Plant Pathology Bulletin 11:215-220, 2002

Screening Strains of *Trichoderma* spp for Plant Growth Enhancement in Taiwan

Chaur-Tsuen Lo [1,2], and Chien-Yih Lin [1]

1 Taiwan Agricultural Research Institute, Wu-Feng, Taichung 413, Taiwan, R.O.C.
2 Corresponding author, E-mail: ctlo@wufeng.tari.gov.tw ; Tel: +886-4-3302301 ; Fax: +886-4-3338162
Accepted for publication: Nov. 5, 2002

ABSTRACT

Lo, C.T., and Lin, C. Y. 2002. Screening strain of *Trichoderma* spp for plant growth enhancement in Taiwan. Plant Pathol. Bull. 11:215-220.

Several strains of *Trichoderma* spp. isolated from rhizosphere soils and rhizoplane of agricultural crops in Taiwan were selected to detect the root growth of bitter gourd, loofah, and cucumber. Some strains of tested species of *Trichoderma* in this study promoted the root growth of these cucurbitaceous crops as compared with untreated plants. Tests in greenhouse showed the strains of the *Trichoderma* species significantly increased of 26 to 61% in seedling height, 85-209% in root exploration, 27 to 38% in leaf area, and 38 to 62% in root dry weight for 15 days after sowing seeds of bitter gourd. Similarly, These *Trichoderma* strains also increased seedling growth of loofah and cucumber. In a test of chlorophyll concentration, the results indicated that the concentration of chlorophyll (mg/cm^2 of leaves) was significantly increased in the plants treated with *Trichoderma*. Based on regression tests, there was no relationship between percentage of root colonization of *Trichoderma* and enhanced plant growth.

Key words: *Trichoderma* spp., biological control, root exploration, chlorophyll

INTRODUCTION

Biological disease control is a promising strategy for control of soilborne and foliar diseases in several crops. Meanwhile, it also provides practices compatible with the goal of a sustainable agricultural system [15]. Among beneficial microorganisms, *Trichoderma* spp. are the most common biocontrol agents of plant pathogenic fungi that caused soil-borne, air-borne, and post-harvest diseases in several crops [7,13,15]. In addition, the increased growth response induced by *Trichoderma* species has also been reported for many kinds of crops such as bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), pepper (*Capsicum annum* L.), carnation (*Dianthus caryophyllus*), maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) [4,6,9,10,14]. However, most the results only reported the effect of the agents on plant height.

In Taiwan, *Trichoderma* spp. has been reported to control Rhizoctonia diseases in some crops [12,24]. However, none reported the enhanced growth response induced by the fungus in Journal. Generally, the beneficial effects obtained with *Trichoderma* to increase plant response have important economic implications, such as shortening the plant growth period and time in the nursery, thereby increasing production capacity. Consequently, the objectives of this study were (i) to report the strains of *Trichoderma* species isolated from Taiwan could promote plant growth, (ii) to expand research to include the responses of other plant species besides cucumber with additional measurements of growth parameters such as root exploration, leaf area, and chlorophyll concentration, and (iii) to determine the correlation between the rhizosphere competence and plant growth response of *Trichoderma* spp.

MATERIALS AND METHODS

Trichoderma spp. and strains

Most strains of *Trichoderma* species used in this study, unless otherwise stated, were isolated from different localities in Taiwan and were used to test their effect on the length of primary root and the amount of lateral roots of per cucumber seedling (*Cucumis sativus* L.) (Table 1). The isolates of *Trichoderma* spp. that increased primary-root length and lateral-root number of cucumber were then selected to evaluate the rhizosphere competence and the growth response by following tests in greenhouse [1,2,20,21]

Strains of *T. harzianum* (designed hereafter as BR16) originally was isolated from a rhizosplane of betel palm (*Areca catechu* L.) in Siou-li. *T. harzianum* strain 1295-22 (ATCC20847) was provided by Dr. Gary E. Harman (New

Table 1. Screening strains of *Trichoderma* spp. isolated from crops of Taiwan, based on length of tap roots and numbers of lateral roots of cucumber [1]

