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Overexpression of the *UGT76E12* gene modulates seed germination, growth, and response to NaCl, mannitol, and abscisic acid

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Abstract

Seed germination and following seedling growth are largely affected by environmental conditions. However, the genes involved in adaptations to these conditions are largely unknown. In this study, we cloned and characterized an *Arabidopsis* uridine diphosphate glycosyltransferase gene *UGT76E12* and investigated its function in seed germination and plant growth under adverse environments. We found that *UGT76E12* gene expression was induced by NaCl, mannitol, and abscisic acid (ABA) treatments. Under these treatments, the *UGT76E12* overexpression lines exhibited a delayed seed germination and cotyledon growth compared with a wild type, and this delayed growth could be restored by treatment with sodium tungstate, an ABA synthesis inhibitor. Further, real-time quantitative PCR analysis reveals that the stress-induced expressions of genes involved in ABA biosynthesis were greatly enhanced in the *UGT76E12* overexpression lines. Therefore, our data suggest that the *UGT76E12* gene plays an important role in regulation of seed germination and growth under adverse environmental conditions by affecting ABA biosynthesis.

Additional key words: adaptation, *Arabidopsis thaliana,* glycosyltransferase, growth inhibition, transgenic plants.

Introduction

Mature seeds are the end point of embryogenesis and the state of the seed itself determines the germination or dormancy (Leubner-Metzger 2003, Kucera *et al*. 2005, Muller *et al*. 2006). Seed germination is the first step of growth, and it is of an immense significance for a final yield. Seed germination requires proper environmental conditions and under adverse conditions, the germination is delayed. However, staggering germination safeguards some seeds and seedlings from suffering damage or death under adverse environmental conditions. Seed dormancy is a mechanism to prevent germination during unsuitable conditions. Many specific biochemical and physiological processes occur in germinating seeds (Kroj *et al*. 2003, Miransari and Smith 2009), and environmental factors, such as temperature, irradiance, pH, soil moisture, and plant hormones, jointly control the balance between seed germination and dormancy (Taylorson 1987, Bewley and Black 1994, Bewley 1997, Chachalis and Reddy 2000). Furthermore, environmental factors ultimately determine the content of major plant hormones (Olszewski *et al.* 2002, Nambara and Marion-Poll 2005). It was indicated that seed germination is mainly regulated by gibberellins and abscisic acid (ABA) (Karssen *et al*. 1989, Groot and Karseen 1992, Matilla and Matilla-Vazquez 2008). While gibberellins activate dormant seeds, ABA inhibits seed germination by affecting the cell cycle (Matilla and Matilla-Vazquez 2008). Thus, gibberellins and ABA play antagonistic roles in seed germination (Bewley 1997, Miransari and Smith 2009). Recent studies indicated that gibberellins and ABA have a complicated signal crosstalk under unfavorable conditions, and DELLA proteins may be a crucial interface in regulating this crosstalk (Golldack *et al.* 2013). Abscisic acid is known to regulate various stress responses in plants such as responses to salt and drought (Hetherington 2001, Lopez-Molina *et al.* 2001, Nambara and Marion-Poll 2003). Abscisic acid affects plants mainly in two areas: seed germination and early seedling development, and regulation of stomatal

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Abbreviations: ABA - abscisic acid; MS - Murashige and Skoog; RT - reverse transcription; q - quantitative; UGT - uridinediphosphate glycosyltransferase; WT - wild type.

