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Expression of pathogenesis-related (*PR*) genes in tomato against *Fusarium* wilt by challenge inoculation with *Streptomyces* NSP3

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Abstract In a previous study, *Streptomyces* strain NSP3 was selected from the strong activity *in vitro* antifungal activity against various plant pathogenic fungi, and *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) isolate *Fol*CK_117. In this study, *Streptomyces* NSP3 was shown to be a biotic elicitor of priming agents that initiated plant defense responses to *Fusarium* wilt disease in tomato cv. 'Bonny Best'. The effects of seed treatment or soil application with the NSP3 and combination of the two methods were compared against challenge inoculation with *Fol*CK_117. The recognition event leading to expression of some plant defense-related genes, including *PR-1a*, *Chi3*, *Chi9* and *CEVI-1*, was analyzed using real-time quantitative PCR (qPCR) and normalized to the *Actin* gene at 0, 3, 6, 12 and 24 h post-inoculation (hpi). The results implied that plants had strategy involved inducible defense reactions which are activated after elicitor applications within 24 hpi. Combination of two described methods above was more effective for induction and accumulation of these *PR* proteins than either alone. Gene expression of *PR-1a* was increased to maximum (73.1 fold) at 3 hpi. Gene expression of *Chi3* was remarkably increased at 24 hpi to 56.1 fold. Gene expression of *Chi9* and *CEVI-1* were likewise increased to maximum at 12 hpi (50.7 and 43.3 fold, respectively). These results suggested that *Streptomyces* NSP3 was a strong elicitor of plant defense responses. Understanding the bioactive component of defense induction may lead to a control strategy for *Fusarium* wilt disease in tomato.

Keywords: elicitor, mRNA expression, *PR* protein, biological control, plant defense mechanism

Introduction

Fusarium wilt of tomato, incited by soil-inhabiting fungus *F. oxysporum* f. sp. *lycopersici* W. C. Snyder & H. N. Hans (*Fol*), is a serious disease and now occurs worldwide. Plants afflicted with *Fusarium* wilt first develop yellowing of the lowest leaves that is often restricted to one side of the plant or a single shoot. The affected leaves wilt and die. Wilting progresses up the stem until

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the foliage is killed and the stem decays. It may cause between 10-90% loss varies in yield depending on the stage of the plant growth at which section occurs and the environmental conditions (Agrios, 1997; Singh, 2005). Since no chemicals are effective to control this disease, it has always been an interest for biological control strategies.

Plants defend themselves are demonstrated as natural resistance to pathogens based on the combined effects of natural barriers and inducible mechanisms that activated upon pathogen attack through a wide variety. There are two different phenomena types represent active plant defense response to phytopathogen attack that have been extensively studied, including induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Keen, 1990; McGovern, 2015). ISR, dependent of the phytohormones ethylene and jasmonate (jasmonic acid), is induced by the exposure of roots to specific strains of plant growth-promoting rhizobacteria and is not associated with the accumulation or transcripts of pathogenesis-related (*PR*) proteins. On the other hand, SAR, dependent of the phytohormone salicylate (salicylic acid), is induced by the exposure of root or foliar tissues to abiotic or biotic elicitors and associated with the accumulation of *PR* proteins (Vallad and Goodman, 2004). *PR* proteins are generally presented constitutively and only increased during severely infection with functions as antimicrobials degrading cell wall of pathogen and also act as messengers to signal pathogen attack, which serve as excellent molecular markers of plant defense (van Loon *et al*., 2006). The better understanding of plant signalling pathways is elicitors or activators that stimulate the synthesis of especially *PR* proteins, one of the most important and effective plant defense mechanisms against various pathogens. Elicitors induce similar defense responses in plants as induced by the pathogen infection and also in use successfully for biocontrol applications. However, both plant defend responses are also effective against a broad spectrum of virulent plant pathogens (van Loon and van Strien, 1999).

