

Asian Journal of Food and Agro-Industry

ISSN 1906-3040

Available online at www.ajofai.info

Induced systemic resistance of biocontrol fungus, *Trichoderma* spp. against bacterial and gray leaf spot in tomatoes

Weerasak Saksirirat^{1,2,3*}, Punyisa Chareerak¹ and Wandee Bunyatrachata⁴

¹Plant Pathology Section, Department of Plant Science and Agricultural Resources, Faculty of Agriculture,

²Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University, Khon Kaen 40002, Thailand.

³Center of Agricultural Biotechnology, Khon Kaen University, Science and Technology Postgraduate Education and Research Development Office, Thailand.

⁴Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

*Author to whom correspondence should be addressed, email: weerasak@kku.ac.th

This article was originally presented at the International Symposium "GoOrganic2009", Bangkok, Thailand, August 2009.

Abstract

The efficiency of *Trichoderma* spp. in inducing acquired systemic disease resistance in tomato was conducted. Systemic induce resistant reaction was evaluated on chitinolytic and β -1,3-glucanolytic activities produced by tomato plant (Sida cultivar) including disease severity of bacterial and gray leaf spot. Fifteen isolates of *Trichoderma* spp., T1, T9, T10, T13, T14, T17, T18, T19, T20, T24, T25, T30, T35, 90 and 103 were inoculated in soil of tomato potted plants. Tomato leaves were collected on 0, 5, 8, 11 and 14 days of interval after inoculation. Crude enzymes were extracted from leaf samples and determined for chitinase and β -1,3-glucanase activities. High chitinase activity was detected from *Trichoderma* isolates in descending order, T1, T9, T13, T18 and T18. For β -1,3-glucanase activities, The isolates T9, T13, T14 and T17 induced to tomato plant in this enzyme activity ranking from high to low, compared to uninoculated plants. Foliar disease resistant induction of *Trichoderma* spp. was tested under screened house condition. The *Trichoderma* isolate T9 (*T. harzianum*), T13 (*T. asperellum*), T17 (*T. asperellum*) and T18 (*T. asperellum*) were evaluated by cultivation in sterilized sorghum grains and inoculated in tomato pot plants. The test plants were inoculated with

bacterial suspension of *Xanthomonas campestris* pv. *vesicatoria* (XCV). The result showed that *T. harzianum* (T9) induced resistance with the best reduction spot numbers (69.32 %). Other isolates T13, T17 and T18 showed reduction in bacterial spot numbers of 34.66%, 37.41% and 44.77%, respectively. On *Stemphylium* gray leaf spot, these 4 isolates of *Trichoderma* spp. reduced number of spots in parenthesis as followed, T18 (19.23%), T9 (7.52%), T13 (3.8%) and T17 (3.69%). *Trichoderma* spp. are shown to have potential in inducing resistant of bacterial spot and gray leaf spot of tomato which varies among the range of isolates evaluated.

Keywords: β -1,3-glucanase; chitinase; induce resistance; *Trichoderma*; tomato

Introduction

Trichoderma spp. is a well-know antagonistic fungus against various plant pathogens. Biological control mechanisms of this fungus are competition, antibiosis and parasitism. Besides, the fungus can colonize plant roots leading to induce growth and nutrient adsorption (Harman et al., 2004). Certain isolates *Trichoderma* spp. invade in the vascular tissue or epidermal cells of plant root, giving rise to accumulation of signal molecules, salicylic acid (SA) and jasmonic acid (JA). These compounds induce the PR gene function coding pathogenesis-related proteins (PR protein), which were expressed by plant to defense pathogen infection (Hurtado, 2004; Wasternack et al., 2006). The PR protein was classified into 14 families, among them the degrading enzymes, chitinase and β -1,3-glucanase that capable to lyses the fungal plant pathogen cell wall. Moreover, *Trichoderma* spp. was used not only for control plant disease, but also stimulated nutritional adsorption (Harman et al., 2004). Our previous reports revealed species diversity of *Trichoderma* spp. in tomato seed production fields and its effectiveness against Fusarium wilt (Saksirirat et al., 2005; Saepaisan, 2006). However, the induced systemic resistant property of those isolates is still not carried out. Therefore, the objective of this study are to investigate chitinase and β -1,3-glucanase activity produced in tomato leaves after challenge by *Trichoderma* isolates and to evaluate the effectiveness of *Trichoderma* spp. to induce systemic resistance against bacterial and gray leaf spot in tomato.

