Four potato (*Solanum tuberosum*) ABCG transporters and their expression in response to abiotic factors and *Phytophthora infestans* infection

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ABSTRACT

Pleiotropic drug resistant (PDR/ABCG) genes are involved in plant response to biotic and abiotic stresses. In this work, we cloned, from *Solanum tuberosum*, four PDR/ABCG transporter genes named StPDR1, StPDR2, StPDR3 and StPDR4, which were differentially expressed in plant tissues and cell cultures. A number of different chemically unrelated compounds were found to regulate the transcript levels of the four genes in cultured cells. In particular, StPDR2 was highly up-regulated in the presence of *Botrytis cinerea* cell walls, NaCl, 2,4-dichlorophenol, sclareol and α-solalin and biological compounds. The expression of the genes was also investigated by real time RT–PCR during infection by *Phytophthora infestans*. StPDR1 and StPDR2 were up-regulated about 13- and 37-fold at 48 h post-infection (hpi), StPDR3 was expressed (4–5-fold) at 24 and 48 hpi and then rapidly decreased, while StPDR4 RNA accumulation was stimulated (about 4-fold) at 12 and 24 hpi, decreased at 48 hpi and increased again at 96 hpi. We discuss the role of StPDR1–4 genes in response to pathogens and abiotic stresses.

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Introduction

The ATP-binding cassette (ABC) transporter superfamily consists of a large group of related proteins whose members have been implicated in the active movement of a variety of substances across cellular membranes in organisms ranging from bacteria to man (Higgins, 2001). These proteins typically contain two core structural elements: a hydrophilic transmembrane domain (TMD) consisting of multiple membrane spanning segments (usually six), and a hydrophilic nucleotide binding fold (NBF) containing the Walker A, Walker B and ABC signature (Rea, 2007; Walker et al., 1982). ABC transporters have been reported to occur both as “half-size,” which contain of one hydrophilic and one hydrophilic domain, and as “full size”, with two domains each (Verrier et al., 2008).

Plants have a large number of ABC proteins that may be associated with the movement of a variety of secondary metabolites. In the absence of a specialized secretory structure, plants need to establish steep concentration gradients to allow the movement of solutes across cellular membranes (Verrier et al., 2008; Yazaki et al., 2006). The sequencing of *Arabidopsis thaliana* and *Oryza sativa* genomes led to the identification of more than 120 genes encoding ABC transporters (Garcia et al., 2004; Lee et al., 2005; Sanchez-Fernandez et al., 2001), while only 50–70 ABC-proteins have been found in the genome of humans, fly (*Drosophila melanogaster*) and worm (*Caenorhabditis elegans*) (Rea, 2007).

Because ABC transporters are involved in cellular detoxification, chlorophyll biosynthesis, stomata opening and closing, they can play a direct or indirect role in plant growth and developmental processes (Klein et al., 2004; Luo et al., 2007; Martinioa et al., 2002; Ticconi et al., 2009; Titapiwatanakun and Murphy, 2009; Zientara et al., 2009).

Plant ABC proteins are divided into 13 subfamilies on the basis of protein size (full, half, or quarter molecules), orientation of TMD and NBF domains, presence or absence of idiotypic transmembrane and/or linker domains, and overall sequence similarity. Some of the best characterized subfamilies are multidrug resistance proteins (MDR/ABC), multidrug resistance-associated proteins (MRP/ABCC), pleiotropic drug resistance proteins (PDR/ABCG) and peroxisomal membrane proteins (PMP/ABCD) (for reviews see: Rea, 2007; Sanchez-Fernandez et al., 2001; Verrier et al., 2008).

The PDR/ABCG subfamily is encoded by more than 15 ORFs in *Arabidopsis* (Jasinski et al., 2003; Sanchez-Fernandez et al., 2001; van den Brule and Smart, 2002) and 23 in rice (Crouzet et al., 2006). Genes encoding for PDR/ABCG homologues have not been identified in animal and prokaryotes, but only in yeasts, fungi and plants. Interest in PDR/ABCG transporters has been particularly stimulated by their involvement in the extrusion of cytotoxic compounds. In fungi, PDR/ABCG transporters have been associated with...
the acquisition of multiple drug resistance (Del Sorbo et al., 2000; Schoonbeek et al., 2001), pathogenicity (Fleissner et al., 2002), self-detoxification and microbial interactions (Ruocco et al., 2009). The capability of some PDR/ABC transporters to excrete xenobiotics (Bauer et al., 1999) has led to the hypothesis that, in plants, they support detoxification from herbicides, fungicides, as well as the secretion of defense compounds (Davies and Coleman, 2000; Luo et al., 2007). However, plant PDR/ABCs have been poorly investigated.

