INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
17–0342/2017/19–6–1497–1504
DOI: 10.17957/IJAB/15.0449
http://www.fspublishers.org

Full Length Article

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

Junbo Du[1,2,3,4*†], Xin Sun[1,2,3,4,†], Mengyuan Sun[1,2,3], Hengke Jiang[1], Yan Li[1,2,3], Chuanyin Wan[1], George Bawa[1,2], Runsheng Wu[1], Kai Shu[1,2,3,4], Jiang Liu[1,2,3,4], Weiguo Liu[1,2,3,4], Feng Yang[1,2,3,4], Taiwen Yong[1,2,3,4], Xiaochun Wang[1,2,3,4], Shu Yuan[5], Zhuang Li[1,4] and Wenyu Yang[1,2,3*]

[1]*College of Agronomy, Sichuan Agricultural University, Chengdu 611130, P.R. China*
[2]*Sichuan Engineering Research Center for Crop Strip Intercropping system, Sichuan Agricultural University, Chengdu 611130, P.R. China*
[3]*Key Laboratory of Crop Eco-physiology and Farming System in Southwest China (Ministry of Agriculture), Sichuan Agricultural University, Chengdu 611130, P.R. China*
[4]*Joint International Research Laboratory of Crop Resources and Genetic Improvement, Ministry of Education, Sichuan Agricultural University, Chengdu 611130, Sichuan, China*
[5]*College of Resources, Sichuan Agricultural University, Chengdu 611130, P.R. China*
[*]For correspondence: junbodu@hotmail.com; mssiyangwy@sicau.edu.cn
[†]These authors contributed equally to this work

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.

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

To cite this paper: Du, J., X. Sun, M. Sun, H. Jiang, Y. Li, C Wan, G. Bawa, R. Wu, K. Shu, J. Liu, W. Liu, F. Yang, T. Yong, X. Wang, S. Yuan, Z. Li and W. Yang, 2017. Identification and expression analysis of *ERECTA* homologous genes in *Glycine max*. *Int. J. Agric. Biol*., 19: 1497–1504

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 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 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 apexes 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: 95ºC for 5 min, 95ºC for 15 s, 50ºC for 30 s, 72ºC 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 95ºC for 3 min, 95ºC for 15 s, 55ºC for 15 s, 72ºC for 20 s, go to step 2 for 39 more cycles. Then increment of 0.5ºC from 65ºC to 95ºC for 5 s was used for melt curve analysis. ΔΔCq 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 *Agrobacteria 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	TGACTTTTAAATAAACGTAGT	
GmERb-qF	AGAAAAGAAAGAGGGAACGGTAAAG	
GmERb-qR	CTTGTGAGAGAGAAGGAGGGAGTT	
GmERc-qF	GAATACTAGTCTCAAACAAAACT	
GmERc-qR	CTCAACAACCACCATCATCAAGC	
GmERd-qF	AGTAGGAGGGAAGCAAATACTATTG	
GmERd-qR	AGATAACTGATCCAAAGCCAGCTA	
GmACT3-qF	GGCTGGATTTGCTGGAGAT	
GmACT3-qR	ATCCTTTTGCCCCATTCC	
GmERap-PB1-3	AAAAAGCAGGCTTCCAGACTCTTCCAGGTTACCCACGGGA	Primers for promoter amplification.
GmERap-PB2	AGAAAGCTGGGTCAGTCAACAACAACAACAACAGC	
GmERbp-PB1	AAAAAGCAGGCTTCTTGTTACCAGACTTGTACCGAAC	
GmERbp-PB2	AGAAAGCTGGGTCAGCCAACAACAACAACAACCG	
GmERcp-PB1	AAAAAGCAGGCTTCAGCATCAACAAATCATTCCAGC	
GmERcp-PB2	AGAAAGCTGGGTCAGCTGCAACTGCAGAACTTTCAAC	
GmERdp-PB1	AAAAAGCAGGCTTCCAATGCTGTGACGCCATATT	
GmERdp-PB2	AGAAAGCTGGGTCAGCTGCAACTGATGAACTTCAAC	

(*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 extracelluar 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 *GmER*s were mostly expressed in the shoot apices, of which *GmERa* showed a highest expression level (Fig. 3). In the trifoliate 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 *GmER*s, 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.

