A vacuole localized β-glucosidase contributes to drought tolerance in Arabidopsis

WANG PengTao, LIU Hao, HUA HongJie, WANG Lei & SONG Chun-Peng*

State Key Laboratory of Cotton Biology, Laboratory of Plant Stress Biology, Department of Biology, Henan University, Kaifeng 475004, China

Received July 21, 2011; accepted September 1, 2011

The phytohormone abscisic acid (ABA) plays a critical role in plant growth, development, and adaptation to various stress conditions. The cellular ABA level is constantly adjusted to respond to changing physiological and environmental conditions. To date, the mechanisms for fine-tuning ABA levels remain elusive. Here, we report that BGLU10, a member of a multigene family of β-glucosidases, contributes to drought tolerance in Arabidopsis. The T-DNA insertion mutant bglu10 exhibited a drought-sensitive phenotype, characterized by an increased rate of water loss, and lower leaf temperature, β-glucosidase activity, ABA content, and expressions of ABA- and drought-responsive genes under drought stress. In contrast, lines overexpressing BGLU10 showed greater drought resistance than that of the wild-type, as shown by decreased water loss via transpiration, higher β-glucosidase activity, ABA level, and expressions of ABA- and stress-responsive genes under drought stress. Transient expression of BGLU10::GFP and γ-TIP1::RFP in mesophyll cell protoplasts showed that the BGLU10 enzyme protein was localized to the vacuole. Meanwhile, BGLU10 was expressed in various organs, and was induced by several abiotic stresses, suggesting that BGLU10 may be involved in a variety of stress responses, and that hydrolysis of ABA-GE produces free ABA in the plant stress response.

Arabidopsis, β-glucosidase, BGLU10, ABA, drought

The plant hormone abscisic acid (ABA) plays a crucial role in plant responses to several abiotic stresses such as drought, salt, and cold. It also plays roles in plant growth and development, including seed dormancy, germination, seedling growth, lateral root development, transition from the vegetative to the reproductive phase, and senescence [1–3]. Cellular ABA levels constantly fluctuate allow plants to adapt to the changing physiological and environmental conditions. In particular, there are dramatic increases in the cellular level of ABA during seed maturation and under abiotic stresses, while there is a distinct decrease in the ABA level during seed germination [4,5].

The cellular ABA level is controlled by complex de novo synthesis pathways, and hydroxylation and conjugation reactions. The de novo synthesis pathway is now well characterized in plants, especially in Arabidopsis [6]. All the steps for de novo ABA synthesis occur in plastids, except for the final two steps involving the conversion of xanthoxin to ABA, which take place in the cytoplasm [7]. The importance of de novo ABA synthesis to increase cellular ABA levels has been well established using biosynthetic mutants with a variety of physiological defects, including precocious germination, wilting, and sensitivity to environmental stresses [8]. A large body of evidence has shown that genes encoding biosynthetic enzymes are upregulated in response to physiological cues or environmental stresses, leading to an increase in de novo ABA synthesis [9,10]. On the other hand, ABA is catabolized into inactive forms by either oxidation or conjugation [6]. The oxidative pathways play a pivotal role in various physiological processes. The major oxidative pathway is catalyzed by ABA 8′-hydroxylase, which converts ABA′ C-8′-hydroxy into 8′-
hydroxy ABA (8′OH-ABA). In the subsequent step, 8′OH-ABA is spontaneously isomerized to form phaseic acid (PA), which is then converted to dihydrophaseic acid (DPA). ABA 8′-hydroxylation is a cytochrome P450 monoxygenase that is induced by ABA and dehydration stress [11]. In addition to 8′OH-ABA, 7′OH-ABA and 9′-OH-ABA were also found as minor catabolites in a variety of plant species [6]. As for the conjugation pathway, the carboxyl (at the C-1) and hydroxyl groups of ABA and its oxidative catabolites are potential targets for conjugation with glucose. Among such conjugates, ABA glucose ester (ABA-GE) is the predominant form [12]. An ABA-specific glucosyltransferase catalyzes the conjugation process [13]. The conventional view of ABA-GE, which accumulates in plant vacuoles or apoplastic spaces during aging and stress treatments [14], is that it is a physiologically inactive end-product of ABA metabolism rather than storage or transport form. However, some other reports have suggested that ABA-GE is a long-distance transport form of the hormone, providing a source of ABA for subsequent hydrolysis [15]. Furthermore, exogenously applied ABA-GE inhibited growth of hypocotyls of Arabidopsis seedlings; since ABA-GE cannot migrate passively through the plasma membrane, this result implied that exogenously applied ABA-GE may be absorbed by roots and then hydrolyzed by ABA-β-D-glucosidase, liberating free ABA. This could then potentially inhibit the growth of Arabidopsis hypocotyls [16]. In 2006, Lee et al. [17] provided direct evidence that ABA-GE can be hydrolyzed in response to stress by the β-glucosidase AtBG1, resulting in an increase in the concentration of active ABA. AtBG1 β-glucosidase is located in the endoplasmic reticulum (ER) and remains in the ER during stress responses. As mentioned above, ABA-GE is located in intracellular storage organelles (vacuoles), in xylem sap, and probably in the cell wall as well [18]. Thus, it was proposed that uncharacterized membrane-localized transporter proteins may be involved in the movement of ABA-GE into the ER.

