



Full Length Article

Identification and Expression Analysis of *ERECTA* Homologous Genes in *Glycine max*

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Abstract

ERECTA (ER), a plant receptor-like kinase, plays important roles in many aspects of plant growth, development and environmental adaptation. Previous studies suggested that three *ER* homologs, *ER*, *ERECTA-LIKE 1* (*ERL1*) and *ERECTA-LIKE 2* (*ERL2*) are in the genome of *Arabidopsis thaliana*. But little is known about the copy numbers and the biological functions of *ER* homologs in other plant species, especially in crops. Here we identified four *ER* homologs in soybean (*Glycine max*) by the basic local alignment search tool (BLAST) with *Arabidopsis* ER sequences. Four *ER* paralogs, *GmERa* (Glyma04g05912), *GmERb* (Glyma06g05900), *GmERc* (Glyma14g11220) and *GmERd* (Glyma17g34380) were predicted to be located on different chromosomes of soybean. Organ-specific expression analyses indicated high transcript levels of soybean *ER* homologs in hypocotyls, petioles, stems and leaves, while the levels in roots and cotyledons were relatively low. Real-time quantitative PCR and *ER* promoter::GUS analyses showed that the four *GmER* genes were differentially induced by the dehydration stress. These results suggest that four soybean *ER* paralogs might play distinct roles in different plant tissues and function differentially after the water stress. Among them, *GmERb* is expressed in almost all tissues and could be induced to the highest level by the dehydration stress. © 2017 Friends Science Publishers

Keywords: Soybean; Receptor-like kinase; RLK; *ERECTA*; Expression; qPCR; Drought

Introduction

Crop production is critical for food security resulted from ever-increasing global population (Farooq *et al.*, 2013). Soybean (*Glycine max*) has become one of the most important economical crops in the world owing to its high protein and oil content (Sun *et al.*, 2013). However, environmental stresses such as water shortage and drought related stresses, significantly affects the growth, yield and grain quality of soybean (Nakayama *et al.*, 2007; Deshmukh *et al.*, 2014). Drought is a major environmental factor which limits soybean production worldwide, the sustainable soybean production has been threatened by persistent drought stress in many parts of world (Kunert *et al.*, 2016). Scientists have made big efforts to improve its environmental stress tolerance thus improving soybean

production (Xue *et al.*, 2007; Seo *et al.*, 2011; Bihmidine *et al.*, 2012; Ku *et al.*, 2013). Maize-soybean strip intercropping is one of the best agricultural practice to improve soybean production and balance a better agricultural sustainability in China (Yang *et al.*, 2014; Yang *et al.*, 2015; Liu *et al.*, 2017). But the water stress and imbalance of water distribution is one of the limiting factor affecting soybean production and further extension of the intercropping model (Rahman *et al.*, 2017). A better understanding of the molecular mechanisms of how soybean perceives and responds to environmental stresses especially drought stress not only can help in the development of stress tolerance and high yielding soybean cultivar but also can improve the total yield of maize-soybean strip intercropping system.

Plants have evolved a set of sophisticated

physiological and biochemical mechanisms to adapt the changeable environmental stresses including drought, salinity, light stress, extreme temperature, nutrient deficiencies and pathogen attacks (Jaleel *et al.*, 2009; Du *et al.*, 2016a). Plasma membrane is an important barrier and bridge for environmental sensing of plant cells. Plant plasma membrane-localizing proteins receptor-like protein kinases (RLKs) are critical in cell-to-cell and cell-to-environment communications, which function in plant growth and development, and environmental sensing (Li, 2010; Du *et al.*, 2016a). A typical RLK contains an N terminal signal peptide, an extracellular domain for signal perception from surrounding environment, a single-pass transmembrane domain for membrane anchoring and a cytoplasmic kinase domain in charge of transduction of extracellular signals to intracellular compartment via a protein phosphorylation manner (Li, 2010). At least 610, 1130, 1416 RLKs and cytoplasmic receptor-like kinases (RLCKs) were identified in Arabidopsis, rice and soybean, respectively (Shiu and Bleecker, 2001; Shiu *et al.*, 2004; Lehti-Shiu and Shiu, 2012). Of all the plant RLKs, leucine-rich repeat RLKs (LRR-RLKs) are the largest family, which contains about 223 and 467 RLK-encoding genes in Arabidopsis and soybean genome, respectively (Gou *et al.*, 2010; Zhou *et al.*, 2016). Currently, only limited functions of several plant RLKs have been elucidated. Some RLKs might play roles in adjustment of plant growth and environmental responses (Marshall *et al.*, 2012; Tameshige *et al.*, 2017).