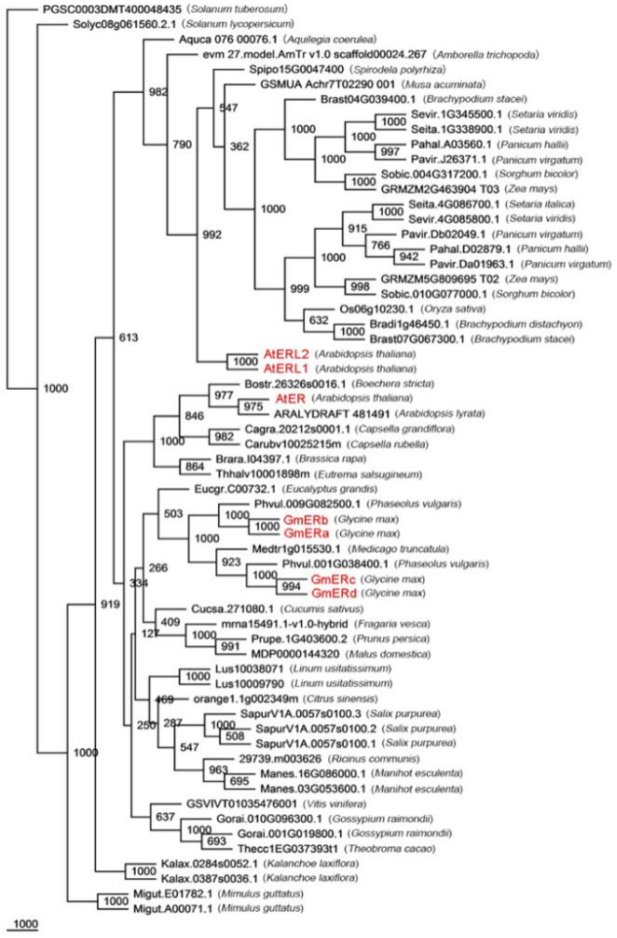

Fig. 1: Phylogenetic tree of ER homologs in plant species
The amino acid sequence of ER homologs was obtained from Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). Repetitions of 1000 were used to examine the bootstrap of the phylogenetic tree

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 *GmER*s (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).

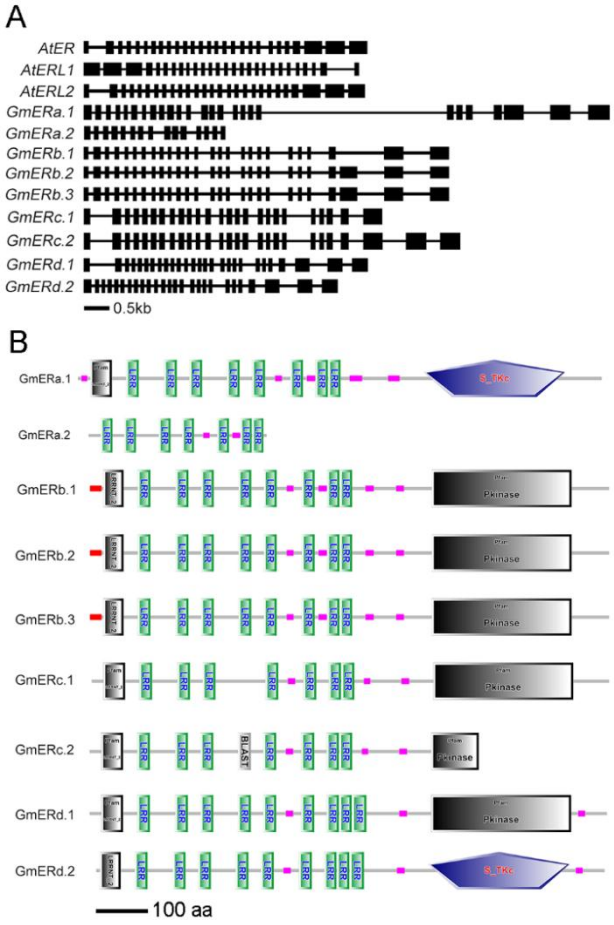

Fig. 2: Comparative analysis shows the differences of genomic DNA structures and protein domains of ER homologs

(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* paralogous genes in soybean under drought stress, we performed RT-qPCR analyses to detect the transcripts of *GmER* paralogous genes in soybean seedlings with 10% PEG6000 treatment

