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Functional cooperation between PR3 and TUB in potato plants infected with late blight

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Abstract

Late blight (LB), caused by the fungus *Phytophthora infestans* is an economical disease of potato worldwide. Little is known about how defense-related genes cooperate to resist potato LB development. In the current work, expression of two genes *PR3* and *TUB* were examined in resistant and susceptible potato cultivars at early stages of LB infection using quantitative real-time PCR (qPCR). The results showed significant variance in the expression patterns of the both genes between resistant and susceptible potato - *P. infestans* interactions as compared to the non-inoculated controls. It is also notable that *PR3* and *TUB* genes were highly expressed in the resistant cultivar as compared to a susceptible one. qPCR analysis revealed strong expression of genes in resistant cultivar when compared to susceptible with expression for *PR3* (6.1 and 2.5-fold) and *TUB* (1.8 and 1.3-fold) respectively, at 72 hours post inoculation. The obtained results suggest that *PR3* and *TUB* genes, positively regulate *P. infestans* — resistance in potato plants during disease progress, which can provide testable hypotheses that will need direct future tests to determine how these changes may be specified in the potato plant cytoskeleton rearrangement during the biotrophic stage.

Keywords: Potato, defense response, *Phytophthora infestans*, real time PCR

1.Introduction

Late blight caused by the oomycete fungus *Phytophthora infestans* (Mont.) De Bary, is one of the most common diseases of potato (*Solanum tuberosum*) worldwide causing substantial yield losses [6, 20]. It is greatly challenging to control *P. infestans* due to a poor understanding of the defense mechanisms and as a resulting, no highly potato resistant genotypes are yet available. Hence, increasing our knowledge of the resistance mechanisms is quiet essential.

P. infestans is hemibiotrophs pathogen, which start infections with *biotrophic* phase but cause necrosis symptoms late during the disease cycle [1]. During the biotrophic stage, it needs living cell to obtain the nutrition via the haustoria [9]. Potato plants exposed to *P. infestans* use complex defense mechanisms developed during infection stages. Many pathogeneses related genes conferring resistance against potato LB have been cloned, and all belong to the nucleotide-binding, leucine-rich-repeat (NLR) class [7]. However, many of their specific functions still remain unknown.

Chitinases (*PR3*) is strongly induced after the pathogen attack of host plant, which has an important weaponry of plants against pathogens and have ability to inhibit fungal growth by degrading heterogeneous polysaccharide (chitin), a major component of the fungi cell wall [18]. In addition, tubulin genes which are highly conserved in structure have function roles in host cytoskeleton rearrangement in infected cells with fungal pathogens [10]. There is also evidence for increased expression of host tubulin gene during infection with these kind of pathogens in potato [12], in cotton [5] and in flax [8]. However, many of *PR3* and *TUB* specific functions during potato infection with *P. infestans* still remain unknown.

Quantitative PCR (qPCR) is considered to be the most accurate method, since it allows the assessing of the relative expression level of a specific transcript and determines its expression after being

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exposed to a specific alteration, such as infection by a pathogen [3].

In this work, we studied the defense responses of two potato cultivars Sponta and Draga, which are integrated in international breeding programs aimed at developing *P. infestans* resistant potato cultivars. Sponta was described as resistant to LB [16], i.e. showed a lower level (compared with Draga) of *LB* symptom progress. We consequently hypothesized that *PR3* and *TUB* genes could drive contrasted levels of resistance in Sponta and Draga, inoculated by the same isolate of the pathogen. Thus, the present study aimed at evaluating the changes in induction of *PR3* and *TUB* expressions in two potato cultivars with different resistance levels against *P. infestans* using PCR (qPCR) approach.

2. Materials and Method

Plant growth

Potato seed tubers (~ 65 g) of LB-*resistant 'Sponta' and susceptible 'Draga' cultivars* [16] *were grown in plastic pots (20-cm in diameter) filled with sterilized peat moss in five replicates.* Pots were kept in a growth chamber set at 20° with a 16 h/8h (light/dark) cycle and *60 ± 5%* % relative humidity.

