Research Paper

BrUGE1 transgenic rice showed improved growth performance with enhanced drought tolerance

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UDP-glucose 4-epimerase (UGE) catalyzes the reversible conversion of UDP-glucose to UDP-galactose. To understand the biological function of *UGE* from *Brassica rapa*, the gene *BrUGE1* was cloned and introduced into the genome of wild type rice ‘Gopum’ using the *Agrobacterium*-mediated transformation method. Four lines which carried a single copy gene were selected and forwarded to T₃ generation. Agronomic traits evaluation of the transgenic T₃ lines (CB01, CB03, and CB06) under optimal field conditions revealed enriched biomass production particularly in panicle length, number of productive tillers, number of spikelets per panicle, and filled spikelets. These remarkably improved agronomic traits were ascribed to a higher photosynthetic rate complemented with higher CO₂ assimilation. Transcripts of *BrUGE1* in transgenic lines continuously accumulated at higher levels after the 20% PEG6000 treatment, implying its probable role in drought stress regulation. This was paralleled by rapid accumulation of soluble sugars which act as osmoprotectants, leading to delayed leaf rolling and drying. Our findings suggest the potential of *BrUGE1* in improving rice growth performance under optimal and water deficit conditions.

Key Words: UDP-glucose 4-epimerase, rice, biomass, drought.

Introduction

Plants possess a sophisticated sugar biosynthetic machinery comprising families of nucleotide sugar interconversion enzymes (Seifert 2004). Nucleotide sugars act as biosynthetic substrates and intermediates in the uptake of free sugars released from the breakdown of nutritional or storage carbohydrates and other sources. Simple sugars generally found in plants are glucose, galactose, xylose, arabinose, rhamnose, glucuronic acid, galacuronic acid, mannose, and fructose (Reiter and Vanzin 2001). These sugars need to be activated to serve as building blocks for cell components. Nucleotide-diphosphate sugars (NDP sugars) are important as high-energy glycosyl donors in the synthesis of most of these polysaccharides, for example, the enzyme starch synthase utilizes uridine diphosphate glucose (UDP-glucose) and adenosine diphosphate glucose (ADP-glucose) as sub-

strates (Kim *et al.* 2009). UDP-glucose is the most important of the NDP-sugars. It can be synthesized from glucose 1-phosphate using the reaction $\text{NTP} + \text{sugar 1-phosphate} \leftrightarrow \text{NDP-sugar} + \text{pyrophosphate}$ or from sucrose using the enzyme sucrose synthase (Nakai *et al.* 1999).

The enzyme catalyzing the reversible conversion/epimerization of UDP-glucose into UDP galactose is UDP-glucose 4-epimerase (UGE; EC.5.1.3.2). The reversible epimerization is catalyzed via an enzyme-bound UDP-4-keto-hexose intermediate (Maitra and Ankel 1971).

Complete sequencing of entire genomes revealed a surprising over-representation of genes encoding putative isoforms of nucleotide sugar interconversion enzymes in plant genomes (Barber *et al.* 2006, Reiter and Vanzin 2001, Seifert 2004), but the functional significance of this apparent genetic redundancy remains to be established (Barber *et al.* 2006). Comparative analyses of genetically encoded isoforms of nucleotide sugar interconversion enzymes (Barber *et al.* 2006, Pattathil *et al.* 2005, Rosti *et al.* 2007, Zhang *et al.* 2006) highlight variations in their biochemical properties, transcriptional regulation, and subcellular regulation which led to speculation of this genetic diversity to be related

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to the regulation of cell wall carbohydrate during development, growth and stress (Bonin *et al.* 2003, Pattathil *et al.* 2005, Rösti *et al.* 2007, Seifert 2004).

