Plant Growth Promotion of *Trichoderma virens*, Tv911 on Some Vegetables and Its Antagonistic Effect on Fusarium Wilt of Tomato

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Soil saprophytic fungi isolated from the waste paper sludge were identified by ITS genes sequencing. Their antagonistic effects on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were tested in vitro, and *Trichoderma virens*, 911 (Tv911) had inhibited 60.35% of the growth compare to control. Moreover, it was selected for investigating plant growth promoting abilities on Japanese mustard spinach, tomato and radish in manufacturer recommended soils, farmer use nursery soil (FNS) and home garden soil (HGS). Plant height of one-month old Japanese mustard spinach and tomato were increased 16.22% and 50.26% in treated FNS and 9.84% and 7.00% in treated HGS in comparison with non-treated control, respectively. Fresh shoot and root weight of radish were also increased 23.83% and 58.86% in treated FNS and 12.77% and 64.45% in treated HGS. Disease suppression of Tv911 against FOL was also examined, and disease severity of fusarium wilt of tomato was significantly reduced. Additionally, the Tv911 colonization was observed as a stable in soil and increased in root tissue over growing period of one month. Therefore, this strain has the potential for plant growth promotion and diseases suppression against FOL, and it would be useful as a biological agent in crop production.

Keywords : biocontrol, biofertilizer, Japanese mustard spinach, radish, tomato

INTRODUCTION

Application of synthetic agro-chemicals for plant growth enhancement and weeds, pest and disease control would cause negative impacts on biodiversity, environment and human health (Debenest et al., 2010; Geiger et al., 2010). Accordingly, organic agriculture has become popular with rising trend throughout the world (Willer et al., 2009). Nowadays, many microbial products are available for pest and disease management in crop production (Berg, 2009), and also available for plant growth promotion as a biofertilizer (Rodriguez and Fraga, 1999; Vessey, 2003).

The genus *Trichoderma* (Teleomorph Hypocreaceae) has attracted an increasing attention and interest because of their economic value, and it is widely used not only as a biocontrol agent especially for soil borne plant pathogenic fungi (Qualhato et al., 2013) but also for enzyme production in industrial usage (Ahamed and Vermette, 2008). Additionally, it has the potential to degrade environmental hazardous chemical residue from the contaminated agricultural soil (Arfarita et al., 2013).

Antagonist mechanisms of *Trichoderma* spp. are competition for nutrient and space, and direct mycoparasitism with antibiotics such as cell wall degrading enzymes to inhibit the growth of plant pathogens (Benitez et al., 2004). Moreover, these species are common and persistent fungi in the rhizosphere soil microbial community in a natural ecosystem (Lidia et al., 2011). Among them, *T. harzianum*, *T. virens*, *T. asperellum*, *T. koningii* and *T. hamatum* were colonizing to the roots and rhizosphere and some strains of each species have been identified as a plant growth promoter of the early growth stage of bean plants (Hoyos-Carvajal et al., 2009). Isolates of *T. harzianum* increased plant height and leaf area of pepper and cucumber seedlings and reduced damping-off disease significantly under commercial growing conditions (Inbar et al., 1994). Isolates of *T. virens* produced plant growth regulator, the auxin-related compounds, and enhanced biomass and root growth of Arabidopsis (*Arabidopsis thaliana*) seedlings (Contreras-Cornejo et al., 2009). The morphological and cultural properties of *T. harzianum* and *T. virens* were not obviously different (Sharma and Singh, 2014), and it can be accurately identified by molecular sequencing of ITS gene (White et al., 1990).

