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Research Article

Isolation and characterization of *Bradh1* gene encoding alcohol dehydrogenase from Chinese cabbage (*Brassica rapa*)

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Abstract Alcohol dehydrogenase (E.C.1.1.1.1) is an enzyme present in higher plants involved in the anaerobic fermentation pathway that catalyzes the reduction of pyruvate to ethanol, resulting in continuous NAD^+ regeneration. It also plays an important role in many plant developments including tolerance to anoxia condition. Here, a cDNA clone encoding alcohol dehydrogenase (ADH) was isolated from Chinese cabbage (*Brassica rapa*) seedlings. The gene named *Bradh1* had a total length of 1,326 bp that contains a single open reading frame of 1,140 bp. The predicted protein consists of 379 amino acid residues with a calculated molecular mass of 41.17 kDa. Expression pattern analysis revealed a tissue-specific expressing gene in different tissues and strongly expressed in the shoot, roots and seeds of Chinese cabbage. *Agrobacterium* transformation of full-length cDNA *Bradh1* into rice Gopumbyeo showed high efficiency. Furthermore, induction of ADH in transgenic rice enhanced tolerance to anaerobiosis stresses and elevated mRNA transcripts. The overexpression of *Bradh1* in rice increases germination under anaerobiosis stresses, implying the possibility of developing new varieties suited for direct seeding or flood-prone rice field.

Keywords alcohol dehydrogenase, ADH, *Bradh1*, transgenic rice, anaerobiosis, germination

Introduction

Rice plant is constantly challenged by environmental stresses that reduce crop yield. With climate change, floods are highly unpredictable and may occur at any growth stage of the crop. Rice can tolerate some submergence as paddy rice or deep water rice. It is well adapted to flooding of the roots because of its ability to transport oxygen efficiently from the aerial parts of the plant to the roots. However, problems occur when the rice plant is completely submerged for periods of time (Drew 1997; Quimio et al. 2000). In direct seeding method of rice planting, poor establishment has been observed if the field is flooded during germination and early seedling development. Anaerobiosis, or the limitation of oxygen, is one of the better characterized environmental stresses endured during flooding. Rice rapidly dies when oxygen availability is limited (Voesenek et al. 2006). Gases diffuse 10,000 times slower in water than in air. Hence growth and survival during submergence of rice is affected by partial (hypoxia) or complete loss (anoxia) of O_2. Reduced supply of O_2 and CO_2 as well as reduced C_2H_4 diffusion limit respiratory activities, photosynthesis and have a negative impact on elongation and growth of rice plants.

Alcohol dehydrogenase (ADH) (E.C.1.1.1.1) is an enzyme present in higher plants including *Arabidopsis* (Chang et al. 1986), barley, maize, pea, pearl millet, sunflower and wheat (Gottlieb 1982) and some prokaryotic organisms. ADH activity is considered essential for the survival of plants during anaerobic conditions (Johnson et al. 1994). Under normal aerobic environments carbohydrates in root cells is catabolized to carbon dioxide and water through glycolysis, the TCA cycle and oxidative phosphorylation. This process provides energy in the form of ATP (36 mole ATP per mole glucose). Under limiting oxygen concentrations, root cells switch from aerobic to anaerobic metabolism, the majority of pyruvate undergoing alcoholic fermentation generating NAD^+ and a limited amount of ATP (2 mole ATP per mole glu-

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cose) (Roberts et al. 1984). Alcoholic fermentation consists of two steps catalyzed by pyruvate decarboxylase and alcohol dehydrogenase, these enzymes converting pyruvate through a toxic intermediate, acetaldehyde, into ethanol.

ADH expression is tissue specific. In general, expression of the *adh* gene has been shown predominantly in roots, as well as seeds (cotyledon and primary root) and pollen, while lacking in green aerial tissues of most plant species (Freeling and Bennett 1985; Dolferus et al. 2003). It has been shown that ADH mRNA level increases with anaerobiosis. This indicates that the ADH enzyme is regulated at the transcriptional level (Xie and Wu, 1989; Lasanthi- Kudahettige et al. 2007). More, its expression is also increased in response to dehydration, low temperatures and to abscisic acid and it plays an important role in many plant developments (Thompson, et al. 2010). Recently, several studies have focused on the effect of overexpression of genes on flooding tolerance. Zhang et al. (2000) overexpressed a gene involved in cytokinin biosynthesis and demonstrated improved flooding tolerance in *Arabidopsis*. Grichko and Glick (2001) overexpressed a bacterial ACC deaminase under the control of three different promoters and were able to produce transgenic tomato (*Lycopersicon esculentum*) plants that had increased flooding tolerance. Pathhuri (2011) reported that alcohol dehydrogenase 1 of barley modulates susceptibility to the parasitic fungus *Blumeria graminis* f.sp. hordei.

