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The Arabidopsis UDP-glycosyltransferase75B1, conjugates abscisic acid and affects plant response to abiotic stresses

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Key message **This study revealed that the Arabidopsis UGT75B1 plays an important role inmodulating ABA activity by glycosylation when confronting stress environments.**

Abstract The cellular ABA content and activity can be tightly controlled in several ways, one of which is glycosylation by family 1 UDP-glycosyltransferases (UGTs). Previous analysis has shown UGT75B1 activity towards ABA in vitro. However, the biological role of UGT75B1 remains to be elucidated. Here, we characterized the function of UGT75B1 in abiotic stress responses via ABA glycosylation. GUS assay and qRT-PCR indicated that *UGT75B1* is significantly upregulated by adverse conditions, such as osmotic stress, salinity and ABA. Overexpression of *UGT75B1* in Arabidopsis leads to higher seed germination rates and seedling greening rates upon exposure to salt and osmotic stresses. In contrast, the big *UGT75B1* overexpression plants are more sensitive under salt and osmotic stresses. Additionally, the *UGT75B1* overexpression plants showed larger stomatal aperture and more water loss under drought condition, which can be explained by lower ABA levels examined in *UGT75B1* OE plants in response to water deficit conditions. Consistently, *UGT75B1* ectopic expression leads to downregulation of many ABA-responsive genes under stress conditions, including *ABI3, ABI5* newly germinated seedlings and *RD29A*, *KIN1*, *AIL1* in big plants. In summary, our results revealed that the Arabidopsis UGT75B1 plays an important role in coping with abiotic stresses via glycosylation of ABA.

Keywords *Arabidopsis thaliana* · Abscisic acid · Glycosyltransferase · Stress response · UGT75B1

Introduction

Abscisic acid (ABA) is one of the most important phytohormones that affects many aspects of plant growth and development, including seed maturation and dormancy, seed germination, postgermination growth etc. (Finkelstein et al. 2002; Xiong and Zhu 2003). More importantly, ABA is also regarded to play a role in mediating the plant response to environmental stresses, such as cold, drought, salinity etc. via ABA-dependent signaling pathway (Hetherington 2001; Tuteja 2007). The plant development and some physiological processes are highly correlated with ABA level and activity. Plants have to adjust ABA levels constantly to cope with changing physiological and environmental conditions (Nambara and Marion-Poll 2005). For example, high level of ABA is required for seed to enter dormancy status. In tobacco, overexpression of ABA synthetic enzyme zeaxanthin epoxidase in seeds leads to increased dormancy, whereas knockout of the gene yields phenotypes that are less dormant (Frey et al. 1999). On the other hand, ABA level must be reduced upon imbibition in order for the seeds to germinate (Gubler et al. 2005). ABA production could be sensitively and rapidly regulated by stresses. Under non-stressful conditions, ABA remains at very low level, which might be required for normal plant growth (Xiong and Zhu 2003). However, when confronting stresses, ABA tends to be rapidly accumulated in vegetative tissues to mediate the plant adaptation to adverse conditions. In addition, ABA could also be degraded or deactivated soon once the stress

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is relieved, so that the plants can resume normal growth (Zhang et al. 2006).

In higher plants, the de novo biosynthesis of ABA starts from the cleavage of a C40 carotenoid, and then the product zeaxanthin is catalyzed into all-trans-violaxanthin, followed by convertion from xanthoxin intermediate to ABA via ABA aldehyde (Seo and Koshiba 2002). With the exception of *SDR1,* all the involving genes in ABA biosynthesis pathway are found to be up-regulated under drought and salinity (Thompson et al. 2000; Iuchi et al. 2001; Xiong et al. 2001). Notably, NCED gene, one of the most crucial genes in ABA biosynthesis, can be quickly induced in response to abiotic stresses in many plant species (Chernys and Zeevaart 2000; Iuchi et al. 2000, 2001). On the other hand, it is essential to balance ABA accumulation and activity in plants, which can be mediated by ABA hydroxylation and conjugation. ABA hydroxylation, catalyzed by *CYP707A* encoding ABA 8′-hydroxylase, appears to be activated by ABA accumulation and stress relief (Xiong and Zhu 2003; Kushiro et al. 2004; Saito et al. 2004). Kushiro et al. (2004) found that the mRNA levels of *CYP707A* genes were moderately induced by dehydration and rapidly upregulated by the following rehydration. ABA could also be transformed into inactive forms by glycosylation, which produces ABA glucose-ester (ABA-GE), the main conjugation form of ABA (Lim et al. 2005). Glycosylation represents a more flexible way for maintaining ABA homeostasis, since ABA-GE can be catalyzed into free ABA by glucosidases and hydrolyses in confronting abiotic stresses. AtBG1*,* a β-glucosidase that hydrolyzes ABA-GE, is also found to be induced by abiotic stresses such as salt, dehydration and cold stresses (Lee et al. 2006; Xu et al. 2012). On the whole, ABA level and activity can be delicately regulated by the biosynthesis, catabolism and modification when coping with environmental stimuli.

ABA glucosylation is catalyzed by the plant family 1 UDP-glycosyltransferases (UGTs). Till now, seven UGTs have been identified to show activities towards ABA in vitro or in planta, including UGT84B1, UGT75B1, UGT84A2, UGT71B6, UGT71B7, UGT71B8 and UGT71C5, with varied specific activities, expression profiles and biological functions (Lim et al. 2005; Dong et al. 2014; Liu et al. 2015). For example, UGT71B6 only recognizes the naturally-occurring ABA enantiomer, (+)-ABA, but not its unnatural relative (-)-ABA. This is in contrast with the other identified UGTs which accept both ABA stereoisomers as substrates (Priest et al. 2006). The roles of UGT71C5 have been characterized in planta*.* Mutation of *UGT71C5* and down-expression of *UGT71C5* in Arabidopsis lead to delayed seed germination and enhanced drought tolerance, indicating that UGT71C5 might play a major role in mediating ABA levels (Liu et al. 2015). Some of these UGTs also show tissue-specific or stress responsive expression patterns. For instance, *UGT84B1* is reported to be preferentially expressed in the endosperm (Rehman et al. 2018). *UGT71B6*, *UGT71B7* and *UGT71B8* were found to be up-regulated by ABA, NaCl and Mannitol (Dong et al. 2014). It is inferred that each UGT involved in ABA conjugation might play specific and distinct roles. It would be interesting to investigate their functions in planta to enrich the knowledge of signaling pathways involving ABA.

Although UGT75B1 was identified to glucosylate ABA in vitro, its function in planta remains to be revealed. In this study, we characterized the biological role of UGT75B1 in Arabidopsis. Firstly, we found that *UGT75B1* is significantly upregulated under salt and osmotic stress conditions, as well as ABA treatment. Overexpression of *UGT75B1* leads to overproduction of ABA-GE in Arabidopsis and declines the active ABA levels, which in turn allowed seed germination and seedling greening under salt, osmotic and ABA treatments. However, during adult stage, *UGT75B1* overexpression plants showed sensitivity to salt and drought stresses. In line with reduced ABA level, *UGT75B1* overexpression plants showed downregulated expression of ABA-responsive genes under salt and osmotic stress conditions. To conclude, our result showed that UGT75B1 is involved in modulating plant response to stresses via modifying ABA glucosylation.