Streptomyces, the largest genus of Antinobaceria (Kämpfer, 2006), are known for their ability to produce biologically active secondary metabolites and particularly antibiotics which inhibit growth activities of many plant pathogenic, resulting in biocontrol of plant pathogenic especially fungi (Lechevalier, 1989). The most interesting property of *Streptomyces* is the ability to produce bioactive secondary metabolites such as antifungals, antivirals, antitumoral, anti-hypertensives, and mainly antibiotics and immunosuppressives (Khan, 2011), which are biologically active compounds. *Streptomyces* can also induce systemic and localized resistance to plant pathogens and improve plant growth and metabolism. According to Conrath *et al*. (2002) who suggested that the non-pathogenic bacteria prime the plant for accelerated and enhanced response

to a second stress stimulus, such as a pathogen, and *Streptomyces* species, resulted in the induction of the plant defense pathway (Conn *et al*., 2008; Lehr *et al*., 2008). Besides, other reports were represented *Streptomyces* elicited a systemic defense response as biotic elicitors to protect many plant diseases. Perhaps, the effective strain, *Streptomyces* NSP3, might be showed antifungal activity to control pathogenic fungi and based on this aim, the examining expression of plant defense related genes mRNA in response to *Streptomyces* NSP3 and challenged inoculation of *F. oxysporum* f. sp. *lycopersici* to tomato plants were demonstrated.

Materials and methods

Experimental strains

The pathogenic fungus *F. oxysporum* f. sp. *lycopersici* (*Fol*) isolate *Fol*CK_117, causal agent of *Fusarium* wilt disease, was isolated from a naturally infected tomato plant and used for artificial inoculations. The strain was previously categorized as the most virulent isolate to tomato seedlings cv. 'Bonny Best'. A microconidia suspension was prepared by pouring 10 ml of sterile distilled water into PDA plates containing a 7-day-old *Fol* culture grown at room temperature (RT) and adjusted to a concentration to 1×10^7 conidia/ml.

The potent antifungal strain of *Streptomyces* isolate NSP3; which exhibited strong antagonism against *Fol*CK_117 under *in vitro* conditions and was isolated from a natural soil sample from Suthep-Pui National Park, Chiang Mai, Thailand, was suspended in 10 ml of sterile distilled water and the population was adjusted to 9×10^8 colony-forming unit (cfu)/ml.

The *Fol*CK_117 and NSP3 were presented to the culture collection of the laboratory of the Department of Entomology and Plant Pathology of Chiang Mai University, Thailand.

Induction of plant defense related gene expression

Plant materials

Seeds of tomato (*Solanum lycopersicum* L.; formerly *Lycopersicon esculentum* Mill.) cv. 'Bonny Best', kindly provided by Hortigenetics Research (S.E. Asia) Limited, were surface-sterilized with 1% sodium hypochlorite (NaOCl) before being separately soaked for 12 h, and then air dried overnight. Treated seeds were sowed in 72-cell plastic seedling trays (28 × 54 cm) containing a peat moss-based growing medium (Klasmann®) mixed with coconut coir dust (1:1 ratio) and maintained in a greenhouse at 30 ± 2 ℃ with a

12 h photoperiod. The experiments were performed with 30-day-old tomato plants with four expanded leaves.

Streptomyces NSP3 application

Detailed methods of *Streptomyces* NSP3 application are described below:

Seed treatment (ST): Ten gram of surface-sterilized tomato seeds were soaked in 10 ml of *Streptomyces* NSP3 suspension for 12 h, and then air dried overnight before sowing and maintained in a greenhouse according to the methods described previously. Seeds soaked in sterile distilled water served as the control.

Soil application (SA): Ten ml of *Streptomyces* NSP3 suspension, containing 9×10^8 cfu/ml, was drenched into each pot at 5 d before *Fol* inoculation. Sterile distilled water served as the control.

Fol inoculation (*Fol*-inoc): Five days after soil application, each seedling was challenge inoculated with 10 ml of *Fol*CK_117 containing 1×10^7 conidia/ml as a soil-drench. Seedlings without prior treatment with *Fol* served as the control.

