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ORIGINAL ARTICLE

Molecular characterization of the UDP-glucose 4-epimerase (*BrUGE*) gene family in response to biotic and abiotic stress in Chinese cabbage (*Brassica rapa*)

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Abstract UDP-glucose 4-epimerase (UGE; EC 5.1.3.2) is an enzyme that plays an essential role in the interconverts UDP-D-glucose (UDP-Glc) and UDP-Dgalactose (UDP-Gal). Five members of the Chinese cabbage (*Brassica rapa*) UGE gene family, designated *BrUGE*1 to *BrUGE*5, have been cloned and characterized. Quantitative 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. All genes showed organ-specific expression pattern, two of which (*BrUGE1* and *4*) actively responded after *Pectobacterium carotovorum* subsp. *carotovorum* infection, while four genes (*BrUGE-1, -3, -4, and -5*) were shown to respond considerably against salt, drought and abscisic acid treatments. To better understand the function of the UGE gene, we constructed a recombinant pART vector carrying the *BrUGE1* gene under the control of the CaMV 35S promoter and nos terminator and transformed using *Agrobacterium tumefaciens*. We then investigated *BrUGE1* overexpressing rice lines at the physiological and molecular levels under biotic and abiotic stress conditions. Bioassay of T_3 progeny lines of the transgenic plants in Yoshida solution containing 120 mM NaCl for 2 weeks, confirmed that the *BrUGE1* enhances salt tolerance to transgenic rice plants. Also T_3 progeny lines of the transgenic plants, when exposed to infection caused by *Xanthomonas oryzae* pv. *oryzae*, showed tolerance to bacterial blight. These results showed that *BrUGE1* can be used as potential genetic resource for engineering *Brassica* with multiple stress resistance.

Keywords Chinese cabbage · Bacterial blight · Gene expression · Plant cell wall · Transgenic plants · UDP-D-glucose 4-epimerase (UGE)

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Introduction

Plant cell walls are chemically complex, diverse structures that are modified throughout the processes of cell division, cell growth, and differentiation, and in response to abiotic and biotic stresses. During the process of cell wall biosynthesis the different glycosyl transferases use varying nucleoside-diphosphate (NDP) sugars to assemble the different cell wall polysaccharides (Feingold and Avigad 1980). The availability of the NDP-sugars is thus an important factor in determining the final polysaccharide composition. Nevertheless, it is unclear to what extent modifications in the pool size of a particular NDP-sugar can affect the presence or structure of cell wall polysaccharides. Plants possess a sophisticated sugar biosynthetic machinery comprising families of nucleotide sugars that

can be modified at their glycosyl moieties by nucleotide sugar interconversion enzymes to generate different sugars (Seifert 2004). UDP- glucose 4-epimerase (also UDP-galactose 4-epimerase, UGE, EC 5.1.3.2) catalyzes the interconversion of UDP-GIc and UDP-Gal (Majumdar et al. 2004). UGEs identified from plants lack transmembrane motifs and signal peptides and appear to exist as soluble entities in the cytoplasm (Barber et al. 2006). Generally, plant UDP-GIc epimerase enzymes are localized to the cytosol, where their substrates UDP-GIc and UDP-Gal are present at high levels (Seifert 2004; Pattathil et al. 2005). UDP-glucose is the most important of the NDP-sugars. It can be synthesized either from glucose 1-phosphate using the reaction NTP + sugar 1 − phosphate ↔ NDP-sugar + pyrophosphate or from sucrose using the enzyme sucrose synthase (Nakai et al. 1999). The gene encoding the UDP-glucose epimerase has been cloned from a variety of organisms (Dörmann and Benning 1998). In *Arabidopsis thaliana*, five UGEs were present and each UGE had different catalytic properties (Seifert 2004; Barber et al. 2006). *AtUGE2* and *AtUGE4* cooperate in providing cell wall biosynthesis and growth, *AtUGE3* is specialized for pollen development, and *AtUGE1* and *AtUGE5* might act in stress situations in *Arabidopsis* (Mayer et al. 1999; Theologis et al. 2000; Rösti et al. 2007). In rice, four UGE genes (*OsUGE1-4*) were activated after drought, salt, or UV irradiation stress (Liu et al. 2007; Kim et al. 2009; Abdula et al. 2013; Joo et al. 2014). Indeed, drought stress induces increased expression of a *UGE* gene, which maps to a root thickness quantitative trait locus (QTL) region (Nguyen et al. 2004). Overexpression of *OsUGE-1* altered raffinose level and tolerance to abiotic stress but not morphology in *Arabidopsis* (Liu et al. 2007). Similarly, two potato UGEs increased the galactose content of potato tuber cell walls (Oomen et al. 2004). The expression of the UGEs isolated from different plant species was found variable in response to different stresses. Until recently, no study has been reported about the UGE in Chinese cabbage, which is one of the important crops in Asia. This study investigated the *UGE* gene subfamily of Chinese cabbage. Five *BrUGE* genes belonging to the same family were identified from the Brassica database. cDNAs were isolated and expression profiling in different organs was analyzed following application of different biotic and abiotic stresses. In particular, this paper describes the expression analysis of these genes under different biotic and abiotic stresses and the overexpression of *BrUGE1* gene in rice plants using the CaMV 35S promoter. The expression patterns and physiological effects in both overexpressing and wild-type plants under biotic and abiotic stress conditions were also investigated.

Materials and methods

Plant tissues sampling

Chinese cabbage (*Brassica rapa* 'SUN-3061') plants were grown at the Department of Horticulture, Hankyong National University, Korea. Fresh roots, stems, leaves, and flower buds of the Chinese cabbage were harvested, immediately frozen in liquid nitrogen, and then stored at −80 °C until subsequent analysis in an organ-specific expression study.

