EXAMPLE 9: DISSERTATION ABSTRACT PAGE

DISSERTATION ABSTRACT

GENETIC MANIPULATION OF

ECTOMYCORRHIZAL

FUNGI

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Genetically Modified strains of ectomycorrhizal fungi could be important for the improvement of plant growth and yield. Methods to genetically alter species of ectomycorrhizal fungi are described. Genetic manipulation was accomplished by the induction of mutations and DNA-mediated transformationusing protoplasts of ectomycorrhizal fungi.

Conditions for the formation and regeneration of protoplasts from selected species of ectomycorrhizal fungi are described. The age of the fungal culture and extent of incubation in a lytic enzyme mixture are critical factors for efficient information of protoplasts. There is a correlation between the distribution of nuclei in protoplasts and the frequency of protoplast regeneration.

Protoplasts of *Laccaria laccata* were transformed at frequencies of 5 to 50 transformants per ug of DNA. Transformation was based on positive selection for resistance to hygromycin B using an *Escherichia coli* aminocyclitrol phosphotransferase gene bracketed by an *Aspergillus nidulans* glyceraldehyde-e-phosphate dehydrogenase promoter and the transcription terminator region of the *Aspergillus nidulans* tryptophan synthetase gene. Southern blot hybridization revealed that hygromycin B-resistant transformants have integrated vector sequences involving one or more insertional events.

These results provide the first evidence for genetic transformation of an ectomycorrhizal fungus. The potential to improve ectomycorrhizal symbiosis through transgenic manipulation of the fungal component can now be realized, provided specific genes beneficial to this symbiosis can be identified.