The importance of ADH for the germination of seeds under anaerobic circumstances is illustrated by *adh1* null mutants which lack ADH activity. These mutants fail to germinate after prolonged periods of hypoxia, whereas wild type, ADH-producing plants, germinates under these conditions. Plants which are more flooding tolerant have a more active alcoholic fermentation pathway and *adh1* null mutants are more flooding sensitive (Kennedy et al. 1992; Roberts et al. 1984). Engineering varieties with tolerance to anoxia stress is very important for direct seeding of crop establishment. Here, we isolated and reported the transformation of the full-length cDNA *Bradh1* gene derived from Chinese cabbage (*Brassica rapa*) and compared its overexpression in rice against anoxia condition.

Materials and methods

Isolation and cloning of *Bradh1* gene

Total RNA was extracted from Chinese cabbage (*Brassica rapa*) with easy-BLUE reagents (iNtRON Biotechnology, Seoul, Korea). For reverse transcription-PCR (RT-PCR), 1 ug of total RNA was treated with RNase-free DNase and first-strand cDNA was synthesized using an oligo (dT) primer (Invitrogen, Carlsbad, CA, USA). This cDNA was used as a template for PCR amplification with degenerate primers of 5'-ATGTCTACCACCGGACAG AT-3' (BR11-Fw) and 5'-TCAAGCACCCATGGTGAT G-3' (BR11-Rv). PCR condition was the followings: denaturation at 94℃ for 5 min, followed by 35 sequential cycles of 94℃ for 40 sec, 55℃ for 40 sec, 72℃ for 2 min, and an extension at 72℃ for 10 min. The RT-PCR products were electrophoresed and visualized on a 1.0 % (w/v) agarose gel with ethidium bromide (EtBr). The PCR products were subsequently cloned into a pGEM T-Easy vector (Promega), and sequenced extensively. On the basis of this cloned fragment of *Bradh1* cDNA, the RACE approach was then employed to amplify the 3' and 5' ends of *Bradh1* cDNA using 5' and 3' RACE kit (TaKaRa, Shiga, Japan). All the inserted sequences were checked for their homology using the BLAST program in NCBI sequence database and another program in the GenBank/EMBL/DDBJ sequence database.

Vector construction and transformation of *Bradh1*

The *Bradh1* full length cDNA was ligated into the *pBig_sfiI* vector (TaKaRa, Shiga, Japan). The recombinant vector with *Bradh1* has been constructed under the control of CaMV 35S promoter and NOS terminator as shown in Figure 1 and transformed using *Agrobacterium tumefaciens*-mediated transformation method.

Production of *Bradh1* transgenic plants

The transformation was carried out according to the method of Toki et al. (2006) with some modifications. Mature rice seeds of japonica rice cultivar Gopumbyeo were dehulled and sterilized with 70% ethanol for 1 min prior to washing in sterile water. Seeds were further sterilized with 2.5% sodium hypochlorite containing one drop of Tween 20 per 50 ml for 15 min then washed five times in sterile water. This step was repeated once without Tween 20. The sterilized seeds were inoculated on N6D medium solidified with 0.4% Gelrite and cultured under continuous light at 32℃ for 5 days.

Agrobacterium strain EHA105 harboring binary Ti plasmid *pFLCIII* (Fig. 1) containing the full-length cDNA of alcohol dehydrogenase gene was cultured on AB

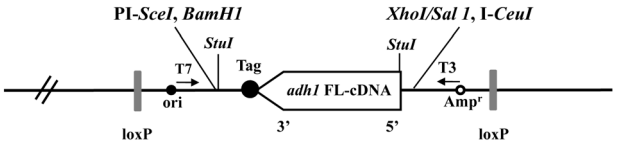

Fig. 1 Schematic diagram of the binary Ti plasmid *pFLCIII* containing the alcohol dehydrogenase full-length cDNA from *Brassica rapa*

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 RE-III medium. Plantlets arising from the calli were transferred to HF medium to induce roots.