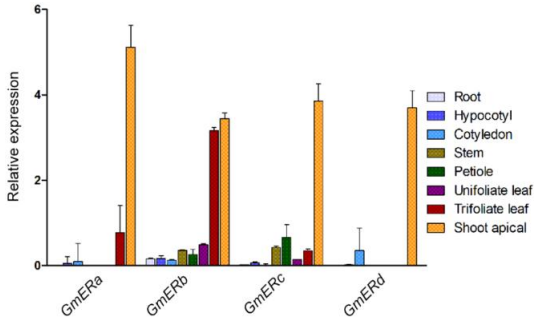

Fig. 3: Differential expression pattern of *GmER* paralogous genes in different tissues of soybean

qPCR analysis shows that of *GmER* paralogous genes are differentially expressed in different tissues of soybean

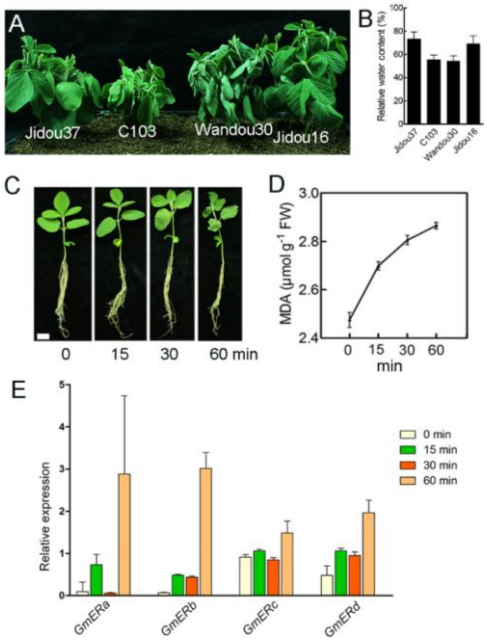

Fig. 4: Soybean *ER* paralogous genes are differentially induced by dehydration stress in drought-sensitive soybean cultivar C103

(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

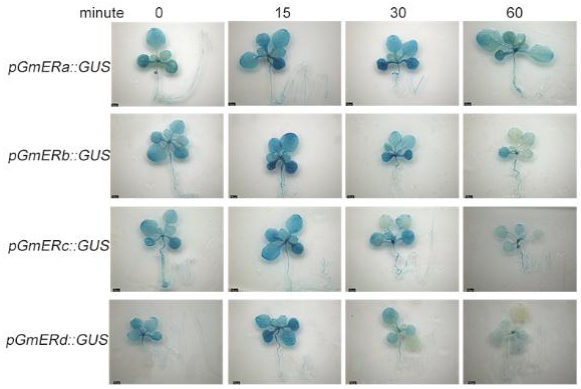

Fig. 5: Promoter::GUS analysis of soybean *ER* paralogous genes in Arabidopsis under drought stress. Scale bars represent 5 mm

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 *GmER*s 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 *GmER*s 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 *GmER*s, *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 Phvul.009G082500 in *Phaseolus vulgaris*, the other is composed of GmERc and GmERd, which is similar to Phvul.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 *GmER*s 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 *GmER*s were quickly up-regulated first and decreased later by dehydration stress in Arabidopsis. It is possible that the high transcript levels of *GmER*s 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 *GmER*s in Arabidopsis. Expression of *GmER*s is increased in soybean seedlings (Fig. 4), while expression levels of *GmER*s 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.

Acknowledgements

This work was supported by National Natural Science Foundation of China grants 31401308, 31671445 and 31371555, China Postdoctoral Science Foundation Grants 2011M501491 and 2012T50830.

References

Bihmidine, S., J. Lin, J.M. Stone, T. Awada, J.E. Specht and T.E. Clemente, 2012. Activity of the Arabidopsis RD29A and RD29B promoter elements in soybean under water stress. *Planta*, 237: 55–64

Blair, M.W., A.J. Cortés and D. This, 2016. Identification of an ERECTA gene and its drought adaptation associations with wild and cultivated common bean. *Plant Sci.*, 242: 250–259

Chinchilla, D., C. Zipfel, S. Robatzek, B. Kemmerling, T. Nurnberger and J.D.G. Jones, 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, 448: 497–500

Clark, S.E., M.P. Running and E.M. Meyerowitz, 1993. CLAVATA1, a regulator of meristem and flower development in Arabidopsis. *Development*, 119: 397–418