ERECTA (ER), an LRR-RLK, was found to play important roles not only in Arabidopsis growth and development but also in response to environmental stimuli (Torii *et al.*, 1996; van Zanten *et al.*, 2009; Shpak, 2013; Tameshige *et al.*, 2017). Genetic analysis has shown that the *er* mutant displays compact inflorescence and short blunt silique phenotypes due to the decrease in cell proliferation and growth (Shpak *et al.*, 2003). Further studies showed that another two ER-family receptor-like kinases, ERL1 and ERL2 play a redundant role in cell proliferation of organ growth and patterning (Shpak *et al.*, 2004) and the LRR-RLK TOO MANY MOUTHS (TMM) negatively regulates ER family proteins in stomatal differentiation (Shpak *et al.*, 2005). Furthermore, ER was found to participate in abiotic stress resistance control. Overexpression of truncated Arabidopsis *ER* in tomato decreased water loss and enhanced drought tolerance (Villagarcia *et al.*, 2012). Overexpression of Arabidopsis *ER* in Arabidopsis, rice and tomato increased plant biomass and improved thermal tolerance independent of water content (Shen *et al.*, 2015). Single nucleotide polymorphism (SNP) analysis has shown that an *ER* homologous gene might be associated with drought adaptation between wild and common bean (Blair *et al.*, 2016). However, whether any *ER* homologous genes exist in the genomes of the important crops like soybean and whether they involve in drought stress tolerance is still unknown. In the present study, we identified several *ER* homologous genes in several plant species including

soybean by the basic local alignment search tool (BLAST) with an Arabidopsis ER sequence. From the results, four paralogous ERs, *GmERa* (Glyma04g05912), *GmERb* (Glyma06g05900), *GmERc* (Glyma14g11220) and *GmERd* (Glyma17g34380) were found on different chromosomes of soybean. Organ-specific expression analyses indicated a high transcript level of soybean *ER* homologs in aerial parts, especially in shoot apical, while the levels in roots were relatively low. Quantitative reverse transcription-PCR (RT-qPCR) analyses showed that the four *GmER* paralogous genes were upregulated differentially by dehydration stress. Expression patterns of the four *GmER* homologous genes in Arabidopsis by promoter::GUS analysis showed that the four *GmERs* are differentially responsive to drought stress. Among them, *GmERb* is expressed in almost all tissues and could be induced to the highest level by the dehydration stress, suggesting that the four *GmERs* might function distinguishingly in drought stress tolerance. These results make a new discovery of *ER* paralogous genes in soybean and also provide an evidence that *GmERs* might play distinct roles in different plant tissues and involve in plant drought stress tolerance.

Materials and Methods

Plant Materials and Growth Conditions

The soybean material in the current studies is *Glycine max* (L.) Merr. Cv. C103 (also named as Nan032-4) (Meng *et al.*, 2016). Soybean seeds were grown under soil condition in a growth chamber (light/dark 16 h/8 h, 948 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 25°C) for two weeks. Two-week-old soybean seedlings were collected and used for PEG6000 treatment. Roots, hypocotyl, cotyledon, unifoliate leaves, trifoliate leaves, petioles, stems and stem apices were collected for total RNA extraction according to Sun *et al.* (Sun *et al.*, 2015).

Sequence Analyses

Arabidopsis protein sequences AtER (At2g26330), AtERL1 (At5g62230) and AtERL2 (At5g07180) were obtained from The Arabidopsis Information Resource (TAIR) database (<http://www.arabidopsis.org/>). Protein sequence of AtER was used to search the plant ER homologs in Phytozome database (<https://phytozome.jgi.doe.gov/>). All the candidate ER homolog sequences were aligned by ClustalX2. The phylogenetic trees of ER homologs were constructed by Phylogeny Inference Package (PHYLIP-3.695, <http://evolution.genetics.washington.edu/phylip.html>) using the aligned sequences.

