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ORIGINAL ARTICLE

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Increased Expression of Beta-Tubulin in Potato Plants Challenged with *Phytophthora infestans*

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Late blight, caused by *Phytophthora infestans* is an important foliar disease of potato worldwide. Relatively little is known about the mechanisms of interaction between potato and this fungal pathogen. In the present work, expression of beta-tubulin (TUB) gene was monitored in resistant and susceptible potato cultivars at four early points of infection using quantitative real-time PCR (qPCR). Data showed significant variation in the expression patterns of the *TUB* gene during potato- *P. infestans* interactions as compared to the non-inoculated controls. It also shows that *β*- tubulin gene has a higher expression and faster induction in the resistant cultivar (0.9-fold) as compared with the susceptible one (0.11-fold), 24 hours post inoculation (hpi). However, the *maximum* expressions were 1.85 –folds in the resistant, and 1.3 –folds in the susceptible one 48 and 72 hpi, respectively. Increased potato TUB expression could be due to plant cytoskeleton rearrangement in response to *P. infestans* up to 72 hpi, and the subsequent decrease in its expression at 96hpi could be due to plant cell disruption resulting from tissue damage during necrosis. The information obtained from this study highlights crucial remarks into the signaling pathway that accounts for *TUB* gene expression changes elicited during potato- *P. infestans* interactions, which can provide testable hypotheses that will need direct future tests to determine how *TUB* changes may be specified in the defense system.

Key words: Solanum tuberosum L., Phytophthora infestans, β- tubulin expression, real time PCR

The fungus *Phytophthora infestans* (Mont.) de Bary, is the causal agent of late blight disease of potato (*Solanum tuberosum L.*), that attack both potato foliage and tubers causing substantial crop losses (Kamoun *et al.,* 2015; Xue *et al.,* 2021). During infection, the pathogen shows an initial asymptomatic biotrophic stage of infection followed by a necrotrophic stage which is characterized by hyphal ramification, water soaking and necrosis (Grenville-Briggs *et al*., 2005). However, resistance of potato plants to this disease often depends on the activation of defense responses that are regulated through different plant signaling pathways genes (Gao and Bradeen 2016; Paluchowska *et al.,* 2022). However, many of their specific functions still remain unknown.

Tubulin genes which are highly conserved in structure and function from alpha, beta, and gamma-*tubulin* families were commonly used as reference genes for reverse-transcribed polymerase chain reaction (RT-PCR) (Ray and Johnson 2014; Jayaswal *et al.,* 2019). However, the expression pattern of plant tubulin has been studied in various species such as in populous (Oakley *et al.,* 2007), in potato (Koo *et al.,* 2009), in flax (Gavazzi *et al.,* 2017) and in cotton (Chen *et al.,* 2021). There is growing evidence, based primarily on microscopy studies, that microtubules and tubulins of the host cytoskeleton are rearranged in infected cells with fungal pathogens (Skalamera and Heath 1998; Kitaeva *et al.,* 2022). There is also evidence for increased expression of host tubulin gene during infection with a mycorrhizal fungus (Plágaro *et al.,* 2021). However, characterization of host plant tubulin is equally important along with tubulin from plant pathogenic fungi.

β-tubulin gene (TUB) has been characterized in potato plants and cloned full length cDNAs isolated from potato leaves using RT-PCR revealed that potato plants beta-tubulin were predicted to encode 451 amino acid long proteins with molecular masses of 60 kDa (Taylor *et al.,* 1994; Koo *et al.,* 2009). Different works showed that the expression of at least one tubulin gene can be altered due to certain external stimuli (Gasic 2022), and perhaps fungal infection could also affect TUB expression. However, it is unknown whether potato plant TUB gene expression could also be altered by *P. infestans* infection. Thus, the present work aimed at evaluating the changes in induction of in a TUB expression in two potato cultivars with different levels of resistance towards *P. infestans* pathogen using PCR (qPCR) approach.

MATERIALS AND METHODS

Plant material

The two Netherlands potato resistant (cv. Sponta) and susceptible (cv. Draga) cultivars (Salima 2015) grown widely in Syria were used in this work. A single seed tuber (~ 60 g) was planted at the center of plastic pots filled with sterilized peatmoss with five replicates. Pots were placed in a growth chamber set at 20° with a 16 h (light) and 8h (dark) cycle and 90 % relative humidity.

Inoculation with *P. infestans*

The virulent *P. infestans* isolate PiSYR1 (Salima 2015) was used in this work. Small pieces of infected potato leaves were placed in Petri dishes under disinfected tuber slices and incubated at 20 °C and 16 h/8h (light/dark) for 7 days. Mycelium was transferred to fresh rye agar after growing on the surface of the potato slice (Caten and Jinks 1968). Inoculation was performed by conidial suspension adjusted to 5×10^4 spores/mL sprayed with a hand sprayer onto the potato seedlings in each pot. The plants sprayed with pathogen-free water served as controls.

RNA manipulations

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

Quantitative RT-PCR analysis

TUB gene expression were analyzed at the four selected times with RT-qPCR assays using SYBR Green Master kit (Roche, USA). The primers sequences 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. The TUB expression level 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 significance 0.05 level. All the experiments were repeated at least twice in triplicate.

RESULTS AND DISCUSSION

In this investigation, four time points 24, 48, 72 and 96 h of *P. infestans* infection, were chosen as being representative of biotrophy and transition to necrotrophy phases. The choice of these time points was based on the stages in the infection cycle, as 24h to 48h the biotrophic stage, and 72h beginning the necrotrophic stage as shown in Table 2 (Xiao *et al.* 2019).