Materials and methods

Plant materials

Arabidopsis thaliana ecotype Columbia-0 was used in this study. Surface sterilized seeds were sown on 1/2 MS medium supplemented with 1% sucrose and stratified at 4 °C for 3 days in the dark prior to germination. Seedlings were grown on 1/2 MS plates or soil under LD (16 h light/8 h dark) condition at 22 °C.

Plasmid construction and plant transformation

To generate vector for prokaryotic expression, the coding region of *UGT75B1* was cloned into the PGEX-2T vector fused with a glutathione-S-transferase (GST) tag. To generate the 35S::*UGT75B1* construct, the coding region of *UGT75B1* was cloned into the pBI121 binary vector driven by the CaMV 35S promoter. For generating *UGT75B1pro*::GUS fusion, the 1539 bp upstream sequence of *UGT75B1* was cloned into the pBI121 binary vector by replacing the CaMV 35S promoter, and fused with the GUS reporter gene.

All the constructs were transformed into Arabidopsis Col-0 by Agrobacterium mediated floral dip method (Clough 2005).

Determination of UGT75B1 activity and ABA-GE contents

The recombinant UGT75B1 protein was expressed in *E. coli* and purified with Glutathione SepharoseTM 4B (GE Healthcare). The glycosyltransferase activity assay was carried out with the conditions described by Hou et al. (2004). The products were analyzed by HPLC on a Shimadzu HPLC system (https://www.shimadzu.com) using a 5 µm C18 column (150 × 4.6 mm, Zorbax; Agilent, https://www.agilent.com). A linear gradient increased concentration of methanol containing 0.1% acetic acid (pH 3.5; triethylamine) from 10 to 40% (v/v) against double-distilled H_2O containing 0.1% acetic acid (pH 3.5; triethylamine) at a flow rate of 1 ml/min over 35 min was used, and the eluate was monitored at 270 nm.

The mass spectrometer operated in a positive electrospray ionization mode with 30 eV and a probe voltage of 5.0 kV. The dry heater was set to 180 °C. The data acquisition and analysis were performed with XCALIBUR 2.0.6. The methods and mobile phases were similar to the HPLC conditions, except that 0.1% formic acid was used instead of 0.1% acetic acid (pH 3.5; triethylamine).

The extraction of ABA-Glc conjugates was performed as following: in brief: 1 g of plant tissue was ground to fine powder and was extracted with 10 ml of 80% methanol. The slurry was left at room temperature for 1 h followed by centrifugation. After filtration, the supernatant was collected, concentrated in vacuo, and then dissolved in 0.1 ml methanol. The supernatant was analyzed with HPLC and LC–MS as described above.

Histochemical analysis of GUS activity

Histochemical localization of GUS activities was analyzed after the *UGT75B1pro*::GUS transgenic plants were incubated overnight at 37 °C in 1 mg/l 5-bromo-4-chloro-3-indolylglucuronic acid, 5 mM potassium ferrocyanide, 0.03% Triton X-100 and 0.1 M sodium phosphate buffer, pH 7.0. The tissues were cleaned with 70% ethanol and then observed. These GUS staining data were representative of at least five independent transgenic lines for each construct.

Seed germination and stress assays

For germination assay under stresses, seeds were surface sterilized with 70% ethanol for 1 min, 2.6% hypochlorite for 10 min, and then rinsed with sterile deionized water. Seeds were sown on MS medium supplemented with different concentrations of mannitol and ABA, and the plates were placed at 4 °C for 3 days in the dark and then transferred to the growth chamber (16 h light/8 h dark) at 22 °C. The seeds were regarded as germinated when the radicles protrude from the seed coat.

For seedling greening calculation, seeds were germinated and kept growing on 1/2 MS medium supplemented with different concentrations of ABA, NaCl and mannitol conditions, respectively. After 2 weeks, the seedlings with green cotyledons were regarded as greening and the rates were calculated.

For drought stress assays in soil, 3-week-old regularly irrigated Arabidopsis seedlings growing in soil were subjected to water deprivation for another 2 weeks and then the phenotypes were observed and photographed.

Calculation of water loss

For water loss calculation, fresh detached leaves from 3-week-old plants were weighed firstly (FW) and dried naturally. Weights of the drying leaves (DW) were taken every 20 min. Water loss was calculated with (FW − DW)/time × FW.

Measurement of stomatal closure

For measuring stomatal closure under drought stress, detached leaves were dried for 1 h on filter papers and the epidermis was peeled for stomatal aperture observing and measuring. Each sample was replicated at least three times.

Analysis of endogenous ABA contents

For turgid tissues, 4-week old plant rosettes were harvested and frozen immediately in liquid nitrogen. The wilting treatment was carried out according to Priest et al. (2006) and Liu et al. (2015). Briefly, detached rosettes were kept in the air at room temperature for 3 h and frozen in liquid nitrogen. ABA contents were measured in Metware company (www.metware.cn).

Quantitative RT-PCR

For quantitative real-time PCR, total RNAs were first extracted from various tissues according to the instructions provided with the Trizol reagent (Takara). Reverse transcription reactions were performed using 5 µg RNA with the PrimeScript RT reagent kit with gDNA Eraser (Takara), according to the supplier's manual. Real-time PCR reactions were performed with a Bio-Rad real-time thermal cycling system using SYBR-Green to detect gene expression abundances. All reactions were done using at least three replicates. Data were analyzed using Bio-Rad CFX Manager software. Primer information for qRT-PCR assay is included in supplementary Table S1.

Results

UGT75B1 is induced by abiotic stresses and ABA

To investigate the expression of UGT75B1, the promoter region of *UGT75B1* was cloned and fused to the GUS reporter gene, and the generated *UGT75B1pro::GUS* transgenic plants was subjected to histochemical staining. It is revealed that *UGT75B1* is extensively expressed in various developmental stages and tissues. We observed strong GUS staining in young or fast-growing tissues, such as germinating seeds, young seedlings and root tip (Fig. 1). As the seedlings grow older, GUS staining becomes weaker (Fig. 1). Additionally, since UGT75B1 was identified as an ABA glycosyltransferase, we exposed the *UGT75B1*pro::GUS transgenic plants to various treatments, including 100 μM ABA, 150 mM NaCl and 250 mM mannitol for 3, 6, 12, 24 h respectively, and GUS activity was gradually enhanced during the increase of the time course (Fig. 2a). To gain further verification of upregulation of *UGT75B1*, we also subjected the 2-week-old wild type (WT) Arabidopsis to the same treatments and evaluated the endogenous *UGT75B1* mRNA levels by qRT-PCR. The result confirmed the strong upregulation of *UGT75B1* by these treatments, reaching approximately fourfolds by ABA treatment, more than 15 folds by mannitol treatment for 3 h, and a similar 15 folds of upregulation by NaCl treatment for 6 h.(Fig. 2b).