Experimental design

The experimental treatments included (T_1) ST + *Fol*-inoc; (T_2) SA + *Fol*-inoc; (T_3) combination of ST + SA + *Fol*-inoc; (C_1) ST, (C_2) SA, (C_3) combination of ST + SA, (C_4) *Fol*-inoc, (C_5) wounding root and (C_6) healthy seedlings. Three replications were arranged in a RCBD, with a plant per replicate. The plants were maintained in the greenhouse at 30 ± 2 ℃ with a 12 h photoperiod until sampling for total mRNA extraction.

Sampling tomato leaves

The 3rd leaf blade (fully expanded) of treated-tomato plant was carefully cut without causing damage to tissue at different time intervals: 0, 3, 6, 12 and 24 h post-inoculation (hpi) sampling one plant at time, and then immediately storing the sample in an ultralow freezer (-80 ℃) until mRNA extraction.

RNA isolation and double-stranded cDNA synthesis

Total RNA was isolated from 0.5 g of tomato leaf samples. Plant tissue was ground in liquid nitrogen, and followed by addition of 1.0 ml of TRIzol® Reagent (Invitrogen™, USA) according to the manufacturer's instructions. Then, double-stranded cDNA was synthesized from 1 ng of total RNA using a

ReverTra Ace® qPCR RT Master Mix (TOYOBO, Japan) according to the manufacturer's instructions. Extracted RNA concentration was determined by measuring absorbance at 260 nm and 280 nm using a NanoDrop™ Spectrophotomer (A260 × dilution × 40 = μg RNA/ml). A260/280 and A260/230 values greater than 1.8 are typically suitable for analysis.

Double-stranded cDNA was then synthesized from 1 ng of total RNA using a ReverTra Ace® qPCR RT Master Mix (TOYOBO, Japan) according to the manufacturer's instructions.

Real-time quantitative PCR (qPCR) analysis of gene expression

Real-time qPCR assays were performed in a C1000™ Thermal Cycler with a CFX96™ Real-Time System (Bio-Rad, USA). The following primer sets were used: *PR-1a* gene primers (*Le*PR-1a-F, 5´-TCT TGT GAG GCC CAA AAT TC-3´; *Le*PR-1a-R, 5´TAG TCT GGC CTC TCG GAC A-3´); acidic chitinase *Chi3* gene primers (*Le*Chi3-F, 5´-TGC AGG AAC ATT CAC TGG AG-3´; *Le*Chi3-R, 5´-TAA CGT TGT GGC ATG ATG GT-3´); basic chitinase *Chi9* gene primers (*Le*Chi9-F, forward, 5´-GAA ATT GCT GCT TTC CTT GC-3´; *Le*Chi9-R, 5´-TCC AAT GGC TCT TCC ACA T-3´); and peroxidase *CEVI-1*gene primers (*CEVI-1*-F, forward, 5´-GCA ACA AGC CCA AAG TAC CG-3´; *CEVI-1*-R, 5´-GAA ACA ACG CCA GGA CAC AC-3´). The *Actin* gene primers (forward, 5´-AGG CAC ACA GGT GTT ATG GT-3´; reverse, 5´-AGC AAC TCG AAG CTC ATT GT-3´) were used as the control to normalize the quantity of each PCR fragment.

Real-time qPCR reactions were carried out with 100 ng of cDNA, 400 μM of each primer, 10 μl of 2x SYBR green master mix (SensiFAST™ SYBR® No-Rox Kit, Bioline™, UK) and nuclease-free water in a final volume of 20 μl. For the negative control, cDNA was replaced by nuclease-free water. The program used for the real-time qPCR reaction was 95℃ for 2 min, 50 cycles of denaturation for 5 sec at 95℃, annealing for 30 sec at 58℃ and extension for 20 sec at 72℃; at the end of which the fluorescence was measured. The *Actin* (a housekeeping gene) was used as an internal standard to normalize each cDNA sample and calculate the relative expression values using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

The analysis of variance (ANOVA) was computed and treatment means were compared using Fisher's Least Significant Difference (LSD) at $P = 0.05$.