Abiotic stress treatments

Chinese cabbage seeds were aseptically grown on half-strength MS (HMS) agar medium in a culture room under a 16 h light photoperiod at 25 °C. After 3 weeks of growth, the seedlings were transferred to fresh liquid HMS medium containing 250 mM NaCl for salt stress, and 100 μM ABA. In addition, drought stress treatment was applied by keeping the seedling on filter paper for 24 h. For each stress, the leaf samples were collected after 0, 30 min, 1, 2, 4, 8, 12, and 24 h of treatment and each sample was collected three times using two individual plants.

Biotic stress treatments

Two of the most important pathogens that constantly challenge Chinese cabbage production worldwide include *Fusarium oxysporum* which causes wilt and *Pectobacterium carotovorum* which causes soft rot. Chinese cabbage (*B. rapa* 'SUN-3061') plants were infected with *F. oxysporum* f.sp. *conglutinans* at the Screening Center for Disease Resistant Vegetable Crops, Korea. The root-dip inoculation (RDI) method was used with some modifications to inoculate the cabbage and Chinese cabbage with the fungus (Ospina-Giraldo et al. 2003). Briefly, 3-week-old seedlings were removed from the soil and immersed in the conidial suspension. Samples were then collected from infected and mock-treated plants at 0, 3, 6, 24 h, 6, 8, and 11 days. The local (fourth) and systemic (fifth) leaves were harvested as samples. Chinese cabbage (*B. rapa* 'SUN-3061') plants were grown for 6 weeks under culture room conditions with 16 h light and 8 h dark maintaining 25 °C temperature prior to treatment. The *P. carotovorum* subsp. *carotovorum* stock (10 μl) was cultured in 25 ml of liquid YEP medium until $OD_{600} = 1.4$ equals 1,170,000 colony forming units (cfu) ml^{-1} and then diluted it to $OD_{600} = 1.19$ equals 1×10^6 CFU ml^{-1} by adding double-distilled water. For pathogen inoculation, 10 μl of *P. carotovorum* subsp. *carotovorum* culture solution (1×10^6 cfu ml^{-1}) was added to the freshly needle wounded site (at

the lower 1/3 position) of the midrib of the upper third leaves, and incubated at 25 °C covering with polyvinyl bags to maintain 80–90 % humidity. All inoculations were performed three times, and the infection was confirmed by observing disease lesion in the leaves of Chinese cabbage plants. About one-third parts from the top of the infected leaves were harvested for RNA extraction at 0, 6, 12, 24, and 72 h after inoculation. Upon collection of tissues for both biotic and abiotic treatments, the samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA isolation.

RNA extraction

Total RNA were extracted using the RNeasy mini kit (Qiagen, USA), after which it was treated with RNase-free DNase (Promega, USA) to remove genomic DNA contaminants. The cDNA was subsequently synthesized using a Superscript® III First-Strand synthesis kit (Invitrogen, USA) according to the manufacturer's instructions.

Amino acid sequence analysis of *BrUGE* genes

We constructed a full-length cDNA library of *B. rapa* cv. Osome (Park et al., 2010) and obtained 3429 expressed sequence tags (ESTs). This dataset was subsequently analyzed for gene ontology and found 1017 ESTs functionally annotated to stress responses. Based on the cluster analysis of these stress responsive genes, 140 are related to biotic stresses, two of which (*BrUGE*1 and *BrUGE*2) were annotated as UDP-glucose 4-epimerase (UGE) proteins (unpublished). Using these two UGE proteins as reference, 3 more genes (*BrUGE3* to *BrUGE5*) belonging to the same family were recovered from the *Brassica* database (http:// brassicadb.org/brad/index.php). The primary structure of genes was analyzed using protParam (http://expasy.org/ tools/protparam.html) and Augustus (http://augustus. gobics.de/submission). An alignment search was conducted using NCBI BLAST (http://www.ncbi.nlm.nih.gov/ BLAST/) and the program BLASTp, with the "nr" database. Typical domains were analyzed using the EMBL web tool (http://smart.embl.de/smart/set.mode.cgiGENOMIC= 1). Multiple protein sequences were aligned using PIR (http://pir.georgetown.edu/pirwww/search/multialn.shtml), and a phylogenetic tree was constructed according to the neighbor-joining method using the ClustalW program (http://www.genome.jp/tools/clustalw/) (Thompson et al. 1994). Accession numbers are as follows: from *A. thaliana* At1g12780, At4g23920, At1g63180, At1g64440, At4g10960 coded as *AtUGE1* t0 *5*, respectively; XP_001698706 (*Chlamydomonas reinhardtii CrUGE1*); O65781 (*Cyamopsis tetragonoloba CtUGE1*); XP_643834 (*Dictyostelium discoideum DdUGE1*); NP_001035389

(zebrafish *DaniorerioDrUGE1*); Q14376 (*Homo sapiens HsUGE1*); from barley *Hordeum vulgare,* AAX49504, AAX49505, AAX49503 (*HvUGE1*–3); from *Medicago truncatula*, ACJ85116 and ACJ84690 (*MtUGE1*–2); BAF51705 (apple *Malus × domestica MxdUGE1*); *OsUGE1* from rice (*Oryza sativa*), Os05g0595100, Os08g0374800, Os09g0526700, Os09g0323000 for *OsUGE1*–4; CAL54894 (*Ostreococcus tauri OtUGE1*); from *Physcomitrella patens* subsp. *patens*, XP_001768301, XP_001777464, XP_001775163, XP_001751529 for *PpUGE1*–4; and AAP68981 (*Zea mays ZmUGE1*).

Expression analysis

RT-PCR was conducted using an AMV one step RT-PCR kit (Takara, Japan). Specific primers for all the genes were used for RT-PCR, and actin primers of *B. rapa* (FJ969844) were used as a control (Table 1). PCR was performed using 50 ng of cDNA from the roots, leaves, stems and flower buds as templates in master mixes composed of 20 pmol of each primer, 150 μM of each dNTP, 1.2 U Taq polymerase, 1 × Taq polymerase buffer, and double-distilled H_2O diluted to a total volume of 20 μl in 0.5 ml PCR tubes. The samples were then subjected to the following conditions: pre-denaturing at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 1 min, with a final extension for 5 min at 72 °C. Real-time quantitative PCR was performed using 1 μl of cDNA in a 25 μl reaction volume employing iTaqTM SYBR® Green Super-mix with ROX (California, USA). The specific primers used for real-time PCR are listed in Table 1. The conditions for real-time PCR were as follows: 10 min at 95 °C, followed by 40 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. The fluorescence was measured following the last step of each cycle, and three replications were used for each sample. Amplification, detection, and data analysis were conducted using a Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Science, Australia).

