TOXICITY OF COTTON PHYTOALEXINS TO THE SEEDLING DISEASE PATHOGEN *RHIZOCTONIA SOLANI*

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Abstract

Certain strains of *Trichoderma* (*Gliocladium*) *virens* are effective agents for controlling cotton seedling diseases caused by *Rhizoctonia solani*. To gain an understanding of the biocontrol mechanisms of *T. virens*, interactions of *R. solani* and *T. virens* with cotton are being investigated. Recently, it has been reported that *R. solani* and *T. virens* cause increased phytoalexin (PA) levels in cotton seedling roots. The PA's that were affected include hemigossypol (HG), desoxyhemigossypol (dHG), hemigossylic acid lactone (HGAL), hemigossypol-6-methyl ether (MHG), desoxyhemigossypol-6-methyl ether (dMHG), gossypol (Goss), gossypol-6-methyl ether (MG) and gossypol-6,6'-dimethyl ether (DMG). It is significant that the PA stimulation effect is greater for biocontrol effective *T. virens* (with or without the added presence of *R. solani*) than it is for *R. solani* alone or ineffective *T. virens*. To determine the possible importance of PA stimulation to the biocontrol mechanism, the toxicities of the cotton PA's towards *R. solani* were evaluated using lethal dose (LD100) and effective dose (ED50) bioassay methods. In the LD100 method, macerated *R. solani* strain J1 was suspended in a buffered nutrient medium, mixed with solutions containing different PA concentrations and then pipetted into 24-well tissue culture dishes. The dishes were incubated for 48h then the fungus+PA suspensions were transferred to potato dextrose agar plates. After 72-96h, the plates were examined for live fungus and the lowest PA concentration that had no live fungus was noted as the LD100 value. The LD100's are 7-10μg/mL for dMHG, 7-10μg/mL for dHG, 10-15μg/mL for MHG and 20-25μg/mL for HG. Due to solubility problems, the LD100's for Goss, MG and DMG could not be determined exactly but the results do indicate that the values are greater than 60μg/mL. In the ED50 bioassay method, the macerated *R. solani* strain J1 was filtered to give a suspension composed predominantly of 75-180μm hyphal fragments in buffered nutrient medium. The hyphal suspension was mixed with PA solutions of different concentrations then pipetted into 96-well microtest culture dishes. After incubation for 72-84h, the optical densities of the fungus+PA suspensions were read at 725nm. At each PA concentration, a fractional growth effect was obtained by comparison of the fungus+PA suspension OD with a fungus-only control OD. The PA concentrations and fractional effect data were used to calculate an ED50 value. The ED50 bioassay results correlate with the previously discussed LD100 test findings. dMHG and dHG have very similar toxicities towards *R. solani* with ED50's of 0.65±0.11μg/mL and 0.87±0.10μg/mL, respectively, while MHG with an ED50 of 3.60±0.55μg/mL is more potent than HG with an ED50 of 5.79±0.72μg/mL. Additionally, it was found that Goss (with an ED50 of 15.6±2.3μg/mL) is much less toxic to *R. solani* than are dMHG, dHG, MHG or HG. As in the LD100 work, PA solubility limitations prevented the determination of ED50 values for MG and DMG; however, the results did show that the ED50's for MG and DMG are greater than 23μg/mL. Therefore, these compounds are less toxic to *R. solani* than their unmethylated Goss parent is.

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