Journal of Plant Physiology 168 (2011) 2225–2233

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

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

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ARTICLE INFO

Article history:
Received 3 May 2011
Received in revised form 12 July 2011
Accepted 21 July 2011

Keywords:
Stress response
ABC transporters
Plant–microbe interactions
Potato
Phytophthora infestans

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-solanin 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.

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 hydrophobic 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 hydrophobic 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 secretor 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; Martinoia 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/ABCB), 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

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doi:10.1016/j.jplph.2011.07.008

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/ABCG 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/ABCGs have been poorly investigated. Smart and Fleming (1996) cloned and characterized *SpTUR2* from the aquatic plant *Spirodela polyrrhiza*, the first gene encoding a homologue of the yeast PDR5. Its expression was induced by environmental stresses caused by low temperature, high salinity and abscissic acid (ABA). Over-expression of *SpTUR2* in *Arabidopsis* confers resistance to the antifungal diterpene sclareol (van den Brule et al., 2002). Similarly, *NpPDR1* (formerly known as *NpABC1*) from *Nicotiana plumbaginifolia* and *AtPDR12* from *A. thaliana* were involved in the secretion of sclareol (Jasinski et al., 2001; van den Brule et al., 2002). In general, the expression of plant PDR/ABCGs 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/ABCG 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, H_2O_2, cadmium sulphate, NaCl, fusaric acid, abscisic acid, α-solanine, *Botrytis cinerea* cell walls), acetone (4,15-diacetoxyscirpenol), or dimethyl sulphoxide (2,4-dichlorophenol, arachidonic acid, sulfometuron methyl, sclareol) and filter sterilized. *B. cinerea* cell walls were extracted according to Schirmbock et al., 1994. They were added to the growth medium at 1:1000 ratio v/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. Heynh) were sterilized in 1% sodium hypochlorite (30 min), washed three times with sterile water, planted in pots containing sterile soil and maintained at constant temperature at 20 °C and 16 h day photoperiod in a climatic chamber (Angelantoni, 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^{-1} casein, 30 g/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 (P31) (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 sclareol; 10 μM 4,15-diacetoxyscirpenol, 100 μM cycloheximide, 1000 ppm fusaric acid, 200 ppm α-solanine, 2 ppm sulfometuron 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. Abscissic acid is an inducer of the *SpTUR2* gene of *Spirodela polyrrhiza* (Smart and Fleming, 1996) at concentrations between 10 μM and 50 nM. Sclareol 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-solanine and α-chaconine of about 50:50 (Nitithamyong 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 have in cells an external extra supply of α-solanine, 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 trichotecenes, 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 trichotecenes. 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. Sulfometuron methyl is a sulfonylurea herbicide, which was the first tested substrate for Pdr5p. Primisulfuron, another