Updated inventory, evolutionary and expression analyses of *G (PDR)* type *ABC* transporter genes of rice

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Abstract

ABC transporters constitute the largest family of transporter proteins in living organisms and divided into eight subfamilies, from A-H. ABCG members, specific to plants and fungi, belong to subfamily G. In this study, we provide updated inventory, detailed account of phylogeny, gene structure characteristics, and expression profiling during reproductive development, abiotic and biotic stresses of members of ABCG gene family in rice along with reannotation and cloning of FL-cDNA of *OsABCG50/PDR23*. We observed that of the 22 ABCGs/PDRs, four genes evolved as a result of gene duplication events and their expression pattern changed after duplication. Analysis of expression revealed seed and developmental stage preferential expression of five ABCG/PDR members. Transcript levels of eight *ABCGs/PDRs* were affected by abiotic and biotic stresses. Expression of seven *ABCG/PDR* genes was also altered by hormonal elicitors. The modulated expression is nicely correlated with the presence of tissue/stress specific *cis*-acting elements present in putative promoter region.

Introduction

Being sessile, plants cannot escape adverse changes in their environment and generally opt for the "overcome" response by implementing specific measures. One such measure includes differential gene expression, which enables adaptation to environmental changes (Chauhan et al., 2011). One of the crucial classes of genes that exhibit differential regulation in their transcription, under environmental stress, comprises transporter proteins, including ATP-binding cassette (ABC) transporters. ABC proteins constitute a superfamily that is ubiquitous in all life forms, such as bacteria, plants, and animals. ABC transporter proteins transport a plethora of compounds that play crucial roles in the entire life of terrestrial plants, such as coating materials (wax), supportive materials (lignin), secondary metabolites (various defense compounds), plant hormones, during gametogenesis, seed development, seed germination, organ formation, and secondary growth (Verrier et al., 2008; Kang et al., 2011; Nuruzzaman et al., 2014; Hwang et al., 2016; Do et al., 2018). ABC transporters also transport complex organic materials against their respective concentration gradients to the regions where the materials are required (Do et al., 2018). In rice and Arabidopsis, 133 and 130 members, respectively, of the ABC transporter superfamily, have been observed; these proteins are responsible for the import or export of a diverse array of substances across the plasma membrane (Verrier et al., 2008; Hwang et al., 2016).

ABC proteins are characterized by the presence of an ABC, also called a nucleotide-binding domain (NBD). This cassette is a hydrophilic domain with five highly conserved motifs, characterized by Walker A and Walker B sequences. A signature C-motif (LSGGQ is a consensus sequence) and a transmembrane domain (TMD), with hydrophobic alpha helices, are the other characteristics features of this transporter class (Bauer et al., 1999). A typical ABC full-size transporter is composed of a core unit containing two NBDs and two TMDs, which can be present as (TMD–NBD)2 and (NBD–TMD)2, while half-size transporter are composed of (TMD–NBD) and (NBD–TMD). NBDs are mainly associated with hydrolysis of ATP to drive active transport, while TMDs are involved in substrate sequestration and translocation (Verrier et al., 2008). The nomenclature of ABC proteins has been a challenging task; various methods are available for ABC protein nomenclature. However, the nomenclature system proposed by Verrier et al. (2008), which is the most frequently followed system, divides plant ABC transporters into eight subfamilies (designated letters A through H). This system is used in the present study.

ABC transporters are known to play a crucial role in maintaining the capacity of plants to withstand constantly changes in the environment. One of the examples of successful involvement of ABC transporters in plants in response to stress is observed in plants that fight against rust disease in cereals. One of the ABC transporters, Lr34, provides durable resistance against leaf rust, stripe rust, and powdery mildew to wheat plants (Krattinger et al., 2009, 2011)..Pleiotropic Drug Resistant or PDR-type ABC transporters belong to subfamily G of ABC transporters and are present only in plants and fungi. They are named after a fungal transporter, found in yeast, which is involved in resistance to weak acids, drugs, and fungicide. The protein is coded in the reverse orientation, which is (NBD–TMD)2.

During evolution from microalgae to green plants, ABC transporter genes are hypothesized to have undergone multiplication and functional diversification to meet the requirements of successful adaptation to dry land through the transport of various critical compounds (Hwang et al., 2016). Many ABC transporters have been reported to play a crucial role in the deposition of sporopollenin during pollen development; *AtABCG26/WBC27* transports sporopollenin from the tapetum to the microspore (Quilichini et al., 2014) and the loss of function of

AtABCG26/WBC27, was reported to result in the formation of a defective exine layer and degeneration of most of the microspores during development. Similarly, in rice, *OsABCG15/WBC15* a putative ortholog of *AtABCG26/WBC27*, exhibited functions similar to those of *AtABCG26/WBC27*, and the presence of the mutated form *osabcg15* caused defects in the exine structure (Niu et al., 2013; Wu et al., 2014). Zhao et al., (2015) reported the collaborative roles of *OsABCG26/WBC26* with *OsABCG15/WBC15* in the developmental regulation of anther cuticle and pollen exine in rice. Double mutant rice plants *osabcg26osabcg15* showed almost complete absence of anther cuticle and pollen exine suggesting collaborative role in rice male development. *OsABCG9/WBC9* plays an essential role in the normal development during early seedlings, particularly root and shoot development. It also plays a role in the transportation of epicuticular wax in rice plants. The mutant line *osabcg9* exhibited the growth retardation and increased sensitivity for drought stress (Nguyen et al., 2018). AtABCG32/PDR4 is a PDR-type transporter and is involved in export of cutin precursors from the epidermal cells in petals; the mutant line *atabcg32* showed a higher cuticle permeability in leaves and petals (Bessire et al., 2011) than the wild-type plants. RCN1/OsABCG5/WBC5 is a half-size transporter, responsible for ABA-mediated stomatal closure by accumulation of the ABA hormone in guard cells (Matsuda et al., 2016). Many ABCG/PDR ABC transporters are induced by biotic stresses (Kang et al., 2011). For example, *AtABCG40*/*PDR12* is induced by fungal and bacterial infections (Campbell et al., 2003). *AtABCG16/WBC16* is induced by bacterial infection and coronatine (Ji et al., 2014). Chemical inducers, such as jasmonic acid (JA), auxins, and heavy metals (e.g., Cd) were reported to induce the expression of *OsABCG36/PDR9* in rice root (Moons, 2003). In the present study, the ABCG/PDR-type ABC transporters were selected for experiments because of their proposed functions during plant development and in diverse conditions, such as mediating resistance against pathogens, heavy metals, and auxinic herbicide compounds (Bauer et al., 1999; Moons, 2003, 2008; Campbell et al., 2003; Lee et al., 2005; Crouzet et al., 2006; Ito and Gray, 2006).

