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Research Article

Isolation and functional characterization of *BrUGT* gene encoding a UDP-glycosyltransferase from Chinese cabbage (*Brassica rapa*)

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Abstract Glycosyltransferases are enzymes (EC 2.4) that catalyze the transfer of monosaccharide moieties from activated nucleotide sugar to a glycosyl acceptor molecule which can be a carbohydrate, glycoside, oligosaccharide, or a polysaccharide. In this study, a UDP-glucosyltransferase cDNA was isolated from *Brassica rapa* using a rapid amplification of cDNA ends (RACE) and subsequently named *BrUGT*. It has a full-length cDNA of 1,236 bp with 119 bp 5′-untranslated region (UTR), a complete ORF of 834 bp encoding a polypeptide of 277 amino acids (31.19 kDa) and a 3′-UTR of 283 bp. BLASTX analysis hits a catalytic domain of Glycos_transf_1 super family (cl12012) that belongs to the Glycosyltransferases group 1 with tetratricopeptide (TPR) regions located between 165 to 350 bp. Expression analysis showed high mRNA transcripts in pistil, followed by petal, seed and calyx of flower. Moreover, expression analysis of *BrUGT* in Chinese cabbage seedlings under stresses of cold, salt, PEG, H_2O_2, drought and ABA showed elevated mRNA transcript. Furthermore, when *BrUGT* gene was transformed into rice using *pUbi-1* promoter, overexpression was evident among the T_1 plants. This study provides insights into the function of *BrUGT* in plants.

Keywords Glycosyltransferase, stresses, Chinese cabbage, overexpression

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Introduction

Plants synthesize a large variety of natural products, known as secondary metabolites, which play important roles in plant defense against microorganisms and herbivores (Wink 1999; Dixon 2001). Plant secondary metabolite UDP-dependent glycosyltransferases (UGTs) catalyze the transfer of a carbohydrate from an activated donor sugar onto small molecule acceptors by the formation of a glycosidic bond (Mackenzie et al. 1998; Li et al. 2001). Glycosylation changes the stability and/or solubility of the aglyca, and even create a higher diversity due to differential and multiple conjugations. These reactions are an important feature of the biosynthesis of many secondary metabolites and in many cases of the regulation of the activity of signaling molecules and defense compounds.

It has been reported that *Arabidopsis thaliana* contains 120 UGTs (Li et al. 2001; Paquette et al. 2003). Several UGTs using different groups of substrates in *A. thaliana* have been characterized *in vitro* but substrates of most UGTs still remain unknown (Bowles et al. 2005). Similarly, rice has more UGTs than *A. thaliana* but their functional characterization is currently under way to elucidate their functional importance (Ko et al. 2006). The UGT superfamily in higher plants is thought to encode enzymes that glycosylate a broad array of aglycones, including plant hormones, all major classes of plant secondary metabolites, and xenobiotics such as herbicides (Vogt and Jones 2000). Indeed, many studies have shown that glucose regulates expression of a broad range of genes involved in primary metabolism, signal

transduction, and metabolite transport (Mita 1995; Price et al 2004; Thibaud et al. 2004). Overexpression of UDP glucosyltransferase increases glucose tolerance in *A. thaliana* (Yan et al 2010). Further, analysis of UGT expression in *Arabidopsis* defense-signaling mutants revealed that their induction was methyl jasmonate independent, but partially salicylic acid dependent. T-DNA tagged mutants (*ugt73b3* and *ugt73b5*) exhibited decreased resistance to *P. syringae* pv *tomato-AvrRpm1* (Langlois-Meurinne et al. 2005). These results emphasize the importance of plant secondary metabolite UGTs in plant-pathogen interactions and provide foundation for future understanding of the exact role of UGTs during the hypersensitive response.