Database searches have revealed a multigene family of β-glucosidases, which has 48 members in Arabidopsis [19]. The AtBG1 protein studied by Lee et al. [17] is BGLU18. In the present study, our results showed that BGLU10, another member of the β-glucosidase family in Arabidopsis, is localized in vacuoles and played roles in drought tolerance.

1 Materials and methods

1.1 Plant materials and growth conditions

Arabidopsis thaliana ecotype Columbia was used in this study. Seeds were sterilized and kept for 3 d at 4°C in the dark to break dormancy. The seeds were then sown on MS medium containing 0.6% agar and incubated at 22±2°C with a 16-h-light/8-h-dark photoperiod and 70% relative humidity (RH). Seedlings were transferred to soil at 7–10 d after germination and placed in a growth chamber at (22±2)°C under a 16-h-light/8-h-dark photoperiod and 70% RH, and watered every other day for normal growth conditions. For drought stress conditions, plants were grown under normal watering conditions for 20 d and then stressed by withholding irrigation for 10 d.

1.2 Isolation of bglu10 mutant

The T-DNA insertion lines for BGLU10 (At4g27830) SALK_101672 were ordered from the Arabidopsis Biological Resource Center (ABRC) (http://www.arabidopsis.org/abrc/). Homozygous mutant plants were screened by PCR amplification using gene-specific primers LBp1 (TTAACGGTCGAAGACAAAGGCC), RP (ATCCCTTATGACTTTCGCG) and vector left-border-specific primers LBb1 (GCGTGGGAACGTTTGCCTG) and LBb1 (GCGTGGGAACGTTTGCCTG). The homozygous lines were backcrossed with wild-type (WT) plants, and the F2 progeny were used for phenotype analysis.

1.3 Plasmid constructs

The gene-specific cDNA fragment of BGLU10 was amplified by PCR, and the following primer pairs were used to construct the super1300+ over-expression plasmid: forward primer CCCCTGCGATGAAACTTTACTCTCTCTTTGCTTCT (and reverse primer CCGGTCCAAGTGATGACGTTTGCCTG). The T-DNA insertion lines for Arabidopsis, BGLU10 (At4g27830) SALK_101672 were ordered from the Arabidopsis Biological Resource Center (ABRC) (http://www.arabidopsis.org/abrc/). Homozygous mutant plants were screened by PCR amplification using gene-specific primers LBp1 (TTAACGGTCGAAGACAAAGGCC), RP (ATCCCTTATGACTTTCGCG) and vector left-border-specific primers LBb1 (GCGTGGGAACGTTTGCCTG) and LBb1 (GCGTGGGAACGTTTGCCTG). The homozygous lines were backcrossed with wild-type (WT) plants, and the F2 progeny were used for phenotype analysis.