The gene encoding the UDP-glucose epimerase has been cloned from a variety of organisms (Dorman and Benning 1998). In *Arabidopsis thaliana*, five *UGE*s were present and each *UGE* had different catalytic properties (Barber *et al.* 2006). *UGE2* and *UGE4* cooperate in providing cell wall biosynthesis and growth, *UGE2* and *UGE3* cooperate in pollen development, and *UGE1* and *UGE5* contribute non-specifically to *UGE* activity and growth under unstressed conditions but might be more specifically involved in stress situations (Rösti *et al.* 2007). Overexpression and down-regulation analyses of *UGE1* in *A. thaliana* revealed no alteration in carbohydrate composition, despite an increase by up to 250% and a decrease to 10% of *UGE* activity compared with the wild type (Dörmann and Benning 1998). The study further demonstrated that *UGE1* transgenics grown in soil showed a normal growth habit and contained wild type amounts of D-galactose in their cell wall material and chloroplast lipids. However, tolerance to galactose was correlated to *UGE1* expression in various transgenic lines (Barber *et al.* 2006). In rice, four *UGE* genes (*OsUGE1-4*) were identified, and *OsUGE1* is activated after drought, salt or UV irradiation stress (Kim *et al.* 2009, Liu *et al.* 2007). A study demonstrated an increased expression of the gene after imposition of drought stress, which maps to a root thickness quantitative trait locus (QTL) region (Nguyen *et al.* 2004). Overexpression of *OsUGE1* altered raffinose level and tolerance to abiotic stress but not the morphology in *Arabidopsis* (Liu *et al.* 2007). A recent study involving constitutive overexpression of *OsUGE1* showed the potential role of the gene in cell wall carbohydrate partitioning during limiting nitrogen conditions in rice (Guevara *et al.* 2014). This study shows that *OsUGE1* transgenic rice lines maintained 18–24% more sucrose and 12–22% less cellulose in shoots compared to wild type when subjected to sub-optimal N-levels, and maintained proportionally more galactose and glucose in the hemicellulosic polysaccharide profile of plants compared to wild type plants under low Nitrogen.

Moreover, two potato *UGE*s were found to increase the galactose content of potato tuber cell walls (Oomen *et al.* 2004). Also, at least two isoforms of this enzyme are expressed in the developing seeds of cluster beans to produce UDP-D-galactose required for the synthesis of the storage polysaccharide galactomannan (Joersbo *et al.* 1999). In previous studies, five members of the Chinese cabbage (*Brassica rapa*) *UGE* gene family, designated *BrUGE1* to *BrUGE5*, were cloned and characterized. Quantitative RT-PCR shows that the *BrUGE1* and *BrUGE4* mRNA are most abundant among other *BrUGE* genes, accounting for more than 55% of total *BrUGE* transcripts in most of the tissues examined (Jung *et al.* 2015).

Expression of the *UGE*s isolated from different plant species varied, more so in their response to different stresses.

This study was undertaken to investigate the function of *BrUGE1* from *Brassica rapa* in rice. Molecular, morphological, and physiological characterization of *Ubi-1::BrUGE1* transgenic rice under optimal and stress conditions are presented in this paper.

Materials and Methods

Isolation of genes, vector construction and plant transformation

The total RNA was extracted from the young leaves of Chinese cabbage (*Brassica rapa* cv Hwangbok) using the RNeasy Plant Mini Kit (Qiagen). For reverse transcription PCR, 1 µg of total RNA was treated with RNase-free DNase I and first-strand cDNA was synthesized using SuperScriptTM III Reverse Transcriptase (Invitrogen). The cDNA was used as a template for PCR amplification with primers Br15-Fw: 5'-GACTACCATGTGATGGATT-3' and Br15-Rw: 5'-GAAGTCAATGTGTATGGAGAC-3'. The PCR product was subsequently cloned into the *pSB11* vector (Komari *et al.* 1996). The recombinant vector carrying *BrUGE1* was constructed under the control of *Ubiquitin-1* promoter and NOS terminator (Fig. 1A). The *pSB11-BrUGE1* construct was electroporated into *Agrobacterium tumefaciens* EHA105 and introduced into rice using the *Agrobacterium*-mediated transformation method with modification (Lee *et al.* 2011).

Fig. 1. (A) Schematic diagram of the binary Ti plasmid *pFLCIII* containing the *BrUGE1*. *pBigs* vector consisted of two different *Sfi* sites. P35S, CaMV 35S promoter; *pUbi-1*, maize Ubiquitin-1 promoter; Tg7 and Nos, polyadenylation signals from gene 7 and nopaline synthase (nos) gene in the T-DNA, respectively; hpt, hygromycin resistance gene. (B) Southern blot analysis of selected transgenic rice. The HPT gene was used as probe using a non-isotope method (dioxigenin). 1) CB01, 2) CB02, 3) CB03, 4) CB04, 5) CB05 and 6) CB06. (C) Relative expression of *BrUGE1* in transgenic lines and wild type.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was isolated from 1-month-old plants using the method described in Abdula *et al.* (2011) and Cho *et al.* (2007). PCR analysis was performed using *HPT*-Fw: 5'-GGATTTTCGGCTCCAATGTCCTGACGGA-3' and *HPT*-Rv: 5'-CTTCTACACAGCCATCGGTCCAGA-3' primers to check the introgression of hygromycin phosphotransferase gene (*HPT*), and *BrUGE1*-Fw: 5'-GCTTTGCGTGCCTTC TTATC-3' and *BrUGE1*-Rv: 5'-GGAGAACCCACTTGGC AAAA-3' to confirm the presence of *BrUGE1* gene.

