Gene Expression and Sequence Analysis of BADH1 Gene in CLSU Aromatic Rice (*Oryza sativa* L.) Accessions Subjected to Drought and Saline Condition

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Summary The BADH1 was characterized by investigating its association to aroma, drought and salinity stress through sequence and gene expression analysis using the selected aromatic rice accessions from Central Luzon State University, Philippines. Polymorphisms including SNPs, were observed in genomic analysis between the resistant check and the susceptible varieties during saline condition. On the other hand, BADH1 transcript level in tolerant varieties revealed that during salt treatment, the salt tolerant check Pokkali and moderately salt tolerant accession Leyte Special have increased transcript level compared to non-treated saline condition relative to actin. The downstream investigation of the BADH1 using genomic and transcriptomic approach is important information to elucidate the molecular mechanism of fragrance development among aromatic rice in CLSU and its response to abiotic stresses.

Key Words BADH1, polymorphism, aroma, genomics, transcriptomics

The 2-acetyl-1-pyrroline (2AP), the known compound that contributes to the aroma in rice is synthesized through the loss of functional enzyme of Betaine Aldehyde Dehydrogenase 2 (BADH2) (1). Interestingly, another gene present in rice, known as BADH1, a homologous gene of BADH2, is reported to have a close correlation with salt tolerance (2), while Singh et al. (3) argued that BADH1 is associated to rice aroma (1). Glycine betaine (GB) is known as an osmolyte or osmoprotectant compound (4) and is synthesized by the plant and other organisms in response to salinity, drought, and temperature stress (3). Similar to this, GABA is also a compound with osmoprotectant properties (5), which suggests that BADH1 might be involved in mechanism responses of rice to different environmental stresses. In this study, the association of BADH1 to aroma, salinity, and other environmental stress, such as drought, was evaluated in genomic and transcriptomic level from selected aromatic rice accessions of CLSU Research Germplasm.

Materials and Methods

Plant Material

The aromatic rice seeds used in this experiment were obtained from the germplasm collection of the Research Office of the Central Luzon State University. The seeds were disinfected with 5% hypochlorite solution for 30 min and soaked in distilled water for 24 h. The seeds were placed in petri dishes lined with moistened filter

paper and were incubated at 30°C for 48 h to allow germination.

Hydroponic Set-up

The seeds were sowed in hydroponics set-up using the protocol of Gregorio et al. (6). The pre-germinated seeds were sowed in the holes of the styrofoam float. Two seeds per hole were placed on the Styrofoam float that was placed in the hydroponic solution.

Imposition of Drought²

Leaf rolling (LeR) and leaf drying (LeD) scores are determined for all the entries when susceptible check already reaches the score of 7 or 9. The scoring of the LeR and LeD response to drought was based on IRRI's Standard Evaluation System (SES) for Rice (7). The entries were scored for green plant recovery and vigor after 6 d from re-watering.

Salinization and Nutrient Solution Treatment

The nutrient solution was salinized (for the treatment only) by adding NaCl (ordinary table salt) while stirring up to the desired electron conductivity (EC) following the IRRI's Standard Evaluation System (SES) for Rice (7).

Sequencing of BADH1 Gene

Leaves from fifteen-day old rice plant were collected and subjected to DNA extraction. The leaf samples were powdered using liquid nitrogen and sterilized mortar and pestle. The DNA extraction was done using CTAB method.

Primer Design and Polymerase Chain Reaction

The sequence of the primers was designed using Primer3 version 0.4.0 (8). The BADH1 gene sequence (*Oryza sativa japonica* cv 'Nipponbare') (AK103582)

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obtained from the NCBI website (www.ncbi.nlm.nih.gov) was used as reference sequence to design forward and reverse primer for the amplification and sequencing of BADH1 gene. The designed primers were used to amplify the BADH1 gene together with other PCR components. The PCR profile consisted of pre-denaturation of DNA at 94°C for 2 min and 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 51 to 55°C for 30 s, extension at 72°C for 60 to 90 s, and final extension at 72°C for 5 min with hold at 10°C for 10 min or until removed from the thermal cycler. The PCR products were checked using gel electrophoresis.

Sequencing and Aligning of the BADH1 Gene2

The PCR product was subjected to gene sequencing. The contigs were then aligned for consensus alignment using CondonCode Aligner Software version 8.0.1 (9). The consensus sequences were then aligned using MUSCLE Alignment (10) and Gblock (11) for the determination of the present single nucleotide polymorphism (SNP), indel, and polymorphisms among the BADH1 sequences.

Gene Expression Analysis of BADH1 Gene

Gene expression of BADH1 gene was evaluated using Real-Time Polymerase Chain Reaction (RT-PCR). Since BADH1 was often associated with salinity stress response, only accession with salinity tolerance response was chosen for gene expression analysis together with the tolerant check Pokkali.

RNA Extraction and cDNA Synthesis2

The total RNA was extracted using TRisure RNA extraction kit (Bioline Inc.) according to the manufacturer's instruction from 100 mg of powdered leaves using mortar and pestle with liquid nitrogen. The RNA was quantified and undergone cDNA synthesis using SensiFAST cDNA Synthesis Kit (Bioline Inc.) following the manufacturer's instruction.