Materials and Methods

Identification of Rice OsABCGs/PDRs, phylogenetic and evolutionary analysis

The identities of rice G type ABC transporters were taken from previous reports (Verrier et al., 2008; Moons, 2008). Additionally, we performed key word ABCG and PDR at TIGR (http://rice.plantbiology.msu.edu/) and RAP-DB (https://rapdb.dna.affrc.go.jp/tools/search) database for related genes to look for any additional member. The nucleotide and protein sequence of Rice OsABCGs/PDRs and their predicted alternative spliced variants were taken from TIGR rice genome project (Kawahara et al., 2013; http://rice.plantbiology.msu.edu/). TIGR locus IDs corresponding to rice *OsABCG/PDR* genes were searched to find the availability of corresponding full-length cDNA. Nucleotide sequences of these genes, their corresponding cDNAs, ESTs and their 1 kb upstream sequences were fetched from same database (Table 1 and Supplementary Table S1). Coordinates of exons and UTR regions were parsed from the GFF3 file, available at Rice Genome Annotation Project FTP. These coordinates were used to show exon intron junctions using Gene Structure Display Server version 2 (Hu et al., 2015; http://gsds.cbi.pku.edu.cn/). For prediction of sub-cellular localization, protein sequences of all the rice *OsABCG/PDR* genes were searched by TargetP program (Emanuelsson et al., 2000) and the position and number of transmembrane helices (TMD) were determined by using TMHMM (version 2.0, Krogh et al., 2001). Phylogenetic analysis of members of *OsABCG/PDR* gene family of rice and Arabidopsis was done by Mega 7.0 software (Kumar et al., 2016), by using Clustal W for multiple sequence alignment and a bootstrap N-J tree was

constructed with default parameters. For this purpose, the respective protein identities of OsABCG/PDR members of both rice and Arabidopsis were taken from (Verrier et al., 2008). For analysis of evolutionary selection pressure, (dNdS ratio) dN and dS values and calculation of their ratio, PAML (http://abacus.gene.ucl.ac.uk/software/paml.html) based calculation was done. A dNdS calculator (Shaw et al., 2012) was used to estimate these values using CDS sequences of the *OsABCG/PDR* genes from rice and Arabidopsis.

Reannotation and cloning of FL-cDNA of *OsABCG50/PDR23*

Based on the TIGR rice genome annotation Release 7, OsABCG50/PDR23 was annotated as two separate protein coding gene loci positioned back to back, LOC_Os12g32814 and LOC_Os12g32820 respectively, each encoding a half-size ABCG transporter protein. Two alternatively spliced transcripts were reported for LOC_Os12g32820 (LOC_Os12g32820.1 and LOC_Os12g32820.2), with two full-length KOME cDNA clones available, J033119C07 (AK103110.1) and J033091K10 (AK102367.1) encoded by the longer transcript of LOC_Os12g32820.1. However, we hypothesize that this could be one gene encoding for a full length OsABCG/PDR type transporter. In order to amplify a full-length *OsABCG50/PDR23* cDNA, total plant RNA was isolated from cv. Nipponbare flag leaves using the TRIZOL® method. 180 µg total plant RNA were used to enrich for poly-A+ RNA with the Qiagen OligoTex mRNA isolation kit and cDNA was synthesized from 150ng poly-A+ RNA in a 12 µl reaction volume using the oligo(d)T primer and the M-MLV enzyme (Promega). PCR amplification of the full-length cDNA was performed on 2µl of 1:6 diluted cDNA using the Herculase enzyme (Stratagene) in a 50ul reaction. The primer pair AATTTCGCAAAGGCAAGAGA(fwd)/ATCAACCTCATGCATTTCGC(rev), located 29 bp 5' of the predicted start codon and 82 bp 3' of the predicted stop codon, was used for full-length amplification. 1µl of the purified primary PCR amplicon was used for a secondary PCR to increase the DNA yield. The purified secondary PCR product was cloned into the StrataClone blunt cloning vector (Agilent Technologies). The complete cDNA sequence in *psc-b(+) OsABCG50/PDR23*_cDNA was verified by sequencing.

Expression analyses in developmental stages and stress treatments

Microarray .cel files from the geo series accession GSE6893, GSE6901 and GSE37557 (Jain et al., 2007; Ray et al., 2007; Garg et al., 2012) were downloaded. These cel files were analyzed in R (https://cran.r-project.org/) version 3.2.3 using bioconductor hosted packages include Affy and GCRMA. Briefly, the .cel files were imported into a project and qualitative analysis was done to check the integrity and any outlier amongst the .cel files. GCRMA normalized expression values were obtained for provided replicates and the average of the $\log_2$ of the expression values was used to plot a heatmap to show the expression profiles exhibited in these samples. To plot the heatmaps, rcolorbrewer and gplot packages of R were used. Manhattan distance across the row were calculated and clustered following ward's rule.

Expression analysis of OsABCG/PDR genes in various tissues through publically available RNA sequencing data at Rice Genome Annotation project (http://rice.plantbiology.msu.edu/)

RNA-seq expression data were taken from RGAP database (http://rice.plantbiology.msu.edu/). Briefly, published data from NCBI sequence read archives were taken and aligned to the recently curated pseudomolecules of RGAP version 7.0. FPKM values generated using selected RNA-seq data were used to plot it in form of heat-map for

easier visualisation. To plot the heat-map, FPKM values were used in R (https://cran.r-project.org/) version 3.2.3 and gplot and r colorbrewer packages. FPKM values were log transformed (log base 2) and were used for plotting the heat map. Stages where FPKM values are '0' (no transcript detected) are shown using 'aquamarine colour on heat-map. FPKM values <1 were converted to '0' to plot on heat-map.

Identification of *cis* acting elements in 1kb upstream region

The information about *cis* elements were downloaded from Place Database (Higo et al., 1998). The data was used to find the location of *cis* acting elements. Whole genome sequences of the Rice cv. Japonica was downloaded from FTP server of RGAP. Transcripts coordinates for all the genes were fetched from locus info file from RGAP version 7(ftp://ftp.plantbiology.msu.). 1Kb upstream coordinate for each gene was calculated and used as putative promoter. The sequences comprising the same were fetched using a custom written perl script employing samtools version 1.1 (Li et al., 2009) and the Chromosome sequences available from RGAP ver. 7.0 (ftp://ftp.plantbiology.msu). The identified *cis* elements were plotted on a to map scale to represent their relative distribution. Additionally, the 1-kb putative promoters of all *OsABCG/PDR* genes were also analyzed for the putative *cis*- acting elements using PLANTCARE database (Lescot et al., 2002).

Plant material, growth conditions, chemical treatment and RNA isolation

Rice*, Orzya sativa* (sp. *indica* cv. IR64), seeds were obtained from Indian Agricultural Research Institute, New Delhi, for the current study. Seeds were allowed to grow for 15-20 days under 16:8 day/night condition at 25^{o}C temperature. Various stress and hormone treatments were given at 3-leaf stage. For various stress treatments, plants were kept with immersed roots in aqueous solutions of 200 mM NaCl (salt stress), 100 mM $CdCl_2$ (heavy metal stress) and 10 mM $CaCl_2$. For heat stress plants were kept at heat 42^{o}C for four hours in a growth chamber. Similarly, 2,4-D (2 µM, Sigma, USA), brassinosteroid (1 µM, Sigma), 6-BAP (2 µM, Sigma), jasmonic acid (10 µM, Sigma), salicylic acid (100 µM, Sigma) treatments were also given for 4 hours. For biotic stress, rice plant grown in polyhouse were infected at flowering stage, with sheath blight fungus (*Rhizoctonia solani*), taken from field grown naturally infected plants. After treatments, the tissues were snap frozen in liquid Nitrogen and kept at -80 ^{o}C till RNA isolation. Total RNA from root, shoot and flower tissues was isolated using SV Total RNA Isolation System (Promega, WI, USA) with on-column RNase-free DNase-I treatment to remove any genomic DNA contamination. For isolation of RNA from developing seeds, method described by (Singh et al., 2003) was used. The integrity of RNA was checked using 1.2% MOPS-formaldehyde denaturing gel. The concentration of RNA was measured using NanoDrop™.

