# Differential gene behavior in resistant potato plants challenged with late blight disease

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## Abstract

Late blight caused by the fungus *Phytophthora infestans*, is one of the most important diseases of potato (*Solanum tuberosum* L.) crop worldwide. However, in spite of its importance, understanding of the pathosystems mediating potato infection by this fungus is still limited. Therefore, to better understand the defense responses initiated by potato resistant plants challenged with *P. infestans*, expression of six well known defense-related genes were monitored at early four-time points of infection. Time-course experiments revealed notable differences in gene expression patterns during potato-pathogen interaction as compared to the non-infected control. It is noteworthy that both *PR3* and *StSYR1* have higher constitutive expressions with 6.1 and 8.1 folds, respectively, at 48 and 72 h post inoculation (hpi) period. It was clear that their expressions were higher at the necrotrophic stage compared with the biotrophic one. According to findings, our data can provide an insight into the signaling pathways that accounts for conventional gene expression changes elicited during potato-pathogen interactions.

**Keywords:** Potato-Late blight -*Phytophthora infestans* -Defense response -PCR (qPCR).

## Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops in the world with a total global production exceeded 370 million metric tons (FAO, 2019). This production is highly affected by late blight, a disease caused by the oomycete *Phytophthora infestans*, which has become the most destructive fungal foliar diseases in many potato growing regions around the world (Kamoun *et al.,* 2015). The fungus can be transmitted from infected seed tubers to newly emerging potato plants, where it produces airborne spores that can move to neighboring plants. It can destroy all potato parts and causes heavy yield losses up to 80% (Nowicki *et al.,* 2012).

The pathogen *P. infestans* has a hemi biotrophic mode of nutrition since initial infection is through a biotrophicmode and later turns towards necrotrophic phase. During the biotrophic phase, the pathogen needs living host plant cell and obtains the nutrition through the haustoria (Lee and Rose, 2010). It produces effectors to attack potato plants which are recognized by trans-membrane spanning, pathogen effector detection receptors genes (Xue *et al.,* 2021).

Infecting potato plants with *P. infestans* activate the defence responses, which are regulated by an intensive expression of diverse plant pathways (Bengtsson *et al.,* 2014). The expression levels of pathogenesis related (*PR*) proteins and other genes like *StSYR1* and *PAL* are low or absent in mature vigorous plants but become raised after pathogen attack (Van Loon *et al.,* 2006). However, the molecular events involved in potato defence response against *P. infestans* are not yet fully understood, although an increasing number of potentially involved components were determined (Harrison, 1995; Duan *et al.,* 2020). Therefore, quantitative PCR (qPCR) would be an efficient approach to be used, since it enables to measuring the levels of expressed genes after being exposed to a specific alteration, such as an infection by *P. infestans* (Derveaux *et al.,* 2010; Osawa *et al.,* 2021).

Understanding the basis of potato resistance towards *P. infestans* would greatly help the development of new control strategies and the identification of pathogen and host factors essential for disease progression. Therefore, the objective of the present research was to evaluate the changes in the induction of some well-known defense-related genes *PR-1, PR-2, PR-3, PR-5, PAL* and *StSYR1* during potato-pathogen interaction deploying qPCR approach.

## Materials and Methods

### Plant material and growing conditions

The resistant potato cultivar Spunta, originated from the Netherlands and widely grown in Syria, was used in this study. A single seed tuber (60–65 g) was grown at the center of plastic pots filled with sterilized peat moss and arranged in a completely randomized design with five replicates. Pots were placed in a growth chamber at

temperatures 18 ºC (day) and 16 ºC (night) in a 12 h light/12 h dark cycle and 85–90% relative humidity.

**Isolation *P. infestans***

The virulent isolate PiSYR1 collected during the 2014 from the middle region of Syria, was used in this study (Salima, 2015). Small pieces from the infected potato leaves were placed in Petri dishes under disinfected tuber slices and incubated in a growing chamber at 18 °C and 16 h in light and 8 h in dark for 6 to 7 days. When mycelium was growing on the top of the potato slices, the mycelium was transferred to fresh rye agar. *P. infestans* mycelia (PiSYR1) were purified by repetitive transfers to rye agar medium and microscopic checks. The zoospore concentration was $5 \times 10^4$ spores $mL^{-1}$ was sprayed with a hand sprayer onto the potato seedlings in each pot, and mock inoculation was performed by spraying plants with pathogen-free water.

