Transformation of Bermudagrass for Improved Fungal Resistance

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Objectives:

1. *Isolate, identify, and characterize chitinases and glucanases and their genes that have high anti-SDS activity.*
2. *Develop an efficient protocol to transform (genetically engineer) bermduagrass.*
3. *Transform and characterize bermdagrass with the antifungal chitinase and/or glucanase genes directed against the spring dead spot casual organism.*

Spring dead spot is a major bermudagrass disease in the Southern United States. The causal agent is *Ophiosphaerella herpotricha* and *Ophioshpearella korrea*. The most susceptible varieties include many high quality vegetatively propagated bermudas. Improvement in resistance in these sexually isolated lines require the development of genetic transformation protocols. The objectives of this work is to first develop an efficient bermudagrass transformation system, second, to isolate genes and/or factors with specific activity against the causal agent of spring dead spot and other fungal diseases, and third, to utilize these agents or genes to increase resistance to fungal diseases.

Transformation of the bermudagrass variety Brazos was performed using the biolistics bombardment with DNA containing an expression cassette that include the bar gene coupled to a constitutive ubiquitin promoter. The bar gene codes for an enzyme that metabolizes the herbicide Liberty. After bombardment, Liberty was used in the selection media to screen for resistant transgenic tissues. Over 671 potential transformants were recovered from bombarded tissues. Current efforts to screen transgenic plants using PCR and Southern hybridization techniques identified three consistent positives (P252, P303, and P319). Of the three positives, P303, showed more intense hybridization possibly indicating incorporation of multiple copies of the bar gene. Furthermore, in an herbicide swipe test P303 showed higher levels of resistance to the herbicide Liberty. Further work to exhaustively screen all 671 transformants is in progress.

A microorganism with potent activity against *0. herpotricha* was recently discovered and characterized. Activity of the antifungal factor was stable *in vitro* over a six month period. The microorganism was identified to the genus level using GC-FAME and Biolog analysis. Activity was due to the secretion of a potent antifungal factor that was stable under the harshest conditions. Isolation of the factor using preparative SDS PAGE and reverse phase chromatography was successful. The compound was identified through mass spectroscopy and other analytical techniques. Currently we are interested in developing either the microorganism or the antifungal factor as a biocontrol agent.

Chitinases are well known enzymes that possess potent antifimgal activity. To determine if chitinases play a role in the resistance mechanism against *0. herpotricha* we inoculated pots with the fungus, grew the plants for more than a month to establish the infection and then transferred the plants to a 15 C temperature, a temperature more conducive to *0. herpotricha* growth. We developed a novel protocol to isolate individual chitinase isozymes and determine enzyme activity. Isozymes were separated according to their isoelectric point. Infection with *0. herpotricha* resulted in a doubling of basic chitanases in resistant Midiron, but not Tifgreen. Futhermore, northern blot analysis clearly shoed an increase in basic chitinase gene expression at 15 C. This data indicates that basic chitinasese may have a role in disease resistance in bermuda crown tissues.