In our previous study, several *Trichoderma* species isolated from waste paper sludge compost abandoned by paper manufacturing company in Saga, Japan during 2014–2016 were characterized by their paper degradation ability. As the results, these isolates are potentially responsible for production of cellulose degrading enzyme (cellulase) and total crude protein in treated paper wastes (Peng et al., 2016). Remarkably, there is no report concerned to the characterization of *Trichoderma* spp. derived from such paper sludge compost and possibility of usage of these isolates as a biological resource of plant growth promotion and antagonist of the plant pathogenic fungi. Although, cel-
lulose is not a major component in cell wall of plant pathogenic fungi (Bartnicki-Garcia, 1968), we assumed that degradation of cellulose residue in soil, and colonization of those saprophytic fungi in soil is helpful for plant growth and disease suppression. Additionally, *Trichoderma* is a potential microbial inoculant to decompose crop residues, and it increase soil fertility status, physico-chemical and microbial condition of the soil for sustainable agricultural production (Devika et al., 2019; Sharma et al., 2012). Therefore, this study aimed to discover beneficial biological agent from paper sludge compost for vegetable production.

**MATERIALS AND METHODS**

*Isolation and identification of fungal resources*

The fungus resources were newly isolated from waste paper sludge compost obtained from Saga Prefecture, Japan by serial dilution method for this experiment. Briefly, 1 g of waste paper sludge compost was dissolved in 1 mL of sterilized distilled water and shaken on rotary shaker (EYELA, MULTI SHAKER MMS) at 150 rpm speed for 30 minutes and 10 times serial dilution was performed to obtained $10^{-4}$ concentration of the original solution. Consequently, the diluted suspension was spread on the sterilized potato dextrose agar (PDA) plates amended with streptomycin. One mL of 10% streptomycin solution was sterilized by using Minisart® SYRINGE Filter (Sartorius Stedim Biotech GmbH, 37070, Goettingen, Germany), and added to 1 L of warm (approximately 50°C) PDA medium after autoclaved sterilization. The PDA plates were incubated at 25°C for 48 hours. The grown fungal colony was transplanted on new PDA plates, and their genus level identification was conducted by visual observation using light microscope (Leica DM 2500) based on morphological characteristics. Single spore culture was done to obtained pure culture for further investigations.

For species identification, representative 6 isolates were selected and genomic DNA of each isolate was extracted by using DNeasy® Plant Mini Kit (QIAGEN, Germany) according to the manufacturer’s instruction and ITS regions of rDNA was amplified by using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTCATATGC-3′) primers (White et al., 1990). PCR was performed in total volume of 25 μL, containing 2.5 μL of 10×reaction buffer, 2 μL of dNTPs (2 mM each), 1 μL of each primer (10 μM), 0.25 μL of Taq polymerase (2.5 units/μL) (Toyobo, Japan), 2 μL of template DNA and 16.25 μL of sterilized distilled water. This PCR reaction was conducted in thermal cycler (Biometra TProfessional basic Thermalcycler gradient) programmed for 95°C for 2 minutes, 39 cycles (95°C for 30 seconds, 55°C for 50 seconds, and 72°C for 1 minute), and 72°C for 5 minutes for final extension. The quality of PCR products were checked with ethidium bromide stained gel electrophoresis using 1.5% agarose gel. PCR products were purified with QIAquick® PCR purification kit (QIAGEN, Germany) according to the manufacture instructions. The purified PCR products were sent to Fasmac Co. (Kanagawa, Japan) for sequencing. Resultant sequences were queried to the BLAST in http://ddbj.nig.ac.jp/blast/blastn, and the sequences were deposited in GenBank with the accession number LC269252 to LC269257.

*Antagonistic effects of collected fungal resources against Fusarium oxysporum f. sp. lycopersici in vitro*

We assessed the biocontrol activities of the collected fungal materials by using a slightly modified dual culture technique proposed by Mbarga et al. (2012). Concisely, the fungal isolates of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (MAFF 103045), *Trichoderma* spp. (Tv811, Tv911 and Th621) and *Clonostachys rosea* (Cr322, Cr411 and Cr15-3-1) were separately grown on PDA plates, and incubated for 5 days at 25°C. Five mm mycelial disc of FOL and each tested fungal isolate was placed in opposite side with 70 mm apart to each other on PDA plate for the assessment of direct antagonistic effect. Moreover, antagonistic effect by volatile compound of tested fungal isolates was also examined by using the seal plate method (Aydin Ben Abdallah et al., 2015). In this method, 5 mm mycelial disc of FOL and each tested isolate of *Trichoderma* spp. and *Clonostachys* sp. were put on PDA separately, the plate containing FOL was inverted over the tested isolate plates, and seal with parafilm to avoid escaping of the volatile compound. The data were collected at 1 week after incubation at 25°C. The effectiveness of biological agents was considered according to their inhibition rate (IR) with the following formula. IR (%) = [(colony diameter of control–colony diameter presence of tested antagonists)/colony diameter of control] ×100. This experiment was carried out with 5 replications, and colony diameter was mean value of two perpendicular measurements of FOL colony.

