academicJournals

Vol. 12(30), pp. 4722-4729, 24 July, 2013
DOI: 10.5897/AJB12.2916
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Genome-wide identification, functional analysis and expression profiling of pleiotropic drug resistance (PDR) sub-family in potato

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Accepted 19 July, 2013

The plant pleiotropic drug resistance (PDR) family of ATP-binding cassette (ABC) transporters has comprehensively been researched in relation to transport of antifungal agents and resistant pathogens. In our study, analyses of the whole family of PDR genes present in the potato genome were provided. This analysis resolves discrepancies of potato PDR proteins and provides an expression analysis of all annotated potato PDR genes based on RNA-seq data. The results indicate that the potato genome contains 76 encoding PDR proteins and that these genes show a specific expression patterns, both at the organ level and in response to various hormonal treatment. These data provide some clues for future molecular genetic analysis of this important subfamily of ABC transporters. In addition, potato PDR genes may also play some important roles in the transportation of antifungal agents and resistant pathogens.

Key words: ABC transporter, potato, pleiotropic drug resistance (PDR), RNA-seq.

INTRODUCTION

ATP-binding cassette (ABC) transporters have some implications in the active movement of a wide variety of substrates across cellular membranes (Higgins, 1992). They are involved in accumulating plants secondary metabolites in specialized organs, such as organic acids, alkaloids, lipids; transport hormones, accumulate detoxification and enhance defence in plant (Yazaki, 2006; Knöller et al., 2011). Meanwhile, plant ABC transporters play important roles in growth and development. The proteins consist of one or two cytosolically oriented nubleotide-binding folds (NBFs) or ATP-binding cassettes (ABCs) linked to multiple (usually six) hydrophobic transmembrane-spanning (TMS) domains. The ABC domains are highly conserved and contain an ATP-binding site consisting of a Walker A box and Walker B box. Every box consists of approximately 120 amino acids (Walker et al., 1982); a consensus sequence specific for ABC transporters is known as the ABC signature between the two boxes (Bairoch, 1992) (Figure 1). These molecular characters are regarded as a modular fashion within the ABC transporter protein. ABC transporters consist of a single TMS-ABC or ABC-TMS module or repetition of these modules. These proteins have been designated as 'half size' or 'full-size' ABC transporters (Higgins, 1992). The full size ABC transporters include four major subfamilies, such as, multidrug resistance [MDR (Gottesman and Pastan, 1993)], MRP (MDR-associated protein (Borst et al., 1999)), ABCA (Broccardo et al., 1999), and pleiotropic drug resistance (PDR). The PDR family characterised by a configuration in the ABC module is closer to the N-terminal end of the protein than the TMS domain (ABC-TMS). In plant, the PDR ABC transporters contain perfectly conserved Walker A motifs with a PDR N-terminal consensus of GPP [GS][SCA]GK[TS] and a C-terminal