No. lateral root (Treated/Control)	Tap root length (cm) (Treated/Control)	No. isolate	Rate (%)
>1.1	>1.1	66	2.8
>1.1	0.9<TR2<1.1	86	3.6
>1.1	<0.9	597	25.2
0.9<LR3<1.1	>1.1	15	0.6
0.9<LR<1.1	0.9<TR<1.1	50	2.1
0.9<LR<1.1	<0.9	613	25.9
<0.9	>1.1	17	0.7
<0.9	0.9<TR<1.1	44	<0.9
<0.9	875	37.0	
Total No.		2363	

[1.] The seeds of cucumber were sowed on water agar plates and placed in incubator at 25℃ for seven days.
[2.] TR = the length of tap roots
[3.] LR = No. of the lateral roots

York State Agricultural Experiment Station, Geneva, NY). Strain of *T. harzianum* (designed as Zts428) was isolated from the rhizosphere soils of ginger (*Zingiber officinale* Rescoe) in Nan-tou. *T. virens* strain R42 was isolated from the rhizoplane of papaya (*Carica papaya* L.) in Lin-nan. These strains were cultured on a potato dextrose agar medium (PDA; Difco) at room temperature for 14 days and used to test plant growth response.

The tested plants in this study included cucumber, loofah (*Luffa cylindrica* L.), and bitter gourd (*Momordica charantia* L.). These cultivars were bought from Know-you Seed Co. (Taiwan).

Plant response measurements

Rice bran/peat mixture (3:100 w/w) adjusted to 50% moisture (w/w) was autoclaved in polyethylene bags (PEB, 45 x 30 cm) for 30 min on two successive days. The substrate in the PEB was inoculated with spore suspension (10^5 spores/ml) and then placed in incubator for 14 days at 27℃. This preparation of *Trichoderma* was mixed with soil to obtain 1-2 x 10^6 cfu/g soil before sowing seeds of different plants. The following plant response parameters were measured in each test plant from the various strain-treatments: *plant height*, each plant was rated from base of stem to apical bud; *leaf area*, five leaves were examined from the lowest to the upper leaf and their area were measured by measuring leaf diameter; *plant root weight*, the roots of per plant were washed under running tap water to remove residual soil from the roots, then dried at 105℃ in an oven. After 24h, the dried weight of plant roots was determined on an analytical balance (Mettler, Greifensee, Switzerland).

Analyzing root architecture in soil

The germinated seeds of cucumber, loofah, and bitter gourd were grown in acrylic boxes (one seed in per box) with 10 replicates for 15 days. Cucumber was grown in 20 x 20 x 3 cm boxes. Loofah and bitter gourd were separately grown in 30 x 30 x 3 cm boxes. The intact root systems incubated for 7-15 days in greenhouse were removed from the thin acrylic boxes by pressing a board with stainless-steel nails in a uniform 1-cm grid into the soil to hold the roots in place, after which the soil was gently washed off. The roots were photographed while in their original orientation. Soil exploration was measured by modified Bjorkman's method from the photographs as the proportion of the 400 x 1-cm and the 900 x 1-cm squares that contained roots of cucumber, loofha and bitter gourd, respectively [(5)]. The experiment was carried out at least twice.

Detection of Chlorophyll concentration of cucumber leaves

Chlorophyll concentration can be measured in vivo by nondestructive spectroscopic techniques that do not require sample preparation. In this experiment, a commercial nondestructive dual-wavelength meter (Model SPAD-502, Minolta Crop) was used to directly estimate chlorophyll content [(17)]. In the mean time, a destructive colorimeter measurement was made using acetone extraction procedure to determine the relationship between the dual-wavelength chlorophyll valve and leaf chlorophyll concentration as a standard curve for translation data by following detection. Leaf discs (1 x 3 cm^2) were taken from cucumber seedlings treated by different strains of *Trichoderma* spp. About 30 mg of leaf material was placed in opaque screw-cap vials containing 10 ml of 80% acetone for 3 hours. Three milliliters of the resulting extract were placed in a quartz cuvette, and absorbance at 663, 645, and 450 nm was measured in a Beckman spectrophotometer (Beckman, Fullerton, Calif.). Chlorophyll concentration was calculated using the equations from Porra *et al.* [(19)]. In this study, the data showed that had positive relationship between the dual-wavelength chlorophyll value and leaf chlorophyll concentration in cucumber (r^2=0.92). The equation was 【chlorophyll concentration (mg/m^2)= -378.31 + 19.16 x SPAD-502 value】