Vector construction and plant transformation

The full-length cDNA of BrUGE1 (Accession No. KF601691) was isolated by PCR from *Brassica* using the primers 5′-CCGCTCGAGATCAAGCTATGTCCGAGAA GG-3′ (*Xho*I site underlined) and 50-CGGGGTACCT CAGG CTGGTCTCTGCACATCTCCT-3′ (*Kpn*I site underlined). The product was ligated into the pART vector (Gleave 1992). The recombinant vector carrying BrUGE1 was constructed under the control of the CaMV 35S promoter and nos terminator. The pART-UGE1 construct was electrophoresed into *Agrobacterium tumefaciens* LBA4404 and then introduced into pre-soaked rice seed using the

Table 1 qRT-PCR primer sequences and product sizes of UDPs-glucose 4-epimerase (UGE) genes

Primer name	Primer pair (5′-3′)	Amplicon size
*BrUGE*1	F: ATCAAGCTATGTCCGAGAAGG	120 bp
	F′: CCAAAGGGTATCCCTAATAACC	405 bp
	R: CTGGTCTCTGCACATCTCCT	
*BrUGE*2	F: GATTGTTTACGCCTCAACAG	122 bp
	F′: GGTTCCAAACAATCTCATGC	430 bp
	R: GGCGTAGCCGTAAGGATTAT	
*BrUGE*3	F: ATGCTTCAACTGAGAGAGC	120 bp
	F′: AATCCTGTTGGAGCTCACGA	469 bp
	R: GCTTTTCATGGAAACCCCAT	
*BrUGE*4	F: GGTGTACGCATCAACCGAAA	120 bp
	F′: AACCTCATGCCTTATGTCCA	422 bp
	R: GAACCGTAACCGAGAGGATT	
*BrUGE*5	F: CTATGAAGCTCTGTCCGAGA	121 bp
	F′: CAATCTCATGCCTTTTGTCC	404 bp
	R: TCAGGCATCAGAGGAATCAT	
BrActin 1	F: CAACCAATCGTCTGTGACAA	106 bp
	R: ATGTCTTGGCCTACCAACAA	
BrActin 2	F: ATTCAGGCCGTTCTTTCTCT	580 bp
	R: CCTTGATCTTCATGCTGCTT	

F Real-time qRT-PCR forward primer, *F′* RT-PCR forward primer, *R* reverse primer for qRT and RT-PCR

method of Lee et al. (2011) with minor modifications (Supplemental Fig. 3).

PCR analysis of transgenic rice plants

Four-week-old rice seedlings were analyzed using PCR amplification to select transgenic lines. DNA was extracted from the leaves of the rice seedlings using the cetyl-trimethyl ammonium bromide (CTAB) method (Rogers and Bendich 1994). The primer set for the PCR amplification and the probe for the Southern blot analysis were *BrUGE*-F2 and *BrUGE1*-R2. The PCR amplification profile consisted of an initial step at 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and a final step at 72 °C for 10 min.

Expression analysis of transgenic lines

Total RNA from leaf tissue was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. The specific primers for the analysis of *BrUGE1* expression of germinating seeds were *BrUGE1*-F3 5-ATGCCTTCTTCTGGAGATCC-3′ and *BrUGE1*-R3 5′-GTTCGTTGGACGATGAAGGT-3′. Real-time PCR was performed using a Bio-RAD I Cycler IQ5 machine as previously described using RT pre-mix (TOYOBO Co., Japan) (Ali-Benali et al. 2005). The threshold cycle (Ct) values of PCR reactions from three independent biological replicates were averaged and the relative quantification of the expression levels was performed using the comparative Ct method for all experiments (Livak and Schmittgen 2001). The fold change in total RNA of a target gene relative to the reference gene (actin gene) was determined by the following formula: fold change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ = (Ct target gene − Ct actin gene) transgenic plants − (Ct target gene − Ct actin gene) wild-type plants.

Bioassay for bacteria blight

Six-week-old plants and controls were inoculated with K3a (race 3) using the leaf-clipping method (Kauffman et al. 1973; Jung et al. 2012). Inoculum suspension density was about ∼1 × 10⁷ cells per milliliter. The inoculated plants were covered with polythene bag for 24 h and incubated under the following conditions: 4 and 23 h following humidity at night. Plant reaction to bacteria was scored on six leaves by measuring the lesion lengths 14 days after inoculation.

Statistical analysis

Data requiring statistical analysis were computed using the Statistix version 8 (http://www.statistix.com). Significant *P* values were analyzed using the two-sided Dunnett's multiple comparisons with the WT Dongjin and MOCK as a reference.