Smart and Fleming (1996) cloned and characterized SpTUR2 from the aquatic plant Spirodela polyrhiza, the first gene encoding a homologue of the yeast PDR5. Its expression was induced by environmental stresses caused by low temperature, high salinity and absicic acid (ABA). Over-expression of SpTUR2 in Arabidopsis confers resistance to the antifungal diterpene scarel cole (van den Brule et al., 2002). Similarly, NpPDR1 (formerly known as NpABC1) from Nicotiana plumbaginifolia and AtPDR2 from Arabidopsis thaliana were involved in the secretion of scarel (Jasinski et al., 2001; van den Brule et al., 2002). In general, the expression of plant PDR/ABCs is promoted by: cycloheximide, brassinolides, herbicides, high salinity, heavy metals (cadmium and zinc), hypoxic stress, jasmonates, auxins, cytokinins, iron starvation and a wide range of microbial elicitors (Ruzicka et al., 2010; Sasabe et al., 2002). Stein et al. (2006) demonstrated that the A. thaliana PEN3/PDR8 gene contributes to non-host resistance against pathogens attempting direct penetration.

In the present paper, we describe the cloning and functional characterization of four PDR/ABC transporter genes from Solanum tuberosum, named StPDR1, StPDR2, StPDR3 and StPDR4. These are the first ABCG genes reported in potato. We provide evidence indicating that they are involved in the response to treatments with a variety of compounds, including chemicals, hormones, phytotoxins and pathogen cell walls. We also analyzed the expression pattern of the four genes following potato leaf infection by Phytophthora infestans.

Materials and methods

Chemicals

Chemicals (obtained from Sigma-Aldrich, Fluka) were dissolved either in water (cycloheximide, H2O2, cadmium sulphate, NaCl, fusaric acid, abscisic acid, α-solane, Botrytis cinerea cell walls), acetonitrile (4,15-diacetoxyscirpenol), or dimethyl sulfoxide (2,4-dichlorophenol, arachidonic acid, sulfoluron methyl, scarel) and filter sterilized. B. cinerea cell walls were extracted according to Schirrmbeck et al., 1994. They were added to the growth medium at 1:1000 ratio w/v of concentrated filter-sterilized stock solutions in 30 mL total growth medium. Control medium was treated with an equal volume of fresh solvent.

Potato plant growth

Potato tubers (Solanum tuberosum cv Desirée L. Heyn) were sterilized in 1% sodium hypochlorite (30 min), washed three times with sterile water, placed in pots containing sterile soil and maintained at constant temperature at 20 °C and 16 h day photoperiod in a climatic chamber (Angelantonio, Massa Martana, Pg, Italy). Plants were regularly irrigated at 5 day intervals. Six- to seven-week-old plants were used to collect roots, stems and leaves for DNA and RNA extraction. Tubers were obtained from greenhouse-grown plants of about three month old and used for RNA extraction. The hypothetical involvement of the oxidation processes on the expression of the four genes was analyzed in tubers by cutting and leaving them at room temperature in a Petri dish for 24 h (Hayashi et al., 2002).

