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ORIGINAL ARTICLE

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Effect of Infection of Potato Plants by *Phytophthora infestans* on Pathway Signaling of *TUB* and *PR5* Genes

Antonious Al-Daoude*, Amina Shoaib and Mohammed Jawhar

[1] *Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria*

*E-Mail: ***ascientific@aec.org.sy***

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Late blight, caused by the pathogen *Phytophthora infestans* is an economical disease of potato worldwide. To better understand mechanisms of potato to resist this fungus, pathway signaling of *TUB* and *PR5* were monitored using quantitative real-time PCR (qPCR) across four time points post infection. Results demonstrated that significant variance in the expression profiles of both genes in infected potato plants as compared to the non-infected controls. It is also notable that *TUB* and *PR5* genes have a higher and faster expression in the resistant cultivar 'Spunta' as compared to the susceptible one 'Draga' with a maximum expression for *TUB* (1.8 and 1.4-fold) and *PR5* (3.5 and 1.2-fold) respectively, at 48 hours post infection. Our data suggest that *TUB* and *PR5* genes, positively regulate *P. infestans*—resistance in potato plants during disease progress, which can provide testable hypotheses that will need direct future experiments to define how these pathway signaling of both genes may be specified in potato defense system.

Key words: Phytophthora infestans, potato, β- tubulin, PR5, real time PCR

Late blight (LB), caused by the fungus *Phytophthora infestans* Mont.) de Bary, is an economically important disease of potato (*Solanum tuberosum L*.) that causes substantial crop losses worldwide (Xue *et al.,* 2021; Dong and Zhou 2022). The pathogen has now spread widely and hence LB resistance breeding is an ongoing program across the world (Angmo *et al.,* 2023). Furthermore, it is highly challenging to control LB due to a poor understanding of the resistance mechanisms and as a consequent, no highly resistant potato cultivar is yet available. Resistance to this disease often depends on the activation of defense responses that are regulated through different plant signaling pathways genes (Paluchowska *et al.,* 2022). However, many of their specific functions still remain unknown.

Potato plants respond to *P. infestans* by activating various defense responses that are regulated through different signalling pathways. Pathogenesis-related (PR) proteins were discovered to primarily accumulate in plants in response to a variety of fungal diseases (Van Loon and Van Strien 1999). They are currently separated into 17 distinct classes (Van Loon *et al.,* 2006), however, the Tubulin (*TUB*) and *PR5* genes in potato plants have not been clearly demonstrated.

TUB genes were commonly used as reference genes for reverse-transcribed polymerase chain reaction (RT-PCR) since they have highly conserved in structure and function *tubulin* families (Jayaswal *et al.,* 2019). However, characterization of host plant tubulin is equally vital along with tubulin from plant fungal pathogens since there is obvious indication, that microtubules and tubulins of the host plant cytoskeleton are rearranged in infected cells with these pathogens (Kitaeva *et al.,* 2022). *TUB* expression has been observed in different plant species such as in cotton (Chen *et al.,* 2021), potato (Koo *et al.,* 2009; Al-Daoude *et al.,* 2023) and in flax (Gavazzi *et al.,* 2017). Furthermore, there is also evidence for increasing tubulin gene expression after infection with a mycorrhizal fungus (Plágaro *et al.,* 2021).

On the other hand, thaumatin-like proteins (*PR5*) has an important function in plant disease resistance and its antifungal activity has been recorded in various plant species. It is well known that *PR5* family includes basic and acidic members according to their isoelectric points, although they show similar activity, however, *PR5* proteins had an antifungal activities that were observed in rice and orange plants after infection with to *Rhizoctonia solani and Phytophthora infestans* (Bachmann et al., 1998). However, *TUB* roles, especially their interactions with the other defense responses in plant cells, are still not fully understood. Quantitative PCR (qPCR) is an effective method for measuring of the relative expression level of a particular genes and determines their expressions after infection by fungal pathogens (Derveaux *et al.,* 2010).

Here, we monitored the defense reactions of two potato cultivars Spunta and Draga, which are included in international breeding programs targeted at developing *P. infestans,* resistant potato cultivars. Spunta was defined as resistant i.e. showed a lower level (compared with Draga) of *LB* symptom development (Salima 2015). We thus hypothesized that *TUB* and *PR5* genes could drive contrasted resistance levels in Spunta and Draga, inoculated by the same isolate of *P. infestans*. Thus, the current work aimed at evaluating the expressions of these both genes in two potato cultivars with varying levels of resistance against *P. infestans* fungus using PCR (qPCR) method.