In addition to the stress and defense roles of UGT, it also involves in the flavonol glycoside biosynthesis *in vivo*, and metabolite profiling of the knock-out mutants of UGT73C6 and UGT78D1 showed no accumulation of quercetin and kaempferol glycosides in leaf and floral tissues (Wang 2009; Jones et al. 2003). UGT74F2 glucosylates anthranilate (Quiel and Bender 2003) and salicylic acid *in vivo* (Dean and Delaney 2008), and UGT75C1 is a functional anthocyanin 5-O-glucosyltransferase (Tohge et al. 2005). In *Medicago truncatula*, UGT71G1 was identified to be involved in saponin biosynthesis, and an integrated transcript and metabolite profiling showed that methyl jasmonate treatment resulted in induction of UGT71G1 in cell cultures and also induced accumulation of triterpene glycosides (Achnine et al. 2005). *Medicago* UGT72L1 is involved in the production of epicatechin 3'-O-glucoside in the seed coat as a key step in proanthocyanidin biosynthesis (Pang et al. 2008). Furthermore, UGTs also play important roles in detoxification of xenobiotics and regulating activity of plant hormones (Bowles et al. 2006). For example, *Arabidopsis* UGT73C5 glucosylates steroid hormone brassinosteroids and reduces their bioactivity (Poppenberger et al. 2005). *Arabidopsis* UGT72B1 is involved in xenobiotic metabolism (Brazier-Hicks et al. 2007).

Plant UGTs have attracted extensive research interest due to their key roles in glycosylation and physiological functions in plants as well as their potential application in biotechnology. In recent years, significant progress has been achieved in structural studies of plant UGTs, advancing our understanding of the UGT mediated glycosylation mechanism and directed the UGT engineering from altering glycosylation patterns to producing bioactive glycosides. It will be essential to integrate data from *in vitro* and *in vivo* studies in many crops to gain more complete picture of the potential biological and molecular roles of UGT in plants. In this study, we reported the molecular characterization of a full-length cDNA of *BrUGT* gene cloned from Chinese cabbage (*Brassica rapa*) and its response to abiotic stresses.

Material and method

Plant materials

Chinese cabbage (*Brassica rapa* var. 'sosongche') plants were grown in the greenhouse at Hankyong National University, Anseong, Korea. Different parts of the plant, i.e. young leaves, stems, seeds, flower buds, flower stalks, pistils, stamens, petals, calyx of flowers and roots (branched side roots) from 2-month-old nursery grown plants were used as plant materials.

Stress treatments

Two-month-old potted mature plants of *Brassica rapa* var. sosongche were subjected to different stress conditions in the following manner. Seedlings at the 5-6 leaf stage were placed in 15% PEG 6000 solution and drought condition to induce drought. For other treatments, seedlings at the 5-6 leaf stage were treated with 100 μM ABA, 250 mM NaCl, 4℃ cold, 3% H_2O_2 by spraying and/or irrigation. Samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h and immediately stored at -80℃.

RNA isolation and real-time PCR

Total RNA from leaf tissue was extracted as described by Lee et al. (2012) with some modifications. The relative purity and concentration of extracted RNA was estimated using NanoDrop-1000 spectrophotometer, and stored at -80℃ freezer. Total RNAs were cleaned using DNaseI, and the first-strand cDNA synthesis was performed by reverse transcription of mRNA using Oligo $(dT)_{20}$ primer and SuperScript TMⅢ Reverse Transcriptase. For RT-PCR analysis, the gene specific primers were: *BrUGT* Fw1 : 5'-GTGG AGAGTCTTGTCGGAGA-3' and *BrUGT* Rv1 : 5'-GACC GGTTTGAGATTGAATG-3'. The PCR amplification program consisted of an initial step at 94℃ for 5 min followed by 30 cycles of 94℃ for 1 min, 58℃ for 1 min, 72℃ for 2 min, and a final step at 72℃ for 10 min. Actin primers were used as a loading control and also used as an internal control for normalization of RT-PCR reaction. For the quantitative RT-PCR, the gene specific primers were : *BrUGT* Fw2 : 5'-CACAGCATCATATCTCCAAA-3' and *BrUGT* Rv2 : 5'-TATAGAGCGTGGATGGTTTT-3'. The cDNA samples

were diluted to 10 ng/µl. Triplicate quantitative assays were performed using 5 µl of each cDNA dilution with the SYBR Green Master mix and the CFX connect real-time system (Biorad, USA). The relative quantification method (△△Ct) was used to evaluate quantitative variation between the replicates. The amplification of actin as an internal control to normalize all data was used.

