthetic rate; E, transpiration rate; gs, stomatal conductance; Ci, intercellular CO_2 concentration; TKW, 1000-kernel weight; MSPGY, main stem panicle grain yield; MSPL, main stem panicle length; SN, stomatal number per unit; MSD, main stem diameter; PH, plant height; MSFW, main stem fresh weight; MSDW, main stem drought weight; JYR, juice yield rate; *r*, correlation coefficient.

probably leading to the variation in photosynthetic index and biomass.

Discussion

The characteristics of gene families are important to the study of their functions. The accuracy and reliability of analysis of the evolutionary characteristics of gene families depend on the genome sequences. Sorghum has two members of the *ER* family (*SbER10* and *SbER4*) located on chromosomes 10 and 4, respectively. The *SbER10* gene sequence in the sorghum genome was long with three spliceosomes, whereas the *SbER4* gene sequence was small with two spliceosomes. The number of exons varied in the three copies of *SbER10*, and this finding is different from previous studies (Li *et al*. 2019). The *ER* family also has two members in rice, wheat, cotton, tobacco and other crops, and each member has different splice bodies, resulting in uneven distribution of introns and exons across the genome (Liu *et al*. 2019). In eukaryotes, the acquisition or loss of introns is one of the evolutionary mechanisms of a gene family (Roy and Penny 2007). Any difference in the number of

Table 3. Correlation coefficient of *SbER10_X1* expressions with photosynthetic index and agronomic traits

Correlation coefficient was evaluated based on nine replications of each trait ($n = 9$); *$P < 0.05$; **$P < 0.01$. Variation range shows the difference between maximum and minimum of each agronomic trait measured with 32 sorghum germplasm resources. A, Photosynthetic rate; E was transpiration rate; gs, stomatal conductance; Ci, intercellular CO_2 concentration; TKW, 1000-kernel weight; MSPGY, main stem panicle grain yield; MSPL, main stem panicle length; SN, stomatal number per unit; MSD, main stem diameter; PH, plant height; MSFW, main stem fresh weight; MSDW, main stem drought weight; JYR, juice yield rate

Code	Trait	Variation range	Correlation coefficient	Code	Trait	Variation range	Correlation coefficient
1	A (μmol m^{-2} s^{-1})	23.8	–0.13	8	SN	50.0	–0.58**
2	E (mmol m^{-2} s^{-1})	2.4	–0.88*	9	MSD (mm)	10.4	0.66*
3	Gs (mmol m^{-2} s^{-1})	148.9	–0.76*	10	PH (m)	1.87	0.31
4	Ci (μmol. mol^{-1})	231.9	0.10	11	MSFW (g)	785.0	0.59**
5	TKW (g)	16.9	0.70*	12	MSDW (g)	593.3	0.57**
6	MSPGY (g)	48.3	0.57*	13	JYR (%)	0.34	–0.18
7	MSPL (cm)	24.3	–0.08				

introns will affect the expression levels of the target genes. When the *AtER* gene of *A. thaliana* lacks introns, the amount of target protein decreases 500–900 times (Karve *et al*. 2011). When the extracellular region of soybean *GmER* reduces the number of LRR tandems (exons decrease), shading treatment can increase the hypocotyl length, leaf area and petiole length (Du *et al*. 2018). Different splice bodies of *SbER* genes in sorghum probably cause functional differences, and need to be further investigated.

The *ER* gene family is a member of the *RLK* gene family and codes for a transmembrane protein that senses external stimuli, activates the expression of intracellular signalling factors, and regulates the physiological response of cells (Shpak *et al*. 2004; Zheng and Hu 2016;). Prior to the emergence of monocotyledonous and dicotyledonous plants, the *ER* family evolved into two large families of *ER* and *ERL*. Multiple copies of the *ER* and *ERL* families of monocotyledonous and dicotyledonous plants have gradually formed with the occurrence of gene replication events (Liu *et al*. 2019). In this work, the *ER* family can be clearly divided into three categories: monocotyledonous, dicotyledonous and *Arabidopsis ERL*. Among them, *SbER10* has a close evolution relationship with maize, and *SbER4* has a close relationship with rice. The *SbER* family and *ER* family of dicotyledonous and monocotyledonous plants have large differences in amino acid residues in the N-terminal signal peptide region and transmembrane region. The most important function of the *ER* gene is phosphorylation. The difference in the amino acid position of the transmembrane region affects the phosphorylation of RLKs; the N-terminal extension region is an integral part of kinase folding and is essential for kinase activity (Kosentka *et al*. 2017). The absence of the kinase region does not affect the increase in the elongation of stalk and pedicel cells but can cause the *A. thaliana* inflorescence to be compact and the silique to shrink. Moreover, this gene has different molecular mechanisms for stomatal development and cell elongation (Shpak *et al*. 2003; Villagarcia *et al*. 2012). After 3 h of IPTG induction, SbER10_kinase protein is completely expressed in prokaryotic cells. This finding provides theoretical guidance for the next synthesis of SbER-specific antibodies.