Materials and Methods

Fungal materials

Fifteen isolates of antagonistic fungus, *Trichoderma* spp. (T1, T9, T10, T13, T14, T17, T18, T19, T20, T24, T25, T30, T35, 90 and 103) were obtained from Plant Pathology Section, Faculty of Agriculture, Khon Kaen University, and identified in to species by Saepaisan (2006). Folia pathogens of tomato, *Xanthomonas campestris* pv. *vesicatoria* (XCV), a causal agent of bacterial spot and *Stemphylium solani*, a causal agent of gray leaf spot were derived from our laboratory.

Determination of chitinase and β -1,3-glucanase activities in tomato leaf

Crude enzyme sample was prepared using 0.5 g of tomato leaves (Sida cultivar) harvested at 0, 5, 8, 11 and 14 days of interval after inoculation of all isolates of *Trichoderma* spp. in to soil, homogenized with 1.2 mL of 0.1 M acetate buffer pH 5.0, centrifuged (14,000 rpm), transferred supernatant and kept at -20 °C until used. Protein content in enzyme solution was determined my method of Bradford (1976) using bovine serum albumin as standard protein. Chitinase activity was evaluated by incubation 1% colloidal protein and enzyme solution in 0.1 M acetate buffer pH 5.0 at 37 °C for 1 hour. Reaction mixture was stopped by boiling aliquot

for 15 min. The amount of reducing sugar released in reaction mixture was calculated by analysis method of Somogyi (1952) using N-acetylglucosamine (GlcNac) as standard. Chitinase activity was expressed as GlcNac equivalent released in reaction mixture per milligram protein per hour. For β -1,3-glucanase activity, enzyme solution was incubated with 0.1 % laminarin in 0.1 M acetate buffer pH 5.0 at 37 °C for 1 hour. Other condition was used as the same manner as chitinase activity evaluation. The enzyme activity was expressed as reducing sugar

Results and Discussion

Chitinase and β -1,3-glucanase activities in tomato induced by Trichoderma spp.

All 14 isolates of *Trichoderma* spp. Induced significantly ($P<0.05$) chitinolytic activities in tomato leaves, especially on 14 days after inoculation, compared to uninoculated tomato plants. On 5, 8 and 11 days after with *Trichoderma* spp., chitinase activity was increased higher than on first day of inoculation. However, the activity was lower than on 14 days. The isolates T1, T9, T13, T17 and T18 showed progressive increasing of enzyme activity. High activities were detected in tomato leaves induced by isolated T13, T18, and 103 with 42.76, 41.28 and 41.56 μ mol (GlcNac)/mg protein/hr, respectively (Fig. 1). For β -1,3-glucanase activity, high activities were detected in tomato leaves at 14 days after inoculated, with *Trichoderma* spp. Isolates, T9, T13, T14, T17 and 103. The enzyme activities detected on 14 days were higher than on the data 5, 8 and 11 days after *Trichoderma* spp. inoculation (Fig. 2).

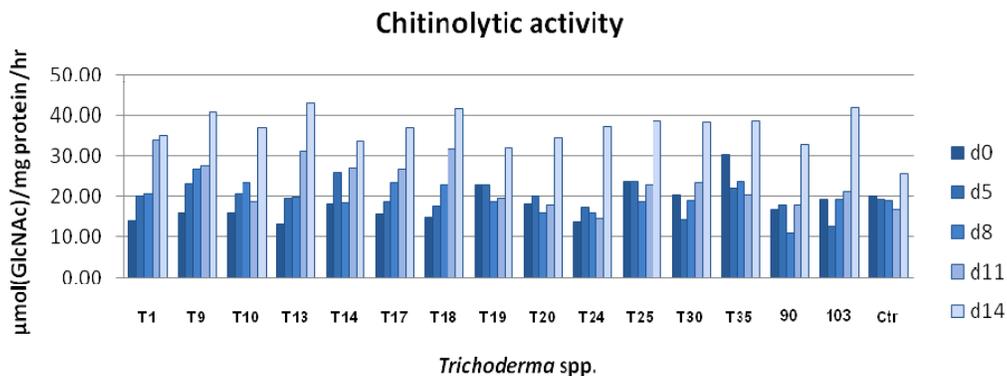


Figure 1: Chitinase activity in tomato leaves after 0, 5, 8, 11 and 14 days of inoculation.