DNA extraction and PCR analysis

Genomic DNA was extracted as described by Cho et al. (2007) with some modifications. 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 waterbath 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 pre-cooled isopropanol was added and mixed well, 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 double-distilled water. The relative purity and concentration of extracted DNA was estimated using NanoDrop-1000. PCR analysis was performed using HPT-Fw (GGA TTT CGG CTC CAA TGT CCT GAC GGA) and HPT-Rv (CTT CTA CAC AGC CAT CGG TCC AGA) primers to check the introgression of hygromycin phosphotransferase gene (*hpt*) and *pBigs_SfiI*-Fw (TAT TCG GAG AGG GTA CGT ATT TTT AC) and *pBigs_sfiI*-Rv (GCA ACA GGA TTC AAT CTT AAG AAA CT) to check the introgression of the full-length cDNA of alcohol dehydrogenase gene from T_1 generations.

Activity and expression of alcohol dehydrogenase

Rice culture technique: Rice seeds were soaked in 1 % sodium hypochlorite for 15 min, washed with sterilized distilled water for 30 minutes until there are no more traces of sodium hypochlorite, and then soaked in sterile water. Seeds were germinated in the dark at 32℃. Microbial contaminations were kept to a minimum by daily water changes. Six-day old etiolated rice seedlings were used for anaerobic induction, ADH activity assays and RNA experiments.

Anaerobic induction: Six-day old etiolated rice seedlings were induced by immersion in 10 mM Tris-HCl (pH 8.0) in flasks. Tris-HCl buffer was boiled to remove any air. When cooled, it was degassed using a vacuum pump and then bubbled with N_2 gas continuously for 30 min to remove any air. The rice seedlings were quickly transferred into the flasks, which were wrapped with aluminum foil. N_2 was again bubbled continuously into the buffer in the flasks for 10 min. The flasks were then put into a degassed N_2-filled sealed chamber.

ADH activity assay: After each twelve-hour period of anaerobic induction, the etiolated rice seedlings were taken out of the flasks, quickly frozen with liquid nitrogen and cut into shoot and roots, each part containing at least 0.5 mg of fresh weight. Each part was separately ground with liquid nitrogen using mortar and pestle. After the liquid nitrogen has evaporated completely, 1 ml ADH grinding buffer was added to the samples and mixed for 5 min, and then centrifuged at 0℃ for 15 min. The supernatant was transferred to a new 1.5 ml tube. ADH enzyme activity was measured after the addition of 10 μl or rice tissue extract and 400 μl ADH

reaction mixture. The activity was calculated from the linear portion of the curve during the accumulation of NADH. The protein concentration of the crude extracts was determined according to the Bradford Method using bovine serum albumin, fraction IV (Sigma) as the standard.

Anaerobic germination analysis: Seeds of transgenic rice T_1 generation that was positive in both HPT and *pBigs_SfiI* primers were selected. They were surface sterilized with 50% (v/v) commercial bleach for 15 min, followed by rinsing in sterile distilled water for 30 min. Seeds were then soaked in degassed sterile distilled water and supplied with N_2 gas for 30 min. They were carefully sealed in petri dishes and not allowing any O_2 to come in. Seeds were then germinated in 32℃. After 2 and 6 days, seedlings were removed from the petri dish, washed with distilled water and placed on moistened filter paper. Shoot length was measured in mm. Survival was based on the ability of the plants to resume growth of the roots and greening of the shoots.

RNA extraction and *c*DNA preparations

Total RNA was extracted from the leaf and root of each *adh1* mutant lines and wild type gopumbyeo plant using the Easy-spin IIP kit (Cat. No.17310) according to the manufacturer's instructions. This process was repeated after 12, 24, 36, 48, 60, and 72 hour of anaerobic treatment. The relative purity and concentration of extracted RNA was estimated using NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Inc. USA), and stored at -80℃ freezer. Total RNAs were cleaned using DNase 1 kit (Cat. No: 18068-015, Invitrogen), and the first-strand cDNA synthesis was performed by reverse transcription of *m*RNA using Oligo $(dT)_{20}$ primer and SuperScript™ Ⅲ Reverse Transcriptase (Cat. No: 18080-051, Invitrogen). The specific sequences of the primer pairs used in a semi-quantitative reverse transcription PCR (RT-PCR) are BR 11-Fw (5'- ATT CTCCATCAACGGCAAAC -3') and BR 11-Rv (5'-CCGAGACCGAAGATAGCAAC -3'). Actin primers were used as loading controls and also used as internal controls for normalization of quantitative RT-PCR reaction