Dehydration and Physiological Parameter Measurement

Soybean seedlings grown in soil were watered evenly for two weeks and after plant roots were harvested and cleaned properly before immersed in 10% PEG6000 solution for 0,

15, 30 and 60 min. Then, leaves of soybean C103 treated with or without PEG6000 were collected to measure MDA as described by Du *et al.* (Du *et al.*, 2011) as well as RNA extraction for reverse-transcription and quantitative PCR analyses.

Reverse Transcription and Quantitative PCR (RT-qPCR) Analyses

Total RNA was extracted using a Plant Total RNA Miniprep Purification Kit (Tiangen, <http://www.tiangen.com/>). cDNA was reversely transcribed from 2 µg of total RNA using an Oligo (dT)₂₀ primer and M-MLV reverse transcriptase (<http://www.invitrogen.com>). First strand cDNAs of reversely transcribed 50 ng of RNA was used for semi-quantitative RT-PCR analyses with *ExTaq* DNA polymerase (TaKaRa) and qPCR with Universal SYBR[®] GREEN qPCR Master Mix (2×) (Gangchi Bio). The parameters of semi-quantitative PCR were as follows: 95°C for 5 min, 95°C for 15 s, 50°C for 30 s, 72°C for 1 kb min⁻¹, go to step 2 for another more cycles according to the expression level of the specific genes. Parameters of the qPCR is 95°C for 3 min, 95°C for 15 s, 55°C for 15 s, 72°C for 20 s, go to step 2 for 39 more cycles. Then increment of 0.5°C from 65°C to 95°C for 5 s was used for melt curve analysis. $\Delta\Delta Cq$ method was used to normalize the qPCR data (Du *et al.*, 2016b). *GmACT3* (Glyma09g17040) was amplified as an internal control. Gene-specific primer pairs were designed using Primer 5.0 (Table 1).

Promoter Cloning and Transformation

Promoter sequences of *GmERa*, *GmERb*, *GmERc* and *GmERd* were amplified from C103 genomic DNA by PCR using primers of GmERap-PB1-3 and GmERap-PB2, GmERbp-PB1 and GmERbp-PB2, GmERcp-PB1 and

GmERcp-PB2 and GmERdp-PB1 and GmERdp-PB2, respectively (Table S1). A Gateway[®] cloning approach was used to construct the promoter sequences into a *pBASTA-GWR-GUS* binary vector. The plasmids were then transformed into *Arabidopsis Col-0* by *Agrobacterium tumefaciens* GV3101-mediated floral dip (Clough and Bent, 1998; Du *et al.*, 2016a).

GUS Staining

Ten-days-old transgenic *Arabidopsis* plants harboring promoter::GUS of *pBASTA-pGmERa-GUS*, *pBASTA-pGmERb-GUS*, *pBASTA-pGmERc-GUS*, *pBASTA-pGmERd-GUS* were treated by 10% PEG6000 for 0, 15, 30 and 60 min and then used for GUS staining according to previous studies (Wu *et al.*, 2016). Gene-specific primer pairs were designed by using Primer 5.0 (Table S1).

Results

Identification of ER Homologs in Soybean

Sequence alignment using *Arabidopsis ER* showed that four ER paralogs, GmERa (Glyma04g05912), GmERb (Glyma06g05900), GmERc (Glyma14g11220) and GmERd (Glyma17g34380) were identified in soybean. GmERa, GmERb, GmERc and GmERd shared 86%, 87%, 89% and 89% similarities, respectively with *Arabidopsis ER*. GmERa showed similarities of 95.1% to GmERb and GmERc showed similarities of 96.9% to GmERd. Phylogenetic tree indicated that soybean ER family proteins is divided into two branches. GmERa and GmERb form one group, whereas GmERc and GmERd form another one, which are similar to *Arabidopsis ER* family proteins. In *Arabidopsis thaliana*, ER and its two paralogs, ERL1 and ERL2 were split into two main groups (Fig. 1). Of all the soybean ER encoding genes, *GmERa* has two splice variants, one of them

