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Effects of *Trichoderma asperellum* on nutrient uptake and Fusarium wilt of tomato

Ying-Tzu Li [a], San-Gwang Hwang [b, c], Yuh-Ming Huang [a, c], Cheng-Hua Huang [a, c, *]

[a] Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan
[b] Department of Horticulture, National Chung Hsing University, Taichung, Taiwan
[c] The MS Program for Plant Medical Science and Safe Agriculture, National Chung Hsing University, Taichung, Taiwan

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), results in severe losses in tomato production. Biological control offers a promising alternative to manage this disease due to its eco-friendly nature compared to pesticides. The objectives of this study were to select a species' strain of *Trichoderma* for containerized-transplant production to reduce Fusarium wilt and to determine the relationship between disease severity and nutrient uptake of tomato plants. Fifty eight isolates of *Trichoderma* were obtained from field soils and commercial composts. Of these 58 isolates, isolate CHF 78 showed the best antagonistic ability against FOL in a dual culture test and was further studied. The taxonomy status of CHF 78 was determined using combined sequences of internal transcribed spacer (ITS) and translation elongation factor-1α gene (TEF1α). The plant growth promoting traits and biocontrol efficiency of CHF 78 were also evaluated. Phylogenetic analyses demonstrated that CHF 78 was *T. asperellum*. This strain also showed several plant growth-promoting traits including the ability to solubilize $Ca_3(PO_4)_2$, and to produce cellulases, chitinases, indole acetic acid (IAA), proteases, and siderophores. In addition, CHF 78 significantly increased dry weight and plant height of tomato plants inoculated with or without FOL compared to those inoculated only with FOL. Inoculation of tomato plants with CHF 78 significantly reduced disease severity of Fusarium wilt, and pre-inoculation of tomato plants with CHF 78 followed by inoculation with FOL significantly promoted nutrient uptake of P, K, Mg and Zn as a result of reducing disease severity and these plant growth promoting traits. Interestingly, there was a significantly negative correlation between disease severity and nutrient uptake of all the elements analyzed in this study. *Trichoderma asperellum* strain CHF 78 can potentially be used to reduce Fusarium wilt and promote plant growth and nutrient uptake under commercial tomato production.

1. Introduction

Fusarium oxysporum f. sp. *lycopersici* (FOL) is the causal agent of Fusarium wilt of tomato, resulting in severe losses in tomato production (Gale et al., 2003; Huang et al., 2012; Jones et al., 1991; McGovern, 2015). The disease is favored by soil and air temperatures of 28 °C and has been reported in at least 32 countries (Jones et al., 1991). Typical symptoms caused by the pathogen contain stunting of infected seedlings, yellowing of older leaves, and browning of vascular tissues (Huang et al., 2012). Dissemination processes for FOL include movement of contaminated seeds, tomato stakes, soils, aerial dispersal of conidia, and infected transplants, which can cause serious outbreaks and introduce new isolates or races of FOL into other tomato fields (Jones et al., 1991; Katan et al., 1997).

Management of Fusarium wilt of tomato is challenging as a result of the phase-out of methyl bromide that was successfully used for control of FOL (Gilreath et al., 2004; Yucel et al., 2009). Although several potential soil fumigation substitutes for methyl bromide have proven efficient to manage Fusarium wilt of tomato (Yucel et al., 2009), the fumigation alternatives may have little success because of rapid re-colonization of fumigated soil by conidia of FOL released from infected plants (Katan et al., 1997). In addition, soil fumigation is costly, and social, environmental, and regulatory concerns are increasingly against the use of such chemicals (Gamliel et al., 2000). In contrast, biological control

* Corresponding author. Department of Soil and Environmental Sciences, National Chung Hsing University, Taichung, Taiwan.
E-mail address: chhuang@nchu.edu.tw (C.-H. Huang).

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through the use of beneficial fungi or bacteria offers a promising alternative to manage Fusarium wilt of tomato due to its eco-friendly nature (Manikandan and Raguchander, 2014; Shanmugam et al., 2015; Son et al., 2009).

Isolates of several *Trichoderma* species have been reported to effectively reduce Fusarium wilt diseases (Marzano et al., 2013; Srivastava et al., 2010). The antagonistic interactions of *Trichoderma* spp. with different plant pathogens determine their biocontrol efficiency of the pathogens. Biocontrol mechanisms of *Trichoderma* spp. include antibiosis, mycoparasitism, competition for nutrients and potential infection courts, and induced systemic resistance in plants (Druzhinina et al., 2011; Harman, 2006; Papavizas, 1985; Segarra et al., 2010). However, the level of biocontrol of diseases caused by *F. oxysporum* using *Trichoderma* spp. may vary because a particular strain of *Trichoderma* expresses high levels of one or another antagonistic mechanism of action (Marzano et al., 2013). Therefore, it is important to select an effective strain and species of *Trichoderma* to control a given plant pathogen. Similarly, it is necessary to select appropriate strains of *Trichoderma* species for containerized-transplant production to obtain a superior biocontrol performance of Fusarium wilt of tomato since growing media have been used for planting tomato seeds before tomato seedlings can be transplanted into field soils (Jones et al., 1991). Once contaminated by pathogenic *Fusarium oxysporum*, the growing media can also be a inoculum source, and the pathogen may be disseminated via wind, water, shoes, tools, and equipment (McGovern, 2015). Therefore, the growing media pre-inoculated with antagonistic *Trichoderma* species may enhance their suppressiveness to Fusarium wilt of tomato and consequently reduce the dissemination of FOL.