The effect of rhizosphere competent strains of *Trichoderma* spp. on plant growth response

In order to determine the relationships among rhizosphere competence and plant response, several strains of *Trichoderma* with different abilities of rhizosphere colonization in cucumber roots were selected to detect their effect on plant growth response of cucumber seedlings.

Data analysis

Plant growth responses were statistically analyzed by ANOVA (SAS institute, Cary, NC). Fisher's protected least significant difference (LSD) was usually calculated for mean

separations within each test using $p<0.05$. All experiments were conducted twice and data from the first trial are presented when the similar results were obtained in the second trial.

RESULTS

Screening strains of *Trichoderma* spp.

Two thousand three hundred and sixty three strains of *Trichoderma* spp. isolated from rhizosphere soils and rhizoplane of agricultural crops in Taiwan were selected to detect the root growth of cucumber. In laboratory tests, only 2.8% of tested *Trichoderma* strains in this study promoted both primary roots and lateral root growth of cucumber as compared with untreated plants (Table 1). The results also indicated that about 3.6% of tested strains could increase the numbers of lateral root, but not affect tap-root lenght. Contrary, only 0.6% promoted the length of primary (tap) root, but not effect lateral root growth, when the treated seedlings were compared with untreated seedlings of cucumber (Table 1).

Assay for rhizosphere competence

Strains of *Trichoderma* species were reisolated from root segments of cucumber by *Trichoderma* selective medium [13] after washing the roots of cucumber seedlings with running tap water. The results indicated that most *Trichoderma* strains were not good rhizosphere competent. Most strains only colonized the roots of cucumber at 1-2 cm of the upper root segments. However, about 8 percent of test isolates with beneficial root growth could colonize the entire root of cucumbers (Fig. 1, Table 2). The results indicated that there was no relationship between rhizosphere competence and enhanced plant growth including plant height and root weight in regression-test (r^2=0.201).

Enhanced growth response in cucumber, loofah, and bitter gourd seedlings

In cucumber (Table 3), a significant increase of plant growth treated separately with *Trichoderma harzianum* strain 1295-22, BR-61, Zts428, and *T. virens* strain PR42 was observed on the measured parameters (plant height, leaf area, plant root dry weight, and soil exploration), in each strain of *Trichoderma* spp. compared to the non-treated seedlings. Particularly, the seedling height of cucumber treated with *T. harzianum* strain BR-61, and *T. virens* strain R42 were as 1.4-fold height as the non-treated seedlings for 15 days after sowing. Similarly, the chlorophyll concentration in leaves of cucumber treated by *Trichoderma* spp. was higher than that of untreated plants (Table 3). In bitter gourd, significant increases in size of emerged plants in soil inoculated with different strains of *Trichoderma* were more uniform than those of control plants. The isolates of *Trichoderma* species significantly increased of 26 to 61% in seedling height, 85-209% in root exploration (Fig. 2), 27 to 38% in leaf area, and 38 to 62% in root dry weight for 15 days after sowing seeds of bitter gourd as compared with untreated plants. Similarly, the dry weights of loofah roots treated with *Trichoderma*

Table 2. Detection of root-colonization ability of 66 isolates of *Trichoderma* spp. which could enhance growth of cucumber in growth chamber [1]

Root colonization (%)	No. isolate
24.4 (ave.)	Untreated plants
<30	7
31-50	18
51-70	11
71-90	14
91-99	8
100	8

[1.] The cucumber seeds were sowed for 7 days.