Quantitative real-time PCR analysis

Transcript levels for all rice *OsABCG*s/*PDRs* under different developmental and stress conditions were quantified by qRT-PCR analysis using Quant Studio 3 system (Thermo Fischer Scientific, USA). The qRT-PCR analysis was done in an array of 28 different tissues (Table 2) representing different developmental stages, abiotic and biotic stresses. Primers were designed using unique region of the cDNAs of rice *OsABCG/PDR* genes by Primer express 3.0 (Applied Biosystems, USA; Supplementary Table-S2). First-strand of cDNA was synthesised by reverse transcription of 1µg of total RNA using high capacity cDNA reverse transcription kit (Thermo Fischer Scientific, USA) as per manufacture's instruction. These cDNA samples were mixed with 5 µM of each primer and PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, USA) in 96-well optical reaction plates

(Applied Biosystems, USA). The identity of the amplicon and specificity of the reaction were verified by melting curve analysis. The relative cDNA level for the each of the *OsABCG/PDR* genes was normalized using *Ubiquitin 5* gene as housekeeping control (Jain et al., 2006). The analysis of expression was based on three replicates of each sample and expression in terms of relative fold change was calculated using ΔΔcT method by using untreated root and shoot as control tissues. And for flower and developing seeds, untreated shoot was used for the calculation of relative fold change. To represent the qPCR expression results, the relative fold change values calculated by ΔΔcT methods were used to make a heat map. In R (version 3.2.3), the values were imported and by using 'gplots' library, these values were plotted as heat-map. To cluster the data, distance was calculated using 'Manhattan' method and 'Ward' clustering method was applied.

Results

Phylogeny, gene structure, and distribution of the rice *OsABCG/PDR* gene family

The subfamily OsABCG/PDR is characterized by the reverse orientation of the NBD and TMD, which is generally repeated twice (NBD–TMD)2. The rice *OsABCG/PDR* gene subfamily is reported to comprise either 20 members (Verrier et al., 2008) or 23 members (Moons, 2008), however after reannotation of *OsABCG33/PDR14* as a retrotransposon, the actual number is 22, including 20 and 2 full-size and half-size transporters, respectively. The characteristic features of rice OsABCG/PDR proteins, such as length, transmembrane domain position, and predicted localization are provided in Table 1 and supplementary table S1, along with The Institute of Genomic Research (TIGR) locus and FL-cDNA accession numbers. The rice *OsABCG/PDR* genes were asymmetrically distributed and were located on only 7 of 12 chromosomes. The maximum seven *OsABCG/PDR* genes were present on chromosome 1 followed by four *OsABCGs/PDR* genes on chromosome 9 (Supplementary Fig. 1). Phylogenetic analysis conducted using the alignment of the deduced full-length protein sequences divided the rice OsABCG/PDR proteins into two major clades (Fig. 1).

OsABCG 36*/PDR9* (*Os01G42380*), *OsABCG35/PDR11* (*Os01G4237*0), and *OsABCG 34/PDR10* (*Os01G42350*), which are all closely located on chromosome 1, appeared to be tandemly duplicated. Of the three, only *OsABCG 36/PDR9* appears to be expressed, as evidenced from the presence of FL-cDNA, many expression sequence tags (ESTs) and expression analyses were conducted in the present study (Table 1 and 2); some expression analyses were previously reported by Moons (2008). Similarly, *OsABCG46/PDR21, 47/19, 52/18,* and *53/20,* present on chromosome 9, that are clubbed together in phylogenetic tree were duplicated (Fig. 1, Table 1). Among these genes, only *OsABCG53/20* was expressed and the others were not expressed; furthermore, no FL-cDNA was available for *OsABCG 46/PDR21, 47/19,* and *52/18*. Notably, a retrotransposon occurred before *OsABCG 46/PDR21, 47/19,* and *52/18*. To determine the evolutionary relatedness between members of dicot and monocot species, we also constructed a similar phylogenetic tree by using rice and Arabidopsis AtABCG/PDR members (Supplementary Fig S2). In the combined tree, we also observed two distinct clades, with considerable similarity as far as the members of these two species were concerned.

The grouping of the members from a species was clear; however, in the subclades, the ABCG/PDR members were grouped together according to their names. For example, OsABCG/PDR 10, 11, and 12 of rice were grouped with AtABCG/PDR 10, 11, and 12 of Arabidopsis. Hence, Arabidopsis exhibits evolutionary conservation. Furthermore, the ratio of nucleotide substitution rates, nonsynonymous (dN) to synonymous (dS), which indicate the selective pressures operating on these genes, indicates evolutionary conservation. In the present analysis, the dN/dS ratio for 13 *OsABCGs/PDRs* was determined (Supplementary Table S3). For all these rice *OsABCG/PDRs*, dN/dS ratio was found to be <1.0, compared with their Arabidopsis homologs. These results indicate that the proteins coded by the aforementioned genes have been subjected to purifying selection during the course of evolution, and selection pressure was applied to retain and conserve the protein sequences; furthermore, mutations in these sequences are of a neutral type. None of the *OsABCG/PDRs* showed positive selection pressure. This also explains the moderately conserved function of these proteins across dicots and monocots.

Most of the *OsABCGs/PDRs*, except for *OsABCG43/PDR5 and OsABCG31/PDR6*, contain at least 10 exons in their genomic sequences (Supplementary Fig. S3). While *OsABCG40/PDR4, OsABCG42/PDR12, OsABCG38/PDR15,* and *OsABCG44/PDR17* contain exons 10–20, the remaining *OsABCGs/PDRs* have more than 20 exons. Notably, the FL-cDNAs of only 12 *OsABCG/PDR* genes were found in TIGR database. No information on the FL-cDNAs of *OsABCG45/PDR1*, *OsABCG41/PDR2*, *OsABCG40/PDR4*, *OsABCG34/PDR10*, *OsABCG35/PDR11*, *OsABCG47/PDR19, OsABCG46/PDR21,* and *OsABCG49/PDR22* was found in the TIGR. Furthermore, most of these genes lacked EST support, except for *OsABCG45/PDR1* and *OsABCG49/PDR22,* and many of the genes, which were duplicated and lacked expression were preceded by retrotransposons (Fig 1 and Table 1). Rice *OsABCG50/PDR23* (*Os12G32820*) was incorrectly annotated as the predicted gene and the corresponding FL-cDNA (AK102367) coded for a protein with only 721 amino acid residues. *OsABCG50/PDR23* is closely related to wheat *Lr34* and shows a high amino acid similarity of 86% (Krattinger et al., 2011). Sequence alignment of the genomic sequence on rice chromosome 12, containing the manually annotated LOC_Os12g32820 and LOC_Os12g32814, with the genomic sequence of *Lr34res* from wheat cv. Chinese Spring allowed the reannotation of these two loci as a single gene. According to this new annotation, the *OsABCG50/PDR23* gene encodes a putative full-size pleiotropic drug resistance ABCG/PDR transporter, spanning a coding sequence of 11,617 bp, which consists of 24 exons and 23 introns. This structure is similar to that of *Lr34*. Experimental evidence for the *Lr34*-based reannotation of *OsABCG50/PDR23* was obtained by amplification of a full-length transcript of 4221 bp coding for 1406 residues (Supplementary Document 1). The deduced protein exhibited all the characteristic features of a full-size OsABCG/PDR transporter, such as two NBDs and two TMD, Walker A and Walker B motifs, and PDR signature sequences (Supplementary Fig S4). TMHMM-based prediction of the presence of TMD and the number of transmembrane helices revealed that 16 OsABCGs/PDRs have two TMDs with six or seven transmembrane helices, while the remaining two members, namely OsABCG43/PDR5 and OsABCG31/PDR6, contain only one TMD with six or seven transmembrane helices. Target peptide analysis predicted that most OsABCGs/PDRs are carrier proteins across the plasma membrane. Some OsABCGs/PDRs, such as OsABCG48/PDR3, OsABCG34/PDR10, and OsABCG35/PDR11, were predicted to be transporter proteins across chloroplast membrane, while OsABCG39/PDR7, OsABCG37/PDR8, and OsABCG32/PDR16 were predicted to carry substrates across mitochondria membrane. Among all the rice OsABCGs/PDRs, only OsABCG44/PDR17 was predicted to be a secretory protein, which is

secreted outside the cell and performs its function outside the plasma membrane. The remaining 12 OsABCGs/PDRs were predicted to be proteins localized on the plasma membrane. While studying their chromosomal distribution, a cluster of four *OsABCGs/PDRs,* namely *OsABCG34/PDR10, OsABCG35/PDR11, OsABCG36/PDR9* and *OsABCG37/PDR8,* was found to be located on the long arm of chromosome 1 (Supplementary Fig. S1). From this cluster, two proteins were predicted to be localized in the chloroplasts, one in the mitochondria, and another one in the plasma membrane, which indicated the absence of correlation between the chromosomal location of the genes coding the proteins and the predicted cellular localization of the proteins. Furthermore, the proteins predicted to be present on the plasma membrane were of different sizes, whereas those predicted to be localized in the mitochondria or chloroplasts exhibited comparable sizes.