To verify further the introgression and activity of the *HPT* gene in transgenic rice, the T₁ seeds were subjected to a germination test on 1/2 MS media treated with 4 mg/L hygromycin. After 14 days, germinated seeds were counted.

Gene expression analysis

Total RNA was extracted from the Chinese cabbage, transgenic rice lines, and wild type plant using the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The relative purity and concentration of extracted RNA was estimated using a NanoDrop-1000 spectrophotometer (NanoDrop Technologies), and stored at -80°C in a freezer. Total RNA were cleaned using DNase I kit (Qiagen), and the first-strand cDNA synthesis was performed by reverse transcription of mRNA using Oligo (dT)₂₀ primer and SuperScript™ III Reverse Transcriptase (Invitrogen). Semi-quantitative reverse transcription PCR (RT-PCR) was performed using the primer pair, BR15-Fw: 5'-GACTACCATGTGATGGATTT-3', BR15-Rv: 5'-GAA CTCAATGTCTATGGAGAC-3' and Actin-Fw: 5'-CCAGC AAGGTCGAGACGAA-3', Actin-Rv: 5'-TGTATGCCAGT GGTCGTACCA-3'. Actin primers were used as an internal control for normalization of the quantitative RT-PCR reaction.

Southern blot analysis

For DNA blot analysis, 10 µg of genomic DNA was extracted as described in Cho *et al.* (2007). A digoxigenin (DIG) probe was synthesized according to the manufacturer's instructions (PCR DIG Probe Synthesis Kit, Roche Molecular Biochemicals, USA). The vector containing the full-length cDNA clone was used as a template for the probe synthesis. The forward/reverse primers used for the synthesis of the probe were 5'-GATTAAGCATCCAAATGTTGT TCA-3' and 5'-CCTACGAGCCTCATCTTCT-3'. The PCR amplification was initiated with a denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 sec, 56°C for 30 sec, and 72°C for 60 sec, and the final elongation step at 72°C for 7 min. Ten µg genomic DNA was digested at 37°C overnight with the restriction enzymes *Bam*HI and *Eco*RI. Digested DNA was subjected to electrophoresis in a 1% agarose gel and was transferred to a Hybond-N⁺ membrane (Amersham Biosciences, USA). The membrane was pre-hybridized at 42°C in a DIG East Hyb buffer for 1 hr. A DIG-labeled probe was then added (2 µl probe/1 ml buffer) and hybridization was carried out overnight at 42°C. The

membrane was then washed twice with a low stringency washing solution (2 × SSC, 0.1% SDS) while incubated at 25°C for 5 min, then high stringency solution (0.1 × SSC, 0.5% SDS) twice at 68°C for 15 min. The DIG-labeled DNA was detected according to the manufacturer's instructions (DIG Nucleic Acid Detection Kit, Roche Molecular Biochemicals, USA).

Crop establishment and drought stress treatment

Three T₃ independent transgenic rice lines (CB01, CB03, and CB06) along with wild type Gopum were sown in planting trays and kept in a greenhouse for three weeks. The seedlings were transplanted in the experimental farm at 30 × 15 cm spacing with one seedling per hill. The experiment was carried out in a randomized complete block design (RCBD) with three replications. Each transgenic rice line was planted in 2 × 2 m² plot size. The fertilizer N-P₂O₅-K₂O was applied at the rate of 90-45-47 kg/ha. Cultivation and management were performed according to the rice cultivation standards of the experimental farm of Chungbuk National University in Korea. The morpho-agronomic characters were evaluated at appropriate growth stages of the transgenic rice (Lee *et al.* 2014).