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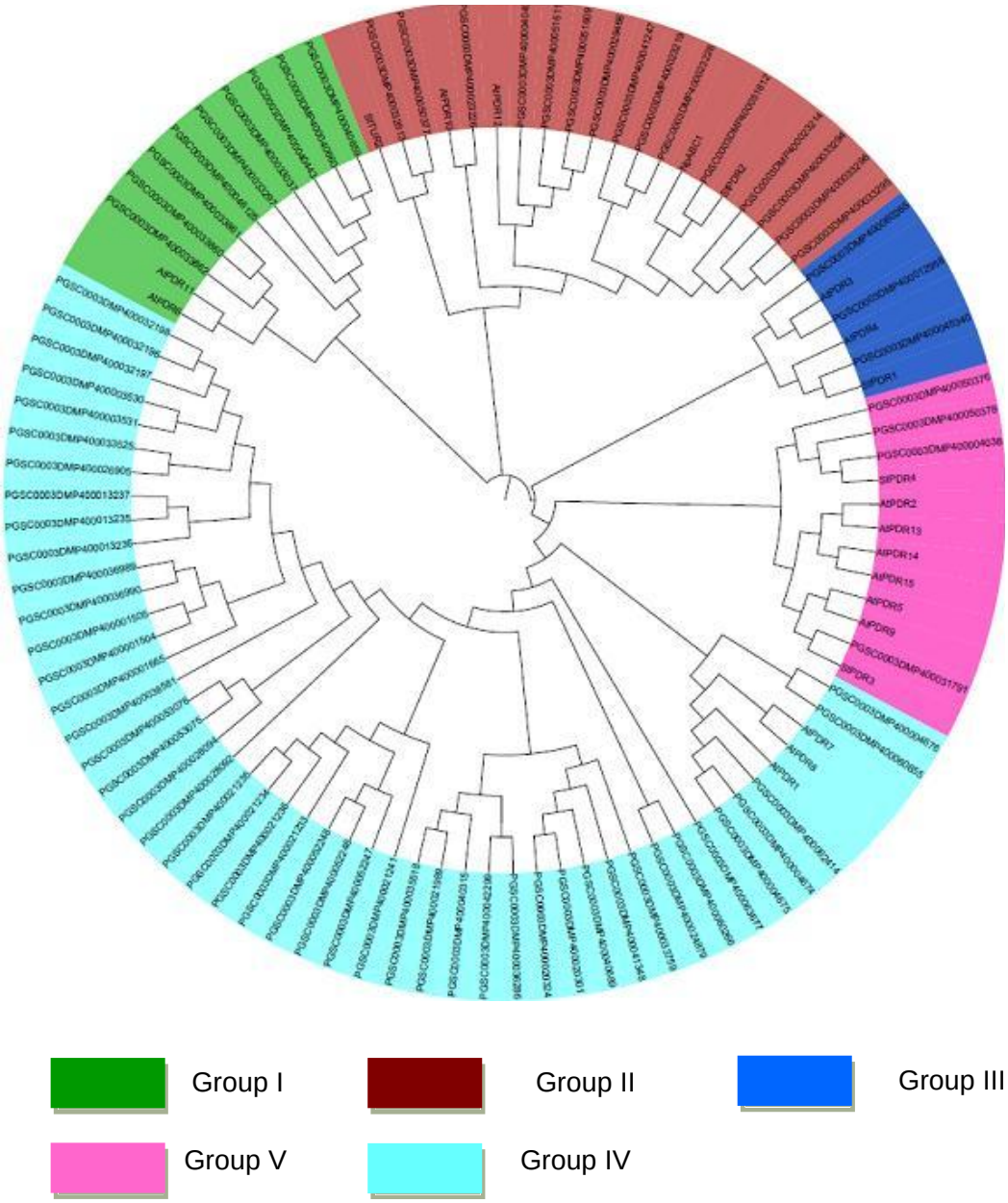

Figure 1. Phylogenetic tree of PDR family genes from potato. The unrooted phylogenetic tree is based on a multiple alignment of 55 polypeptide sequences of PDR subfamily proteins produced by the CLUSTALW program. Distance matrix, phylogenetic tree and bootstrap values were calculated with CLUSTALW. Bootstrap analysis was manipulated by Interactive Tree Of Life. *At, Arabidopsis thaliana*, *St*, *Solanum tuberosum*

consensus of G[VIS]SG[AR]GKT. The N-terminal PDR Walker B motif is [ATV][LF][FL]MD and the C-terminal is I[ILV]F[ML]D(van den Brule and Smart, 2002).

The PDR subfamily has important roles in transporting antifungal agents with specific modular configuration. The PDR genes have been researched to have many biology functions, such as exporting xenobiotics (Kolaczkowski et al., 1996) and antifungal drug resistance (Kolaczkowski et al., 1996). In plant, PDR subfamilies are necessary for pathogenicity (Urban et al., 1999). The first plant PDR gene, *SpTUR2* was identified from the water plant, *Spirodela polyrrhiza (*Smart and Fleming, 1996*)*. And the expression of *SpTUR2* was associated with the ABA level. The expression of the *SpTUR2* transporter is related to the acquisition of resistance to sclareol in *Arabidopsis* (van den Brule et al., 2002). So in plants, PDR protein may play a key role in plants' interactions with fungi (Crouzet et al., 2006).

