Short Communication

A Pathogen-Responsive cDNA from Potato Encodes a Protein with Homology to a Phosphate Starvation-Induced Phosphatase

Julia Petters 1, Cornelia Göbel, Dierk Scheel and Sabine Rosahl 2

Institute of Plant Biochemistry, Department of Stress and Developmental Biology, Weinberg 3, D-06120 Halle / Saale, Germany

Infiltration of potato leaves with the phytopathogenic bacteria Pseudomonas syringae pv. maculicola induces local and systemic defense gene expression as well as increased resistance against subsequent pathogen attacks. By cDNA-AFLP a gene was identified that is activated locally in potato leaves in response to bacterial infiltration and after infection with Phytophthora infestans, the causal agent of late blight disease. The encoded protein has high homology to a phosphate starvation-induced acid phosphatase from tomato. Possibly, decreased phosphate availability after pathogen infection acts as a signal for the activation of the potato phosphatase gene.

Keywords: Phytophthora infestans — Pseudomonas syringae — Solanum tuberosum.

Plants defend themselves against pathogens by activating a multicomponent defense response which includes the reinforcement of cell walls, generation of antimicrobial compounds and hypersensitive cell death (Scheel 1998). Recognition of pathogen-derived structures by the plant triggers signal transduction chains that lead to the activation of defense genes (Nürnberg and Scheel 2001). Among the defense gene products are proteins with antifungal activity, enzymes involved in the synthesis of antimicrobial and signaling compounds as well as regulatory proteins that orchestrate the defense response (Scheel 1998). The importance of pathogen-activated defense genes is demonstrated by the increased resistance of transgenic products (Scheel 1998). The importance of pathogen-activated defense genes is demonstrated by the increased resistance of transgenic plants constitutively expressing single or several defense genes (Punja 2001).

In potato, infiltration of the phytopathogenic bacteria Pseudomonas syringae pv. maculicola induces the rapid development of necroses on the infiltrated leaves as well as local and systemic activation of defense genes. These processes correlate with the expression of systemic acquired resistance (SAR) which is manifested by a decrease in lesion number and size upon subsequent pathogen attack (Kombrink et al. 1996). Genes expressed in SAR-primed potato comprise “classical” pathogenesis-related (PR) genes such as those encoding chitinases and glucanases (Büchter et al. 1997, Kombrink and Schmelzer 2001). In order to identify novel genes that are activated specifically after pathogen infection, cDNA-AFLP was performed. One of the genes activated locally encodes a protein with high homology to a recently described phosphate starvation-induced acid phosphatase from tomato (Baldwin et al. 2001), suggesting that decreased phosphate levels due to pathogen infection might act as a signal for defense gene activation.

Potato plants (Solanum tuberosum L. cv. Désirée) were kindly provided by E. Tacke (Bioplant GmbH, Ebstorf) and grown as sterile plants in a phytochamber with 16 h of light [200 µE] at 22°C. After transfer to soil, plants were kept in a phytochamber with 16 h of light [200 µE], 20°C and 60% humidity for 5 weeks. Lower leaves were infiltrated with a suspension of P. syringae pv. maculicola at a concentration of 10⁸ cfu ml⁻¹ or, as a control, 10 nM MgCl₂-solution as described (Kombrink et al. 1996). Alternatively, lower leaves were inoculated with P. infestans by pipetting 10–20 droplets of 10 µl of a P. infestans zoospore-suspension in water (10⁵ zoospores ml⁻¹) onto the abaxial leaf surface and keeping the plants at 100% humidity for the duration of the experiment. As controls, water was pipetted onto the leaves. Induction of gene expression by signaling compounds was performed by either incubating detached leaves of potato plants in 10 or 100 µM solutions of salicylic, jasmonic or arachidonic acid for 24 h or by infiltration of 10 or 100 µM solutions of these compounds into potato leaves.