UGT75B1 is involved in catalyzing glucosylation of ABA

To confirm the specific activity of UGT75B1 towards ABA, we purified the GST-UGT75B1 recombinant protein and determined its activity towards a wide variety of substrates, including plant hormones such as ABA, GA3, IBA, 6-BA, SA, SA-ME, and small molecules such as kaempferol, quercetin, naringenin, coumaric acid, and found that UGT75B1 has high activity towards ABA in vitro (Fig. 3a), and has nearly no activity towards other substrates examined in this study (Fig. S1). According to Jackson et al.'s study (2001), UGT75B1 could also conjugates IAA and some phenolic compounds in vitro. Thus, ABA is one of the endogenous substrates of UGT75B1, and it is necessary to further investigate its activity in planta. Thus, we generated transgenic Arabidopsis overexpressing *UGT75B1* driven by CaMV 35S promoter. Two transgenic lines OE1 and OE5 with high *UGT75B1* expression levels were selected for further analysis (Fig. 3b). To determine whether steady-state levels of UGT*75B1* correlates with UGT activity, soluble protein extracts of WT, *UGT75B1*OE1 and OE5 transgenic plants were assayed in vitro with the substrates ABA and UDP-glucose, and accumulation of the products were measured. It is found that the protein extracted from OE1 and OE5 has relatively higher ABA-catalyzing activity than that from WT (Fig. 3c). Then the reaction products of UGT75B1 in catalyzing ABA were analyzed by LC–MS, and the corresponding ABA glucose-ester (ABA-GE) was identified (Fig. 3d). Additionally, to analyze whether endogenous ABA-GE is accumulated upon *UGT75B1* overexpression, the 3-week-old WT, *UGT75B1* OE1 and OE5 plants were subjected to ABA treatment for 12 h, and ABA-GE was extracted and determined by LC–MS. The result showed that the endogenous ABA-GE levels were higher in *UGT75B1 OE1* and *OE5* plants compared with that of WT (Fig. S2), which further verified the glucosylation activity of UGT75B1 towards ABA in planta.

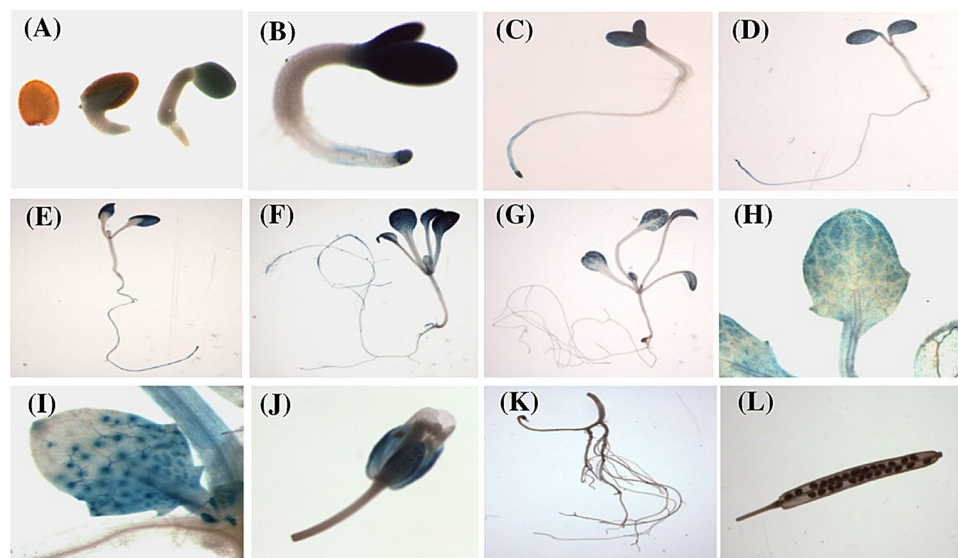

Fig. 1 Spatio-temporal expression of *UGT75B1* in Arabidopsis evaluated by staining *UGT75B1*pro::GUS plants. **a** Germinating seeds; **b** 2-day-old seedling; **c** 3-day-old seedling; **d** 5-day-old seedling; **e** 7-day-old seedling; **f** 14-day-old seedling; **g** 21-day-old seedling; **h** rossete leaf; **i** new born leaf; **j** inflorescence; **k** root; **l** silique

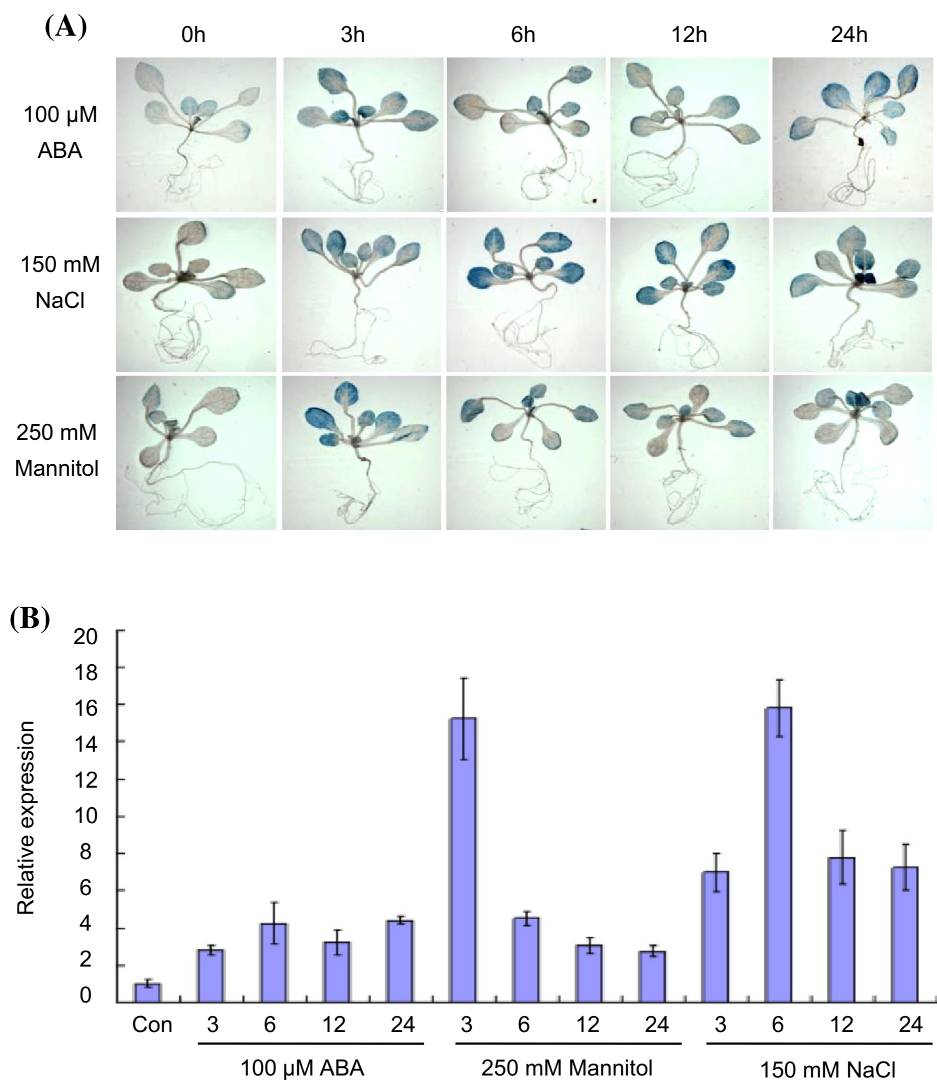

Fig. 2 *UGT75B1* is induced by NaCl, mannitol and Abscisic acid. **a** For GUS staining, 14-day-old *UGT75B1*pro::GUS seedlings were subjected to the same treatments and were stained with X-Gluc for GUS expression analysis. **b** For qRT-PCR, 14-day-old Arabidopsis seedlings were subjected to NaCl, mannitol and Abscisic acid treatment for 3 h, 6 h, 12, 24 h, *UGT75B1* mRNA levels were assessed