DNA Extraction and PCR Analysis

Genomic DNA was extracted as described by Abdula et al. (2011) with some modifications. Point two gram (0.2 g) of ground powder of fresh rice leaf tissue was placed in 2 ml microcentrifuge tube, and 900 µl of 65℃ preheated extraction buffer (pH7.8-8.0) was added. After incubation in water bath at 65℃ for 30 min, 700 µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added into the tube and mixed for 10 min, and centrifugation was carried out at 13,000 rpm for 10 min. The upper phase was transferred into a new 2 ml tube and equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed for 10 min. The mixed samples were centrifuged at 13,000 rpm for 10 min and the upper phase was transferred into a new 1.5 ml tube containing 4 µl of 10 mg/ml RNase1. After incubation at 37℃ for 30 min, 0.7 volume of precooled isopropanol was added and mixed by vortexing, and centrifuged at 13,000 rpm at 4℃ for 15 min. The supernatant was discarded and 1 ml of 70% ethanol was applied to wash the DNA pellet and centrifuged at 13,000 rpm for 5 min. DNA pellet was air-dried and suspended in 50 µl of 0.5 M TE buffer. The relative purity and concentration of extracted DNA was estimated using NanoDrop-1000 (NanoDrop Technologies, Inc. USA). PCR analysis was performed using HPT-Fw (5’-ATGAAAAAGCCTGAACT CACCGC-3’) and HPT-Rv (5’-CTATTTCTTTGCCCTCG GACGA-3’) primers to check the introgression of hygromycinphosphotransferase gene (hpt), and *BrUGT* Fw1 and Rv1 primers to check the introduction of the full-length cDNA of *BrUGT* gene.

Vector Constructs and Rice Transformation

The *BrUGT* full length cDNA was ligated into the *pBig_sfiI* vector (TaKaRa, Shiga, Japan). The recombinant vector with *BrUGT* has been constructed under the control of *pUbi-1* promoter and NOS terminator and transformed using *Agrobacterium tumefaciens*-mediated transformation method (Lee et al. 2011). *Agrobacterium* strain EHA105 harboring binary Ti plasmid *pFLCIII* containing the full-length cDNA of *BrUGT* gene was cultured on AB medium containing 50 mg/L kanamycin sulfate solidified with 1.5% agar for 3 days at 28℃ in the dark. *Agrobacterium* culture was scraped from the plates and suspended in AAM medium to yield an OD_{600} of approximately 0.3. Pre-cultured seeds were immersed in the *Agrobacterium* suspension by gently inverting the tube for 1.5 min, blotted dry with a sterilized filter paper to remove excess bacteria. The seeds were transferred onto a sterilized filter paper that had been moistened with 0.5 ml of AAM medium placed on 2N6-AS medium solidified with 0.4% gelrite. After 3 days of co-cultivation at 25℃ in the dark, seeds were washed five times in sterilized water and then washed once in sterilized water containing 500 mg/L carbenicillin to remove *Agrobacterium.* The seeds were rapidly blotted dry on a sterilized filter paper and cultured on N6D medium containing 50 mg/L hygromycin and 400 mg/L carbenicillin under continuous light at 32℃ for 2 weeks. Proliferating calli arising from the scutellum were transferred to SF medium. Plantlets arising from the calli were transferred to RF medium to induce roots.

Results

Characterization of *BrUGT* gene

Analysis of the *Brassica rapa* UDP-glucosyltransferase (*BrUGT*) showed a full-length cDNA of 1,236 bp with 119 bp 5′-untranslated region (UTR), a complete open reading frame (ORF) of 834 bp encoding a polypeptide of 277 amino acids (31.19 kDa) and a 3′-UTR of 283 bp. BLASTX analysis of the deduced amino acid sequences showed a glycosyltransferase_GTB_type superfamily located in the N-terminal region (Fig. 1). The members of this family share a common GTB topology, one of the two protein topologies observed for nucleotide-sugar-dependent glycosyltransferases. Moreover, *BrUGT* showed 83% amino acid identity to *Arabidopsis lyrata* (Accession No. XP 002879117), and *Arabidopsis thaliana* (Accession No. AAM 61749), 66% to *Ricinus communis* (Accession No. XP 002523692), and 59% in *Dianthus carypophyllus* (Accession No. BAF 75888). Furthermore, phylogenetic tree analysis using the deduced amino acid of *BrUGT* showed close divergence to *Arabidopsis lyrata* and *Arabidopsis thaliana* (Fig. 2). This result is not surprising since Chinese cabbage belong to the *Brassicaceae* (*Cruciferae*) family which includes both the *A. lyrata* and *A. thaliana* (Abe et al. 2011).