Clark, S.E., R.W. Williams and E.M. Meyerowitz, 1997. The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. *Cell*, 89: 575–585

Clough, S.J. and A.F. Bent, 1998. Floral dip: a simplified method forAgrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J.*, 16: 735–743

Deshmukh, R.K., H. Sonah, G. Patil, W. Chen, S. Prince, R. Mutava, T. Vuong, B. Valliyodan and H.T. Nguyen, 2014. Integrating omic approaches for abiotic stress tolerance in soybean. *Front. Plant Sci.*, 5: 244

Du, J., Y. Gao, Y. Zhan, S. Zhang, Y. Wu, Y. Xiao, B. Zou, K. He, X. Gou, G. Li, H. Lin and J. Li, 2016a. Nucleocytoplasmic trafficking is essential for BAK1- and BKK1-mediated cell-death control. *Plant J.*, 85: 520–531

Du, J., S. Yuan, Y. Chen, X. Sun, Z. Zhang, F. Xu, M. Yuan, J. Shang and H. Lin, 2011. Comparative expression analysis of dehydrins between two barley varieties, wild barley and Tibetan hulless barley associated with different stress resistance. *Acta Physiol. Plant.*, 33: 567–574

Du, J., B. Zhao, X. Sun, D. Zhang, S. Zhang and W. Yang, 2016b. Identification and characterization of multiple intermediate alleles of the key genes regulating brassinosteroid biosynthesis pathways. *Front. Plant Sci.*, 7: 1893

Farooq, M., A.A. Bajwa, S.A. Cheema and Z.A. Cheema, 2013. Application of allelopathy in crop production. *Int. J. Agric. Biol.*, 15: 1367–1378

Gou, X., H. Yin, K. He, J. Du, J. Yi, S. Xu, H. Lin, S.D. Clouse and J. Li, 2012. Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. *PLoS Genet.*, 8: e1002452

Gou, X.P., K. He, H. Yang, T. Yuan, H.H. Lin and S.D. Clouse, 2010. Genome-wide cloning and sequence analysis of leucine-rich repeat receptor-like protein kinase genes in *Arabidopsis thaliana*. *BMC Genom*ics, 11: 19

He, K., X. Gou, T. Yuan, H. Lin, T. Asami, S. Yoshida, S.D. Russell and J. Li, 2007a. BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. *Curr. Biol.*, 17: 1109–1115

He, K., X.P. Gou, T. Yuan, H.H. Lin, T. Asami and S. Yoshida, 2007b. BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid independent cell-death pathways. *Curr. Biol.*, 17: 1109

Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Aljuburi, R. Somasundaram and R. Panneerselvam, 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.*, 11: 100–105

Ku, Y.S., W.K. Au-Yeung, Y.L. Yung, M.W. Li, C.Q. Wen, X. Liu and H.M. Lam, 2013. *Drought Stress and Tolerance in Soybean. A Comprehensive Survey of International Soybean Research - Genetics, Physiology, Agronomy and Nitrogen Relationships*", James E. Board, InTech, Rijeka, Croatia

Kunert, K.J., B.J. Vorster, B.A. Fenta, T. Kibido, G. Dionisio and C.H. Foyer, 2016. Drought stress responses in soybean roots and nodules. *Front. Plant Sci.*, 7: 1015

Lehti-Shiu, M.D. and S-H. Shiu, 2012. Diversity, classification and function of the plant protein kinase superfamily. *Phil. Trans. Royal Soc. B.*, 367: 2619–2639

Li, J., 2010. Multi-tasking of somatic embryogenesis receptor-like protein kinases. *Curr. Opin. Plant Biol.*, 13: 509–514

Li, J., J. Wen, K.A. Lease, J.T. Doke, F.E. Tax and J.C. Walker, 2002. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell*, 110: 213–222

Liu, X., T. Rahman, C. Song, B. Su, F. Yang, T. Yong, Y. Wu, C. Zhang and W. Yang, 2017. Changes in light environment, morphology, growth and yield of soybean in maize-soybean intercropping systems. *Fied Crops Res.*, 200: 38–46