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opening (Pandey *et al.* 2006). *Via* analyzing ABA-insensitive mutants, several important modules have been found to be controlled by ABA (Hegedus *et al.* 2003). Among them, ABI1 (Leung *et al.* 1994) and ABI2 (Rodriguez *et al.* 1998) are negative regulators of ABA signaling, which influences seed dormancy, germination, and guard cell movements (Allen *et al.* 1999). In addition, other transcription factors of the ABI family, including ABI3, ABI4, and ABI5, are also involved in the regulation of the ABA signaling pathways and play an active role in post-germination growth (Finkelstein *et al.* 2002). Actually, there are various types of mechanisms for regulation of seed germination and dormancy apart from ABA and gibberellins mentioned above. Brassinosteroids, ethylene, and other hormones were also reported to regulate seed dormancy and germination (Kucera *et al*. 2005). For example, addition of ethylene is able to rescue the germination of gibberellin deficient mutants (Karssen *et al*. 1989, Matilla and Matilla-Vazquez 2008) as well as to affect the rate of seed germination of some plants such as wheat, corn, soybean, and rice (Pennazio and Roggero 1991, Zapata *et al*. 2004). Brassinosteroids could stimulate seed germination *via* enhancing the growth of embryo (Leubner-Metzger, 2001). Furthermore, both brassino-steroids and indoleacetic acid could promote the production of ethylene (Cooke 1993, Arteca and Arteca 2008,). Generally, it is thought that gibberellins, ethylene, and brassinosteroids are able to induce seed germination by controling the inhibitory effects of ABA (Finch-Savage and Leubner-Metzger 2006). Therefore, a complex regulatory network between various hormones is likely responsible for the progress of seed germination.

When plants cope with adverse conditions, among plethora of metabolic modifications, one important process is modification of certain molecules by glycosylation. Uridinediphosphate (UDP) glycosyl-transferases (UGTs) are the responsible enzymes for transferring sugar moieties from activated donor molecules (UDP-sugars) to specific acceptor molecules. It had been shown that UGTs have many biological functions including plant adaptation to stresses (Lim *et al.* 2004, Bowles *et al.* 2005, 2006). Recently, several studies reported that UGTs could promote seed germination and growth under stress conditions, and enhance resistance to unfavorable environments (Liu *et al.* 2015, Li *et al.* 2017a,b, 2018). By contrast, it is still not clear whether UGTs are also involved in the inhibition of seed germination and growth under stress conditions so that seeds can survive in the adverse environments.

To further investigate the function of plant UGTs, we analyzed the published *Arabidopsis* microarray data, and isolated several abiotic stress-induced UGT genes. Our aim is to determine whether UGTs are also involved in the inhibition of seed germination and growth under adverse environments.

Materials and methods

Plant, growth, and treatments: *Arabidopsis thaliana* L. plants were of the Columbia (Col-0) ecotype background. Seeds were sown on autoclaved soil or on a medium containing Murashige and Skoog (MS) salts and 3 % (m/v) sucrose and cultivated in a growth chamber under an irradiance of 100 μmol·m^{-2} s^{-1}, a 16-h photoperiod, and a temperature of 22 °C.

For seed germination and early seedling growth, surface sterilized seeds were sown on an MS solid medium without (control) or with different concentrations of NaCl, mannitol, or ABA. The Petri-dishes were transferred to a growth chamber after stratification at 4°C in the dark for 3 d. The seeds were regarded as germinated when radicles protruded from the seed coat.

For post-germination assays, seeds were cultured in MS medium as a control or in an MS medium containing 300 mM mannitol and/or 0.15 mM sodium tungstate after stratification. Two weeks later, the rate of seedling growth was determined. At least 50 seeds per treatment were used.

The generation of *UGT76E12* overexpression lines: The full-length cDNA of the *UGT76E12* gene (At3g46660) was amplified using reverse transcription (RT) PCR method from total RNA of wild type *Arabidopsis* plants. The cDNA of *UGT76E12* was then cloned into a pBI121 binary vector and driven by the CaMV 35S promoter. The construct was transformed into *Arabidopsis* plants through *Agrobacterium*-mediated floral dip method (Clough and Bent 1998). Homozygous transgenic plants were analyzed for *UGT76E12* expression using RT-qPCR method.

Reverse transcription quantitative PCR: For RT-qPCR, total RNA was extracted from different tissues using a *Trizol* reagent (*TaKaRa*, Dalian, China). A DNase-treated RNA (5 μg) was used for reverse transcription reactions with a *PrimeScript* RT reagent kit (*TaKaRa*) according to the supplier's manual. The RT-qPCR was performed with a *Bio-Rad* thermal-cycling system *CFX Connect* (Hercules, CA, USA) using a *SYBR-Green Ex Taq II* kit (*TaKaRa*) to detect gene expression. The *ACTIN2* (At3g18780) was used as an internal reference gene. The relative expression was calculated using the comparative ΔΔCt method (Livak and Schmittgen 2001). Primer information for the PCR assay is included in Table 1 Suppl.

