A NOVEL NONHEME HALOPEROXIDASE GENE FOR DISEASE RESISTANCE IN TRANSGENIC COTTON AND TOBACCO

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Abstract

Genetic transformation of cotton and tobacco were carried out with a novel gene, chloroperoxidase (CPO), from *Pseudomonas pyrrocinia.* Chloroperoxidases and other haloperoxidases convert hydrogen peroxide to much more potent antimicrobial compounds, hypochlorous acid and peracetic acid.

We tested the potential of chloroperoxidase as an antifungal agent using transgenic tobacco model system. A large number of transgenic tobacco plants producing chloroperoxidase were obtained by *Agrobacterium*-mediated transformation. Successful transformation was shown by *npt* II ELISA, PCR, Southern, northern and western blot analyses. Using the substrate, monochlorodimedon, the halogenating activity of the chloroperoxidase enzyme was also demonstrated in transgenic tobacco leaf extracts. Plant extracts from tobacco plants transformed with the chloroperoxidase gene significantly reduced the number of fungal colonies arising from germinating conidia of both *Aspergillus flavus* and *Verticillium dahliae* compared to the extracts from the non-transformed control. For example, leaf extracts from transformed tobacco inhibited the growth of germinating conidia of *A. flavus* by more than 90%. Leaf extracts from transgenic tobacco also reduced the number of colonies arising from germinating conidia of *Fusarium moniliforme*, although the results were not significant. The transformed tobacco plants showed greater levels of disease resistance *in planta*, against a fungal pathogen, *Colletotrichum destructivum*, which causes anthracnose and a bacterial phytopathogen, *Pseudomonas syringae* pv. *tabaci*, which causes the disease, fireblight.

Parallel transformation experiments with cotton have also been carried out. Analyses to date indicate successful transformation as shown by *npt* II ELISA, PCR-Southern and by the demonstration of enzymatic activity in transgenic cotton cells. We have just begun assaying the chimeric cotton callus cells for antifungal activity. Preliminary results with extracts from selected cotton callus colonies indicate significant antifungal activity against germinated spores of *A. flavus* and *V. dahliae*. Several of the transgenic callus colonies have been induced to form somatic embryos and we are in the process of regenerating plantlets for further evaluation in the greenhouse.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:446-446 (1999)
National Cotton Council, Memphis TN