Results

Treatment effects on the expression of defense related genes in tomato 0, 3, 6, 12 and 24 h post-*Fol* inoculation (hpi) were determined and validated using the *Actin* gene as the internal control. Tomato seedlings responded to the *Streptomyces* NSP3 treatments. The levels of transcripts encoding the *PR* proteins observed, *PR-1a*, *Chi3*, *Chi9* and *CEVI-1* genes, they increased to the maximum following treatment with *Streptomyces* NSP3 after challenge-inoculation with *Fol* (T_1 – T_3) within 24 hpi. These *PR* proteins were also provided by the wounding treatment (C_5), but they expressed lower level. In contrast, the accumulation of these genes was always lower in other controls (C_1 – C_4), comparable to healthy seedlings (C_6). Statistical analysis showed significant differences in the relationship between the treatments and time-sampling in observed *PR* proteins (Figure 1).

PR-1a

The maximum accumulation of *PR-1a* was detected at 3 hpi in combination of seed treatment and soil application before challenge inoculation with *Fol* (T_3) (73.1 fold) before found to be stable at 6 -12 hpi (average 51.8 fold), and then decreased to average 34.9 fold until 24 hpi. Soil application before challenge inoculation with *Fol* (T_2) showed a maximum relative *PR-1a* expression of 14.2 fold (at 3 hpi), then found to be stable until 24 hpi (average 12.5 fold). Seed treatment before challenge inoculation with *Fol* (T_1) showed a stable *PR-1a* expression from 3 – 6 hpi (average 10.2 fold), before increased to maximum at 12 hpi (13.4 fold) and slightly decreased at 24 hpi (12.5 fold). Although the accumulation of *PR-1a* in treatment of wounding roots (C_5) was increased, the expression was reaching a maximum only 4.9 fold at 24 hpi. However, significantly lower *PR-1a* expression levels were found in four different control treatments in plants inoculated with *Streptomyces* NSP3 or *Fol* alone (C_1 – C_4), comparable to healthy seedlings (C_6) (Fig 1A).

Chi3

The maximum accumulation of *Chi3* was also detected in the combination of seed treatment and soil application before challenge inoculation with *Fol* (T_3); the relative *PR-1a* expression was found to be relatively constant at 3 – 6 hpi (average 19.3 fold), before increased to 41.9 fold (12 hpi) and reached a maximum at 24 hpi (56.1 fold). Soil application before challenge inoculation with *Fol* (T_2) showed a stable of *Chi3* expression during 3 – 12 hpi (average 12.3 fold), then increased to a maximum 19.0 fold at 24 hpi. The

expression of *Chi3* gene in seed treatment before challenge inoculation with *Fol* (T_1) was found to be stable until 6 h after treatment (average 8.2 fold), then increased to 16.0 fold at 12 hpi before increasing to be a maximum at 24 hpi (18.6 fold). Although the accumulation of *Chi3* in treatment of wounding roots (C_5) was increased, the expression was reaching a maximum only 5.2 fold at 24 hpi. However, significantly lower *Chi3* expression levels were found in four different control treatments in plants inoculated with *Streptomyces* NSP3 or *Fol* alone ($C_1 – C_4$), comparable to healthy seedlings (C_6) (Fig. 1B).

Chi9

The maximum accumulation of *Chi9* was detected in the combination of seed treatment and soil application before challenge inoculation with *Fol* (T_3); the *Chi9* gene expression was successively increased at 3 and 6 hpi (23.7 and 31.3 fold, respectively) before reaching a maximum at 12 hpi (50.7 fold), the decreased slightly at 24 hpi (42.8 fold). The accumulation of *Chi9* in soil application before challenge inoculation with *Fol* (T_2) showed a stable expression from 3 – 6 hpi (average 14.9 fold), before increasing to a maximum until 24 hpi (average 18.9 fold). The *Chi9* gene expression of seed treatment before challenge inoculation with *Fol* (T_1) was found to be relatively constant at 3 – 6 hpi (average 10.3 fold), then reaching a maximum at 12 hpi (13.29 fold) before decreased slightly at 24 hpi (12.0 fold). In the same way, the accumulation of *Chi9* in treatment of wounding roots (C_5) was increased to maximum only 4.7 fold at 24 hpi. However, significantly lower *Chi*9 expression levels were found in four different control treatments in plants inoculated with *Streptomyces* NSP3 or *Fol* alone ($C_1 – C_4$), comparable to healthy seedlings (C_6) (Fig. 1C).