Potato cell cultures and treatments

Potato cell suspensions (S. tuberosum L., dihaploid clone SVP11) were started and maintained as liquid cultures, under darkness at 28 °C, and 100 rpm conditions in 250–mL flasks (Leone et al., 1994), on Murashige-Skoog (MS) basal medium added with 29 μM thiamine, 48 μM nicotinic acid, 37 μM pyridoxine, 2 g/L L-1 casein, 30 g/L L-1 sucrose, 23 μM 2,4-D and 1 μM kinetin, and adjusted to pH 5.8. Potato cell suspensions were transferred weekly to fresh suspension medium (P3) (Tavazza and Ordas, 1998). All the substrates used for gene induction were added to a 5-day-old cell culture. The final concentrations for each compound were: 50 μM 2,4-dichlorophenol, 1 μM abscisic acid, 0.33 μM arachidonic acid, 100 μM cadmium sulphate, 1000 μM hydrogen peroxide, 100 mM NaCl, 500 μM scarel; 10 μM 4,15-diacetoxyscirpenol, 100 μM cycloheximide, 1000 ppm fusaric acid, 200 ppm α-solane, 2 ppm sulfoluron methyl and 100 mg B. cinerea cell wall, previously freeze-dried and suspended in 500 μL of sterile water. The concentration of each compound was established on the basis of previous studies. Abscisic acid is an inducer of the SpTUR2 gene of Spirodela polyrhiza (Smart and Fleming, 1996) at concentrations between 10 μM and 50 mM. Scarel is an antifungal diterpene secreted by NpPDR1 gene, which strongly induces NpPDR1 transcription (Jasinski et al., 2001) at concentrations between 20 and 500 μM. Solanin is a toxic glycoalkaloid produced by potato which is potentially involved in transcription of potato ABC transporters. The total concentration of steroid glycoalkaloids in potato is influenced by several factors and ranges between 0.3 and 0.7 mg/g dry weight, with a ratio between α-solane and α-chacbone of about 50:50 (Nitinhampong et al., 1999). If we assume that live potato cells contain about 80% water, a concentration of 0.1 mg/mL could be “physiological”. In order to cells an external extra supply of α-solane, we increased its concentration up to 0.2 mg/mL Fusarium sambucinum, one of the casual agents of potato dry rot, was found to produce a trichotenes, 4,15-diacetoxyscirpenol (DAS) up to 700 mg/g dry mycelium (Altomare et al., 1995). We tested whether StPDR1–4 are involved in defense of potato against trichotenes. We used a concentration 10 ppm of DAS, since the EC50 for Artemia salina is 1 ppm or 10 μM, which can inhibit or strongly disturb growth of tobacco plants (Muhitch et al., 2000). Arachidonic acid is a strong elicitor of hypersensitive reaction (HR) in potato (Knight et al., 2001). In previous studies with potato tuber discs, a concentration of 0.33 μM was sufficient to enhance transcription of genes involved in phytoalexin biosynthesis, such as rishitin and lubimin, which could be potentially secreted by ABC transporters. Hydrogen peroxide is generated and accumulated in cells during hypersensitive response (HR) in concentration around 30 μM (Delledonne et al., 2001). The elicitor-induced resistance is based on the expression of multiple resistance genes. Fungal cell walls contain and release a number of substances which could enhance expression of resistance genes and ABC transporters genes. In fact, water-soluble low-molecular-weight (3–10 kDa) chitosan, obtained by enzymatic degradation of high-molecular-weight chitosan, as well as its deaminated derivatives, can be used as elicitors of late blight resistance in potato (Vasiukova et al., 2000). Cadmium, which can be administered as sulphate, is a toxic heavy metal and we used 100 μM cadmium sulphate, as reported by Yazaki et al. (2006). The 2,4-dichlorophenol (2,4-D) is the degradation product of a number of agricultural compounds (i.e. the herbicide 2,4-D as well as some fungicides) and is quite recalcitrant to further degradation. It is also similar to dioxin, an environmental pollutant deriving from anthropic activities. The concentration tested was derived from the work of Smart and Fleming (1996), who observed a weak inducing effect of 2,4-D at 10 μM on the SpTUR2 transcription. Sulfoluron methyl is a sulfonylurea herbicide, which was the first tested substrate for Pdr5p. Primisulfonyl, another...
sulfonylurea herbicide, at 20 nM, strongly induces the expression of the EST2 of Arabidopsis thaliana (Tommasini et al., 1997). We tested 2 ppm of sulfometuron methyl, since it was found that 0.2 ppm and 5 ppm inhibit the growth of a yeast strain by 50% and 98%, respectively. Osmotic stress with NaCl at 100 mM was found to strongly induce the SpTUR2 gene of S. polyrhiza (Smart and Fleming, 1996). Controls included cell cultures untreated or treated with DMSO at a 1:1000 ratio, since DMSO was used to dissolve some of the tested compounds.

