A Multigene-Transfer Strategy to Improve Disease and Environmental Stress Resistance in Creeping Bentgrass

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Objectives:

1. *Enhance the expression (increase level of pest resistance) of the American elm chitinase gene in creeping bentgrass.*
2. *Transfer two drought-resistance genes controlled by either a constitutive or an ABA-inducible promoter into creeping bentgrass.*
3. *Determine disease resistance of transgenic plants expressing different levels of the chitinase gene and transgenic plants containing single-versus multiple-inserted genes under green house and field conditions.*
4. *Determine environmental stress resistance of transgenic plants containing single-versus multiple-inseted genes grown under greenhouse and field condiditons.*
5. *Evaluate transgenic creeping bentgrass clones for turf quality characteristics under field conditions.*
6. *Release transgenic creeping bentgrass germplasm with combined improvements in turf quality and pest and stress resistance to Pure Seed Testing, Inc. and/or other sectors for use in their field testing and commercial breeding program.*

Major biotic and abiotic problems associated with the management of creeping bentgrass turf include several pathogenic disorders and certain environmental extremes such as drought, heat, and cold stress. Environmental extremes such as drought can influence the health of the plant and its ability to resist infection by biotic agents.
Resistance to biotic and abiotic stresses in plants has been reported to be associated with relatively complex genetic factors. Most biotechnological approaches of the last two decades, especially those related to the control of insects and diseases, have concentrated on transferring a single gene to plants. The single gene approach may sound attractive over a short period of time, however, this approach may result in more serious problems over longer periods of time as populations of biotic agents develop resistance to the single gene. Our long term goals include development of transgenic turfgrasses with improved resistance to pathogens and drought tolerance.
Previously, Sticklen's research team developed creeping bentgrass clones that contain: a glufosinate ammonium (Liberty) herbicide resistance gene; a chitinase gene; a proteinase inhibitor gene; and a mannitol dehydrogenase (*mt*1D) gene for drought and salt tolerance. So far, our research team confirmed that glufosinate ammonium has fungicidal as well as herbicidal properties. Therefore, we have been able to simultaneously control turfgrass pathogens (mainly *Sclerotinia ulnocarpal* and *Rhizoctonia solani*) as well as weeds by

spraying this herbicide on transgenic creeping bentgrass expressing the herbicide resistance gene under greenhouse conditions.

Studies have shown that chitinase genes can make transgenic plants resistant to pathogenic fungi such as *R. solani*. Research by Dr. Vargas' laboratory has shown that our transgenic creeping bentgrass clone 711, transcribing the elm chitinase gene controlled by the cauliflower mosaic virus 35S promoter, has improved resistance to *R. solani* under controlled environmental conditions. Dr. Sticklen's laboratory has constructed a plasmid containing the elm chitinase gene controlled by a rice actin promoter and transformed creeping bentgrass with this construct. Molecular analysis is in progress to test the expression of the chitinase gene regulated by this grass-specific promoter in creeping bentgrass.

The mannitol I-phosphate dehydrogenase gene for drought tolerance (*mt*1D) that we have used to transform creeping bentgrass is also associated with salt tolerance. Experiments performed by Dr. Baird's laboratory have not shown any drought tolerance in transgenic plants, nor have they shown accumulation of mannitol in transgenic plants. Antibodies were made against the enzyme, mannitol dehydrogenase, using synthetic peptides. Western blotting will be performed to test the level of expression of this gene in different transgenic lines.