MATERIALS AND METHODS

Host Plant

The two potato resistant (cv. Spunta) and susceptible (cv. Draga) cultivars from Netherlands grown commonly in Syria were used in this work (Salima 2015). A single seed tuber (~ 65 g) was planted at the center of each plastic pot filled with sterilized peatmoss with five replicates. Pots were placed in a growth chamber set at 20° (16h light/ 8h dark) and 85-90 % relative humidity.

Infection with P. infestans

The Syrian virulent isolate PiSYR1 (Salima 2015) was used in the experiments. It was isolated from small infected potato leaves and placed in Petri dishes under disinfected tuber slices and incubated in a growing chamber for 7 days at 20 °C and 16 h/8h (light/dark). When mycelium was growing on the surface of the

potato slice, the mycelium was transferred to fresh rye agar (Caten and Jinks 1968). The conidial suspension was adjusted to 5×10^4 spores/mL and sprayed with a handheld sprayer onto the potato seedlings. The control plants were sprayed with fungus-free water.

RNA isolation and cDNA synthesis

RNA was isolated from Potato primary leaves of infected and no-infected leaves after 24, 48, 72 and 96 hours post infection (hpi) using Trizol Reagent (Macherey-Nagel, Germany) based on the manufacturer's guidelines. The control samples were collected at the similar time periods. cDNA was created using the QuantiTect Reverse Transcription Kit (Qiagen) following the manufacturer's instructions.

Quantitative RT-PCR analysis

TUB and *PR5* genes were assayed at each time point with RT-qPCR assays using SYBR Green Master kit (Roche, USA). The used primers used are presented in Table 1. 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. Gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method using *EF1α* as a reference gene (Livak and Schmittgen 2001). Means were compared using Tukey's test. All experiments were repeated in triplicate.

RESULTS AND DISCUSSION

In this study, two potato cultivars with varying resistance levels against the fungus *P. infestans* were used. The pathogen caused more severe symptoms on the susceptible cv. 'Draga' as compared to the resistant one 'Spunta'. *P. infestans* produced blackish-brown lesions on leaves and stems and developed latter to water-soaked or have chlorotic borders and the entire leaf becomes become necrotic, and these symptoms were more severe on the susceptible cv. 'Draga' after 10 days of infection (Table 2).

To better understand these interactions, *TUB* and *PR5* expressions were monitored at four early time points of potato infection by *P. infestans*. Data showed that at 24 hpi, the both genes were significantly upregulated after *P. infestans* infection in resistant and susceptible cultivars (Figs. 1 and 2), suggesting that robust and distinct defense responses are early initiated. However, the expressed gene patterns of both resistant and susceptible potato plants were recorded cooperative functions which occur after 24, 48, 72 and 96 hours attacking by *P. infestans*. For instance, the results revealed that maximum gene expressions in resistant and susceptible were 48 hours post inoculation for *TUB* (1.8 and 1.4-fold) and *PR5* (3.5 and 1.2-fold), respectively (Figs. 1 and 2).

However, considering that the two used potato cultivars had high different levels of resistance to *P. infestans* (Salima 2015; Al-Daoude *et al.,* 2023). The *TUB* and *PR5* were up-regulated 24 hpi in inoculated potato plants as compared to non- inoculated plants, which might indicate that their roles are related to the severity of LB symptoms rather than to resistance. These upregulations were higher in the resistant cultivar than in the susceptible cultivar (Figs. 1 and 2).

Our findings are in line with Van Loon *et al.* (2006) who reported that some PR proteins might have important functions against oomycetes fungi such as *P. infestans*. On the other hand, PR5 was increased in both potato cultivars during the time of infection up 96h (Fig. 2). It is well known that *PR5* exert their antifungal activity through a very fast and dramatic increase in the permeability of the pathogen's plasma membrane, by disrupting the lipid bi-layer and creating trans-membrane pores (Vigers *et al.,* 1992; Krebitz), this incident might explain its roles in potato cell during infection with *P. infestans.*

On the other hand, increased potato *TUB* expression at 48 and 72 hpi could be due to plant cytoskeleton rearrangement in response to biotrophic infection, and the subsequent decrease in *TUB* expression at 96 hpi could be due to plant cell disruption resulting from tissue damage during necrosis. Gasic *et al.* (2019) found differential expressions of tubulins due to a result of a post-transcriptional regulation of tubulin mRNA. Our results are in same with those of Kobayashi *et al.* (1994) who observed new arrangements of TUB in flax cells after flax rust infection.