Results

Cloning and sequence analysis of *Bradh1* cDNA

To isolate *Bradh1* from Chinese cabbage plants, the sequence search in the cDNA library of other related to *adh* sequences was performed using the database of NCBI nucleic acid sequence. The sequence degenerate primers of 5'-ATGTCTACCACCGGACAGAT-3' (BR11-Fw) and 5'-TCAAGCACCCATGGTGATG-3' (BR11-Rv) were designed and used for cloning of a 1,326 bp cDNA that contains a single open reading frame of 1,140 bp *Brassica rapa adh1*gene (*Bradh1,* JF682847) (Fig. 2). The predicted protein consists of 379 residues with a calculated molecular mass of 41.17 kDa. It was found out that high sequence similarity to *Arabidopsis adh1* (NM106362.2)(*Atadh1*, 88% on the nucleic acid level) and *Brassica oleracea adh1* (AF110433.1) (*Boadh1*, 97%) (Fig. 3). Sequence similarity to *Oryza sativa adh1* (EF122490.1) (*Osadh1*) and *Zea maize adh1* (NM0011 11939.1) (*Zmadh1*) is 76%. *Adh1* gene has been cloned and isolated in other crops. In *Arabidopsis*, the DNA sequence contains an open reading frame similar to the *Bradh1* capable of encoding a polypeptide the same length as maize *adh1* and *adh2* (379 amino acids) and having 80% homology with both maize enzymes (Chang and Meyerowitz 1986).

Development of transgenic rice overexpressing *Bradh1*

Oryza sativa L. cv Gopumbyeo was transformed through

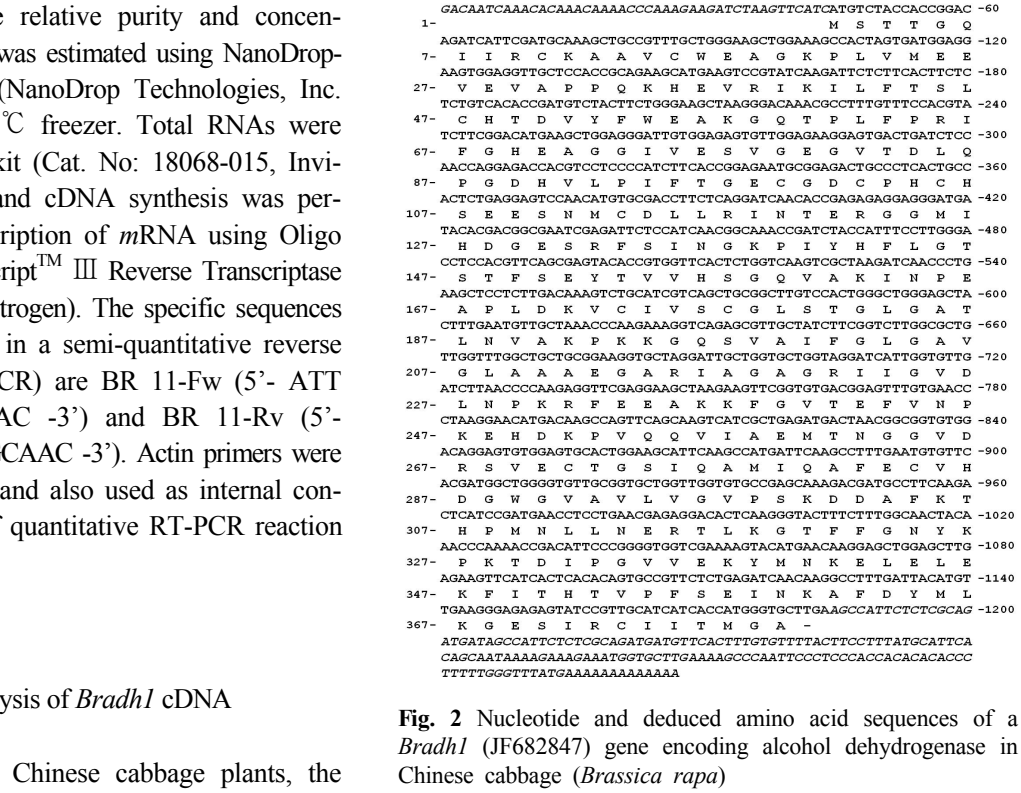

Fig. 2 Nucleotide and deduced amino acid sequences of a *Bradh1* (JF682847) gene encoding alcohol dehydrogenase in Chinese cabbage (*Brassica rapa*)