Statistical analysis: For all experiments in this study, at least three independent biological replicates (each including three technical replicates) were done for each treatment. Significance of differences were calculated with the Student *t*-test.

Results

Publicly available microarray data show that *UGT76E12* expression is induced by abiotic stresses. To verify this result, we conducted RT-qPCR analysis to determine the mRNA content of *UGT76E12* in the germinating seeds exposed to the solutions containing NaCl (150 mM), mannitol (250 mM), and ABA (100 μM) for 12 h. Our analyses indicate that *UGT76E12* was strongly induced in germinating seeds by all these treatments (Fig. 1).

To further analyze the role of UGT76E12 in seed germination under stress conditions, we generated the cauliflower mosaic virus 35S promoter-driven *UGT76E12* overexpression lines. Among nine independent T3 homozygous lines, *OE10* and *OE20* were selected for further study (Fig. 1 Suppl.). Two days after sowing the seeds of wild type (WT), *OE10*, and *OE20* on a medium containing NaCl or mannitol, we observed that overexpression of *UGT76E12* caused a significantly reduced germination rate as compared to WT (Fig. 2 and Fig. 2 Suppl.). Moreover, the inhibition of germination by overexpressing *UGT76E12* enhanced with the increase of NaCl or mannitol concentration. These observations suggest that *UGT76E12* was potentially involved in modulating seed germination in response to abiotic stresses.

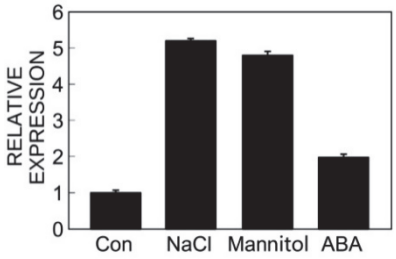

Fig. 1. The expression of uridinediphosphate glycosyltransferase (*UGT*) *76E12* induced by250 mM mannitol, 150 mM NaCl, and 100 μM abscisic acid (ABA) treatments lasting for 12 h. The expression of non-treated samples (Con) were set to 1.0. Means ± SDs from three replicates.

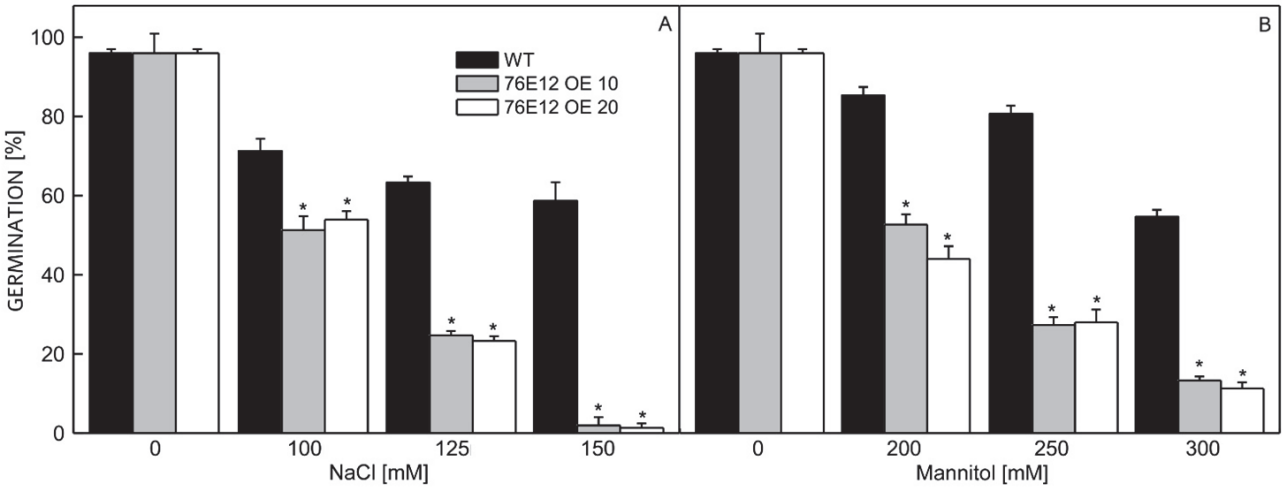

Fig. 2. The germination rate of wild type (WT) and uridinediphosphate glycosyltransferase (*UGT*) *76E12* overexpression lines (76E12 OE 10 and 76E12 OE 20) under NaCl (*A*) and mannitol (*B*) treatments for 2 d. Means ± SDs of three biological replicates. *Asterisks* indicate significant differences relative to WT (the Student *t*-test at $P < 0.05$).