Table 1: Primers used for RT-PCR analyses and *GmERa* CDS amplification

Primer name	Primer sequence (5'-3')	Note
GmERa-qF	TGAGAATCAAATTCAGAATGAA	Primers for qPCR analysis.
GmERa-qR	TGACTTTTAAATAAACGTTAGT	
GmERb-qF	AGAAAAGAAAGAGGGAACGGTAAAG	
GmERb-qR	CTTGTGAGAGAGAAGGAGGGAGTT	
GmERc-qF	GAATACTAGTCTCAAACAAAAC	
GmERc-qR	CTCAACAACCACCATCATCAAGC	
GmERd-qF	AGTAGGAGGGAAGCAAATACTATTG	
GmERd-qR	AGATAACTGATCCAAAGCCAGCTA	
GmACT3-qF	GGCTGGATTTGCTGGAGAT	
GmACT3-qR	ATCCTTTTGGCCCATTC	
GmERap-PB1-3	AAAAAGCAGGCTTCCAGACTCTTCCAGGTTACCCACGGGA	Primers for promoter amplification.
GmERap-PB2	AGAAAAGCTGGGTCAGTCAACAACAACAACAACAGC	
GmERbp-PB1	AAAAAGCAGGCTTCTTGTACCAGACTTGTACCGAAC	
GmERbp-PB2	AGAAAAGCTGGGTCAGCCAACAACAACAACACCG	
GmERcp-PB1	AAAAAGCAGGCTTCCAGCATCAACAAATCATTCCAGC	
GmERcp-PB2	AGAAAAGCTGGGTCAGCTGCAACTGCAGAACTTTCAAC	
GmERdp-PB1	AAAAAGCAGGCTTCCAATGCTGTGACGCCATATT	
GmERdp-PB2	AGAAAAGCTGGGTCAGCTGCAACTGATGAACCTCAAC	

(*GmERa.1*) has twenty-six exons with a intron of 3676 bp between the 19th and 20th exons, which predicted to encoding a full RLK protein similar to that of Arabidopsis ER, while the other one (*GmERa.2*) has only fifteen exons, which encodes a putative protein lack a intercellular kinase domain. *GmERb* has three splice variants with 26 (*GmERb.1*) and 27 exons (*GmERb.2* and *GmERb.3*), *GmERc* has 25 (*GmERc.1*) and 27 exons (*GmERc.2*), *GmERd* has 28 (*GmERd.1*) and 26 exons (*GmERd.2*), respectively (Fig. 2A). Except for *GmERa.2*, the predicted protein domains of *GmERs* are consist of an extracellular domain a transmembrane domain and a kinase domain, which are consistent of Arabidopsis ER homologs (Fig. 2B). These results suggest that *GmERs* might have similar functions to that of Arabidopsis ER.

Organ-specific Expression Patterns

To investigate whether there are any dissimilarities among the ER paralogous genes, we queried the expression pattern of *GmERs* soybean eFP browser (<http://bar.utoronto.ca/efpsoybean/cgi-bin/efpWeb.cgi>) but only the expression of *GmERb*, *GmERc* and *GmERd* could be obtained at one developmental stage. All the three genes showed a high expression level in the leaves and a highest expression level in the shoot apical meristem. *GmERb* showed a highest expression level in the green pod than that of *GmERc* and *GmERd*. In the flower, *GmERc* shows a highest expression level than the other two genes. *GmERd* was expressed at a lower expression level in the flower but a higher expression level in the green pods than *GmERc*. These results suggest that the *GmER* paralogous genes might play different roles in *planta*.

To further explore the differentiation of the *GmERs*, we performed qPCR to detect the expression patterns of the four soybean ER paralogous genes in different soybean tissues. The results showed that all the four *GmERs* were mostly expressed in the shoot apices, of which *GmERa* showed a highest expression level (Fig. 3). In the trifoliolate leaves, *GmERa*, *GmERb* and *GmERc* were remarkably expressed higher than *GmERd* of which *GmERb* showed a highest expression level. Furthermore, expression of *GmERb* was higher than *GmERa*, *GmERc* and *GmERd* in most tissues except for shoot apical as a whole (Fig. 3). The transcripts of *GmERa* were relatively high in shoot apices and true leaves rather than other tissues of the seedlings, while the expression of *GmERb* was relatively high in true leaves. A high expression level of *GmERc* was detected in stems, petioles, shoot apices, while in cotyledons and shoot apices for *GmERd*. Of all the *GmERs*, expression of *GmERa*, *GmERc* and *GmERd* were detected in the aerial parts whereas *GmERb* was detectable in the roots, suggesting that *GmERa* and *GmERc* and *GmERd* may mainly function in the aerial parts of soybean plants but not in the roots during the early development.