To investigate the expression of *BrUGT*, quantitative

RT-PCR analysis was conducted in different parts of Chinese cabbage. mRNA transcript of *BrUGT* gene was detected in 10 different tissues (seed, pitil, stamen, flower bud, petal, caly of flower, flower stalk, leaf, stem and root) with highest levels in pistil and petal but no or barely detectable in stamen, bud, stem and root indicating that *BrUGT* is tissue-specific and may function on different tissues (Fig. 3).

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      ACATTACAACACAACAACAAACAACAACAACATTACATTTTACATTCTACAACTACATC
      TAGAGGCCAAATCGGCCATCACGATATCTCTCGCTCCCCCTCTTGAAGATCCCAATTTTA
  1-  ATGGCGGACGTTAAAAACCCTAACAAACACCATAAACATCATCGTCTTCATGCTCTCTTG
       M  A  D  V  K  N  P  N  K  H  H  K  H  H  R  L  H  A  L  L  -20
 61-  ATCCCATACCCATTCCAAGGACATGTAAACCCGTTCGTACACTTAGCCATCAAGCTCGCG
       I  P  Y  P  F  Q  G  H  V  N  P  F  V  H  L  A  I  K  L  A  -40
121-  TCGCAGGGGATCACCGTCACATTCGTCAACACTCATTACAACCACCACCAGATCAACTCG
       S  Q  G  I  T  V  T  F  V  N  T  H  Y  N  H  H  Q  I  N  S  -60
181-  GGGGATATTTTCGCCGGAGTTAGATCGGAGTCTGGTCTTGACATACGTTACGCTACGGTT
       G  D  I  F  A  G  V  R  S  E  S  G  L  D  I  R  Y  A  T  V  -80
241-  TCCGACGGACTTCCTCTCGGGTTTGACCGGTCTTTGAACCATGACCAGTACCAATCTGCG
       S  D  G  L  P  L  G  F  D  R  S  L  N  H  D  Q  Y  Q  S  A  -100
301-  TTGCTGCACGTGTTCTCGGCTCACGTGGAGGAGCTTGTGGAGAGTCTTGTCGGAGAAGGT
       L  L  H  V  F  S  A  H  V  E  E  L  V  E  S  L  V  G  E  G  -120
361-  GTGAATGTGATGATCGCAGACACGTTTTTTGTTTGGCCGTCGGTGGTTGCGAGGAAGTTC
       V  N  V  M  I  A  D  T  F  F  V  W  P  S  V  V  A  R  K  F  -140
421-  GGTTTGGTTTGTGTCTCGTTTTGGACCGAAGCTGCTTTGGTTTTTTCTCTCTATTACCAT
       G  L  V  C  V  S  F  W  T  E  A  A  L  V  F  S  L  Y  Y  H  -160
481-  ATGGATCTGCTTCGGATTCATGGTCATTTTGGTGCTCAAGAAACCCGAAGAGATCTAATC
       M  D  L  L  R  I  H  G  H  F  G  A  Q  E  T  R  R  D  L  I  -180
541-  GATTACATCCCCGGGGTCGAAGCAATAAACCCGAAAGACACAGCATCATATCTCCAAAAA
       D  Y  I  P  G  V  E  A  I  N  P  K  D  T  A  S  Y  L  Q  K  -200
601-  ACCGACATCTCCTCCGTGGTCCACCAAATCATCTTCGAAGCATTCAAAGACGTGAAGAAA
       T  D  I  S  S  V  V  H  Q  I  I  F  E  A  F  K  D  V  K  K  -220
661-  GTCGACTTCGTCCTCTGCAACACCATCCACCAATTCGAATCCAAAACCATCCACGCTCTA
       V  D  F  V  L  C  N  T  I  H  Q  F  E  S  K  T  I  H  A  L  -240
721-  TACTCCAAAATCCCCTTCTACGCGATCGGACCGATATCCCATTCAATCTCAAACCGGTCT
       Y  S  K  I  P  F  Y  A  I  G  P  I  S  H  S  I  S  N  R  S  -260
781-  TCGGTCACACCAGTCTCTGATCAGAATCTGATTGCACGCATGGCTCAACACTAAACAGGT
       S  V  T  P  V  S  D  Q  N  L  I  A  R  M  A  Q  H  -           -277
      CGATCAGTTTATACATTTCTTCGAGCTACTCGCACGTGACATGAAGACTAGTCGAGATCG
      CTACTGGATCTCGTAAGCAAATGACTCGTGTGGTTGATCGTCCGAATCGTCAGCTCCGAC
      GAATGAACCATGCGCTGGTCGATCGAGCCGAAACACAGAGACGGATCATGTGTTCGATAA
      GAGCTCCATCAACTCGAGTGAGCATGGGTGGACTACTTGGACATGGCGACGACTGCCTTG
      GAGCAGATATCAACTGGTAATGATGGAACTTTTGACA
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Fig. 1 Nucleotide and deduced amino acid sequence of the full-length cDNA of *BrUGT* gene