UGT75B1 overexpression promoted seed germination and postgermination growth under abiotic stress conditions

In light of substantial upregulation of *UGT75B1* by abiotic stress and ABA treatments (Fig. 2), next we evaluated the performances of *UGT75B1* overexpression plants under NaCl, mannitol and ABA. Seeds of the wild type, two *UGT75B1* overexpression lines OE1 and OE5 were germinated on MS medium supplemented with different concentrations of NaCl, mannitol and ABA, and the germination rates was calculated in the following seven days. We clearly saw that the two *UGT75B1* overexpression plants showed higher germination rates than WT plants under any treatments, and especially, more discrepancies were seen under higher concentrations of NaCl, mannitol and ABA, while they showed no differences under non-treatment condition (Fig. S3). Then, we also observed the postgermination growth of the plants, and found that OE1 and OE5 had more greening seedlings than WT. The greening rates of WT, OE1 and OE5 under 150 mM NaCl treatment were 8%, 18% and 30%, respectively. Under harsh treatments, such as 150 mM NaCl, 250 mM Mannitol and all concentrations of ABA, postgermination growth of WT arrested. Under 1 μM and 2 μM ABA, *UGT75B1*OE1 and OE5 also displayed growth arrest (Fig. 4). These findings indicate that *UGT75B1* overexpression alleviates the sensitivity of the plants to abiotic stresses. To further analyze UGT75B1 role, we generated *ugt75b1* single mutant by crispr-cas9 strategy, and it has no phenotypic changes in response to ABA treatment (Fig. S3). In addition, its homolog gene UGT75B2, which locates in the same branch with UGT75B1 in the phylogenetic tree (Li et al. 2001), has negligible specificity towards ABA. The *ugt75b1/75b2* double mutant also showed no phenotypic changes in response to ABA treatment (Fig. S3).

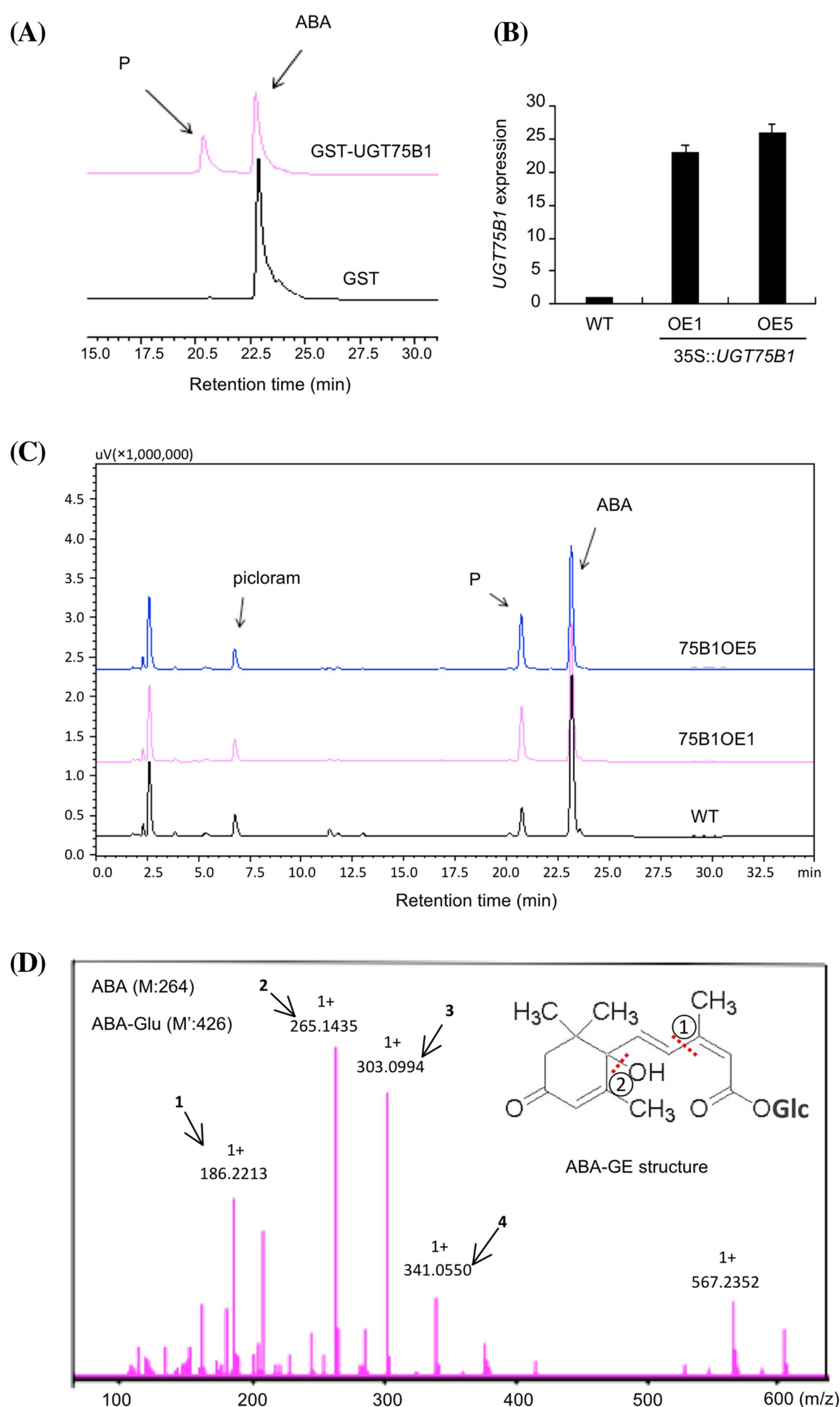

Fig. 3 UGT75B1 catalyzing activity towards ABA analyzed by HPLC and LC–MS. **a** Reaction products of purified UGT75B1 enzyme towards ABA in vitro analyzed by HPLC. P represents the product ABA-Glu ester. **b** *UGT75B1* expression levels in 35S::*UGT75B1* transgenic plants detected by qRT-PCR. **c** ABA catalyzing activity of the total proteins extracted from WT and *UGT75B1* overexpression plants. **d** LC–MS confirmation of the endogenous ABA-Glu ester from *UGT75B1* overexpression plants. 1: m/z 186.22(M'-①+H+-②+Na); 2: m/z 265.14 (M+H+); 3: m/z 303.09 (M+K); 4: m/z341.05(M'-①)

