BIOLOGICAL CONTROL OF DAMPING-OFF IN SUGAR BEET SEEDLINGS WITH *TRICHODERMA* SPECIES

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ABSTRACT

Isolates of *Trichoderma virens* and other *Trichoderma* species are effective biocontrol agents for diseases of several crops. Control of damping-off caused by *Rhizoctonia solani* has been observed in a number of crop species. To test for biocontrol activity on sugar beet, *Trichoderma* strains were grown in 5% ground wheat bran and 1% ground peat moss. The preparation was air dried and applied to seed with a latex sticker. Seeds were planted in greenhouse potting mix with either sterile vermiculite or *R. solani* grown on sterile barley and mixed with vermiculite. The survival of sugar beet (FC403) seedlings under severe *Rhizoctonia* pressure was compared for different *Trichoderma* isolates in the greenhouse at 7, 14, and 21 days. Seedling roots were collected, washed and plated to determine fungal colonization. All of the *Trichoderma* isolates colonized sugar beet radicles and young roots well. Some fungal isolates significantly improved seedling emergence and survival in greenhouse tests. There was no significant correlation between *in vitro* antibiosis against *R. solani* and biological control activity. However, a biocontrol-effective strain induced a higher level of peroxidase activity in seedling roots grown in sterile glass dishes than did a biocontrol-ineffective strain.

INTRODUCTION

Rhizoctonia solani Kühn causes damping-off of sugarbeet (*Beta vulgaris* L.) seedlings (Whitney, E.D. & Duffus, J.E., 1986).. While resistance to *R. solani* is available in sugarbeet (Ruppel, E.G. & Hecker, R.J., 1994), this resistance is poorly expressed in seedlings. Thus other control methods are desirable. Species of the fungal genus *Trichoderma* have biocontrol activity against a number of plant pathogenic fungi on various hosts (Papavizas, G.C., 1985). Control of seedling damping off caused by *R. solani* on various crops has been reported with *Trichoderma (Gliocladium) virens* Miller et al. (Howell, C.R. et al., 2000; Lumsden, R.D. & Locke, J.C., 1989). In addition, *Trichoderma harzianum* Rifai aggr. has reported activity against Rhizoctonia root rot on sugarbeet, but not on sugarbeet seedlings (Ruppel, E.G. et al. 1983).

Several possible biocontrol mechanisms have been proposed for *Trichoderma*. Biocontrol strains of *Trichoderma* often produce antibiotics with activity against fungal pathogens (Dunlop, R.W. et al., 1989; Ghisalberti, E.L. & Rowland, C.Y., 1993; Howell, C.R. et al., 1993). In addition, biocontrol-effective *T. virens* strains induce the production of defense compounds, such as phytoalexins and peroxidase, in the roots of cotton seedlings; an induction that correlates with

biocontrol activity against *R. solani* (Howell et al. 2000). The object of this research was to examine strains of *Trichoderma* species for biocontrol activity against seedling *R. solani* on sugarbeet and to investigate factors that may play a role in biocontrol activity.

1.- MATERIALS AND METHODS

1.1.- BIOCONTROL AGENTS EXAMINED

Several Isolates used included *T. virens* strains G6, an isolate from Texas cotton field soil with good biological control activity on cotton (Howell, C.R. et al., 1993); SB-1, an isolate from CO sugar beet seed; LH-2, an isolate from CO sugar beet root; G6-4, a UV mutant of isolate G6 that has lost biocontrol activity; and *T. koningii* strain Tk7, an isolate from Texas wheat field soil.

1.2.- TESTING BIOCONTROL ACTIVITY

Biocontrol activity was tested by growing *Trichoderma* isolates in shake cultures of 100 ml of 5% wheat bran and 1% peat moss (WBPM, pH 4.0) as described by Howell, C.R. *et al.* (1997). The solid fractions from 7-day-old cultures were air dried, ground to a fine powder, and stored at 4°C. WBPM preparations were applied with a latex sticker to sugar beet seed ['FC403' (Hecker, R.J. & Lasa, J. M., 1992)] at 0.01 g/seed and the seeds were planted in trays containing non-sterile soilless planting mix. Nine seeds were planted per tray, with three trays per treatment. An air-dried and ground preparation of *R. solani* [AG2-2, strain R9 (Pierson, V.G. & Gaskill, J.O., 1961)], grown on sterile barley seed at 25EC in the dark, was mixed with ground sterile vermiculite and sprinkled over the seeds before covering with planting mix. Trays were randomly placed in the greenhouse and watered daily. Emerged seedlings were counted weekly and, at the end of three weeks at 75EC, the number of surviving seedlings was recorded for each tray. Controls were coated with latex sticker and sterile WBPM and grown with or without the pathogen.