The T-DNA insertion lines for BGLU10 (At4g27830) were used as donors for plant transformation experiments. The T-DNA was introduced into Agrobacterium tumefaciens. Biologically active DNA of BGLU10 was transformed into Agrobacterium tumefaciens XHC101 (lacking Ti plasmid) by electroporation and then introduced into Arabidopsis thaliana ecotype Columbia. For plant transformation and protoplast transfection, the constructs were introduced into Agrobacterium tumefaciens.
ciens strain GV3101 and transformed by floral infiltration into WT Arabidopsis (Columbia ecotype) for gene overexpression and GUS staining assays. To observe the subcellular localization of the proteins of interest, protoplasts isolated from Arabidopsis leaves were transformed with plasmid combinations BGLU10-pHBT-GFP and γ-TIP1-pHBT-DsRed as described previously [20]. After incubation for 16 to 20 h, the fluorescence of protoplasts was detected under a laser scanning confocal microscope.

1.5 Confocal imaging for BGLU10 subcellular localization

The fluorescence of the protoplasts was observed under a laser scanning confocal microscope (FV1000, Olympus, Japan). The protoplasts were excited at 488 nm and fluorescence was detected at 500–530 nm for GFP and 650–750 nm for autofluorescence of chloroplasts. GFP was excited at 543 nm and detected at 560–630 nm, and transmitted fields were collected simultaneously for use in merged images. Magnification at 60× using an oil immersion lens was used to obtain high resolution images of cells.

1.6 Histochemical detection of GUS activity

Ten independent transgenic lines containing the BGLU10 promoter-GUS constructs were tested for GUS activity by incubation of excised tissues in GUS staining buffer (3 mmol/L 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, 0.1 mol/L sodium phosphate buffer, pH 7.0, 0.1% Triton X-100, and 8 mmol/L β-mercaptoethanol) at 37°C in the dark overnight. The staining was terminated by acidification of the staining solution with 70% ethanol solution, and samples were stored at 4°C until microscopic observations.

1.7 Semi-quantitative RT-PCR and real-time quantitative RT-PCR

Plants were grown in chamber for 15 d at 22±2°C under a 16-h-light/8-h-dark photoperiod and 70% RH, and watered every other day for normal growth conditions as control. For drought stress treatments, plants were grown under well-watered conditions (22±2°C, 60%–70% RH, 16 h photoperiod, plants watered every other day). Drought stress was initiated by withholding water for 3 d. Thermal images were obtained within the growth chamber (22±2°C, 60%–70% RH, 16 h photoperiod) using a ThermaCAM SC3000 infrared camera (FLIR System, USA). Images were analyzed using version 5.31 of the public domain image-analysis program IRWinReport.

1.8 Infrared thermography imaging

Thermal imaging of drought-stressed plantlets was performed as described previously [21]. Briefly, plantlets were first grown under well-watered conditions (22±2°C, 60%–70% RH, 16 h photoperiod, plants watered every other day). Drought stress was initiated by withholding water for 3 d. Thermal images were obtained within the growth chamber (22±2°C, 60%–70% RH, 16 h photoperiod) using a ThermaCAM SC3000 infrared camera (FLIR System, USA). Images were analyzed using version 5.31 of the public domain image-analysis program IRWinReport.

1.9 Determination of β-glucosidase activity

Plants of the WT, bglu10, and BGLU10-OE lines were grown under normal watering conditions for 20 d or stressed by withholding water for 10 d. Then, plant tissue (2 g) of each type of plants was ground in 100 mL 0.1 mol/L (pH 6.6) phosphate buffered saline before centrifugation at 6000×g for 10 min. The supernatant was then adjusted to pH 5.0 with 0.1 mol/L HCl. The homogenate were centrifuged at 6000×g for 10 min, and supernatant stored at 4°C before use (within 24 h).

β-glucosidase activity was measured by the release of p-nitrophenol from the substrate p-nitrophenyl-β-D-glucopyranoside as described previously [18]. The standard enzyme assay contained 400 μL 100 mmol/L citrate buffer adjusted to pH 4.8 with KOH. The stock solution consisted of p-nitrophenyl-β-D-glucopyranoside dissolved in dimethylformamide (200 mg/mL). For assays, 10 μL substrate stock solution and usually 12.5 μL enzyme solution were added to the buffer. After 1 h of incubation at 37°C, the reaction was terminated by addition of 1 mL 200 mmol/L Na2CO3 solution. The amount of liberated p-nitrophenol was quantified spectrophotometrically at 405 nm using the molar extinction coefficient, ε=18300 ΔAbs×1× (mol cm)−1.

