



Molecular Biology

Genome-wide expression analysis of rice ABC transporter family across spatio-temporal samples and in response to abiotic stresses



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ARTICLE INFO

Article history:

Received 19 October 2013

Received in revised form 28 April 2014

Accepted 13 May 2014

Available online 10 June 2014

Keywords:

ABC transporters

Abiotic stress responses

Meta-analysis of tissue specific expression profiles

Rice

Phylogenomic analysis

ABSTRACT

Although the super family of ATP-binding cassette (ABC) proteins plays key roles in the physiology and development of plants, the functions of members of this interesting family mostly remain to be clarified, especially in crop plants. Thus, systematic analysis of this family in rice (*Oryza sativa*), a major model crop plant, will be helpful in the design of effective strategies for functional analysis. Phylogenomic analysis that integrates anatomy and stress meta-profiling data based on a large collection of rice Affymetrix array data into the phylogenetic context provides useful clues into the functions for each of the ABC transporter family members in rice. Using anatomy data, we identified 17 root-preferred and 16-shoot preferred genes at the vegetative stage, and 3 pollen, 2 embryo, 2 ovary, 2 endosperm, and 1 anther-preferred gene at the reproductive stage. The stress data revealed significant up-regulation or down-regulation of 47 genes under heavy metal treatment, 16 genes under nutrient deficient conditions, and 51 genes under abiotic stress conditions. Of these, we confirmed the differential expression patterns of 14 genes in root samples exposed to drought stress using quantitative real-time PCR. Network analysis using RiceNet suggests a functional gene network involving nine rice ABC transporters that are differentially regulated by drought stress in root, further enhancing the prediction of biological function. Our analysis provides a molecular basis for the study of diverse biological phenomena mediated by the ABC family in rice and will contribute to the enhancement of crop yield and stress tolerance.

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Introduction

ATP-binding cassette transporter family (ABC-transporter) constitutes one of the largest protein families with transporter activity and is conserved in all organisms. This family plays roles in the export or import of various substrates across biological membranes. Minimal structure of ABC transporter family requires highly conserved nucleotide-binding domains (NBD) and a less conserved trans-membrane domain (TMD). The former consists of Walker A and Walker B motifs for ATP binding and H- and Q-loops for the hydrolysis. The latter forms the ligand binding sites by several α -helices and determines the specificity of substrates. A typical membrane-bound ABC transporter contains four domains: two TMDs and two NBDs. ABC transporter is formed from single

gene or two genes (heterodimers or homodimers) (Kang et al., 2011).

By the difference in protein size, orientation (the organization of domain), and TMDs sequence (idiotypic and/or linker), plant ABC transporter family is currently classified to eight subfamilies. From ABCA to ABCD, subfamilies are characterized in forward TMD–NBD organization, whereas ABCG subfamily contains reverse orientation NBD–TMD. The soluble subfamilies E and F only encode two NBDs but no TMD, and the members of ABCI subfamily retain single domain such as NBD or accessory domain. Even TMD sequences were selected as criterion for the subfamily classification except soluble ABC transporters, high analogy among their sequences do not entirely indicate similarity in their function. However, a few closely linked members in their phylogenetic tree are more likely to play similar or redundant functions. For example, *AtABCB1* and its closest homolog *AtABCB19*, participate in auxin transport (Sidler, 1998), or *AtABCC1* and *AtABCC2* exhibit redundant function in the translocation of plant vacuolar phytochelatin (Song et al., 2010). Beyond the range of species, functions of genes with homology are frequently conserved. For example, Qin et al. (2013) provided evidence that *OsABCG15* is essential for post-meiotic anther and pollen exine development, like the homolog of *OsABCG15* in Arabidopsis,

Abbreviations: ABA, abscisic acid; ABC transporter, ATP-binding cassette transporter; As, arsenate; Cd, cadmium; COV, coefficient of variation; Cr, chromium; NBD, nucleotide binding domain; Pb, lead; RT-PCR, reverse transcription polymerase chain reaction; TMD, trans-membrane domain.

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AtABCG26/WBC27 (Quilichini et al., 2010). Another example is *AtABCG32/PEC1* and *OsABCG31* that are involved in the formation of the cuticular layer of the cell wall to protect from losing water (Bessire et al., 2011; Chen et al., 2011). Therefore, phylogenetic analysis among different species provides us useful clues for the functional study among homologous genes. Even if the structural feature has been well studied, the identification of biological functions in plants is very limited. In case of Arabidopsis ABC transporters, there are 128 family members and functions of 26 ABC transporters have been characterized (Kang et al., 2011), and the number for rice ABC transporters with known functions is much smaller than that of Arabidopsis. Until now, three ABC transporters related to water retention in leaf, pollen exine development, and production of phytic acid in rice have been functionally characterized (Chen et al., 2011; Qin et al., 2013; Xu et al., 2009).

Water scarcity causes a huge damage on crop production. Besides their developmental roles, ABC transporters perform the function in rescuing plants which are exposed to various abiotic stresses, especially drought. So far, the identification of both the importer (Kang et al., 2010) and exporter (Kuromori et al., 2010) of ABA in Arabidopsis enhanced our knowledge about ABA signaling which is one of the most important phytohormone pathways associated with drought stress responses. ABC transporters also have functions related to guard cell responses (Klein et al., 2004; Kuromori et al., 2011) or required for functional cuticle formation, associated with water retention in the leaf like *AtABCG32/PEC1* and *OsABCG31* as mentioned earlier. Genome-wide analysis of ABC transporters in rice associated with water scarcity has not been carried out and will be required to provide novel insight of ABC transporter for rescuing water scarcity.