*Plant growth promotion effects of Tv911 on some vegetables*

*Trichoderma virens* isolate Tv911 was used as a resource of plant growth promotion test on three kinds of vegetables; Japanese mustard spinach (*Brassica rapa* var. *perviridis*), tomato (*Solanum esculentum* cv. *Monterosa*) and radish (*Raphanus sativus* cv. *Saradakomachi*) in this experiment. The tested crops were grown in two type of soil, namely Farmer use nursery soil (FNS) and Home garden soil (HGS), commercial products of Oishi Bussan Co., Ltd., Fukuoka. These soils were named by the company according to usage of the customers, where FNS is used by the farmers and HGS is mainly used in home garden, and the trade name is Monsaku and HB-101, Biyoudo, respectively.

Firstly, the inoculum was prepared from 10 days old culture of Tv911 on PDA by harvesting conidial suspension with sterilized distilled water. The conidial suspension was adjusted 10⁷ conidia/mL concentration, and mixed thoroughly with the soil materials using soil mixer machine with the rate of 1 mL spore suspension kg⁻¹ of soil. The inoculated soil materials were put in 20 L plastic bag, and it was sealed and incubated for one month at room temperature to obtain stable survival fungal population at plant sowing time. Two kg of inoculated and non-inoculated soil were put in pots and the seeds were sown at 1 cm depth. The experiment was performed with three rep-
Disease suppression effects of Tv911 against tomato fusarium wilt caused by FOL

In this experiment, tomato cv. Monterosa was used as the test plant, and seeds were surface sterilized by 2% NaOCl solution for 3 minutes and washed three times with sterilized water and sown in HGS soil. Two weeks old seedlings were transplanted into pots containing 1.5 kg of autoclaved sterilized HGS soil. The treatments were non-inoculated control (mock), inoculated with FOL only, FOL+Tv911 and Tv911 only. Ten mL of spore suspension of FOL and Tv911 were inoculated, where the concentration was adjusted to obtain 1×10^5 conidia/mL. The experiment was done as completely randomized designed (CRD) with four replications and each replication contained three plants. Irrigation was carried out as necessary. The disease severity was recorded as index of leaf damage (ILD) at 4 weeks after transplanting with the formula, ILD = Σ grades/max grade where the grades are from 0 to 4 score scale (0= asymptomatic leaves, 1= leaves wilted, 2= leaves moderate yellowing, 3= leaves with necrosis and 4= dead leaves) according to the method of Selim et al. (2014).

The colonization of Tv911 was estimated by using real-time PCR using DNA binding dye including standard melt curve method. The species specific primers were designed base on ITS gene sequence of Tv911 in Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Two primer sets; Tv911-1F (5'-CGTGGGCGTTCGAA AATG-3') and Tv911-1R (5'-GGCTGAAATGACGC TCGG-3'), and Tv911-2F (5'-GTTGGGATCGGCC TTTAC-3') and Tv911-2R (5'-CACCGGGTATCTCTA CCTGA-3') were selected based on the dissimilarities to DNA sequences of other fungi. Several conventional PCR trials were performed to determine the selectivity and sensitivity of the primer sets to target species using template genomic DNA of Trichoerma virens, Trichoderma harzianum, Clonostachys rosea, Phomopsis asparagi, and Diaportha melonis.