To induce drought stress in greenhouse, twenty 2-week-old seedlings of T₃ generation were treated with 20% polyethylene glycol 6000 (PEG6000) solution. The set up was supplied with new and fresh PEG6000 solution after eight days. Leaf samples were collected at 0, 6, 12, 24, 36, and 48 hours after treatment for biochemical analyses. Leaf rolling and drying were evaluated when the susceptible variety showed symptoms of drought after three weeks. Plant samples were evaluated using the International Rice Research Institute (IRRI) standard evaluation system for rice (IRRI 2007). The scales were as follows: leaf rolling at vegetative stage; 0 = healthy leaves, 1 = leaves start to fold (shallow), 3 = leaves folding (deep V-shape), 9 = leaves tightly rolled. For leaf drying scale; 0 = no symptom, 1 = slight tip drying, 3 = tip drying extended up to 1/4 of leaves, 9 = all plants apparently dead. Shoot biomass was measured by getting the dry weight of each sample. Plant samples were oven-dried at 60°C to a constant weight. Drought tolerance was also determined three weeks after the treatment by measuring the shoot water status of freshly harvested leaf samples and the dry weight.

Evaluation of agronomic traits

At maturity, ten plants for each transgenic line and wild type were evaluated for plant height, number of productive tillers, 1000-grain weight, percent filled grains, culm length, number of spikelets per panicle, panicle length, and days to heading. Evaluation was similar to that described in Cho *et al.* (2007).

Soluble sugar analysis

Total soluble sugars in leaves were determined using the modified phenolsulfuric acid method DuBois *et al.* (1956).

About 0.1 g of leaves were homogenized in 8 ml of double-distilled water and boiled twice in a water bath at 100°C for 30 min. The extract (about 500 µl) was transferred to a new microcentrifuge tube, with 1.5 ml of double-distilled water, 1 ml of 9% (v/v) phenol, and 5 ml of sulfuric acid added, and kept at room temperature for 30 min. The absorbance was measured at 485 nm in spectrophotometer Optizen Series (Model 2120UV). All measurements were replicated three times.

Photosynthesis measurement

Leaf CO₂ assimilation rate, stomatal conductance, and internal CO₂ concentration (Ci) were measured using an LI-6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE), between 10 AM and noon under saturating PPFD (Photosynthetic Photon Flux Density) (1,500 mmol m⁻² s⁻¹). Ten fully expanded leaves were selected and measured for each transgenic and control plants at the maximum tillering stage. An average of three replication experiments (in each replica average of photosynthesis data of 10 leaves per transgenic and control plants) was taken. Measurements were made for 20 sec immediately after stable decrease in CO₂ concentration inside the chamber was achieved.

Statistical analysis

Data requiring statistical analysis were computed using the Statistix version 8. Significant *P* value was further analyzed using the two-sided Dunnett's multiple comparisons or the least significant difference (LSD) with the wild type as control.

Web-based analysis of the gene

All inserted sequences were checked using the BLAST program in the NCBI sequence database. The open-reading frame (ORF) and conserved domain were generated by the NCBI BLASTN program. Sequence alignment, ORF translation, molecular weight calculation of the predicted proteins, and a structural analysis of the deduced proteins were carried out by DNASTar's Lasergene sequence analysis software.

Results

Characterization of *BrUGE1* gene

During the large scale screening of rice full-length cDNA over-eXpressor gene (FOX) hunting system to abiotic stresses tolerance (Abdula *et al.* 2013), *BrUGE1* (KF601691) was selected for showing improved agronomic traits and elevated tolerance to polyethylene glycol 6000 (PEG6000) induced stress. The full-length cDNA of *BrUGE1* was isolated using degenerative primers revealing a 1,328 bp length including 120 bp 5'-untranslated region (UTR) and 152 bp 3'UTR (Supplemental Fig. 1A). The open-reading frame (ORF) was 1,056 bp encoding a polypeptide of 351 amino acid residues with a predicted molecular weight of 39 kDa. The sequence of *BrUGE1* protein has the characteristic of active site tetrad and NAD-binding motif of the extended

short chain dehydrogenase/reductase (SDR) superfamily (Supplemental Fig. 1B). Extended SDR are distinct from classical SDRs. Phylogenetic tree analysis showed 90.2% identity with *Arabidopsis lyrata* (XM002892666.1) and *Arabidopsis thaliana* (NM101148.3), 44% with *Oryza sativa* Japonica group (AB087745.1), 27.9% with *Hordeum vulgare* (AY943955.1), and 49.9% with *Zea mays* (EU961891.1) (Supplemental Fig. 1C).

