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ORIGINAL ARTICLE

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Comparative Analysis of Osmotin-Like *PR5* and *PA13* Proteins in Potato Infected by Late Blight (*Phytophthora infestans*)

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Induction and accumulation of osmotin-like proteins are crucial components of innate immune responses in plants challenged with pathogens. One of these proteins, *PR5* and *PA13*, are abundantly secreted and are able to elicit plant defenses, however, their native function in potato plants infected with *Phytophthora infestans* remains unknown. Here, the pathway signaling of the two genes were monitored in potato plants at early points of infection with *P. infestans* using quantitative real-time PCR (qPCR). Our data demonstrated significant gene expression variance of the two genes in infected plants as compared to the non-infected controls. It is also notable that *PA13* gene had higher expression than *PR5* in the resistant cultivar 'Sponta' as compared to the susceptible one 'Draga' with a maximum expression for *PR5* (3.5 and 1.2-fold) and *PA13* (8.2 and 2.7-fold) respectively, at 48 hours post infection. The obtained results suggested that *PR5* and *PA13* genes, positively regulate *P. infestans*—resistance in potato plants during disease progress, which can offer testable hypotheses that will need straight upcoming experiments to define how the *PR5* and *PA13* pathway signaling may be specified in potato defense system.

Key words: Potato, defense response, Phytophthora infestans, PR5, PA13, PCR (qPCR)

Late blight (LB), caused by the fungus *Phytophthora infestans* Mont.) de Bary, is a dangerous disease of potato (*Solanum tuberosum L.*) causing significant yield losses worldwide (Dong and Zhou 2022; Angmo *et al.,* 2023). The complicated systemic responses of potato to fight LB necessitate attention due to the potential for yield protection in field, and the immune responses during these interactions, however, are highly complex and often differ between genotypes (Salima 2015; Al-Daoude *et al.,* 2023). Therefore, it is highly challenging to control LB due to a poor understanding of the resistance mechanisms since plants resist this disease through initiation of diverse signaling pathways (Paluchowska *et al.,* 2022).

P. infestans begins potato infection through a biotrophic phase which needs living cells to get the nutrition through the haustoria (Kagda *et al.,* 2020), and they respond by activating different signaling pathways including osmotin-like proteins. However, many of their specific functions still remain unknown. The proteins members of the *PR5* and *PA13* family play vital functions in plant defense responses (Liu *et al.,* 1994). *PR5* family includes basic and acidic members according to their isoelectric points, although they show similar activity, however, *PR5* proteins had an antifungal activities in rice and orange plants after inoculation with to the two fungal pathogens *Rhizoctonia solani and Phytophthora infestans* (Bachmann *et al*., 1998).

In addition, osmotin-like proteins have *in vitro* antifungal activity against the *P. infestans* (Woloshuk *et al.,* 1991). Zhu *et al.* (1995) found high levels of the *PA13* osmotin-like protein in transgenic potato plants that infected with this pathogen at different stages of infection. However, relatively little is known about gene expression of *PR5* and *PA13* during potato interaction with *P. infestans.*

Quantitative PCR (qPCR) is an accurate technique for assessing plant gene expression levels under various biotic and abiotic stresses (Bates *et al.,* 2001). In previous work, the potato resistant cv. Sponta was exhibited a lower level of *LB* symptoms progress comparing with the susceptible cv. Draga (Salima 2015). We accordingly hypothesized that *PR5* and *PA13* genes could drive differentiated levels of resistance in these two cultivars, inoculated by the same isolate of *P. infestans*.

Hence, the objective of the present work was to evaluate the changes in induction of the two osmotin-Like *PR-5* and *PA13* proteins in the two selected potato cultivars with different resistance levels against *P. infestans* using PCR (qPCR) technique.

MATERIALS AND METHODS

Host Plant

The resistant potato (Sponta) and susceptible (Draga) cultivars from Netherlands grown commonly in Syria were used. A single tuber was planted in plastic pot filled with sterilized peatmoss with three replicates. Pots were placed under a growth chamber conditions at 20° (16h light/ 8h dark) and 85-90 % relative humidity.

Inoculation with P. infestans

The Syrian *P. infestans* virulent isolate PiSYR1 (Salima 2015) was used in the experiments. It was isolated from small infected potato leaves and grown in Petri dishes under disinfected tuber slices for 7 days at 20 ± 2 °C under 16 h/8h light/dark photoperiod. Once mycelium was growing on the surface of the potato slice, the mycelium was transferred to fresh rye agar (Caten and Jinks 1968). A conidial suspension with 5×10^4 spores/mL were sprayed onto the potato seedlings, whereas, control plants were sprayed with fungus-free water.

