Grass Seed Cropping Systems For A Sustainable Agriculture (GSCSSA)
2006 Progress Report

Title: DNA Sequence-based Seed Tests for Rapid Differentiation of Ryegrass Growth Types and Increased Economic Returns to Growers

Objectives:

1) Isolate and characterize vernalization candidate gene(s) in *Lolium sp*. and genetically map in the MF population to determine their association with responses to vernalization.
2) Compare the sequences of vernalization candidate genes from a panel of annual and perennial ryegrass cultivars to identify DNA-based markers, which will distinguish the growth types.
3) Identify DNA sequence differences in superoxide dismutase (SOD) and phosphoglucose isomerase (PGI) genes from both annual and perennial ryegrass cultivars to determine the genetic basis of the observed isozyme differences.
4) Develop procedures for a DNA-based seed test based on differences in the vernalization genes, SOD and PGI genes between growth types.
5) Train interested grass seed testers in the procedures for conducting DNA-based tests.

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Abstract of 2006 Progress:

We have made considerable progress in developing the prototype DNA Sequence-Based Multiplex Seed Test. Three DNA markers for genes from Lolium that are involved in the flowering or vernalization responses are being integrated into the protocol for the seed lab test. Additional markers to improve accuracy and optimize test efficiency and lower costs are being examined. The test is being validated on 20 SRF-tested ryegrass cultivars, including nonfluorescent plants and 'Gulf' plants as controls. This large panel (~900 plants) is being used to refine the test parameters to help reduce cost to growers and to increase sample throughput for seed labs.

Justification:

More accurate detection of seed lot contamination would benefit seed growers by reducing incorrect price reductions, and would benefit turf growers by reducing off-type plants in the turf. The Grow Out Test (GOT) delays time to market and does not fully estimate growth-type (over-estimates perennial-type plants). Gene specific markers would allow breeders to select for annual or perennial growth type at the seed or seedling stage rather than in the mature plant in the field. This would also increase the number of genetically variable individuals breeders could examine and enable faster progress in developing improved cultivars.

Project Status Report:

In the past few months, we have made considerable progress in developing the prototype DNA Sequence-Based Multiplex Seed Test for implementation in commercial seed laboratories. As stated in the objectives outlined in the proposal, we have designed a protocol that involves the following steps: 1) germinate seeds, 2) harvest seedling leaf material, 3) extract DNA, 4) perform PCR analysis on the DNA, and 5) visualize and score band profile results. A seed sample (currently 400 seeds) is germinated and the seedling root fluorescence (SRF) assay is performed, normally run for 14 days, but seedling leaves can be harvested at anytime after SRF is expressed. A leaf from individual seedlings having fluorescent roots is sampled for DNA extractions. The DNA extraction step is a routine laboratory procedure that can be performed in a number of ways depending upon the constraints of time, money and labor. Many commercial kits are available from various suppliers, which can save time and reduce labor costs, but cost more initially. Another factor to consider in choosing a DNA extraction method will be the number of samples that are being handled. Once the DNA is extracted, quantified and quality checked, the PCR (polymerase chain reaction) analysis will be performed. This procedure involves taking a small amount of the extracted DNA sample, adding a DNA polymerase enzyme and a few other ingredients and running the reactions on a thermocycler or PCR Machine. The products of these reactions are then digested with a second specific enzyme (commercially available) and the products are visualized by gel electrophoresis, a process that separates the DNA fragments by size and visualizes them as "bands" on a gel. The pattern of bands on the gel is scored to determine the allele type present. There are a number of commercially available systems that are designed for safety, reliability and speed of analysis. By combining the results from our various markers, we will be able to make a more accurate prediction of the ryegrass growth types than by using one measure alone.

Objectives and Timeline:

1) **Isolate and characterize vernalization candidate gene(s) in *Lolium sp*. and genetically map in the MF population to determine their association with responses to vernalization**.

July- Dec 2006

We have isolated and characterized three vernalization (LpVrn-1) or flowering- related (LpCO and ID1) genes from Lolium. These have been genetically mapped in the MF population. We are continuing to develop additional markers that will be useful in discriminating the Lolium growth types in a multiplex test.

2) **Compare the sequences of vernalization candidate genes from a panel of annual and perennial ryegrass cultivars to identify DNA-based markers which will distinguish the growth types. - In progress**

July- Dec 2006

Based on the sequences of the flowering-related genes (LpCO and ID1), we have identified DNA sequence differences that can be easily detected using standard laboratory procedures. For the vernalization gene (LpVrn-1), a sequence difference was identified between the annual cultivar 'Gulf' and the perennial cultivar 'Manhattan'. We are currently adapting the existing test so that it can be performed in the same procedure along with the LpCO and ID1 markers, further improving the accuracy of the test.

3) **Identify DNA sequence differences in superoxide dismutase (SOD) and phosphoglucose isomerase (PGI) genes from both annual and perennial ryegrass cultivars to determine the genetic basis of the observed isozyme differences**.
This work is in progress and expected to be completed by March 2007.

4) Develop procedures for a DNA-based seed test based on differences in the vernalization genes, SOD and PGI genes between growth types

July –Dec 2006

We assayed the LpCO and ID1 markers in at least ten individuals each from a panel of 8 annual and 13 perennial cultivars. The LpCO SNP was universally absent in the perennial cultivars tested, except for Derby Supreme (present in 2 of 12 individuals) and was present in approximately 50% of the annual types. Variation for the LpCO marker shows it is a good marker for perennial growth types, but is less consistent within annual-type cultivars.

The LpID1 is showing good potential to be used in combination with LpCO to predict ryegrass growth type. The LpID1 marker was present in 100% of the individuals from annual type cultivars and was absent in the perennial cultivars tested. We are working to further refine this marker and combine the assays to improve accuracy and decrease costs and time requirements of the test.

We are currently testing the *LpCO* and ID_1 markers on a larger panel of 20 ryegrass cultivars, including nonfluorescent controls and also a number of "Gulf" plants as controls. This larger panel (~874 plants) is being used to refine the test parameters.

5) Train grass seed testers in the procedures for conducting DNA-based tests.

This will be the last phase of the project and will be done in May-June 2007 and as interest warrants from grass seed testers. Several individuals have already visited our lab to observe progress and become familiar with the approach we are developing. During such visits we have discussed equipment that will need to be available in a seed testing lab and estimated cost per sample that will be determined largely by the equipment and pre-prepared supply options chosen by each particular lab.

Interactions with other researchers conducting related activities

We have ongoing interaction and collaboration with several other researchers in the turfgrass genetics research area, including Dr. Rebecca Nelson-Brown, from the University of Rhode Island and Dr. Scott Warnke, from the USDA-ARS in Beltsville, MD. Our cultivar panels being used to validate marker effectiveness are being performed on seed from actual SRF and grow-out tests performed by the OSU Seed Testing Lab and AgriSeed Testing, Inc.

Publications, Reports and Presentations

Cooper, L.D., R.E. Barker, S.E. Warnke and R.N. Brown. Using advances in plant genomics to develop a DNA-based test to benefit the ryegrass seed industry. p. 48-51. In W.C. Young III (ed.). 2005 Seed Production Research. Dept. Crop and Soil Sci. Ext/CrS 125, 4/06. 2006.

Cooper, Laurel D., Reed E. Barker, Rebecca Brown, Scott Warnke and Jim Dombrowski, DNA sequence-based multiplex seed test for rapid differentiation of ryegrass growth types. In Annual meetings abstracts [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI. 2006.