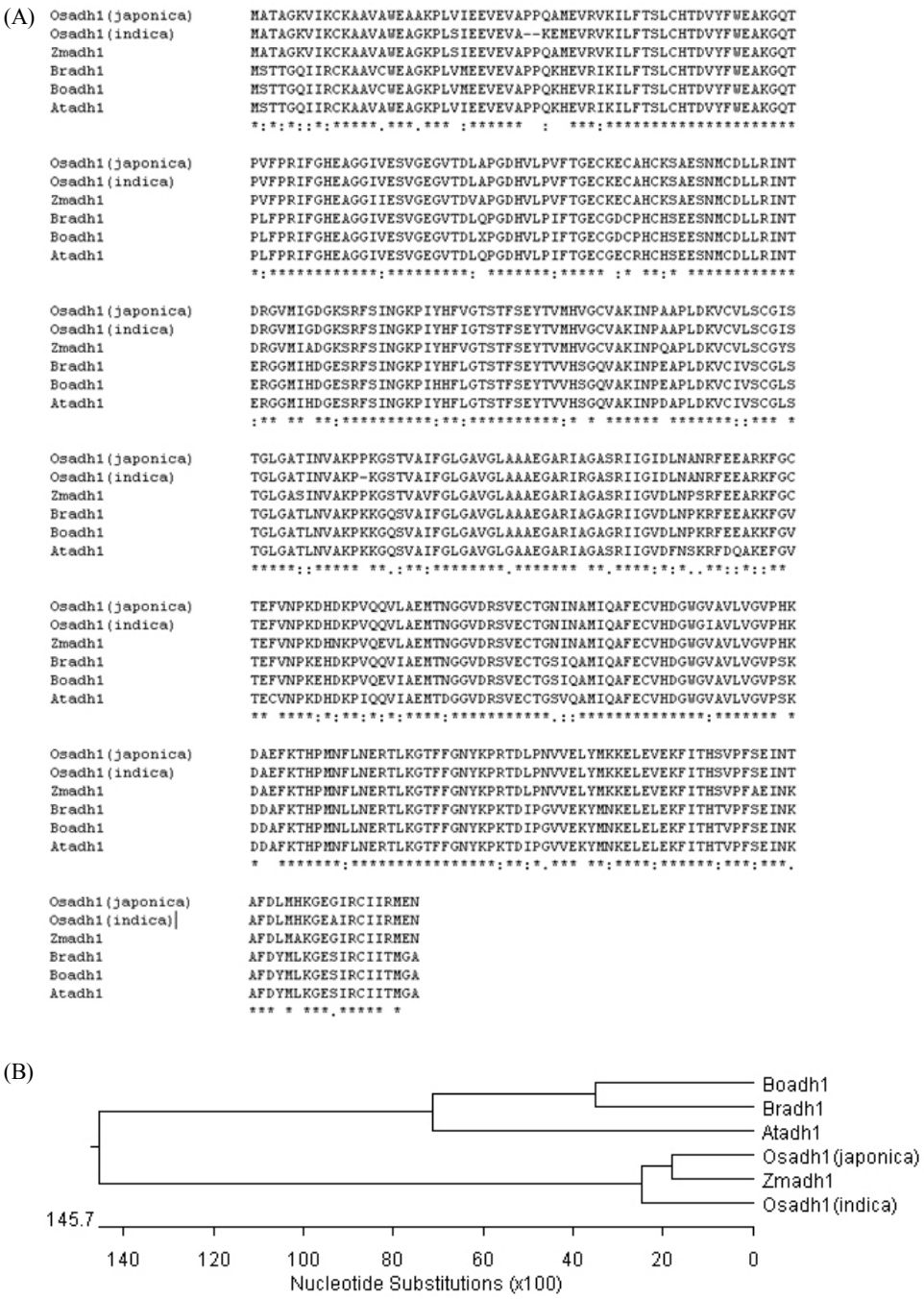

Fig. 3 Multiple alignment of deduced amino acid sequences of *Bradh1* with other related proteins and phylogenetic tree analysis. (A) Alignment of deduced amino acid sequences of *Bradh1*(JF682847) gene, *Boadh1* (AF110433.1), *Atadh1* (NM106362.2), *Osadh1*(japonica) (EF122490.1), *Zmadh1* (NM001111939.1). Putative signal peptide and active site domain are shaded. (B) Phylogenetic tree of alcohol dehydrogenase genes by ClustalW. The numbers next to the nodes give bootstrap values from 100 replicates