Marshall, A., R.B. Aalen, D. Audenaert, T. Beeckman, M.R. Broadley, M.A. Butenko, A.I. Caño-Delgado, S. de Vries, T. Dresselhaus, G. Felix, N.S. Graham, J. Foulkes, C. Granier, T. Greb, U. Grossniklaus, J.P. Hammond, R. Heidstra, C. Hodgman, M. Hothorn, D. Inzé, L. Østergaard, E. Russinova, R. Simon, A. Skirycz, Y. Stahl, C. Zipfel and I. De Smet, 2012. Tackling Drought Stress: RECEPTOR-LIKE KINASES Present New Approaches. *Plant Cell*, 24: 2262–2278

Masle, J., S.R. Gilmore and G.D. Farquhar, 2005. The ERECTA gene regulates plant transpiration efficiency in Arabidopsis. *Nature*, 436: 866–870

Meng, Y., F. Chen, H. Shuai, X. Luo, J. Ding, S. Tang, S. Xu, J. Liu, W. Liu, J. Du, J. Liu, F. Yang, X. Sun, T. Yong, X. Wang, Y. Feng, K. Shu and W. Yang, 2016. Karrikins delay soybean seed germination by mediating abscisic acid and gibberellin biogenesis under shaded conditions. *Sci. Rep.*, 6: 22073

Nakayama, N., H. Saneoka, R.E.A. Moghaieb, G.S. Premachandra and K. Fujita, 2007. Response of growth, photosynthetic gas exchange, translocation of 13C-labelled photosynthate and N accumulation in two soybean (*Glycine max* L.) *Merrill)* cultivars to drought stress. *Int. J. Agric. Biol*., 9: 669–674

Nam, K.H. and J. Li, 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell*, 110: 203–212

Rahman, T., X. Liu, S. Hussain, S. Ahmed, G. Chen, F. Yang, L. Chen, J. Du, W. Liu and W. Yang, 2017. Water use efficiency and evapotranspiration in maize-soybean relay strip intercrop systems as affected by planting geometries. *PLoS ONE*, 12: e0178332

Roux, M., B. Schwessinger, C. Albrecht, D. Chinchilla, A. Jones, N. Holton, F.G. Malinovsky, M. Tör, S. de Vries and C. Zipfel, 2011. The Arabidopsis leucine-rich repeat receptor–like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell*, 23: 2440–2455

Schmutz, J., S.B. Cannon, J. Schlueter, J. Ma, T. Mitros, W. Nelson, D.L. Hyten, Q. Song, J.J. Thelen, J. Cheng, D. Xu, U. Hellsten, G.D. May, Y. Yu, T. Sakurai, T. Umezawa, M.K. Bhattacharyya, D. Sandhu, B. Valliyodan, E. Lindquist, M. Peto, D. Grant, S. Shu, D. Goodstein, K. Barry, M. Futrell-Griggs, B. Abernathy, J. Du, Z. Tian, L. Zhu, N. Gill, T. Joshi, M. Libault, A. Sethuraman, X-C. Zhang, K. Shinozaki, H.T. Nguyen, R.A. Wing, P. Cregan, J. Specht, J. Grimwood, D. Rokhsar, G. Stacey, R.C. Shoemaker and S.A. Jackson, 2010. Genome sequence of the palaeopolyploid soybean. *Nature*, 463: 178–183

Searle, I.R., A.E. Men, T.S. Laniya, D.M. Buzas, I. Iturbe-Ormaetxe, B.J. Carroll and P.M. Gresshoff, 2003. Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science*, 299: 109–112

Seo, J.S., H.B. Sohn, K. Noh, C. Jung, J.H. An, C.M. Donovan, D.A. Somers, D.I. Kim, S-C. Jeong, C-G. Kim, H.M. Kim, S-H. Lee, Y.D. Choi, T.W. Moon, C.H. Kim and J-J. Cheong, 2011. Expression of the Arabidopsis AtMYB44 gene confers drought/salt-stress tolerance in transgenic soybean. *Mol. Breed.*, 29: 601–608

Shen, H., X. Zhong, F. Zhao, Y. Wang, B. Yan, Q. Li, G. Chen, B. Mao, J. Wang, Y. Li, G. Xiao, Y. He, H. Xiao, J. Li and Z. He, 2015. Overexpression of receptor-like kinase ERECTA improves thermotolerance in rice and tomato. *Nat Biotech.*, 33: 996–1003

Shiu, S.H., W.M. Karlowski, R. Pan, Y.H. Tzeng, K.F.X. Mayer and W.H. Li, 2004. Comparative analysis of the receptor-like kinase family in arabidopsis and rice. *Plant Cell*, 16: 1220–1234