Results

Identification and sequence analysis

In this study, we identified 5 UGE, designated *B. rapa* UDP-glucose 4-Epimerase (*BrUGE 1–5*). Gene sequences were analyzed, and are presented in Table 2. The size of the 5 *BrUGE*s varies from 343 to 351 amino acids (~37.7–39.1 kDa) and the predicted isoelectric points vary from 5.28 to 6.61 (Table 2). All *BrUGE*s contained the conserved β-NAD+ binding domain (GXXGXXG, where X is any amino acid) at the N terminal. The catalytic triad (YXXXK) conserved in UGEs (Weirenga et al. 1986) was found in all *BrUGE*s. Genomic DNA sequences of all *BrUGE* genes were isolated from the *B. rapa* chromosome sequences and the introns and exons were identified upon sequence analysis. Structural information pertaining to the 5 *BrUGE* genes is presented in Fig. 1. The sequence data revealed that the identified genes are UGEs which contain conserved β-NAD+ binding domain. For comparison of these *BrUGE*s with other published UGEs, an alignment search was carried out using a BLAST search of the NCBI database. The deduced amino acid sequences of 5 BrUGEs shared high homology, UGEs of *A. thaliana* and some other homologous species (Table 3). Amino acid sequence among these 5 *BrUGE*s of *B. rapa* was showed 60–80 % homology (Supplemental Fig. 1). We again retrieved 32 UGE sequences of different plant species from NCBI and a phylogenetic tree was constructed with the deduced amino acid sequences of 5 UGEs of *B. rapa* using the NJ method (Fig. 2). Results revealed two groups; *BrUGE1* and *3* together with 16 UGEs from other crops formed Group I, and *BrUGE2*, *4* and *5* among other 14 UGEs fell under Group II. In addition, phylogenetic relationships of *BrUGE1* with the enzymes from other origins including mammals, insects, fungi, and bacteria were analyzed (Fig. 2). Together with *AtUGE1*, *AtUGE3*, *OsUGE3*, and several other plant enzymes, *BrUGE1* formed a plant subgroup apart from mammalian and bacterial enzymes.

Organ-specific expression analysis

Although all *BrUGE* genes showed considerable expressions in roots, stems, leaves, and flower buds, expression pattern of each gene seemingly occurred in an organ-specific manner. In *BrUGE*1, 2 and 3, transcripts were

Table 2 Overall analysis of UDP-glucose 4-epimerase (UGE) genes identified from cDNA library of *Brassica rapa* cv. Osome and collected from the *Brassica* database

Name	Accession number	ORF (bp)	Chromosome number	Protein				
				Length (aa)	UGE domain start and end		Mol. wt. (kDa)	PI
					Catalytic motifs	Active site		
*BrUGE*1	Bra016757	1056	A08	351	13–19	162–167	39.1	6.61
*BrUGE*2	Bra013744	1047	A01	348	9–15	157–162	38.3	6.52
*BrUGE*3	Bra027626	1056	A09	351	13–19	162–167	38.9	5.87
*BrUGE*4	Bra027732	1032	A09	343	9–15	157–162	37.7	5.97
*BrUGE*5	Bra035262	1044	A08	347	10–16	158–163	38.1	5.28

*Brassica* database (http://brassicadb.org/brad/index.php) accession number

Fig. 1 Gene structure of 5 UDP-glucose 4-epimerase (UGE) genes of *Brassica rapa*. *Solid boxes* and *lines* indicate exons and introns, respectively. The figures above the *solid boxes* and the *lines* indicate the length of exons and introns, respectively

Table 3 Homology analysis of 5 UDP-glucose 4-epimerase (UGE) genes of *Brassica rapa*

Gene	Top matched clones	Name of protein	Identify	Top homologous species	References
*BrUGE*1	NP172738	UGE	96 %	*A. thaliana*	Theologis et al. (2000)
*BrUGE*2	NP194123	UGE	94 %	*A. thaliana*	Mayer et al. (1999)
*BrUGE*3	NP564811	UGE	94 %	*A. thaliana*	Theologis et al. (2000)
*BrUGE*4	NP176625	UGE	93 %	*A. thaliana*	Theologis et al. (2000)
*BrUGE*5	NP192834	UGE	95 %	*A. thaliana*	Mayer et al. (1999)

Analyzed using BLAST from NCBI, http://www.ncbi.nlm.nih.gov/BLAST/

Fig. 2 Phylogenetic tree showing the relatedness of the deduced full-length amino acid sequences of 5 *BrUGEs* and 32 UGE proteins of other plant species. The phylogenetic tree was generated using ClustalW program. The species names and the GenBank protein accessions numbers are described in materials and methods. Enzymes shown to have high UDP-glucose 4-epimerase activity are boxed. The bootstrap values for the respective branches are shown. The *bar* indicates 0.1 substitution per site
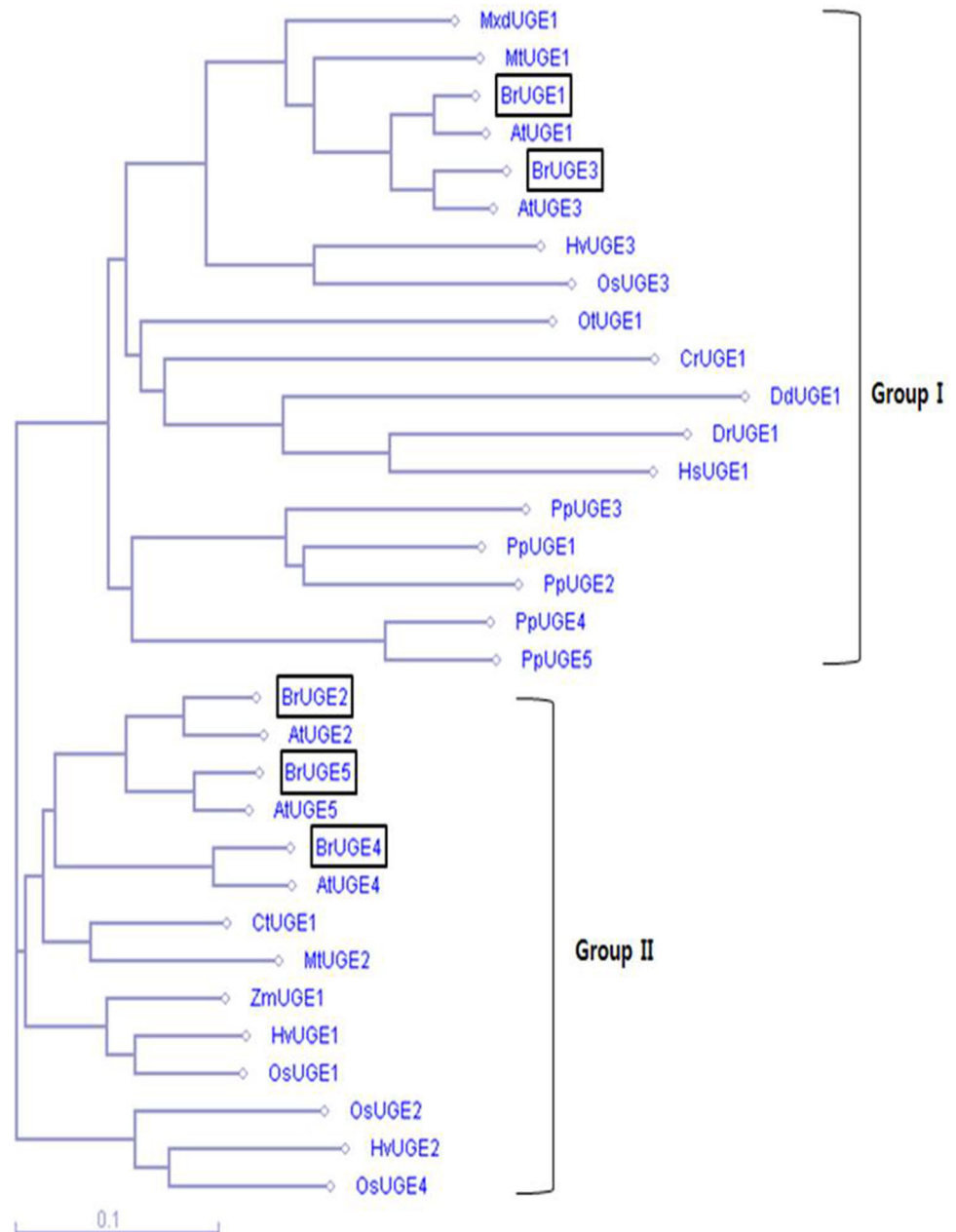
highest in the leaves. In *BrUGE4*, gene expression was highest in roots, while in *BrUGE5*, the expression peaked in flower buds. Among the organs tested, expression in stem was the lowest for *BrUGE1*, *3* and *5*, whereas mRNA of *BrUGE2* and *4* were very low in the flower buds (Fig. 3). It is also worth considering that overall expression of these genes was higher in leaves and roots than in stems and flower buds.