Agrobacterium containing the full-length cDNA of alcohol dehydrogenase gene 1 from *Brassica rapa*. A total of 58 regenerated plants from co-cultivation of rice seeds were analyzed by PCR. Of the 58 T_1 plants, 98% and 70% were positive with *hpt* and *pBigs_sfl* primers, respectively (Fig. 4). The size of the introduced full-length alcohol dehydrogenase cDNA is about 1 kb. All of the amplified products for both *hpt* and *pBigs_SfiI* primers were single bands. Here, our transformation efficiency is relatively high (70%). The advantages of *Agrobacterium*-mediated transformation over other methods are high efficiency of transformation, the transfer of

pieces with defined ends, the transfer of relatively large segments of DNA, and the absence of requirement protoplast-culture techniques. This method has demonstrated the stable integration of foreign DNA in rice chromosomes (Chan et al. 1993; Hiei et al. 1994).

Expression level of *Bradh1* gene

In order to explain the expression pattern of the *Bradh1* gene in the different organs, we performed an RT-PCR analysis on different parts of *Brassica rapa* plants using the pistil, stamen, bud, calyx, rachis, petal, leaf, shoot, root, and seed (Fig. 5). *Bradh1* mRNA transcripts were detected in all organs, however, the highest levels were in shoot, root, and seed. In transgenic rice, *Bradh1* mRNA transcript is highly expressed in root, followed by seed and seedlings (Fig. 6). The same expression pattern was observed in both *Brassica rapa* and transgenic rice. This result revealed that *Bradh1* gene is tissue specific and that mRNA transcript is highly regulated.

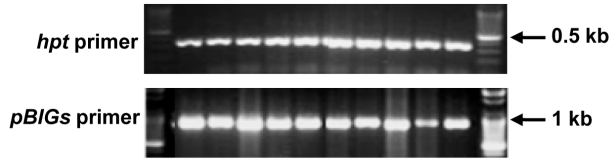

Fig. 4 Representative of genomic PCR analysis of transformants (T_1 generation). Upper level is the *hpt* (hygromycin resistant) and the lower level is *pBigs_SfiI* primers. PCR products were run in 1 % agarose gel by electrophoresis. Bands were viewed using ethidium bromide under UV light

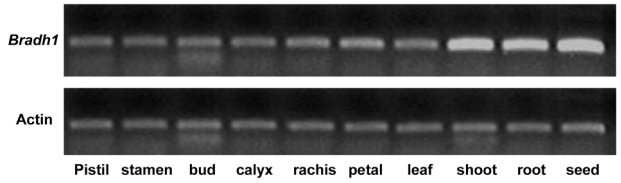

Fig. 5 Expression of *Bradh1* gene in *Brassica rapa*. Upper panel shows expression level and lower panel shows internal actin used for loading adjustment

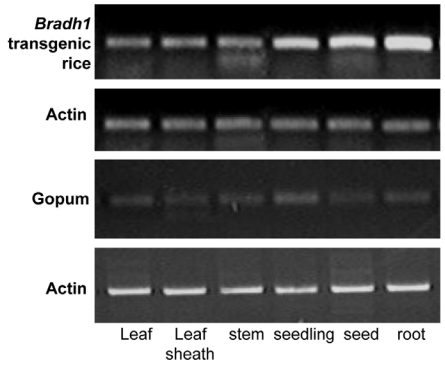

Fig. 6 Expression of *Bradh1* gene in transgenic rice plants and wild type, Gopumbyeo

Bradh1 activity and expression under anaerobiosis

ADH activity was induced in *Bradh1* transgenic rice lines and wild type, Gopumbyeo. Six-day old etiolated rice seedlings were used for the anaerobic induction. The ADH activity for both transgenic rice lines and wild type were both increasing as the length of anaerobic induction increases. However, the transgenic rice lines showed higher ADH activity in both roots and shoots than wild type (Fig. 7, 8). Interestingly, significant differences in roots between mutants and wild type were observed after 48 hr with t-test value of 2.17 indicating that *Bradh1* gene played an important role during