Post-germination growth under NaCl and manninol induced stresses was also investigated. The seeds of WT, *OE10*, and *OE20* were treated with NaCl, mannitol, and ABA for two weeks, and then the ratio of the number of seedlings with green and expanded cotyledons to the total number of seedlings was determined (Fig. 3 and Fig. 3 Suppl.). The growth of WT seedlings was inhibited under the stress conditions, while both overexpression lines showed a much more inhibition of seedlings with green cotyledons under stress conditions. Similar to the results of germination under the stress conditions, the stress-induced inhibition of seedling growth by *UGT76E12* over-expression was enhanced along with increased NaCl, mannitol, and ABA concentrations.

To explore whether a UGT76E12-mediated germination response to stresses is related to ABA signaling pathway, we applied mannitol together with sodium tungstate, an inhibitor of ABA biosynthesis, to WT and *OE10* seedlings. Sodium tungstate treatment barely affected the growth of both WT and *OE10*, whereas mannitol inhibited growth of *OE10* seedlings to a larger extent than WT seedlings (Fig. 4 and Fig. 4 Suppl.). Intriguingly, when seedlings were treated with mannitol along with sodium tungstate, the ratio of seedlings with green cotyledons of *OE10* was restored to the level of WT (Fig. 4, Fig 4 Suppl.). These results suggest that UGT76E12-mediated growth inhibition of seeds under osmotic stress conditions requires ABA biosynthesis.

To investigate how UGT76E12 affects ABA signaling pathway under stress conditions, the relative expressions of ABA biosynthesis related genes in *OE10* and WT seedlings were detected. The total RNA was extracted from seedlings germinating in the presence or absence of NaCl, mannitol, or ABA. The examined genes included *ABI3* and *ABI5* (regulator genes of ABA signaling) (Lopez-Molina *et al.* 2002), *ABA3* and *NCED3* (ABA biosynthetic genes), and *AAO3* (a gene coding for ABA aldehyde oxidase) (Iuchi *et al.* 2001, Nambara and Marion-Poll 2005). As is shown in Fig. 5, the expressions of these genes were similar in WT and in *OE10* seedlings under control conditions. Differences of stress-induced changes in *ABI3* and *ABI5* expressions between WT and *OE10* were only marginal. On the other hand, although NaCl treatment showed no clear effect, the mannitol-induced expressions of ABA synthetic genes *ABA3*, *AAO3*, and *NCED3* in *OE10* were significantly higher than in WT (Fig. 5) indicating that UGT76E12 could promote osmotic-stress-induced ABA synthesis.

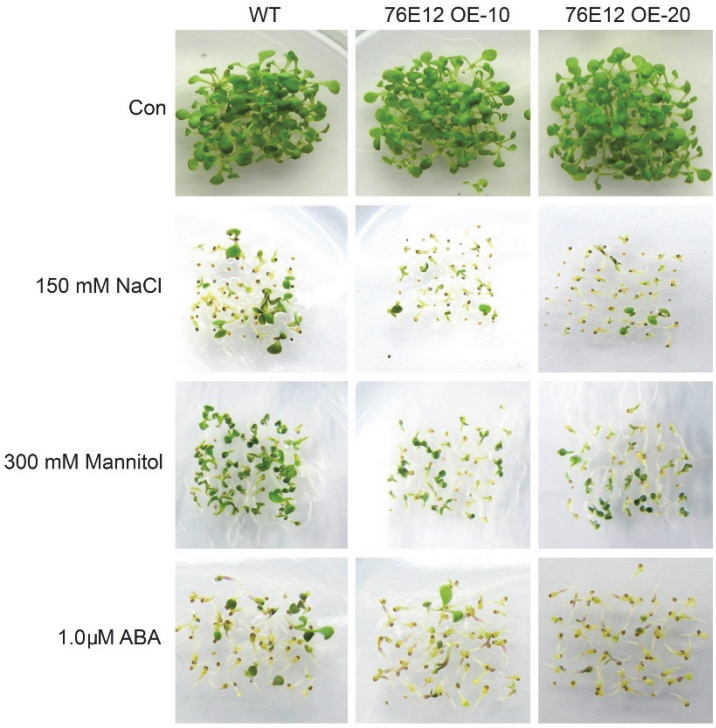

Fig. 3. Cotyledon greening and growth of wild type (WT) and *UGT76E12* overexpression lines (76E12 OE 10 and 76E12 OE 20) under control conditions and NaCl, mannitol, and ABA treatments for two weeks.