CEVI-1

The maximum accumulation of *CEVI-1* was also detected in the combination of seed treatment and soil application before challenge inoculation with *Fol* (T_3), which found to be stable at 3 – 6 hpi (average 25.6 fold), then increased to maximum (43.3 fold) at 12 hpi before decreasing thereafter (37.1 fold at 24 hpi). The *CEVI-1* gene expression in soil application before challenge inoculation with *Fol* (T_2) showed 16.7 fold upregulation at 3 hpi, then relatively unchanging *CEVI-1* gene expression until 12 hpi (average 18.9 fold), then increased to a the maximum of 26.0 fold at 24 hpi. Seed treatment before challenge inoculation with *Fol* (T_1) showed *CEVI-1* gene expression stable until 6 hpi (average 14.9 fold), then gene expression increased to a maximum at 12

hpi (27.7 fold) before decreasing to 20.5 fold at 24 hpi. There were similarly to other *PR* gene expression in treatment of wounding roots (C_5); although the accumulation of *CEVI-1* in was increased, the expression was reaching a maximum only 6.8 fold at 24 hpi. However, significantly lower *CEVI-1* expression levels were found in four different control treatments in plants inoculated with *Streptomyces* NSP3 or *Fol* alone (C_1 – C_4), comparable to healthy seedlings (C_6) (Fig. 1D).

Discussion

Plants have endogenous defense mechanisms or latent defensive systems that are induced upon response to attack by insects and pathogens, were activated. Induced resistance in plants refers to a state of heightened defensive capacity created by a prior stimulus. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy (Kuc, 1995; McGovern, 2015). Systemic acquired resistance (SAR) is induced systemically throughout the plant in response to a pathogen infected plant, conferring a long-lasting and broad-spectrum of pathogen resistance against (Durrant and Dong, 2004). The development of SAR is associated with the induction of *PR* proteins, which are mostly of low molecular weight, preferentially extracted at low pH, resistant to proteolysis, and localized predominantly in the intercellular spaces of leaves (Taheri and Tarighi, 2012). The hypothesis was that the non-pathogenic rhizobacteria have been shown to enhance disease resistance by stimulating the systemic defense pathways. Besides, many reports have demonstrated the efficiencies of *Streptomyces* spp. in controlling plant diseases caused by pathogenic fungi. However, little is known about the ability of *Streptomyces* to trigger SAR in tomato against *Fol.*

The aim of the present study was to investigate the accumulation of transcript encoding some induced *PR* proteins in tomato plants response towards *Streptomyces* NSP3 challenge inoculated with or without *F. oxysporum* f. sp. *lycopersici Fol*CK_117 causing *Fusarium* wilt. In this study, four *PR* proteins, including *PR-1a, Chi3, Chi9* and *CEVI-1*, were investigated the upregulation by real-time qPCR. The *PR-1* genes have been frequently used as marker genes for SAR in many plant species as previously described by Mitsuhara *et al*. (2008). This study exhibited the strongly increased of *PR-1a* expression immediately after *Fol*-inoculation. This result was in accordance with those already published by Conn *et al*. (2008) who reported that inoculation *Arabidopsis thaliana* seeds with *Streptomyces* sp. strain EN27 and EN28 challenged inoculation with *F. oxysporum* resulted in activation of the

SAR pathway 4 day after pathogen inoculation via induction of the *PR*-1 gene, which increased expression 850 and 47.4 fold respectively, compared with the uninoculated control. Related to further reports of Berrocal-Lobo and Molina (2004) demonstrated the induction of the *PR-1* gene in *Arabidopsis thaliana* transcript 4 days after infection with *F. oxysporum*, lead to activation of the SAR pathways. However, the function of *PR-1* gene is still unclear (van Loon *et al.*, 2006) as described by Silvar *et al*. (2008) showed that *PR-1* and sesquiterpene cyclase genes were up-regulated in infected stems of the pepper markedly by 24 hpi especially in resistant cultivars and suggested that the precise biological role of the *PR-1* proteins remains unknown, perhaps they appear to be important role in restricting pathogen colonization in resistant cultivar and related to biosynthesis of defense-related sesquiterpene phytoalexins.