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Microarray expression profiles of rice *OsABCG/PDR* genes across developmental stages and in hormone- and stress-treated samples

Techniques in functional genomics, such as microarray and RNA-seq, enable simultaneous monitoring of thousands of genes. In this analysis, previously generated data were analyzed to obtain expression profiles exhibited by rice *OsABCG/PDR* genes. Five clusters of *OsABCG/PDRs* genes were identified based on their expression correlation (Fig. 2). A major cluster of genes, namely *OsABCG45/PDR1, OsABCG36/PDR9, OsABCG48/PDR3, OsABCG42/PDR12, OsABCG47/PDR19,* and *OsABCG53/PDR20,* have shown relatively high expression in reproductive stages as well as in the stress- and hormone- (IAA and BAP treatments) treated samples. *OsABCG42/PDR12* and *OsABCG44/PDR17* showed relatively high expression in late panicle developmental stages and early seed developmental stages. By contrast, *OsABCG45/PDR1* showed higher accumulation levels of transcripts at late seed developmental stages. A cluster of three *OsABCGs/PDRs,* including *OsABCG34/PDR10*, *OsABCG35/PDR11*, and *OsABCG46/PDR21* showed negligible expression among the tissues that were studied (Fig. 2). This suggest that they might have some very specific role and their expression is not perturbed in the selected stages. Similar to the aforementioned cluster, two genes, namely *OsABCG49/PDR22* and *OsABCG50/PDR23,* were identified with mild level expression across all the stages. Another cluster of three genes, namely *OsABCG31/PDR6*, *OsABCG38/PDR15,* and *OsABCG47/PDR19,* showed moderate expression levels across in the studied stages. The fifth group of *OsABCG/PDRs,* which comprised *OsABCG41/PDR2*, *OsABCG40/PDR4,* and *OsABCG39/PDR7,* showed relatively high expression levels in vegetative developmental stages or in response to hormone treatments.

Expression analysis of rice OsABCG/PDR genes in various tissue through RNA-seq data available at Rice Genome Annotation Project

RNA-seq FPKM value showed that, there were high expression of *OsABCG45/PDR1* and *OsABCG31/PDR6* during seed development as also revealed by microarray analysis. During pre-emergence and post-emergence inflorescence *OsABCG31/PDR6* has shown higher expression as seen in both RGAP RNA-seq and microarray analysis. In shoot and leaf tissue, there was high expression of *OsABCG42/PDR12* gene observed by RNA-seq analysis while there was moderate expression of this gene in young leaf as revealed in microarray analysis.

We found comparable similarities in expression data of RNA-seq and microarray for many tissues (Fig 2 and Supplementary Fig S5). Such as, in leaf, *OsABCG53*/*PDR20* was highly expressed and *OsABCG40/PDR4* and *OsABCG42*/*PDR12* were moderately expressed in both RNA-seq and microarray analysis. In caryopsis, *OsABCG45*/*PDR1* and *OsABCG31*/*PDR6* showed high expression levels in both analyses. Our experimental results also showed the expression levels of *OsABCG45/PDR1, OsABCG31/PDR6,* and *OsABCG37*/*PDR8* were high during seed development. Furthermore, we found that the genes (*OsABCG/PDR11, OsABCG52/PDR18*, *OsABCG47/PDRl9,* and *OsABCG46/PDR21),* which were not expressed or showed the lowest expression levels in microarray analysis also showed similar expression in RNA sequencing.

Expression profiling of rice *OsABCG/PDRs* genes through qPCR in different developmental stages and under various stresses

Expression analysis of rice *OsABCG/PDRs* genes was performed through qRT-PCR in 28 tissues representing abiotic and biotic stresses, key plant developmental stages, and treatments with plant growth regulators and chemicals. The results in terms of "relative fold change" are presented in Table 2 and in a heat map (Supplementary Fig S6). For this analysis, we sampled tissues representing reproductive phase of rice plant from flowering to maturity at different time points in their reproductive phase. We found that six *OsABCG s/PDRs* are expressed in all the four types of test conditions (abiotic stress, biotic stress, reproductive development, and chemical inducers), while another six are expressed in abiotic stresses, developmental stages, and chemical inducers (Fig. 3). In this analysis, we identified 10 and 9 *OsABCG/PDR* genes with preferential expression in the flowering stage and in seed development, respectively. In the flowering stage, *OsABCG43/PDR5* and *OsABCG31/PDR6* exhibited the highest transcript levels. *OsABCG31*/*PDR6* expression was recorded to be the highest at 3 and 14 days after pollination (DAP). *OsABCG37/OsPDR8* and *OsABCG45/PDR1* were found to have the highest transcript levels at the later stages, namely 7 and 21 DAP, respectively. In general, *OsABCG31PDR6, OsABCG37/PDR8,* and *OsABCG53/PDR20* were expressed across all seed developmental stages (Table 2, Fig 2 and Supplementary Fig S6

Eight *OsABCG/PDRs* genes were upregulated in root tissue with a relatively high induction of *OsABCG36/PDR9*, while seven *OsABCG/PDR* genes were upregulated in shoot tissue with very high expression levels of *OsABCG44/PDR17* under drought conditions (Table 2). Similarly, seven and 10 *OsABCG/PDRs* genes were upregulated in the root and shoot, respectively. Salinity stress exhibited a relatively mild effect on *OsABCG/PDR* gene expression because only *OsABCG43/PDR5* and *OsABCG36/PDR9* exhibited high expression levels in the root, whereas *OsABCG37PDR8* is mildly induced in shoot. Expression profiles of these genes were also influenced by hormones and heavy metal stress. The synthetic auxin 2,4-D was found to regulate the expression of five *OsABCG/PDRs* genes in the root, including highly upregulated *OsABCG36/PDR9* and *OsABCG45/PDR1*. BAP (cytokinin) treatment also induced the expression of 10 *OsABCG/PDRs* genes in the root, including *OsABCG53/PDR20, OsABCG32/PDR16*, *OsABCG44/PDR17*, *OsABCG42/PDR12,* and *OsABCG45/PDR1,* with the highest induction levels. Furthermore, nine genes, including *OsABCG36/PDR9*, *OsABCG37/PDR8*, *OsABCG44/PDR17*, *OsABCG43/PDR5,* and *OsABCG45/PDR1,* were highly induced in the shoots. JA induced nine *OsABCG/PDR* genes in the roots among which *OsABCG36/PDR9*, *OsABCG43/PDR5,* and

OsABCG45/PDR1 were the highly expressed, and five *OsABCG/PDRs* genes in the shoots. Similarly, salicylic acid also induced the expression of *OsABCG36/PDR9*, *OsABCG37/PDR8,* and *OsABCG45/PDR1* in the root and *OsABCG40/PDR4* and *OsABCG43/PDR5* in the shoot. Brassinosteroids (BR) also induced the expression of six *OsABCG/PDRs* genes in the roots, with exceptionally high expression of *OsABCG36/PDR9,* and seven *OsABCG/PDRs* genes in the shoots. Under $CaCl_2$ treatment, *OsABCG36/PDR9* was highly expressed in the roots followed by *OsABCG45/PDR1*, while in the shoots moderate expression of *OsABCG43/PDR5* was observed. The heavy metal salt $CdCl_2$ induced extremely high expression of *OsABCG36/PDR9* in the roots and high expression of *OsABCG44/PDR17* in the shoots. In general, we found that under most of the stress conditions, several *OsABCG/PDRs* genes were induced in the root tissues, except for heat stress, where a relatively high number of *OsABCG/PDR* genes were induced in the shoot (Table 2). This can be explained by the observation that heat stress directly affects shoot, while the remaining stress treatments were provided by immersing the roots in aqueous solutions. As far as biotic stress of sheath blight disease of rice caused by fungus *Rhizoctonia solani* is concerned, we found upregulation the seven *OsABCG/PDRs* genes by this infection and a high expression of *OsABCG45/PDR1*, *OsABCG48/PDR3, OsABCG37/PDR8,* and *OsABCG51/PDR13* was detected in both diseased leaf sheath and inflorescence tissues (Table 2 and Supplementary Fig S6).