LB inoculation

The Syrian virulent isolate PiSYR1 [16] was used in this study. Small pieces from infected potato leaves were placed separately in sterile Petri dishes under disinfected tuber slices and incubated in a growing chamber 16 h/8h (light/dark) at 20 °C for a week. When mycelium was growing on the surface of the potato slice, the mycelium was transferred to fresh rye agar [4]. Conidial suspension was adjusted to 5 × 10^4 spores/mL and sprayed with a hand sprayer onto the potato seedlings in each pot. Control plants were sprayed with distilled water.

RNA isolation and cDNA synthesis

Total RNA was isolated from Potato leaves of inoculated as well as uninoculated leaves after 24, 48, 72 and 96 hpi using Trizol Reagent (Macherey-Nagel, Germany). cDNA was synthesized with the QuantiTect Reverse Transcription Kit (Qiagen) following the manufacturer's instructions. The control samples were collected at the same time points.

Quantitative RT-PCR analysis

Three plants of each time were analyzed with RT-qPCR assays using SYBR Green Master kit (Roche, USA). The primers used for PR3 and TUB gene are given in Table 2. The PCR conditions were 95° for 5 min, followed by 40 cycles of 95° for 10 s, 60° for 20 s, and 72° for 20 s. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method using *EF1α* as an internal reference gene according to Livak and Schmittgen (2001) [13]. Means were compared using Tukey's test at the significance 0.05 level. All qRT-PCR tests were repeated in triplicate.

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3.Results and discussion

In the current study, two potato cultivars Sponta and Draga with different resistance levels to *P. infestans* infection were used.

LB severity was always higher in the susceptible cultivar as compared with the resistant one (Table 1). These remarks are in agreement with those noted under natural field infections [16].

Table 1. LB symptoms on two potato cultivars used in this work

Cultivar	Disease*	LB symptoms
Reaction		
Sponta	R	Small blackish-brown lesions on leaves and stems
Draga	S	Water-soaked or have chlorotic borders and the entire leaf becomes necrotic

*Disease reaction and symptoms as described by Salima (2015), where; R: resistant and S: susceptible

Table 2. List of genes studied with accession number and corresponding primers used for RT-qPCR

Gene	Accession No.	Sequence
EF1α	AT1G07920	TGGATTTGAGGGTGACAACA CCGTTCCAATACCACCAATC
PR3	AT3G12500	GGGGCTACTGTTTCAAGCAA GCAACAAGGTCAGGGTTGTT
TUB	NM_001288449	TCTGCAACCATGAGTGGTGT ATGTTGCTCTCGGCTTCAGT

In order to get insight into the early responses of *PR3* and *TUB* genes in potato inoculated with *P. infestans* four time points 24, 48, 72 and 96 h of *P. infestans* infection, were chosen as being representative of *biotrophic* and *necrotrophic* phases [2]. Data showed that selected genes in both potato cultivars displayed different expressions at $P = 0.001$, and it was remarkable that these genes had high expression and faster induction in the resistant cv. 'Sponta' as compared to a susceptible one' Draga' (Fig. 1).

The data proved that at 24 hpi, the two genes were significantly upregulated after *P. infestans* inoculation in both resistant and susceptible cultivars (Fig. 1), suggesting that robust and distinct defense responses are initiated early. However, based on the supposition that disease infection involves the early recognition of the invading pathogen, the expressed genes in both resistant and susceptible potato plants were recorded a cooperative function which occur 24, 48, 72 and 96 hours after *P. infestans* attack. The results also revealed higher gene expression in resistant cultivar than susceptible ones with expression for *PR3* (6.1 and 2.5-fold) and *TUB* (1.8 and 1.3-fold) respectively, at 72 hours post inoculation. (Fig. 1).

The induced expression of *PR3* proteins in this work after infection suggests that it is part of a plant defence mechanism through inhibit fungal growth by degrading heterogeneous polysaccharide of the fungi cell wall [1]. A major component of the *P. infestons* cell wall is 1,3-ß-glucan but chitin is lacking. Therefore only 1,3-ß-glucanases are potentially capable of hydrolysing the cell wall of *P. infestans* [14]. This incident may be the cause of potato cell wall damage during infection with *P. infestans*. Our results in agreement with Van Loon *et al.* (2006) [19] who reported that PR family proteins might have biochemical and biological properties against oomycetes such as *P. infestans*.

