INCONSISTENT GROWTH PROMOTION OF CABBAGE AND LETTUCE FROM *TRICHODERMA* ISOLATES

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ABSTRACT

Trichoderma isolates were evaluated for growth promotion effects on cabbage seedlings (*Brassica oleracea* L.) in glasshouse trials. Dipping transplants in spore suspensions (10^7 conidia/ml) of *T. longipile* (6Sr4 and 3Sr4-2) and *T. tomentosum* (5Sr2-2) increased ($P < 0.05$) leaf area (58-71%), shoot dry weight (91-102%) and root dry weight (100-158%) compared with the untreated control in one trial but not in a second. In a field trial, yield of lettuce (*Lactuca sativa* L.) treated with *T. longipile* 6Sr4 was also increased.

Keywords: cabbage, lettuce, growth promotion, *Trichoderma longipile*, *T. tomentosum*.

INTRODUCTION

In addition to their biocontrol activities, *Trichoderma* spp. have been reported to promote plant growth. Possible explanations of this phenomenon include: control of minor pathogens leading to stronger root growth and nutrient uptake (Ousley *et al.* 1993), secretion of plant growth regulatory factors such as phytohormones (Windham *et al.* 1986; Chang *et al.* 1986; Baker 1988) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley *et al.* 1994a).

In the present study, *Trichoderma* isolates which had shown biocontrol activity against *Sclerotinia* on cabbage, were evaluated for their effects on leaf area and shoot and root dry weights of cabbage seedlings in two glasshouse trials. *Trichoderma longipile* 6Sr4 was also tested on lettuce in a field trial.

METHODS

Preparation of fungal inoculum

Trichoderma longipile isolates 6Sr4, 3Sr4-2, 8Sr4-2, *T. virens* GV4 and *T. tomentosum* 5Sr2-2 were grown on oatmeal agar (33 g/litre), *T. virens* B19 and *T. longipile* S1BYG were grown on potato dextrose agar (PDA, 39 g/litre) and incubated for 21 days at 20°C under a 12 h light (irradiance 1.05 W/m²)/12 h dark regime. Conidial suspensions (100 ml) of each isolate were obtained by flooding plates with 0.2% (v/v) Triton X-100 (BDH Ltd., UK) in sterile distilled water (SDW) and the concentration of conidia was adjusted to 5×10^7 spores/ml.

Cabbage glasshouse trial 1

Cabbage seeds (*Brassica oleracea* var. Leprechaun mini) were grown in germination trays containing standard potting mix for 28 days. Roots of seedlings were then immersed in conidial suspensions of each isolate (100 ml) for 30 min before transplanting into 125 mm diameter pots containing potting mix (80% composted bark; 20% sand; 2 kg/m³ N:P:K (15:4.8:10.8); 4 kg/m³ dolomite). The untreated control was 0.2% Triton X-100 in SDW. There were seven fungal treatments and one

untreated control. The experiment was a randomized complete block design with 10 replicate blocks, with one plant per treatment per block. The glasshouse experiment ran for 42 days. The ambient air temperature in the glasshouse ranged from 5.4 to 46.6°C with a mean of 17.7±7.5°C. Total growing degree days (°Cd) accumulated 28 days after planting (DAP), were 498°Cd (above a base temperature of 0°C). A tube solarimeter was used to determine the percentage of shade in the glasshouse. Total solar radiation inside the glasshouse for 28 DAP was 173 MJ/m².

At 28 and 42 DAP, leaf area of five randomly selected plants in each treatment was measured. Washed roots, leaves and shoots were then dried to constant weight. Variables were analysed using ANOVA (SAS Institute Inc. 1989) and treatment means were separated using Fisher's protected LSD test (FLSD).

Cabbage glasshouse trial 2

Treatments, measurements and statistical analyses used were as described for trial 1 with the exception that there were two plants per treatment per replicate block. The glasshouse temperature ranged from 7.5 to 54.9°C with a mean of 21.1±7°C over the 28 days of the trial. Total growing degree days were 561°Cd and total solar radiation inside the glasshouse was 410 MJ/m².