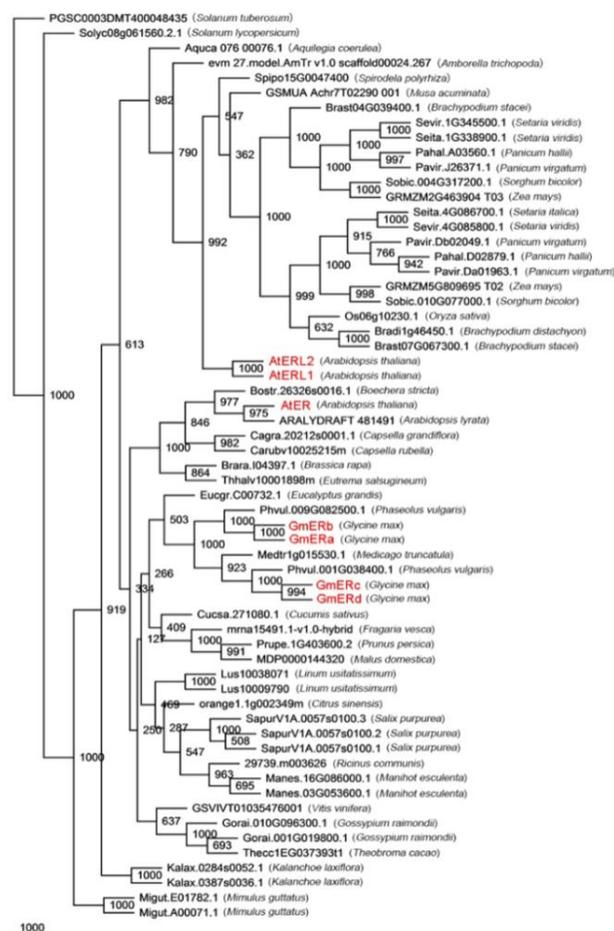


Fig. 1: Phylogenetic tree of ER homologs in plant species. The amino acid sequence of ER homologs was obtained from Phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>). Repetitions of 1000 were used to examine the bootstrap of the phylogenetic tree

Expression of *GmERa* and *GmERd* were relatively lower than *GmERb* and *GmERc* in the aerial parts of the plants besides in the shoot apical, implying that *GmERa* and *GmERd* might principally function in cotyledons, hypocotyls, stems, petioles and true leaves rather than *GmERb* and *GmERc* (Fig. 3). Expression of *GmERd* was less detectable in the tissues except for cotyledons and shoot apices than the other three *GmERs* (Fig. 3). These results suggest that the four soybean *GmER* homologs might play distinct roles in different organs of soybean seedlings in their developmental stage.

GmER Paralogous Genes are Differentially Expressed under Dehydration Stress

In order to further explore the functions of *GmERs* in soybean four cultivars were used to examine the sensitivity of drought stress. The results show that soybean cultivars C103 and Wandou30 are more sensitive to drought stress than Jidou16 and Jidou37 (Fig. 4A and B).