Fertilization is vital for tomato production for obtaining a high level of fruit yield and quality since the nutrient contents of plants can affect their susceptibility to disease (Jones et al., 1991; McGovern, 2015). However, few studies have demonstrated the relationship between Fusarium wilt of tomato and nutrient contents in tomato plants. Fusarium wilt of tomato is favored by low soil pH, high NH_4-N, high P, high Mg and all supplied micronutrients (Woltz and Jones, 1973). It has been reported that silicon, applied as sodium metasilicate, significantly reduces Fusarium crown and root rot of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici*, a closely related *forma specialis* of FOL. In addition, increased Si content of roots is significantly correlated with disease suppression (Huang et al., 2011). Apart from fertilization, it has been suggested that *T. asperellum* reduces Fusarium wilt of tomato due to competition for Fe with FOL (Segarra et al., 2010), but the Fe concentration in tomato plants is not significantly associated with the *T. asperellum* inoculation. Further studies are needed to elucidate the relationship between disease severity of Fusarium wilt and nutrient uptake of tomato plants inoculated with *Trichoderma* species.

The objectives of this study were: (i) to isolate a *Trichoderma* species with a high level of antagonistic activity against FOL and (ii) to determine the relationship between disease severity of Fusarium wilt and nutrient uptake of tomato plants grown in a potting medium.

2. Materials and methods

2.1. Fungal isolates

Isolate FOL146 (race 2) of *F. oxysporum* f. sp. *lycopersici* (FOL) was used in this study because it is dominant in Taiwan and its pathogenicity has been previously confirmed (Huang et al., 2016). Species of *Trichoderma* were isolated using a selective medium as previously described (Chung and Hoitink, 1990) from two tomato-growing field soils and three commercial manure composts made from chicken manure, swine manure, or both mixed with spent mushroom waste. Fifty-eight isolates with morphological characters of *Trichoderma* were single-spored and then stored in 30% glycerol at −80 °C for long-term storage. Of these 58 isolates, isolate CHF 78 showed the best antagonistic ability against FOL in a dual culture test. Therefore, isolate CHF 78 was used for the following experiments.

2.2. DNA extraction and PCR reaction

Genomic DNA was extracted from fresh mycelia of CHF 78 using the hexadecyltrimethylammonium bromide method as previously described (Ausubel et al., 1998). DNA was stored in Tris-EDTA buffer (10 mM Tris and 1 mM EDTA, pH 8.0) at −20 °C. Primers and amplification conditions for internal transcribed spacer (ITS) and translation elongation factor-1α gene (TEF1α) fragments were employed as previously described (Komoń-Zelazowska et al., 2007; White et al., 1990). Amplifications were carried out in a FlexCycler^2 thermocycler (Analytik Jena AG, Jena, Germany). Amplicons were sequenced by Genomics and Bioscience and Technology Co., Ltd. (New Taipei City, Taiwan) using BigDye Terminator Cycle Sequencing Chemistry and ABI 3730 XL DNA Sequencer (Applied Biosystems, Foster City, CA). Sequences were edited using CodonCode Aligner version 5.0.2 (CodonCode Corporation, Centerville, MA). Accession numbers of the sequences obtained in this study for ITS and TEF1α are KX377621 and KX377622, respectively.

2.3. Phylogenetic analyses

Representative sequences of ITS and TEF1α of *Trichoderma* were downloaded from GenBank (Table 1) for alignment with sequences of CHF 78 in this study. The alignment was performed using Clustal X version 2.0.6 (Larkin et al., 2007) and adjusted by eye using BioEdit version 7.2.5 (Hall, 1999). Gaps were considered missing data. Parsimony analyses were conducted using MEGA6 (Tamura et al., 2013). The most parsimonious tree was obtained using the close-neighbor-interchange algorithm with search level 1 in which initial trees were obtained with the random addition of sequences (1000 replicates). Clade stability was evaluated using 1000

Table 1
Trichoderma species and their gene accession numbers used for phylogenetic analysis in this study.

Species name	Strain	GenBank accession number	
		ITS	TEF1α
T. asperelloides	GJS 99-6	DQ315464	DQ109550
T. asperelloides	GJS 04-116	GU198301	GU248412
T. asperelloides	GJS 04-187	JN133553	JN133571
T. asperellum	CHF 78	KX377621	KX377622
T. asperellum	CBS 433.97	AY380912	AY376058
T. asperellum	GJS 90-7	GU198317	EU338333
T. asperellum	GJS 04-15	GU198311	GU198290
T. asperellum	GJS 06-294	GU198307	GU198235
T. asperellum	GJS 91-162	FJ442224	FJ463285
T. asperellum	GJS 01-294	EU856297	EU856323
T. asperellum	GJS 05-328	GU198318	EU248627
T. hamatum	GJS 98-170	DQ109530	DQ109544
T. hamatum	GJS 04-203	EU883567	EU883565
T. hamatum	GJS 04-325	EU856293	EU856318
T. hamatum	GJS 05-262	EU856292	EU856317
T. hamatum	GJS 05-334	EU856291	EU856316
T. paucisporum	GJS 01-13	DQ109526	DQ109540
T. paucisporum	GJS 03-69	DQ109527	DQ109541
T. theobromicola	Dis 85f	DQ109525	DQ109539
T. theobromicola	Dis 376f	EU856296	EU856322
T. viride	GJS 05-104	DQ841741	DQ841727

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