The *ER* family participates in light-induced subtropical growth (van Zanten *et al*. 2010), improves the drought resistance of maize (Li *et al*. 2019), and regulates rice blast non-host resistance (Takahashi *et al*. 2016). Combined with ABA and methyl jasmonate, the gene family regulates the quantitative resistant traits of *A. thaliana* (Häffner *et al*. 2014), and inhibits cell division and promotes cell elongation (Qu *et al*. 2017). In this study, the promoter of the *SbER* family contains ABA (ABRE), drought, low temperature, methyl jasmonate, anaerobic induction and other core elements, implying that the gene improves plant stress resistance. However, the mechanism of low temperature response and anaerobic induction reaction has not been reported. For the light response mechanism, *ER* genes are involved in mediating hypocotyl elongation, shortening the cycle of the plant biological clock, and reducing the range of leaf surface movement under the interaction of blue and dark light (van Zanten *et al*. 2009). Those authors also reported that *ER* affects the carboxylation rate and photoelectron transfer ability of ribulose diphosphate carboxylase and increases the photosynthetic ability of *A. thaliana* (van Zanten *et al*. 2009). *SbER* has four *cis*-acting elements that are all involved in the photoreaction, and the *SbER10_X1* protein is located on the cell membrane and chloroplast. This finding indicates that this gene is involved in plant photosynthesis and has great application potential for improving sorghum photosynthesis and increasing biological yield.

The *ER* family is involved in signal regulation networks mediated by IAA (Qu *et al*. 2017), BR (Wang *et al*. 2017), GA_3 (Ragni *et al*. 2011) and ABA (Häffner *et al*. 2014). *SbER2-1* can improve the drought resistance and water use efficiency of maize (Li *et al*. 2019), and *VvER* (in grape) has a positive response to hormone, drought and pathogenic bacteria treatment (Liu *et al*. 2019). This study found that *SbER10* genes actively respond to hormone induction. This finding is consistent with previous research results and suggests that *SbER* has similar functions in plant growth and development and stress resistance regulation.

In *A. thaliana* and wheat, the *ER* gene is hardly expressed in plant roots (Shpak *et al*. 2004; Zheng and Hu 2016), and the expression level of *SbER10* in sorghum roots is low. The expression levels in tissues such as young panicles and pedicels are extremely high, indicating that *SbER* mainly regulates the development of young tissues and organs above ground. Among different growth stages, *SbER* has

the highest expression level in the new leaves at booting stage and has low expression at the seedling stage and after heading. This finding is different from the tissue expression specificity of wheat *TaER* gene (Zheng and Hu 2016), probably because germinal leaves develop from the seed embryo at the germination stage of wheat, whereas the new leaves of sorghum develop from the growth cone of stem tissues after jointing stage. Transcriptional level of *SbER* was high in young tissues, displaying abundant transcript at booting stage and a reduced trend with leaves senescence process.

Of the 32 sorghum germplasm resources of different regions of China used to assess variation in *SbER10_X1* expression, analysis found that *SbER10_X1* is abundant in only a few varieties, most of which come from mountainous areas with sufficient light, heat and water resources. In other regions, *SbER10_X1* expression is relatively low, indicating that differences in the natural environment greatly influence the *SbER* transcription level in sorghum. *SbER10_X1* expression was positively correlated with MSFW, MSDW, MSD, MSPGY and TKW, but negatively correlated with E, gs and SN; this concurs with previous studies (Zheng *at al*. 2015). However, the strongest correlation of *SbER10_X1* expression was with MSFW and MSDW, which greatly contribute to the biomass of forage sorghum. The link between *SbER* expression with photosynthetic index and agronomic traits confers another strategic target for breeding or biotechnological approaches to increase sorghum biomass.

Conclusions

SbERECTA (*SbERs*) family has two members, *SbER10* with three copies (*SbER10_X1*, *SbER10_X2*, and *SbER10_X3*) and *SbER4* with two copies (*SbER4_X1* and *SbER4_X2*), in sorghum genome. *SbER10* has a close evolutionary relationship with maize, and *SbER4* with rice. The promoter of the *SbER* family contains ABA, low temperature, anaerobic induction and other core elements, and *SbER10* genes actively respond to hormone induction. *SbER10_X1*, functioning on the cell membrane and chloroplast, showed abundant transcript in only a few mountain varieties experiencing strong light, heat and water supply among 32 sorghum germplasm resources. *SbER10_X1* expression was significantly and positively correlated with plant biomass, providing a strategic target for forage sorghum breeding.

Conflicts of interest

The authors declare no conflicts of interest.

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