**RNA isolation and cDNA synthesis**

Potato primary leaves were harvested at 24, 48, 72 and 96 hpi and were immediately frozen in liquid nitrogen. Mock-inoculated control samples were collected at the same time points. Total RNA was extracted using the Nucleotrap mRNA mini kit (Macherey-Nagel, Germany) following the manufacturer's protocol. cDNA was synthesized with the QuantiTect Reverse Transcription Kit (Qiagen, Germany) following the manufacturer's instructions and the obtained cDNA was stored at −20 °C.

**Gene expression profiling**

The expression levels of six well known defense-related genes *viz. PR-1, PR-2, PR-3, PR-5, PAL* and *StSYR1* were compared at four time points: 24, 48, 72 and 96 hpi. The choice of these time points was based on the stages in the infection cycle (Avrova *et al.,* 2007). The time point 0 h corresponded to inoculation, 24 h to the biotrophic stage, 48 h to the beginning of the necrotrophic stage and 72 and 96 h to the necrotrophic stage. At each time point, three plants for each of the four times were analyzed with RT-qPCR assays using SYBR Green Master kit (Roche, USA) and the primers used for each gene are given in Table 2. All the qRT-PCR reactions were performed in triplicate for each cDNA sample with an annealing temperature of 60 °C and a total of 40 cycles of amplification. The expression level of each gene was calculated according to Livak and Schmittgen (2001) method using *EF1α* as an internal reference. Standard deviation was calculated from the replicated experimental data. The treated means were compared using Tukey's test at the 0.05 level. All the experiments were repeated at least twice in triplicate.

## Results and Discussion

In this investigation, the resistant potato cv. Spunta to *P. infestans* was used. The pathogen produced late blight symptoms 48 hpi as small, light to dark green spots on the infected plants (Fig. 1). Four different stages were chosen here to cover early potato responses to late blight disease which leads within 96 h to a visible hypersensitive cell death on plants by considering the observations of Avrova *et al.* (2007) with potato susceptibility to *P. infestans*.

In order to determine the defense responses exhibited by potato plants to overcome *P. infestans* pathogen infection, the induction of well-known defense-related genes *viz. PR-1, PR-2, PR-3, PR-5, PAL* and *StSYR1* was assayed in potato leaves (Tables 1 and 2). The data demonstrated the patterns of expressed genes at the beginning of the late blight inoculation test represent the normal set of active genes in a resistant plant after four hours of being sprayed with water. At 24 hpi, the six defense genes were significantly upregulated after *P. infestans* inoculation (Fig. 2). Based on the assumption that disease infection involves the early recognition of the invading pathogen, the expressed gene patterns of resistant potato plants were recorded a cooperative functions, which occur 24, 48, 72 and 96 h after *P. infestans* attack.

Our analysis showed that *PR-1, PR-2, PR-3, PR-5, PAL* and *StSYR1* genes in the resistant potato exhibited a differential expression by $P = 0.05$, and were inversely regulated during different times point post inoculation (Fig. 2). However, the expression of the *PR1* gene was observed to fluctuate, both up and down, throughout the course of the experiment in plants challenged with *P. infestans*. *PR1* has been found to be linked to partial resistance to *P. infestans* in solanum species (Vleeshouwers *et al.,* 2000). Recent researches demonstrated that both acidic and basic *PR-1s* bind sterols, however, since *Phytophthora* species contain sterols in their membranes, and the pathogens need sterols from the plants they infect and are particularly inhibited by *PR-1* binding, which depletes sterol pools required by the pathogen (Gamir *et al.,* 2017; Kattupalli *et al.,* 2021).

