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Short communication

Effect of *Trichoderma* colonization on auxin-mediated regulation of root elongation

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Abstract

The biocontrol fungus *Trichoderma harzianum* 1295-22 increases root growth in addition to roles in suppressing disease. Its agricultural use could be expanded if the mechanism of growth enhancement were known. Among the proposed mechanisms of growth enhancement is that the fungus counteracts auxin inhibition of root-cell elongation. We tested whether there was evidence for a secreted auxin inhibitor, for enhanced auxin degradation, or for altered auxin sensitivity. Our results provide no support for any of these mechanisms. *Trichoderma* secretions inhibited growth, whereas an auxin inhibitor would increase growth. Auxin inhibited growth to the same extent in colonized and uncolonized roots, indicating no change in auxin sensitivity. Endogenous auxin levels maintained growth closer to the maximum in uncolonized roots, indicating stronger auxin limitation of growth in colonized roots. These tests indicated that *Trichoderma*-colonized roots had a faster maximum growth rate, but an unchanged response to auxin.

Abbreviations: IAA – indole acetic acid

Introduction

The root-colonizing fungus *Trichoderma harzianum* Rifai 1295-22 enhances root growth (Björkman et al. 1998; Harman and Björkman 1998), and is used for this purpose in crop production. A number of mechanisms for growth enhancement have been proposed (Harman et al. 2004). Among these, fungal interaction with auxin signaling has not been examined despite auxin being a major agent of growth regulation. This investigation examined the effect of *Trichoderma* on auxin inhibition and sensitivity, as well as the auxin-independent maximum growth rate, of root tips.

Materials and methods

Sweet corn (*Zea mays* L. 'Supersweet Jubilee') seedlings were used when the roots were about 5 cm long. Roots were colonized with *T. harzianum* strain 1295-22, known commercially as T-22, by inoculating dry seeds with 10^5 cfu spores (RootShield, Bioworks Inc, Geneva, NY). This moderate-vigor *sh2* corn variety has a pronounced growth response to such *Trichoderma* colonization (Björkman et al. 1998).

In vitro *auxin assay*

Growth responses to auxin were measured on excised root tips to eliminate endogenous auxin

(Scott and Wilkins 1968). The apical 40.0 mm of uncolonized seedling roots were incubated in test medium for 4 h, then remeasured. The test medium contained 2% sucrose, 5 mM KCl, 0.5 mM $CaCl_2$, and 0.1 mM MES-KOH, pH 6.0 (Björkman and Cleland 1988). Seventeen roots were used for each treatment, and the entire experiment was repeated three times with comparable results. The greatest power to detect relief of auxin inhibition was at 10^{-7} M indole acetic acid (IAA), which reduced growth to 10–20% of control (data not shown).

Culture filtrate addition

In tests for auxin inhibitor production, the test medium was supplemented with 10^{-7} M IAA or a 5% sucrose–yeast extract medium (Altomare et al. 1999) in which *T. harzianum* 1295-22 had been grown to sporulation and the nutrients depleted. Roots were not colonized by *Trichoderma*.

Auxin inactivation test

To test for changes in response to exogenous auxin, the test medium was supplemented with 10^{-7} M IAA in the auxin treatment. Both colonized and uncolonized roots were used.

Maximum growth

To test the effect on maximum growth rate, intact seedlings were mounted vertically in a humidified box. The apical 5 mm were submerged in the test medium for 30 min, then growth over the next 4 h measured. Maximum growth rate was obtained by supplementing test medium with 3 μM fusicoccin (Pilet 1976). Two-way analysis of variance was used to determine main effects and interaction.