Samples were harvested 3h after the induction treatment, frozen in liquid nitrogen, freeze-dried, ground to a fine powder and stored at −70 °C until further use.

Phytophthora infestans infection of potato plants

P. infestans strains 772 (mating type A1), and 779 (mating type A2) were kindly provided by Prof. Gennaro Cristinzio (University of Naples “Federico II”, Italy). They were grown on V8p agar (tomato juice 50 mL−1, peas 75 g L−1, CaCO3 0.5 g L−1 and agar 20 g L−1) at 20 °C. Virulence was maintained by infecting potato leaves and re-isolating the pathogen every 3–4 months on V8p agar added with vancomicina 150 mg L−1, neomicina 10 mg L−1, pimaricina 10 mg L−1, benomyl 10 mg L−1 and nistatina 10 mg L−1 at 20 °C. Zoospores were released by flooding 10- to 14-day-old cultures with 10 mL cold distilled water and incubating at 4 °C for 2–3 h.

Six- to seven-week-old plants grown as above described were inoculated with the strains of P. infestans by spraying with freshly prepared spores at 1 × 106 spore/mL. Leaflets from second to fourth apical leaves were used for all experiments. After inoculation, plants were maintained at 20 °C and a 16h day photoperiod under saturating humidity. To maintain the high humidity required for infection, plants were covered with Plexiglas boxes having the inner surfaces sprayed with water. Control plants were sprayed with water and covered in the same way. Leaves (infected and control) were collected immediately before inoculation and after 6, 12, 24, 48, 72, 96 h, frozen in liquid nitrogen and stored at −70 °C prior to RNA extraction. For each experiment, infected leaves were

maintained on the plant for four more days, to control the infection process.

DNA sequencing and sequence analysis

Degenerated primers were the same Pdasp1, Pdasp2, Pdsp1, and Pdsp2 used by Ruocco et al. (2009) (Table 1). PCR amplification was performed, unless indicated otherwise, by using 100 ng of S. tuberosum DNA as template. Amplicons of approximately 500 bp were excised from the gel and cloned in the pGEMT Easy vector (Promega Corp., Madison, WI, USA). Plasmid DNA was purified from Escherichia coli cultures using an alkaline lysis method (Sambrook et al., 1989) or a Qiagen spin miniprep kit (Qiagen, Hilden, Germany) following the manufacturer’s directions. The resulting plasmids were sequenced by MWG Biotech AG (Ebersberg, Germany) and subjected to BLASTX analysis. Sequences were analyzed using the DNAstar package (DNASTAR). Similarity searches were conducted with BLAST programs at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

For the cloning of the SpPDR1–4 complete cDNA sequence, in addition to the primers reported in Table 1, two more primers (Table 2) were designed against the genomic sequences at 5’ and 3’ as found at the Potato Genome Sequencing Consortium website (PGSC http://www.potatogenome.net/index.php/Main_Page). PCR amplicons obtained by using cDNA as a template and primer pairs described in Table 2, were cloned in the pGEMT Easy vector and the resulting plasmids were sequenced by GENOPOM DNA sequence service (Portici, Italy).

DNA isolation and gel blot analysis

Genomic S. tuberosum DNA (gdNA) extraction was performed from leaf tissues according to Reader and Broda (1985). Briefly, 1g of freeze-dried leaves was ground with a mechanical bead grinder. Ten milliliters of extraction buffer (0.5 M NaCl, Tris–HCl 10 mM pH 7.5, EDTA 10 mM, SDS 1%), 10 mL phenol and 10 mL chloroform:iso-amyl alcohol, were added. Samples were incubated at room temperature for 1 hour. After centrifugation for 10 min at

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13,000 × g, 3 mL of supernatant were mixed with an equal volume of isopropanol and centrifuged at 13,000 × g for 10 min. The pellet was washed twice with 500 μL ethanol 70% and resuspended in 500 μL of water. Ten micrograms of S. tuberosum gDNA were digested overnight with Sall and NotI restriction enzymes (Promega) and subjected to DNA gel blot analysis as previously described by Ruocco et al. (2009).