BrUGE expression in response to abiotic stresses

As UGEs have been shown to express in response to abiotic stress conditions (Rajam et al. 2007; Alam et al. 2014), responses of the 5 *BrUGEs* identified in this study, were investigated after salt, drought, and ABA stresses on seedling plants (Fig. 4). Among the 5 *BrUGEs*, four showed differential expression pattern. The genes that were upregulated throughout the whole observation time (0–24 h) include *BrUGE1* and *5* during salt stress treatment, *BrUGE4* and *5* in response to drought stress, and *BrUGE4* in response to ABA stress. On the other hand, expression of *BrUGE2* was down regulated during early observation time in three treatments. Specifically,

expression response of this gene spiked only after 12 h of treatments both in drought and ABA stress, while it was only expressed mildly against salt stress. Notable strong expression at 12 h of ABA treatment was observed with *BrUGE3*, *4* and *5*, and 12 h of drought condition with *BrUGE2* and *5*, as indicated by high intensity band (Fig. 4).

BrUGE genes expression under biotic stresses

In addition to abiotic stresses, responses of the 5 *BrUGEs* were also investigated against the infection of *F. oxysporum* f.sp. *conglutinans* in Chinese cabbage at various times after exposure. Here, no responsive expression was observed (Supplemental Fig. 2), indicating that the genes may not be effective against broad range of pathogens. To explore their possible role against phytobacteria, analysis of expression of 5 *BrUGEs* was performed after inoculating *P. carotovorum* subsp. *Carotovorum,* a necrotroph bacterium that causes soft rot, a serious problem in *Brassica* production. Expression of *BrUGE1* and *4* were relatively higher among other *BrUGEs*, with levels that peaked at 6 h post inoculation (hpi). Low levels of expression were