Further studies of potato interactions with *P. infestans* by measuring TUB gene expression at four early time points after pathogen challenge, the data demonstrated that at 24 hpi, the TUB expression 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. It is also noteworthy that *TUB* gene has a higher expression and faster induction in the resistant cultivar (0.9-fold) as compared with the susceptible one (0.11-fold), at 24 hours post inoculation (hpi). However, the *maximum* expressions were 1.85 –folds in the resistant, and 1.3 –folds in susceptible one after 48 and 72 hpi, respectively (Fig. 1).

This increase in TUB expression corresponded to the conversion from the biotrophic to necrotrophic stage of infection in potato leaves which was observed between 48 and 72 hpi. By 96 hpi, the pathogen appears to have heavily colonized the plant tissue. 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. Yen *et al.* (1988) and Gasic *et al.* (2019) reported that the differential expressions of tubulins due to a result of a post-transcriptional regulation of tubulin mRNA. This mechanism, known as tubulin autoregulation, is a negative feedback loop that involves indirect cotranslational regulation of the stability of mature spliced, but not unspliced, tubulin pre-mRNA by unpolymerized tubulin.

Our results are in good agreements with those of Kobayashi *et al.* (1994) who found new arrangements of TUB have been observed in flax cells responding to the flax rust infection, and with Swiecicka *et al*. (2009) increase in the expression of tubulin and microtubule-associated proteins during nematode infection. Also, on line with Haikonen *et al.* (2013), who stated involvement of microtubule protein in infection of plants with a potyvirus, *Potato virus A* (PVA).

Table 1. Properties and nucleotide sequences of primers used in this study.

Gene	Gene description	Accession No.	Sequence	Amplified fragment (bp)
EF1α	Elongation factor-1 Alpha	AT1G07920	5' TGTTGTCACCCTCAAATCCA 3' 5' GATTGGTGGTATTGGAACGG 3'	153
TUB	beta-tubulin protien	NM_001288449	5' TCTGCAACCATGAGTGGTGT 3' 5' ATGTTGCTCTCGGCTTCAGT 3'	150

Table 2. Sampling time-points according to the developmental stages of *P. infestans*

Sampling time point	Hours after inoculation (hpi)
Germination of conidia and beginning the biotrophic phase	24hpi
The biotrophic phase, germinating sporangia	48hpi
Invading hyphae penetrating plant tissue	72hpi
Hyphal growth and apparent of mycelial branching	96hpi
Water soaking (gray-green) visible, indicating the transition from biotrophy to necrotrophy	

The developmental stages of the fungus as described Xiao *et al.* (2019).

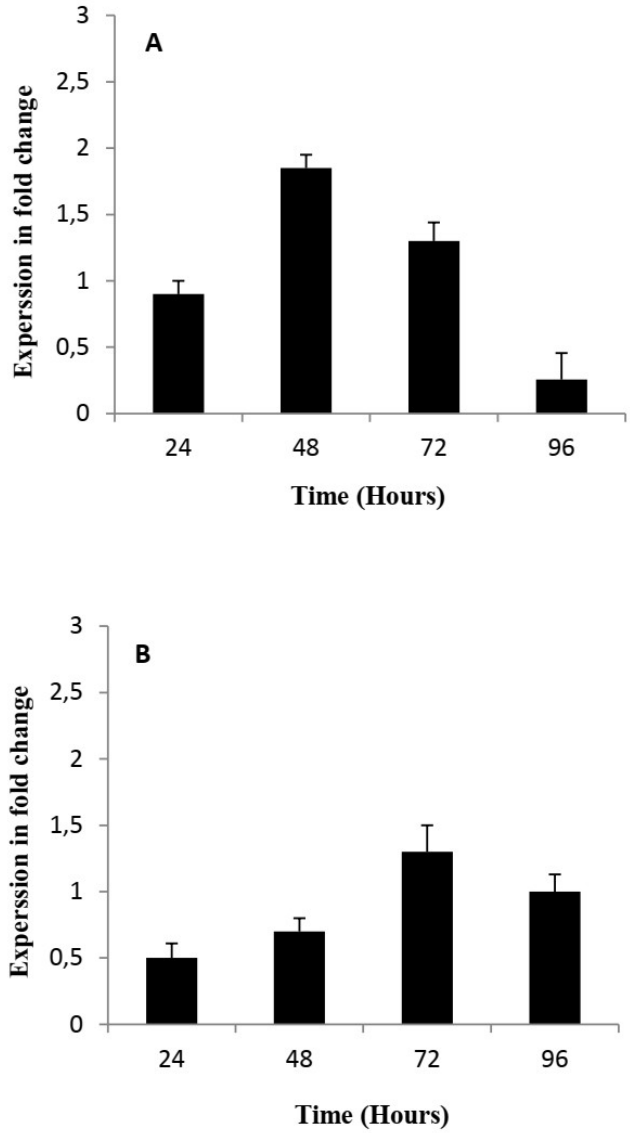

Figure 1. Relative expression profiles of TUB gene in the resistant cv. Spunta (A) and in the susceptible cv. Draga (B) during the time course following infections with late blight disease. Error bars are representative of the standard error (Mean ± SD, n = 3). Data are normalized to Elongation factor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

CONCLUSION

In this work, TUB gene showed different patterns of expression with an initial increase following potato infection with *P. infestans* in both resistant and susceptible cultivars indicating that this gene is activated directly after the pathogen attack. It is noteworthy that TUB has higher expression and faster induction in the resistant cultivar as compared with the susceptible one. However, increased TUB expression could be due to plant cytoskeleton rearrangement in response to biotrophic infection, and the subsequent decrease in its expression could be due to plant cell disruption resulting from tissue damage during necrosis. The data can provide testable hypotheses that will need further tests to determine how *TUB* changes may be specified in the defense system.

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CONFLICTS OF INTEREST

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

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