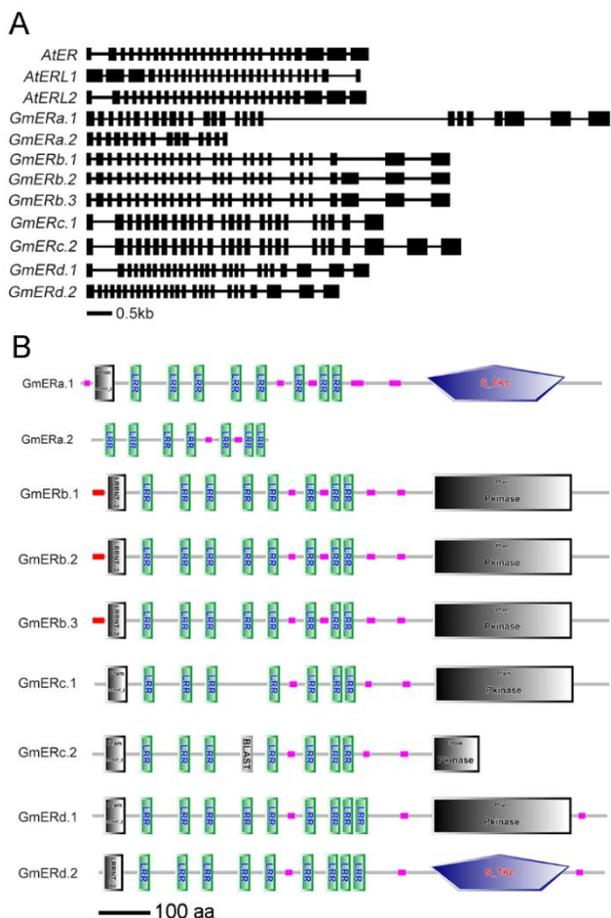


Fig. 2: Comparative analysis shows the differences of genomic DNA structures and protein domains of ER homologs

(A) DNA structures of GmERs. Black boxes represent exons and black lines represent introns. All the ER homologous genes were obtained from the Phytozome database

(B) Protein domain prediction of GmERs. Protein domains were predicted by Simple Modular Architecture Research Tool (SMART) (<http://smart.embl-heidelberg.de/>)

Thus, we then chose the drought-sensitive cultivar C103 as a material for further exploration of GmERs response to drought stress.

Water deficiency can increase malondialdehyde (MDA) of plants. Throughout the PEG6000 treatment period, soybean seedlings suffered varying degrees of dehydration (Fig. 4C). After 60 min of PEG6000 treatment, the leaves and stems became wilted and MDA content rapidly raised (Fig. 4D). These results indicate that the PEG6000 treatment triggers a physiological response of soybean seedlings which are appropriate to examine drought responsive gene expression.

In order to further explore the four GmER paralogs genes in soybean under drought stress, we performed RT-qPCR analyses to detect the transcripts of GmER paralogs genes in soybean seedlings with 10% PEG6000 treatment

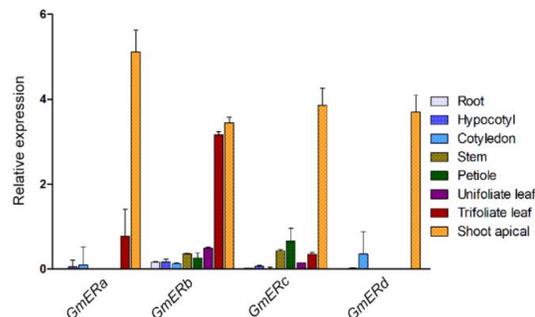


Fig. 3: Differential expression pattern of GmER paralogs genes in different tissues of soybean qPCR analysis shows that of GmER paralogs genes are differentially expressed in different tissues of soybean

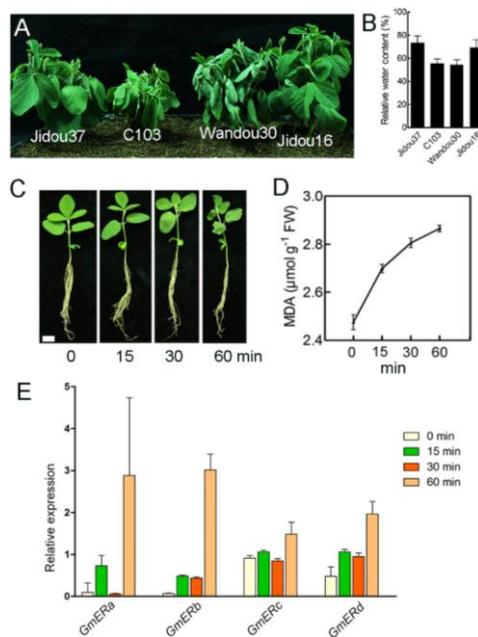


Fig. 4: Soybean ER paralogs genes are differentially induced by dehydration stress in drought-sensitive soybean cultivar C103