Tv911 colonization in rhizospheric soil and root tissue was quantified by real-time PCR assay using SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA). The measurements were conducted at 1, 2 and 4 weeks after transplanting. Total genomic DNA from 100 mg of washed and blotted dried fresh plant root tissue and 500 mg of soil were extracted by using DNeasy Plant mini kit (QIAGEN, Germany) and ISOIL DNA extraction kit (Nippon Gene, Japan), respectively. The standard curve for DNA calibration was constructed by 10 times serial dilutions (from 4×10^7 ng to 4×10^-3 ng concentration) of known concentration of DNA from Tv911 pure culture, which were simultaneously run with subjected samples in each real-time PCR assay. The PCR reaction mixture was prepared to total 10 μL volume containing 5 μL of 2× SYBR Green Supermix, 100 nM of forward primer (Tv911-2F), 150 nM of reversed primer (Tav911-2R), 1 μL of template DNA, and sterilized distilled water for volume makeup. The assay was performed in the condition of 1 cycle of 96°C for 3 minutes, followed by 40 cycles of 95°C for 20 seconds, 60°C for 25 seconds, and 72°C for 25 seconds (Agilent Technologies, AriaMx Real-Time PCR System). The melt curve obtained by 0.5°C resolution with 5 seconds soak time for data acquisition from 65°C to 95°C. Data were analyzed using AriaMx Software version 1.6.

Identification of fungal isolates from waste paper sludge

This study discovered total of three fungal species from the waste paper sludge compost (Table 1). Firstly, it can be differentiated into genus (Trichoderma and Clonostachys) based on the morphological characteristics. Then, the studied isolates could be identified as the following species by performing the molecular characterization. The nucleotides sequences of ITS regions rDNA of isolate, Tv811 and Tv911 were 100% identical to the Trichoderma virens (GenBank accession JX908731), three isolates of Cr322, Cr411 and Cr15-3-1 were 99% identical to Clonostachys rosea (GenBank accession: KY365588) and isolate Th621 was 99% identical to Trichoderma harzia-num (GenBank accession: KY750364), respectively.

Antagonistic effect of fungal isolates on vegetative mycelial growth of FOL in vitro

The antagonistic effect of obtained isolates against

| No. | Species                | Isolate | GenBank accession no. | Inhibition (%) by direct* | Inhibition (%) by volatile* |
|-----|------------------------|---------|-----------------------|--------------------------|---------------------------|
| 1   | Trichoderma virens     | Tv811   | LC2690256             | 60.55 a                  | 19.53 b                   |
| 2   | T. virens              | Tv911   | LC269257              | 64.27 a                  | 27.41 a                   |
| 3   | T. harzianum           | Th621   | LC269254              | 59.06 a                  | 18.08 b                   |
| 4   | Clonostachys rosea     | Cr322   | LC269252              | 9.68 b                   | 7.00 c                    |
| 5   | C. rosea               | Cr411   | LC269253              | 10.92 b                  | 3.50 c                    |
| 6   | C. rosea               | Cr15-3-1| LC269255             | 9.19 b                   | 4.37 c                    |

* Number followed by the same letter do not differ significantly by LSD value at P≤0.05.
soil borne plant pathogenic fungus, FOL was determined with inhibition rate (IR) in two experiments of direct metabolites and volatile compound in vitro (Fig. 1). The mean values of IR of tested isolates on FOL in both direct metabolites and volatile compound tests were significantly differed among the isolates ($P \leq 0.05$), but the growth inhibition by direct metabolites was higher than the inhibition by volatile compound at 7 days after incubation. In the direct growth inhibition, the highest IR (64.27 %) was observed in Tv911 and there were no significantly differences among the Trichoderma isolates. IR of Trichoderma isolates was significantly higher than C. rosea isolates; Cr322, Cr411 and Cr15-3-1 in both tests (Table 1). Conse-

![Image](image1)

**Fig. 1** Antagonism of *Trichoderma virens*, Tv911 against the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (a) direct metabolites test, (b) control check of FOL culture in direct metabolites test, (c) volatile compound test, (c) control check of FOL culture in volatile compound test.

![Image](image2)

**Fig. 2** Mycelial coiling of *Trichoderma virens* (Tv911) around the host *F. oxysporum* f. sp. *lycopersici* (FOL) in the inhibition zone between the growth of two fungi (Bar=50 μm).