Response to abiotic stresses

To characterize the expression patterns of *BrUGT* gene in Chinese cabbage at the molecular level, we quantitatively measured the expression of mRNA transcript on Chinese cabbage plants after abiotic stress treatments of cold (4℃), 250 mM NaCl, 15% PEG6000, 3% H_2O_2, 100 μM ABA and drought (Fig. 4). Using a 4-week-old seedling, the mRNA transcript of *BrUGT* gene was elevated in all stresses at different onset of expression. For the cold stress, expression was noticed as early as 15 min and relatively constant until 1 hr, whereas in the salinity stress, expression was observed from 0.5~8 hr after NaCl imposition. Furthermore, in PEG, mRNA expression was elevated from 1~4 hr after treatment, whereas 24 hr in the hydrogen peroxide stress. For drought stress, no trend was observed in the expression, whereas, mRNA expression in the ABA treatment was variable. The results on abiotic stress mRNA expression were variable

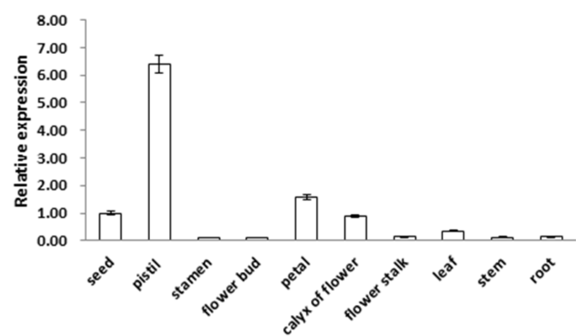

Fig. 3 Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of *BrUGT* was conducted in various tissues collected from *Brassica rapa*. Actin transcripts served as a positive control and were detected in all tissues

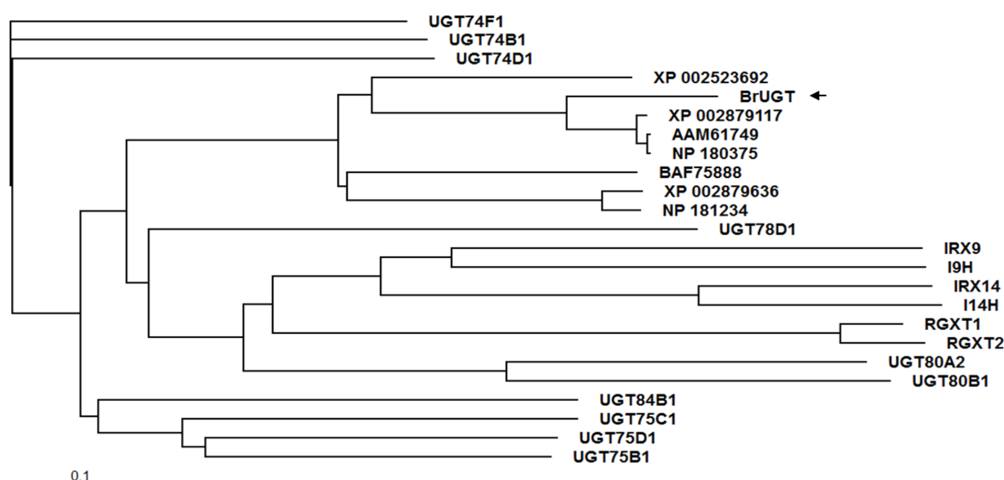

Fig. 2 A rooted neighbour-joined tree comparing the phylogenetic sequence relationship of the *BrUGT* gene domains