(A) Phenotypes of 3-week-old soybean cultivars Jidou37, C103, Wandou30 and Jidou16 by drought stress

(B) Relative water content of soybean cultivars by drought stress in (A)

(C) Phenotype of two-week-old C103 soybean seedlings treated with 10% PEG6000 solution for 0, 15, 30, 60 and 120 minutes. Bar represents 1 cm

(D) Changes of MDA of soybean seedlings by treatment of 10% PEG6000 solution for 0, 15, 30 and 60 minutes

(E) Relative expression of GmERs induced by drought stress in C103

for 0, 15, 30 and 60 min. Expression of GmERa was up-regulated by dehydration at 15 min and down-regulated at 30 min then increased again. Moreover, expression of GmERb was also up-regulated at 15 min and reach a highest level at 30 min. GmERc showed a relatively steady expression level even by dehydration stress. Expression of GmERd is up-regulated by a 15 min dehydration and to a

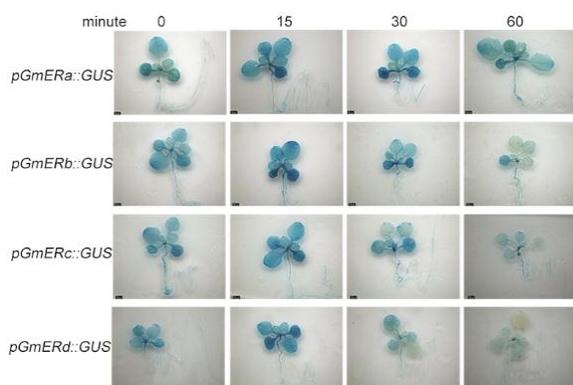


Fig. 5: Promoter::GUS analysis of soybean *ER* paralogous genes in Arabidopsis under drought stress. Scale bars represent 5 mm

highest level after 60 min (Fig. 4E). Among them, *GmERb* is expressed in almost all tissues and could be induced to the highest level by the dehydration stress, suggesting that the four *GmERs* might play distinct roles in drought stress response.

***GmERs* are Differentially Regulated by Drought Stress in Arabidopsis by Promoter::GUS Analysis**

In order to further explore the expression patterns of *GmERs* under drought stress, ten-day-old Arabidopsis transgenic plants harboring *GmER* promoter::GUS were analyzed by 10% PEG6000 treatment. The results show that all the four *GmERs* were principally expressed in the aerial parts of the transgenic Arabidopsis. Expression of the four *GmER* paralogous genes in the leaves increased drastically after 15 min of dehydration stress. Among all the *GmERs*, *GmERa* and *GmERb* displayed a highest expression level compared to *GmERc* and *GmERd* after 15 min PEG6000 treatment. *GmERb*, *GmERc* and *GmERd* decreased gradually at 30 min whereas, *GmERa* decreased gradually after 30 min. The results indicates that the four *GmER* homologous genes are differentially responsive to dehydration stress and *GmERa* and *GmERb* might make more contribution to drought response (Fig. 5).

Discussion

Plant RLKs, located on the plasma membrane, play important roles in sensing the environment usually as original sensors. Since the first RLK was found in maize in 1990 (Walker and Zhang, 1990) more and more RLK-encoding genes have been cloned from different plant species in recent years. However, only partial functions of a few RLKs have been identified until now. Our current studies suggest that ER homologs might play similar function to Arabidopsis ER and make a distinct contribution to plant resistance to drought stresses. Firstly, the four

soybean ER paralogs are on the same branch with AtER and divided into two groups, which are closed to the homologs in *Phaseolus vulgaris*. One group is composed of GmERa and GmERb showing a high similarity to PhvuI.009G082500 in *Phaseolus vulgaris*, the other is composed of GmERc and GmERd, which is similar to PhvuI.001G038400 in *Phaseolus vulgaris*. These results suggest that soybean ERs might have similar functions to Arabidopsis ER. Moreover, GmERa and GmERb, rather than GmERc and GmERd bear a closer genetic relationship with AtER. While AtERL1 and AtERL2 located on another branch, which is closed to the ER homologs of many monocots (Fig. 1). These results suggest that GmERa and GmERb, rather than GmERc and GmERd might have similar function to AtER, such as in plant development and environmental stress tolerance and GmERs might functionally substitute AtERL1 and AtERL2.