![Image](image3)

**Fig. 3** (a) Plant height of Japanese mustard spinach, (b) plant height of tomato and (c) fresh shoot and root weight of radish in *Trichoderma virens*, Tv911 treated and non-treated Farmer use nursery soil (FNS) and home garden soil (HGS) at 4, 4, and 6 weeks after sowing, respectively. Symbols of *, ** and ns mean significantly different at $P<0.05$, $P<0.01$ and not significantly different, respectively.

consequently, *Trichoderma virens*, Tv911 isolate was selected for the next experiments. The growth inhibition zone of direct antagonistic test was observed by light microscope, where mycelia of *Trichoderma* were coiling to those of host FOL (Fig. 2).

**Plant growth promotion effect of Tv911**

Growth promotion effect of Tv911, plant height of Japanese mustard spinach was not significantly different between treated and non-treated of both FNS and HGS (Fig. 3-a). However, the plant growth was increased
## PLANT GROWTH PROMOTION

### Table 2  Summary statistics of the effects of *Trichoderma virens* (Tv911) on the growth of three vegetables.

| Treatment  | Japanese mustard spinach plant height (cm)† | Tomato plant height (cm) † | Radish fresh shoot weight (g/plant) † | Radish fresh root weight (g/plant) † |
|------------|--------------------------------------------|-----------------------------|---------------------------------------|---------------------------------------|
|            | FNS | HGS | FNS | HGS | FNS | HGS | FNS | HGS |
| Treated    | 16.3±1.9 | 14.4±1.0 | 71.8±2.4 | 64.0±8.0 | 24.8±5.4 | 19.1±4.4 | 81.1±14.4 | 70.5±7.0 |
| Control    | 14.1±1.4 | 13.1±1.3 | 47.8±5.6 | 59.8±3.2 | 20.0±11.7 | 16.9±5.9 | 46.8±12.3 | 42.9±13.8 |

Increased over control (%)  
Treated: 16.22% | 9.84% | 50.26% | 7.00% | 23.83% | 12.77% | 58.86% | 64.45%  
Control:  

† Mean values of three replications, and each replication contained 3 plants.  
‡ Mean values of three replications, and each replication contained 4 plants.  
§ Mean values of three replications, and each replication contained 5 plants.

16.22% and 9.84% in Tv911 treated FNS and treated HGS, respectively. The plant heights of tomato plant were significantly different among the treated and non-treated soils, where treated FNS was significantly higher than non-treated FNS (Fig. 3-b). The plant heights of tomato plant were promoted at 50.26% and 7.00% in both treated FNS and HGS, respectively. In the experiment of effect of Tv911 on the growth of radish plants, fresh shoot and root weight were measured. There were no significant differences in the fresh shoot weight among the treatments. However, the root weights were significantly different between treated and non-treated soils (Fig. 3-c), and the average root weight in treated FNS and HGS were 58.86% and 64.45% increased over non-treated FNS and HGS, respectively (Table 2).

### Disease control effect of Tv911 against fusarium wilt of tomato caused by FOL

The index of leaf damage (ILD) in FOL and FOL+Tv911 was significantly higher than mock and Tv911 treatments. FOL+Tv911 was significantly lower ILD than the FOL only. Moreover, the plant height of FOL+Tv911 was significantly higher than FOL only, and the Tv911 reduced the amount of disease damage by the FOL (Fig. 4 and Table 3).