Lettuce field trial

A conidium suspension of *T. longipile* 6Sr4 (450 ml of 10⁷ conidia/ml) was incorporated into 4.5 kg of potting mix to give a concentration of 10⁶ conidia/g potting mix. Germination trays each consisting of 135 cells (30 ml volume/cell) were filled with inoculated potting mix. Non-infested potting mix served as the experimental control. Lettuce seeds (*Lactuca sativa* cv. Yates lake) were sown into each cell and grown in the glasshouse for 28 days at 21°C.

A field site at Lincoln University was prepared using conventional cultivation and the trial ran for 9 weeks. The design was a randomised complete block with two treatments and four replicates. Each block consisted of two 1 m² plots (25 plants at 0.2 x 0.2 m spacing) to which the two treatments (untreated control or *T. longipile* 6Sr4 inoculated to transplant potting mix) were applied. Plants were watered using an overhead sprinkler system through to harvest. For the first 28 days of the trial the mean ambient temperature was 16.6°C (range 12.8-21.7°C), the growing degree days were 480°Cd and total solar radiation was 637 MJ/m².

Mean fresh weight of harvestable head was recorded and data analysed using t-tests (SAS Institute Inc. 1989).

RESULTS

Cabbage glasshouse trial 1

Three isolates of *T. longipile* (6Sr4, 8Sr4-2, 3Sr4-2) and *T. tomentosum* 5Sr2-2 increased ($P<0.05$) leaf area by 71, 32, 58 and 71%, respectively compared with the untreated control at 28 DAP (Table 1). *Trichoderma longipile* (6Sr4, 3Sr4-2) and *T. tomentosum* (5Sr2-2) also increased ($P<0.05$) shoot dry weight by 94, 91 and 102% and root weight by 142, 100 and 158% respectively, compared with the untreated control. There was no effect ($P>0.05$) of fungal treatments on shoot:root ratio (4.9-5.9) compared with the untreated control (6.1) and growth promotion was not apparent at 42 DAP.

Cabbage glasshouse trial 2

Mean leaf area and shoot and root dry weights of cabbage at 28 DAP in control plots were 7.1, 3.8 and 0.5. Higher values for leaf area, shoot and root dry weight were recorded for *T. longipile* 6Sr4-2 and *T. tomentosum* 5Sr2-2 but these were not different ($P>0.05$) to those for the untreated control plants. There was no effect ($P>0.05$) of fungal treatment on any plant parameter at 28 DAP.

Lettuce field trial

Mean weight of harvestable heads was greater ($P<0.05$) for *T. longipile* 6Sr4 treated plots (4.1 kg) than for untreated plots (2.9 kg).

TABLE 1: Mean leaf area, shoot and root dry weights (dry wt.) of cabbage plants for different fungal treatments at 28 and 42 days after planting.

Treatment	Leaf area area ($\times 10^{-2} \text{ m}^2$)	28 days after planting		Shoot: root ratio	42 days after planting	
		Shoot dry wt. dry wt. (g)	Root dry wt. dry wt. (g)		Shoot dry wt. (g)	Root dry wt. (g)
Untreated control	3.1 $\pm$ 0.5 bc ¹	1.16 $\pm$ 0.3 d	0.19 $\pm$ 0.04 d	6.1	6.51 $\pm$ 0.3	0.76 $\pm$ 0.13
<i>T. longipile</i> 6Sr4	5.3 $\pm$ 0.7 a	2.25 $\pm$ 0.3 ab	0.46 $\pm$ 0.08 ab	4.9	5.69 $\pm$ 0.5	0.89 $\pm$ 0.06
<i>T. longipile</i> 8Sr4-2	4.1 $\pm$ 0.3 a	1.63 $\pm$ 0.2 abcd	0.32 $\pm$ 0.05 abcd	5.1	5.51 $\pm$ 0.6	0.65 $\pm$ 0.12
<i>T. longipile</i> 3Sr4-2	4.9 $\pm$ 0.4 a	2.22 $\pm$ 0.2 ab	0.38 $\pm$ 0.06 abc	5.8	5.93 $\pm$ 0.6	0.75 $\pm$ 0.06
<i>T. longipile</i> S1BYG	3.3 $\pm$ 0.2 bc	1.37 $\pm$ 0.2 cd	0.26 $\pm$ 0.06 bcd	5.2	5.92 $\pm$ 0.5	0.77 $\pm$ 0.1
<i>T. tomentosum</i> 5Sr2-2	5.3 $\pm$ 0.7 a	2.35 $\pm$ 0.5 a	0.49 $\pm$ 0.11 a	4.8	6.0 $\pm$ 0.6	0.66 $\pm$ 0.13
<i>T. vires</i> B19	2.7 $\pm$ 0.2 bc	0.95 $\pm$ 0.1 d	0.16 $\pm$ 0.03 d	5.9	4.6 $\pm$ 0.3	0.29 $\pm$ 0.06
<i>T. vires</i> GV4	3.2 $\pm$ 0.4 bc	1.18 $\pm$ 0.2 d	0.21 $\pm$ 0.06 d	5.6	3.1 $\pm$ 0.5	0.48 $\pm$ 0.12
P-value	P<0.05	P<0.05	P<0.05	P>0.05	P>0.05	P>0.05
LSD	1.2	0.8	0.2	2.19	3.6	0.5