In contrast, *PR2* was up-regulated during the time points of inoculation up to 96 hpi (Fig. 2), in agreement with Van Loon *et al.* (2006) who reported that *PR-1* and *PR-2* family proteins have biochemical and biological properties associated with activity against oomycetes such as *P. infestans*. Boava *et al.* (2011) reported that *PR2* family has b-1, 3-endoglucanase properties and hydrolyses b-1, 3-glucans, a major component of the cell wall of oomycetes. *PR2*, which codes for a b-glucosidase, is often linked to the salicylic acid (SA) pathway, which is an important compound required for the late blight disease resistance against potato plants (Halim *et al.,* 2007). The other families like *PR5* (osmotins) was increased during the time of inoculation up 96 h, which is very important against the oomycetes as a membrane permeabilizing agent (van Loon *et al.,* 2006).

On the other hand, data showed the both *PR3* and *StSYR1* genes have expression with 6.1 and 8.1 folds higher respectively at the 48 and 72 h post inoculation (hpi) and it was clear that their expressions were higher at the necrotrophic stage (24–48 hpi) as compared with the other genes. *PR3* proteins can play a function role for protecting cell damage against fungal pathogens attack (Ali *et al.,* 2017). On the other hand, Rivas-San Vicente and Plasencia (2011) reported that *StPR1* gene expression was correlated with increasing SA levels and enhanced defense responses against *P. infestans*. Eschen-Lippold *et al.* (2012) found anactivation of defense against *P. infestans* in potato by down-regulation of syntax in gene expression.

In addition, the data showed that *PAL* expression levels were increased during the times of inoculation and these relative expressions fluctuated less over the course of the infection than the *PR* genes (Fig. 2). This is in line with the results of Gallou *et al.* (2011) who reported that *PAL* can play an important function in potato plants challenged by *P. infestans*. It is known that *PAL* catalyses the non-oxidative deamination of phenylalanine to *trans*-cinnamate. This is the first step in the phenylpropanoid pathway which is an essential regulation point between primary and secondary metabolism (Huang *et al.*, 2010; Vogt, 2010). This phenomenon may be the cause of potato cell wall leakage during *P. infestans* infection.

# Conclusion

This work sheds some light on the relative contributions of six important defense-related genes during the *P. infestans*-potato interactions. Results showed that resistant potato revealed a remarkable discrepancy in the gene expression patterns against this fungus. It is noteworthy that both *PR3* and *StSYR1* have higher constitutive expressions with 6.1 and 8.1 folds higher respectively at the 48 and 72 hpi, and that their expressions were higher at the biotrophic stage (24–48 hpi) comparing with the other genes. This consistency in the defense mechanisms could be in agreement with the well-accepted hypotheses that defense responses are extremely intense in resistant plants. Our work provided helpful information for understanding late blight resistance mechanism of potato.

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**Table 1:** The pathogenesis-related proteins used in the study.

| Family | Type member | Properties |
|---|---|---|
| *PR1* | *PR*1a | Unknown |
| *PR2* | *PR*2 | ß-1,3-glucanase |
| *PR3* | Tobacco P, Q | Chitinase class I, II, IV-VII |
| *PR5* | Tobaco S | Thaumatin-lik |
| *PAL* | Phenylpropanoid pathway | Phenylalanine ammonia lyase (PAL; E.C 4.3.1.5) |
| *StSYR1* | Syntaxin-related1 | Q-SNARE proteins |

**Table 2:** List of genes studied with accession number from Sol Genomics Network or NCBI and corresponding primers used for RT-qPCR.