RT-PCR

Semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) reactions were performed in a one-step procedure using the SuperScript™ One-Step RT-PCR (Invitrogen) with total RNA obtained from potato cell suspension or potato tissues (roots, leaves, stems and tuber). RT-PCR reactions were performed with primers described above (Table 1). The amplicons generated were sequenced to confirm their identity. Transcript levels of all samples were normalized to the 18S rRNA level, and fold induction was calculated by dividing the signal strength of the experimental sample by the signal strength of the control (cell suspension not induced). All experiments were performed in duplicate.

Real-time RT-PCR quantification of transcript levels during plant–pathogen interaction

Fifty microliter samples were prepared by mixing 2 μL cDNA solution with 25 μL Sybr™ Green Mastermix (Applied Biosystems, Foster City, USA) and the appropriate primers (final concentration 300 nM). Real-time PCR reactions were run in three replicates per sample on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). After 2 min at 50 °C followed by a 10 min denaturation step at 95 °C, samples were run for 40 cycles of 15 s at 95 °C and 1 min at 54 °C. After each run, a dissociation curve was acquired to check for amplification specificity by heating the samples from 60 to 95 °C. The sequences of the primers were designed using the Primer Express® Software version 3.0 (Applied Biosystems, Foster City, CA, USA) for each target sequence, detailed in Table 1. SYBR® Green I dye chemistry was used for a two-step real-time PCR. All reaction mixtures were analyzed by agarose gel electrophoresis to confirm that only one PCR product was synthesized. In all experiments, appropriate negative controls containing no template DNA or RNA were subjected to the same procedure to exclude or detect any possible contamination or carryover.

The results were normalized using the Cts obtained for the 18S RNA amplifications run on the same plate.

Serial dilutions of pure cDNA from a sample of known quantity were used to trace standard curves for each gene, which were used to quantify the transcript levels of the four ABC transporters during the plant–pathogen time courses. A linear relationship was obtained by plotting the threshold cycle against the logarithm of known amount of initial template. The equation of the line that best fits the data was determined by regression analysis. The R² value was calculated for each data set to estimate the accuracy of the real-time PCR with SYBR® Green I dye as a quantification method.

For quantification normalized to an endogenous control, standard curves were prepared for both the target and the endogenous control. For each sample, the amounts of target and endogenous control were determined by extrapolation from the linear regression of the standard curve. Then, the target amount was divided by the endogenous control amount to obtain a normalized target value.

Real-time quantitative PCR controls were performed to check for the linearity of amplification over the dynamic range. A linear regression analysis showed an almost perfect correlation between the recorded fluorescence signal and the initial cDNA amount both after amplification of serial dilutions from 60 to 480 ng of S. tuberosum cDNA with StPDR1–4 specific primer pair, and with primers RT 18SFor/RT 18Srev, which are specific for the S. tuberosum 18S ribosomal subunit, used as endogenous gene control.

All experiments were performed in triplicate.

Phylogenetic analysis

The phylogenetic relationships of the four genes were inferred by using the Maximum Likelihood method based on the Wheelan and Goldman + Freq. model (Wheelan and Goldman, 2001). The bootstrap consensus tree obtained from 100 replicates is taken to represent the relationships of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) is shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise, the BIONJ method with an MCL distance matrix was employed. A discrete Gamma distribution was used to model evolutionary rate differences among sites

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(5 categories (+G, parameter = 1.5180). The rate variation model allowed for some sites to be evolutionarily invariable [+]I, 0.0000% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 20 amino acid sequences (Table 3). All positions with less than 0% site coverage were eliminated. That is, fewer than 100% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 902 positions in the final dataset. Evolutionary analyses were conducted in MUSCLE 3.6 (Edgar, 2004).