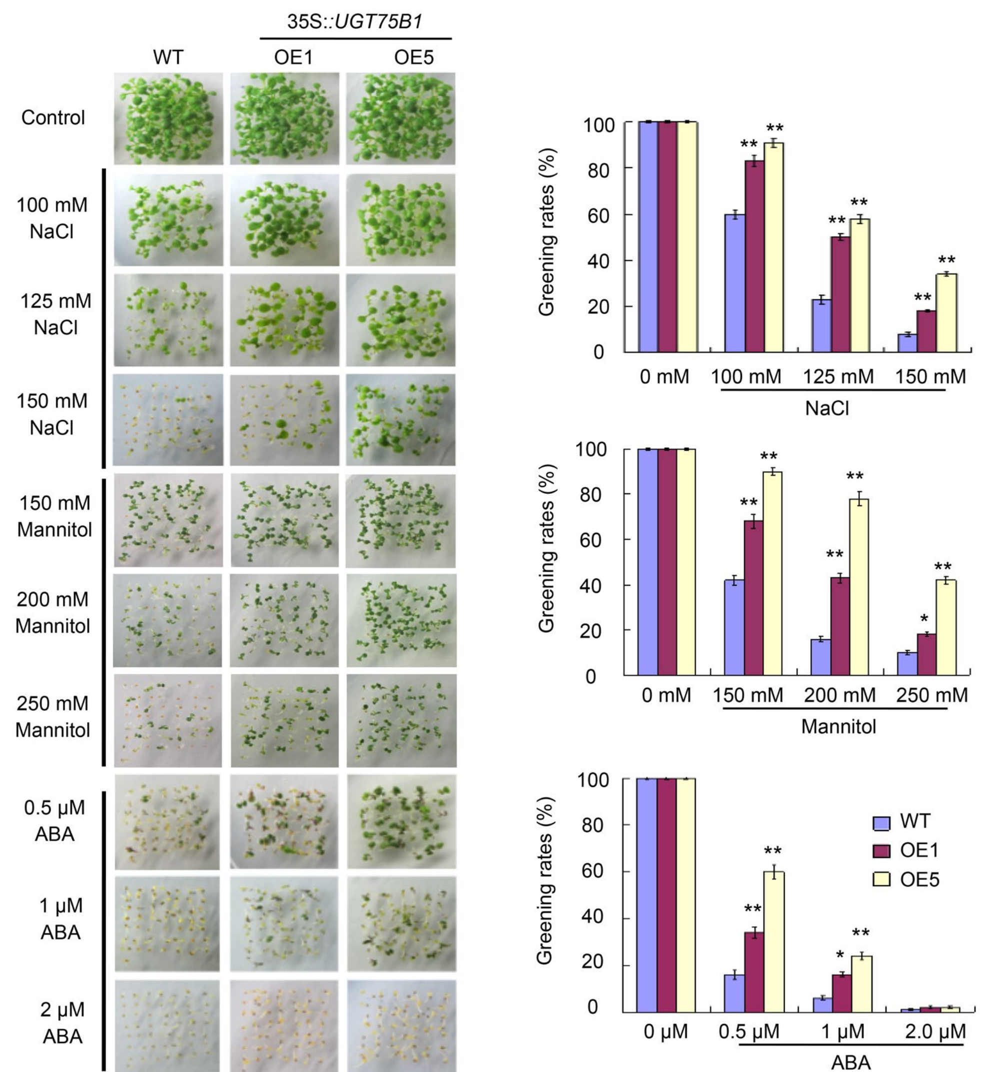

Fig. 4 Seedling greening of WT and two *UGT75B1* overexpression lines under NaCl, mannitol and ABA treatments. Fifty seedlings were studied in this experiment and at least three biological replicates were done. Error bars represent standard deviation (SD) from three biological replicates. Asterisks indicate significant difference relative to control condition (student's *t* test, $*P<0.05$, $**P<0.01$)

UGT75B1 overexpression plants showed sensitivity to abiotic stresses during adult stage

To see the growing of adult plants under abiotic stresses, 3-week-old WT and *UGT75B1*OE1 and OE5 plants growing in soil were exposed to salt and drought stresses. After the treatments, we clearly saw that the *UGT75B1* overexpression plants were more yellow and wilted than that of WT (Fig. 5). The survival rates of WT, OE1 and OE5 after salt treatment were 70%, 32% and 23%, respectively; and after rehydration following drought treatment were 79%, 50% and 42%, respectively (Fig. 5).

Since ABA is known to mediate stomatal movement, next we analyzed the stomatal opening of the 4-week old WT and *UGT75B1* overexpression plants in response to drought stress. Under normal conditions, no difference in stomatal aperture was observed for the three lines. After exposed to drought stress for 1 h, detached leaves of *UGT75B1*OE1 and OE5 transgenic plants showed larger stomatal opening under microscope (Fig. 6a). The stomatal aperture (indicated by width/length) of WT was approximately 0.25, while OE1 and OE5 were around 0.37 and 0.40, respectively (Fig. 6b). Consistently, detached leaves of *OE1* and *OE5* showed higher water loss than WT within 120 min, likely due to having larger stomatal opening (Fig. 6c). To determine the influence of UGT75B1 to endogenous ABA levels, the hormone was evaluated in WT and the two UGT75B1 OE lines. In turgid rosettes, ABA contents showed no differences in the three plants. As expected, after drying in the air for 3 h, ABA was significantly increased in wilted rosettes, and the two OE lines accumulated less ABA than WT (Fig. 6d). These observations demonstrate that UGT75B1 might mainly function in stressed conditions. The decreased ABA levels also accounted for the larger stomotal opening of *UGT75B1 OE* plants.