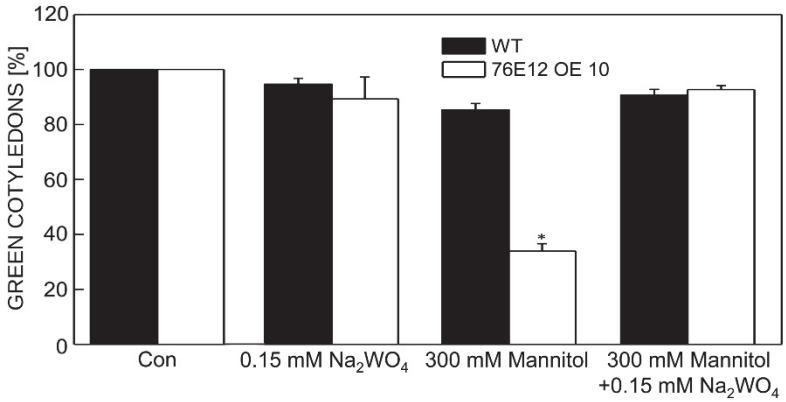

Fig. 4. The ratio of the number of greening cotyledons to the total number of cotyledons in wild type (WT) and *UGT76E12* overexpression line (76E12 OE 10) seedlings under mannitol and sodium tungstate (Na_2WO_4) treatments. For this data, at least 50 seeds were used per treatment and at least 3 independent biological replicates and 3 technical replicates were done. Means ± SDs of three biological replicates. *Asterisks* indicate significant differences relative to WT (the Student *t*-test at $P < 0.05$).

To conclude, our results suggest that UGT76E12 inhibited seed germination and seedling growth under stress conditions *via* promoting endogenous ABA synthesis.