As a result of the sequencing of the *Arabidopsis* genome, some PDR genes of *Arabidopsis* were identified

and annotated (Davies and Coleman, 2000; Sanchez-Fernandez et al., 2001; Martinoia et al., 2002; van den Brule and Smart, 2002). The gene function of the PDR family in plant has been researched (Sasabe et al., 2002; Campbell et al., 2003; Moons, 2003; Ducos et al., 2005). Having the Potato Genome Sequence Consortium (PGSC) finished in 2011(Xu et al., 2011), it is very useful to identify genes and characterize their function. Four PDR genes had been identified as well as their expression in response to abiotic factors and phytophthora infestans infection (Ruocco et al., 2011).

Potato is one of the major crops in the world. The United Nations (FAO) reported that the world production of potatoes in 2011 was about 374 million tonnes and China is now the world's largest potato-producing country. But the yield of potato has been lost because of pathogenic stress and abiotic stress. Based on the previous research result on *Arabidopsis* and other crops, PDR may play a key role in response to abiotic and pathogenic stress. In this paper, we provide an inventory of all the potato PDR proteins so far characterised and a detailed analysis of the annotated PDR genes in potato genome. We also provide an analysis of the expression pattern of all the annotated PDR genes in potato at the organ level and in response to various environmental, hormonal and chemical factors. These data provide some information for finding out the potential function of the individual PDR genes in potato.

MATERIALS AND METHODS

Identification of potato PDR genes and sequences search

To identify all PDR genes, the annotation of potato was downloaded from Potato Genome Sequencing Consortium (PGSC) database (http://www.potatogenome.net). To identify all PDR genes sequences, the protein sequences were downloaded from PGSC. All Arabidopsis PDR genes protein sequences were downloaded from TAIR database (http://www.arabidopsis.org).

Sequence alignment and phylogenetic analysis

All downloaded amino acid sequences of all of the PDR ABC transporters were aligned and subjected to phylogenetic analysis using ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Thompson et al., 1997). Neighbour-joining method was used. The circle analysis was performed by using Interactive Tree Of Life (http://itol.embl.de/) (Letunic and Bork, 2011).

PDR genes expression pattern analysis

Two genotype RNA-Seq Gene Expression Data were downloaded from PGSC Data Release (Xu et al., 2011) (http://potatogenomics.plantbiology.msu.edu/index.html). One was *Solanum tuberosum* Phureja DM1-3 516 R44 referred to as DM and from a homozygous line derived by using classical tissue culture techniques. The other was *Solanum tuberosum* group *Tuberosum* RH89-039-16 referred to as RH and from a heterozygous diploid breeding line. All PDR genes expression was analyzed, using Mev 4.8 version (Saeed et al., 2003).

RESULTS

Identification of 76 potato PDR proteins by sequence analysis

Potato genome annotation files were downloaded. Then Mapman was used to identify the gene function. A total of 76 PDR protein sequences were identified according to function annotation (Table 1). We downloaded the potato protein sequence and searched the PDR ABC transporter protein sequences. A total of 20 PDR protein sequences were more than 1000 amino acids, regarded as full PDR genes; while a total of 30 PDR protein sequences were more than 500 amino acids, which were regarded as half PDR genes. The rest of the PDR genes were less than 500 amino acids, which were not really the PDR family members possibly. The amino acid sequences of *SpTUR2* were from *Sprirodela polyrrhiza*; whereas, the 15 *Arabidopsis thaliana* proteins sequences were searched in Arabidopsis Database. To clarify the phylogenetic relationships of PDR family proteins between potato in this research and *A. thaliana*, all the PDR protein sequences were performed by using ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Thompson et al., 1997) with multiple alignment analysis and neighbour-joining method. The raw tree file was got from ClustalW2 program. Then the circle tree was performed by using Interactive Tree Of Life (http://itol.embl.de/) (Letunic and Bork, 2011). Sequence alignment showed that all of the potato PDR genes were divided into five groups: I, II, III, IV, V. The genes of group V had more than other groups, with 53 PDR genes and *AtPDR3*.