Generation and selection of transgenic plant

To understand the functional roles of *BrUGE1*, we introduced the gene (Fig. 1A) into a rice variety, Gopum, using the *Agrobacterium*-mediated transformation method. A total of nine regenerated plants analyzed through PCR were confirmed to carry both *BrUGE1* and *HPT* genes (data not shown). The copy number of the gene in the transgenic lines was determined by Southern blot. Of the six independent lines tested, four were confirmed as having a single copy of the gene (Fig. 1B). These lines were further advanced to next generations (up to T₃) for all the tests and experiments performed in this study. In each generation, a PCR assay targeting both *HPT* and *BrUGE1* genes was performed to check their introgression. Genomic DNA of each independent transgenic rice line was digested with *Bam*HI enzyme. Moreover, the stable inheritance of transgene to the T₁ progeny was confirmed by a resistance test to hygromycin. The segregation ratios between resistant and susceptible plants were 3:1 (71:24, 68:26, 52:17) as expected (Supplemental Table 1). Confirmed plants were used to determine the expression pattern of the *BrUGE1* gene in transgenic rice and wild type. Transcriptional analysis of *Ubi-1::BrUGE1* plants showed an enhanced expression of the *BrUGE1* gene compared with the wild type (Fig. 1C). The levels of expression varied among the transgenic plants. Finally, three uniform lines (CB01, CB03, and CB06) showing strong gene expression were selected. These lines were used in the subsequent experiments.

Photosynthesis rate and agronomic data

Photosynthesis rate (PS), internal CO₂ concentration (Ci), and stomatal conductance (SC) data (Table 1) revealed that transgenic rice lines outperformed the wild type control showing significantly higher levels in the three parameters (P-value <0.01) at 1% level of significance (calculated by

Table 1. Photosynthetic rate of *Ubi-1::BrUGE1* transgenic lines compare with Gopum (WT)

Lines	PS (Photosynthesis rate) [µmol m ⁻² s ⁻¹]	Ci (Internal carbon concentration) [µmol mol ⁻¹]	Stomatal conductance [cm s ⁻¹]
Gopum	20.5 ± 2.09	200 ± 10.05	0.91 ± 0.15
CB01	30.5 ± 1.96*	300 ± 10.09*	1.81 ± 0.24*
CB03	29.2 ± 1.92*	285 ± 12.13*	1.62 ± 0.29*
CB06	30.1 ± 2.01*	270 ± 15.05*	1.74 ± 0.36*

Asterisk (*) means significantly different by LSD at 5% relative to the Gopum.

Table 2. Agronomic traits of *BrUGEI* overexpressing lines and wild type under optimal field conditions at appropriate growth stages

Lines	Chlorophyll content	Days to heading	Plant height	Culm length	Panicle length	Number of tillers	No. of spikelets/ panicles	% filled grains	1000 grain weight
CB01	44.5 ± 2.5	108.0 ± 2.0	105.0 ± 4.0	82.7 ± 2.5	24.5 ± 0.9*	11.3 ± 1.2*	161.0 ± 2.7	93.7 ± 1.8*	22.7 ± 0.8
CB03	44.7 ± 1.2	110.3 ± 0.6	102.0 ± 2.7	82.2 ± 3.5	23.3 ± 0.6*	7.3 ± 1.2	163.3 ± 10.0*	92.1 ± 1.0*	23.1 ± 1.1
CB06	47.6 ± 3.1	108.7 ± 0.6	100.3 ± 2.1	81.6 ± 4.5	22.8 ± 0.7*	7.7 ± 0.6	172.7 ± 15.5*	90.9 ± 2.3	23.2 ± 1.1
Gopum (WT)	41.8 ± 0.9	113.0 ± 1.0	97.3 ± 1.5	80.3 ± 2.0	20.1 ± 0.4	7.3 ± 1.5	154.0 ± 9.2	89.6 ± 1.6	22.6 ± 0.3
LSD _{0.05}	3.9	20.8	7.4	6.4	1.8	2.5	8.4	2.8	2.4
CV(%)	3.54	5.63	2.89	3.10	3.15	11.88	6.90	1.19	4.22

Asterisk (*) means significantly different by Dunnett's multiple comparison with the wild type Gopum.