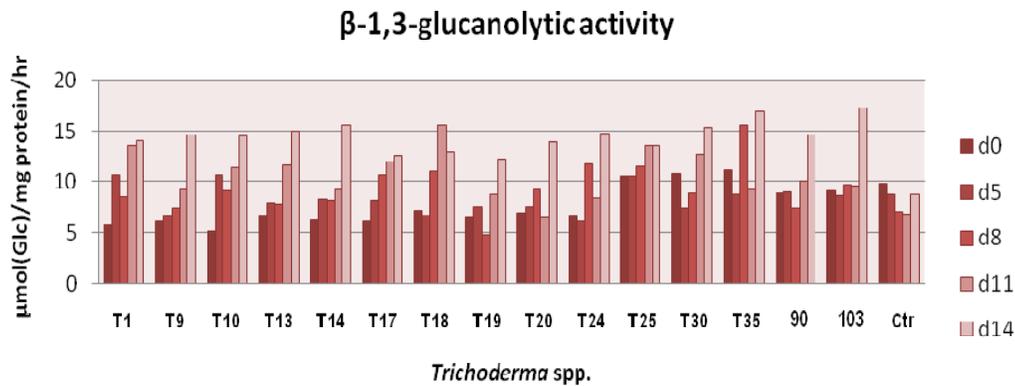


Figure 2: β -1,3-Glucanase activity in tomato leaves after 0, 5, 8, 11 and 14 days of inoculation.

Induce systemic test of tomato against Bacterial and gray leaf spot

One isolated of *T. harzianum* (T9) and 3 isolated of *T. asperellum* (T13, T17 and T18) selected to apply in pot soil (2% W/W). Induce resistance in tomato plants. The isolate T9 showed the best property in reducing bacterial spot of 62.30%. Other isolates, T13, T17 and T18 reduced spot of 34.66%, 34.41% and 44.78%, respectively (Table 1). On gray leaf spot, T18 induce significantly ($P>0.01$) tomato plant in reducing spot numbers of 19.23% more than other isolates (T9, T13 and T17) reduced leaf spot ranking 3.69-7.52% (Table 2).

Table 1: Bacterial spot numbers and spot reduction of tomato induced resistant by *Trichoderma* spp. in 7, 10 and 14 days post inoculation.

<i>Trichoderma</i> spp.	average spots / plant					Spot reduction (%)
	7 dpi2/	Spot reduction (%)	10 dpi	Spot reduction (%)	14 dpi	
T9 (<i>T. harzianum</i>)	71.50 c	61.68	85.63 c	54.12	97.88 d	69.32
T13 (<i>T. asperellum</i>)	115.75 b	37.97	139.88 b	25.04	169.63 b	34.66
T17 (<i>T. asperellum</i>)	125.63 b	32.68	150.88 b	19.15	162.50 bc	37.41
T18 (<i>T. asperellum</i>)	99.12 bc	46.89	120.88 bc	35.23	143.38 c	44.77
non <i>Trichoderma</i>	186.63 c	0	245.75 a	0	259.63 a	0
C.V. (%)	18.90		17.68		15.87	

Means followed by same letter in a column are not significantly different ($P>0.05$, DMRT).

Table 2: Gray leaf spot numbers and spot reduction of tomato induce resistant by *Trichoderma* spp. in 7 and 10 days post inoculation.

Treatments	Number of spot / leaf			Spot reduction (%)
	7 dpi2	Spot reduction (%)	10 dpi	
T9 (<i>T. harzianum</i>)	19.96 bc	16.69	23.12 b	7.52
T13 (<i>T. asperellum</i>)	20.78 ab	13.27	25.04 abc	3.8
T17 (<i>T. asperellum</i>)	21.69 abc	9.47	25.18 abc	3.69
T18 (<i>T. asperellum</i>)	18.23 c	23.91	21.36 c	19.23
control	23.96 a	0	26.07 a	0
C.V. (%)	21.1		17.03	

Means followed by same letter in a column are not significantly different ($P>0.05$, DMRT).