1.2.1.- ROOT COLONIZATION

Root colonization was determined by plating three 1-cm sections from washed roots of two plants per tray. They were plated on potato dextrose agar (PDA) containing streptomycin sulfate and penicillin G at 25EC. Plates were examined daily for fungal growth. One week after plating, root sections were examined under the microscope to determine the presence of *R. solani* and the biocontrol agents.

1.2.2.- ANTIBIOSIS

Antibiosis was examined by plating four 4 mm plugs of the *Trichoderma* strains equidistant from a 6 mm plug of *R. solani* on PDA. Plates were incubated at 24 C and examined daily. Antibiosis was measured as the reduction in growth of *R. solani* towards *Trichoderma* plugs compared to growth toward PDA plugs with no *Trichoderma*.

1.2.3.- PEROXIDASE ACTIVITY

To determine peroxidase activity, sugarbeet seed ('FC403') was coated with a WBPM preparation of the biocontrol-effective strain G6 or a biocontrol ineffective strain, G6-4, as above. Seeds were planted in autoclaved moist vermiculite. Seedlings were harvested five days later. Five replicates of 12 randomly selected seedlings were used for each treatment. Roots and shoots were separated, weighed, and ground in 0.1 M sodium phosphate buffer (pH 6.0) at 2µl buffer per mg tissue. Ground material was centrifuged at 12,000 rpm in a microcentrifuge at 7 C for 15 min. Supernatants were assayed for peroxidase activity by the guiacol assay as described by Smit, F. & Dubery, I.A. (1997).

2.- RESULTS

Isolates differed significantly in biocontrol activity, as measured by seedling emergence and survival at 3 weeks post emergence (Table 1).

Table 1 Sugarbeet seedling emergence and survival in planting mix infested with Rhizoctonia solani AG2-2 strain R9.

Treatment	Percent emergence*	Percent survival*
R. solani control	36.5 c**	31.7 c
G6	100.0 a	93.7 a
SB-1	82.5 a	82.5 a
LH-2	60.3 b	60.3 b
G6-4	37.0 c	35.0 c
Tk-7	40.5 bc	40.3 bc

** All numbers are given as percent of emergence in the absence of R. solani (which was 63%).*

*** Values with different letters are significantly different p=0.05.*

1. All the *Trichoderma* isolates colonized sugar beet roots. No visual differences were seen among isolates.
2. *Trichoderma* isolates G6, SB-1, and Tk-7 inhibited *R. solani* growth on PDA, while G6-4 and LH-2 did not.
3. No significant difference was found in peroxidase activity of sugarbeet shoots following treatment with a *T. virens* biocontrol strain versus an ineffective strain, but activity was significantly higher in roots treated with a biocontrol-effective strain than in roots treated with an ineffective strain (Table 2).

Table 2 Effect of seed treatment with T. virens or a control on peroxidase activity in sugar beet roots and shoots.

Treatment	Root*	Shoot*
G6-4 (biocontrol ineffective)	14.74 ± 1.12 pkat	8.62 ± 0.97 pkat
G6 (biocontrol effective)	17.57 ± 1.34 pkat	9.65 ± 0.39 pkat
t-test	P = 0.04	P = 0.13

**Results are the average of five replicates of 12 plants each.*

CONCLUSION

1. Isolates of *Trichoderma virens* show biocontrol activity against sugarbeet seedling damping-off caused by *Rhizoctonia solani* in the greenhouse.
2. All isolates colonized roots well, so differences in activity were unlikely to be due to differential colonization.
3. Some biocontrol-effective isolates showed antibiosis against *R. solani* while others did not. Similarly, some biocontrol-ineffective isolates showed antibiosis, while others did not. Antibiosis is unlikely to be important in biocontrol activity.
4. Peroxidase activity was significantly higher in roots of plants treated with a biocontrol effective strain than in roots treated with an ineffective strain. Stimulation of resistance responses by biocontrol strains may play a role in biocontrol activity.

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