MINITAB 13 software package). In particular, the CB01 line exhibited the highest gain with 48% more photosynthesis rate, 50% more internal CO₂ concentration, and 98% more stomatal conductance than the wild type.

Three independent lines (CB01, CB03, and CB06) were evaluated for agronomic characters under optimal field conditions (Table 2). Results showed that these transgenic lines were not significantly different from the wild type in terms of days to heading, plant height, culm length, and 1,000 grain weight. The chlorophyll content, although, not significantly different, was slightly elevated in the transgenic lines. Interestingly, a significant increase was observed in panicle length, number of productive tillers, number of spikelets per panicle, and percent filled grains in transgenic lines. The transgenic lines produced at most 4 more tillers, 7 to 18.7 more spikelets per panicle, at most 4.1% more filled grains, and were 2.7 to 4.4 cm-longer in panicle length than the wild type. On average, the wild type was reported to show 86% ripened grain (Choi *et al.* 2007), but in this experiment it showed 89.6%.

Drought evaluation

Confirmed *BrUGEI* transgenic rice lines (CB01, CB03 and CB06) were subjected to osmotically induced drought stress using 20% PEG6000. Phenotypic measurement was done when the susceptible check, Sangnam, showed severe rolling and drying of the leaves. All transgenic lines exhibited significant differences from the wild type (LSD_{0.05}) (Table 3) while their phenotype resembled the tolerant check, Gaya, which displayed a healthy and vigorous growth (Fig. 2). Visual phenotypic observations using the standard evaluation system (SES) for rice (IRRI 2007) showed that the wild type displayed a deep V-shape (moderately severe) leaf folding (5.2 score) while the transgenic lines displayed shallow folding of leaves (1.6~2.1 score). Scores among the transgenic rice plants were not significantly different from each other. The leaf drying scores of these transgenic lines were also lower (2.1~2.2; slight tip drying) than the wild type (5.5; tip drying extended up to 1/2 of leaves). In terms of water content, the highest amount was recorded in CB01 with 79.4%, relatively higher than the wild type (40.3%). The biomasses of these transgenic lines were relatively higher (0.36~0.39 g) than in wild type (0.19 g).

Table 3. Response of *Ubi-1::BrUGEI* rice lines and wild type plants after application of 20% PEG6000 for 14 days using 2-week-old seedlings

Lines	Leaf rolling score ¹	Leaf drying score ²	Water content (%)	Biomass (g)
Gopum	5.2 ^a	5.5 ^a	40.3 ^a	0.19 ^a
CB01	1.7 ^c	2.1 ^b	79.4 ^c	0.39 ^b
CB03	2.1 ^b	2.2 ^b	65.5 ^b	0.36 ^b
CB06	1.6 ^b	2.1 ^b	64.7 ^a	0.38 ^b

¹ Leaf rolling score 0 to 9: 0 = leaves healthy and 9 = leaves tightly rolled.

² Leaf drying score 0 to 9: 0 = no symptoms and 9 = seedlings dead. Means followed by a letter (a, b, and c) in a column are not significantly different at 5% level using LSD.

Fig. 2. Visual phenotypic observations of *BrUGEI* overexpression lines along with control plants under water deficit treatment.

Temporal expression of *BrUGEI* under drought conditions in rice

Transcripts of *BrUGEI* accumulated at different levels after 20% PEG6000 treatment both in transgenic lines and wild type (Fig. 3). About a 19-fold change (highest level) was recorded in the transgenic lines (CB06) 6 h after the treatment which continued to increase after 12, 24, 36 h, and reached the peak at 48 h with level highest in CB01. The wild type also showed an increasing trend but only at a minimum level, which peaked earlier (~7-fold change) after 36 h and started to diminish at 48 h.

Soluble sugar analysis under PEG6000 treatment

To determine osmoprotectants related to drought tolerance, osmolytes sugars were measured in the three transgenic lines. No significant difference was observed among the

Fig. 3. Temporal expression of *BrUGE1* in transgenic lines as induced by PEG treatment. Real-time quantitative PCR expression analysis of *BrUGE1* gene was determined at 0, 6, 12, 24, 36, and 48 h after PEG treatment. *Actin* was used as an internal control.