We noted a strong correlation among the result of microarray, RNA-seq, and q-PCR-based expression analyses. The *OsABCG/PDR* genes, namely *OsABCG34/PDR10, OsABCG35/PDR11, OsABCG47/PDR19, OsABCG46/PDR21, OsABCG49/PDR22, OsABCG50/PDR23, and OsABCG52/PDR18,* showed minimal or no expression in various tissues tested in both microarray and qPCR (Fig. 2 and Table 2). By contrast, the *OsABCG/PDR* genes *OsABCG45/PDR1, OsABCG48/PDR3, OsABCG36/PDR9, OsABCG42/PDR12*, *OsABCG44/PDR17,* and *OsABCG53/PDR20* were highly expressed in various tissues, included in both microarray and qPCR based expression analysis (Fig 2 and Table 2). *OsABCG45/PDR1*, *OsABCG31/PDR6,* and *OsABCG53/PDR20* these three genes were expressed in the flowers and developing seeds, while *OsABCG44/PDR17* was induced under drought and saline conditions as confirmed using microarray and qPCR analysis. The putative promoter region of *OsABCG44/PDR17* contains drought response elements (DRE).

Distribution of functional *cis*-elements in the upstream regulatory region of rice *OsABCG*/*PDR* genes

Analysis of *cis*-elements in 1-kb upstream region gives an idea about the regulatory regimen followed by the downstream genes. Some of these elements with conserved pattern were identified using PLACE and PlantCARE databases (Supplementary Table S4 and S5). In total, 24 different types of *cis*-acting regulatory elements were identified in the promoter of *OsABCG/PDR* genes (Supplementary Fig- S7).

Top ten over-represented *cis*-acting regulatory elements in these promoters includes I_box_light_regulated (GATAA), GT-1_Motif_Salt_induced (GAAAAA), ABRE (ACGTCGGC), DREB/CBF1_binding_site (GCCGAC), CarG_motif_AGL15 (CATATTTTTG), RD22_binding_site (CACATG), LTRE (CCGAAA), Root_Hair-specific_cis-elements (TCACGA), ASF1-Auxin/Salicylic_Acid (TGACG), A_box_Sugar_repression (TACGTA). Additionally, some of the *cis*-acting regulatory elements with conserved pattern could be identified using the PlantCARE database [29]. We also analyzed the putative promoter sequence for stress-related and developmental-specific *cis*-acting regulatory elements because several genes were induced by both biotic and abiotic stress and at different developmental stages. A total of 13 such associated elements were identified

(Supplementary Table S5). Many of them exhibited a strong positive correlation with its presence in the promoter and upregulation of transcript levels in the qPCR data (Table 2). Five *cis*-acting regulatory elements that control gene expression in abiotic stress responses including *HSE*, low temp, ABA, Drought, and Anoxic stress were identified. A total of 9, 6, 11, 12 and 1 genes harbor these elements in their promoters, respectively. However, only 7, 0, 8, 12, 0 have shown modulation in their expression in response to heat, ABA, and draft abiotic stresses. Similarly, seven biotic stress responsive (fungal pathogen) *cis*-elements were also identified in the upstream region of *OsABCG48/PDR3, OsABCG39/PDR7, OsABCG36/PDR9, OsABCG46/PDR21, OsABCG40/PDR4, OsABCG35/PDR11* and *OsABCG50/PDR23,* and four of them have also shown expression induction in fungus infected tissues (Table 2). Six hormone regulated *cis*-elements, namely auxin, salicylic acid (SA), methyl jasmonate (MeJA), ethylene, gibberellin, and zeatin, were identified in the upstream regions of 5, 7, 10, 5, 8 and 8 *OsABCGs/PDRs,* respectively, and induction of 4, 5, and 9 *OsABCGs/PDRs* were reported by treatment of auxin, SA, and MeJA. Four *cis*-acting regulatory element regulating expression in a specific developmental stage (seed) were identified of which three were functional. This analysis indicates that expression of *OsABCG/PDR* genes is often correlated with the presence of known *cis*-acting regulatory elements.

Discussion

ABC transporters are found in all living organisms and constitute one of the largest protein families in plants (Hennikoff, 1997). PDR-type ABC transporters are specific to plants and fungi and belong to the subfamily G. In the present investigation, we conclude that there are 22 members in ABCG/PDR gene family of rice. .

The members of the ABCG/PDR subfamily with a reverse (NBD–TMD) 2 configuration are mostly localized to the plasma membrane (Crouzet et al., 2006). In the present investigation, we also observed that most of the ABCGs/PDRs are predicted to be localized to the plasma membrane. The only two exceptions were OsABCG48/PDR3, which were predicted to be localized to chloroplasts, and OsABCG44/PDR17, a secretory protein (Table 1). Thus far, ABCG/PDR proteins have not been found to localize in the chloroplast, but several ABC transporters, which are localized to plastids and the endoplasmic reticulum and involved in the transportation of lipids (Roston et al., 2012; Kim et al., 2013).

ABC transporter genes might have undergone multiplication and diversification events during evolution. Specifically, the ABCG/PDR-type ABC transporter genes might have evolved recently (Hwang et al., 2016). Andolfo et al (2015) studied duplication in the ABCG/PDR gene family in different plant species including rice and Arabidopsis. They reported that in rice *ABCGs/PDRs 34/10, 35/11,* and *36/9* are part of a block duplication event. Notably, we observed that only for *ABCG36/PDR9*, an *FL-cDNA* has been submitted and that only *OsABCG36/PDR9* is expressed, as revealed by qPCR and microarray analysis (Fig. 2 and Table 2). We hypothesized that the regulatory mechanism of *OsABCG34/PDR10* and *OsABCG35/PDR11* were altered during duplication events. Similarly, *OsABCG47/PDR19* and *OsABCG53/PDR20* are part of another block duplication and *OsABCG37/PDR8* and *32/16* are tandemly duplicated (Andolfo et al., 2015). The FL-cDNA for *OsABCG46/PDR21* and *OsABCG47/PDR19* is not available, and no or little expression observed has been observed with these genes in micro array and qPCR expression analysis. In the case of tandemly duplicated pair of *OsABCG37/PDR8* and *OsABCG32/PDR16*, the expression level of *OsABCG37/PDR8* is higher than that of *OsABCG32/PDR16* (Fig. 2 and Table 2). Furthermore, most of the duplicated and non-expressing *OsABCG/PDR*

genes have retrotransposons in their vicinity, which could explain the nonavailability of FL-cDNA and markedly reduced expression (Table 1 and Supplementary Fig 1). From the discussion and our results, we can conclude that although the expansion of the *OsABCG/PDR* gene family occurred through duplication, the expression pattern was also altered in the duplicated genes.