The colonization of Tv911 in rhizospheric soil and root tissue were detected by quantification PCR products with fluorometric real-time PCR at 1, 2 and 4 weeks after transplanting. For this experiment, the species selective

![Mock FOL FOL+Tv911 Tv911](image)

**Fig. 4** Effect of Tv911 on the fusarium wilt of tomato in greenhouse experiment at 4 weeks after transplanting.

### Table 3  Disease control effect of Tv911 on fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at 4 weeks after transplanting.

| Treatment   | ILD†   | Plant height† (cm) | Soil pg DNA of Tv911/mg soil ‡ | Root pg DNA of Tv911/mg fresh tissue ‡ | 1 WAT | 2 WAT | 4WAT |
|-------------|--------|-------------------|---------------------------------|----------------------------------------|-------|-------|------|
| Mock        | 0.04±0.02 c* | 48.1±4.8 b*      | NA                              | NA                                     | NA    | NA    | NA   |
| FOL         | 0.45±0.05 a  | 33.4±3.5 c       | NA                              | NA                                     | NA    | NA    | NA   |
| FOL+Tv911   | 0.17±0.03 b  | 54.5±1.5 ab      | 6.66±0.5                       | 5.80±0.4                               | 0.05±0.01 | 0.12±0.01 | 0.19±0.02 |
| Tv911       | 0.02±0.02 c  | 57.0±2.6 a       | 6.13±0.6                       | 6.37±0.9                               | 0.06±0.02 | 0.12±0.02 | 0.23±0.08 |

† DNA of Tv911 was quantified by real-time PCR using SYBR Green and Tv911-2/F/Tv911-2/R primers after extraction from soil and root tissue at 1 WAT, 2 WAT and 4 WAT, respectively. NA = not detectable fluorescence signal in PCR reaction.

‡ Mean value of plant height at 4 WAT from four replications.

§ Mean value of index of leaf damage at 4 weeks after transplanting (WAT) from four replications.

© Number followed by the same letter are not different significantly by LSD at $P≤0.05$. 

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primers were designed based on the ITS gene sequence, and the selectivity of the primers (Tv911-2F/Tv911-2R) was checked using conventional PCR. Only T. virens template DNA can be amplified and the expected PCR products (171 bp) were obtained. Other template DNAs from T. harzianum, C. rosea, P. asparagi, and D. melonis were not amplified by the primer set. Primer dimers were not also observed in non-template DNA control.

In the PCR assay using above primer sets, the quantification of the PCR products was carried out based on the standard curve. The amount of input template DNA was linearly correlated to the cycle threshold, Cq (AR) as expressed by the equation Cq = -3.335x + 15.36, \( R^2 = 0.999 \) where x is input amount (ng) of template DNA of Tv911 (Fig. 5-a). The results revealed that the detection limit of the assay using this primer set was approximately \( 4 \times 10^{-5} \) ng of template DNA per sample. The primer dimers and amplification of non-target regions were not generated, and there was a single peak in melt-curve (Fig. 5-b). In this experiment, the significant fluorescence signal was not observed in extracted DNA from both soil and root samples of mock and FOL treatments, respectively. There was no significant difference between FOL+Tv911 and Tv911 treatments in each sampling time. However, amount of DNA of Tv911 in rhizospheric soil was not obviously changed over the one month growing period of tomato, and those of Tv911 in root tissue as endophytes was increasing about four times from 0.05 pg/(mg root tissue) to 0.19 pg/(mg root tissue) in FOL+Tv911 and 0.06 pg/(mg root tissue) to 0.23 pg/(mg root tissue) in Tv911 treatments (Table 3).