Additionally, the *PR-3* (*Chi3*) and *PR*-4 (*Chi9*) genes are comprised of chitinases, which well-known that are constitutively expressed at low levels in plants, but are dramatically induced when plants respond to infection by fungal, bacterial, or viral pathogens (van Loon and van Strien, 1999). Chitinases have the potential to hydrolyse chitin, which is a major component of fungal cell walls. Chitin and glucan oligomers released during degradation of fungal cell walls act as elicitors that elicit various defence mechanisms in the plants (Frindlender *et al*., 1993). In this study, *Chi3* mRNA was maximum expressed at 3 hpi, while *Chi9* was investigated during 6 – 12 h. It was previously shown by Taheri and Tarighi (2012) that observed resistance in tomato plants against *Rhizoctonia solani* between in 'Sunny 6066' (cv. resistant) and 'Rio Grande' (cv. susceptible). The results revealed the highest elevated levels of chitinase gene (*LOC544149*) expression in both cultivars at 24 hpi. However, higher level of *LOC544149* gene expression and earlier upregulation were observed in 'Sunny' compared to 'Rio Grande' cultivar at 12 hpi. Similarly in tomato, investigations of Chen *et al*. (2009) revealed the involvement of chitinase in defense of the plants against *Botrytis cinerea*. Also, Cachinero *et al*. (2002) demonstrated that inoculation of chickpea with protective strains of *F. oxysporum* resulted in an increased accumulation of chitinase, *β*-1,3-glucanases and peroxidase activities in roots correlated with plant resistance to *Fusarium wilt*. Similarly, Ito *et al*. (2005) showed an enhanced expression of acidic chitinase gene (*Chi3*) in tomato plants inoculated with strains belonging to *formae speciales* non-pathogenic on tomato. In The result indicated that *PR-3* and *PR*-4 encoding chitinase gene may play an important role in host plant defense.

The *PR-9* or peroxidases (*CEVI-1*) are key enzymes in the cell wall building process, and it has been suggested that extracellular or wall-bound

peroxidases would enhance resistance in various plant species against phytopathogens by the construction of a cell wall barrier that may hamper pathogen ingress and spread in plant cells. They often increase in response to stress and one of the principal roles of peroxidase appears to be cellular protection from oxidative reactions imposed by various stresses. Plant peroxidase produces antimicrobial phenolic compounds in the chemical defense systems against plant pathogens (Siegel, 1993; Taheri and Tarighi, 2012). In this study, *Chi9* mRNA expression was increased to maximum after 3 hpi. Resembly, Taheri and Tarighi (2012) investigated the expression level of peroxidase gene (*CEVI-1*) the time point of upregulation in tomato plants against *Rhizoctonia solani* between in 'Sunny 6066' (cv. resistant) and 'Rio Grande' (cv. susceptible). The results showed that peroxidase gene increased at 12 hpi and reached its maximum at 48 hpi in the 'Rio Grande' plants. However, an increase in peroxidase gene expression at 6 hpi and peaking at 18 hpi was observed in cv. 'Sunny'. Additionally, Xue *et al*. (1998) reported that resistance of bean plants, against the root rot pathogen *Rhizoctonia solani* and the anthracnose pathogen *Colletotrichum lindemuthianum*, were elicited after inoculated with nonpathogenic *Rhizoctonia* species by released peroxidases, *β*-1,3-glucanases and chitinases at 48 h after challenged inoculation. Recently, Boominathan and Sivakumaar (2013) found that *Bacillus megaterium* strain AUM72, a plant growth promoting rhizobacteria (PGPR), had ability to control rhizome rot in turmeric (*Curcuma longa* L) by increasing activities of peroxidase, *β*-1,3-glucanase, chitinase, phenylalanine ammonia lyase and polyphenol oxidase.