1.10 Measurement of endogenous ABA by gas chromatography-mass spectrometry

The level of endogenous ABA was quantified by gas chromatography-mass spectrometry (GC-MS) as described previously [22–26]. Plants were immediately frozen in liquid nitrogen. Frozen samples were lyophilized and stored at −70°C until extraction. Plant samples were extracted with 30 mL of extraction solution (95% isopropanol: 5% glacial
acetic acid). The filtrate was concentrated using a rotary evaporator. The residue was dissolved in 4 mL 1 mol/L NaOH solution, and then washed 3 times with 3 mL methylene chloride to remove lipophilic materials. The aqueous phase was brought to approximately pH 3.5 with 6 mol/L HCl and partitioned three times into EtOAc. EtOAc extracts were then combined and evaporated. The dried residue was dissolved in phosphate buffer (pH 8.0) and then run through a polyvinylpolypyrrolidone (PVPP) column. The extracts were dried and methylated by adding diazomethane for GC-MS analysis on a 6890N Network GC system, equipped with a 5973 Network Mass Selective Detector (Agilent). The amount of endogenous ABA was calculated by dividing the endogenous peak area by the internal standard peak area multiplied by amount of internal standard divided by weight.

2 Results

2.1 Deficiency of BGLU10 results in drought-sensitive of Arabidopsis

In 2006, Lee et al. [17] were the first to show that the cleavage of glucose-conjugated ABA by an ABA-specific β-glucosidase, AtBG1, produced bioactive ABA in response to dehydration stress. BGLU10, which was studied in this work, belongs to the multigene family of β-glucosidases in Arabidopsis [19]. To examine the role of BGLU10 in the response of plants to drought stress, we identified a mutant Arabidopsis line, bglu10, from the SALK collection [27]. A T-DNA insertion in the BGLU10 gene (At4g27830). The T-DNA is inserted in the third exon of BGLU10 (Figure 1(a)). The insertion was expected to eliminate or strongly reduce the transcription of BGLU10, and this was confirmed by reverse transcriptase-PCR (RT-PCR) analyses (Figure 1(b)). As shown in Figure 1(c), there were no obvious differences between the mutant bglu10 and the WT when grown under normal watering conditions (16-h-light/8-h-dark photoperiod, 70% RH and watered every other day), but bglu10 was more sensitive than the WT to drought stress when irrigation was withheld (Figure 1(d)). At 10 d of drought treatment, the WT plants showed slight wilting, whereas the bglu10 plants were severely wilted or dead. Correspondingly, the leaf temperature of bglu10 plantlets was 0.6°C lower than that of the WT under drought treatment, as recorded by infrared thermography (Figure 1(e) and (g), significant difference according to the Stell-Dwass test: \( P < 0.05 \)). We then compared the rates of water loss from rosette leaves. The bglu10 mutant leaves lost water slightly faster than did WT leaves. At 200 min after leaves were cut, leaves of bglu10 and WT plants had lost water by 38% and 29%, respectively, of their initial moisture (Figure 1(h), significant difference according to the Stell-Dwass test: \( P < 0.05 \)). These temperature differences may reflect the role of BGLU10 in the regulation of the ABA content and the stomatal aperture to control transpirational water loss. These results imply that BGLU10, like AtBG1, might also contribute to drought tolerance in Arabidopsis.

2.2 Enhanced drought tolerance in plants overexpressing BGLU10

Plants typically synthesize ABA in response to drought. The ABA triggers the closing of stomata, reducing water loss and enhancing drought resistance [28]. To further test whether BGLU10 might be involved in plant drought stress responses, we generated BGLU10-OE lines in which the BGLU10 gene was upregulated by almost 30 times compared with its expression in the WT (Figure 2(a)). The bglu10 mutant, overexpression lines, and WT plants were grown in soil for 10 d under normal watering conditions, before drought stress was imposed by withholding irrigation for 5 d. In response to the drought treatment, the mutant plants began to display visible symptoms of dehydration, and WT plants also displayed dehydration symptoms with only temporary wilting. In contrast, BGLU10-OE lines showed more normal growth than the WT (Figure 2(b)). Moreover, when the bglu10 mutant, BGLU10-OE lines, and WT plants were rewatered, all leaves of the BGLU10-OE lines recovered from dehydration symptoms, whereas the recovery of WT plants was somewhat poorer than that of BGLU10-OE lines. In the bglu10 mutant, only upper leaves were able to recover from dehydration symptoms (Figure 2(c)). Furthermore, the rates of water loss in rosette leaves were consistent with the drought-resistant phenotype (Figure 2(d)). These results implied that the drought tolerance in transgenic plants overexpressing BGLU10 was increased compared with that of WT plants, and greatly increased compared with that of the mutants.