Fig. 3 Real-time quantitative PCR expression analysis of 5 *BrUGE* genes in leaves, roots, flower buds, and stems of *Brassica rapa* plants were determined by qRT-PCR in leaves, roots, flower buds, and stems *Brassica rapa* "SUN-3061"SUN-3061′ plants. The *error bars* represent the standard error of the means of three independent replicates. *Letters* indicate significant differences between tissues for each gene
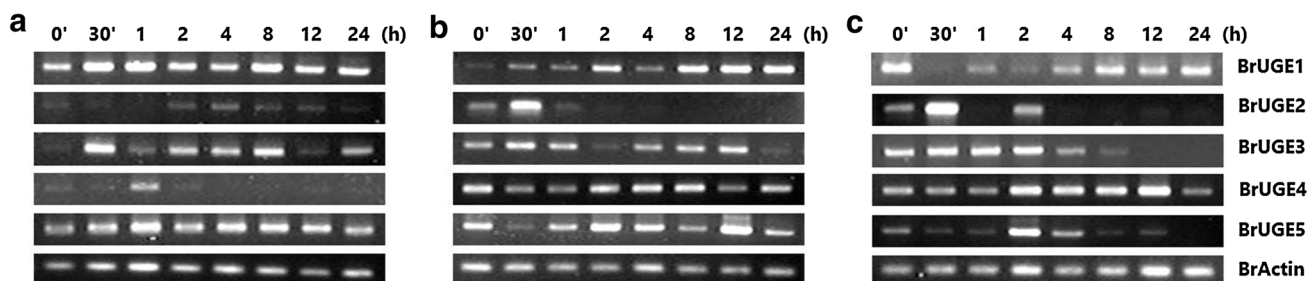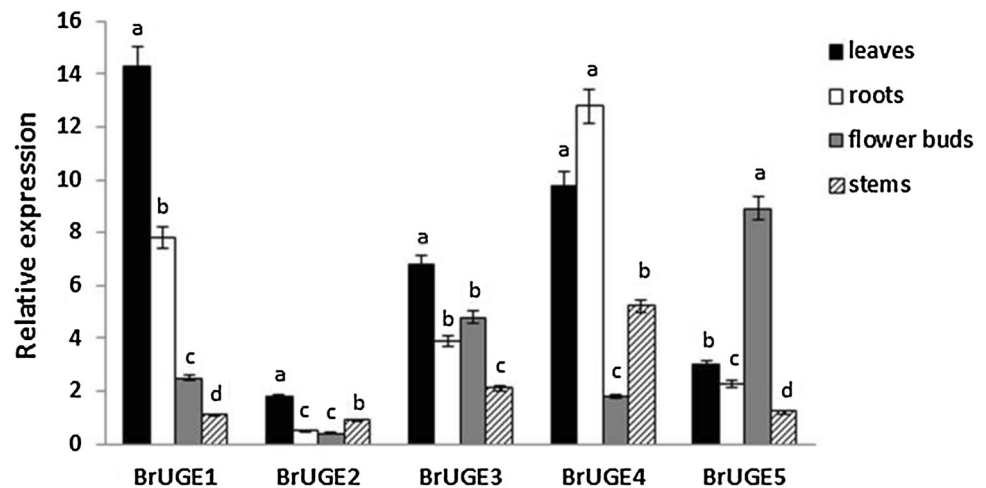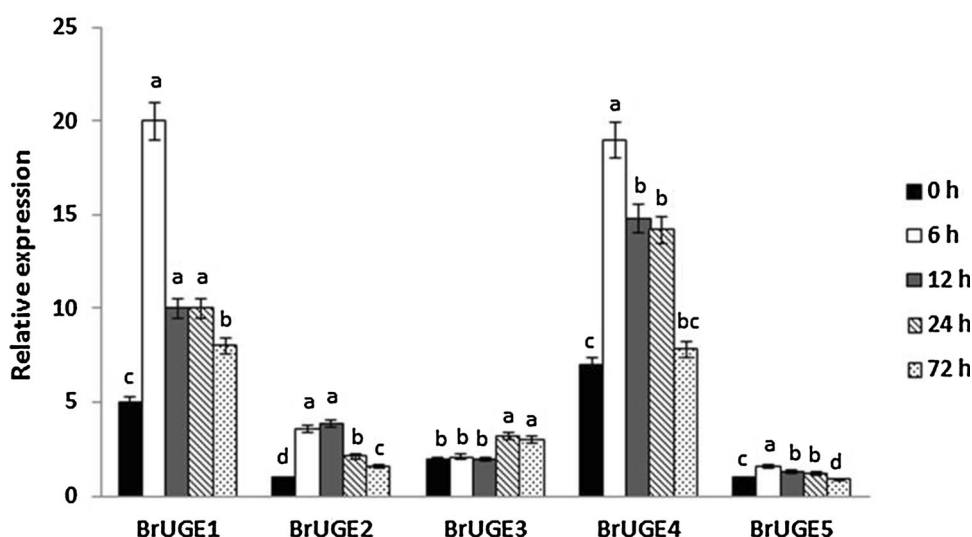

Fig. 4 Semi-quantitative RT-PCR expression of the 5 *BrUGE* genes in leaves of seedling *Brassica rapa* after application of 250 mM of NaCl (**a**), drought (**b**), or 100 μM of ABA (**c**)

observed for *BrUGE2,3,* and *5*, which initially showed an increasing pattern, reached the plateau at 6–24 h and which eventually declined until 72 h (Fig. 5). The *BrUGE1* and *3* in Group I were responsive to the salt stress as sensitive markers, while there was no clear pattern regarding the response of three *BrUGEs* in Group II to various abiotic and biotic stresses except for drought stress where *BrUGE4* and *5* were responsive.

Characterization and expression of UGE1 transgenic rice

A total of 35 regenerated plants were analyzed by PCR. We confirmed that 86 % of the 35 T_0 plants were positive with the UGE1 gene-specific primers. mRNA transcript analysis of CaMV 35S::BrUGE1 plants showed an enhanced expression of the *BrUGE1* gene compared to that of the WT, Dongjin. Although the transgenic plants showed *BrUGE1* overexpression, variability in the degree of expression was observed. Along with this, we selected five uniform lines with strong expression and named these as BrUGE1-OX5. These selected lines were used in all subsequent experiments. Furthermore, we investigated mRNA expression in various organs. BrUGE1-OX1-5 was selected for expression analysis in young leaves among the transgenic lines. The results showed that the *BrUGE1* mRNA transcript was highly expressed in young seedlings except for BrUGE-OX1 and BrUGE-OX5 (Fig. 6). As expected, Dongjin (WT) also showed an mRNA transcription was not expressed in young seedling (Fig. 6). To examine NaCl treatment's effect in UGE1-overexpressing (TG#1 and TG#2) and WT plants, 2-week-old seedlings were grown in hydroponics treated with 300 mM NaCl and supplied with nutrient solution. No significant differences were observed in the survival rates between the WT and transgenic plants,
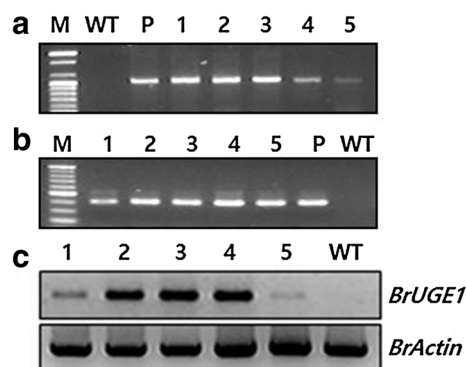