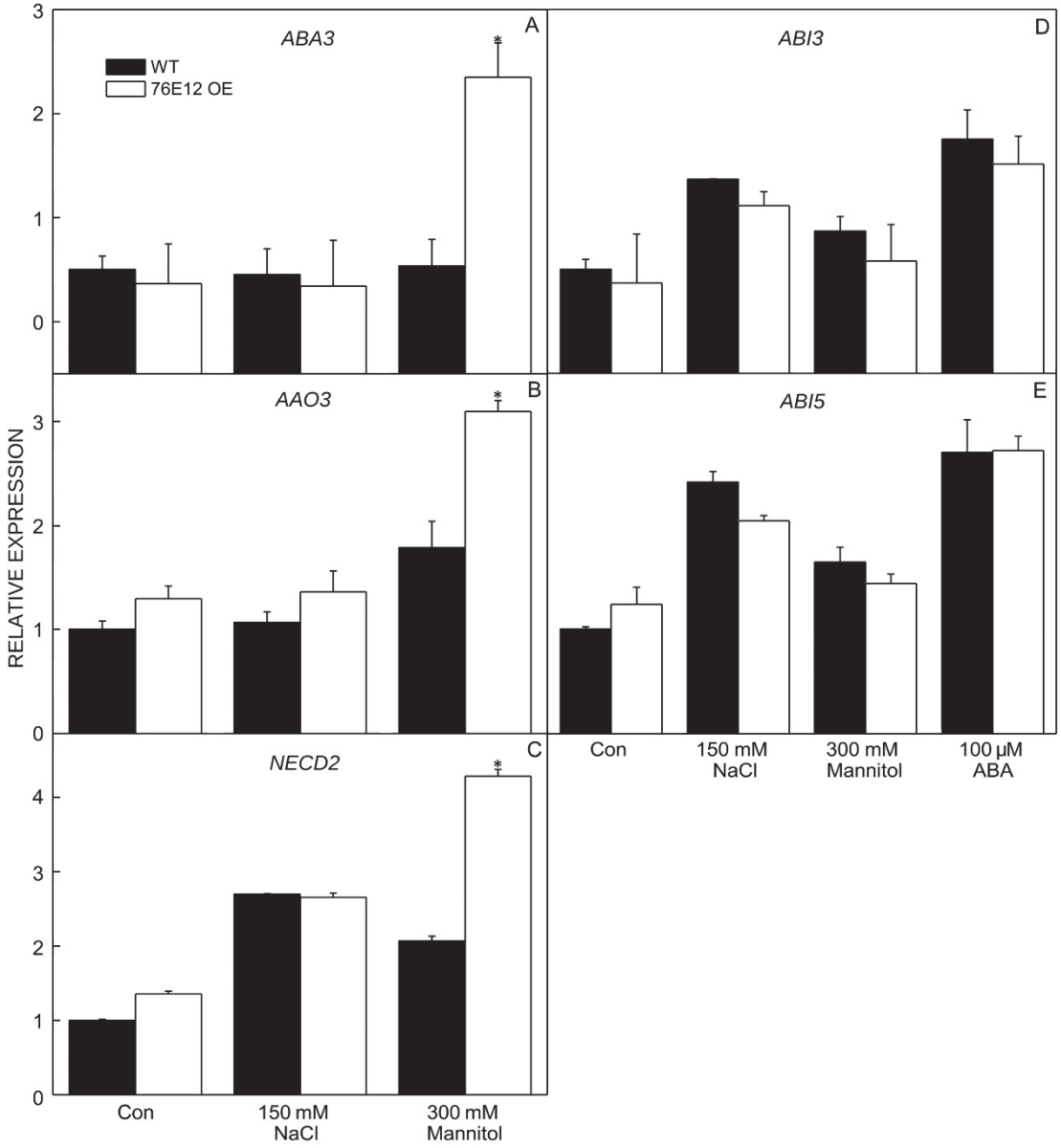

Fig. 5. The expressions of abscisic acid (ABA) biosynthesis related genes in a wild type (WT) and a *UGT76E12* overexpression line (76E12 OE 10) detected by reverse transcriprtion quantitative PCR. Seeds were germinated on a control medium or a medium with mannitol, NaCl, or ABA for 2 d. Means ± SDs of three biological replicates. *Asterisks* indicate significant differences relative to WT (the Student *t*-test at $P < 0.05$).

Discussion

Under biotic and abiotic stresses, plants could adjust their physiological and biochemical processes to maintain proper growth and development. At the same time, plants also evolve an ability to avoid unfavourable conditions including delayed seed germination and retarded growth so that the plants could save energy to cope with future challenges. In this study, *Arabidopsis* UGT76E12 was found to be involved in the seed germination control under stress conditions. Over-expression of *UGT76E12* delayed seed germination and post-germination growth under stress conditions. This suggests that UGT76E12 is involved in the control of germination under harsh

environments. Our further experiments reveal that UGT76E12-mediated growth inhibition in response to stresses was caused by enhanced ABA biosynthesis. Therefore, this finding not only increases our understanding of the molecular mechanism on seed germination control under adverse environments, but also deepens our knowledge on the physiological function of the glycosyltransferase family in plants.

Our previous study reported that UGT76E11 is involved in a plant response to abiotic stresses (Li *et al.* 2018). However, differing from the role of UGT76E12 reported in this study, UGT76E11 could promote germination and post-germination growth under stress conditions. The UGT76E11 was shown to catalyze the glucosylation of flavonoids, thus enhancing the ability of plants to scavenge reactive oxygen species under adverse conditions (Li *et al.* 2018). The UGT76E11 and UGT76E12 are closely related in the UGT family, yet we did not find the activity of UGT76E12 toward flavonoids. Moreover, instead of promoting seed germination under stress conditions, overexpression of *UGT76E12* led to a delayed seed germination. Therefore, UGT76E12 and UGT76E11 are likely to play different roles in coping with harsh environments through different mechanisms. In crop breeding, different strategies can be adopted to enhance seed germination as well as seedling survival at the early development stage under stress conditions. Our finding provides another possibility that by activating *UGT76E12,* seeds could delay germination to avoid unfavourable conditions, thus save energy for future growth and development.

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