In Arabidopsis, both ubiquitous and tissue-preferential expression of *AtABCG*/*PDR* genes have been reported. For example, *AtABCG36/PDR8* is expressed in all tissues, *AtABCG40/PDR12* is expressed in the inflorescence and root while *AtABCG30/PDR2* and *AtABCG34/PDR6* are specific to the root (Van den Brule et al., 2002). Similarly, Nguyen et al., (2014) showed the tissue specific expression of *OsABCG/PDR* genes in various rice tissues like root (*OsABCG48/PDR3*), shoot (*OsABCG40/PDR4*), leaf (*OsABCG47/PDR19*), and endosperm (*OsABCG45/PDR1*). We also found high expression levels of *OsABCG43/PDR5* in inflorescence, while *OsABCG45/PDR1*, *OsABCG31/PDR6,* and *OsABCG37/PDR8* was specifically expressed during seed developmental stages of rice. *OsABCG/PDR* genes have been shown to be regulated by abiotic stress like heat, salt, drought, cold and heavy metals (Nguyen et al., 2014). They showed the upregulation of *OsABCG36/PDR9* gene under the salt stress condition, and our expression study was also in agreement with them in rice root. Nguyen et al., (2014) observed that, during drought stress in leaf, there were upregulation of *OsABCGs/PDRs* (37/8, 45/1, 48/3 and 36/9) genes. Similarly, we found that there were upregulation of *OsABCG36/PDR9* and *OsABCG37/PDR8* genes during drought stress in rice shoot tissues and marked increase in expression of *OsABCG36/PDR9* in rice root tissue.

Many reports have suggested that while the upregulation of some *PDRs* is both tissue- and stress-specific, others respond to a wide range of stress conditions (Moons, 2008; Verrier et al., 2008; Stein et al., 2006; Hwang et al., 2016). We also observed that several *OsABCG/PDR* genes, such as *OsABCG37/PDR8, OsABCG36/PDR9,* and *OsABCG44*/*PDR17,* showed significantly higher expression levels in abiotic stresses such as heat, drought, and chemical treatments.

Not only, abiotic stresses were found to affect the expression of *OsABCG/PDR* genes in rice, but also their transcript level, which were altered by biotic stress. Similarly, in Arabidopsis, *AtABCG40/PDR12* was differentially induced in the plants challenged with compatible and incompatible fungal pathogen as well as treatment with biotic-stress-related hormones, such as salicylic acid, ethylene, and MeJA, thus suggesting its role in multiple defense pathways (Campbell et al., 2003).

Jasmonates have been reported to play essential roles in plant defense and secondary metabolism (Moons, 2008). In the rice root, *OsABCG36/PDR9* is highly induced by JA, whereas upregulation of its expression by heavy metals, polyethylene glycol, high salt, hypoxic stress, and redox perturbations indicated that *OsABCG36/PDR9* plays a general role in abiotic stress responses (Moons, 2003, 2008). A study reported that antioxidants, such as dithiothreitol and ascorbic acid, markedly induced the expression of *OsABCG36/PDR9* in rice root, while hydrogen peroxide, the strong oxidant, also induced the expression but to lower extent than did the antioxidants (Moons, 2003). Our experimental results also showed a marked increase in the induction of *OsABCG36/PDR9* by JA, BR, SA, the secondary messenger $CaCl_2$, and heavy metal salt $CdCl_2$ in rice roots. .

The wheat *Lr34* gene, which provides durable, multipathogen resistance, codes for an ABC transporter belonging to the ABCG/PDR subtype and the resistant allele of *TaLr34* evolved after wheat domestication through two gain

of function mutation in the first transmembrane domain of ABC (Krattinger et al., 2009, 2016). The resistant allele of *TaLr34* has been successfully transferred to several important crop plants, such as wheat (Rinaldo et al., 2017), barley (Chauhan et al., 2015), rice (Krattinger et al., 2016), and maize (Sucher et al., 2017), and found to impart multipathogen resistance in transgenic plants. Rinaldo et al., (2017) reported that *TaLr34,* expressed in transgenic durum wheat seedlings, provides robust seedling resistance to pathogens causing wheat leaf rust, stripe rust, and powdery mildew. Chauhan et al (2015) demonstrated the strong constitutive, reprogramming of metabolism in transgenic barley plants expressing *TaLr34*. Transgenic barley showed an increased level of expression of multiple defense pathways genes as well as high levels of JA, SA, lignin, and hordatines. The transgenic rice plant expressing *TaLr34* also showed increased resistance against *Magnoporthe oryzae*, the causal agent of rice blast disease (Krattinger et al., 2016). Sucher et al., (2017) showed the maize hybrid Hi-II expressing *TaLr34* exhibited increased resistance against rust and northern corn leaf blight fungal diseases, furthermore transgenic maize was reported to develop a phenotype of late leaf tip necrosis without a negative effect on plant growth. Krattinger et al., (2019) found that ABA phytohormone act as a substrate of LR34 ABC transporter and *Lr34res*-expressing rice line had increased ABA accumulation, which causes the induction of ABA regulated genes, physiological alterations and disease resistance. In the present study we cloned the FL-cDNA and reannotated rice *OsABCG50/PDR23*, however, whether it functions in similar way as wheat Lr34 remains to be studied. We also found that expression of *OsABCG45/PDR1, OsABCG43/PDR5, OsABCG37/PDR8, OsABCG51/PDR13,* and *OsABCG53/PDR20* was markedly upregulated in infected sheath and spike tissues in rice infected by the sheath blight fungus *Rhizoctonia solani*. Whether any of these rice *OsABCG/PDR* gene can be used as a source of enhanced resistance to fungal pathogen(s) should be investigated.

Conclusion

There are examples across the plant kingdom showing the involvement of PDR transporters in mediating stress response against different stresses like physical, hormonal, abiotic and biotic stresses. Because plant ABC transporters transport molecules that are important not only for plant growth and development but also for survival under adverse conditions, use of these genes in molecular breeding through transgenic approaches may form the basis for developing improved crops. A better understanding of ABC transporters that are involved in stress and growth hormones can be used to develop crops with improved stress resistance and improved yield and biomass, respectively. Our experiments showed the rice *OsABCG45/PDR1, OsABCG36/PDR9*(abiotic stress) and *OsABCG48/PDR3*, OsABCG53/PDR13 (biotic stress) genes could be the candidate genes for the development of transgenic in rice as well as in other cereal crop with increased tolerance to the abiotic stress and biotic stress respectively.

Author contribution statement

HC conceptualized and supervised the study, DA, BBG, VKB, LS, SGK did the experiments, HC, DA, BBG, SKM, PS, AKP, RC, VKB, SGK and RK analyzed the data and wrote the manuscript.

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Conflict of Interest: The authors declare that they have no conflict of interest

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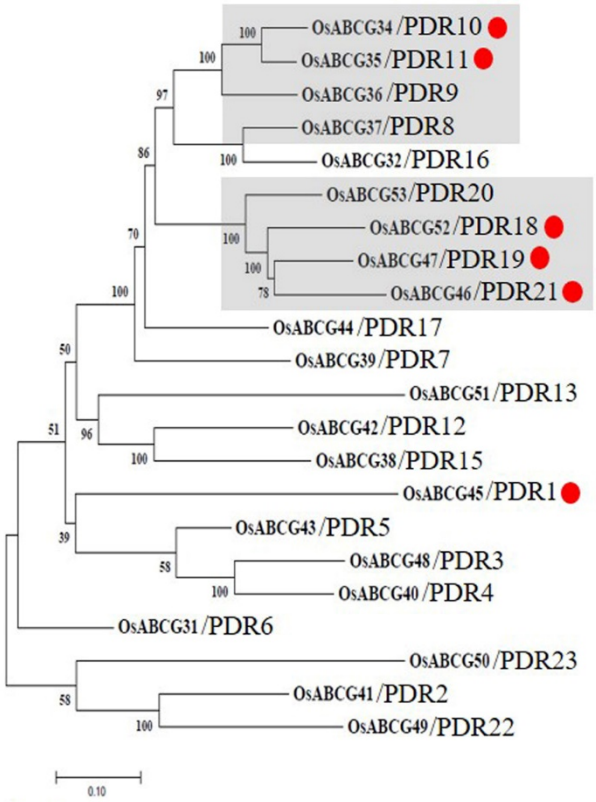

Fig 1

Fig 1: Phylogenetic analysis of members of OsABCG/PDR type ABC transporter of rice. The tree was constructed in MEGA7 by using Clustal W for multiple sequence alignment and a bootstrap N-J tree was constructed with default parameters. The OsABCG/PDRs shown in shaded box represents duplicated members and the red dot represent presence of retrotransposon ahead of the gene.