Five-day-old suspension cultured potato cells (cv. Désirée, Göbel et al. 2001) were washed with MS-medium without phosphate. Three g of cells were transferred to flasks containing 50 ml of MS-medium, either with 1 mM or no phosphate. Cells were harvested at different time points and total RNA was isolated and analyzed as described (Schmidt et al. 1999). For elicitation experiments, suspension cultured potato cells were treated with a crude elicitor preparation from P. infestans culture filtrate (CF) as described (Schmidt et al. 1999).

Total RNA was isolated from potato leaves infiltrated with P. syringae pv. maculicola or MgCl₂ after 3 and 12 h, and polyA⁺-RNA was prepared using magnetic beads (Dynal, Hamburg, Germany). cDNA-AFLP was performed using the displayProfile™ Kit (Qbiogene, Heidelberg, Germany). Bands

1 Present address: Martin-Luther-University, Institute of Pharmaceutical Biology and Pharmacology, D-06099 Halle / Saale, Germany.
2 Corresponding author: E-mail, srosahl@ipb-halle.de; Fax, +49-345-5582-1409.
Pathogen-responsive phosphatase from potato

Fig. 1 Comparison of the deduced amino acid sequence of StPPP1 with the deduced amino acid sequence of the tomato cDNA for a phosphate starvation-induced phosphatase (Baldwin et al. 2001). Dots indicate identical amino acids. Conserved phosphatase motifs are boxed.

corresponding to differentially accumulating transcripts were extracted from the gel, amplified, subcloned and sequenced. cDNA was prepared with the Pharmacia Time Saver Kit (Amersham Pharmacia Biotech, Freiburg, Germany) and ligated to lambda ZAPII EcoRI cut arms (Stratagene, Amsterdam, Netherlands). After packaging the recombinant DNA with Gigapack Gold II (Stratagene, Amsterdam, Netherlands), the cDNA library was plated and screened using a 292 bp long fragment as a probe that had been isolated in the differential display experiment. Ten clones were identified in the cDNA library and subjected to in vivo excision. The inserts of the respective plasmids were sequenced on a LICOR automatic sequencer (MWG Biotech, Ebersberg, Germany). Database searches were done according to Altschul et al. (1997).

For expression analyses, 20 μg of total RNA were subjected to formaldehyde gel electrophoresis, blotted onto nylon membranes and hybridized to the NorI insert of the StPPP1 cDNA clone or the EcoRI inserts of StPR1 and StPAL as well as to a ribosomal probe as a loading control (Göbel et al. 2001). Hybridization and washing conditions were as described (Schmidt et al. 1999).

Southern analyses were performed as described (Schmidt et al. 1999). Hybridizations were carried out at 42°C in 5× SSPE, 5× Denhardt’s, 0.1% SDS, 50% formamide and 100 μg ml\(^{-1}\) denatured salmon sperm DNA. Filters were washed three times at 65°C with 3× SSC, 0.1% SDS and analyzed using the phosphorimager (Molecular Dynamics, Freiburg, Germany).