Results

Cloning and sequence analysis of potato genes encoding ABCG transporters

Degenerated primers (Table 1) used to amplify S. tuberosum genomic DNA produced four fragments with strong similarity to genes encoding ABC transporters of the PDR/ABCG subfamily, which were named StPDR1 (S. tuberosum PDR transporter 1), StPDR2, StPDR3 and StPDR4 (Table 4). The partial sequence of StPDR1 was 547 bp. The relative genomic sequence resulted 7394 bp long with 17 putative introns. The obtained cDNA sequence of 3099 bp encodes a putative protein of 1032 aa with a (NBF-TMD6)2 topology. StPDR1 showed high similarity with ABC transporter genes of the plant PDR/ABCG subfamily (Verrier et al., 2008), especially with the A. thaliana ATP4D/ATP4G gene (75% similarity) of unknown function. The partial sequence of StPDR2 was 550 bp long, while the complete DNA sequence of 9241 bp contained 17 putative introns. The obtained cDNA sequence was 4132 bp and encodes a putative protein of 1378 aa with a (NBF-TMD6)2 topology. StPDR2 was highly similar to plant PDR/ABCG transporters and, in particular, to NpPDR1 from N. plumbaginifolia (91% similarity). NpPDR1 encodes an ABC transporter (NBF-TMD6)2 that is involved in the secretion of the antifungal terpenoid sclareol (Jasinski et al., 2001). The partial sequence of StPDR3 was 565 bp long, with a complete DNA sequence of 9687 bp, interrupted by 20 putative introns. The obtained cDNA sequence of 3519 bp encodes a putative protein of 1172 aa with a (NBF-TMD6)2 topology. It showed high similarity (86%) to N. tabacum NtPDR3, an ABC transporter gene inducible by iron-deficiency (Ducos et al., 2005), and to A. thaliana AtPDR9/AtPDR9 gene (75%) encoding a putative ABC transporter of the (NBF-TMD6)2 superfamily. The partial sequence of StPDR4 was 546 bp long, with a complete DNA sequence of 7068 bp and 24 putative introns. The obtained cDNA sequence of 4272 bp encodes a putative protein of 1423 aa with a (NBF-TMD6)2 topology. StPDR4 showed high similarity to NpPDR1 (Jasinski et al., 2001) and O. sativa and A. thaliana genes coding for putative ABC transporters belonging to (NBF-TMD6)2 superfamily.

All the domains characteristic of the PDR/ABCG subfamily are present and well conserved in the four genes: the ATP binding site domain, the ABC signature, the Walker A and Walker B, the Q-loop and H-loop, and the PDR domain (data not shown). Molecular phylogenetic analysis (Fig. 1) with sequences of the PDR/ABCG

Table 4

<table>
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<th>Gene name</th>
<th>DNA partial sequence length in bp</th>
<th>DNA partial sequence Acc. No.</th>
<th>DNA complete sequence length in bp</th>
<th>DNA complete sequence PGSC0003DM5 Acc. No.</th>
<th>Predicted introns No.</th>
<th>cDNA length in bp</th>
<th>cDNA sequence Acc. No.</th>
<th>Putative protein sequence length in AA</th>
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<td>1423</td>
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</table>

subfamily genes confirmed the similarities obtained with Blastp analysis. Three distinct subgroups were formed, one containing all the half-size proteins of the subfamily ABCG/WBC (white–brown complex), such as AtWBC16, AtWBC20, AtWBC25, AtWBC28 and AtWBC29. The four StPDR sequences divided into 2 sub-groups, one containing StPDR1,2 and 4 and the other containing StPDR3. Amino acidic sequence comparison indicated that the four S. tuberosum transporters are more closely related to each other than to PDR5 from S. cerevisiae, the prototype of the PDR family (data not shown). DNA gel blot analysis demonstrated that StPDR1, 2 and 4 are single-copy genes, while StPDR3 has more than one copy (Fig. 2).

Expression in potato tissues and cell cultures

We used RT-PCR analysis to determine expression levels of the four PDR/ABCG genes in S. tuberosum roots, tubers, stems and leaves. StPDR1 and StPDR4 were expressed in all tissues, StPDR2 in roots and leaves, and StPDR3 mainly in roots (Fig. 3). Expression levels of the StPDR1–4 genes in tuber slices left to air oxidize for 24 h were similar to those obtained from fresh tissue (data not shown).

The results obtained by RT-PCR performed on total RNA from potato cell cultures are reported in Fig. 4. Transcript levels for all samples were normalized to that of the 18S RNA. The designed primers (Table 1) produced amplicons of 258, 280, 290 and 242 bp in size for the StPDR1–4 genes, respectively. Expression of StPDR1, which was low in cell cultures, was unaffected by most of the added compounds and down-regulated by some of them, such as B. cinerea cell walls, DAS and sclareol. StPDR2 was up-regulated by sclareol, B. cinerea cell walls, 2,4-dichlorophenoxyacetic acid, sulfometuron

![Fig. 2](image1.png)

![Fig. 3](image2.png)

![Fig. 4](image3.png)
methyl and sodium chloride. StPDR3 was constitutively expressed and apparently down-regulated by most of the added compounds, with the exception of NaCl, DMSO and α-solanine. StPDR4 was only slightly altered, in most cases up-regulated, by the tested compounds.