Fig. 6 Identification of transferred gene in transgenic rice lines. PCR amplification using primer set of hygromycin (**a**) and *BrUGE1* gene (**b**). **c** Expression analysis of *BrUGE1* gene. The amplification products were separated using a 1.5 % agarose gel. *Lane M* DNA ladder, *Lane P* PCR product generated from the DNA template of the vector plasmid that contains the gene, *WT* Wild-type, *Lane 1–5* independent transgenic lines

although yellowing of the leaf margin was observed in the WT plants after the NaCl treatment. When growth on media was monitored 7 days after planting, a growth reduction was noticed in all plants, including the WT, which showed a higher reduction than the BrUGE-OX plants (TG#1 and TG#2) (Fig. 7). The growth reduction of shoots was 80 % for WT and 30–45 % for TG#1 and TG#2 plants. In the case of the roots, the WT plants exhibited a 40 % growth reduction, whereas the TG#1 and TG#2 plants showed only 35 % reductions in growth. To analyze the resistance to pathogenic bacteria, TG#1, TG#2, and WT plants were inoculated with Xoo (*Xanthomonas oryzae* pv. *oryzae*, K3a). The K3a diluted with ~1 × 10⁷ cultured in an LB liquid medium. Rice was infected with the three leaf stage. T3 progeny lines compared with WT and MOCK showed many symptoms noticeable against infection

Fig. 7 Salt tolerance assays of *BrUGE1* transgenic rice. 3-week-old seedlings were watered with 250 mM NaCl solution for 24 h (**a**), and then recovered for 20 days (**b**). Survival rates of the WT and transgenic rice lines (**c**). WT; Dongjin rice, Mock; transferred only vector plasmid, TG #1–2; transgenic plant lines. The data are the mean ± SE of three independent experiments. The values with significant differences according to *t* tests are $P < 0.05$
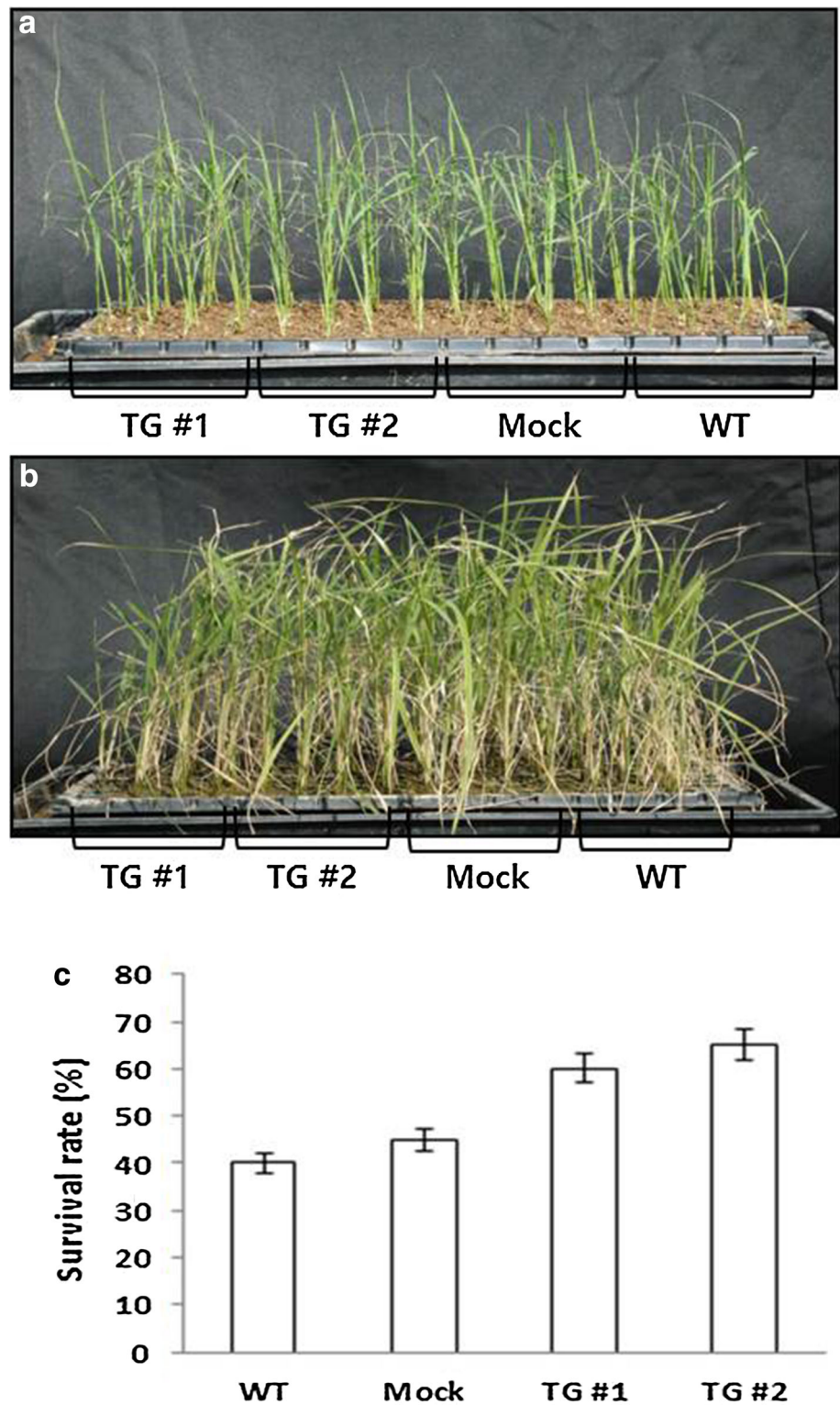
disease. Three weeks later, the leaves of WT and MOCK were significantly increased the size of the lesion to 8.3 and 8.8 cm, respectively, the transgenic lines (TG#1 and TG#2)

showed a lesion of the low value to 5.8–6.8 cm, which indicated enhanced tolerance to bacterial blight disease (Fig. 8).