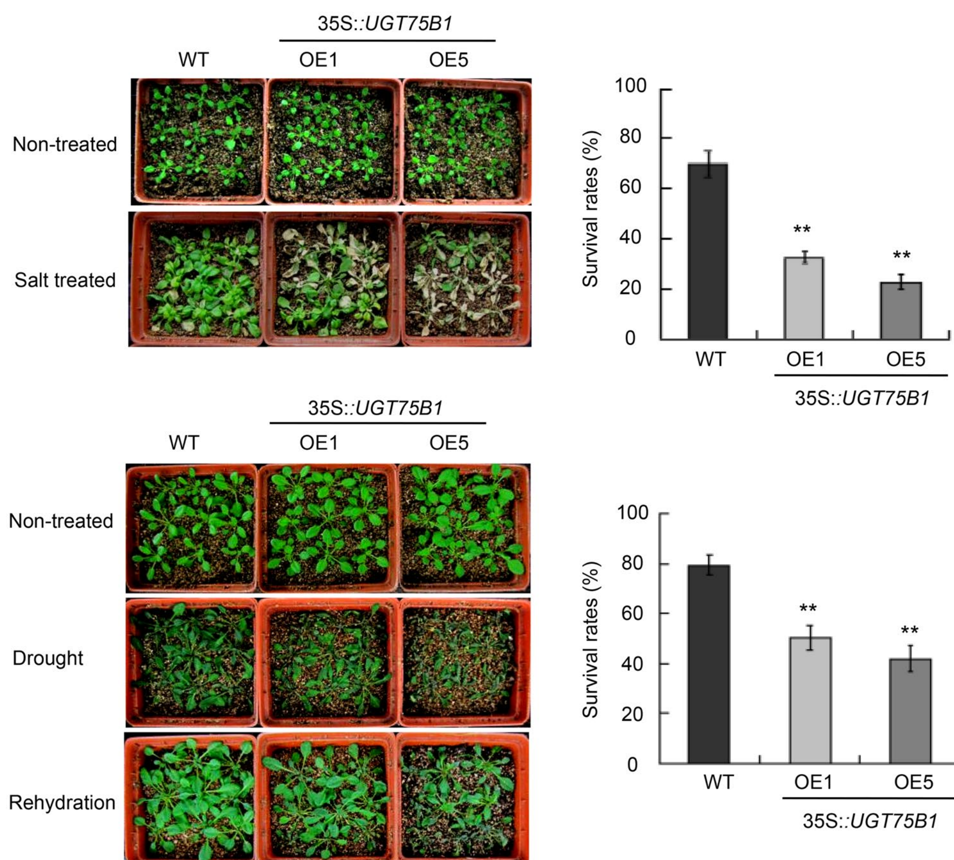

Fig. 5 *UGT75B1* overexpression plants exhibit salt and drought sensitive phenotype. The plants were incubated at 22 °C for 3 weeks and then treated with salt and dehydration. Error bars represent standard deviation (SD) from three biological replicates. Asterisks indicate significant difference relative to control condition (student's *t* test, $*P<0.05$, $**P<0.01$)

UGT75B1 overexpression leads to reduced ABI3 and ABI5 expression of ABI3 and ABI5 during postgermination growth

ABI3 (ABA-insensitive 3) and ABI5 (ABA-insensitive 5) are important ABA-responsive genes that mediate the ABA-dependent growth arrest during seed germination (Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000; Lopez-Molina et al. 2002). During the first three days of seed germination, the expression of *ABI3* and *ABI5* gradually decreased as the degradation of ABA to enable the germination of seeds and greening of seedlings (Lopez-Molina et al. 2002; Nakashima et al. 2006). Overexpression of *ABI3* or *ABI5* significantly blocks post-germination growth, whereas knockout of *ABI3* or *ABI5* allows the continued greening of seedlings in the presence of ABA (Lopez-Molina et al. 2001, 2002). Since *UGT75B1* overexpression lines showed promoted germination rate and post-germination growth, we detected the expression of *ABI3* and *ABI5* in newly generated WT and *UGT75B1* overexpression seedlings under stress conditions. The results showed that both *ABI3* and *ABI5* were downregulated upon *UGT75B1* overexpression especially under NaCl and mannitol conditions (Fig. 7). This result implied that likely via mediating ABA level and activity, UGT75B1 promotes post-germination growth of the newly germinated seedlings.

UGT75B1 overexpression downregulated the ABA-dependent pathway genes in stress conditions

To further explore the effect of elevated UGT75B1 level to the stress-related ABA signaling pathways, three abiotic stress-related marker genes were selected for expressional analysis, including *RESPONSIVE TO DESSICATION 29A (RD29A)*, *ABA INDUCIBLE LEA* (*AIL1*), *KINASE1 (KIN1)*, all of which belong to ABA-dependent stress pathway genes. Two-week-old WT and *UGT75B1* overexpression plants were subjected to NaCl and mannitol treatments, and the expression of these genes were evaluated by quantitative real-time PCR (qRT-PCR). The result showed that under non-stressed condition, the mRNA levels of these genes were similar in WT, OE1 and OE5 plants. In response to NaCl and mannitol treatment, the transcript levels of these genes were obviously upregulated, while the upregulation of selected genes were much prominent in WT than that in *UGT75B1* overexpression plants (Fig. 8). This attenuated response of *AIL1*, *RD29A* and *KIN1* under stress conditions further supports the suppressive role of *UGT75B1* on ABA

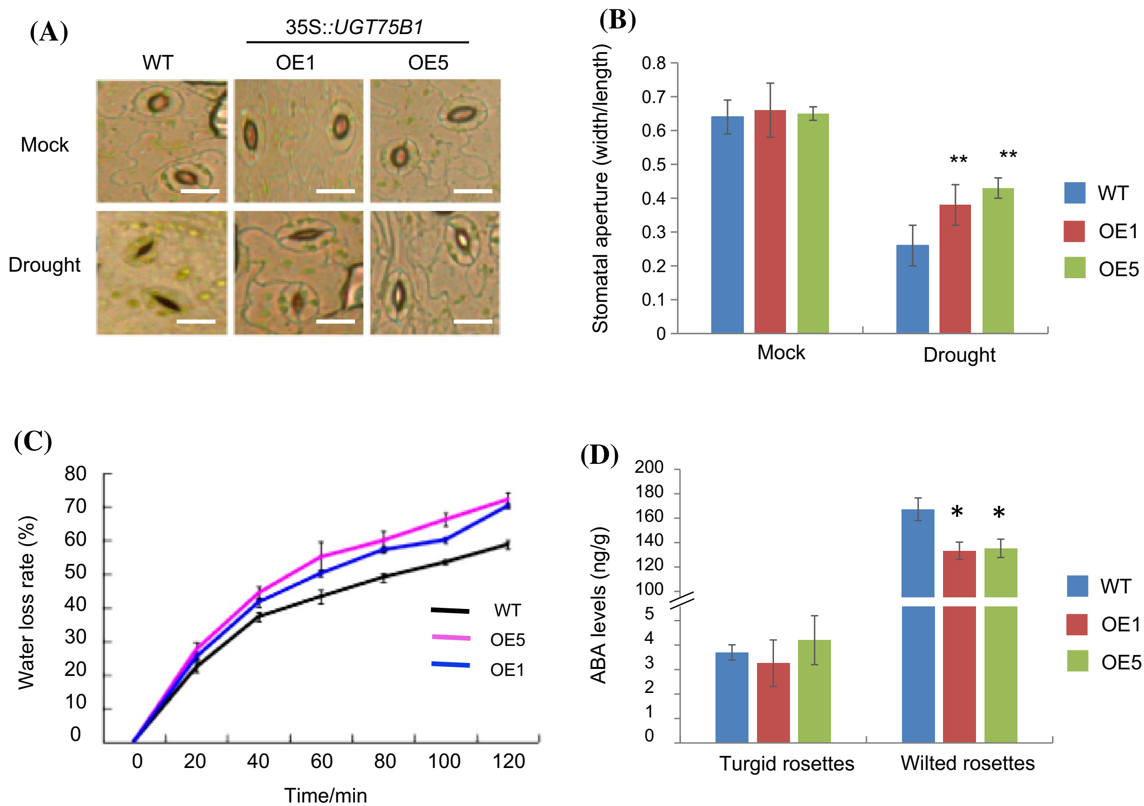

Fig. 6 Stomatal behavior and water loss of wild type and *35S::UGT75B1* OE1 and OE5 plants. **a** Detached leaves from the 4-week-old plants were dried for 1 h on filter papers, and stomatal closure was observed. **b** The stomatal aperture was measured by width/length value. Scale bar represents 20 µm. **c** Water loss of detached leaves from WT, OE1 and OE5 plants during 120 min. **d** Endogenous ABA levels in WT and *UGT75B1* overexpression plants in turgid and wilted tissues. Error bars represent standard deviation (SD) from three biological replicates. Asterisks indicate significant difference relative to mock (student's *t* test, $*P<0.05$, $**P<0.01$)

activity, which in turn caused the sensitive phenotype of the *UGT75B1* overexpression lines under stress conditions.