Table 3. Increased plant growth response in cucurbitaceous seedlings treated by *Trichoderma* spp. [1]

	Leaf area diameter (cm)			Root dry weight (mg/plant)			Root exploration (%)			Plant height (cm)			Chlorophyll concentration (mg/m^2)		
Treatment	C.	L.	B.	C.	L.	B.	C.	L.	B.	C.	L.	B.	C.	L.	B[2].
T. harzianum 1295-22	8.1	6.3	5.3	130	110	100	49	46	33	15.2	23	22.5	510	ND	ND
T. harzianum BR16	7.9	6.1	5.8	125	120	116	49	50	65	14.1	22	23.9	520	ND	ND
T. harzianum Zts428	7.2	ND	5.4	110	ND	100	38	ND	38	13.2	ND	19.0	ND	ND	ND
T. virens R42	8.0	6.1	5.7	130	125	116	49	45	61	15.4	24	24.2	550	ND	ND
Substrate [3]	6.9	4.2	4.1	62	75	72	29	22	21	10.3	15	15.0	465	ND	ND
Control	4.1	4.2	4.3	47	58	57	18	15	13	4.2	12	7.2	450	ND	ND
LSD ($P<0.05$)	0.9	1.1	0.9	28	26	28	7.6	7.6	8.6	2.6	3.2	2.7	32	ND	ND

[1.] All cucurbitaceous seeds were sowed in soil boxes for 15 days in greenhouse.
[2.] ND= No Detection.
C=cucumber; L=loofah; B=bitter gourd.
[3.] Substract = rice bran/peat preparation.

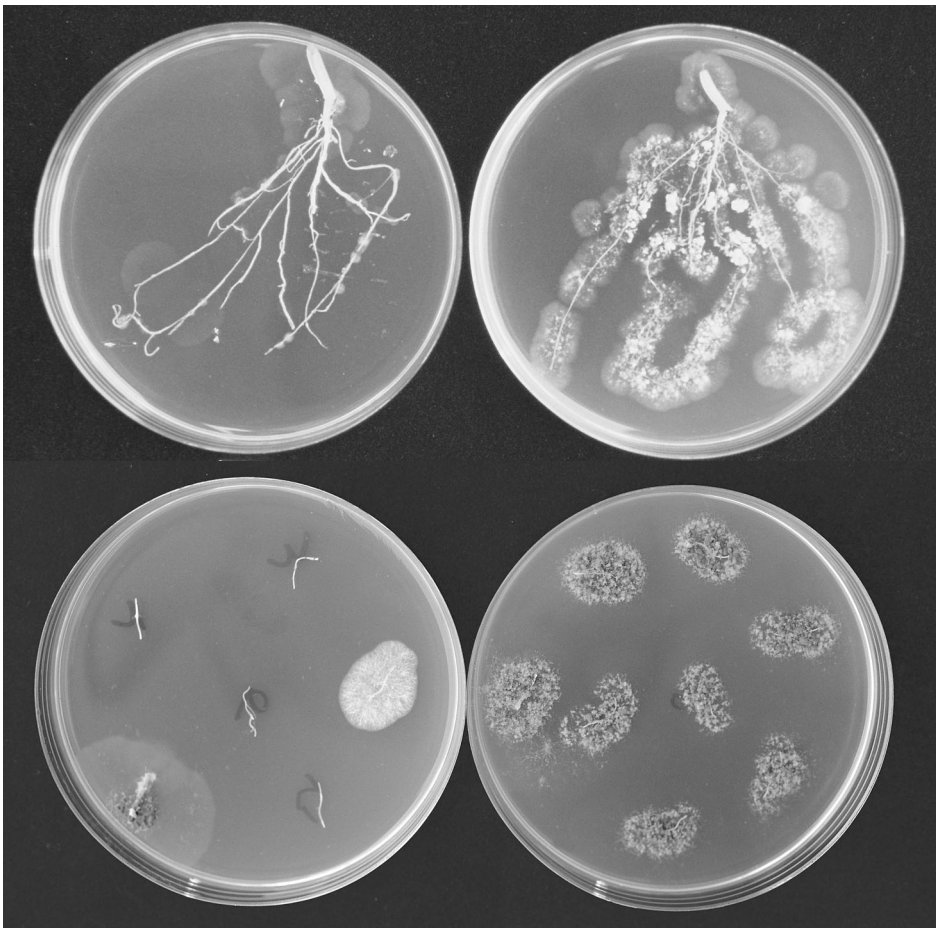

Fig. 1. Reisolation of *Trichoderma* spp. in cucumber roots. (Left column, untreated control which colonized only a few part of roots; right column, *Trichoderma*-colonization on entire root)