Expression patterns of the potato PDR genes in RH and DM

A total of 55 PDR unigenes were identified from 76 PDR potato protein sequence (Table 1). In order to investigate potato PDR genes expression pattern in different tissues, we downloaded RH and DM expression data from PGSC Data Release (Xu et al. 2011) (http://potatogenomics.plantbiology.msu.edu/index.html). Then, we used Mev 4.8 version (Saeed et al., 2003) to analyze all genes expression (Figure 2). In RH and DM, 33 and 30 PDR genes had high expression level in different tissues, respectively. *PGSC0003DMG402029631* (*StPDR2*), *PGSC0003DMG400011469*, *PGSC0003DMG401002262* (*StPDR4*), *PGSC0003DMG400018249* (*StPDR3*), *PGSC0003DMG400019166*, and *PGSC0003DMG400026543* were only expressed in water-stressed leaf, flower, whole *in vitro* plant, root and root in RH, respectively. *PGSC0003DMG400000787*, *PGSC0003DMG400019476*, *PGSC0003DMG400020888*, *PGSC0003DMG401016070*, and *PGSC0003DMG400011469* were only expressed in se-

Table 1 . PDR genes of expression levelenhanced twice after different hormone treatments in DM.

Gene ID	peptide ID	BAP	ABA	IAA	GA3	Group
PGSC0003DMG400012432	PGSC0003DMP400021989	5.2	5.3	0.7	2.7	V
PGSC0003DMG400018249	PGSC0003DMP400031791	4.8	0.0	0.9	3.3	IV
PGSC0003DMG400018818	PGSC0003DMP400032813	4.5	0.0	2.1	2.7	III
PGSC0003DMG400007465	PGSC0003DMP400013235	1.4	1.5	1.0	1.8	V
PGSC0003DMG400023490	PGSC0003DMP400040660	1.1	1.9	0.9	1.2	I
PGSC0003DMG400002613	PGSC0003DMP400004674	0.7	1.8	0.8	1.1	IV
PGSC0003DMG400023388	PGSC0003DMP400040443	0.0	1.8	0.7	1.3	V
PGSC0003DMG400011482	PGSC0003DMP400020324	0.0	2.6	1.2	2.5	V
PGSC0003DMG400021343	PGSC0003DMP400036990	0.7	3.6	1.0	1.6	V
PGSC0003DMG400023918	PGSC0003DMP400041348	1.9	3.5	0.7	2.1	V
PGSC0003DMG400023506	PGSC0003DMP400040689	2.1	3.0	0.8	1.8	V
PGSC0003DMG402029631	PGSC0003DMP400051612	0.3	6.6	0.4	1.3	II