Fig. 4. Soluble sugar contents of *BrUGE1* overexpression lines under PEG treatment. Significant difference (*) with wild type at 5% level of Dunnett's comparison.

plants tested (Fig. 4) prior to stress treatment (0 h). At 6 h, a significant increase was observed in the transgenic plants exhibiting a several fold times change in expression higher than the wild type. CB01 line showed the highest accumulation of soluble sugars.

Discussion

UDP-glucose 4-epimerase (EC 5.1.3.2), also known as UDP-galactose 4-epimerase or GALE, is a nucleotide inter-conversion enzyme (Shaw *et al.* 2003) which performs the final step in the Leloir pathway of galactose metabolism, catalyzing the reversible conversion of UDP-galactose to UDP-glucose (Holden *et al.* 2003).

To our knowledge, this is the first report on *UGE1* from *Brassicca rapa*. The gene was closely related to *UGE1* in

Arabidopsis thaliana, the dominant isoform in green plant parts (Dormann and Benning 1998) which was previously reported to function in carbohydrate catabolism (Barber *et al.* 2006).

The recombinant vector carrying *BrUGE1* was constructed under the control of *Ubiquitin-1* promoter and NOS terminator as shown in Fig. 1A and transformed into rice using the *Agrobacterium*-mediated transformation method (Lee *et al.* 2011).

Under optimal field condition, these transgenic lines (CB01, CB03, and CB06) showed an improved panicle length, number of tillers, number of spikelets per panicle, and percentage filled grains (Table 2). This finding was paralleled with higher photosynthesis rates in the leaves of overexpressing lines contributed by higher stomatal conductance and higher internal CO₂ concentrations (Table 1). Our data on spatial expression of the gene in the source material, *Brassica rapa*, likewise showed an abundance of the gene's transcript in leaf tissue (Supplemental Fig. 2) supporting the idea of its probable role in photosynthesis. Recent studies have implicated the important function of UGE in photosynthesis, although not in CO₂ fixation but in the biosynthesis of mono- and digalactosyldiacylglycerol (MGDG), which account for the majority of the total thylakoid lipids that are crucial for a well-developed chloroplast thylakoid system (Li *et al.* 2011). This tempts us to speculate the probable extended role of this gene in the Calvin system.

Since PEG is a flexible, water-soluble polymer, it can be used to create very high osmotic pressures and is unlikely to have specific interactions with biological chemicals (Hsiao *et al.* 1984). By taking into account leaf rolling and drying, the two most obvious phenotypic signs of a water deficit condition, the transgenic lines displayed enhanced tolerance (Fig. 2, Table 3), showing a lower degree of leaf rolling and tip drying than the wild type. Delayed leaf rolling was postulated as a mechanism in plants to escape drought (Dingkuhn *et al.* 1991) by adjusting their leaf water potential to allow them to absorb soil water in a more efficient manner; and this process, being uncommon for better-adjusting plants does not prohibit their photosynthesis (Bunnag and Pongthai 2013). Accordingly, the water content and dry matter in transgenic lines were significantly higher than in wild type under PEG stress (Table 3). Moreover, our data suggest that *BrUGE1* improved tolerance to PEG stress by regulating soluble sugar induction which was found to accumulate immediately following imposition of stress in transgenic lines at continuously increasing levels (Fig. 4). Under drought stress, cell turgidity collapsed as a result of limited water. Plants resorted to many adaptive strategies in response to this stress such as dehydration and excessive osmotic pressure which include changes in physiological and biochemical processes. Among these is the accumulation of compatible solutes which is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars (Mohammadkhani and Heidari 2008). Soluble sugars act as nutrients and metabolite signaling

molecules that activate specific or hormone-crosstalk transduction pathways, thus resulting in important modifications of gene expression and proteomic patterns. Their involvement has been ascribed in reactive oxygen species balance and responses to oxidative stress in plants (Couée *et al.* 2005).

In summary, *BrUGE1* performs overlapping functions previously demonstrated by several *UGE* isoforms in *A. thaliana*. It plays a role in enriching growth development in rice seemingly by increasing the photosynthesis rate. In depth information on how this gene regulates photosynthesis, however, remains to be seen. Moreover, *BrUGE1* enhances tolerance to drought stress by rapid accumulation of soluble sugars which act as osmoprotectants, leading to delayed leaf rolling and drying.

Acknowledgments

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