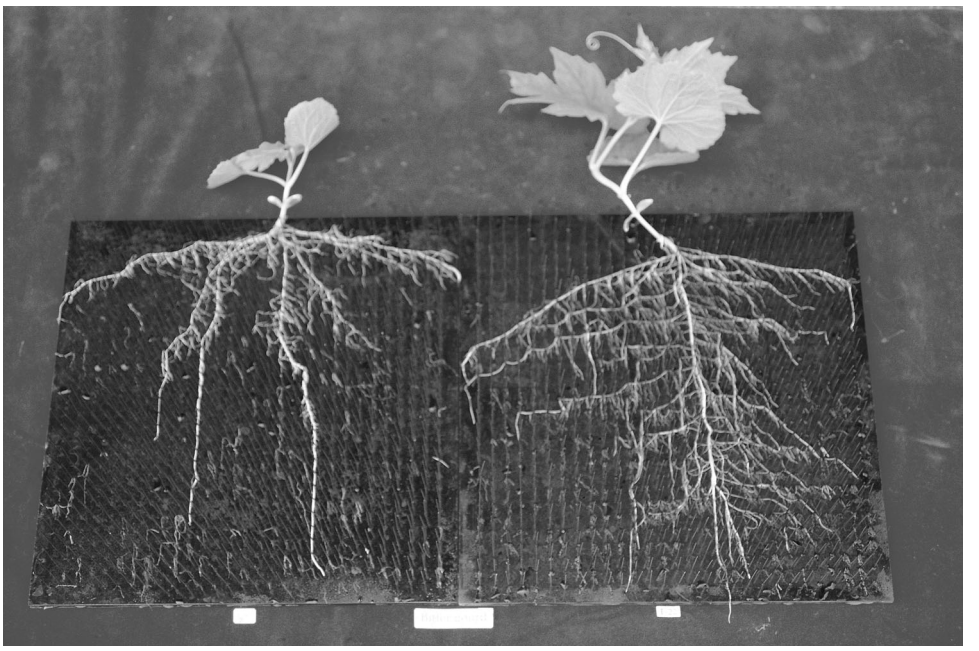

Fig. 2. Enhanced root exploration in bitter gourd seedlings. The the area of root distribution of *Trichoderma*-treated seedlings (right) were over twice as wide as that of untreated seedlings (left). The seedlings were cultured for 15 days in greenhouse.

species, increased to 47-67% of the untreated control (substrate). In other parameters, the tested isolates of *Trichoderma* species also significantly increased 46 to 60% in seedling height, 100-127% in root exploration, and 14 to 50% in leaf area (Table 3).

DISCUSSION

Trichoderma species are well known as biocontrol agents for control of several crop diseases [7,14,18]. The enhanced growth response of several plants following application of *Trichoderma* spp. has also been well documented [4,11,21]. Consequently, the strains of *Trichoderma* species with biocontrol ability and increased plant growth will be a desirable trait for selection of biocontrol agents in Taiwan. Although several papers reported that *Trichoderma* spp could enhance crop growth [1,4,8], in this study, we only found that a few isolates (about 2.8%) of the selected isolates of *Trichoderma* spp. increased the root-length and lateral root numbers of cucumber seedlings. Moreover, rice bran/peat preparation of the promoting-growth isolates of *Trichoderma* species also significantly enhanced plant growth of cucumber and other cucurbitaceous seedlings relative to untreated seedlings in greenhouse tests.