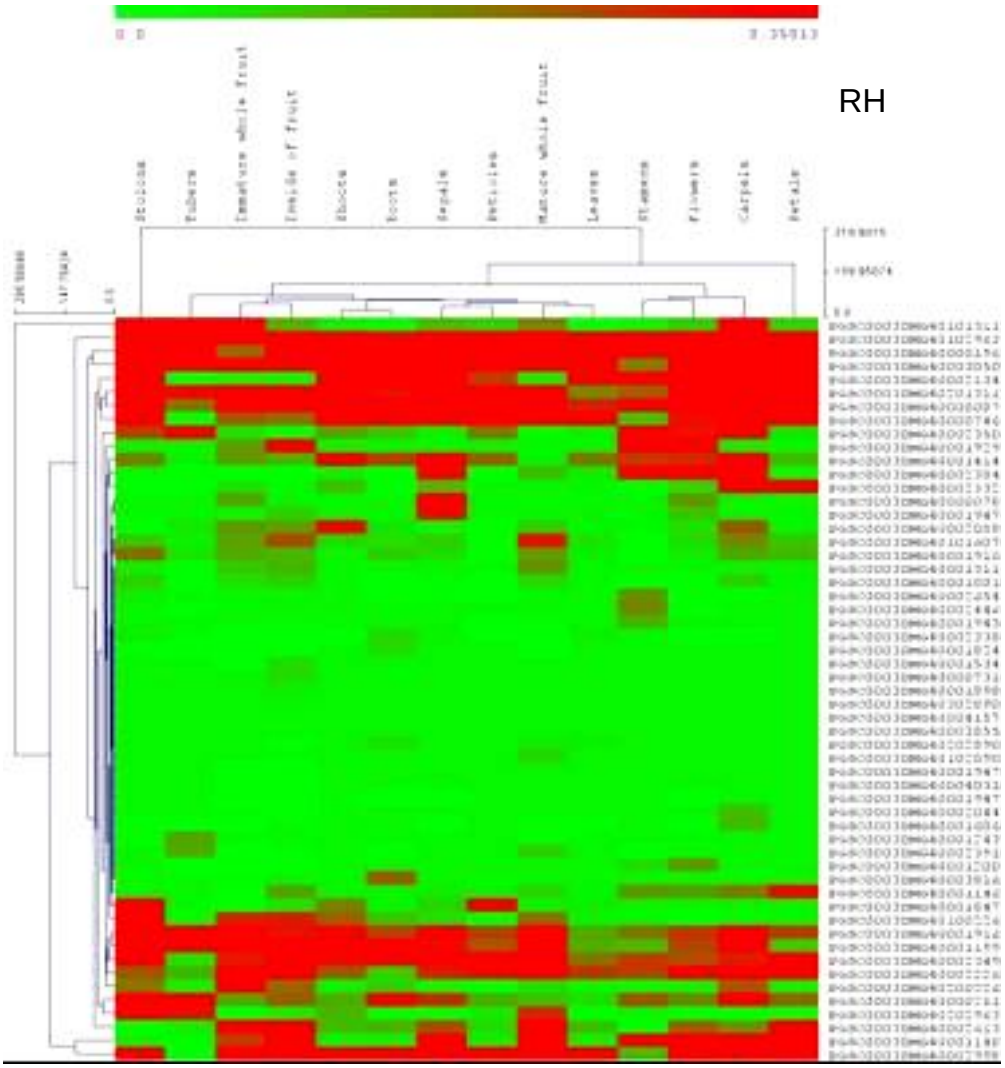

Figure 2. Potato PDR genes exhibit differential expression across different tissues in RH and DM. The pattern of relative transcript accumulation of each of 55 PDR genes as determined by RNA-seq analysis are presented as a heatmap, with red indicating higher levels and green indicating lower levels of transcript accumulation. Each column represented a discreet biological sample.