In the present experiment revealed that the treatments of *Streptomyces* NSP3 challenge inoculated with *F. oxysporum* f. sp. *lycopersici Fol*CK_117 was greatly exhibited high levels of studied *PR* proteins either seed treatment or soil application. Moreover, the combination of these two methods elicited the highest of interested *PR* genes expression. Induction resistance to *Fol* mediated by *Streptomyces* NSP3 occurred primarily by the SAR pathway. The studied *PR* genes in the plant challenged inoculate with *Fol*CK_117 and NSP3 were responded at the higher level than plants challenged inoculate with NSP3 alone or *Fol*-inoculation plants. Furthermore, no upreguration of *PR* proteins were found in healthy plants. Related to Ramamoorthy *et al*. (2002) the protective strain *Pseudomonas fluorescens* isolate Pf1 was found to protect tomato plants by exhibited *PR* proteins and phenolics, including PAL, peroxidase, PPO, chitinase and TLP. The accumulation was higher responded after challenged inoculate with the pathogen *Fol*. Results of this study indicated that the selected *Streptomyces* NSP3 severed as excellent trigger in defense mechanism against *Fusarium* wilt disease in tomato plants as described by Suwan *et al*.

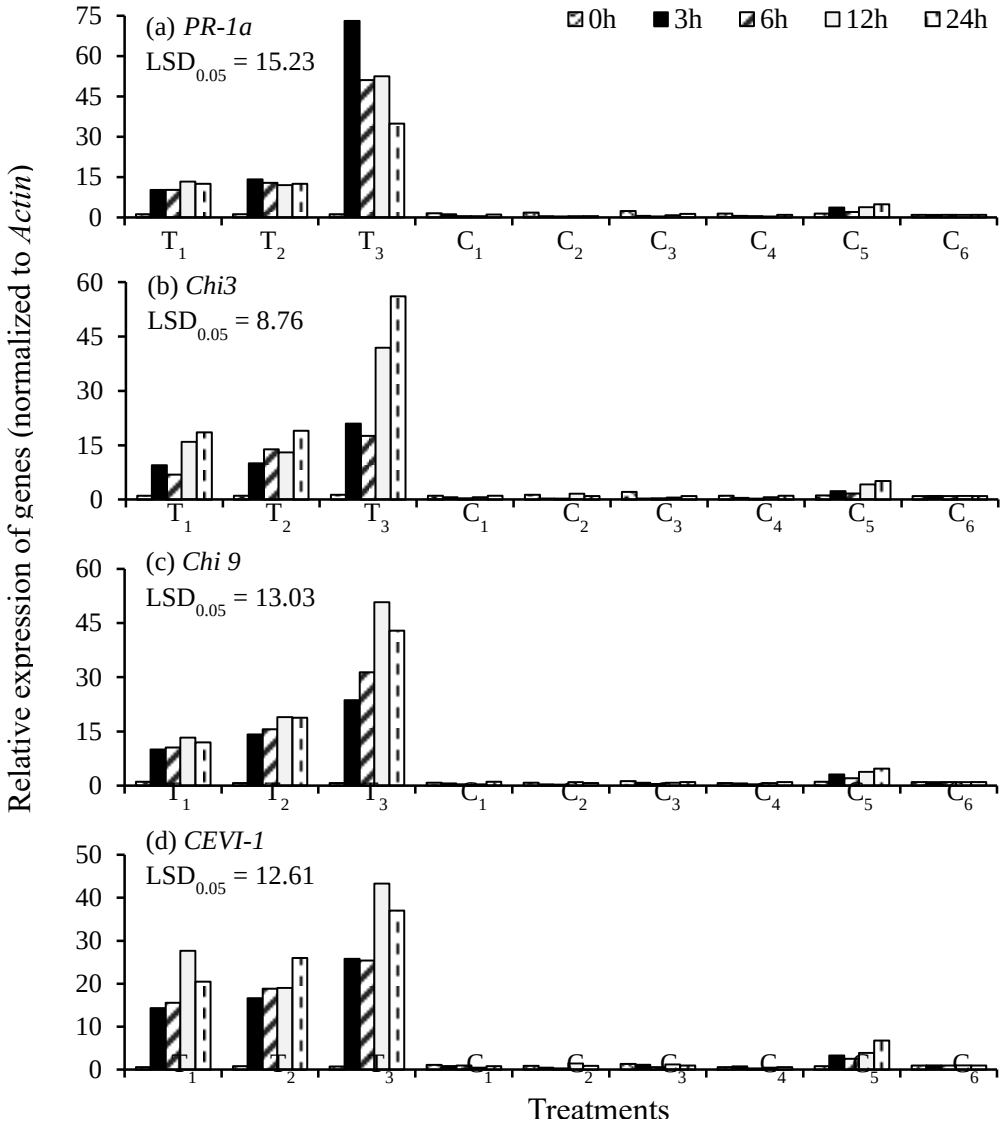

Figure 1 Real-time qPCR analysis of *PR* gene expression in tomato leaves in response to application of *Streptomyces* NSP3 with or without challenge inoculation with *Fusarium oxysporum* f. sp. *lycopersici Fol*CK_117 causing *Fusarium* wilt. Control plants were treated with sterile distilled water. Bars represent relative expression levels of transcripts assessed 0, 3, 6, 12 and 24 h post-*Fol* inoculation. The mean of three replications were analysed by 2-factor ANOVA. Significant treatment effects were determined by LSD at $P \leq 0.05$ (treatment × time-sampling interaction), while ns = non significantly different. Experimental treatments included (T_1) Seed Treatment + *Fol*-inoc, (T_2) Soil Application + *Fol*-inoc, (T_3) combination of Seed Treatment + Soil Application + *Fol*-inoc, (C_1) ST, (C_2) SA, (C_3) combination of ST + SA, (C_4) *Fol*-inoc, (C_5) wounding root and (C_6) healthy seedlings.