Discussion

ABA glycosylation is one of a key catabolism mode in regulating cellular ABA level, which is critical for the plant growth and development. Although UGT75B1 was previously identified to catalyze ABA glucosylation in vitro (Lim et al. 2005), the in vivo evidence of UGT75B1 activity towards ABA is still missing. In this study, we focused on investigating the biological role of UGT75B1 in planta. Firstly, we found that *UGT75B1* could be significantly induced by ABA treatment as well as salinity and drought stress (Fig. 2), which links its role to stress response. During seed germination, ABA is gradually degraded to enable germination of seeds and postgermination growth. However, once encountering environmental stresses during this period, ABA will be rapidly synthesized to inhibit the seed germination and arrest the postgermination growth. Actually, it is regarded as a protective mechanism that can help the seeds and greening seedlings to evade unfavorable conditions (Lopez-Molina et al. 2001). In this study, it is observed that overexpression of *UGT75B1* substantially promoted seed germination and postgermination growth under stress conditions (Fig. S3; Fig. 4). Under normal condition, ABA level declines upon seed imbibition to allow the seeds to germinate and develop into seedlings. However, under abiotic stress conditions, ABA level remains high, arresting seed germination and seedling establishment (Reyes and Chua 2007). *ABI3* and *ABI5* are two ABA-induced marker genes whose expressions are limited to a narrow window of 72 h after seed stratification, and ABI3 acts upstream of ABI5 (Lopez-Molina et al. 2001, 2002). Consistent with enhanced seed germination and post-germination growth (Fig. 4), we observed that the expressions of *ABI3* and *ABI5* were downregulated in the *UGT75B1* overexpression plants under abiotic stresses (Fig. 7). Moreover, we also found that the adult *UGT75B1* overexpression plants are sensitive to salt and drought stresses (Fig. 5), and the ABA-related stress pathway genes *RD29A*, *KIN1* and *AIL1* are downregulated in the transgenic plants in response to stresses

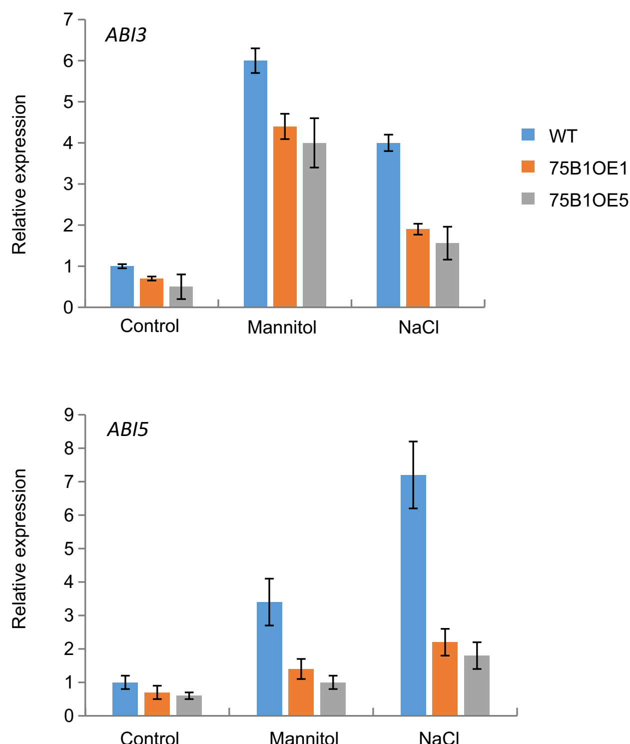

Fig. 7 Expression of *ABI*3 and *ABI5* in WT, *UGT75B1*OE1 and OE5 plants during seed germination. Seeds were germinated on water-moistened filter paper for 24 h after stratification, and then were transferred onto filtered paper moistened with 200 mM Mannitol and 125 mM NaCl, respectively. After treated for 12 h, the samples were harvested and RNA was extracted. Here, three biological repeats were done and each qRT-PCR was also performed three times

(Fig. 8). Induced ABA accumulation by water deficit can rapidly promote stomatal closure to reduce the transpiration rate and water loss (MacRobbie, 1998; Niu et al. 2018). In this study, we also measured the ABA levels in response to drought stress, and found that *UGT75B1 OE* lines showed lower ABA levels, which further validated UGT75B1 activity towards ABA in planta. The reduced ABA accumulation in *UGT75B1 OE* plants under drought condition likely contributes to the impeded stomatal closing and downregulation of ABA-responsive genes.

It is well known that the ABA concentration increases up to 100-fold when plants suffer drought stress, and then rapidly falls to normal level when stressful conditions relieved (Zeevaart and Creelman 1988; Harris and Outlaw 1991; Zeevaart and Yang 2005). We showed that in line with ABA accumulation, *UGT75B1* is also substantially induced by stresses, implying that it might be involved in buffering the stress-induced ABA accumulation to a reasonable level in case it is accumulated too much to exert normal effect. Under stress conditions, the induction of *UGT75B1* shall represent a more rapid response in modulating ABA levels compared with other non-stress-responsive ABA glycosyltransferases. Thus, UGT75B1 might play a critical role in modulating the ABA levels under abiotic stress conditions.

Till now, two groups of ABA-modifying UGTs have been characterized in Arabidopsis: UGT71C5 and UGT71B6 group (including UGT71B6, UGT71B7 and UGT71B8). It is found that loss of function *ugt71c5* mutant showed an ABA-excessive phenotype during the whole life cycle. When exposing *ugt71c5* mutant to drought stress, it showed obvious resistance to the treatment, indicating that UGT71C5 plays a major role in modifying ABA in Arabidopsis. *ugt71b6* single mutant has no phenotypic difference in comparison with WT (Priest et al. 2006). However, Dong et al. (2014) reported that cosuppression of *UGT71B6*, *UGT71B7* and *UGT71B8* by RNA interference displayed an ABA-excessive phenotype, indicating that these three members are highly redundant in functions. *ugt75b1* single mutant displayed no phenotypic changes either (Fig. S3), Lim et al. (2005) found that UGT75B1 showed relatively high specificity towards ABA than UGT84B2 and UGT71B6. However, its homolog gene UGT75B2 has nearly no activity towards ABA. The *ugt75b1/75b2* double mutant also showed no phenotypic changes (Fig. S4). These observations indicates that the function of UGT75B1 might be complemented by other ABA related UGTs.