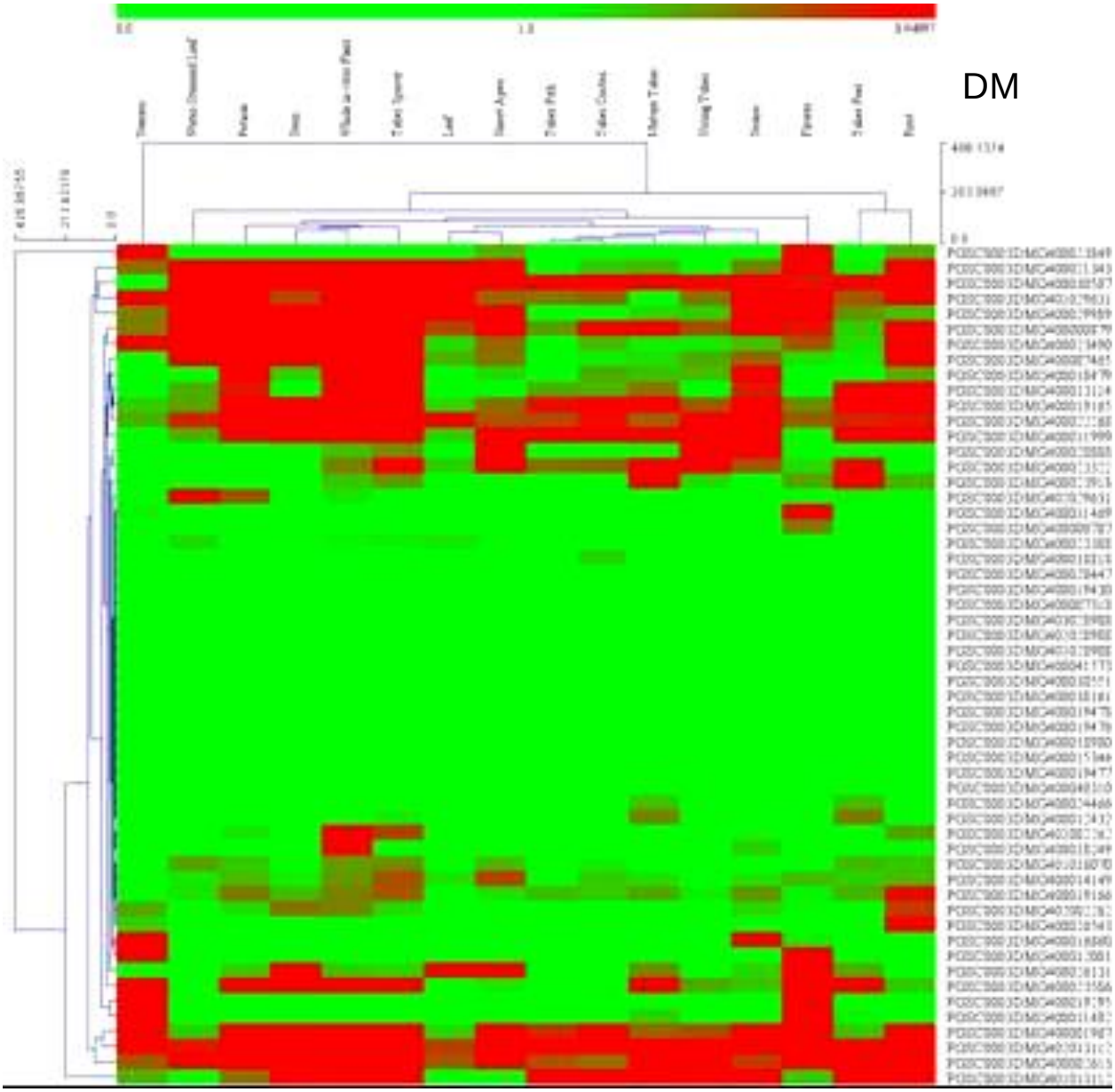

Figure 2. Contd.

pals, shoots, natural whole fruit and petals in DM, respectively. *PGSC0003DMG400011469* was expressed in flower in RH and DM. *PGSC0003DMG400011469* was in the same group with *AtPDR3*. Other PDR genes were expressed in two or more tissues.

PDR genes expression associated with hormone

PDR genes are usually associated with exporting xenobiotics (Kolaczkowski et al., 1996) and antifungal drug resistance (Kolaczkowski et al., 1996). 12 PDR genes enhanced their expression level more than about twice after using BAP, ABA, IAA and GA3 treatments (Figure 3). It was indicated that potato PDR gene was associated with exporting xenobiotics, antifungal drug resistance or biotic stress. *PGSC0003DMG400023490*, *PGSC0003DMG4000026131* (*StPDR1*), *PGSC0003DMG400023388*, *PGSC0003DMG400021343*, and *PGSC0003DMG2029631* were enhanced more about twice than control after using ABA treatment (Figure 3). These PDR genes were clustered into Group I, Group V, Group V, Group V, and Group II. *AtPDR11, AtPDR6*, *AtPDR3*, *AtPDR4* and *AtPDR12* were clustered into Group I, Group V, and Group II, respectively. *PGSC0003DMG400007465* was enhanced twice more