2.3 Contribution of BGLU10 to drought tolerance

Endogenous ABA levels increase dramatically in response to drought stress in plants, and regulate a complicated gene network system to increase resistance to stresses [9,29]. To further confirm the function of BGLU10 in drought responses, we examined total β-glucosidase activity under normal growth conditions or drought stress in the bglu10 mutant, BGLU10-OE lines, and WT plants. The total β-glucosidase activity was greater under drought stress conditions than under normal growth conditions in all plants, but in terms of overall activity, the BGLU10-OE lines showed the highest activity, and the mutant line bglu10 showed the lowest (Figure 3(a), significant difference between each pair of lines according to the Steel-Dwass test: \( P < 0.05 \)). Consistent with the results of the β-glucosidase assay, the ABA level showed similar trends under stressed and non-stressed conditions in the bglu10 mutant, BGLU10-OE lines and WT plants (Figure 3(b)). Previous studies
Figure 1 Deficiency of BGLU10 results in drought-sensitive plants. (a) Insertion positions of T-DNA in the BGLU10 gene; (b) reverse transcriptase PCR analysis of BGLU10 expression. Actin2 served as the control; (c) plants were grown under normal watering conditions for 30 d after transfer of seedlings to soil at 10 d after germination; (d) plants were grown under normal watering conditions for 20 d, and then stressed by completely withholding water for 10 d; (e) false-color infrared images of drought-stressed plantlets. Thermal images show leaf temperature profiles of 14-d-old WT and bglu10 plantlets after drought treatment for 3 d; (f) photograph shows seedlings in (e); (g) temperature of the leaf surface in bglu10 mutant and WT quantified by infrared thermal imaging. Data are means±SD (data are from ~40 measurements of square pixels from each plant, 2 central pixels each leaf); (h) transpirational water loss in WT and bglu10 lines at indicated time points after detachment. Water loss is shown as a percentage of initial fresh weight (FW). Values are means±SD of 4 samples (each sample had 6 leaves).

Figure 2 Enhanced drought stress tolerance in plants overexpressing BGLU10. (a) Real-time quantitative PCR analyses of expression levels of BGLU10 in BGLU10-OE and WT plants; (b) dehydration symptoms of bglu10 mutant, BGLU10-OE lines, and WT plants after withholding water for 5 d; (c) recovery of bglu10 mutant, BGLU10-OE lines, and WT plants after rewatering in (b); (d) transpirational water loss in bglu10 mutant, BGLU10-OE lines, and WT plants at indicated time points after detachment. Water loss is expressed as a percentage of initial fresh weight (FW). Values are means±SD of 4 samples (each sample had 6 leaves).
showed that the expression of \textit{ATHB6} and \textit{RD22} were induced by ABA and drought [30–32]. To determine whether the expressions of ABA- and drought-responsive genes were affected by the \textit{BGLU10} and ABA levels, we used real-time PCR analysis to compare expressions of stress-induced genes among the WT, \textit{BGLU10-OE} lines, and mutants. The results showed that the steady state levels of both \textit{ATHB6} and \textit{RD22} gene transcripts were lower in the mutant than in the WT, whereas greater expressions were observed in \textit{BGLU10-OE} lines than in WT and \textit{bglu10} (Figure 3 (c) and (d)). These findings suggest that \textit{BGLU10} may have a general role in regulating the ABA content and ABA-mediated stress gene expression in plants.

