Genome-wide analysis and expression profiling of half-size ABC protein subgroup G in response to abiotic stress and phytohormone treatments

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Abstract

The roles of the proteins encoded by half-size adenosine triphosphate-binding cassette transporter subgroup G (ABCG) genes in abiotic stress responses are starting to be established in the dicot model Arabidopsis thaliana. In the monocot model rice, the functions of most half-size ABCG proteins in abiotic stress responses are unknown. Ren1/OsABCG5 is an essential transporter for growth and development under abiotic stress, but its molecular function remains largely unclear. Here, we present a comprehensive overview of all 30 half-size ABCG genes in rice, including their gene structures, phylogeny, chromosome locations and conserved motifs. Phylogenetic analysis revealed that the half-size OsABCG proteins were divided into four classes. All seven rice intronless genes, including Ren1/OsABCG5, were in Class III, like the 12 intronless ABCG genes of Arabidopsis. The EST and FL-cDNA databases provided expression information for 25 OsABCG genes. Semi-quantitative and quantitative RT-PCR analyses demonstrated that seven OsABCG genes were up-regulated in seedlings, shoots or roots following treatments with abiotic stresses (6°C, 17°C, 42°C, mannitol and abscisic acid). Another 15 OsABCG genes were up-regulated under at least one of the abiotic stress conditions and other phytohormones besides abscisic acid. Hierarchical clustering analysis of gene expression profiles showed that expression of the OsABCG genes could be classified into four clusters. The Ren1/OsABCG5 cluster was up-regulated by abscisic acid and included OsABCG2, 3, 13 and 27. This study will provide a useful reference for further functional analysis of the ABCGs in monocots.

Results

1. Phylogenetic analysis of half size ABCG proteins.

Phylogenetic tree of rice and Arabidopsis half-size ABCG proteins (a). Full-length sequences of 30 ABCG proteins of rice were conserved for sequences for 28 members of Arabidopsis. ABCG proteins to construct a tree with Mega 6 software using the Neighbor-Joining (NJ) method (15,000 replicates). Exon–intron organization of corresponding ABCG genes (b). Exons and introns are represented by black boxes and lines, respectively. Open boxes indicate untranslated regions.

2. Expression of part of the rice ABCG protein genes

Expression profiles of OsABCG2, OsABCG3, Ren1/OsABCG5, OsABCG13, OsABCG14, OsABCG22, and OsABCG27 are determined by quantitative RT-PCR reactions using total RNA from shoot and root samples harvested from controls and in response to abiotic stress conditions. Relative amount of amplified product was normalized against OsUBQ10 transcripts. Each value represents the average of two or more biological replicate experiments in which each value is the mean of three separate quantitative RT-PCR reactions (±SE). Asterisks indicate significant differences (p<0.05) compared with control.

3. Hierarchical clustering analysis of rice ABCG genes

Hierarchical cluster of expression profiles of rice half-size OsABCG genes. OsABCG genes were considered to be expressed if the normalized intensity value exceeded 0. Expression levels are illustrated by graded color scale, representing relative expression levels of -3, -2, -1, 0, 1, 2, and 3. Red, green, and black represent positive, negative, and zero, respectively.

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