To sum up, in this study, we proposed a UGT75B1 working model based on our data. In response to abiotic stresses, ABA production rapidly increases, and *UGT75B1* expression is induced simultaneously. To avoid the overaccumulation of ABA and buffer ABA activity, UGT75B1 catalyzes excess ABA into its glucose-ester, which is inactive. Strong ABA activity blocks the seed germination and postgermination growth, meanwhile promotes the established seedlings to adapt to abiotic stresses via ABA-dependent pathway genes. On the other hand, induced ABA accumulation by water stress can rapidly promote stomatal closure in adult plants, which in turn reduces the transpiration rate and water loss. Thus, *UGT75B1* overexpression attenuates ABA activity/ABA signaling via glucosylation modification, which promotes seed germination and postgermination growth, and on the other hand leads to high sensitivity of the adult plants to stresses. In addition, the decreased ABA activity in *UGT75B1* overexpression plants also leads to impeded stomatal closure, and thus plays a negative role in stress tolerance (Fig. 9).

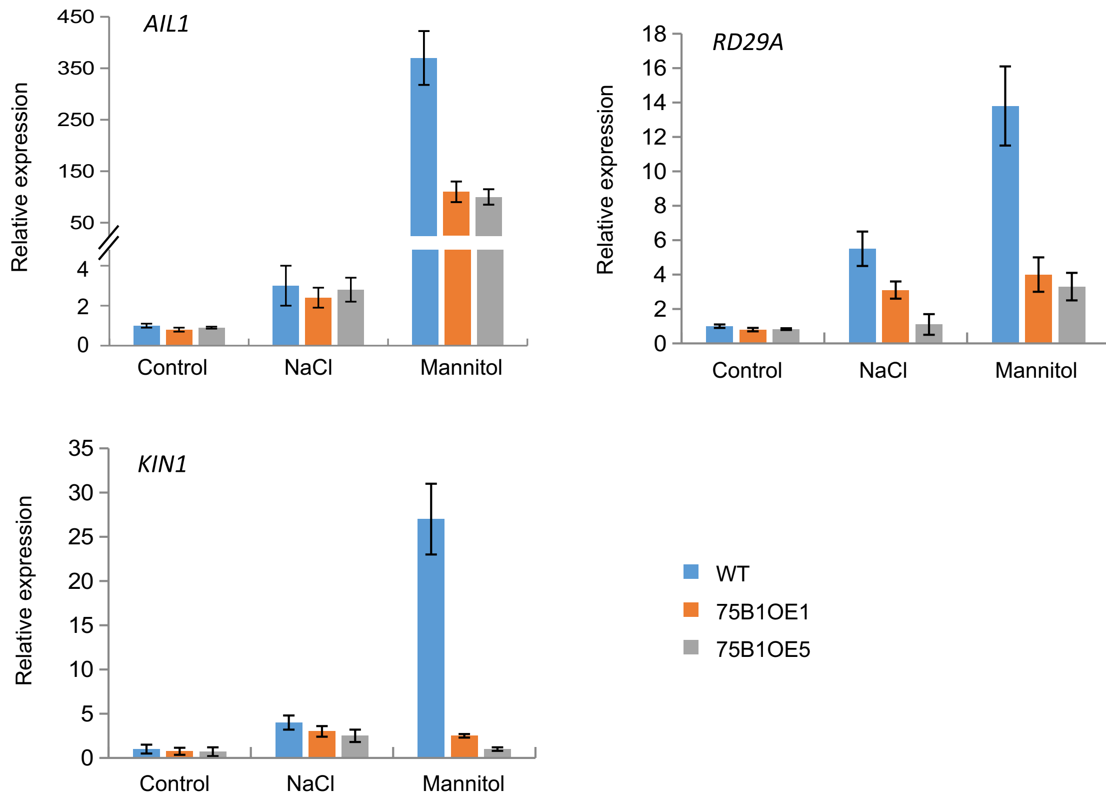

Fig. 8 qRT-PCR analysis of stress-responsive genes in *UGT75B1* overexpression plants when exposed to salt and osmotic stresses. Two-week-old seedlings were subjected to NaCl and mannitol treatment for 12 h. The relative expression of the ABA-dependent pathway genes was normalized using *UBC9* transcript.

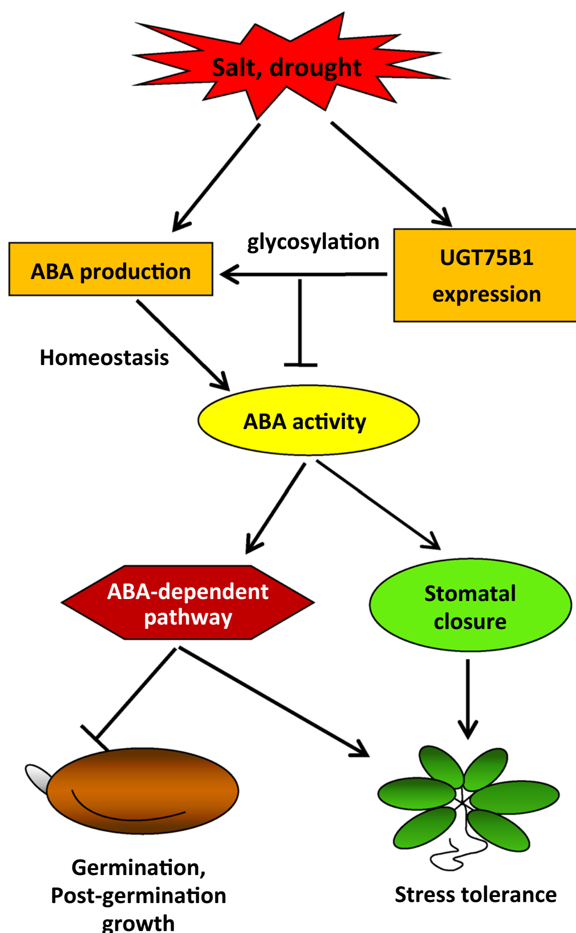

Fig. 9 UGT75B1 working model upon exposure to abiotic stresses. In response to abiotic stresses, ABA production soon increases, and *UGT75B1* expression is induced simutanously. To avoid the overaccumulation of ABA and buffer ABA activity, UGT75B1 catalyzes excess ABA into its glucose-ester, which is inactive. Strong ABA activity blocks the seed germination and postgermination growth, meanwhile promotes the established seedlings to adapt to abiotic stresses via ABA-dependent pathway genes. On the other hand, induced ABA accumulation by water stress can rapidly promote stomatal closure in adult plants, which in turn reduces the transpiration rate and water loss. Thus, *UGT75B1* overexpression attenuates ABA activity/ABA signaling via glucosylation modification, which promotes seed germination and postgermination growth, and on the other hand leads to high sensitivity of the adult plants to stresses. Also, the decreased ABA activity in *UGT75B1* overexpression plants also leads to impeded stomatal closure, and thus plays a negative role in stress tolerance

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