Shiu, S.H. and A.B. Bleecker, 2001. Plant Receptor-Like Kinase Gene Family: Diversity, Function and Signaling. *Sci. STKE* 2001: re22

Shpak, E.D., 2013. Diverse roles of ERECTA family genes in plant development. *J. Integr. Plant Biol.*, 55: 1238–1250

Shpak, E.D., C.T. Berthiaume, E.J. Hill and K.U. Torii, 2004. Synergistic interaction of three ERECTA-family receptor-like kinases controls Arabidopsis organ growth and flower development by promoting cell proliferation. *Development*, 131: 1491–1501

Shpak, E.D., M.B. Lakeman and K.U. Torii, 2003. Dominant-Negative Receptor Uncovers Redundancy in the Arabidopsis ERECTA Leucine-Rich Repeat Receptor–Like Kinase Signaling Pathway That Regulates Organ Shape. *Plant Cell*, 15: 1095–1110

Shpak, E.D., J.M. McAbee, L.J. Pillitteri and K.U. Torii, 2005. Stomatal Patterning and Differentiation by Synergistic Interactions of Receptor Kinases. *Science*, 309: 290–293

Sun, X., T. Lei, J. Du and W. Yang, 2015. Identification and characterization of two paralogous plastid terminal oxidase genes in soybean. *Int. J. Agric. Biol.*, 17: 477–482

Sun, X., M. Sun, X. Luo, X. Ding, W. Ji, H. Cai, X. Bai, X. Liu and Y. Zhu, 2013. A Glycine soja ABA-responsive receptor-like cytoplasmic kinase, GsRLCK, positively controls plant tolerance to salt and drought stresses. *Planta*, 237: 1527–1545

Tameshige, T., S. Ikematsu, K.U. Torii and N. Uchida, 2017. Stem development through vascular tissues: EPFL–ERECTA family signaling that bounces in and out of phloem. *J. Exp. Bot.*, 68: 45–53

Torii, K.U., N. Mitsukawa, T. Oosumi, Y. Matsuura, R. Yokoyama and R.F. Whittier, 1996. The Arabidopsis ERECTA gene encodes a putative receptor protein kinase with extracellular leucine-rich repeats. *Plant Cell*, 8: 735

Van Zanten, M., L.B. Snoek, M.C. Proveniers and A.J. Peeters, 2009. The many functions of ERECTA. *Trends Plant Sci.*, 14: 214

Villagarcia, H., A.C. Morin, E.D. Shpak and M.V. Khodakovskaya, 2012. Modification of tomato growth by expression of truncated ERECTA protein from Arabidopsis thaliana. *J. Exp. Bot.*, 63: 6493–6504

Walker, J.C. and R. Zhang, 1990. Relationship of a putative receptor protein kinase from maize to the S-locus glycoproteins of Brassica. *Nature*, 345: 743–746

Wang, X., U. Kota, K. He, K. Blackburn, J. Li, M.B. Goshe, S.C. Huber and S.D. Clouse, 2008. Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Dev. Cell*, 15: 220–235

Wu, Y., Q. Xun, Y. Guo, J. Zhang, K. Cheng, T. Shi, K. He, S. Hou, X. Gou and J. Li, 2016. Genome-wide expression pattern analyses of the Arabidopsis leucine-rich repeat receptor-like kinases. *Mol. Plant.*, 9: 289–300

Xue, R.G., B. Zhang and H.F. Xie, 2007. Overexpression of a NTR1 in transgenic soybean confers tolerance to water stress. *Plant Cell Tiss. Organ Cult.*, 89: 177–183

Yang, F., S. Huang, R. Gao, W. Liu, T. Yong, X. Wang, X. Wu and W. Yang, 2014. Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red:far-red ratio. *Fied Crops Res.*, 155: 245–253

Yang, F., X. Wang, D. Liao, F. Lu, R. Gao, W. Liu, T. Yong, X. Wu, J. Du, J. Liu and W. Yang, 2015. Yield response to different planting geometries in maize–soybean relay strip intercropping systems. *Agron. J.*, 107: 296–304

Zhou, F., Y. Guo and L.J. Qiu, 2016. Genome-wide identification and evolutionary analysis of leucine-rich repeat receptor-like protein kinase genes in soybean. *BMC Plant Biol.*, 16: 1–13

(Received 28 March 2017; Accepted 17 July 2017)