By cDNA-AFLP analyses, transcripts were identified that specifically accumulate after pathogen infection in potato. Using RNA from potato leaves infiltrated with P. syringae pv. maculicola for 3 and 12 h, several bands were detected that correspond to genes which show increased expression in response to bacterial infiltration (data not shown). Apart from cDNAs encoding known PR proteins, such as PR2 and PR5, as well as those encoding enzymes of the secondary metabolism, such as phenylalanine ammonia lyase (PAL, EC 4.3.1.5), one cDNA fragment was obtained which exhibited significant sequence similarity to a phosphate starvation-induced phosphatase cDNA from tomato (Baldwin et al. 2001). Since phosphatases might be important components of defense responses (Rodriguez 1998), this cDNA-AFLP fragment was chosen for further analyses. A cDNA library was constructed and screened with the cDNA-AFLP-fragment as a probe and 10 independent clones were isolated. Sequence analysis revealed that none of the cDNA clones contained the entire coding region. Therefore, a full-length clone was assembled from two clones which were identical in their overlapping region of 363 bp and which contained either the predicted start or the stop codon as well as the corresponding untranslated regions. This clone was named StPPP1 (Solanum tuberosum phosphate starvation- and pathogen-induced phosphatase). At the nucleotide level, the potato clone is 91% identical to the tomato acid phosphatase sequence (LePS2, accession number AF305968), and the deduced amino acid sequences are 90% identical (Fig. 1). StPPP1 has an insert of 921 nucleotides which codes for a protein of 272 amino acids with a predicted molecular mass of 30.848 Da. The calculated pI is 5.9 and computer analyses predict the protein to be localized in the cytoplasm. There are two motifs in the StPPP1 sequence (DFDKT and GDGIGD) that are characteristic of the haloacid dehalogenase (HAD) superfamily of “DDDD”-type phosphohydrolases (Aravind et al. 1998, Thaller et al. 1998). The first motif, DXDX(T/V), is conserved among non-specific phosphohydrolases (Aravind et al. 1998). Despite the homology to P-type ATPases (Aravind et al. 1998), the second motif characteristic of the HAD superfamily is D, is located 100–200 amino acids further towards the C-terminus and is preceded by a conserved lysine residue (Aravind et al. 1998). This lysine and two aspartate residues, corresponding to the amino acids K154, D9, and D178 of StPPP1, are proposed to be involved in the catalysis in P-type ATPases (Aravind et al. 1998). Despite the homology to members of the HAD superfamily of phosphatases, the substrates for StPPP1 or for LePS2 (Baldwin et al. 2001) are not known.

Expression analyses using RNA from P. syringae pv.
Pathogen-responsive phosphatase from potato 1051

maculicola-infiltrated potato leaves showed that the StPPP1 genes are specifically expressed in pathogen-infected, but not buffer-infiltrated leaves (Fig. 2A). The highest transcript levels are observed 6 and 12 h after infiltration, which interestingly correlates with the initiation of necrosis formation (data not shown). Genes encoding pathogenesis-related protein 1 (StPR1) are activated later than StPPP1. In addition, StPPP1 expression was analyzed in the interaction of potato plants with the oomycete P. infestans, the causal agent of late blight disease (Fig. 2B). Similar to the infiltration of potato with P. syringae pv. maculicola, which does not lead to disease, infection of potato plants with the oomycete P. infestans results in significant expression of StPPP1, which is most pronounced 3 and 4 dpi and corresponds to StPR1 expression (Fig. 2B). In contrast, treatment of suspension-cultured potato cells with a crude elicitor preparation from P. infestans (Schmidt et al. 1998), which activates defense genes such as StPAL, did not induce, but rather appeared to repress StPPP1 expression (Fig. 2C).

Since the tomato homologue of StPPP1, LePS2 (Baldwin et al. 2001), has been identified as a phosphate starvation-induced cDNA, we examined whether expression of StPPP1, in addition to being pathogen-inducible, is also responsive to phosphate deficiency. Five-day-old suspension-cultured potato cells were therefore transferred into medium containing either 1 mM or no phosphate, respectively, and StPPP1 transcript levels were determined at different time points after transfer. RNA analyses revealed that transfer of cells into medium without phosphate rapidly up-regulated StPPP1 expression within 3 h (Fig. 3A), whereas 1 mM phosphate repressed StPPP1 expression (Fig. 3B). StPPP1 transcripts detected in cultured potato cells prior to transfer (Fig. 3B) are indicative of phosphate starvation conditions after five days of subculturing. A similar effect has been described for tomato suspension cultured cells, which have undetectable levels of inorganic phosphate 3 to 4 d after the beginning of subculturing (Stenzel 1998). These results indicate that StPPP1 genes are up-regulated by phosphate starvation and rapidly down-regulated in the presence of phosphate.

The tissue-specific expression of StPPP1 genes was analyzed using RNA from greenhouse-grown potato plants. As shown in Fig. 4, only low levels of StPPP1 transcript were observed. Roots appeared to contain slightly higher amounts of StPPP1 transcripts than leaves, possibly due to phosphate limitation. For the tomato homologue, LePS2, low transcript levels have been reported in leaves and roots of plants grown in the presence of phosphate (Baldwin et al. 2001).

