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MODIFICATION OF A BACTERIAL CHITOSANASE GENE FOR PLANT EXPRESSION AND ENHANCEMENT OF ANTIFUNGAL DEFENSE RESPONSES

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Abstract

Presently, farmers rely on fungicides and cultural practices to minimize losses due to pathogenic fungi, but in the future, other more economical and environmentally friendly options may become available. By utilizing a plant's own signaling and defense pathways it may be possible to create plants that are resistant to fungal attack. There is much to be learned about the specifics of these complex pathways, but many compounds capable of initiating plant defense responses have been identified. Our study investigates the practical applications of one such compound, chitosan. Chitosan is one of the key structural elements found in many fungal cell walls and has been shown to be an effective elicitor when applied to cucumbers, tomatoes, tobacco and other plants. It may be possible to exploit this response pathway by genetically modifying plants to over-express an extra-cellular chitosanase. This enzyme is capable of degrading the chitosan found in fungal cell walls and may be able to stimulate, via the generation of oligomeric cell wall fragments, a plant defense response that could ward off the invading fungi. To meet these ends, a highly active bacterial chitosanase has been cloned and sequenced. An *Arabidopsis thaliana* extra-cellular chitinase transit peptide was added to the mature protein region by primer extension, and the 35S Cauliflower Mosaic Virus promoter and nopaline synthase terminator were added to regulate *in planta* expression. The entire cassette was fused into a plant transformation vector, pPZP 211, that facilitated *Agrobacterium tumenfaciens* mediated leaf disk transformation of *Nicotiana tabacum.* Several high expressing T_0 transformants were selected and will be assayed for enhanced signal transduction and fungal resistance. If the strategy is verified in the tobacco model system, cotton will be transformed with the same or similar gene construct.