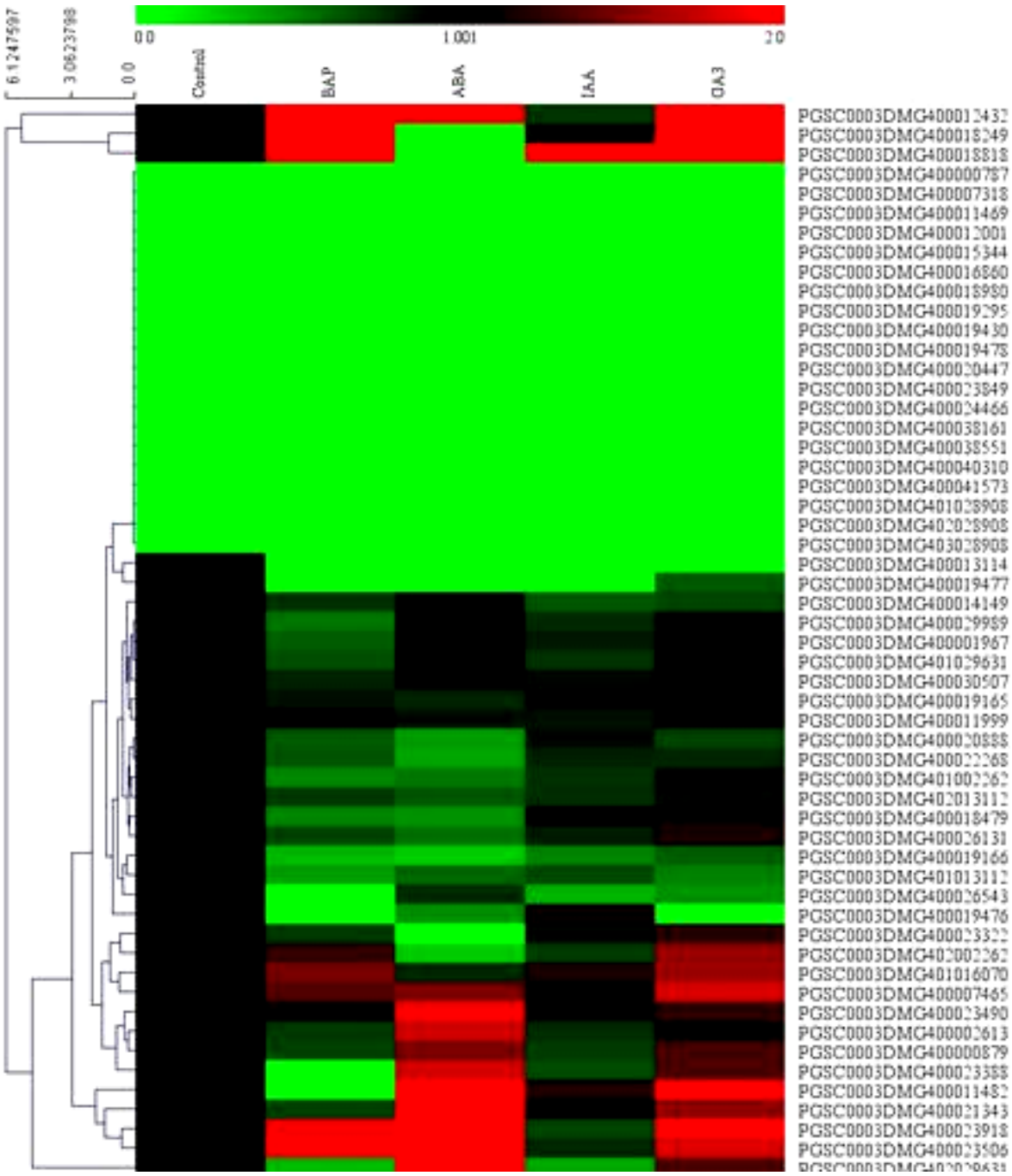

Figure 3. Potato PDR genes exhibit differential expression across different hormone treatments in DM. The pattern of relative transcript accumulation of each of 55 PDR genes as determined by RNA-seq analysis are presented as a heatmap, with red indicating higher levels and green indicating lower levels of transcript accumulation. Each column represented a discreet biological sample.

than control after using GA3 treatment (Figure 3), which was clustered into Group V.