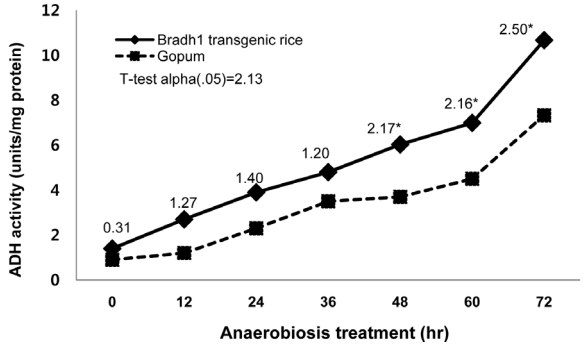

Fig. 7 *Bradh1* transgenic rice and Gopumbyeo ADH activity isolated from 6-day old roots under anaerobiosis. Units of ADH enzyme per mg protein were calculated from the increase of NADH during the assay. The numbers above the line indicates the t-test value. Asterisk (*) means significant at α(0.05)

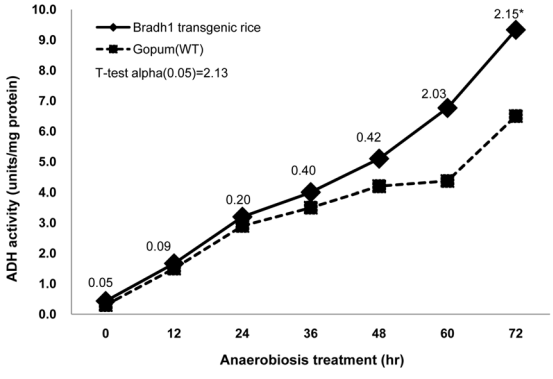

Fig. 8 Transgenic rice and Gopumbyeo ADH activity isolated from 6-day old etiolated shoots under anaerobiosis. Units of ADH enzyme per mg protein were calculated from the increase of NADH during the assay. The numbers above the line indicates the t-test value. Asterisk(*) means significant at α(0.05)

anaerobiosis. Though the shoot increases overtime, significant differences were only observed after 72 hr of anaerobiosis. Overall, shoots and roots showed an almost ten-fold increase in ADH activity compared to the basal level (0 h).

The induction of rice *Bradh1* mRNA, as seen in the RT-PCR analysis (Fig. 9), correlated with the induction of ADH activity under anaerobiosis. RT-PCR analysis showed an increasing expression from 0 h of treatment up to 72 hr. The transgenic rice lines also showed over expression of the *Bradh1*gene under anaerobiosis as compared to wild type. This supports the data showing higher ADH activity in transgenic rice line than the wild type.

Anaerobic germination analysis

Pre-germinated seeds of transgenic rice and wild type, Gopumbyeo, were tested for survival under anoxia condition (Fig. 10). Results showed that rice can germinate in an anaerobic condition as manifested in the 100% survival after 2 day of anaerobic stress. However, after prolong period of anoxia (6 day) the transgenic rice lines have 6 ~ 8% higher seedling survival rates compared to wild type, Gopumbyeo. Throughout the anaerobic germination test, root growth was arrested and shoot growth was limited (data not shown). Most of the shoot growth took place in the second and fourth day but they continued to grow until the sixth day. Some of the transgenic rice lines exhibited longer shoots which suggests that transgenic rice line can adapt better to anaerobic conditions.

Rice is well-known example plant species capable of germinating under totally anaerobic conditions. It can germinate in anaerobic conditions presumably by using energy produced primarily through fermentative metabolism (Kennedy et al. 1992). When rice seeds are germinated in N_2 or under water, only a shoot is produced. It is limited to coleoptiles extension (Turner et al. 1981; Atwell et al. 1982). The transgenic rice lines showed longer coleoptiles compared to the wild type. This data is in accordance with the RT-PCR analysis showing over expression of *Bradh1* in transgenic lines compared to wild type.

