GENOME-WIDE IDENTIFICATION, FUNCTIONAL ANALYSIS AND EXPRESSION PROFILING OF PLEIOTROPIC DRUG RESISTANCE (PDR) SUB-FAMILY IN POTATO

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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 nucleotide-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
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 [ILV][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
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 Spirodela polyrhiza; 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. PGSC0003DMG40000078, PGSC0003DMG400019476, PGSC0003DMG400020888, PGSC0003DMG401016070, and PGSC0003DMG400011469 were only expressed in se-
Table 1. PDR genes of expression level enhanced twice after different hormone treatments in DM.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>peptide ID</th>
<th>BAP</th>
<th>ABA</th>
<th>IAA</th>
<th>GA3</th>
<th>Group</th>
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<tr>
<td>PGSC0003DMG400012432</td>
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<td>5.2</td>
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<td>2.7</td>
<td>V</td>
</tr>
<tr>
<td>PGSC0003DMG400018249</td>
<td>PGSC0003DMP400031791</td>
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<td>0.0</td>
<td>0.9</td>
<td>3.3</td>
<td>IV</td>
</tr>
<tr>
<td>PGSC0003DMG400018818</td>
<td>PGSC0003DMP400032813</td>
<td>4.5</td>
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<td>2.1</td>
<td>2.7</td>
<td>III</td>
</tr>
<tr>
<td>PGSC0003DMG400007465</td>
<td>PGSC0003DMP400013235</td>
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<td>1.5</td>
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<tr>
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<td>0.9</td>
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<tr>
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<td>PGSC0003DMP40004674</td>
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<tr>
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<td>V</td>
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<tr>
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<td>0.4</td>
<td>1.3</td>
<td>II</td>
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</table>

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.
pals, shoots, natural whole fruit and petals in DM, respectively. \textit{PGSC0003DMG400011469} was expressed in flower in RH and DM. \textit{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. \textit{PGSC0003DMG400023490}, \textit{PGSC0003DMG400026131} (StPDR1), \textit{PGSC0003DMG400023388}, \textit{PGSC0003DMG400021343}, and \textit{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, AtPDR8, AtPDR4 and AtPDR12 were clustered into Group I, Group V, and Group II, respectively. \textit{PGSC0003DMG400007465} was enhanced twice more
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, PGSC0003DMG402029631 and PGSC0003DMG400007465 were clustered into Group V with AtPDR3. PGSC0003DMG400023490, PGSC0003DMG4000026131, PGSC0003DMG400023388, PGSC0003DMG400021343 and PGSC0003DMG4029631 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 environmental 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 scarelare. 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.

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REFERENCES