On the other hand, data also showed that sharp increasing in *TUB* gene expression was observed which is in line with Swiecicka *et al.* (2009) [17] who found an increasing in tubulin expression and microtubule related proteins after nematode infection, and with Kobayashi *et al.* (1994) [11] who found new arrangements of TUB have been observed in flax cells responding to the flax rust infection. This event might support its role in potato cell wall leakage during *P. infestans* infestation. However, it is clear that in comparison to potato interactions, in resistant interactions host cells respond much faster to *P. infestans* attack by activating *PR3 and TUB* genes higher than the susceptible interactions. This leads to rapid browning and subsequent death of host cells in the surrounding infected area hypersensitivity reactions (HR) shortly after penetration. As a result, further development of the biotrophic fungus is arrested. In a susceptible host, the rapid HR reaction does not occur [15].

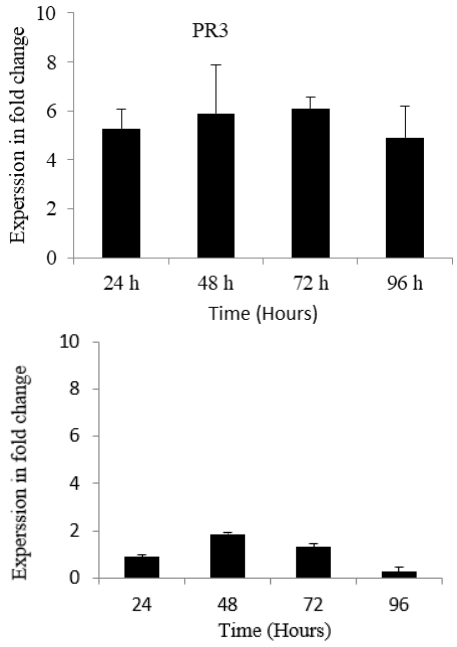

Figure. 1. Relative expression profiles of *PR3* and TUB in resistant (Sponta) cultivar during the time course of late blight infections. *Error bars are representative of the standard error (mean ± SD, n = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).*

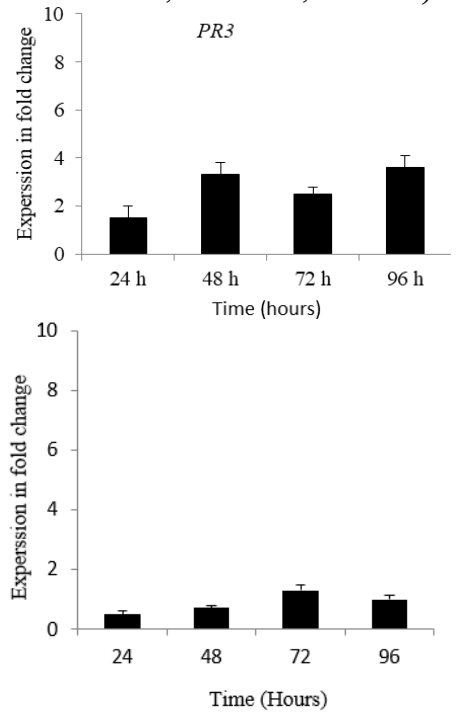

Figure. 2. Relative expression profiles of *PR3* and TUB in susceptible (Draga) cultivar during the time course of late blight infections. *Error bars are representative of the standard error (mean ± SD, n = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).*

4.Conclusion

This work revealed that, significant increases in *PR3* and *TUB* expression were initiated after potato challenged with *P. infestans*. It is also notable that the both genes have highly and faster expressions in the resistant cultivar as compared to a susceptible one. These defense mechanisms could be in agreement with the well-accepted conception that defense responses are very intense in potato resistant plants.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

Conflicts of interest: The authors declare that they have no potential conflicts of interest.

