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RESISTANCE TO RHIZOCTONIA SOLANI AND ALTERNARIA ALTERNATA IN TRANSGENIC COTTON EXPRESSING AN ENDOCHITINASE GENE FROM TRICHODERMA VIRENS

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Abstract

Expression of anti-fungal proteins in plants offers a possibility to develop resistance against fungal diseases. Transgenic expression of the endochitinase genes isolated from a mycoparasitic fungus, *Trichoderma harzianum* has been shown to confer resistance to several fungal pathogens in potato, tobacco, apple, grape, broccoli and petunia. We examined the effectiveness of constitutive expression of several endochitinase genes from *T. virens* in protecting cotton from fungal diseases. Three cDNA clones (*Tv-ech1, Tv-ech2, and Tv-ech3*) and a genomic clone (*Tv-ech1g*) isolated from *T. virens* were tested individually for their expression in transgenic cotton and tobacco. In both species, the transgenic endochitinase activity was observed only in the plants that were transformed with the *Tv-ech1* construct. Eighty-two independent cotton lines transformed with *Tv-ech1* clone were examined for transgene expression using a quantitative, fluorometric method. Seventy-one of the tested lines showed endochitinase activities higher than the background levels observed in untransformed control plants. Southern hybridization analysis on some of the lines showed the presence of one to three copies of the endochitinase transgene. The expression of the transgene was confirmed by Northern blot analysis by using the cDNA fragment of the endochitinase gene as the probe. A gel-based, fluorescence assay confirmed the presence of the 42 kDa endochitinase band in the protein extracts from the lines showing high level expression of the transgene. The quantitative, fluorometric assay was used also to examine transgene expression in various tissues from three selected lines over three generations. Transgenic endochitinase activity was observed in leaves, roots, hypocotyl-root junctions, basal hypocotyl segments, and the seeds suggesting a constitutive expression of the transgene.

Homozygous T_2 seedlings from three selected, high endochitinase-expressing lines were subjected to disease resistance analyses. Thirty seeds each from the transgenic lines and 60 seeds from a non-transgenic control were germinated in soil infested with *Rhizoctonia solani* and the disease severity was examined after one week or two weeks following seeding in two separate experiments. The control seedlings showed severe disease symptoms after one- or two-week assay with the disease indices (mean±SE) of 73.3 ± 4.5 and 91.1 ± 0.9, respectively. However, the seedlings from all the three transgenic lines, in both experiments, showed a significant level of resistance to *R. solani* with the disease indices (mean±SE) ranging from 10.0 ± 1.9 to 26.7 ± 6.7. In the third experiment, 30 seeds each from a control and one of the transgenic lines were germinated in soil infested with double the *R. solani* inoculum pressure. After nine days of exposure to the fungus, 70% of the germinated control seedlings died as a result of severe disease and the remaining seeds failed to germinate. Significantly, 50% of the seedlings from the transgenic line remained healthy, while 33.3% seedlings showed a range of symptoms. Ten percent of the transgenic seedlings died following germination and 6.6% of the seeds did not germinate. In this experiment, the disease indices (mean±SE) for the control and the transgenic line were 88.3 ± 1.0 and 28.9 ± 3.6, respectively.

In addition to the soil-borne pathogen *R. solani*, the transgenic plants were also tested for resistance to *Alternaria alternata*, a foliar pathogen. The disease resistance assay for *A. alternata* was performed by placing agar plugs containing the fungal mycelia on detached leaves from three to four week-old plants. The disease severity was estimated by measuring the percentage of leaf area showing the disease symptoms two weeks following inoculation. In the first experiment, five leaves each from control plants and plants from three different transgenic lines were tested with two agar plugs on each leaf. Severe disease symptoms were observed on control leaves [mean percentage area (±SE) showing disease symptoms = 87.3 ± 14.2], while the transgenic leaves showed varying levels of resistance to *A. alternata*. The mean percent disease area (±SE) for the three transgenic lines were 49.2 ± 17.4, 32.2 ± 8.9, and 26.1 ± 20.9. In the second experiment, 10 leaves each from control plants and plants from three different transgenic lines were tested with one agar plug on each leaf. In this experiment with the lower level of infection pressure, the mean percent disease area (±SE) for the control was 49.9 ± 13.4. Again, the leaves from the three transgenic lines showed significant levels of resistance with mean percent disease area (±SE) of 1.7 ± 0.7, 1.1

± 0.3, and 1.5 ± 0.6, respectively. These results with *R. solani* and *A. alternata* provide the first convincing demonstration of the usefulness of a mycoparasite-derived endochitinase gene in conferring resistance to two types of fungal pathogens in cotton (Emani *et al*., 2003, Plant Biotech. J. 1:321-336).