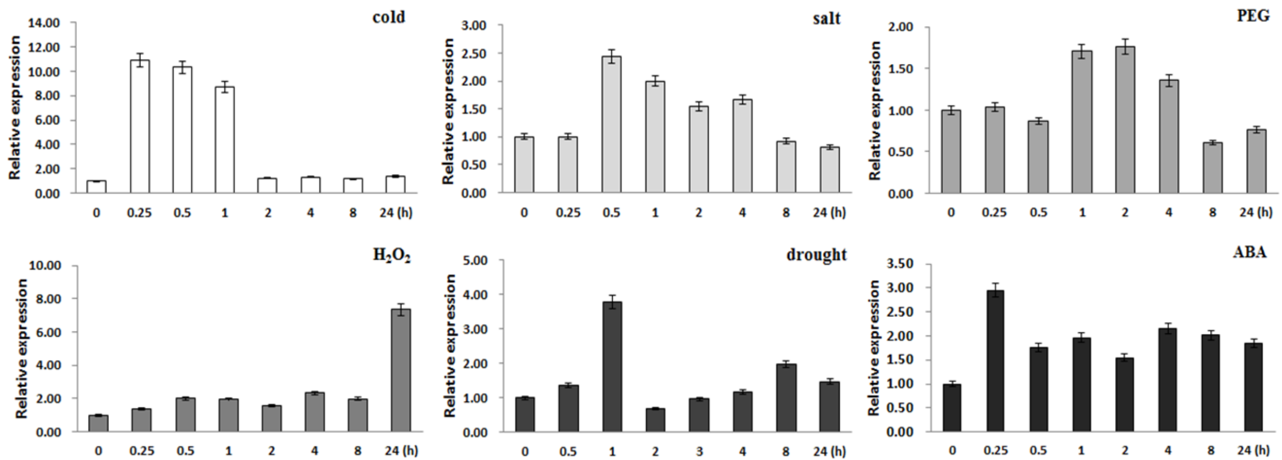

Fig. 4 Quantitative RT-PCR analysis after application of different stresses. Expression of *BrUGT* in the Chinese cabbage seedlings under 4℃ cold, 250 mM NaCl, 15% PEG6000, 3% H_2O_2, drought and 100 μM ABA. Actin transcripts served as a positive control and were detected in wild type plant