RNA isolation and cDNA synthesis

RNA was isolated from Potato leaves after 24, 48, 72 and 96 hours post infection (hpi) using Trizol Reagent (Macherey-Nagel, Germany). Controls were collected at the similar time points. cDNA was generated using the QuantiTect Reverse Transcription Kit (Qiagen) due to the manufacturer's instructions.

RT-qPCR analysis

PR5 and PA13 genes were assessed during the four time points by RT-qPCR assays using SYBR Green Master kit (Roche, USA). The used primers are shown in Table 1. 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 comparisons were carried out using Tukey's test. All experiments were repeated three times.

RESULTS AND DISCUSSION

In this investigation, two potato cultivars with different resistance levels towards *P. infestans* were used. Four time points 24, 48, 72 and 96 hpi, were chosen as being illustrative of biotrophy and switch to necrotrophy phases (Xiao *et al.,* 2019). *L*B symptoms were more severe on the susceptible cultivar as compared to the resistant one (data not shown).

To increase our understanding to these interactions, *PR5* and *PA13* expressions were evaluated at the four early time points of potato infection by *P. infestans*. Our results presented that at 24 hpi, the two genes were significantly upregulated after infection in both resistant and susceptible cultivars (Figs. 1 and 2), proposing that distinct defense responses were early activated, and the expressed gene patterns were recorded cooperative functions which occur after 24, 48, 72 and 96 hpi infecting by *P. infestans*. It is also remarkable that *PA13* gene had higher expression than *PR5* and that maximum expressions in resistant and susceptible were 48 hpi for *PR5* (3.5 and 1.2-fold) and *PA13* (8.2 and 2.7-fold), respectively (Figs. 1 and 2).

However, considering that the two used potato cultivars had high variant resistance levels to *LB* (Salima 2015), the both *PR5* and *PA13* genes were up-regulated 24 hpi in infected plants comparing to the non- infected ones, which might indicate that their functions are associated to the LB severity symptoms rather than to resistance as it obvious in the resistant cultivar than in the susceptible one (Figs. 1 and 2).

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

Gene	Accession No.	Sequence
EF1α	AT1G07920	TGGATTTGAGGGTGACAACA CCGTTCCAATACCACCAATC
PA13	P50701	CATGGGTTATCAATGCGCCA GTTGCTGAACTGGTCCAAGG
PR5	AT1G75040	GGGGCTACTGTTTCAAGCAA GCAGACTGTGGCGGTCTAAG

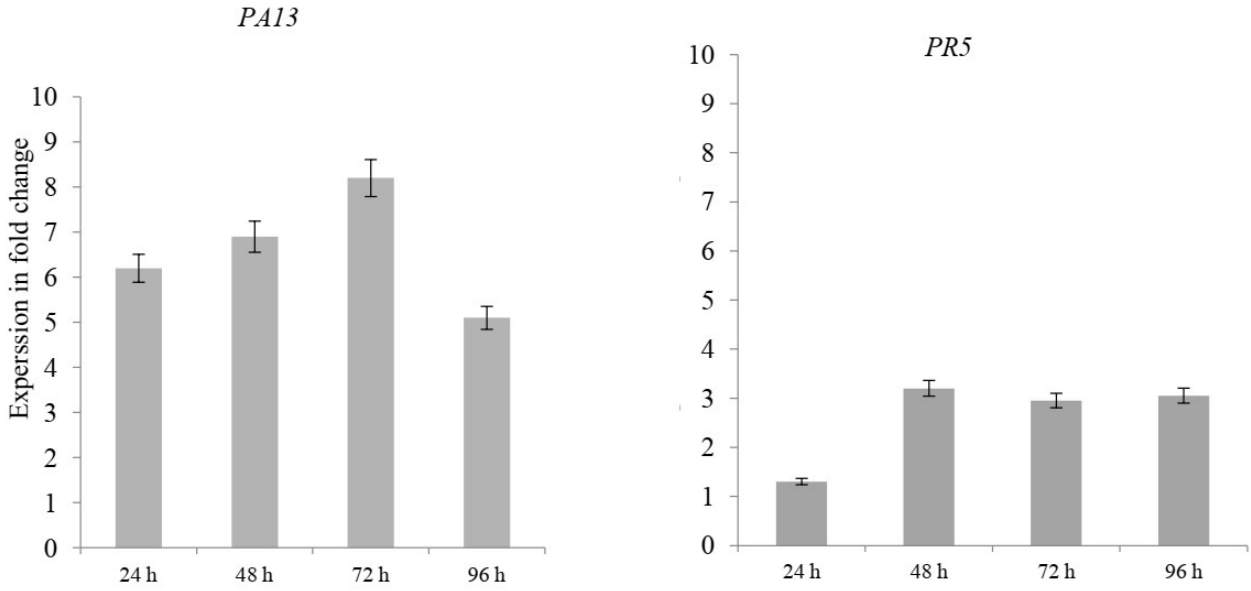

Figure 1. Relative expression profiles of *PR5* and *PA13* in resistant cv. Sponta cultivar during the time course of LB infections. Error bars are representative of the standard error (mean ± SD, n = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).