DISCUSSION

The PDR genes encode a subfamily of ABC transporter in plants (Sanchez-Fernandez et al., 2001; Martinoia et al., 2002). The data presented in this paper provide a definitive annotation of the genomic sequences encoding this family of transporter in potato and indicate that potato contains 76 gene encoding PDR proteins. Phylogenetic analysis allows the grouping of similar PDR genes and these grouping are broadly supported by comparison of

genomic structure, suggesting that acquisition and loss of introns has underpinned the evolution of the plant PDR family. Example, the clustering of *AtPDR6*/*AtPDR11* into Group I and *AtPDR8*/*AtPDR7*/ *AtPDR5*/*AtPDR9* / *AtPDR2*/*AtPDR13* /*AtPDR14*/ *AtPDR15*/ *StPDR3*/ *StPDR4* into Group IV in the phylogenetic analysis is clearly reflected in their similar genomic structure. *StPDR2*, *StPDR3* and *StPDR4* had been identified with response to abiotic factors and *Phytophthora infestans* infection (Ruocco et al., 2011). In our study, *PGSC0003DMG4000026131,* *PGSC0003DMG402029631*, *PGSC0003DMG400018249* and *PGSC0003DMG401002262* were identified as *StPDR1*, *StPDR2*, *StPDR3* and *StPDR4*, respectively. In these groups, the PDR genes of potato have similar genomic structure. However, these similarities at the level of gene structure and protein sequence were not always reflected at the level of transcript accumulation, indicating that even highly similar AtPDR genes can show distinctive patterns of gene expression. For example, in Arabidopsis, *AtPDR5* and *AtPDR9* are both mainly expressed in roots, but only *AtPDR5* is also found in stems and only *AtPDR9* is up-regulated by cycloheximide (van den Brule and Smart, 2002). In potato, *PGSC0003DMG4000026131*, *PGSC0003DMG400023388*, *PGSC0003DMG400021343*, *PGSC0003DMG2029631* and *PGSC0003DMG400007465* were clustered into Group V with *AtPDR3*. *PGSC0003DMG400023490*, *PGSC0003DMG4000026131*, *PGSC0003DMG400023388*, *PGSC0003DMG400021343* and *PGSC0003DMG2029631* were enhanced about twice more than control after using ABA treatment (Figure 3). *PGSC0003DMG400007465* was enhanced twice more than control after using GA3 treatment (Figure 3). Interestingly, *PGSC0003DMG402029631* was expressed in water-stressed leaf in RH and DM; it also found out that *PGSC0003DMG2029631* was enhanced about twice more than control after using ABA treatment (Figure 3). So *PGSC0003DMG402029631* (*StPDR2*) may be an important gene to regulate by chemical and environment stresses. Taken together, these data are consistent with the idea of duplicating particular PDR gene that occurs during evolution and the concomitant acquisition of specific patterns of gene regulation and /or specific functions.

The analysis of transcript profiles for RH, DM, and hormone treatment indicates that PDR genes in potato are subject to complex regulation by endogenous and exogenous factors. PDR genes are also involved in different tissues development and environmental stress. However, some specificity in organ expression and hormonal and environmental induction is observed. These specificities provide clues to the endogenous function of the individual family members. Example, in Arabidopsis, none of the annotated AtPDR genes showed an increased level of transcript in response to ABA, but in potato, *PGSC0003DMG402029631* was expressed in water-stressed leaf in RH and DM; it also found out that *PGSC0003DMG2029631* was enhanced about twice more than control after using ABA treatment (Figure 3). *PGSC0003DMG402029631* may be an important role in resisting some potato pathogens. This result showed that PDR genes in potato may be involved in pathogenic stress induction, on one hand and that PDR genes in potato have different functions with *Arabidopsis,* on the other hand. In the future, we will focus on how to respond to pathogenic stress using molecular technologies, especially *StPDR2*.

ABA can induce some PDR gene expression level up-regulation. It is shown that some PDR proteins have the potential to be involved in exporting xenobiotics and antifungal drug resistance. Previous data from distantly related species (Jasinski et al., 2001; van den Brule et al., 2002) indicated that the PDR proteins *NpABC1* and *SpTUR2* play a role in the excretion of sclareol. In Arabidopsis, *AtPDR12* (Lee et al., 2005), AtPDR8 (Gepstein et al., 2003; Stein et al., 2006; Humphry et al., 2010), *AtPDR11* (Xi et al., 2012) were involved in pathogen resistance. So by analyzing the PDR gene transcript, it is very important to investigate the mechanism and function of PDR genes in the transport of antifungal agents and pathogen resistance.

ACKNOWLEDGEMENT

This work was supported by special fund of the National Modern Agricultural Industry Technology System (CARS-10).

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