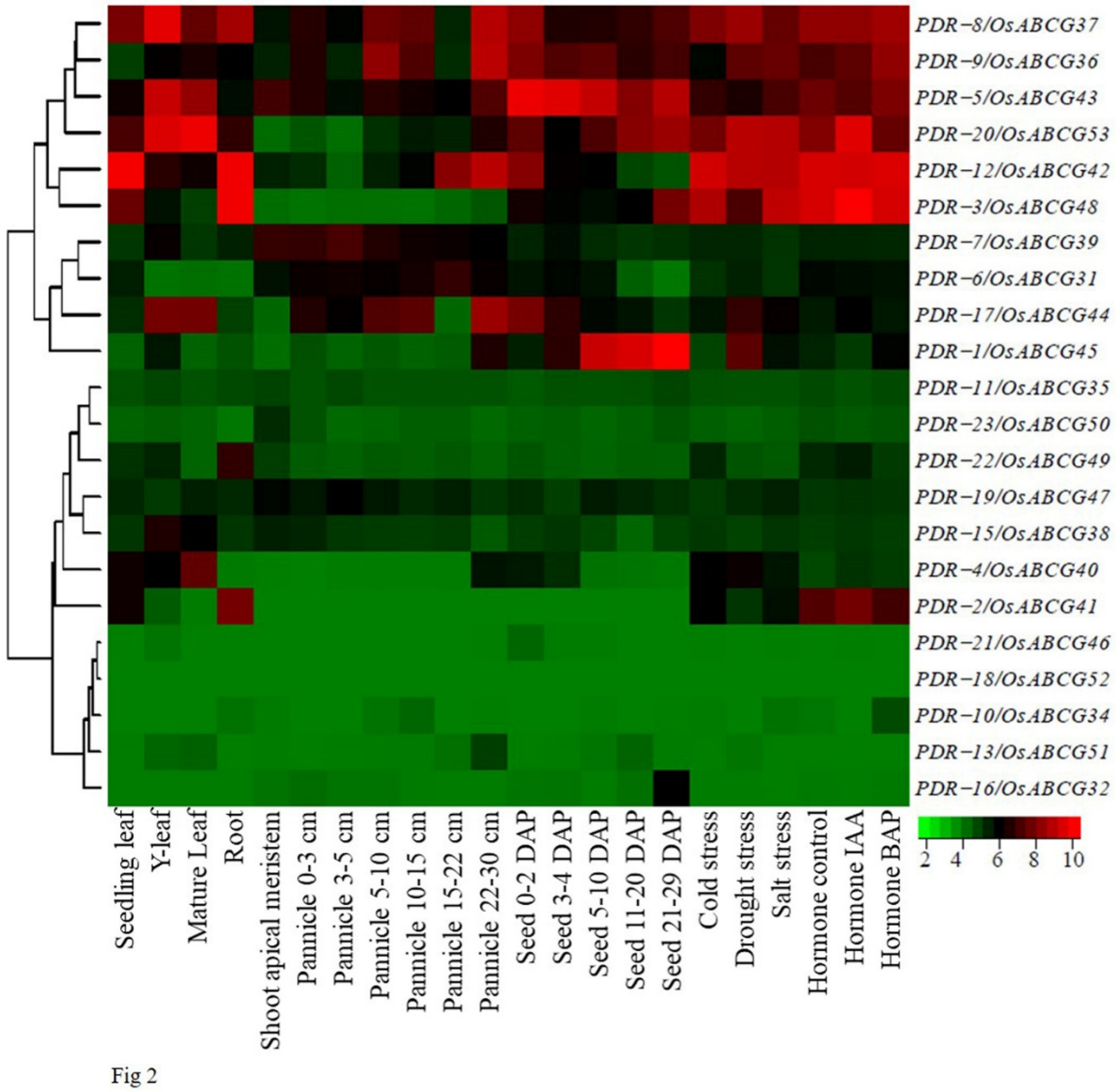

Fig 2: Heat map showing micro array based expression analysis of members of *PDR* gene family of rice in various tissues (Jain et al. 2007; Ray et al. 2007; Garg et al. 2012).

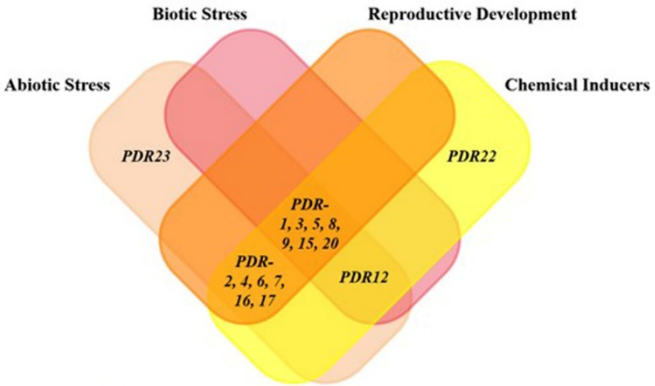

Fig 3: Venn diagram showing overlapping expression of PDR genes of rice in various conditions. The Venn diagram was created by using FunRich tool [38], (http://funrich.org).