Results and discussion

Three potential mechanisms whereby enhanced growth of *Trichoderma*-colonized roots could be mediated by auxin were investigated. First, does the fungus secrete a compound that antagonizes the growth inhibition of root elongation by auxin? Second, does the fungus reduce the activity of auxin? Third, does the fungus change the growth rate by reducing auxin sensitivity? The alternative

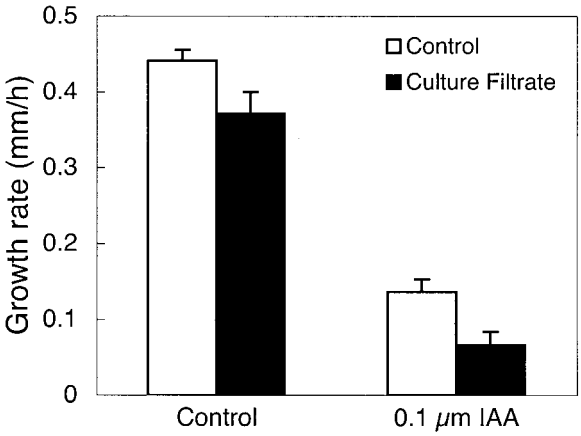

Figure 1. Growth response of *Z. mays* root segments to culture filtrate from *T. harzianum* 1295-22 in the presence and absence on 0.1 μm IAA in the incubation medium. Apical root segments were incubated for 4 h in medium supplemented according to treatment with 0.1 μm IAA and 5% of a nutrient depleted medium in which *T. harzianum* 1295-22 had grown to sporulation.

is that metabolic effects unrelated to auxin increase the maximum growth rate.

Root growth would be enhanced by colonization if *T. harzianum* 1295-22 secretes antiauxin. At endogenous concentrations, auxin regulates growth to be at a rate lower than the maximum (Audus and Das 1955). An antiauxin would increase the growth rate. A variety of compounds secreted by fungi are antiauxins. For example, the benzaldehyde derivative, epoxydon (Arie et al. 1998); pyruvoylaminobenzamide (Kimura et al. 1973); and the indole, hypaphorine (Reboutier et al. 2002). *Trichoderma* culture filtrate slightly inhibited growth rather than increasing it (Figure 1). Elongation was reduced 0.31 mm/h by 10^{-7} M IAA ($F = 2143$, $P < 0.0001$) and 0.07 mm/h by culture filtrate ($F = 11.5$, $P = < 0.005$). The interaction was 0.00 mm/h ($F = 0$, $P = 1$). Whether growing rapidly in buffer or slowed by IAA the roots grew slower in the presence of culture filtrate. The lack of growth enhancement is evidence that *T. harzianum* 1295-22 does not act by secreting antiauxin compounds.

The potential mechanisms of growth enhancement through physiological interaction can be placed in three categories. Those affecting auxin sensitivity, auxin activity, and auxin-independent increases in the maximum with the actual growth rate regulated by auxin. Colonization by *T. harzianum*

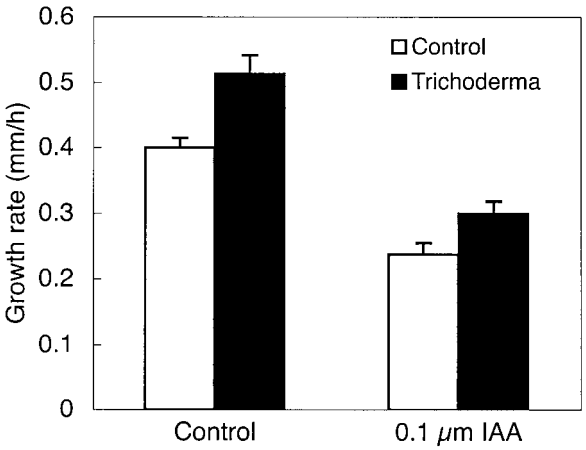

Figure 2. Effect of colonization by *T. harzianum* 1295-22 on the growth response of *Z. mays* root segments to 0.1 μm IAA. Apical segments of roots that were either uncolonized, or colonized since imbibition with *T. harzianum* 1295-22, were incubated for 4 h in medium supplemented according to treatment with 0.1 μm indole acetic acid.