Chang, *et al*. reported that the responses of enhancing plant growth were not observed in all crops by one strain in their experiments [6]. In our studies, the strains of different-*Trichoderma* species also showed a various degree of increased plant response among cucumber, loofah, and bitter gourd. The results suggested that various unknown factors might interact to mediate responses. The factors might result in rhizophere affinity or survival ability of these strains in different crops [4]. Enhanced root growth and plant vigor has also been observed following application of *Trichoderma* spp. to other crops. For example, Lo *et al*., [14] reported that *T. harzianum* strain 1295-22 increased plant vigor of bentgrasses. Bojrkman *et al*., [5] also reported that the fungus increased both root and shoot growth of corn.

The increased growth response induced by *Trichoderma* spp. is not fully understood. However, several possible mechanisms have been suggested to explain this phenomenon of increase plant growth. These factors may include (i) control of deleterious root microorganisms, those were not causing obvious diseases, (ii) direct production of growth-stimulating factors (i.e., plant hormones or growth-factors), (iii) increased nutrients uptake through enhanced root growth or promoted availability of necessary nutrients, (iv) reduction of the concentrations of substances in soil that are inhibitory to plant growth [11,22,23]. The increased growth response inoculated by *Trichoderma* has been reported that it is not only the result of one but also more factors [11]. Harman [8,9] also reported that *T. harzianum* strain 1295-22 could improve nitrogen use efficiency in maize and also could also solubilize a number of poorly soluble nutrients, such as Mn^{4+}, Fe^{3+} and Cu^{2+} etc. [3]. In this works, increased root exploration in soils by different-*Trichoderma* species may be one of important factors for enhancing seedling-growth of cucumber, loofah, and bitter gourd because the plants could increase the chance for nutrients uptake through the root exploration in soils. Certainly, other mechanisms still need more studies for plant growth response of *Trichoderma* spp. in this works.

The association of rhizosphere competence of *Trichoderma* strains with different crops and increase in plant growth response has not yet been determined [1,2,16,20]. Recently, plants grown from seeds treated with *T. harzianum* strain 1295-22 have been reported to produce longer roots than those grown from seeds treated with its parental strains because of its stronger rhizosphere competence. Meanwhile, strain 1295-22 also exhibited a significant improvement in the biological control of *P. ultimum* [7]. However, in this work, we found that there was no relationship between rhizosphere competence and increased plant growth response, even though there is a positive correlation between the rhizosphere competence and rhizoctonia disease control (Lo, unpublished data). Thus, rhizosphere competence may only be one of factors for enhancing plant growth by the fungal strains. Consequently, more detail studies in the various strains of *Trichoderma* different species from Taiwan are still needed in order to provide a better understanding of the mechanisms of promoting plant growth responses and of rhizosphere competence.

ACKNOWLEDGMENTS

This work was supported in part of grant (NSC89-2313-B005-011) from National Science Council of Taiwan, ROC.

LITERATURE CITED

1. Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology 77:182-189.
2. Ahmad, J. S., and Baker, R. 1988. Implications of rhizosphere competence of *Trichoderma harzianum*. Can. J. Microbiol. 34:229-234.
3. Altomare, C., Norvell, W. A., Bjorkman, T., and Harman, G. E. 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65:2926-2933.
4. Baker, R. 1991. Induction of rhizosphere competence in the biocontrol fungus *Trichoderma*. Pages 221-228. in: The Rhizosphere and Plant Growth. D. L. Keister, and P. B. Cregan, eds., Kluwer Academic Publishers, Boston.
5. Bjorkman, T., Price, H. C., Harman, G. E., Ballerstein, J., and Nielsen, P. 1994. Improved performance of shrunken-2 sweet corn using *Trichoderma harzianum* as a