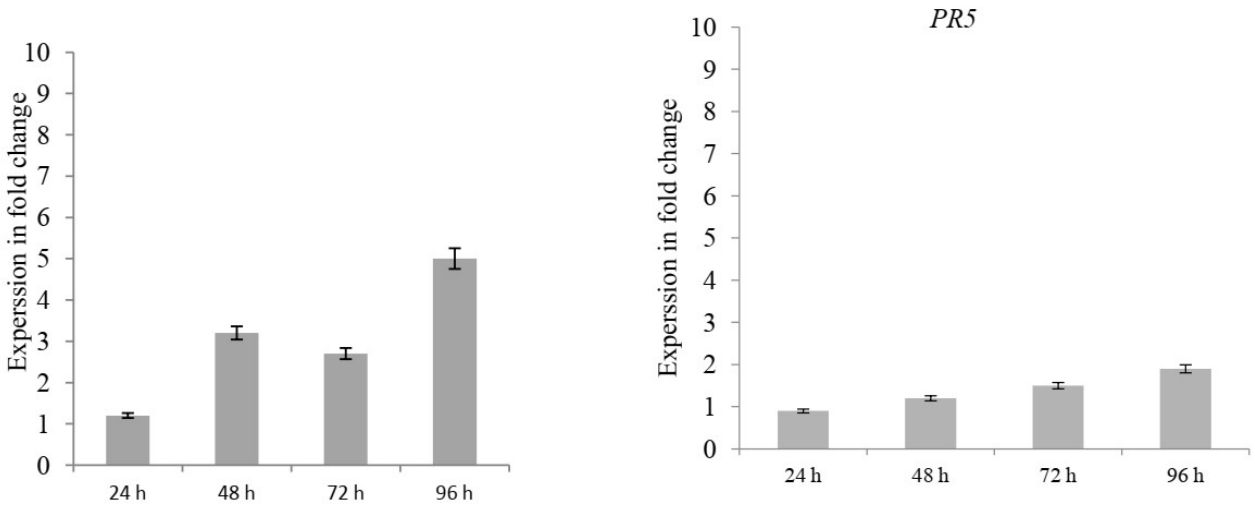

Figure 2. Relative expression profiles of *PR5* and *PA13* in susceptible cv. Draga cultivar during the time course of LB infections. Error bars are representative of the standard error (mean ± SD, n = 3). Data are normalized to Elongation factor 1α (EF-1α) gene expression level (to the calibrator, Control 0 h, taken as 0).

It has been reported that the osmotin-like proteins are cysteine-rich proteins that could increase protein stability under various conditions (Zhao et al., 2020). Therefore, they play a vital role in plant defense through signal transduction pathways for inhibiting the defensive cell wall barriers and rises its cytotoxic efficiency. This might elucidate the changes of the both genes *PR5* and *PA13* during *P. infestans* –potato interaction. Zhu *et al.* (1995) and Sanju *et al.* (2015) reported overexpression of osmotin like proteins in potato response under biotic and abiotic stresses. In addition, de Freitas *et al.* (2011) found that osmotin like protein purified from *Calotropis procera latex* had a strong antifungal activity against *Fusarium solani* and *Colletotrichum gloeosporioides*.

CONCLUSION

Our data showed that significant increases in *PR5* and *PA13* expressions were found upon potato challenged with the *P. infestans*, which can contribute to LB resistance, because these signaling responses induced together in each resistant and susceptible cultivars. It is also notable that *PA13* had higher expressions than *PR5* in the resistant cultivar as compared to the susceptible one with a maximum for *PR5* (3.5 and 1.2-fold) and *PA13* (8.2 and 2.7-fold) respectively, at 48 hpi. These results could be in line with the well-accepted concept that defense responses are very intense in potato resistant plants. Furthermore, we painted the fact that the osmotin-like *PR5* and *PA13* proteins signaling pathways might be activated in response to the same isolate of *P. infestans* in different potato cultivars.

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CONFLICTS OF INTEREST

The author declare that he has no potential conflicts of interest.

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