¹Means followed by same letter are not different (Fisher's protected least significant different test, P<0.05).

DISCUSSION

Cabbage plants treated with *Trichoderma longipile* 6Sr4 and 3Sr4-2 and *T. tomentosum* 5Sr2-2 had greater leaf areas (58-71%), greater shoot weights (91-102%) and greater root weights (100-142%) compared with the untreated plants in trial 1, after 28 days. There was no increase in the shoot:root ratios (dry weight basis) compared with the untreated plants which implies that the observed increment of shoot and root tissue weights occurred proportionately. This contradicts previous reports of increases in leaf area of petunia with an application of *Trichoderma* spp. being correlated with a higher shoot:root ratio (Ousley *et al.* 1994b). The growth promotion effect was not maintained through to the 42 day assessment and was not reproduced in trial 2.

The inconsistency in growth promotion may be related to differences in growing conditions as indicated by growth of the untreated control plants. In trial 1, control plants grew less vigorously than in trial 2 with lower values for leaf area and shoot and root dry weights recorded. Thus, control plants in trial 1 were probably not growing to their full potential, possibly due to suboptimal conditions even though water and nutrients were not limiting.

Over the 28 day trial period, the accumulated growing degree days was similar between trials indicating a similar temperature environment. However, the total solar radiation available was only 173 MJ/m² in trial 1 compared with 410 MJ/m² in trial 2. Thus, the energy available for photosynthesis in trial 1 was only half that in trial 2 and it seems likely any growth promotion effect would be more apparent in the lower light environment.

We postulate that when plants are grown under optimal conditions, expression of growth promotion is unlikely, whereas under suboptimal conditions, enhanced growth can be achieved. For example, Bjorkman *et al.* (1998) reported a growth promotion effect of *Trichoderma* in sweetcorn when low-medium vigour plants were treated, but

not when high vigour plants were treated. Observations for field grown lettuce support this hypothesis, with increased growth of plants treated with *T. longipile* 6Sr4. In this situation, plants were grown under suboptimal conditions due to the presence of minor pathogens (e.g. *Sclerotinia minor*).

Growth promotion by *Trichoderma* is not species dependent. In this study, two different species (*T. longipile* and *T. tomentosum*) gave a transient growth promotion effect and numerous other species such as *T. harzianum* (Chang *et al.* 1986; Inbar *et al.* 1994), *T. viride* (Ousley *et al.* 1993, 1994b) and *T. koningii* (Windham *et al.* 1986) have also been reported to promote plant growth. Similarly, within species in our study, not all isolates were capable of inducing a growth promotion effect, e.g. *T. longipile* isolate S1BYG did not show growth promotion activity. Similar results have been reported by other workers, with different strains of *T. harzianum* or *T. viride* acting differently on various host plants (Ousley *et al.* 1993, 1994b). Further, some *Trichoderma* spp. have also been reported as pathogens of maize seedlings (Edwards 1940; Falloon 1982).

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