Secondly, our current study demonstrated that all the four *GmERs* showed a tissue-specificity and are principally and differentially expressed in the aerial parts. the expression of *ER* family genes in Arabidopsis shows higher levels in the shoot, whereas almost no expression in the root (Shpak et al., 2004). It is reported that Arabidopsis ERs could improve transpiration efficiency in previous studies (Masle et al., 2005). Recent studies shows that Arabidopsis ER could enhance heat tolerance and drought tolerance of tomato and rice (Shen et al., 2015). However, whether soybean homologs are involved in drought tolerance is still unknown.

Thirdly, relative expression of the GmERs were differentially induced by drought stress. Pieces of evidence showed that some of the RLKs are redundant and act as a protein complex in the same signaling pathway. One of RLKs from the protein complex often has the principal function. As one of the example, the receptor-like kinase BAK1 and its homologs BKK1/SERK3 involve in brassinosteroid perception as the co-receptor of BRI1, as well as in spontaneous cell-death control and pathogen defense (Li et al., 2002; Nam and Li, 2002; He et al., 2007a; Roux et al., 2011; Gou et al., 2012; Du et al., 2016a). This might be one of the reasons why expression of the four GmER homologous genes from our identification were differentially responsive to drought stress. In addition, some studies also showed that RLK homologs have diverse function in different plant species. The RLK CLAVATA1 (AtCLV1) functions in shoot and floral meristem establishment and maintaining in Arabidopsis (Clark et al., 1993; Clark et al., 1997), while its homologous GmNARK in soybean controls long-distance signaling in nodulation and lateral root primordia formation (Searle et al., 2003). Two duplications of soybean genome result in multiple copies of nearly 75% genes (Schmutz et al., 2010), in which the paralogous genes may have distinct function from that in Arabidopsis. Previous studies showed that the AtER has diverse function in plant growth and development and environmental stress tolerance, including thermotolerance and drought tolerance (Villagarcia et al.,

2012; Shen *et al.*, 2015). The diversity of ER function promotes our identification of soybean ER homologs and their unknown functions.

Finally, ectopic expression of promoter:GUS of *GmERs* were quickly up-regulated first and decreased later by dehydration stress in *Arabidopsis*. It is possible that the high transcript levels of *GmERs* might contribute to drought tolerance in the early stage of dehydration in *Arabidopsis*. From the previous drought stress, destructions and death of tissues and cells might occur, thus decreasing ectopic expression of *GmERs* in *Arabidopsis*. Expression of *GmERs* is increased in soybean seedlings (Fig. 4), while expression levels of *GmERs* are decreased after dehydration for 30 min (Fig. 5). One of the probable reasons is that ten-day-old *Arabidopsis* seedlings are more susceptible to be damaged by 10% PEG6000 than two-week-old soybean seedlings, which leads to severe cell damage and prevents the normal expression of genes. Previous evidence showed that receptor-like kinases often function as a complex of homodimers or heterodimers (Chinchilla *et al.*, 2007; Wang *et al.*, 2008; Roux *et al.*, 2011) and some known RLKs such as BAK1 and BKK1 control a spontaneous cell death triggered by reactive oxygen species (He *et al.*, 2007b; Du *et al.*, 2016a). One possibility is that *GmERs* might bind to each other or unknown RLKs to form a protein complex to maintain cellular homeostasis. As the four *GmERs* show a high similarity with *AtER*, we speculate that all the *GmERs* might differentially participate in drought response or *GmERs* form a heterodimers to control the balance of cellular homeostasis.

Conclusion

Our studies identified the receptor-like kinase ER homologs in numerous plant species by sequence BLAST. Four ER homologous genes were found in soybean genome and distribute on two branches on the phylogenetic tree. All the *GmERs* showed more sequence similarity to *Arabidopsis* ER but less to *Arabidopsis* ERL1 and ERL2. Expression patterns of *GmERs* were gene- and tissue-specific and differentially regulated by dehydration. Future studies will focus on elucidating on the molecular mechanisms of the four soybean paralogs differentially function in plant development and drought tolerance.

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