Discussion

Flooding can result to anaerobic stress that can eventually lead to yield loss. Rice is considered the most

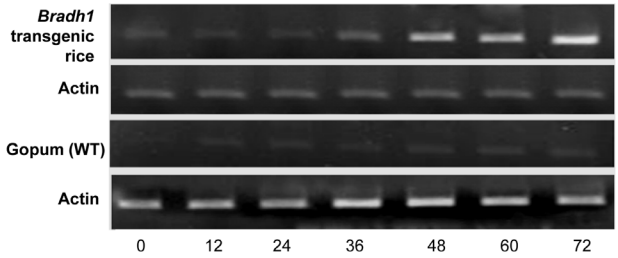

Fig. 9 Expression of *Bradh1* gene in transgenic rice plants and wild type, Gopumbyeo under anoxia conditions. Upper panel shows *m*RNA expression level of *Bradh1* gene under anaerobic stress at different time intervals (0, 12, 24, 36, 48, 60, 72 h) and lower panel shows internal actin used for loading adjustment

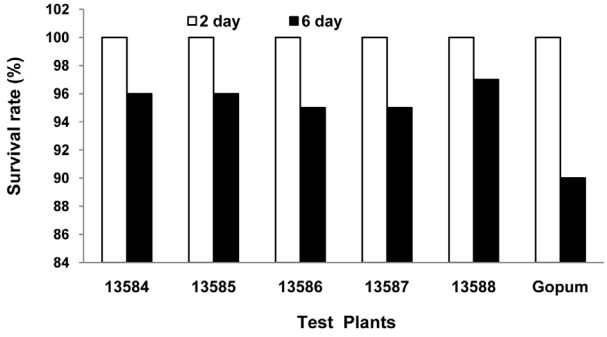

Fig. 10 Ability of transgenic rice and Gopumbyeo seedlings to survive after a subsequent period of anaerobiosis

flooding tolerant plant, but it can only survive up to a certain point. Alcohol dehydrogenase is considered essential for anoxia survival because it recycles NAD^+ for continued glycolysis in the absence of oxygen (Roberts et al. 1984; Johnson et al. 1994). *adh1* has been cloned and well studied in several crops (Chang et al. 1986; Gottlieb 1982). However, *adh1* in Chinese cabbage has not been explored. In this study, *Bradh1* from Chinese cabbage was cloned and subsequently transformed into rice. In the sequence analysis, *Bradh1* contains an open reading frame of 1,140 bp similar to the other *adh1* capable of encoding a polypeptide the same length as maize and having 80% homology with both maize enzymes (Chang and Meyerowitz 1986). Also, *Boadl1* showed high nucleotide similarities with *Bradh1* (Fig. 2). This high similarity is reflected in the phylogenic tree analysis where *Atadh1, Boadh1* and *Bradh1* clade in the same group (Fig. 3). Other related species, *Osadh1* (Japonica), *Zmadh1*, and *Osadh1* (indica) form other clade. The high sequence similarity usually implies significant functional or structural similarity.

Transgenic rice lines inserted with alcohol dehydrogenase gene from *Brassica rapa* were developed through *Agrobacterium*-mediated transformation with high efficiency.

T_1 generations with the *Bradh1* full-length cDNA and hygromycin phosphotransferase marker gene were successfully integrated into the rice Gopumbyeo.

Expression level of *Bradh1* mRNA transcript was assayed to be higher than the wild type, Gopumbyeo, and highly expressed in seedling, root, and seed (Fig. 5, 6). This result was similar to the findings in maize by Freeling and Bennett (1985), and in *Arabidopsis* by Dolferus et al. (2003). The ADH enzyme is therefore regulated at the transcriptional level (Xie and Wu 1989; Lasanthi-Kudahettige et al. 2007). It is not brought about by the conversion of an inactive ADH into an active enzyme (Kadowaki et al. 1988), or by the decline of the inactivator activity itself (Shimomura and Beevers 1983).

The ADH activity for both transgenic rice lines and wild type was both increasing as the length of anaerobic induction increases (Fig. 7, 8). This ADH induction is reflected to the enhanced mRNA gene expression (Fig. 9), higher seedling survival (Fig. 10) compared to the wild type. Collectively, these results clearly demonstrated that the transgenic rice lines transformed with a full-length cDNA of alcohol dehydrogenase (*Bradh1*) showed overexpression compared to wild type, Gopumbyeo. Overexpression of *Bradh1* will be useful for future development of rice varieties that are flood-tolerant or can be planted through direct seeding. It will also be very informative for further functional gene analysis.

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