DISCUSSION

Trichoderma species have been generally recognized as a cellulose decomposer, biological control agents and plant beneficial symbiotic fungus for plant growth promotions (Harman et al., 2004; Ahamed and Vermette, 2008; Contreras-Cornejo et al., 2009). In the present study, we obtained fungal isolates from the waste paper sludge compost and studied their competitiveness to one of the harmful soil fungal plant pathogen \textit{in vitro}. Clonostachys rosea has shown the antagonistic effects on the fungal plant diseases, such as \textit{Sclerotinia sclerotiorum}, \textit{Fusarium culmorum} and \textit{Alternaria} spp. in the previous study (Jensen et al., 2004; Roberti et al., 2008; Rodriguez et al., 2011). The \textit{C. rosea} isolates, Cr322, Cr411 and Cr15-3-1 showed fewer effect on the mycelial growth of FOL in this experiment. These results are consistent with the previous study of \textit{in vitro} assay that \textit{C. rosea} showed lower level of fungal growth inhibition (1–23%) than \textit{Trichoderma} strains (40–68%) against \textit{Fusarium cincinatum} (Moraga-Suazo et al., 2011). In the present study, \textit{Trichoderma} isolates effectively inhibited the growth of FOL in both tests of direct metabolites and volatile compound, but lower inhibition rate (IR) was found in latter. In the antagonistic activities, \textit{Trichoderma} spp. produce siderophores, antibiotics, hydrolytic enzymes and carbon and nitrogen permeases (Benitez et al., 2004). At 7 days after incubation, the cessation of the growth of FOL by Tv911 isolates and direct parasitism of mycelial coiling at the inhibition zones were observed in this experiments (Fig. 2). In another study, different species of \textit{T. viride} inhibited the mycelial growth of \textit{F. oxysporum} f. sp. adzuki and \textit{Pythium arrhenomanes} \textit{in vitro} (John et al., 2010). In this study, we observed that the disease severity of FOL in tomato was significantly reduced by means of Tv911 application.
Tv911 in roots and soil was quantified by using ITS sequenced based species selective primers with real-time PCR assay. Such primers and methodology were simple and effectively used for quantification of specific fungal species, such as *Phomopsis sclerotioides*, in plants and soil (Shishido et al., 2010; 2013). The colonization of Tv911 in rhizosphere soil of tomato roots was not significantly fluctuated at 1, 2, and 4 weeks after transplanting, and it was increased approximately 4 times in tomato roots.

Trichoderma spp. are well known biological agents to promote the plant growth either indirectly, by modifying rhizosphere environment, increasing nutrient availability, enhancing the crop defensive mechanisms, or directly by mycoparasitism to plant pathogenic fungi (Benítez et al., 2004; Matarese et al., 2012; Qualhato et al., 2013). In the current research, *T. virens* (Tv911) increased the growth of three types of vegetables from 7.00% to 64.45% compare to non-treated control in two types of soil, FNS and HGS. There were the differences of effects of Tv911 on plant growth promotion between FNS and HGS in the greenhouse pot experiment. The enhancement of Tv911 in plant height of Japanese mustard spinach and fresh shoot weight of radish between FNS and HGS is not obviously different, where as those of plant height of tomato in FNS was clearly higher than HGS. In another study, *T. harzianum* increased the dry weight of cucumber 17% in vermiculite and 43% in sandy loam soil at 15 days after planting, the root colonization of *T. harzianum* also varied between 40 and 90% depending on the test vegetables (Kleifeild and Chet, 1992). Moreover, *T. harzianum* enhanced 23.8% and 17.2% in the seedling height of cucumber and pepper at 18 to 30 days after planting, respectively (Inbar et al., 1994). *T. virens* had plant growth promotion effects on soybean including shoot and root system, and fruit yield at 12 weeks after planting in the pot experiment (John et al., 2010). Contreras-Cornejo et al. (2009) also demonstrated that plant growth promotion activities through the auxin-related compounds produced by *T. virens* in Arabidopsis seedlings. Application of Trichoderma to the growing media in the nursery has beneficial effects on the crop growth and biological control activities to soil borne pathogens (Inbar et al., 1994).

In conclusion, two species of Trichoderma; *T. virens* (Tv911 and Tv811) and *T. harzianum* (Tv621) have significantly suppressed the growth of FOL, the causal organism of fusarium wilt of tomato by means of direct confrontation in *vitro* and Tv911 reduced the disease severity of fusarium wilt of tomato in the greenhouse test. Additionally, Tv911 isolate has plant growth promotion effects on the growth of these vegetable, Japanese mustard spinach, tomato and radish in two type of growing soil, FNS and HGS in greenhouse pot experiment. Therefore, Tv911 has the potential for plant growth promotion and disease control as a biological agent for organic crop production. Further investigation of extracellular enzymes of Tv911, which seem to be involved in antagonistic mechanism to the plant pathogenic fungi, and enhancement of nutrient uptake ability from soil to plants are required. Moreover, it is necessary to conduct the effect of Tv911-derived metabolites on the metabolic changes of host crop under field condition before commercialization.

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