*Identification of Creeping Bentgrass (*Agrostis palustris *Huds.) Cultivars Using Simple Sequence Repeats (SSRs)*

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Objectives:

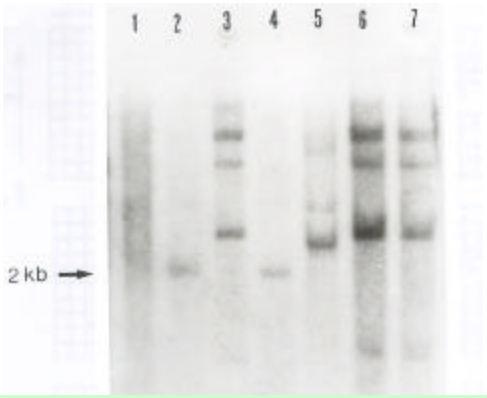
1. Construct a DNA library enriched for selected simple sequence repeats (SSRs).
2. Isolate and characterize approximately 30 loci from the SSR-enriched creeping bentgrass DNA library.
3. Use SSRs to explore diversity within closely and distantly related creeping bentgrass cultivars.
4. Explore genetic relationships among creeping bentgrass cultivars.
5. Determine the optimum sample size needed to represent cross pollinated cultivars.
6. Assess the utility of isolated SSR loci for use within other *Agrostis* species.

Start Date: 2000
Project Duration: 2 years
Total Funding: $49,880

Current methods of cultivar identification (RAPDs, RFLPs, isozymes, and morphological characteristics) within creeping bentgrass have failed to discriminate with certainty among closely related individuals. There is a need for more sensitive methods to distinguish creeping bentgrass cultivars and assess genetic relationships. Simple sequence repeats (SSRs) are a class of molecular markers that have proven to be useful genetic tools in countless plant species.

SSRs are tandemly repeated DNA sequences (e.g. GAGAGAGAGA) found at many locations within the genomes of eukaryotic organisms. SSRs consist of a core repeating unit (e.g. GA) that can range from one (e.g. A ...) to six base pairs long (e.g. GACTGT ...). SSR polymorphisms, or differences among individuals, exist due to variation in the length of the SSR repeat. This variation can be monitored with ease using a technique called the polymerase chain reaction (PCR), which utilizes short sequences or primers that flank the SSR.

Four general features make SSRs useful genetic markers. First, they are widely dispersed throughout eukaryotic genomes, making them useful for linkage mapping. Second, they can be assayed on automated DNA sequencers, making them relatively easy to score. Third, unlike other PCR-based markers, SSRs are co-dominant (i.e. both maternal and paternal contribution can be scored). Co-dominance is useful for identifying heterozygous individuals, making SSRs the tool of choice for genetic analysis and marker-assisted selection. Finally, some SSR loci can be highly polymorphic. Other molecular-marker-based fingerprinting techniques (RAPDs, RFLPs, isozymes) have been criticized for their lack of polymorphism among closely related germplasm. However, the polymorphism exhibited at SSR loci has proven useful for distinguishing among even the most closely related cultivars.



Cultivar identification has been done using isozymes and RAPDs, but both methods fail to discriminate with certainty among closely related individuals.

Over a two-year period, we are isolating simple sequence repeats (SSRs) for cultivar identification in creeping bentgrass. Once isolated, the SSRs are being used to study diversity within and among creeping bentgrass cultivars. We also are testing the utility of SSRs across *Agrostis* species for future synteny mapping. We have already studied the utility of SSRs for cultivar identification in perennial ryegrass and found them useful for establishing genetic relationships and for distinguishing closely related perennial ryegrass clones. This work should be applicable to our studies in creeping bentgrass.

We currently have GT, GA, GCT, and AAT enriched creeping bentgrass DNA libraries. We have isolated 47 polymorphic SSR loci, and the level of polymorphism for each SSR locus has been assessed on a small population. Based on our assessments, the 30 most polymorphic loci have been chosen to genetically profile 11 creeping bentgrass cultivars. So far 11 cultivars (each represented with 30 individual plants) have been profiled at 10 SSR loci. Data from an additional 20 SSR loci is currently being generated. However, our initial analysis indicates that SSRs will be more than adequate for distinguishing among both closely and distantly related cultivars and clones.

Summary Points

- Forty-seven polymorphic SSR loci have been isolated. Thirty loci have been used to profile 11 creeping bentgrass cultivars at 10 SSR loci. Data for the remaining 20 loci is currently being generated.
- Initial analysis indicates SSRs will be more than adequate to distinguish among both closely and distantly related creeping bentgrass cultivars and clones.
- Twenty-four of the 47 creeping bentgrass SSR loci were tested on several colonial bentgrass, dryland bentgrass, redtop, and velvet bentgrass clones. Eighteen of the 24 creeping bentgrass SSR loci are successfully amplified in all of the *Agrostis* species mentioned above.
- These results indicate that there might be a use for these SSRs for the detection of hybrids within species and for synteny map studies.