**Transcript levels of StPDR1–4 during potato–P. infestans interaction**

Transcript levels of StPDR1–4 in potato leaves infected with *P. infestans* are shown in Fig. 5. StPDR1 and StPDR2 were up-regulated about 13- and 37-fold in comparison to untreated controls at 48 hpi, which corresponds to the necrotrophic phase of the infection process. StPDR3 and StPDR4 showed different expression patterns. The first was activated (4- to 5-fold) at 24 and 48 hpi and then its expression rapidly decreased. StPDR4 mRNA accumulation was increased about 4-fold at 12 and 24 hpi, decreased at 48 hpi and increased again at 96 hpi.

**Discussion**

StPDR1–4 were the first PDR/ABCG transporter genes reported for *S. tuberosum*. They encode plant ABC transporters with high homology to the well characterized yeast PDR5-like gene products, NpPDR1 from *N. plumbaginifolia*, SpTUR2 from *S. polyrrhiza* and AtPDR12 from *A. thaliana* (Campbell et al., 2003; Jasinski et al., 2001; Tommasini et al., 1997). StPDR1, 2, 3 and 4 grouped with full size ABCG, while the half size proteins belong to another group (Fig. 1). However, StPDR3 clustered in a group different from that of StPDR1, 2, and 4. It is interesting to note that, like StPDR3, all genes coding for the ABCGs present in this group are mainly expressed in roots. To our knowledge, only another ABC transporter gene, *PMRD1* of the ABCB subfamily, has been studied in potato. The expression of this gene was found to be constitutive in all organs examined, with higher levels in the stem and stolon tip; further, it reached a maximum during tuber initiation and decreased during tuber development (Wang et al., 1996). Previous studies (Kolaczkowski et al., 1998; Tommasini et al., 1997) demonstrated that the plant PDR5 homologues are subject to complex environmental regulation and may confer resistance to microbial compounds produced during plant–pathogen defense responses (Campbell et al., 2003; Jasinski et al., 2003). This hypothesis is supported by evidence that the wheat gene *Lr34*, used for more than 50 years by breeders to introduce resistance to leaf rust, stripe rust and powdery mildew, encodes a protein that resembles adenosine triphosphate-binding cassette transporters of the PDR/ABCG subfamily (Krattinger et al., 2009).

StPDR1–4 expression levels were determined on total RNA from different tissues (root, tuber, stem and leaf) of *S. tuberosum* cv Desireé. In roots and leaves, all four genes are expressed (in leaf, StPDR3 is lightly expressed); while in tubers and stems, only transcripts of StPDR1 and StPDR4 were found. StPDR3 was highly expressed in roots as were *N. tabacum* NpPDR3 (Ducos et al., 2005), *Arabidopsis* PDR9 (Ruzicka et al., 2010; Strader et al., 2008) and PDR2 (Ticconi et al., 2004, 2009) homologues. ABC genes can be expressed in different plant organs. Jasinski et al. (2009), for example, by analyzing the 19 genes of the ABCB subfamily present in *Medicago truncatula*, observed that MtABCG3, MtABCG4, MtABCG7 and MtABCG14 are expressed in roots, while MtABCG13 is expressed in flowers. However, close homologous genes of this family can be expressed in different organs, where they can have similar roles or diverse substrates (Gaedeke et al., 2001; Jasinski et al., 2009). In *M. truncatula*, the majority of the ABCG genes were expressed in...
roots. Since in rhizosphere very complex and dynamic interactions between roots and the surrounding environment take place, this suggests that ABCG genes may play an important role by providing, for example, efficient transport through cellular membranes of different molecules, such as root exudates.