Completion of the rice genome sequence has revealed a large number of ABC transporters (IRGSP, 2005; Verrier et al., 2008). The main obstacle to their functional identification is the functional redundancy within this supergene family and the lack of information guiding functional analysis. Thus, a systematic overview of the ABC family in rice might be a useful starting point for further functional analyses under diverse stress conditions as well as for unique developmental process. Phylogenomics is a phylogenetic approach to predict the biological functions of members of large gene families by assessing the similarity among gene products (Cao et al., 2012; Jung et al., 2010a). The power of estimating functional redundancy is even more enhanced by combining phylogenetic analysis with a large amount of transcriptome data, such as integrating analysis of gene expression data related to SUB1A-mediated submergence tolerance with the phylogenetic tree of the AP2 family (Jung et al., 2010b). Recently, we demonstrated that selection of the predominant gene family member in light conditions has better performance in identifying defective phenotypes through the related loss-of-function study than selecting non-predominant family members (Jung et al., 2008).

In this study, we performed phylogenomic analysis of ABC transporter family members to provide functional clues of individual family members. The anatomy or stress meta-expression profiles based on a large collection of Affymetrix data were then integrated into the phylogenetic tree context of the ABC transporter family. As a result, we identified 38 genes that showed tissues/organ preferred expression, 3 genes that were ubiquitously expressed, 47 genes that responded to heavy metal, 16 genes associated with nutrient deficiency, and 51 genes associated with abiotic stress. Of them, expression patterns of 14 genes in response to drought stress in root are confirmed by quantitative RT-PCR analysis. We developed a functional gene network associated with nine ABC transporter genes differentially expressed in root after drought treatment and this network suggests a hypothetical model to elucidate the underlying molecular mechanisms, which might in turn facilitate future agricultural applications in rice for enhancing water use efficiency.

Materials and methods

Multiple sequence alignment and phylogenetic tree building

To perform phylogenomic analysis of ABC transporters in rice and *Arabidopsis thaliana*, we collected 125 family members with locus IDs from the Rice Genome Annotation Project (RGAP, <http://rice.plantbiology.msu.edu/>) (Table S1) and 128 Arabidopsis family members from a previous report (Verrier et al., 2008). We selected a representative transcript encoding the ABC protein for each locus in rice as suggested in RGAP. Multiple amino acid sequences were aligned using ClustalX program version 2.0.11 (Higgins et al., 1996). The phylogenetic analysis was performed by using MEGA 5.2 and under the following parameters: neighbor-joining tree method, complete deletion, and bootstrap with 500 replicates (Tamura et al., 2011). We developed a phylogenetic tree with rice and Arabidopsis ABC transporters. Rice ABC proteins are currently classified into eight subfamilies (Figs. 1 and S1) in accordance with new nomenclature, based on RGAP annotation release 7 (http://rice.plantbiology.msu.edu/downloads_gad.shtml). We also generated a phylogenetic tree for each subfamily except for ABCD and ABCE, which have only 2 and 3 members, respectively (Fig. S1).

Supplementary Table S1 and Fig. S1 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jplph.2014.05.006>.

Meta-analysis of tissue specific expression profiles and stress responsive expression analysis

Integration of transcriptomes into the context of the phylogenetic tree might be a good reference to direct useful experimental strategies for further functional analysis. Therefore, we used meta-analysis of tissue specific expression profiles based on 983 Affymetrix array data downloaded from the NCBI gene expression omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) (Cao et al., 2012). We then uploaded the log₂ normalized intensity data in tab-delimited text format into Multi Experiment Viewer (MEV, <http://www.tm4.org/mev/>) and illustrated them by using heat maps (Figs. 2–4 and S2 and Table S4). We separated the tissue samples into two groups according to development stage: vegetative stage and reproductive stage. We then selected genes that have at least 2-fold higher expression level in each tissue type relative to the other tissues with a *P*-value less than 0.05. In addition, we generated a heat map image based on log₂ fold-change data in response to abiotic stresses and then integrated the heat map into the phylogenetic tree context. We selected genes showing up-regulation under diverse stresses with log₂ changes greater than 1.5-fold or lower –1.5-fold for down-regulation with a coefficient of variation (COV) less than 1, compared with normal conditions (Fig. 5, Tables S2–3).

Supplementary Tables S2–S4 and Fig. S2 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jplph.2014.05.006>.

Promoter analysis

To analyze the promoter regions for four loci (*OsABCG30*, *OsABCG41*, *OsABCG47*, and *OsABCG48*) differentially expressed between *japonica* and *indica*, we extracted 2 kb upstream sequences from start codon of four loci in both *japonica* and *indica* genomes from Gramene (http://plants.ensembl.org/Oryza_sativa/Info/Index) and EMBL (http://ensembl.gemene.org/Oryza_indica/Info/Index). Comparison of transcription factor binding sites (TFBSs) was processed by Plant promoter analysis navigator, Plantpan (<http://plantpan.mbc.nctu.edu.tw/>) (Chang et al., 2008).

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