Genomic Southern analyses showed the presence of several bands hybridizing to the StPPP1 cDNA (Fig. 5), which correlates with the data reported for tomato (Baldwin et al. 2001). Thus, in both plants, the phosphatase is encoded by a small multigene family of at least three members.

We have identified StPPP1, a pathogen- and phosphate starvation-induced phosphatase cDNA from bacteria-infiltrated potato leaves. Interestingly, StPPP1 is not induced by compounds which are known to act as signal molecules in plant
Pathogen-responsive phosphatase from potato

Hybridized to the radioactively labeled electrophoresis and transferred onto a nylon membrane. The filter was Genomic Southern analysis of StPPP1 expression in potato cell cultures in response to phosphate availability. Total RNA was isolated from suspension cultured potato cells after transfer into medium without (A) or with 1 mM phosphate (B) at the time points indicated (hpt, hours post transfer) and subjected to Northern analyses. Hybridization was carried out using the radioactively labeled StPPP1 cDNA as a probe (upper panels). To check equal loading, rRNA was visualized by staining the gel with ethidium bromide (lower panels, rRNA).

**Fig. 3** StPPP1 expression in potato cell cultures in response to phosphate availability. Total RNA was isolated from suspension cultured potato cells after transfer into medium without (A) or with 1 mM phosphate (B) at the time points indicated (hpt, hours post transfer) and subjected to Northern analyses. Hybridization was carried out using the radioactively labeled StPPP1 cDNA as a probe (upper panels). To check equal loading, rRNA was visualized by staining the gel with ethidium bromide (lower panels, rRNA).

**Fig. 4** Tissue-specific expression of StPPP1. RNA from flowers (f), young leaves (yl), old leaves (ol), petioles (p), stems (s), roots (r) and tubers (t) was separated by gel electrophoresis, blotted and hybridized to the radioactively labeled insert of StPPP1. To control loading, the filter was hybridized to a radioactively labeled probe derived from ribosomal RNA (rRNA).

**Fig. 5** Genomic Southern analysis of StPPP1 genes. Genomic DNA from potato leaves was digested with BamHI (lane 1), EcoRI (lane 2), EcoRV (lane 3), HindIII (lane 4) and XbaI (lane 5), subjected to gel electrophoresis and transferred onto a nylon membrane. The filter was hybridized to the radioactively labeled EcoRI insert of StPPP1. Numbers on the right indicate the position of size markers.

Pathogen interactions. Thus, neither the incubation of detached potato leaves in solutions of salicylic acid nor jasmonic acid induced accumulation of StPPP1 transcripts (data not shown). Moreover, we tested whether pathogen-derived elicitors had any effects on StPPP1 expression. Neither the culture filtrate of *P. infestans* (Schmidt et al. 1998), nor arachidonic acid (Bostock et al. 1981), were able to induce accumulation of StPPP1 transcripts either in cultured potato cells or in potato leaves (Fig. 2C, data not shown). Thus, StPPP1 appears to be expressed only in response to infection by intact organisms during pathogenesis and not as a result of the recognition of pathogen-derived factors by the plant and the subsequent signal transduction leading to the activation of defense genes. Therefore, in this respect, StPPP1 is not a “classical” defense gene. The fact that StPPP1 responds to phosphate starvation moreover suggests that a deficiency in phosphate caused by the pathogen might be the actual signal for the activation of StPPP1 expression. It remains to be shown whether phosphate starvation-induced genes in general respond to pathogen infection.

**Acknowledgments**

We thank Angelika Weinel for excellent technical assistance and Anja Grohnert, Martina Kausch and Ralf Horbach for their help with the cell culture experiments. Margret Köck and Justin Lee are acknowledged for valuable discussions and critical reading of the manuscript. This work was supported by the “Deutsche Forschungsgemeinschaft” (Schc 235/9) and the “Fonds der Chemischen Industrie”.

**References**


Punja, Z.K. (2001) Genetic engineering of plants to enhance resistance to fun-
Pathogen-responsive phosphatase from potato


(Received March 19, 2002; Accepted June 11, 2002)