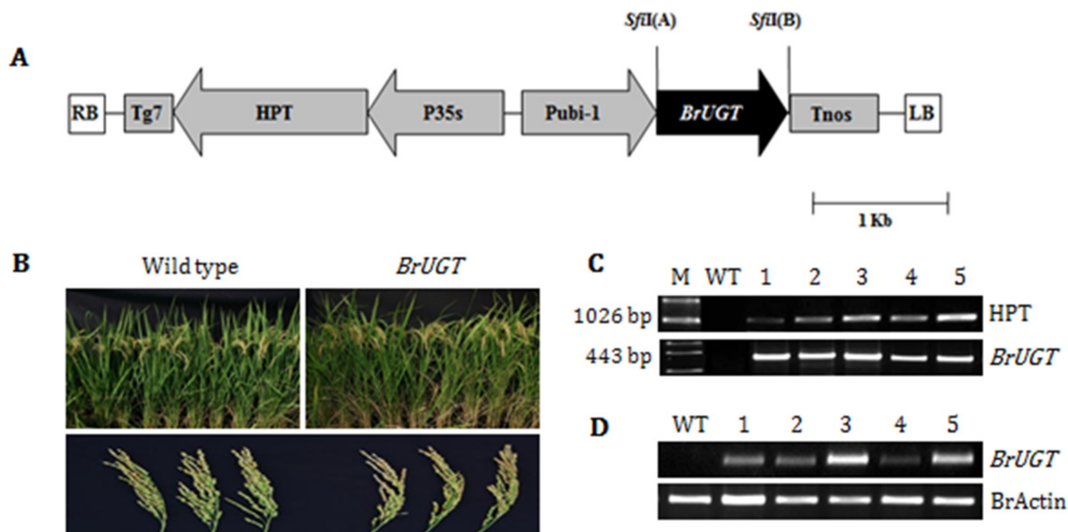

Fig. 5 Development of transgenic rice overexpressing *BrUGT* gene. (A) Schematic diagram of the binary Ti plasmid pFLCIII containing the *UGT* full length cDNA from *Brassica rapa*. pBigs vector consisted by using two different *SfiI* sites [SfiI(A) and SfiI(B)]. P35S, CaMV 35S promoter; *pUbi-1*, maize ubiquitin-1 promoter; Tg7 and Tnos, polyadenylation signals from gene 7 and nopaline synthase (nos) gene in the T-DNA, respectively; *hpt*, hygromycin resistance gene; LB, left border; RB, right border (B) Transgenic plants (C) Expression analysis of *pBigs::BrUGT* in rice. PCR amplification of transgenic rice lines (D) RT-PCR analysis of transgenic rice. The amplification products were separated using a 1.5% agarose gel. Lane M; DNA 100 bp ladder, Lane WT; PCR generated from the DNA or cDNA template of the wild type plant, Lane 1~5; independent transgenic lines

indicating that the gene may act differently in each of the stresses (Fig. 4).

Rice Transformation

To determine the expression of *BrUGT* gene in rice, we directly inoculated *Agrobacterium* containing a *BrUGT* gene under the control of *ubiquitin*-promoter and Tnos terminator in the *pBigs* vector into the pre-soaked seeds in N6D media for 24 hours (Fig. 5A). To confirm its integration into the rice (Fig. 5B), genomic PCR analysis was conducted. As shown in Figure 5C, the DNA amplification was clearly detected whereas no band was seen in the wild type. Further investigation of mRNA showed an overexpression in all independent transgenic plants (Fig. 5D). The magnitude of mRNA expression, however, differs from each independent rice lines. Further analysis of *BrUGT* function is currently undertaken in rice plants.

Discussion

Glycosyltransferases (GT) (EC 2.4.x.y) catalyzes the transfer of sugars to a wide range of acceptor molecules (Ross et al. 2001) and might have an important role in plant defense and stress tolerance (Vogt and Jones 2000). Although these enzymes have been studied intensively for many years, to date only a handful of plant GTs have been characterized and mostly from *Arabidopsis*. So far, our study demonstrated for the first time to report on the characterization of GT in Chinese cabbage (*Brassica rapa*). Like GT in *Arabidopsis*, *BrUGT* shares a common GTB topology, one of the two protein topologies observed for nucleotide-sugar-dependent glycosyltransferases. GTB proteins have distinct N- and C- terminal domains each containing a typical Rossmann fold. *BrUGT* is closely related to *Arabidopsis thaliana* and *Arabidopsis lyrata* (Fig. 2). The substrate profile, catalytic properties, and tissue distribution in plants has been studied but limited describing their mechanism and function.

Despite intensive research efforts aimed at resolving the roles and mechanisms of GTs in plant defense and stress tolerance, little is known regarding the functions of GT expression profiling in plant responses to abiotic stress. Analysis of mutants and overexpressing lines of GTs provides a useful tool to reveal their functions both at the physiological and molecular level. Thus, we investigated the *BrUGT* by overexpression with *pUbi-1* promoter (Fig. 5A). The variation in the expression level may be due to the position effects of *Agrobacterium* transformation which transferred genes anywhere in the somatic cells. Moreover, *BrUGT* is highly expressed in pistil (Fig. 3) indicating that it is a pistil tissue specific gene. Recently, many studies have demonstrated that the expression pattern of GTs can be affected by various biotic and abiotic stresses, including sucrose, ABA, GA, PEG8000, ethylene, NAA, NaCl, wounds, and cold temperatures (Wu et al. 2007; Xu et al. 2002; Korobczak et al. 2005). As seen in Figure 5, overexpression of *BrUGT* gene was confirmed in rice. Further analysis, however, showed different expression level in the mRNA in each of the selected transgenic rice.

In our study, we found that the expression of *BrUGT* was induced by these stresses at the molecular level (Fig. 4). However, the expression level varies considerably in different stresses indicating that *BrUGT* may function differently in these stresses. Further studies on the mechanism and function would be necessary. In summary, the overexpression of *BrGUT* might have an influence on the abiotic stress tolerance in rice. Further functional analysis would be conducted. This study, however, provide insights into the function of *BrUGT* in plants.

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