| Sequence | Source | Accession No. | Gene |
|---|---|---|---|
| TGGATTTGAGGGTGACAACA | *Arabidopsis thaliana* (Mouse-ear cress) | AT1G07920 | *EF1α* |
| CCGTTCCAATACCACCAATC | | | |
| ACTACCTTTCACCCCACAACGC | *Triticum aestivum* | AY005474 | *PR1* |
| TTTCTGTCCAACAACATTCCCG | | | |
| TCATCCCTGAACCTTCCTTG | *Arabidopsis thaliana* (Thale cress) | AT3G57260 | *PR2* |
| GGGGCTACTGTTTCAAGCAA | | | |
| GGGGCTACTGTTTCAAGCAA | *Brachypodium distachyon* (Purple false brome) | AT3G12500 | *PR3* |
| GCAACAAGGTCAGGGTTGTT | | | |
| GGAGACTGTGGCGGTCTAAG | *Arabidopsis thaliana* (Thale | AT1G75040 | *PR5* |

| | | | |
|---|---|---|---|
| | cress) | | |
| GCGTTGAGGTCAGAGACACA | | | |
| CCATTGATGAAGCCAAAGCAAG<br>ATGAGTGGGTTATCGTTGACGG | *Arabidopsis thaliana* (Thale cress) | AT2G14610 | *PAL* |
| | *Arabidopsis PENETRATION1 (AtPEN1)* | | *StSYR1* |


**Fig. 1:** Late blight symptoms on potato cv. Spunta after four-time points of infection

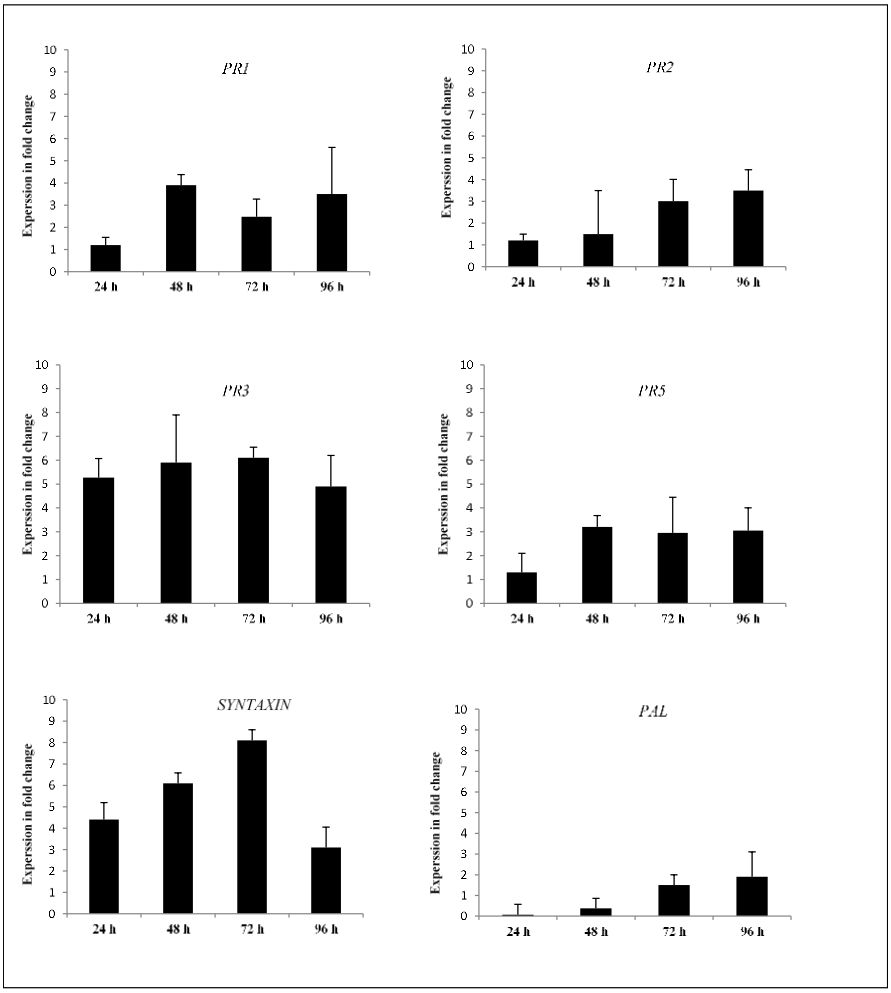


**Fig. 2:** Relative expression profiles of marker genes in the resistant potato cv. Spunta during the time course after infections with *P. infestans.* Error bars are representative of the standard error (Mean ± SD, $n$ = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).

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