bioprotectant. HortScience 29:471.
6. Chang, Y-C., Chang, Y-C., Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70:145-148.
7. Harman, G. E., Taylor, A. G., and Stasz, T. E. 1989. Combining effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatments. Plant Dis. 73:631-637.
8. Harman, E. G. 2000. The dogmas and myths of biocontrol. Changes in perceptions based on research with *Trichoderma harzianum* T22. Plant Dis. 84:377-393.
9. Harman, E. G. 2001. Microbial tools to improve crop performance and profitability and to control plant diseases. Page 71-81. In: Proceeding of International Symposium on Biological Control of Plant Diseases for The New Century-mode of action and application technology. D.S.Tzeng and J.W. Huang (ed.). NCHU, Taichung, Taiwan.
10. Inbar, J., Abramsky, M., Cohen, D., and Chet, I. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European J. Plant pathology 100: 337-346.
11. Kleifeld, O.,and Chet, I. 1992. *Trichoderma harzianum*-interation with plants and effect on growth response. Plant and soil 144:267-272.
12. Liu, S. D. 1991. Biological control of adzuki-bean root rot disease caused by *Rhizoctonia solani*. Plant Prot. Bull. 33:63-71.
13. Lo, C. T., Nelson, E. B., and Harman, G. E. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum*. Plant Dis. 80:736-741
14. Lo, C. T., Nelson, E. B., and Harman, G. E. 1997. Improved the biocontrol efficacy of *Trichoderma harzianum* 1295-22 for controlling foliar phases of turf diseases by spray applications. Plant Dis. 81:1132-1138
15. Lo, C. T. 1998. General mechanisms of action of microbial biocontrol agents. Plant Pathol. Bull. 7:155-166.
16. Lo, C. T., Nelson, E. B., Hayes, C. K., and Harman, G. E. 1998. Ecological studies of transformed *Trichoderma harzianum* strain 1295-22 in the rhizosphere and on the phylloplane of creeping bentgrass. Phytopathology 88:129-137
17. Monje, O. A., and Bugbee, B. 1992. Inherent Limitations of nonderstructiove chlorophyll meters: a comparison of two types of meters. HortScience 27:69-71.
18. Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54.
19. Porra, R. J., Thompson, W. A., and Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: Verification of concentration of the chlorophyll standards by atomic absorption Spectroscopy. Biochem. Biophys. Acta 975: 384-394.
20. Scher, F. M., Ziegle, J. S., and Kloepper, J. W. 1984. A method for assessing the root colonization capacity of bacteria on maize. J. Can. Microbiol. 30:151-157.
21. Sivan, A., and Harman, G. E. 1991. Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum*. J. Gen. Microbiol. 137:23-30.
22. Wang, C., Knill, E., Glick, B. R., and Defago, G. 2000. Effect of transferring 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase genes into *Pseudomonas fluorescens* strain CHA0 and its gacA derivative CHA96 on their growth-promoting and disease-suppressive capacities. Can J. Microbiol.46: 898-907.
23. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518-521.
24. Wu, W. C. 1991. Control of sclerotinia rot of sunflower and chrysanthemum. Plant Prot. Bull. 33:45-55.

摘 要

羅朝村[1,2]、林俊義[1]. 2002. 篩選可促進瓜類生長之木黴菌. 植病會刊11:215-220. ([1.] 台中縣霧峰鄉 農委會農業試驗所；[2.] 聯絡作者：電子郵件：ctlo@wufeng.tari.gov.tw ; 傳眞：+886-4-23338162)

由台灣各地區之作物根圈與根圈土壤分離出來的木黴菌菌株，經直接針對胡瓜種子發芽與根系生長所作的測試；顯示約有 2.8% 的木黴菌菌株可促進胡瓜主根生長與增加側根之數量。這些有益木黴菌菌株經與土壤混合至10^6 cfu/g soil 後，於溫室中作接種試驗，發現多株*Trichoderma harzianum*及*T. virens* 等主要種類菌株，除可增加苦瓜、絲瓜及胡瓜根系分佈外；植株高度、葉面積及根乾重量亦有提高之現象。另外測試瓜葉片之葉綠素亦發現有木黴菌處理者其濃度較無處理者有顯著性提高；然測試根圈纏繞能力與促進瓜類生長關係則無顯著之相關性。

關鍵詞：木黴菌、生物防治、根分布、葉綠素