Table 1: List of genes studied with accession number and corresponding primers used for RT-qPCR

Gene	Accession No.	Sequence
EF1α	AT1G07920	TGGATTTGAGGGTGACAACA CCGTTCCAATACCACCAATC
PR5	AT1G75040	GGGGCTACTGTTTCAAGCAA GCAGACTGTGGCGGTCTAAG
TUB	NM_001288449	GCGTTGAGGTCAGAGACACA ATGTTGCTCTCGGCTTCAGT

Table 2: Late blight symptoms on two potato cultivars used in this study

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

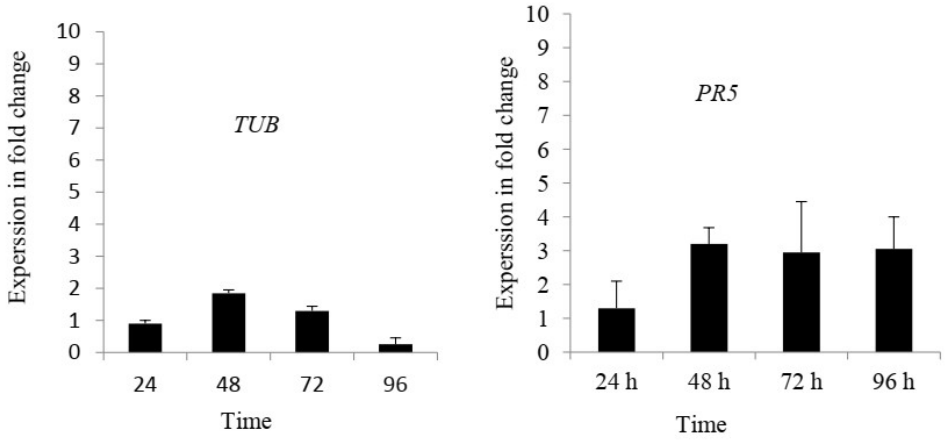

Figure 1. Relative expression profiles of *TUB* and *PR5* genes in the resistant cv. Spunta during the time course following *infections with late blight disease.* Error bars are representative of the standard error (Mean ± SD, *n* = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).

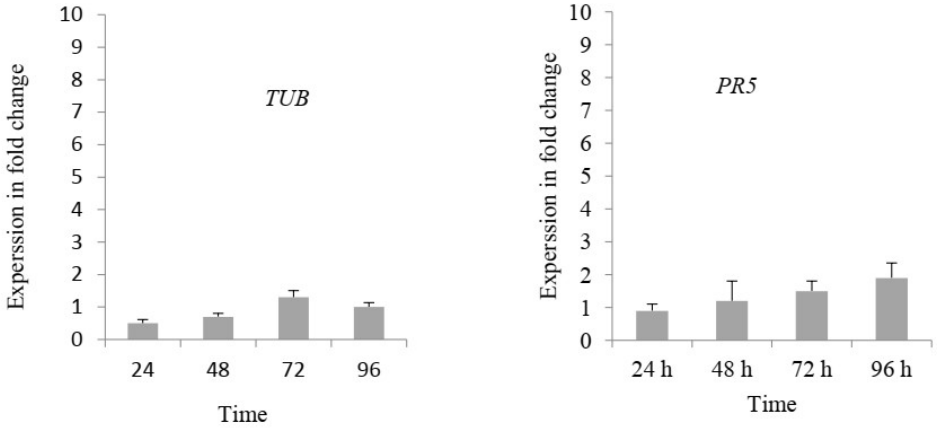

Figure 2. Relative expression profiles of TUB and *PR5* genes in the susceptible cv. Draga during the time course following *infections with late blight disease.* Error bars are representative of the standard error (Mean ± SD, *n* = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).

CONCLUSION

In this investigation, our data showed that significant increases in *TUB* and *PR5* expressions were found upon potato challenged with the *P. infestans*, which can contribute to LB resistance, since these signaling responses induced together in each cultivar. It is also noteworthy that *both genes* have higher and faster expressions in the resistant cultivar as compared to the susceptible one with a maximum for *TUB* (1.8 and 1.4-fold) and *PR5* (3.5 and 1.2-fold) respectively, at 48 hours post inoculation. These findings could be in agreement with the well-accepted notion that defense responses are very intense in potato resistant plants. In addition, we highlighted the fact that the two different signaling pathways may be induced in response to the same isolate of *P. infestans* in different potato cultivars.

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CONFLICTS OF INTEREST

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

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