Table -1 Rice PDR genes and features

Name	TIGR LOCUS	NCBI Gene Symbol	FL cDNA	Amino Acids	Position of TMD and number of transmembrane helices	Localization Prediction	Comments
OsABCG45/PDR-1	Os08g43120	LOC4346210	--	1324	425-676 (6) & 1069-1316 (7)	Plasma Membrane	Retrotransposon ahead
OsABCG41/PDR-2	Os02g32690	LOC9268937	--	1412	535-668 (4) & 1155-1404 (7)	Plasma Membrane	Expressed as many ESTs are available
OsABCG48/PDR-3	Os11g37700	LOC4350807	AK121517	1455	553-807 (6) & 1200-1447 (6)	Chloroplast	
OsABCG40/PDR-4	Os02g21340	LOC4329158	--	1123	280-446 (6) & 868-1155 (6)	Plasma Membrane	Expressed as many ESTs are available
OsABCG43/PDR-5	Os07g33780	LOC4343412	AK105311	588	332-579 (6)	Plasma Membrane	Putative half PDR
OsABCG31/PDR-6	Os01g08260	LOC4323865	AK061474	612	355-604 (7)	Plasma Membrane	Putative half PDR
OsABCG39/PDR-7	Os02g11760	LOC4328687	AK073104	1444	521-773 (6) & 1191-1436 (7)	Mitochondria	
OsABCG37/PDR-8	Os01g42410	LOC4327730	AK121722	1443	519-768 (6) & 1189-1435 (7)	Mitochondria	
OsABCG36/PDR-9	Os01g42380	LOC4327728	AK 271618	1457	534-784 (6) & 1206-1449 (7)	Plasma Membrane	
OsABCG34/PDR-10	Os01g42350	LOC9272130	--	1479	547-797 (6) &1215-1471 (7)	Chloroplast	Tandem duplicated gene, not expressed, Retrotransposon ahead
OsABCG35/PDR-11	Os01g42370	LOC4327727	--	1444	541-793 (6) & 1191-1436 (7)	Chloroplast	Tandem duplicated gene, not expressed, Retrotransposon ahead
OsABCG42/PDR-12	Os06g36090	LOC4341281	AK058448	1167	217-470 (6) & 912-1159 (7)	Plasma Membrane	
OsABCG51/PDR-13	Os10g13830	LOC4348271	CAD59564	1441	525-772 (6) & 1184-1431 (7)	Plasma Membrane	Re-annotated at NCBI
OsABCG38/PDR-15	Os01g52560	LOC4324722	AK288334	1158	211-461 (7) & 903-1050 (7)	Plasma Membrane	
OsABCG32/PDR-16	Os01g24010	LOC4326812	AK106603	1451	530-779 (6) & 1197-1443 (7)	Mitochondria	
OsABCG44/PDR-17	Os08g29570	LOC4345450	AK100858	791	16-122 (3) & 539-783 (7)	Secretory	
OsABCG52/PDR-18	Os09g16449 Os09g16458			1388	501-752 (7) & 1136-1380 (6)	Plasma Membrane	This is re-annotated at NCBI with predicted cDNA XM_015755308 , not expressed, Retrotransposon ahead
OsABCG47/PDR-19	Os09g16380	LOC107276397	--	1386	500-753 (7) & 1134-1378 (6)	Plasma Membrane	Tandem duplicated gene, not expressed, retrotransposon ahead
OsABCG53/PDR-20	Os09g16330	LOC9269190	EU682752	1447	521-771 (6) & 1184-1431 (7)	Plasma Membrane	
OsABCG46/PDR-21	Os09g16290		--	1352	427-683 (7) & 1099-1344 (6)	Mitochondria	Tandem duplicated gene, not expressed, Retrotransposon ahead
OsABCG49/PDR-22	Os12g13720	LOC9268322	--	1227	329-573 (6) & 969-1219 (7)	Plasma Membrane	
OsABCG50/PDR-23	Os12g32820	LOC4352332	This report	1407	505-754 (5) & 1151-1398 (6)	Plasma Membrane	Previously annotated as two half transporter Os12g32820 and Os12g32814

OsABCG33/PDR-14 (Os01g33260) is re-annotated as retrotransposon and described as pseudogene by Moons, 2008, hence removed from the list

Table 2- Expression of *OsABCG/PDR* genes in different tissues of rice as measured by q-PCR. (The values represents average "relative fold change" of 3 biological replicates and presented with respective standard deviation. S represents shoot and R represents root, DAP is days after pollination, for tissues representing shoot, flower, developing seeds, diseased sheath and spike, the relative fold change is calculated by taking non-treated shoot as control, for various root tissues non-treated root was taken as control. The values are presented to nearest complete digit and standard deviation with two decimal points, - - represents fold change ≤ 1.5)

Tissue/stress				**OsABCG/PDR Transporter**											
Shoot Tissues	45/1	41/2	48/3	40/4	43/5	31/6	39/7	37/8	36/9	42/12	51/13	38/15	32/16	44/17	53/20
NaCl	2±0.11	--	--	--	--	--	2±0.2	3±0.21	--	--	5±0.25	2±0.32	--	2±0.11	--
Drought	--	--	--	3±0.22	5±0.52	2±0.22	--	4±0.32	4±0.12	--	--	2±0.22	--	14±0.56	--
Heat	5±0.25	--	5±0.4	2±0.15	2±0.21	2±0.34	6±0.51	12±0.9	2±0.11	2+0.22	--	--	--	2±0.36	--
$CdCl_2$	--	--	--	--	2±0.15	--	--	2±0.21	3±0.11	--	--	--	--	6±0.45	--
2,4-D	--	--	--	3±0.23	3±0.18	3±0.32	--	2±0.21	--	2±0.23	--	3±0.21	--	3±0.24	--
6-BAP	5±0.21	--	3±0.11	3±0.34	6±0.13	--	2±0.12	7±0.35	17±0.59	--	--	2±0.22	--	7±0.36	--
JA	--	--	--	4±0.31	--	2±0.12	--	2±0.12	--	--	--	2±0.21	--	2±0.11	--
SA	--	--	--	2±0.17	--	2±0.23	--	--	--	--	--	--	--	--	--
BR	2±0.15	--	2±0.2	3±0.17	6±0.21	--	--	2±0.14	--	--	--	2±0.21	--	3±0.24	--
$CaCl_2$	--	--	--	2±0.16	4±0.22	2±0.45	--	3±0.12	--	--	--	2±0.29	--	2±0.21	--
Root Tissues															
NaCl	2±0.12	--	--	2±0.24	6±0.14	2±0.24	4±0.32	--	6±0.28	2±0.21	--	--	2±0.13	4±0.11	--
Drought	4±0.09	--	--	--	4±0.19	5±0.39	2±0.21	2±0.12	14±0.57	--	--	--	2±0.21	6±0.12	--
Heat	6±0.12	2±0.21	--	--	--	2±0.42	2±0.22	--	8±0.65	2±0.21	--	--	--	2±0.11	--
$CdCl_2$	3±0.15	2±0.19	--	--	--	4±0.23	--	--	12±0.87	2±0.32	--	--	--	--	--
2,4-D	11±0.5	--	--	--	--	2±0.23	2±0.14	--	14±0.45	5±0.35	--	--	--	--	--
6-BAP	6±0.30	--	3±0.23	2±0.14	4±0.25	2±0.11	3±0.21	4±0.23	--	8±0.46	--	--	13±0.8	9±0.24	3±0.12
JA	9±0.45	2±0.3	3±0.19	--	16±0.75	--	2±0.11	5±0.21	17±0.15	5±0.49	--	--	--	2±0.26	--
SA	5±0.25	--	--	--	--	--	2±0.11	9±0.31	20±0.95	3±0.25	--	--	--	--	2±0.08
BR	5±0.19	--	--	--	2±0.11	2±0.25	--	2±0.11	51±1.21	3±0.32	--	--	--	--	--
$CaCl_2$	6±0.41	--	--	--	3±0.12	--	2±0.12	3±0.12	27±0.85	3±0.32	--	--	--	--	--
Flower	2±0.17	--	2±0.11	--	45±0.58	26±1.2	3±0.11	5±0.15	7±0.24	--	--	3±0.25	2±0.21	5±0.35	--
3 DAP	--	3±0.3	--	2±0.18	4±0.24	21±0.83	--	5±0.21	--	--	--	--	--	--	--
7 DAP	--	2±0.32	--	2±0.17	--	7±0.12	--	20±0.85	--	--	--	--	--	--	--
14 DAP	4±0.20	--	--	--	--	19±0.87	--	8±0.23	--	--	--	--	--	--	2±0.16
21 DAP	24±1.9	--	--	--	--	6±0.21	--	5±0.24	3±0.35	--	6±0.16	--	--	2±0.21	4±0.11
28 DAP	18±1.6	--	--	--	--	2±0.12	2±0.14	13±0.80	--	--	8±0.82	--	--	--	3±0.12
Diseased spike	5±0.2	--	2±0.35	--	--	--	--	3±0.12	--	4±0.18	9±0.38	--	--	--	4±0.14
Diseased Sheath	4±0.21	--	10±0.7	--	6±0.23	--	--	9±0.45	2±0.32	--	14±0.35	--	2±0.21	--	5±0.08

OsABCG/PDR (34/10, 35/11, 33/14, 52/18, 47/19, 46/21, 49/22, 50/23) genes removed from the table-2, because of lack in actual expression in the tissues studied