The result of this study indicated that the antagonistic fungus *Trichoderma* spp. induced tomato plants produced high activities of chitinase and β -1,3-glucanase. The tested of *Trichoderma* spp. in this study were effective against Fusarium wilt pathogen of tomato (*Fusarium oxysporum* f.sp. *lycopersici*) as reported by Saksirirat et al. (2005) and Saepaisan (2006). These

two enzymes were known that they play important role in degrading fungal cell wall. Besides, these enzymes were classified as pathogenesis related protein (PR protein) in various plants including tomato (Van Loon and Van Stien., 1999). Our present result exhibited correlated to other works that the *Trichoderma* spp. can induce resistance in tomato ((Harman et al., 2004). Furthermore, the *Trichoderma* spp. isolates, i.e. T1, T9, T13 and T18 showed good inducing property to enhance PR protein in tomato Sida cultivar. In the experiment of induce resistance, the *Trichoderma* spp. isolate T9 (*T. harzianum*) reduced bacterial spot 62.30% and T18 (*T. asperellum*) showed spot reduction of 19.23% in gray leaf spot. These 2 isolates may induce the tomato plant on PR protein production. However, *Trichoderma* induce plant not only PR protein but also jasmonic acid (JA), which plays a role in induce systemic resistance.

In principle, the antagonistic fungus, *Trichoderma* spp., is able to control various plant diseases, especially the soil-borne diseases. It affects plant pathogens with different mechanisms such as competition, antibiosis and parasitism. In this study, the tomato plant was induced resistance against folia diseases, bacterial spot and gray leaf spot. This mechanism is also a mechanism involved in biological control property of *Trichoderma*. The result of this present study correlated with the report of Harman et al. (2004). In case of bacterial spot, it is possible that the tomato plants produced salicylic acid, jasmonic acid or other substances, phytoalexin or proteinase inhibitor, which suppressed plant pathogens.

Conclusions

The tested isolate T9 (*T. harzianum*) induced resistance of tomato against XCV with spot reduction 69.32 %. The isolate T18 (*T. asperellum*) reduce gray leaf spot 19.23%. The result of this study showed evidently that not only the biological control property against Fusarium wilt in tomato but also induce resistant against bacterial and gray leaf spot diseases.

Acknowledgements

For financial support, we thank the Thailand Research Fund (TRF), Master of Science program in Science and Technology. Thanks are also extended to Agricultural Biotechnology Research Center for Sustainable Economy, Khon Kaen University, Thailand and Center of Agricultural Biotechnology (CAB-CHE) Thailand, for applying the equipments. The Cultivation and Product Development Research Group of Wild silkmoths is acknowledged for other facilities.

References

- Harman, G.E., Howell, C.R., Viterbo, A., Chet, I. and Lorito, M. (2004). *Trichoderma* species opportunistic, avirulent plant symbionts. **Microbiology**, Vol. 2, pp. 43-56.
- Hurtado, O. (2004). Study and manipulation of the salicylic acid-dependent defense pathway in plants parasitized by *Orobanche aegyptiaca* Pers. Master of Sciences Thesis, Plant Physiology, Virginia Polytechnic Institute and State University, USA.
- Saepaisan, S. (2006). Extracellular degrading enzymes, nucleotide sequence relationship of ITS1-5.8S-ITS2 of rDNA and chitinase gene cloning of *Trichoderma* spp. Master of Science Thesis in Plant Pathology, Graduate School, Khon Kaen University, Thailand.
- Saksirirat, W., Chuebandit, M., Sirithorn, P. and Sanoamung, N. (2005). Species diversity of antagonistic fungus, *Trichoderma* spp. from seed production fields and its potential for control

Fusarium wilt of tomato and cucurbits. **The IV Int. Conf. on Biopesticides**. 13-15 Feb 2006, Imperial Maeping, Chiang Mai, Thailand.

van Loon, L.C. and van Stien, E.A. (1999). The families of pathogenesis relate proteins, their activities and comparative analysis of PR-1 type proteins. **Physio. Mole. Pl. Pathol.**, Vol. 55, pp. 85-97.

Wasternack, C., Stenzel, I., Hause, B., Hause, G., Kutter, C., Maucher, H., Neumerkel, J., Feussner, I. and Miersch, O. (2006). The wound response in tomato-Role of jasmonic acid. **J. Pl. Physiol.**, Vol. 163, pp. 297-306.