Real-Time Polymerase Chain Reaction (RT-PCR)2

To determine the gene expression of BADH1 to salt stress, quantitative RT-PCR was conducted using Power SYBR® Green PCR Master Mix. The designed primer pair using Primer3 version 0.4.0 (8) for BADH was 5'-ATTCTGGGAAGCCTCTGGAT-3' (forward) and 5'-AGCTTCTGCCAGATCAGCAT-3' (reverse) while the primer pair for Actin gene (AK060893) 5'-GAGTATGATGAGTCGGGTCCAG-3' (forward) and 5'-ACACAACAATCCCAACAGAG-3' (reverse). Each sample and target genes were triplicated. Gene expression analysis was done using StepOne Software version 2.3 and the confidence interval was calculated. The differences between the values were considered significant at 95% confidence interval (RQ Min and RQ Max) were non-overlapping.

Result

Drought Stress Screening

After 24 h water, Pokkali and Rataria accession showed less leaf rolling response compared to all the aromatic accessions. On the other hand, the leaf drying score showed that Pokkali has less leaf drying response compared to all the aromatic rice accession including

Table 1. Discovery of Polymorphism in BADH1.

POLYMORPHISM	EXON	INTRON	TOTAL
Single Nucleotide Polymorphism (SNP)	7	21	28
Insertion	0	3	3
Deletion	1	1	2

Fig. 1. Mean BADH1 gene transcript level values of Leyte Special.

Fig. 2. Mean BADH1 gene transcript level values of Pokkali.

susceptible check IR-64.

Salinity Tolerance Evaluation

The salt-tolerant check Pokkali showed high salt tolerance response while Leyte Special showed moderate tolerance to salt stress following the score of Pokkali and the other accessions showed highly susceptible response to salinity stress.

Polymorphism in BADH1

A total of twenty-eight (28) SNPs were identified

within the sequence of BADH1 gene; seven (7) were located at the exon region and the rest were all located at the intron region. A total of three (3) insertion occurrences were identified all located at the intron region. Only two (2) cases of deletion were within the BADH1 gene (Table 1). One was located at the exon, while the other one was found at the intron region. Since there were polymorphism identified in the exon region within the sequence of BADH1, the exon sequence of all the accessions and Pokkali were translated to amino acid sequence using EXpasy Tool (12) software. The polymorphism found in the exon region resulted to the substitution of amino acid in the coding sequence.

Gene Expression Analysis

Leyte Special (Fig. 1) and Pokkali (Fig. 2) subjected to saline condition show significantly higher transcript level, therefore, higher expression of BADH1 gene compared to normal condition without salt.

Discussion

Drought tolerance response is required in plants when subjected to water deficiency condition. Plants tolerant of drought can maintain water within the tissues or can be able to survive with low water content and recover completely after re-watering (13). Physiological responses such as closure of stomata and osmotic adjustment limit the water loss. Compatible solutes aid the osmotic adjustment in response to water stress conditions (14). Glycine betaine is a known compatible solute with significant importance to cell's homeostasis having the mechanism of protecting and regulating cell structures, proteins stability, membranes and other simple and complex parts of the cell. This compound was also reported to respond by protecting the photosynthetic activity of PSII complex and RuBisCO in the plants during stress condition. Another compound with osmotic properties is the gamma-aminobutyric acid (GABA) (5). It is reported to have an important role in the effects of abiotic stress (15, 16).

In saline condition, osmotic stress is experienced by the plants as osmotic potential decreases which resulted to the decrease in water uptake by the plant. This condition generates the same effects in drought stress that triggers water stress (17). As a response to water stress, plants adapt by the mechanism of osmotic adjustment through accumulation of solutes. Osmotic adjustment helps the plant to prevent the loss of turgor potential, causing the stomata to stay open and continue to aid gas exchange for photosynthesis. Osmotic adjust is facilitated by compatible solutes. These compatible solutes have similar properties and do not affect the cell's processes. One of the known compatible solutes is the glycine betaine and proline (14). As proposed by Fitzgerald et al. (18), the pathway to synthesize glycine betaine is from the oxidation of betaine aldehyde catalyzed by BADH. The BADH1 allows conversion of betaine aldehyde to glycine betaine, a powerful osmoprotectant compound. On the other hand, another pathway of BADH1 is the oxidation of gamma-aminobutyraldehyde to form gamma-aminobutyric acid, a compound with osmoprotectant properties (18).

protectant properties (18).

Discovery of SNPs Substitutions were also observed in the study of Singh et al. (3) where Lysine becomes Asparagine and Lysine to Glutamine. In their study, the validation of effect of these identified substitutions was done by adding protein modeling and ligand docking experiment. The two substitutions resulted to the reduction in the affinity of the BADH1 enzyme to catalyzed gamma-aminobutyraldehyde (GABald), the known precursor of 2-acetyl-1-pyrroline (2AP).

The same result in the study of Hasthanasombut et al. (2) with the expression of BADH1 to salt-treated cultivars was also reported in Pokkali (salt-tolerant) and KDML105, moderately salt-tolerant Thai jasmine rice. The expression of BADH1 gene in both rice cultivars increased in correlation with salt stress and was shown to be much higher compared to the normal condition (0% NaCl).

Disclosure of State of COI

No conflicts of interest to be declared.

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