References

1. Ali S, Ganai BA, Kamili AN, Bhat AA, Mir ZA, Bhat JA, Tyagi A, Islam ST, Mushtaq M, Yadav P, *et al.*, Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiol. Res.*, **2018**, 212–213, 29–37.
2. Birch PRJ, Avrova A, Armstrong MR, Venter E, Taleb *N et al*. The potato-*Phytophthora infestans* interaction transcriptome. *Can J. Plant Pathol.* **2003**, *25*, 226–231.
3. Caballero-Solares A, Hall JR, Xue X, Rise ML., Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR) for gene expression analyses. *Methods Mol. Biol.*, **2022**, *2508*, 319-340.
4. Caten CE, Jinks JL. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I.Cultural variation. *Can. J. Bot.* **1968**, *46*, 329-347.
5. Chen B, Zhao J, Fu G. *et al*.. Identification and expression analysis of *Tubulin* gene family in upland cotton. *J. Cotton Res.*, **2021**, *4*, 20.
6. Dey T, Saville A, Myers K, *et al*. Large sub-clonal variation in *Phytophthora infestans* from recent severe late blight epidemics in India. *Sci Rep.*, **2018**, *8*, 4429.
7. Jo KR, Visser RG., Jacobsen E, Vossen JH. Characterization of the late blight resistance in potato differential Ma*R9* reveals a qualitative resistance gene, *R9a*, residing in a cluster of *Tm-2(2)* homologs on chromosome IX. *Theor. Appl. Genet.*, **2015**, *128*, 931–941.

8. Gavazzi F, Pigna G, Braglia L, *et al.* 2017. Evolutionary characterization and transcript profiling of β- tubulin genes in flax (*Linum usitatissimum* L.) during plant development. *BMC Plant Biol.* **2017**, *17*, *237*.
9. Kagda MS, Martínez-Soto D, Ah-Fong AMV, Judelson HS 2020. Invertases in *Phytophthora infestans* localize to haustoria and are Programmed for infection-specific expression. *mBio*, **2020**, *11*(5), e01251-20.
10. Kitaeva AB, Gorshkov AP, Kusakin PG, Sadovskaya AR, Tsyganova AV, Tsyganov VE. Tubulin cytoskeleton organization in cells of determinate nodules. *Front. Plant Sci.* **2022**, *26*, 823183.
11. Kobayashi I, Kobayashi Y, Hardham AR. Dynamic reorganization of microtubules and microfilaments in flax cells during the resistance response to flax rust infection. *Planta*, **1994**, *195*, 237– 47.
12. Koo BS, Kalme S, Yeo SH, Lee SJ, Yoon MY. Molecular cloning and biochemical characterization of alpha- and beta-tubulin from potato plants (*Solanum tuberosum* L.). *Plant Physiol. Biochem.*, **2009**, *47*, 761-8.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, **2001**, *25*, 402-408.
14. Mcleod A, Smart CD, Fry WE. Characterization of 1,3- glucanase and 1,3;1,4-glucanase genes from *Phytophthora infestans*. *Fungal Genet. Biol*. **2003**, *38*, 250-63.
15. Mishra AK, Sharma K, Misra RS. Elicitor recognition, signal transduction and induced resistance in plants. *J. Plant Interact.* **2012**, *7*, 95-120.
16. Salima NI. Surveying the distribution of late blight *Phytophthora infestans* on potato in Syria and Studying the physiological and molecular variability of the pathogen. *Thesis, University of Damascus, Faculty of Agriculture*, **2015**, *61*.
17. Swiecicka M, Filipecki M, Lont D, van Vliet J, Qin L, Govers A, Bakker J, Helder J., Dynamics in the tomato root transcriptome on infection with the potato cyst nematode Globodera rostochiensis. *Mol. Plant Pathol.*, **2009**, *10*, 487– 500.
18. Vaghela B, Vashi R, Rajput K, Joshi R., Plant chitinases and their role in plant defense: A comprehensive review. *Enzyme Microb. Technol.* **2022**, *159*, 110055.
19. Van Loon LC, Rep M, Pieterse CM., Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* **2006**, *44*, 135–162.
20. Zhang S, Zheng X, Reiter RJ, Feng S, Wang Y, Liu S, Jin L, Li Z, Datla R, Ren M., Melatonin attenuates potato late blight by disrupting cell growth, stress tolerance, fungicide susceptibility and homeostasis of gene expression in *Phytophthora infestans*. *Front Plant. Sci.* **2017**, *8*, 1993.