Discovery of Stress-responsive Cynodon *Genes by cDNA Sequences and Expression Profiles*

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Objectives:

1. Build the first Internet-accessible "gene encyclopedia" for *Cynodon* (bermudagrass).
2. Identify genes responsible for natural adaptation of different *Cynodon* taxa to diverse environments, especially genes associated with drought resistance.

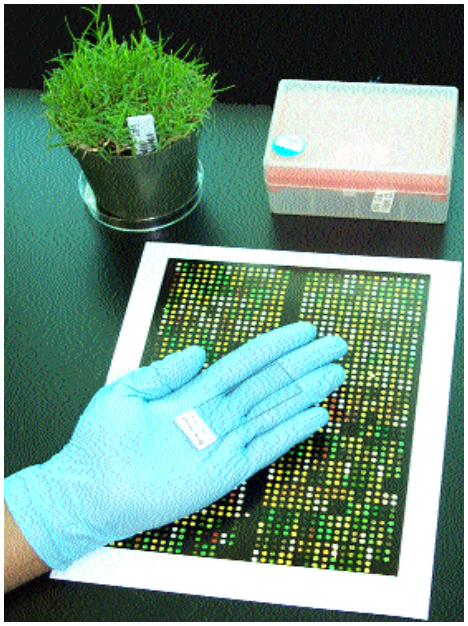
Start Date: 2003
Project Duration: three years
Total Funding: $90,000

Building on our development of the first "genetic map" of the *Cynodon* chromosomes, we will conduct the first large-scale exploration of the "sequences" (spelling) of large numbers of genes in any turfgrass, and use these resources to identify genes that are turned on or off in response to environmental stresses, emphasizing drought.

Our analytical approach will leverage a large body of existing data from other plants to determine the functions of many *Cynodon* genes. We will also leverage recent NSF grants to Dr. Paterson of $4 million for grass genomics, and $600,000 for informatics, in applying bioinformatic methods to the *Cynodon* ESTs, and making the resulting information available to the research community.

At present, very few gene sequences are known in any turfgrass. The world's leading DNA sequence repository, GenBank, presently contains only 269 *Cynodon* sequences, as compared to 247,980 for maize and 109,878 for sorghum. Our lab provided about half of the *Cynodon* gene sequences that are in GenBank. This project will increase the number of *Cynodon* gene sequences by about 15-fold to about 4,000.

We are engaged in the first crucial step - the production of a high-quality cDNA library for the *C. dactylon* genotype T89. A new graduate student, Mr. Changsoo Kim, working with Assistant Research Scientist (Terry Kamps), has produced a normalized cDNA library (i.e. abundance of individual clones is relatively even so sequencing will be efficient) and is presently picking a sampling of 36,864 clones for permanent archival by cryopreservation as a community resource. A



University of Georgia scientists are identifying genes responsible for natural adapataion of different Cynodon *taxa to diverse environments, especially genes associated with drought resistance.*

sampling of clones have been sequenced (about 200) and we intend to sequence the remaining clones over the next few months. The library has been amplified once, amplified samples frozen in small aliquots at -80^{o} C, and stored for future distribution to other interested researchers.

The primary cDNA library was made using specialized techniques to maximize the number of clones that are 'full-length', representing the entire transcribed region of a gene. In addition, the library was 'normalized' to reduce redundancy, and enhance the efficiency of discovering genes that are expressed at relatively low levels.

A total of 36,864 clones (96 384-well plates) from the library have been picked using a Genetix 'QBOT' (in the Paterson lab), and archived as a permanent resource. It is noteworthy that this is twice the number proposed to USGA. These have been replicated for insurance, and can be replicated again (at a cost of about $1000) if other researchers wish to have a set in their own labs. In addition, the library itself has been amplified, and amplified samples stored at -80^{o} C for future distribution to other researchers. Together, these resources provide a means to screen for the *Cynodon* homologs ('versions') of genes whose identities are known from research in a wide variety of other plants, or even animals.

A minimum of 4,608 clones will be sequenced and subjected to comparison to one another and to known sequences from other organisms. Sequencing is in progress. In addition, we partnered with A. Guenzi (OSU) to contribute low-cost sequencing to additional *Cynodon* cDNAs isolated in his research project described separately.

In addition, we plan a number of bio-informatic applications to the sequences, in particular identifying those that resemble genes with known functions and also using the new sequences to clarify the impact of an ancient whole-genome duplication in other grasses on the genome of *Cynodon*.

Summary Points

- At present, very few gene sequences are known in any turfgrass. The world's leading DNA sequence repository, GenBank, presently contains only 269 *Cynodon* sequences, as compared to 247,980 for maize and 109,878 for sorghum. This lab provided about half of the *Cynodon* gene sequences that are in GenBank. This project will increase the number of *Cynodon* gene sequences by about 15-fold to about 4,000.
- Once they have a library of satisfactory quality, 18,432 clones (48 384-well plates) will be picked using a Genetix 'QBOT' (in the Paterson lab). These will be replicated and maintained as a long-term resource that will be available to other researchers.