2.4 \textit{BGLU10} is localized in the vacuole

Previously, it was predicted that \textit{BGLU10} might be localized in vacuole based on the potential signal sequences in the deduced precursor proteins, as determined using SignalP V2.0 [19]. We investigated the subcellular localization of \textit{BGLU10} by transient gene expression analysis in mesophyll protoplasts of \textit{Arabidopsis} with the \textit{BGLU10-pHBT-GFP} plasmid. The green fluorescence of the \textit{BGLU10::GFP} fusion protein only appeared in compartments of the protoplast that lacked chloroplasts (Figure 4 (a3)). To further examine whether the localization of \textit{BGLU10::GFP} is vacuolar or not, a tonoplast intrinsic protein \(\gamma\)-TIP1 was chosen as a fluorescent reporter protein for the vacuole [33], and a \(\gamma\)-TIP1::pHBT-DsRed vector was constructed for use in co-localization imaging analyses. The green fluorescence of the \textit{BGLU10::GFP} fusion protein tightly co-localized with the red fluorescence of the \(\gamma\)-TIP1::DsRed fusion protein (Figure 4 (b3)–(e3)). Meanwhile, only the green fluorescent protein was localized throughout the cells when the protoplasts were transformed with the pHBT-GFP vector control (Figure 4 (a1)–(d1)). Similarly, only the red fluorescent protein was localized throughout the cells when protoplasts were transformed with the pHBT-DsRed vector control (Figure 4 (a2)–(d2)). This result further indicated that \textit{BGLU10} is localized in the vacuole. This pattern of locali-
zation suggests that ABA-GE stored in the vacuole could be hydrolyzed to release bioactive free ABA, thus increasing concentrations of active ABA.

2.5 \textit{BGLU10} is induced by different abiotic stresses, and expressed in various tissues

We conducted detailed analyses of \textit{BGLU10} expression by fusing the \textit{BGLU10} putative promoter region to the \(\beta\)-glucuronidase (GUS) reporter. GUS staining analysis of organ-specific expression of \textit{BGLU10} showed that this gene was transcribed in various organs, including roots, stems, leaves, flowers, and siliques, especially in mature conducting tissues (Figure 5(a)). RT-PCR analysis of the mRNAs in the detached organs of roots, leaves, stems, and flowers confirmed the results of GUS staining (Figure 5(b)). In addition, \textit{BGLU10} was induced by ABA, salt, and drought stresses (Figure 5(c)), suggesting that the \(\beta\)-glucosidase \textit{BGLU10} may be involved in variety of stresses and in the hydrolysis of ABA-GE, producing free ABA during plant stress responses.

3 Discussion

The hormone abscisic acid (ABA) regulates the development and survival of plants in response to environmental stresses. The level of ABA in plant tissues is not only controlled via its biosynthetic rate, but also by the rate of its catabolism [6]. It has long been thought that ABA-GE is the only non-active storage form of ABA [34]. In 2006, Lee et al. [17] first reported that the T-DNA insertion mutant \textit{atbg1}, a member of \(\beta\)-glucosidase family in \textit{Arabidopsis}, was sensitive to drought stress, and exogenously applied ABA
partly restored the normal phenotype. This work indicated that there is another way of producing bioactive ABA in response to dehydration stress in plants.

In this study, our data provide genetic evidence that BGLU10 also plays a significant role in drought resistance in Arabidopsis. The T-DNA insertion homozygous mutant bglu10 showed faster wilting and death than the WT under drought stress (Figure 1(c) and (d)). Infrared thermography imaging analysis (Figure 1(e)) showed that stomata of bglu10 tended to retain a larger aperture than those of the WT, which led to greater water loss (Figure 1(g) and (h)). By contrast, the lines overexpressing BGLU10 were more drought tolerant than the WT (Figure 2(b)–(d)). Determination of ABA content in plants under both normal watering and drought conditions showed that BGLU10-OE lines contained higher ABA levels than the WT, but the ABA content in bglu10 was slightly lower than in the WT (Figure 3(b)). Research has established that the ABA-mediated response to water deficit and osmotic stress conditions is associated with the transcriptional up-regulation of specific genes. Transcripts of ATHB6 are upregulated in response to limited water supply, osmotic stress, and exogenous ABA [35]. The expression of RD22, a dehydration-responsive gene, was induced by the application of exogenous ABA and drought stress in Arabidopsis [30,36]. In the present work, the expressions of the marker genes ATHB6 and RD22 were induced by dehydration stress; their expressions were highest in BGLU10-OE lines and lowest in the bglu10 mutant (Figure 3(c) and (d)). Furthermore, the total β-glucosidase activity was positively correlated with drought resistance in the bglu10 mutant, WT, and BGLU10-OE plants (Figure 3(a)). In addition, BGLU10 was transcribed in various organs, including roots, stems, leaves, flowers, and siliques, especially in mature conducting tissues (Figure 5(a) and (b)), and was induced by ABA, salt, and drought (Figure 5(c)). These results are also helpful to explain the relationship between ABA content and drought tolerance, and suggest that BGLU10, like ATBG1, may function to hydrolyze ABA-GE and release free ABA to enhance the stress tolerance of plants.