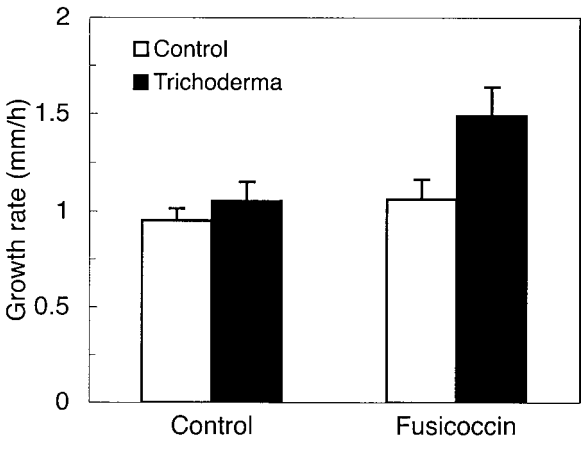

Figure 3. Effect of colonization by *T. harzianum* 1295-22 on the maximum growth rate of intact *Z. mays* roots. Fusicoccin caused roots to maximize their growth rate. Root tips of intact seedlings were incubated in water or 3 μM fusicoccin for 30 min, then grown for 4 h. Roots were either uncolonized or colonized with *T. harzianum* 1295-22 since seed imbibition.

1295-22 could reduce auxin activity by conjugation (LeClere et al. 2002; Ljung et al. 2001, 2002), catabolism (Kerk et al. 2000) or reduced transport to the sensitive tissue (Ljung et al. 2001). Microbes have such effects: conjugation of IAA by a microbial enzyme reduces its activity (Romano et al. 1991); some fungi catabolize auxin (Tramier and Gueselaine 1977; Reddy and Reddy 1987); and fungal metabolites disrupt polar auxin transport (Kim et al. 2000). The maximum growth rate can be affected by many fungal processes, such as a less oxidative environment, improved metal uptake, induced resistance, and wall-degrading enzymes (Harman et al. 2004).

Effects on auxin sensitivity can be distinguished from the other two mechanisms by examining the effect of added auxin. If sensitivity is affected, exogenous auxin will reduce the growth rate less in colonized than in control roots. If only the maximum growth rate is increased, exogenous auxin would affect growth to the same extent. If activity is reduced, uncolonized roots will grow faster in the presence of exogenous auxin to an extent dependent on how effectively auxin activity is reduced. Auxin suppressed growth of colonized and uncolonized roots to the same extent (Figure 2). From an initial growth rate of 0.40 mm/h, elongation was reduced 0.19 mm/h by 10^{-7} M IAA ($F = 12.3$, $P < 0.001$) and increased 0.09 mm/h by *Trichoderma* colonization ($F = 2.7$, $P = 0.11$). There was no significant positive interaction (interaction $= -0.002$ mm/h, $F = 0.22$, $P = 0.64$). This result is not consistent with a change in sensitivity, but is consistent with the last two mechanisms.

An auxin-independent increase in the maximum growth rate can be distinguished from reduced auxin activity by driving the roots to their maximum growth rate with the addition of fusicoccin. Fusicoccin maximizes acid growth of maize roots for at least 8 h (Pilet 1976) by enhancing the activity of expansins in the cell walls (Link and Cosgrove 1998), bypassing auxin regulation. If the enhanced growth is due to lower auxin sensitivity, the fusicoccin-stimulated elongation rate would be equal in colonized and control roots. Colonized roots would also grow closer to maximum (fusicoccin-stimulated) rate. If colonization increases the maximum growth rate, colonization would increase FC-stimulated growth. Fusicoccin-stimulated growth was 40% higher in intact roots colonized by *T. harzianum* 1295-22 than uncolonized roots (Figure 3). Furthermore, they did not grow closer to their maximum growth rate. The actual growth rate was 89% and 71% of the maximum in uncolonized and colonized roots, respectively (Figure 3). These data support the model that auxin sensitivity is unaffected by colonization, while the fusicoccin-stimulated maximum growth rate is raised.

None of these tests found evidence for it being mediated by a change in auxin regulation of root growth. The increased growth of *Trichoderma*-colonized roots can be explained by an ability to sustain a higher elongation rate. This conclusion is consistent with the observation of other metabolic enhancements following colonization by this strain of *Trichoderma* (Harman and Björkman 1998; Harman et al. 2004).

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