Fig. 8 Disease development in *BrUGE1* transgenic lines inoculated with *Xanthomonas oryzae* KACC10859 strain. **a**, **b** Disease scoring was conducted 10 days after inoculation. WT; Dongjin rice, Mock; transferred only vector plasmid, TG #1–2; transgenic plant lines
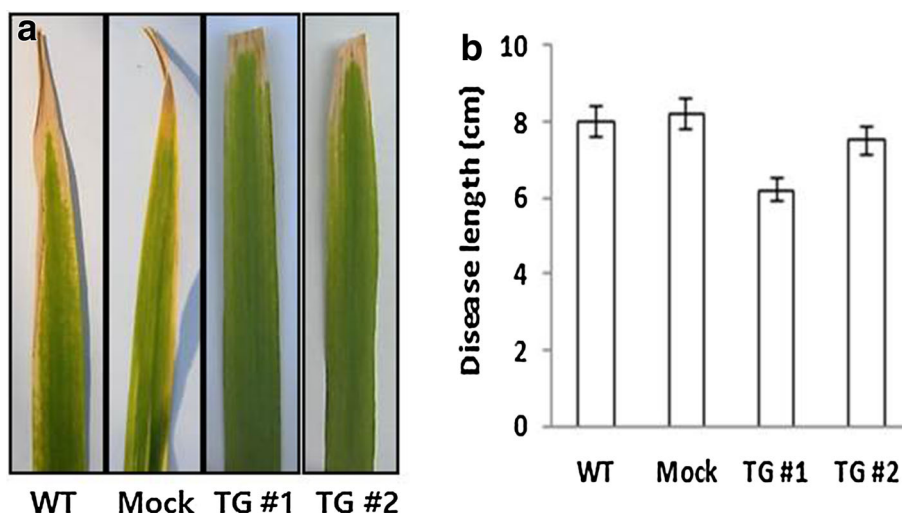
Discussion

Enzyme catalyzing the reversible conversion/epimerization of UDP-glucose into UDP-galactose is the UDP-glucose 4-epimerase (UGE; EC.5.1.3.2) (Majumdar et al. 2004). The reversible epimerization is catalyzed via an enzyme-bound UDP-4-keto-hexose intermediate (Maitra and Ankel 1971). The gene encoding the UDP-glucose epimerase has been cloned from a variety of organisms (Dörmann and Benning 1998). UGE freely interconverts UDP-glucose and UDP-galactose, and a family of five UGE isoforms is encoded in the Arabidopsis genome. *UGE1* to *UGE5* show in vitro variations in substrate affinity, cofactor requirement, and metabolite inhibition profile (Barber et al. 2006). Their global expression pattern suggests that *UGE1* and *UGE3* are coregulated with carbohydrate catabolic enzymes, while *UGE2*, *UGE4*, and *UGE5* are coregulated with carbohydrate biosynthetic enzymes. All isoforms can act in vivo in both directions, as indicated by overexpression experiments (Barber et al. 2006). Expression levels and experiments with *UGE1* antisense lines both suggest that *UGE1* might be the dominant isoform in green plant parts (Dörmann and Benning 1998). Antisense interference of *UGE1*, however, neither induces a morphological phenotype nor alters cell wall polymers or any other galactose-containing carbohydrate. This suggests that other isoforms are required for the biosynthesis of glycoconjugates. In rice, four UGE genes (*OsUGE1-4*) were activated after drought, salt or UV irradiation stress (Liu et al. 2007; Kim et al. 2009). Indeed, drought stress induces increased expression of a *UGE* gene, which maps to a root thickness QTL (quantitative trait locus) region (Nguyen et al. 2004). The 5 BrUGE genes isolated from *B. rapa* was showed specificity expression in different tissues, cluster with 2 groups (Fig. 2) and could play distinct catabolic functions

for Arabidopsis (Barber et al. 2006). For example, as *BrUGE2* and *BrUGE4* are very homologous to *AtUGE2* and *AtUGE4* of Arabidopsis (Rösti et al. 2007). The transcriptional analysis in different tissues and after different stresses can provide clues about the in vivo functions of *BrUGE*s. UDP-sugars are sugar donors for various UDP-dependent glycosyltransferase (UGTs). UGTs from plants are highly specific for UDP-sugars, but microbial UGTs have broad UDP-sugar ranges (Vogt and Jones 2005). In addition, depending on the sugar, the physical and biological activities of some antibiotics and flavonoids are changed (Hollman et al. 1999). Thus, expression analysis of *BrUGE* genes during biotic and abiotic stresses supports the idea that PR-proteins belong to a general plant stress response pathway rather than being specific to distinct stresses, as often hypothesized (Van Loon et al. 2006). The *BrUGE1* and *3* in Group I showed to associate with tolerance to salt stress, while there was no clear pattern regarding the response of three *BrUGE* genes in Group II to various abiotic and biotic stresses except for drought stress where *BrUGE4* and *5* were responsive (Figs. 3, 4). Recent studies showed that UGEs are involved in tolerance against abiotic stresses (Liu et al. 2007; Kim et al. 2009). Overexpression of *OsUGE1* in *A. thaliana* increased the content of raffinose, which resulted in tolerance against abiotic stresses (Liu et al. 2007). The increased level of UDP-galactose resulted in an increased level of raffinose because raffinose is synthesized from UDP-galactose and sucrose (Pridham and Hassid 1965). Transgenic rice overexpressing a putative UDP-galactose epimerase from *Paspalum vaginatum* also showed tolerance against salt (Endo et al. 2005). To better understand the function of the *BrUGE1* gene, we constructed a recombinant pART vector carrying the *BrUGE1* gene under the control of the CaMV 35S promoter and nos terminator and transformed using *A.*

tumefaciens. We then investigated *BrUGE1* overexpressing rice lines at the physiological and molecular levels under biotic and abiotic stress conditions (Figs. 7, 8). Bioassay of T_3 progeny lines of the transgenic plants in Yoshida solution containing 120 mM NaCl for 2 weeks, confirmed that the *BrUGE1* enhances salt tolerance to transgenic rice plants (Fig. 7). Also T_3 progeny lines of the transgenic plants, when exposed to infection caused by *Xanthomonas oryzae* pv. *oryzae*, showed enhanced tolerance to bacterial blight (Fig. 8). These results showed that *BrUGE1* can be used as potential genetic resource for engineering *Brassica* with multiple stress resistance.

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