In plants, ABA-GE is located in intracellular storage organelles (vacuoles), in xylem sap, and probably in the cytosol and cell wall as well [18]. Lee et al. [17] showed that the AtBG1 β-glucosidase was located in the endoplasmic reticulum (ER) and remained in the ER during stress responses. Thus, the authors hypothesized that dehydration stress acts as a signal to transport ABA-GE stored in the vacuole or apoplastic space to the ER. However, the transport systems involved in the movement of ABA-GE have not been identified yet. By using fluorescence co-localization of BGLU10-GFP and γ-TIP1-RFP transiently expressed in mesophyll protoplasts, we observed that BGLU10 was localized in the vacuole (Figure 4 (a3) – (e3)). This result validates the predictions based on bioinformatics analyses in previous reports [19]. More importantly, the vacuolar localization of BGLU10 suggests that it is in a position to hydrolyze ABA-GE and produce free ABA directly, without transportation. Admittedly, our results raise some new questions; for example, is ABA-GE constantly hydrolyzed to release free ABA within the vacuole, or is it rapidly hydrolyzed at particular growth stages or under specific stress conditions? What is the signal that triggers the enzymatic function of BGLU10? In addition, it will be interesting to determine the mechanism by which BGLU10 is activated under abiotic stress conditions.

We thank Professor XIAO LiangTao of Hunan Agricultural University for help with ABA analyses. This work was supported by the National Natural Science Foundation of China (90817106, 31170253 and 30800075).

1 Finkelstein R R, Gampala S S L, Rock C D. Abscisic acid signaling in seeds and seedlings. Plant Cell, 2002, 14(Suppl): S15–S45
2 Christmann A, Moes D, Himmelbach A, et al. Integration of abscisic acid signalling into plant responses. Plant Biol (Stuttgart), 2006, 8: 314–325
3 Seki M, Umezawa T, Urano K, et al. Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol, 2007, 10: 296–302
4 Ali-Rachedi S, Boinot D, Wagner M H, et al. Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of Arabidopsis thaliana. Planta, 2004, 219: 479–499
5 Chiwocha S D, Cutler A J, Abrams S R, et al. The etr1-2 mutation in Arabidopsis thaliana affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. Plant J, 2005, 42: 35–48
6 Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol, 2005, 56: 165–185
7 Wasilewska A, Vlad F, Sirichandra C, et al. An update on abscisic acid signaling in plants and more.... Mol Plant, 2008, 1: 198–217
Hey S I, Byrne E, Halford N G. The interface between metabolic and stress signalling. Ann Bot, 2010, 105: 197–203

Shinozaki K, Yamaguchi-Shinozaki K. Gene networks involved in drought stress response and tolerance. J Exp Bot, 2007, 58: 221–227

Urano K, Maruyama K, Ogata Y, et al. Characterization of the ABA-regulated global responses to dehydration in Arabidopsis by metabolomics. Plant J, 2009, 57: 1065–1078

Mezawura T, Okamoto M, Kishiro T, et al. CYP707A3, a major ABA 8'-hydroxylase involved in dehydration and rehydration response in Arabidopsis thaliana. Plant J, 2006, 46: 171–182

Cutler A J, Krochko J E. Formation and breakdown of ABA. Trends Plant Sci, 1999, 4: 472–478

