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EVALUATION OF A TRANSGENIC CHITOSANASE AS AN ANTI-FUNGAL AGENT

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Chitosanase is an enzyme, similar to chitinase, capable of hydrolyzing the β-1,4-linkages between D-glucosamine residues or between N-acetyl-D-glucosamine and D-glucosamine residues in partially acetylated chitosan polymers found in fungal cell walls. When attacked by pathogenic fungi, many plants exploit this hydrolytic action as a component in a broader, post-attack defense response, but these enzymes may also play a role in the initial plant-pathogen interaction via the generation of elicitors resulting from the hydrolysis of fungal cell walls. To gain insight into these mechanisms, a *Paenbacillus* chitosanase was cloned, sequenced, and modified for plant expression in a tobacco (*Nicotiana tabacum* L. cv. *Xanthine*) model system, for ease of tissue transformation and regeneration. The modified gene was delivered to tobacco leaf disks via *Agrobacterium tumenfaciens*-mediated transformation, and whole plants were regenerated from the transformed cells. The putative GMOs were tested for transgene integration, transcription, and translation. Confirmed transformants were then screened for enhanced responses to a *Rhizopus* spp. cell wall preparation by measuring time-course production of hydrogen peroxide and phenylalanine ammonia lyase. These compounds play roles at different points in a pathogenesis-related signal transduction pathway and, thus, allow for an initial assessment of the global defense response. Transgenic tobacco constitutively expressing the *Paenbacillus* chitosanase exhibited enhanced activation of pathogenesis-related defense responses. These results suggest cell-wall degrading enzymes play a role in pathogensis-related signal transduction, and that constitutive expression of chitosanase increases the rate at which a challenged plant can respond to attack.