(2012) that the selected NF-*Streptomyces* NSP-167 (*Streptomyces* NSP3 in this study) might be activated the plant defense genes in the absence of a pathogen inoculation, suggesting that are detected as "minor" pathogens which do not trigger a full resistance response on their own, because they do not show pathogenic determinants, and this may result in more effective priming of the defense response against *Colletotrichum gloeosporioides* isolate TPCMCg60 causing chili anthracnose. In the result of this chapter indicated that whole interested *PR* proteins may play an important role in host plant defense. These results suggest that induction of *PR* proteins involved in SAR pathway might have contributed to restriction of invasion of *F. oxysporum* f. sp. *lycopersici* in tomato plants.

It is concluded that tomato plants have acquired defense mechanisms to counteract pathogens, and the strategy involves inducible defense reactions that are activated after elicitor application. Applications of *Streptomyces* NSP3, including seed treatment, soil application and combination of these two methods were investigated for the activation of four plant defense related genes. The average accumulation of *PR-1a* was found at the highest level, followed by *Chi3* encoding acidic chitinase, *Chi9* encoding basic chitinase and *CEVI-1* encoding peroxidase. The results implied that these *PR* proteins appeared earlier and accumulated to higher levels (within 24 h) when plants were treated with *Streptomyces* NSP3 and challenge inoculated with *F. oxysporum* f. sp. *lycopersici Fol*CK_117 compared to non-treated plants or those treated with *Streptomyces* NSP3 alone, or the pathogen *Fol* alone. Combination of seed treatment and soil application is more effective for accumulation of these *PR* proteins than either method alone. *Streptomyces* NSP3 was shown to be a good elicitor of plant defense responses. Their bioactive component may represent an alternative resource for the biocontrol of plant diseases and could provide an interesting lead for further development of novel fungicides.

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