Priest D M, Ambrose S J, Vaistij F E, et al. Use of the glucosyltransferase UGT71B6 to disturb abscisic acid homeostasis in Arabidopsis thaliana. Planta, 1986, 168: 559–562

Lehmann H, Glund K. Abscisic acid metabolism-vascular/extravascular distribution of metabolites. Planta, 1986, 168: 559–562

Sauter A, Dietz K J, Hartung W. A possible stress physiological role of abscisic acid conjugates in root-to-shoot signalling. Plant Cell Environ, 2002, 25: 223–228

Kato-Noguchi H, Tanaka Y. Effect of ABA-beta-D-glucopyranosyl ester and activity of ABA-beta-D-glucosidase in Arabidopsis thaliana. J Plant Physiol, 2008, 165: 788–790

Lee K H, Piao H L, Kim H Y, et al. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. Cell, 2006, 126: 1109–1120

Dietz K J, Sauter A, Wichert K, et al. Extracellular β-glucosidase activity in barley involved in the hydrolysis of ABA glucose conjugate in leaves. J Exp Bot, 2000, 51: 937–944

Xu Z, Escamilla-Treviño L, Zeng L, et al. Characterization of the ABA-beta-D-glucosidase activity. Plant J, 2007, 54: 343–367

Yoo S D, Cho Y H, Sheen J. Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat Protoc, 2007, 2: 1565–1572

Merlot S, Mustilli A C, Genty B, et al. Use of infrared thermal imaging to isolate Arabidopsis mutants defective in stomatal regulation. Plant J, 2002, 30: 601–609

Browning G, Wignall T A. Identification and quantitation of indole-3-acetic and abscisic acids in the cambial region of Quercus robur by combined gas chromatography-mass spectrometry. Tree Physiol, 1987, 5: 235–246

Qi Q, Rose P A, Abrams G D, et al. (+)-Abscisic acid metabolism, 3-ketoacyl-coenzyme A synthase gene expression, and very-long-chain monounsaturated fatty acid biosynthesis in Brassica napus embryos. Plant Physiol, 1998, 117: 979–987

Kamboj K S, Browning G, Blake P S, et al. GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. Plant Growth Regul, 1999, 28: 21–27

Kang D J, Seo Y J, Lee J D, et al. Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. J Agron Crop Sci, 2005, 191: 273–282

Seo H S, Kim S K, Jang S W, et al. Effect of jasmonic acid on endogenous gibberellins and abscisic acid in rice under NaCl stress. Biol Plant, 2005, 49: 447–450

Alonso J M, Stepanova A N, Leisse T J, et al. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science, 2003, 301: 653–657

Iuchi S, Kobayashi M, Taji T, et al. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J, 2001, 27: 325–333

Ni F T, Chu L Y, Shao H B, et al. Gene expression and regulation of higher plants under soil water stress. Curr Genomics, 2009, 10: 269–280

Abe H, Yamaguchi-Shinozaki K, Urao T, et al. Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. Plant Cell, 1997, 9: 1859–1868

Söderman E, Hjellström M, Fahlson J, et al. The HD-Zip gene ATHB6 in Arabidopsis is expressed in developing leaves, roots and carpels and up-regulated by water deficit conditions. Plant Mol Biol, 1999, 40: 1073–1083

Uno Y, Furihata T, Abe H, Yoshida R, et al. Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependant signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA, 2000, 97: 11632–11637

Hunter P R, Craddock C P, Di Benedetto S, et al. Fluorescent reporter proteins for the tonoplast and the vacuolar lumen identify a single vacuolar compartment in Arabidopsis cells. Plant Physiol, 2007, 145: 1371–1382

Hartung W, Sauter A, Hose E. Abscisic acid in the xylem: Where does it come from, where does it go to? J Exp Bot, 2002, 53: 27–32

Deng X, Phillips J, Bräutigam A, et al. A homeodomain leucine zipper gene from Craterostigma plantagineum regulates abscisic acid responsive gene expression and physiological responses. Plant Mol Biol, 2006, 61: 469–489

Yamaguchi-Shinozaki K, Shinozaki K. The plant hormone abscisic acid mediates the drought-induced expression but not the seed-specific expression of rd22, a gene responsive to dehydration stress in Arabidopsis thaliana. Mol Gen Genet, 1993, 238: 17–25