We also investigated the expression of the four cloned PDR/ABC transporters in leaves during the interaction potato–P. infestans. Wang et al. (2005), on the basis of the expression of genes involved in the whole infection process, clearly identified three distinct stages of the P. infestans–potato interaction: the early stage (2–6 hpi), mid stage (8–24 hpi), and late stage (36–72 hpi). At 24 hpi, the biotrophic phase ends and the necrotrophic phase starts. Looking at the expression patterns of leaf potato genes during the three stages they found that, from 24 up to 72 hpi, more than 80% of the genes were up-regulated and 9.8% of them were related to secondary metabolite pathways. We extended our experiments up to 96 hpi to examine whether some of the PDR/ABC genes were also involved in the late stage of the infection process. StPDR1–4 genes were up-regulated during the infection, but in different ways. StPDR1 and StPDR2, which showed the strongest activation among the four genes (13- and 37-fold, respectively), were induced only at 48 hpi, during the passage from the mid to the late phase of the infection (Fig. 5). The StPDR2 sharp induction at 48 hpi could be due to the presence of a specific compound(s). Stukens et al. (2005) found that NpPDR1, a N. plumbaginifolia gene showing high homology with StPDR2, was constitutively expressed in the whole root, leaf glandular trichomes, and flower petals. In trichomes, NpPDR1 expression was related to secretion of sclareol, a major diterpene constituent of N. plumbaginifolia foliar exudates, with fungal toxic activity (Jasinski et al., 2001).

StPDR3 and StPDR4 were activated during the early phases of the interaction. However, StPDR3 activation increased by reaching a maximum at 48 hpi and then decreased, while StPDR4 was induced up to 24 hpi, returned to normal level at 48 hpi and was up-regulated again at 96 hpi (very late stage). This indicates that the latter gene can have a role in the transport of compounds produced both during biotrophic and necrotrophic stages.

To identify the substrates for StPDR1–4, the expression of these genes was determined in cell culture suspensions added with a number of compounds selected by considering the possible involvement of ABC transporters in response to biotic and abiotic stresses. These experiments were based on the assumption that there is a direct correlation between the cellular concentration of some molecules and the expression level of the transporter that secrete them (Del Sorbo et al., 2000; Fleissner et al., 2002; Muñoz et al., 2000). Direct treatment of S. tuberosum cell culture suspension proved to be a very convenient tool; it provided a homogenous sample in which cells are in direct contact with the surrounding medium and responded evenly (Jasinski et al., 2001).

In cell culture, StPDR1 was weakly expressed. Addition of hydrogen peroxide, 2,4-dichlorophenoxyacetic, dimethyl sulfoxide, fusaric acid and α-solaneine were ineffective, while B. cinerea cell walls, sodium chloride, cadmium sulphate, sulftometuron methyl, 4,15-diacetoxyescipenol, abscisic acid and scareol down-regulate its expression. We found that addition of scareol, B. cinerea cell walls, sodium chloride, 2,4-dichlorophenoxyacetic, sulftometuron methyl and α-solaneine strongly induced StPDR2 expression in cell suspensions. This result is in good agreement with the up-regulation shown by this gene during the potato–P. infestans interaction and strongly indicates that StPDR2 could be involved in plant defense responses to biotic and abiotic stresses. Further StPDR2 may be functionally related to the previously identified ABC transporters StPTrU2, AtPDR12 and NpABC1, which transport scareol and are involved in general defense responses (Campbell et al., 2003; Sasabe et al., 2002; Smart and Fleming, 1996). A similar role has also been hypothesized for PEN3/PDR8 of A. thaliana, which exports a family of chemically related toxic compounds (Stein et al., 2000).

The result that in cell culture only StPDR2 expression is up-regulated could depend on the fact that none of the tested compounds is a substrate for StPDR1, StPDR3 and StPDR4. Nevertheless, we cannot exclude the possibility that StPDR1, StPDR3 and StPDR4 expression requires induction times longer than those used in our study, or that the activation patterns of these genes could be different in cell cultures and plant organs. Dorey et al. (1999) found that elicitin exhibits different behavior in cell cultures compared to leaves.

This is, to our knowledge, the first study on several PDR/ABC genes’ expression in S. tuberosum that also shows that the activation of all one (StPDR2) is correlated with the pathogen (P. infestans) infection. We hypothesize that the StPDR1–4 genes could be part of a complex marshalling interacting components that modulate and regulate the potato response to pathogens and abiotic stresses.

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References


