*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, and isozymes) within creeping bentgrass fail to discriminate with certainty among closely related individuals. There is a need for more sensitive methods to distinguish cultivars and assess genetic relationships.

Simple sequence repeats (SSRs) are a class of genetic marker that have proven to be used in plants. SSRs are tandemly repeated DNA sequences with a core repeat motif length six base pairs or less. SSR polymorphisms, or differences among individuals, exist due to variation in the length of the SSR repeat. This variation can be easily monitored using a technique called the polymerase chain reaction (PCR), which utilizes short sequences or primers that flank the SSR.

Four features make the 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 the maternal and paternal contribution can be scored). Finally, SSRs can be highly polymorphic, and this polymorphism is useful for distinguishing among closely related varieties and for assessing genetic relationships among individuals.

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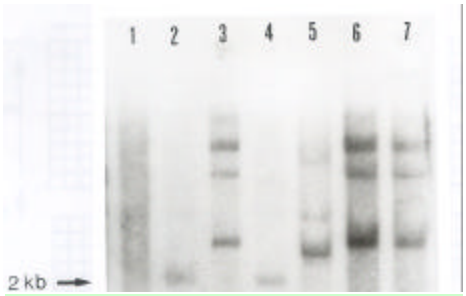
The demand for new and improved cultivars of bentgrass results in the development and release of several new cultivars each year. Reliable and definitive cultivar ientification becomes critical to maintain varietal purity and to protect breeder and consumer rights.

Over a two-year period we have proposed to isolate genetic markers called simple sequence repeats (SSRs) for cultivar identification in creeping bentgrass. Once isolated, the SSRs will be used to study diversity within and among creeping bentgrass cultivars. We also propose to test the utility of the SSRs across *Agrostis* species.

Simple Sequence Repeats have not been used for distinguishing creeping bentgrass cultivars. Cultivar identification has been done using isozymes and RAPDs, but both methods fail to discriminate with certainty among closely related individuals. In addition, isozymes, RAPDs, and RFLPs often lack enough alleles at a given locus to be used as linkage traits for 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. We have also determined that the optimum sample size



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

needed to represent a perennial ryegrass cultivar is at least 20 individuals and the optimum number of SSR loci necessary to represent a cultivar is 15. This work should be applicable to our studies in creeping bentgrass.

We hope this research will result in tools that will help with the following: 1) maintenance of varietal purity 2) protection of breeder and consumer rights 3) assessment of diversity within and among creeping bentgrass cultivars as well as other *Agrostis* species, 4) identification of persisting clones in an overseeded green. and 5) once initially isolated, SSRs will also be useful for linkage mapping which will make marker assisted breeding possible.

Summary Points

- Only two bentgrass SSRs have been produced. More than 30 are needed to detect differences between closely related cultivars.
- The optimum sample size needed to represent a perennial ryegrass cultivar is at least 20 individuals and the optimum number of SSR loci necessary to adequately distinguish the cultivar is 15.